Advance Research Trends in Biology

First Edition

Dr. R.B. Tripathi
Dr. N. Aarthi
Dr. P. Madhiyazhagan

Thanuj International Publishers,
Tamil Nadu, India

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Preface

Advance Research in Trends in Biology have brought about remarkable progress in various fields, including Zoology, Vermicomposting, Bioinstrumentation, Herpetology, Entomology, Toxicology, Microbiology, Biological fertilizers, Ecosystems, Endocrinology, Phycology, Plant pathology, Mycotoxins, Biopesticides, Microbiology, Chromatography, Bioinformatics, Conservation Biology, Pharmacognosy, Marine pollution and Oceanography. We aim to foster scientific curiosity, inspire further research and contribute to the advancement of knowledge in these fields.

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About Editors

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Dr. P. Madhiyazhagan is currently serving as an Assistant Professor in the Department of Zoology at J. K. K. Nataraja College of Arts and Science. With 8 years of combined teaching and research experience, he has established himself as an expert in the field of entomology, particularly focusing on green Nano-based mosquito and agricultural pest control. He has an impressive publication record, having authored 62 research articles and 9 book chapters in highly reputable journals. His contributions have garnered a total impact factor of 84.5, with 3389 citations, h-index of 30, and i-index of 42. In addition to his research accomplishments, he has actively participated in the academic community. He has served as a reviewer and editorial board member for prestigious publications, including Springer and Elsevier. He is also a lifetime member of various scientific organizations. Furthermore, he has shared his expertise as a resource person in seminars and short-term courses. His commitment to both research and education underscores his significant contributions to the field of Entomology.

Dr. N. Aarthi is currently serving as an Assistant Professor in the Department of Zoology at Sri Sarada College for Women (Autonomous), Salem With 7 years of combined teaching and research experience, she has established herself as an expert in the field of entomology and endocrinology, particularly focusing on biology and role of insects in the environment and the significance of hormones for the well being of human. She has an impressive publication record, having authored 20 research articles and 4 book chapters in highly reputable journals. Her contributions have garnered with 910 citations, h-index of 8, and i-index of 8. In addition to her research accomplishments, she has actively participated in the academic community. She has served as a reviewer and editorial board member for prestigious publications, including Hindawi. She is also a lifetime member of various scientific organizations. Furthermore, she has shared his expertise as a resource person in seminars and webinars. Her commitment to both research and education underscores her significant contributions to the field of Entomology.

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General organization and Affinities of Crocodilia

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Introduction

Crocodilia is an order of mostly large, predatory, semiaquatic reptiles, known as **crocodilians**. They first appeared 95 million years ago in the Late Cretaceous period (Cenomanian stage) and are the closest living relatives of birds, as the two groups are the only known survivors of the Archosauria. Members of the order's total group, the clade Pseudosuchia, appeared about 250 million years ago in the Early Triassic period, and diversified during the Mesozoic era.

3 families are crocodiles (family Crocodylidae),the alligators and caimans (family Alligatoridae),and the gharial and false gharial (family Gavialidae). Although the term 'crocodiles' is sometimes used to refer to all of these, crocodilians are a less ambiguous vernacular term for members of this group. Large, solidly built, lizard-like reptiles, crocodilians have long flattened snouts, laterally compressed tails, and eyes, ears, and nostrils at the top of the head. They swim well and can move on land in a "high walk" and a "low walk", while smaller species are even capable of galloping. Their skin is thick and covered in non-overlapping scales. They have conical, peg-like teeth and a powerful bite. They have a four-chambered heart and, somewhat like birds, a unidirectional looping system of airflow within the lungs, but like other living reptiles they are ectotherms.

Crocodilians are found mainly in lowlands in the tropics, but alligators also live in the southeastern United States and the Yangtze River in China. They are largely carnivorous, the various species feeding on animals such as fish, crustaceans, molluscs, birds, and mammals; some species like the Indian gharial are specialised feeders, while others like the saltwater crocodile have generalised diets. Crocodilians are typically solitary and territorial, though cooperative feeding does occur. During breeding, dominant males try to monopolise available females. Females lay eggs in holes or in mounds and, unlike most other reptiles, care for their hatched young.

Some species of crocodilians are known to have attacked humans. The largest number of attacks comes from the Nile crocodile. Humans are the greatest threat to crocodilian populations through activities that include hunting, poaching, and habitat destruction, but farming of crocodilians has greatly reduced unlawful trading in wild skins. Artistic and literary representations of crocodilians have appeared in human cultures around the world since Ancient Egypt.

Characteristic Features:

1. They are carnivorous and freshwater reptiles. They swim by the undulation of their powerful tail.
2. The limbs are not powerful as the tail and are used in carrying the body on land.
3. The forelimbs are shorter than the hind and have five digits in the forelimbs and four digits inthe hind limbs. The digits of the forelimbs are webbed.
4. The body is elongated and the skin bears epidermal scales which are supported by dermal bones or scutes. The scales are supported by dermal plates osteoderms.
5. The tail is laterally compressed.
6. The cloacal aperture is longitudinal, i.e., elongated in the direction of the long axis of the body.
7. Males are provided with a single and median erectile copulatory organ.
8. A clitoris occurs in female.
9. The teeth are thecodont (advanced feature) and are borne on premaxillae, maxillae and dentaries.
10. The teeth contain persistent pulp.
11. The oesophagus can be distended to store food.
12. The stomach suggests a bird's gizzard for the muscular walls are strong, and glandular pyloric end is twisted upward. Very often stones measuring about 2.5 cm in diameter are found inside stomach. The cavity of the mouth is bounded behind by two soft transverse membranes which meet when the animal draws the prey.
13. The nostrils are situated at the tip of the snout.

14. The external narial openings are opened by longitudinal dilator muscle and closed by a constrictor muscle. During submersion in water the external narial openings are closed. The muscle fibres comprising both these muscles are un-striated and are controlled by the sympathetic nervous system.
15. The internal nasal aperture is situated at the back of the mouth.
16. The lungs are invested by pleural sacs.
17. An incipient diaphragm (advanced feature) is situated between thoracic and abdominal wall.
18. The heart is four-chambered and the inter-ventricular septum is complete (advanced feature).
19. The roots of the left and right aortic arches are twisted and communicated by the foramen of Panizza (Fig. 8.59) through which an interchange of blood does not take place.
20. The crocodilians are ectothermic animals.
21. The brain is well-formed. The cerebellum shows the development of a median lobe (vermis) and two lateral lobes (flocculi).
22. The eyes are provided with pecten (advanced feature).
23. The nervous system and the sense organs show many avian features.
24. The auditory organs have a substantial lagena.
25. The tympanic membrane is sunk in a pit (advanced feature) and protected by two scalymovable flaps. These flaps are operated by special muscle and shut the external auditory meatus when the crocodiles dive.
26. The eggs are laid in excavated burrows and need no incubation.
27. The skin glands are situated on the margin of lower jaw, round cloacal aperture and on dorsal scutes. Secretion of the glands smells like musk and becomes very strong during breeding season.
28. The skull is highly sculptured with persistent sutures29. Inter-orbital septum with large alisphenoid is present.
30. Inter-orbital septum is well-developed.
31. Maxillae, palatine and pterygoids meet at the middle line of the roof of the skull and determine the position of posterior nares.

32. Transpaiatine bone is present.
33. The quadrate is large and immovable.
34. The inter-parietal foramen is absent.
35. Internal nasal aperture is single.
36. A bony secondary palate is present which is formed by the fusion of shelves grown out fromthe maxillae, palatines and pterygoids (advanced feature).
37. Lower jaw is composed of a cartilaginous articular working on quadrate and five membranebones.
38. The vertebral column is divisible into cervical, thoracic, lumbar, sacral and caudal regions.
39. Vertebrae are either amphicoelous or procoelous excepting the first two cervicals, sacrals andfirst caudal.
40. There is a pro-atlas in between skull and atlas.
41. The first caudal has convexity at both ends.
42. There are two sacral vertebrae.
43. The caudal vertebrae are provided with chevron bones.
44. The anterior thoracic vertebrae bear bifid and elongated transverse processes.
45. Sternal and abdominal ribs (gastralia) are present.
46. Sternal ribs have uncinate process. Most of the ribs are double-headed (advanced feature).
47. The pectoral girdle consists of dorsal scapulae and ventral coracoids.
48. The clavicles are absent.
49. The epicoracoids are thin strips between sternum and coracoid.
50. The episternum is feebly developed.
51. The coracoids are perforated.
52. The pelvic girdle consists of large ilia, pubes and ischia.
53. The epipubis is present and the symphysis is ischio-pubic in nature.

54. The pubes are small and do not participate in the formation of the acetabulum.

Origin:

The common term “crocodile” comes from the Greek Krokodeilos meaning lizard. Crocodilians may have descended from some group of Triassic thecodonts, the stem group of all archosaurian reptiles and birds. The oldest crocodilian fossil is Proterochampsa barrionuevoi which has been discovered from Triassic beds of Western Argentina. Another fossil of uppermost Triassic, Protosuchus, has discovered from Arizona. These two fossils with others formed a group, called Protosuchia. The protosuchians were about 1.5 m in length. The mesosuchians were evolved from the protosuchian ancestors and were represented by Teleosaurus and Steneosaurus. They lived in huge numbers from lower Jurassic to the end of Cretaceous. The nasal passage of the mesosuchians was completely separated from the mouth cavity. During Cretaceous period, the advanced crocodilians, the Sebacosuchians and the Eusuchians, arose from the Mesosuchians. The sebacosuchians line of evolution was represented by Sabacus and Baurusuchus. These animals were restricted to South America and soon became extinct. The eusuchian line of evolution represents three modern groups of Crocodiles — Crocodylidae, Alligatoridae and Gavialidae. Among the major groups of reptiles, the Order Crocodilia includes the largest forms of living reptiles.

Distribution and Habitats:

Crocodilians are amphibious reptiles, spending part of their time in water and part on land. The last surviving fully terrestrial genus, Mekosuchus, became extinct about 3000 years ago after humans had arrived on its Pacific islands, making the extinction possibly anthropogenic.Typically they are creatures of the tropics; the main exceptions are the American and Chinese alligators, whose ranges consist of the south-eastern United States and the Yangtze River, respectively. Florida, in the United States, is the only place that crocodiles and alligators live side by side. Most crocodilians live in the lowlands, and few are found above 1,000 metres (3,300 ft), where the temperatures are typically about 5 °C (9 °F) lower than at the coast. None of them permanently reside in the sea, though some can venture into it, and several species can tolerate the brackish water of estuaries, mangrove swamps, and the extreme salinity of hypersaline lakes. The saltwater crocodile has the widest distribution of any crocodilian, with a range extending from eastern India to New Guinea and northern Australia. Much of its success is due to its ability to swim out to sea and colonise new locations, but it is not restricted to the marine environment and spends much time in estuaries, rivers, and large lakes.

Various types of aquatic habitats are used by different crocodilians. Some species are relatively more terrestrial and prefer swamps, ponds, and the edges of lakes, where they can bask in the sun and there is plenty of plant life supporting a diverse fauna. Others spend more time in the water and inhabit the lower stretches of rivers, mangrove swamps, and estuaries. These habitats also have a rich flora and provide plenty of food. The Asian gharials find the fish on which they feed in the pools and backwaters of swift rivers. South American dwarf caimans inhabit cool, fast flowing streams, often near waterfalls, and other caimans live in warmer, turbid lakes and slow moving rivers. The crocodiles are mainly river dwellers, and the Chinese alligator is found in slow-moving, turbid rivers flowing across China's floodplains. The American alligator is an adaptable species and inhabits swamps, rivers, or lakes with clear or turbid water. Climatic factors also affect crocodilians' distribution locally. During the dry season, caimans can be restricted to deep pools in rivers for several months; in the rainy season, much of the savanna in the Orinoco Llanos is flooded, and they disperse widely across the plain. Desert crocodiles in Mauritania have adapted to their arid environment by staying in caves or burrows in a state of aestivation during the driest periods. When it rains, the reptiles gather at gueltas. Dry land is also important as it provides opportunities for basking, nesting, and escaping from temperature extremes. Gaping allows evaporation of moisture from the mouth lining and has a cooling effect, and several species make use of shallow burrows on land to keep cool. Wallowing in mud can also help prevent them from overheating. Four species of crocodilians climb trees to bask in areas lacking a shoreline. The type of vegetation bordering the rivers and lakes inhabited by crocodilians is mostly humid tropical forest, with mangrove swamps in estuarine areas. These forests are of great importance to the crocodilians, creating suitable microhabitats where they can flourish. The roots of the trees absorb water when it rains, releasing it back slowly into the environment. When the forests are cleared to make way for agriculture, rivers tend to silt up, the water runs off rapidly, the water courses can dry up in the dry season and flooding can occur in the wet season. Destruction of forest habitat is probably a greater threat to crocodilians than hunting.

Morphology

Crocodilians range in size from the *Paleosuchus* and *Osteolaemus* species, which reach 1–1.5 m (3 ft 3 in – 4 ft 11 in), to the saltwater crocodile, which reaches 7 m (23 ft) and weighs up to

2,000 kg (4,400 lb), though some prehistoric species such as the late Cretaceous *Deinosuchus* were even larger at up to about 11 m (36 ft) and 3,450 kg (7,610 lb). They tend to be sexually dimorphic, with males much larger than females. Though there is diversity in snout and tooth shape, all crocodilian species have essentially the same body morphology. They have solidly built, lizard-like bodies with elongated, flattened snouts and laterally compressed tails. Their limbs are reduced in size; the front feet have five digits with little or no webbing, and the hind feet have four webbed digits and a rudimentary fifth. The skeleton is somewhat typical of tetrapods, although the skull, pelvis and ribs are specialised; in particular, the cartilaginous processes of the ribs allow the thorax to collapse during diving and the structure of the pelvis can accommodate large masses of food, or more air in the lungs. Both sexes have acloaca, a single chamber and outlet at the base of the tailinto which the intestinal, urinary and genital tracts open. It houses the penis in males and the clitoris in females. The crocodilian penis is permanently erect and relies on cloacal muscles for eversion and elastic ligaments and a tendon for recoil. The gonads are located near the kidneys.

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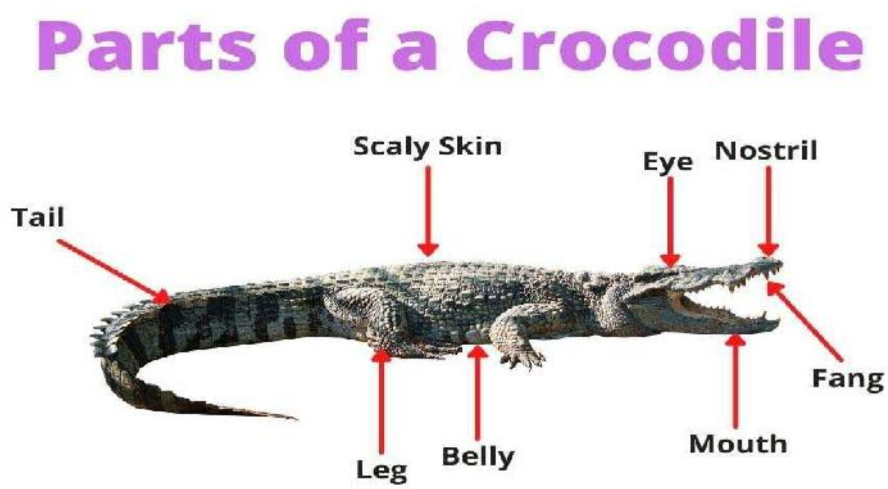
The eyes, ears and nostrils of crocodilians are at the top of the head. This allows them to stalk their prey with most of their bodies underwater. Crocodilians possess a *tapetum lucidum* which enhances vision in low light. While eyesight is fairly good in air, it is significantly weakened underwater. The fovea in other vertebrates is usually circular, but in crocodiles it is a horizontal bar of tightly packed receptors across the middle of the retina. When the animal completely submerges, the nictitating membranes cover its eyes. In addition, glands on the nictitating membrane secrete a salty lubricant that keeps the eye clean. When a crocodilian leaves the water and dries off, this substance is visible as "tears".

The ears are adapted for hearing both in air and underwater, and the eardrums are protected by flaps that can be opened or closed by muscles. Crocodilians have a wide hearing range, with sensitivity comparable to most birds and many mammals. They have only one olfactory chamber and the vomeronasal organ is absent in the adults indicating all olfactory perception is limited to the olfactory system.



Skin and Scales

The skin of crocodilians is thick and cornified, and is clad in non-overlapping scales known as scutes, arranged in regular rows and patterns. These scales are continually being produced by cell division in the underlying layer of the epidermis, the stratum germinativum, and the surface of individual scutes sloughs off periodically. The outer surface of the scutes consists of the relatively rigid beta-keratin while the hinge region between the scutes contains only the more pliable alpha-keratin.

Many of the scutes are strengthened by bony plates known as osteoderms, which are the same size and shape as the superficial scales but grow beneath them. They are most numerous on the back and neck of the animal and may form a protective armour. They often have prominent, lumpy ridges and are covered in hard-wearing beta-keratin. The head and jaws lack actual scales and are instead covered in tight keratinised skin that cracks due to stress. The skin on the neck and flanks is loose, while that on the abdomen and underside of the tail is sheathed in large, flat square scutes arranged in neat rows. The scutes contain blood vessels and may act to absorb or radiate heat during thermoregulation. Research also suggests that alkaline ions released into the blood from the calcium and magnesium in these dermal bones act as a buffer during prolonged submersion when increasing levels of carbon dioxide would otherwise cause acidosis.

Some scutes contain a single pore known as an integumentary sense organ. Crocodiles and gharials have these on large parts of their bodies, while alligators and caimans only have them on the head. Their exact function is not fully understood, but it has been suggested that they may be mechanosensory organs. Another possibility is that they may produce an oily secretion that prevents mud from adhering to the skin. There are prominent paired integumentary glands in skin folds on the throat, and others in the side walls of the cloaca. Various functions for these have been suggested. They may play a part in communication, as indirect evidence suggest that they secrete pheromones used in courtship or nesting. The skin of crocodilians is tough and can withstand damage from conspecifics, and the immune system is effective enough to heal wounds within a few days.

Locomotion

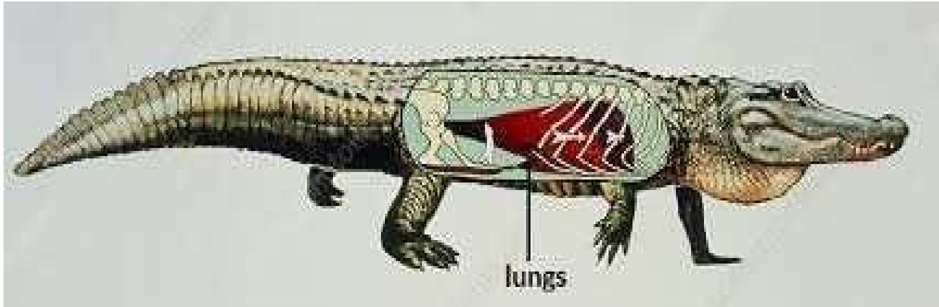
Crocodilians are excellent swimmers. During aquatic locomotion, the muscular tail undulates from side to side to drive the animal through the water while the limbs are held close to the body to reduce drag. When the animal needs to stop, steer, or man oeuvre in a different direction, the limbs are splayed out. Crocodilians generally cruise slowly on the surface or underwater with gentle sinuous movements of the tail, but when pursued or when chasing prey, they can move rapidly. Crocodilians are less well-adapted for moving on land, and are unusual among vertebrates in having two different means of terrestrial locomotion: the "high walk" and the "low walk”. Their ankle joints flex in a different way from those of other reptiles, a feature they share with some early archosaurs. One of the upper row of ankle bones, the astragalus, moves with the tibia and fibula. The other, the calcaneum, is functionally part of the foot, and has a socket into which a peg from the astragalus fits. The result is that the legs can be held almost vertically beneath the body when on land, and the foot can swivel during locomotion with a twisting movement at the ankle.

The high walk of crocodilians, with the belly and most of the tail being held off the ground, is unique among living reptiles. It somewhat resembles the walk of a mammal, with the same sequence of limb movements: left fore, right hind, right fore, left hind. The low walk is similar to the high walk, but without the body being raised, and is quite different from the sprawling walk of salamanders and lizards. The animal can change from one walk to the other instantaneously, but the high walk is the usual means of locomotion on land. The animal may push its body up and use this form immediately, or may take one or two strides of low walk before raising the body higher. Unlike most other land vertebrates, when crocodilians increase their pace of travel they increase the speed at which the lower half of each limb (rather than the whole leg) swings forward; by this means, stride length increases while stride duration decreases

Though typically slow on land, crocodilians can produce brief bursts of speed, and some can run at 12 to 14 km/h (7.5 to 8.7 mph) for short distances. A fast entry into water from a muddy bank can be effected by plunging to the ground, twisting the body from side to side and splaying out the limbs. In some small species such as the freshwater crocodile, a running gait can progress to a bounding gallop. This involves the hind limbs launching the body forward and the fore limbs subsequently taking the weight. Next, the hind limbs swing forward as the spine flexes dorsoventrally, and this sequence of movements is repeated. During terrestrial locomotion, a crocodilian can keep its back and tail straight, since the scales are attached to the vertebrae by muscles. Whether on land or in water, crocodilians can jump or leap by pressing their tails and hind limbs against the substrate and then launching themselves into the air.

Respiration

Crocodilians were traditionally thought to breathe like mammals, with airflow moving in and out tidally, but studies published in 2010 and 2013 conclude that crocodilians breathe more like birds, with airflow moving in a unidirectional loop within the lungs. When a crocodilian inhales, air flows through the trachea and into two primary bronchi, or airways, which branch off into narrower secondary passageways. The air continues to move through these, then into even narrower tertiary airways, and then into other secondary airways which were bypassed the first time. The air then flows back into the primary airways and is exhaled. These aerodynamic valves within the bronchial tree have been hypothesised to explain how crocodilians can have unidirectional airflow without the aid of avian-like air sacs.



The lungs of crocodilians are attached to the liver and the pelvis by the diaphragmaticus muscle (analogous of the diaphragm in mammals). During inhalation, the external intercostal muscles expand the ribs, allowing the animal to take in more air, while the ischiopubis muscle causes the hips to swing downwards and push the belly outward, and the diaphragmaticus pulls the liver back. When exhaling, the internal intercostal muscles push the ribs inward, while the rectus abdominis pulls the hips and liver forwards and the belly inward. Because the lungs expand into the space formerly occupied by the liver and are compressed when it moves back into position, this motion is sometimes referred to as a "hepatic piston". Crocodilians can also use these muscles to adjust the position of their lungs; thereby controlling their buoyancy in the water. An animal sinks when the lungs are pulled towards the tail and floats when they move back towards the head. This allows them to move through the water without creating disturbances that could alert potential prey. They can also spin and twist by moving their lungs laterally.

Swimming and diving crocodilians appear to rely on lung volume more for buoyancy than oxygen storage. Just before diving, the animal exhales to reduce its lung volume and achieve negative buoyancy. When submerging, the nostrils of a crocodilian shut tight. All species have a palatal valve, a membranous flap of skin at the back of the oral cavity that prevents water from flowing into the throat, oesophagus, and trachea. This enables them to open their mouths underwater without drowning. Crocodilians typically remain underwater for fifteen minutes or less at a time, but some can hold their breath for up to two hours under ideal conditions. The maximum diving depth is unknown, but crocodiles can dive to at least 20 m (66 ft).

Diet and Digestion

Jaws and Teeth

The snout shape of crocodilians varies between species. Crocodiles may have either broad or slender snouts, while alligators and caimans have mostly broad ones. Gharials have snouts that are extremely elongated. The muscles that close the jaws are much more massive and powerful than the ones that open them, and a crocodilian's jaws can be held shut by a person fairly easily. Conversely, the jaws are extremely difficult to pry open. The powerful closing muscles attach at the median portion of the lower jaw and the jaw hinge attaches to the atlanto-occipital joint, allowing the animal to open its mouth fairly wide.

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Crocodilians have some of the strongest bite forces in the animal kingdom. In a study published in 2003, an American alligator's bite force was measured at up to 2,125 lbf (9.45 kN). In a 2012 study, a saltwater crocodile's bite force was measured even higher, at 3,700 lbf (16 kN). This study also found no correlation between bite force and snout shape. Nevertheless, the gharial's extremely slender jaws are relatively weak and built more for quick jaw closure. The bite force of *Deinosuchus* may have measured 23,000 lbf (100 kN), even greater than that of theropod dinosaurs like *Tyrannosaurus*.

Crocodilian teeth vary from blunt and dull to sharp and needle-like. Broad-snouted species have teeth that vary in size, while those of slender-snouted species are more uniform. The teeth of crocodiles and gharials tend to be more visible than those of alligators and caimans when the jaws are closed. The easiest way to distinguish crocodiles from alligators is by looking at their jaw line. The teeth on the lower jaw of an alligator fit into sockets in the upper jaw, so only the upper teeth are visible when the mouth is closed. The teeth on the lower jaw of a crocodile fit into grooves on the outside of the top jaw making both the upper and lower teeth visible when the mouth is closed.

Crocodilians are homodonts, meaning each of their teeth are all of the same type (they do not possess different tooth types, such as canines and molars) and polyphyodonts are able to replace each of their approximately 80 teeth up to 50 times in their 35 to 75-year lifespan. They are the only non-mammalian vertebrates with tooth sockets. Next to each full-grown tooth there is a small replacement tooth and an odontogenic stem cell in the dental lamina in standby, which can be activated when required. Tooth replacement slows significantly and eventually stops as the animal grows old.

Diet: By the structure of jaws and teeth, it appears that crocodilians are efficient predators but most of the crocodiles are nocturnal hunters and spend at most of the day time by basking. So field observations during day do not reveal a large number of prey species. The variety of prey species is counted by the examination of stomach contents.

The prey species is markedly different with the change of age, size and habitat. Diet of young crocodilians includes insects, small fishes, snails, crabs, shrimps, tadpoles and frogs. Food of sub adult and adult crocodilians consists of a bulk of fish (about 70%), crabs, terrapins and turtles, birds and small and large mammals.

With the change of the habitat, their food also changes. In the brackish-water and in the estuarine zone, the diet of the crocodilians includes mud or fiddler crabs, mud skippers, prawns, shrimps, insects, molluscs and a variety of fishes. Crocodilians of the swampy and freshwater system feed on tadpoles, frogs, snails and a variety of freshwater fishes.

The food of long snouted crocodilians such as Gharial (Gavialis gangeticus), False Gharial (Tomistoma schegeli), African Long Snouted Crocodile (Crocodylus cataphractus) etc. is mainly fish. Crabs, frogs, birds and small mammals are also reported. The Estuarine crocodile (C. porosus) of Indo-Pacific region subsists on cattle, buffaloes, crab-eating monkeys, squirrels, goats, sheep wallabies and some birds.

Fish also forms a part of the diet. Diet of adult Mugger (C. palustris) includes frogs, snakes, fish, birds and small mammals. Among mammals, monkeys, sambars (Cervus unicolor) and gaur (Bos gaurus) are recorded. Large adult Nile Crocodile (C. niloticus) eats antelope, zebra, warthog, man and large domestic animals. Adult American Alligator (Alligator mississippiensis) consumes turtles, snakes, fish, birds and small mammals. Very occasionally eating of man has been reported.

Crocodilian teeth are adapted for seizing and holding prey, and food is swallowed unchewed. The digestive tract is relatively short, as meat is a fairly simple substance to digest. The stomach is divided into two parts: a muscular gizzard that grinds food, and a digestive chamber where enzymes work on it. The stomach is more acidic than that of any other vertebrate and contains ridges for gastroliths, which play a role in the mechanical breakdown of food. Digestion takes place more quickly at higher temperatures. Crocodilians have a very low metabolic rate and consequently, low energy requirements. This allows them to survive for many months on a single large meal, digesting the food slowly. They can withstand extended fasting, living on stored fat between meals. Even recently hatched crocodiles are able to survive 58 days without food, losing 23% of their bodyweight during this time. An adult crocodile needs between a tenth and a fifth of the amount of food necessary for a lion of the same weight, and can live for half a year without eating. *Amona glabra* (Pond-apple tree) seeds recovered from the stomach of an *Alligator mississippiensis* (American alligator) captured in the Florida Coastal Everglades were nonviable under ideal germination conditions and were likely destroyed by stomach acids. Alligators are unlikely to be dispersers of Pond-apple seeds and may instead act as seed predators.

Circulation

The crocodilian has perhaps the most complex vertebrate circulatory system. It has a fourchambered heart and two ventricles, an unusual trait among extant reptiles, and both a left and right aorta which are connected by a hole called the Foramen of Panizza. Like birds and mammals, crocodilians have heart valves that direct blood flow in a single direction through the heart chambers. They also have unique cog-teeth-like valves that, when interlocked, direct blood to the left aorta and away from the lungs, and then back around the body. This system may allow the animals to remain submerged for a longer period, but this explanation has been questioned. Other possible reasons for the peculiar circulatory system include assistance with thermoregulatory needs, prevention of pulmonary oedema, or faster recovery from metabolic acidosis. Retaining carbon dioxide within the body permits an increase in the rate of gastric acid secretion and thus the efficiency of digestion, and other gastrointestinal organs such as the pancreas, spleen, small intestine, and liver also function more efficiently.

When submerged, a crocodilian's heart rate slows down to one or two beats a minute, and blood flow to the muscles is reduced. When it rises and takes a breath, its heart rate speeds up in seconds, and the muscles receive newly oxygenated blood. Unlike many marine mammals, crocodilians have little myoglobin to store oxygen in their muscles. During diving, muscles are supplied with oxygen when an increasing concentration of bicarbonate ions causes haemoglobin in the blood to release oxygen.

Nervous System

The crocodile is part of the class of reptiles and all brains and nervous systems in this class are fairly similar. Out of the reptiles the crocodile is considered to have one of the most advanced nervous systems. The crocodile has very strong sensory organs, the strongest being its sense of touch. The crocodiles have small bumps around their body that are essentially bundled up nerve tissue. These bumps detect even the slightest movements in the water, enhancing the crocodiles effectiveness in survival and as a predator.

Excretory System

The kidneys and excretory system are much the same as in other reptiles, but crocodilians do not have a bladder. In fresh water, the osmolality (the concentration of solutes that contribute to a solution's osmotic pressure) in the plasma is much higher than it is in the surrounding water. The animals are well-hydrated, and the urine in the cloaca is abundant and dilute, nitrogen being excreted as ammonium bicarbonate. Sodium loss is low and mainly takes place through the skin in freshwater conditions. In seawater, the opposite is true. The osmolality in the plasma is lower than the surrounding water, which is dehydrating for the animal. The cloacal urine is much more concentrated, white, and opaque, with the nitrogenous waste being mostly excreted as insoluble uric acid.

Reproduction:

Courtship

Courtship starts in December and mating takes place in January and February. The females first indicate the willingness of mating by raising the snout upward. Crocodilians are generally polygynous, and individual males try to mate with as many females as they can. Monogamous pairings have been recorded in American alligators. Dominant male crocodilians patrol and defend territories which contain several females. Males of some species, like the American alligator, try to attract females with elaborate courtship displays. During courtship, crocodilian males and females may rub against each other, circle around, and perform swimming displays. Copulation typically occurs in the water. When a female is ready to mate, she arches her back while her head and tail submerge. The male rubs across the female's neck and then grasps her with his hindlimbs, placing his tail underneath hers so their cloacas align and his penis can be inserted. Mating can last up to 15 minutes, during which time the pair continuously submerge and surface. While dominant males usually monopolise reproductive females, multiple paternity is known to exist in American alligators, where as many as three different males may sire offspring in a single clutch. Within a month of mating. The females make nest on a high, steep sandy bank of rivers. Depending on the species, female crocodilians may construct either holes or mounds as nests, the latter made from vegetation, litter, sand, or soil. Nests are typically found near dens or caves. Those made by different females are sometimes close to each other, particularly in hole-nesting species. The female deposits about 35-100 eggs in the hole at night.

Crocodilian eggs are protected by hard shells made of calcium carbonate. The incubation period is two to three months. The temperature at which the eggs incubate determines the sex of the hatchlings. Constant nest temperatures above 32 °C (90 °F) produce more males, while those below 31 °C (88 °F) produce more females. However, sex in crocodilians may be determined in a short interval, and nests are subject to changes in temperature. Most natural nests produce hatchlings of both sexes, though single-sex clutches do occur.

The young may all hatch in a single night. Crocodilians are unusual among reptiles in the amount of parental care provided after the young hatch. The mother helps excavate hatchlings from the nest and carries them to water in her mouth. Newly hatched crocodilians gather together and stay close to their mother. Both male and female adult crocodilians will respond to vocalizations by hatchlings. For spectacled caimans in the Venezuelan llanos, individual mothers are known to leave their young in the same nurseries, or crèches, and one of the mothers guards them. Hatchlings of many species tend to bask in a group during the day and disperse at nightfall to feed. The time it takes young crocodilians to reach independence can vary. For American alligators, groups of young associates with adults for one to two years, while juvenile saltwater and Nile crocodiles become independent in a few months.

The mode of courtship before copulation is complex and most advanced type among reptiles. Both courtship and copulation take place in water. Sexual dimorphism in size occurs in most of crocodilians, in which males grow faster and attain maturity than females.

Male Indian Mugger (Crocodylus palustris) becomes mature in about 10 years old. Before courtship the jaw slap or head slap of the males attracts the female, and the female responds with her own jaw slap. In the month of January or February, the female initiates courtship by swimming around a male with head upraised.

The male also responds by snout rubbing circling and submerging. Within 40 days after copulation the female chooses a bank site and digs a "L shaped hole" at night. She lays about 2530 eggs in the hole but sometimes 46 eggs are known. After egg laying, the mother mugger guards the nest from any intruder for about two months.

Female Salt water crocodiles (C. porosus) of Indo-Pacific region lay eggs on mound nest. Clutches of eggs include 60-80. Females make nest only in winter season and parental care has been observed. Mound nest for egg laying is found in most of the crocodiles. Snout rubbing, mounting by both partners, circling are the signals of courtship in Gharials (Gavialis gangeticus).

Caimans and Alligators make mound nests by fresh vegetation soil and leaf litter. Visual signals and vocal communication use during courtship. Normally at the time of courtship the males lift their heads high and hold their tails vertically out of the water.

Egg Laying and Parental Care

Females American Alligator (Alligator mississippiensis) lay about 45 eggs. Para-natal care has been noticed. Depending on the species, female crocodilians may construct either holes or mounds as nests, the latter made from vegetation, litter, sand, or soil. Nests are typically found near dens or caves. Those made by different females are sometimes close to each other, particularly in hole-nesting species. The number of eggs laid in a single clutch ranges from ten to fifty. Crocodilian eggs are protected by hard shells made of calcium carbonate. The incubation period is two to three months. The temperature at which the eggs incubate determines the sex of the hatchlings. Constant nest temperatures above 32 °C (90 °F) produce more males, while those below 31 °C (88 °F) produce more females. However, sex in crocodilians may be determined in a short interval, and nests are subject to changes in temperature. Most natural nests produce hatchlings of both sexes, though single-sex clutches do occur.

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Thermoregulation

Crocodilians are ectotherms, producing relatively little heat internally and relying on external sources to raise their body temperatures. The sun's heat is the main means of warming for any crocodilian, while immersion in water may either raise its temperature by conduction, or cool the animal in hot weather. The main method for regulating its temperature is behavioural. For example, an alligator in temperate regions may start the day by basking in the sun on land. A bulky animal, it warms up slowly, but at some time later in the day it moves into the water, still exposing its dorsal surface to the sun. At night it remains submerged, and its temperature slowly falls. The basking period is extended in winter and reduced in summer. For crocodiles in the tropics, avoiding overheating is generally the main problem. They may bask briefly in the morning but then move into the shade, remaining there for the rest of the day, or submerge themselves in water to keep cool. Gaping with the mouth can provide cooling by evaporation from the mouth lining. By these means, the temperature range of crocodilians is usually maintained between 25 and 35 °C (77 and 95 °F), and mainly stays in the range 30 to 33 °C (86 to 91 °F).

The ranges of the American and Chinese alligator extend into regions that sometimes experience periods of frost in winter. Being ectothermic, the internal body temperature of crocodilians falls as the temperature drops, and they become sluggish. They may become more active on warm days, but do not usually feed at all during the winter. In cold weather, they remain submerged with their tails in deeper, less cold water and their nostrils just projecting through the surface. If ice forms on the water, they maintain ice-free breathing holes, and there have been occasions when their snouts have become frozen into the ice. Temperature sensing probes implanted in wild American alligators have found that their core body temperatures can descend to around 5 °C (41 °F), but as long as they remain able to breathe, they show no ill effects when the weather warms up.

Osmoregulation

No living species of crocodilian can be considered truly marine; although the saltwater crocodile and the American crocodile are able to swim out to sea, their normal habitats are river mouths, estuaries, mangrove swamps, and hypersaline lakes, though several extinct species have had marine habitats, including the recently extinct Ikanogavialis papuensis, which occurred in a fully marine habitat in the Solomon Islands coastlines. All crocodilians need to maintain the concentration of salt in body fluids at suitable levels. Osmoregulation is related to the quantity of salts and water exchanged with the environment. Intake of water and salts takes place across the lining of the mouth, when water is drunk, incidentally while feeding, and when present in foods. Water is lost from the body during breathing, and both salts and water are lost in the urine and faeces, through the skin, and via salt-excreting glands on the tongue, though these are only present in crocodiles and gharials. The skin is a largely effective barrier to both water and ions. Gaping causes water loss by evaporation from the lining of the mouth, and on land, water is also lost through the skin. Large animals are better able to maintain homeostasis at times of osmotic stress than smaller ones. Newly hatched crocodilians are much less tolerant of exposure to salt water than are older juveniles, presumably because they have a higher surface-area-to-volume ratio.

Communication

Crocodilians can communicate with various sounds, including bellows, roars, growls, grunts, barks, coughs, hisses, toots, moos, whines, and chirps. Young start communicating with each other before they are hatched. It has been shown that a light tapping noise near the nest will be repeated by the young, one after another. Such early communication may help them to hatch simultaneously. Once it has broken out of the egg, a juvenile produces yelps and grunts either spontaneously or as a result of external stimuli and even unrelated adults respond quickly to juvenile distress calls.Vocalisations are frequent as the juveniles disperse, and again as they congregate in the morning. Nearby adults, presumably the parents, also give signals warning of predators or alerting the youngsters to the presence of food. The range and quantity of vocalisations vary between species. Alligators are the noisiest, while some crocodile species are almost completely silent. Adult female New Guinea crocodiles and Siamese crocodiles roar when approached by another adult, while Nile crocodiles grunt or bellow in a similar situation. The American alligator is exceptionally noisy; it emits a series of about seven throaty bellows, each a couple of seconds long, at ten second intervals. It also makes various grunts and hisses. Males create vibrations in the water to send out infrasonic signals that serve to attract females and intimidate rivals. The enlarged boss of the male gharial may serve as a sound resonator. Another form of acoustic communication is the head slap. This typically starts with an animal in the water elevating its snout and remaining stationary. After some time, the jaws are opened sharply then clamped shut with a biting motion that makes a loud slapping sound, and this is immediately followed by a loud splash, after which the head may be submerged and copious bubbles produced. Some species then roar, while others slap the water with their tails. Episodes of head slapping spread through the group. The purpose varies, but it seems to be associated with maintaining social relationships, and is also used in courtship. Dominant individuals may also display their body size while swimming at the water surface, and a subordinate will submit by holding its head at an acute angle with the jaws open before retreating underwater.

Growth and Mortality

Mortality is high for eggs and hatchlings, and nests face threats from floods, overheating, and predators. Flooding is a major cause of failure of crocodilians to breed successfully: nests are submerged, developing embryos are deprived of oxygen, and juveniles get washed away. Numerous predators, both mammalian and reptilian, may raid nests and eat crocodilian eggs. Despite the maternal care they receive, hatchlings commonly fall to predation. While the female is transporting some to the nursery area, others are picked off by predators that lurk near the nest. In addition to terrestrial predators, the hatchlings are also subject to aquatic attacks by fish. Birds take their toll, and in any clutch there may be malformed individuals that are unlikely to survive. In northern Australia, the survival rate for saltwater crocodile hatchlings is only twenty-five percent, but with each succeeding year of life this improves, reaching sixty percent by year five.

Mortality rates are fairly low among subadults and adults, though they are occasionally preyed on by large cats and snakes. The jaguar and the giant otter may prey on caimans in South America. In other parts of the world, elephants and hippopotamuses may kill crocodiles defensively. Authorities differ as to whether much cannibalism takes place among crocodilians. Adults do not normally eat their own offspring, but there is some evidence of subadults feeding on juveniles and of adults attacking subadults. Rival male Nile crocodiles sometimes kill each other during the breeding season.

Growth in hatchlings and young crocodilians depends on the food supply, and sexual maturity is linked with length rather than age. Female saltwater crocodiles reach maturity at 2.2–2.5 m (7–8 ft), while males mature at 3 m (10 ft). Australian freshwater crocodiles take ten years to reach maturity at 1.4 m (4 ft 7 in). The spectacled caiman matures earlier, reaching its mature length of 1.2 m (4 ft) in four to seven years. Crocodilians continue to grow throughout their lives. Males in particular continue to gain in weight as they get older, but this is mostly in the form of extra girth rather than length. Crocodilians can live 35–75 years, and their age can be determined by growth rings in their bones.

Points	Crocodiles	Alligators	Gharials
1. Shape of the head	Elongated.	Blunt and broad.	Elongated and narrow.
2. Teeth	Almost similar in size.	Slightly different.	Strictly similar.
3. Mandibular symphysis	Extend up to 8th tooth.	Extend up to 8th tooth.	Extend up to 15th tooth.
4. External nostril	Bounded mainly by premaxillae.	Bounded by premaxillae and partly by nasals.	Bounded only by the premaxillae.

Order Crocodilia include 3 families, namely:

(1) Crocodylidae (crocodiles)

(2) Alligatoridae (alligators and Caimans)

(3) Gavialidae (ghariais)

Family 1. Crocodylidae

The crocodiles include three genera: Crocodylus, Osteolaemus and Osteoblepharon. The genus Crocodylus is found in Africa to South China, Australia, New Guinea, Western Pacific and Southern United States to Venezuela. This genus is characterised by having nasal bones dividing the nasal aperture into two.The genus, Osteolaemus, is found in Western Africa and is characterised by having undivided nasal aperture and the snout turned up in front. The genus, Osteoblepharon, is found in Congo having close similarities with Osteolaemus but the snout is not turned up.

Family 2. Alligatoridae

The alligators are placed in two genera: Alligator and Caiman. The genus Alligator is widespread in southern parts of the United States and Southern China. The nasal bones divide the nasal aperture in this -genus. The other genus, Caiman, is found in tropical South America. The nasal aperture is undivided in this genus. The biggest alligator — Alligator mississippiensis is about 6 metres or 20 feet in length, but harmless.

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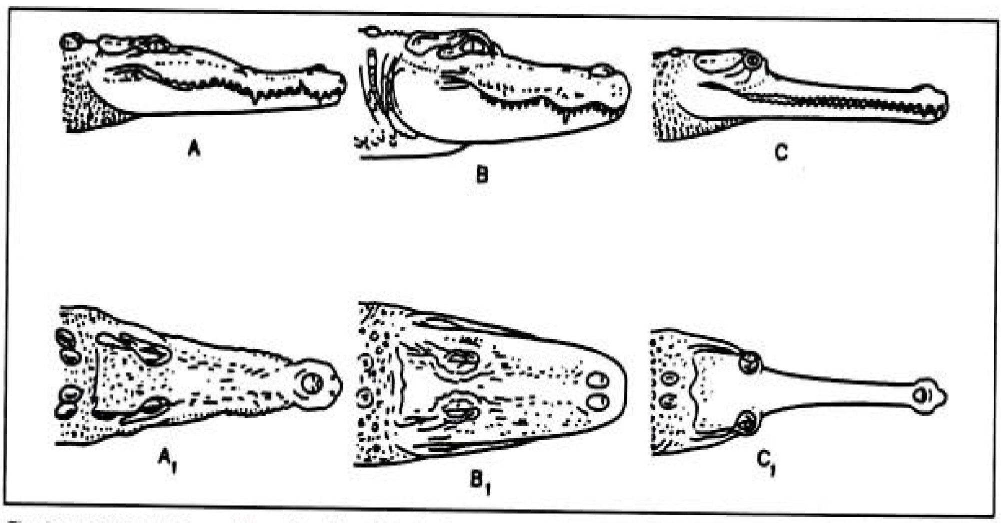

Fig. 8.61 : Different Crocodilians (head region). A. Crocodile (lateral view), A_1. Crocodile (dorsal view); B. Alligator (lateral view), B_1. Alligator (dorsal view); C. *Gavialis* (lateral view), C_1. *Gavialis* (dorsal view).

Family 3. Gavialidae

The ghariais are included under two genera: Gavialis and Tomistoma. The genus Gavialis is found in northern part of India and is characterised by having 27-29 teeth on each side of the upper jaw. The genus Tomistoma is abundant in Borneo and Sumatra, and differs from Gavialis by having 20-21 teeth on each side of the upper jaw.The Indian gharial, Gavialis gangeticus has been recorded to exceed about 6 m in length. There is no accurate record as to the longevity of different forms. An American alligator has been recorded to have lived to the age of about 56 years.

Affinities

With Sphenodon Similarities:

(1) The quadrate is immovable.

(2) Caudal ribs are fused with vertebra.

(3) Urinary bladder is present.

(4) A process from parietal reaches the squamosal.

(5) Pecten is absent.

Dissimilarities:

(1) In Sphenodon the vomer is paired but in Chelonia it is unpaired.

(2) In Sphenodon sternum is present but it is absent in Chelonia.

(3) Anal opening is trans¬verse in Sphenodon but longitudinal in Chelonia.

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(4) The penis is absent in Sphenodon but present in Chelonia.

(5) The oviduct in Sphenodon opens dorsally but in Chelonia the opening is ventral.

Remarks:

Some degree of similarities is apparent between Sphenodon on one hand and Crocodilia and Chelonia on the other. But the dissimilarities are more pronounced. Considering these it will not be justified to place the Sphenodon in the same taxonomic rank as that of the orders of Crocodilia and Chelonia.

With Chelonia: Similarities:

(1) The quadrate is immovable.

(2) Pro-atlas is present.

(3) The skull is of diapsid type. (4) Cochlear process is tubular.

(5) Ribs bear uncinate process.

(6) Caudal ribs are fused with vertebrae.

(7) Abdominal ribs are present.

(8) Chevron bones are present.

Dissimilarities:

(1) The teeth are acrodont in Sphenodon but thecodont in crocodile.

(2) The nasal-opening is double in Sphenodon but single in crocodile.

(3) The vertebrae are amphicoelus in Sphenodon but procoelus in crocodile.

(4) Clavicle is present in Sphenodon but absent in crocodile.

(5) Pecten is absent in Sphenodon but present in crocodile.

(6) Penis is absent in Sphenodon but present in crocodile.

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The basis of Vermicomposting and Vermiculture

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Introduction

Environmental degradation caused by anthropogenic activities, such as overexploitation of natural resources and pollution, is a major global issue. The continuous growth of the human population has led to an increase in waste generation, which significantly contributes in the environmental degradation. Waste Paraphrased as any material that no longer required by the owner, producer, or processor and has lost its utility. Based on the source, waste differ from one another.

There are several types of waste, including municipal solid waste, agricultural waste, biomedical waste, and industrial waste. Municipal solid waste had generated from household and commercial activities consists of food waste, paper, plastic, metal, glass, and other materials. Source of agricultural waste includes livestock waste, agricultural crop residues, and agro-industrial by-products and tourism waste. Industrial waste has generated during manufacturing or industrial activities in factories, mills, and mines.

Agricultural waste can be recycled back into the soil as organic matter and nutrients through techniques such as composting, anaerobic digestion, and biogas production. However, industrial waste may be hazardous or non-hazardous. The proper management of industrial waste involves prevention of environmental pollution, waste reduction, and recycling measures, such as closed-loop production systems and eco-design.

Waste generation is increasing at an alarming rate and has become a global issue. The total quantity of waste generated annually in the world has predicted to reach 2.2 billion tons by 2025. In 2016 alone, 242 million tons of

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plastic waste was generated globally Rate of waste generation in small towns of India is 0.41 kg/capita/day, and in rural areas, 0.3 to 0.4 million metric tons of solid waste is generated on daily basis .Mostly of the waste is organic in nature, consisting of cattle dung and crop residues. The increase in waste generation attributed to various factors, such as industrialization, urbanization, population explosion, and unsustainable economic development. Moreover, higher increase had predicted in lower-income countries

It has been reported that over 75% of waste is managed through the landfilling method globally .However, landfills generate leachate, which contaminates the surface and groundwater .Solid waste decay contributes to about 5% of greenhouse gas emissions including methane, which in turn exacerbating the climate change .

However, every component of solid waste used if it is converted into a functional product through a suitable scientific method. Therefore, this review highlights the need for efficient waste management techniques that utilize scientific approaches to reduce the impact of waste on the environment .Hence, waste management has become critical aspect of environmental protection and sustainable development that needs to be addressed through scientific methods, awareness, and proper management techniques. Failure to do so will lead to a further increase in waste generation and environmental threats. It involves the adoption of scientific and effective strategies to reduce waste generation, promote waste reduction and recycling, and prevents contamination of environmental.

The maintenance of a healthy ecosystem in nature depends on the management of solid waste. There are several issues with the environment related to the large production and buildup of organic waste. It damages the fertility of the land, pollutes the water and the soil, and has a negative impact on human health. Preprocessing the organic waste before releasing it into the natural environmentavoids all the environmental hazards. The process of organic biodegradation is required to produce high-quality goods and the safe disposal of organic waste. Specific type of species in earthworms were used in a technique known as "Verm technology," it is possible to process the breakdown of organic waste by saprophytic organisms (eating dead and decaying stuff). Vermicomposting and vermiculture are two subcategories of verm technology.

Vermiculture

Vermiculture is a technique based on utilizing some species of earthworms to convert organic waste into Vermicompost that is again, the product of decomposition by various worms. A practice of harvesting worms that take part in decomposing organic waste and turning it into nutrient-rich fertilizer. The worms consume the decomposing organic material and flush it out of their system, often referred to as worm manure.

Earthworms commonly used in Vermiculture are, *Eisenia Andrei*, *Eiseniafetida*, and *Lumbricusrubellus* horticultural in temperate climates and *Pheretima perionyx*, *Hawanya excavatus* and *Eudrilus eugeniae* and in the tropical areas.In short, Vermiculture and Vermicomposting are the cultivation of earthworms and the use of earthworms to decompose organic wastes into nutrient-rich fertilizers.

Vermiculture meaning

In General, terms, Vermiculture means the cultivation of earthworms in order to use them to convert organic waste to nutrient and beneficial microorganism rice fertilizer. It allows us to grow organically rich compost year-round. A biology teacher, Mary Appelhif, first introduced Vermiculture in the 1970s. She developed the idea of using red wiggler worms (*Eiseniafetida*) for composting in indoor and outdoor systems to convert kitchen waste to worm compost.

Vermiculture techniques

There are three major techniques in Vermiculture for harvesting worms. These are

1. Manual
2. Migration
3. Mechanical

Manual method of harvesting

Framers for small-scale businesses of selling worms generally use this method. The worms had harvested from the soil directly by using hands. The organic material, which contains earthworms, has kept on a flat surface and exposed to sunlight. It mightnote that earthworms are sensitive to light, so once they had exposed to sunlight they dive below the surface. The harvester will then remove the organic layer above and once the worms had seen they had harvested.

Migration method of harvesting

This method takes advantage of the earthworm's tendency to migrate to a new location for food and for this reason; onion bags and screens had used for harvesting. At the bottom surface of the screen, a box had constructed where the worms would be collected. The migration method has carried out in two ways.

The downward migration method is where the worms had forced to move downwards in the organic material with the use of light. They will go through the screen mesh and be collected in the container box below which has filled with peat moss. The process has repeated until the required quantity of worms had achieved. It hastimesaving process and could seen in multiple locations.

In the upward migration method, a worm bed would replace the mesh bottom of the box. The box has filled with peat moss and food, which attracts the worms. Generally, coffee grounds and manure from fresh cattle had used as food for worms and they will move towards it and be collected in the box.

Mechanical method of harvesting

In this method, a mechanical harvester has used to collect worms. A trammel screen, which has called a rotary screen that, has used to separate materials. It is around 11ft long and 4ft in diameter and has a cylindrical shape.

The walls of the cylinder are made of screening materials with different sized meshes. The cylinder has powered by an electric motor. The device would be set at an angle at the top side of the trammel. After that, the castings and the worm beds have added. When the rotation starts the castings of the worms will drop through the screen and the worms will move across the trammel device and enter into the wheelbarrow.

Vermiculture process

The method used by farmers to multiply earthworms is by mixing high amounts of organic wastes, including the plant materials, cattle dung in a proportion of 1:1. Once the substrate medium has been made, around 40-50 earthworm species are released into the medium and it is protected from various environmental factors.

Regular maintenance is important for the growth of earthworms. The temperature should be between 15 to 25-degree centigrade and the moisture level should be at 80-90%. Within one to two months, the earthworms can multiply up to 300 times relying on this process and factors affecting the process. After the process, earthworms have harvested.

Vermicompost

Vermicompost is an organic fertilizer rich in nutrients that has created through the vermicomposting process, which involves earthworms breaking down organic materials. Vermicompost plays crucial role in maintaining long-term soil fertility and sustainability also it is an essential component of organic farming. It is the result of earthworms and the microorganisms in their digestive system breaking down organic waste. Vermicompost demonstrates a number of distinctive qualities that make it advantageous for agricultural applications. It has a pleasant earthy smell and a dark, crumbly texture. Depending on the feedstock used and the circumstances surrounding the vermicomposting process, the composition of vermicompost can change. However, compared to conventional compost, it typically contains higher levels of vital plant nutrients like nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg). In addition, vermicompost is a rich source of enzymes, humic substances, beneficial microorganisms, and plant growth-promoting compounds, all of which contribute to its overall fertility-improving abilities.

Vermicompost works well as a soil amendment because of its physical and chemical characteristics. Due to its high waterholding capacity, the soil is better able to retain moisture and experience less water runoff. In order to allow for better root penetration and nutrient uptake by plants, vermicompost also improves soil structure by increasing porosity and improving aggregation. Additionally, the presence of advantageous microorganisms in vermicompost fosters soil biology, improving nutrient cycling and suppressing plant diseases. Vermicomposting is a method for turning organic waste into nutrient-rich vermicompost by utilizing the synergistic activity of earthworms and microorganisms. There are several crucial steps in the process. At the outset, suitable earthworm species had chosen. Examples include *Eisenia fetida* and *Lumbricus rubellus*. To create an ideal habitat for the earthworms, a bedding material—such as shredded newspaper or cardboard—is prepared .The vermicomposting system had then supplemented with organic waste materials, such as kitchen scraps, yard waste, and livestock manure, to provide the earthworms with food. The process depends heavily on maintaining the right moisture levels (roughly 70–90% moisture content) and making sure there is enough aeration. The decomposition process had maintained through periodic feeding and system monitoring. Vermicompost has harvested by separating it from the remaining organic waste and earthworms using techniques like hand sorting or screen sieving after several weeks to months.

Earthworms

The segmented, elongated, thin, cylindrical, metamerically symmetrical invertebrate organism. The body has a thin cuticle layer, is lustrous, and is dark brown in color. They weigh between 700 and 1400 milligrams after ten weeks. Their strong gizzard grinds food down to a size of 2-4 microns. The intestines of the earthworm are home to millions of rotting bacteria. They are hermaphrodite in nature, thus, they commonly cross-fertilize. Before parting, the worms may copulate for almost an hour. A cocoon ejected by each worm's clitellum, into which sperms crawl to fertilize the eggs. Up to three cocoons per worm had produced each week. In each cocoon, 10 to 12 tiny worms haveproduced. Earthworms grow throughout their lifetimes, with new segments continually multiplying in a region called the "growth zone" directly in front of the anus. Up to 70 to 80% of the dry weight of earthworms made up of protein rich in lysin.

They have used as animal feed. A typical earthworm lives between three and seven years, depending on the species and the environment. Earthworms are commonly classified as saprophagous, although they can also be separated into geophages and detrivores based on how they consume. At or near the soil surface, detrivores eat dead roots, plant litter, other plant debris, or animal excrement. Worms of the epigeic and anecic types had collectively referred to as humus formers and also known as detrivorous. Endogeic earthworms known as geophagousworms consume a significant portion of the biologically rich soil below the soil's surface while feeding. Examples of detritivorous and geophagous.

Earthworm growth stages

Vermiculture produces vermicompost using three main types of earthworms. Litter, topsoil, and subsoil are all common places to find these earthworms. Most of the criteria used to classify the groups are their behavior and habitat. Anecic, epigeic, and endogeic are the three types. Epigeic is a Greek word that means "from the soil." This species, which inhabits places with a lot of organic materials, feeds on decaying plant roots, leaf litter, and animal excrement. These earthworms do not dig long-term burrows. Due to their black skin, epigeic species' worms are highly pigmented.

The vermicomposting process primarily uses these kinds of earthworms. For instance, Perionyx excavates and *Eisenia fetida* excavates. Greek for "inside the ground," endogeic. This species subsisted underground and consumed decayed organic materials and dirt. To travel around, they dig horizontal burrows in the ground. For instance, *Octochaetonathurstoni* and *Metaphireposthuma*. Anecic: A Greek name meaning "from the earth." This species digs long-lasting vertical burrows in the ground and emerges at night to feed. They have darker heads and are paler toward the end of their tails. Consider *Lumbricus terrestris*.

Ecology of the earthworms

As burrowing creatures, earthworms physically eat their path through the soil to create tunnels. Earthworm dispersal in the soil had influenced by things like soil moisture and the availability of organic matter. The amount of available organic matter and the soil's pH (6.5-7.5) all affect the dispersion of earthworms in the soil. They have found in a variety of environments, especially those that are moist and dark. Some species find a great attraction in organic materials like humus, cow dung, and kitchen scraps. The touch, light, and dryness are all particularly sensitive to earthworms. They may rise to the surface of the soil and become waterlogged conditions. Temperature plays a key role in earthworms’ sustainability and their metabolism, rate of reproduction, and growth of earthworms. The temperature below 10 °C leads to stress on earthworms and affects their growth. The temperature is more than 35 °C, which leads to the death of earthworms. The optimum temperature range of earthworms is 15 °C to below 35 °C. The earthworms have high growth and

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reproductive at 25 °C. The earthworms take a breath through the skin with the help of moisture in the soil, which contains 75 to 90% water. Less than 50% of water can reduce the respiration rate of earthworms because need to maintain the moisture. The optimum moisture for earthworms is 70% to 80%.

Earthworms as waste managers

Earthworms made a large contribution to the total weight or biomass of invertebrates in soil. Aristotle first drew attention to their role in turning over the soil and called them intestines of the earth. By utilizing various vermiculturetechniques, we can not only manage our wastes but also have a check on the environmental pollution. The basic aim of composting is to bring about decomposition of organic wastes without undue loss of nutrients and the production of product rich in plant nutrients. Vermicompost substitution with fertilizers input will reduce the economic input in agriculture. The main source of food for earthworms is the organic waste such as agro-horticultural crop waste, weeds, forest leaf litter; agroindustrial wastes etc. and defecates the faucal pellets known as vermicompost. This is rich in plant nutrients such as macro and secondary elements, beneficial micro flora and plant growth regulators. Survival and development of earthworms is highly influenced by environmental factors like temperature, bed moisture, rainfall, relative humidity, which determines the population in field. Constant high temperature is detrimental for the development of worms even though all other conditions are favorable.

Production of worm biomass and vermicompost in open field vermicomposting sites had influenced by seasonal variation. Vermiculture technology have employed for composting of various non-toxic organic wastes. Earthworms serve as versatile natural bioreactors to harness the beneficial soil micro flora and destroy pathogen, thus converting organic waste into valuable products such as bio fertilizers, bio pesticides, vitamins, enzymes, antibiotics, growth hormones and proteinaceous worm biomass. Hundreds of tons of biodegradable organic waste have generated in cities and towns in the country, creating disposal problems. This waste have converted into valuable compost by applying vermi-composting technology. This approach reduces pollution and provides a valuable substitute for chemical fertilizers. Earthworms have used for centuries as a means of decomposing wastes and improving soil structure. Increasing numbers of businesses worldwide are successfully employing vermiculture technology and marketing vermicompost as an excellent soil conditioner, to farmers and gardeners. The breeding and propagation of earthworms and the use of its castings has become an important method of waste recycling throughout the world and now it has also gaining importance in various regions of the country.

Vermiculture technology is more efficient, economically viable, environmentally positive, practically feasible recycling process and involves the use of earthworms as versatile natural bioreactors for effective recycling of non-toxic organic solid and liquid wastes in the soil. It had used for the management of weeds and wastes from agriculture, industries, kitchen etc. Research on vermicomposting of a variety of wastes is gaining momentum throughout the world .Waste degradation & composting by earthworms is proving to be economically & environmentally preferred technology over the conventional composting technologies as it is rapid and nearly odorless process, reducing composting time by more than half and the end product is both 'disinfected', 'detoxified' and 'highly nutritive'. Scientist found that most earthworms consume, at the best, half their body weight of organics in the waste in a day. *Eiseniafetida* can consume organic matter at the rate equal to their body weight every day. Earthworm participation enhances natural biodegradation and decomposition of organic waste from 60 to 80 %. In addition, as the worms double their population every 60-70 days, the process becomes faster with time. Given the optimum conditions of temperature (20-30 °C) and moisture (60-70 %), about 5 kg of worms (numbering approx.10, 000) can vermiprocess 1 ton of waste into vermicompost in just 30 days.

Phase of vermicomposting

Phase 1:

Processing involving collection of wastes, shredding, mechanical separation of the metal, glass and ceramics and storage of organic wastes.

Phase 2:

Pre digestion of organic waste for twenty days by heaping the material along with cattle dung slurry. This process partially digests the material and fit for earthworm consumption. Cattle dung and biogas slurry mighthad used after drying. Wet dung had not used for vermicompost production.

Phase 3:

Preparation of earthworm bed. A concrete base is required to put the waste for vermicompost preparation. Loose soil will allow the worms to go into soil and while watering; all the dissolvable nutrients go into the soil along with water.

Phase 4:

Collection of earthworm after vermicompost collection. Sieving the composted material to separate fully composted material. The partially composted material will be again put into vermicompost bed.

Phase 5:

Storing the vermicompost in proper place to maintain moisture and allow the beneficial microorganisms to grow.

The five essential compost worms need:

1. A hospitable living environment, usually called "bedding"
2. A food source
3. Adequate moisture (greater than 50% water content by weight)
4. Adequate aeration
5. Protection from temperature extremes

Procedure

1. To prepare compost, either a plastic or a concrete tank had used. The size of the tank depends upon the availability of raw materials.

2. Collect the biomass and place it under the sun for about 8-12 days. Now chop it to the required size using the cutter.

3. Prepare cow dung slurry and sprinkle it on the heap for quick decomposition.

4. Add a layer (2 – 3 inch) of soil or sand at the bottom of the tank.

5. Now prepare fine bedding by adding partially decomposed cow dung, dried leaves and other biodegradable wastes collected from fields and kitchen. Distribute them evenly on the sand layer.

6. Continue adding both the chopped bio-waste and partially decomposed cow dung layer-wise into the tank up to a depth of 0.5-1.0 ft.

7. After adding all the bio-wastes, release the earthworm species over the mixture and cover the compost mixture with dry straw or gunny bags.

8. Sprinkle water on a regular basis to maintain the moisture content of the compost.

9. Cover the tank with a thatch roof to prevent the entry of ants, lizards, mouse, snakes, etc. and protect the compost from rainwater and direct sunshine.

10. Have a frequent check to avoid the compost from overheating. Maintain proper moisture and temperature.

Precautions during the process

The ideal species for the preparation of vermicompost are African species of earthworms, *Eiseniafetida* and *Eudrilus eugane*. Indian species are not suitable for the preparation of vermicompost. While preparing vermicompost, only plantbased materials such as grass, leaves or vegetable peelings had used. Animal origin substances such as chicken droppings, eggshells, meat, bone etc. are not appropriate for preparing vermicompost. For rearing the earthworms *Gliricidialoppings* and tobacco leaves are not preferable. The earthworms should be secured from rats, birds, and termites. During the process, suitable moisture had preserved. Either absence of moisture or dirty water could kill the earthworms. When the process is complete, the vermicompost should be takeout from the bed in a meantime and restore by fresh waste materials.

Significance of vermiculture

Vermiculture is the culture of earthworms. It is a beneficial way of improving the fertility of the plant and soil. Vermiculture mainly focuses on the breeding of worms toincrease their population. Vermicompost is then prepared to promote the growth and development of crops. It also causes disease in plants along with increasing water retention and the porosity of the soil. This greatly reduces the need for chemical fertilizers and encourages organic matter.

Advantages of vermicompost

1. Vermicompost is rich in all essential plant nutrients.

2. Provides excellent effect on overall plant growth, encourages the growth of new

3. Shoots / leaves and improves the quality and shelf life of the produce.

4. Vermicompost is free flowing, easy to apply, handle and store and does not have bad odour.

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5. It improves soil structure, texture, aeration, and waterholding capacity and prevent soil erosion.

6. Vermicompost is rich in beneficial micro flora such as a fixers, Psolubilizers,cellulose decomposing micro-flora etc in addition to improve soil environment.

7. Vermicompost contains earthworm cocoons and increases the population and activity of earthworm in the soil.

8. It neutralizes the soil protection.

9. It prevents nutrient losses and increases the use efficiency of chemical fertilizers.

10. Vermicompost is free from pathogens, toxic elements, weed seeds etc.

11. Vermicompost minimizes the incidence of pest and diseases.

12. It enhances the decomposition of organic matter in soil.

13. It contains valuable vitamins, enzymes and hormones like auxins, gibberellins etc**.**

Pests and diseases of vermicompost

Compost worms are not subject to diseases caused by microorganisms, but they are subject to predation by certain animals and insects (red mites are the worst) and to a disease known as "sour crop" caused by environmental conditions.

Disadvantages of vermicomposting

Following are the important disadvantages of vermicomposting:

1. It is a time-consuming process and takes as long as six months to convert the organic matter into usable forms.

2. It releases a very foul odour.

3. Vermicomposting is high maintenance. The feed has added periodically and care had taken that the worms are not flooded with too much to eat.

4. The bin should not be too dry or too wet. The moisture levels monitored periodically.

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5. They nurture the growth of pests and pathogens such as fruit flies, centipede and flies.

Conclusion

The vermicomposting technology has an advantage over composting. This is generally due to 'humus' content in vermicompost ejected by earth-worms and in conventional composting system it takes a very long time to form humus, because of slow rotting process of organic matter. On my personal opinion, vermicomposting on organic waste will be very useful in resolving the waste discarding problem. The recycling plant nutrients process reduces the use of inorganic fertilizers. In case of the earthworm, African species are better than the Indian species. The vermicomposting is a productive, easy, environment-friendly, and viable method. It can be easily expand a variety of useful products from the grape marc, for industrial applications yielding. It is important to parallel this production with suitable utilization and industrial application of coffee by-products. From the environmental point of view, value has fixed by valorizing these by-products.

In sustainable development in agriculture biotechnology, vermiwash discovered possible application with respect to its origin, cost-effectiveness, easy accessibility, timesaving, reproducibility, dependability, and ecofriendliness. The quality of soils enhances with the application of vermicompost in the field by increasing microbial activity and microbial biomass that are key components in nutrient cycling, production of plant growth regulators, and protecting plants from soil-borne diseases and insect-pest attacks. "Cent Vermicompost Scheme" is the scheme for the farmers run by the government. The purpose of the scheme is to help the farmers for setting up and run their vermicompost units and helps in investment and working capital requirements of farmer.

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Advances in Instrumentation for Biological Sciences: Unveiling the Secrets of Life

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Abstract

In the relentless pursuit of understanding the intricacies of life, recent advancements in biological instrumentation have ushered in a new era of exploration and discovery. This chapter, titled "Advances in Instrumentation for Biological Sciences: Unveiling the Secrets of Life," provides a comprehensive overview of groundbreaking tools and methodologies that have revolutionized various facets of biological research. Spanning imaging technologies, omics methodologies, single-cell analysis, structural biology, biosensors, mass spectrometry, high-throughput screening, and computational biology, this abstract encapsulates the transformative impact of cutting-edge instrumentation on unravelling the mysteries of life.

The chapter commences with an exploration of imaging technologies, where traditional limitations in resolution and observation have been shattered. Techniques such as super-resolution microscopy and cryo-electron microscopy have provided unprecedented insights into cellular dynamics and structures, enabling researchers to observe intricate molecular events in real-time.

Omics technologies take center stage as the blueprint of life is deciphered with remarkable precision. Next-generation sequencing platforms, epitomized by Illumina and Oxford Nanopore technologies, have democratized genomics, while the CRISPR-Cas9 system has empowered precise genetic editing. In the realm of proteomics, mass spectrometry has become a linchpin for unravelling complex signalling networks and protein interactions.

Single-cell analysis emerges as a paradigm shift, revealing the remarkable heterogeneity within seemingly homogeneous cell populations. Advances in single-cell RNA sequencing, coupled with proteomics and

metabolomics, offer a granular understanding of cellular diversity, unlocking insights into development, disease, and beyond.

Structural biology takes a leap forward with refined techniques such as cryo-electron microscopy, providing high-resolution images of biological macromolecules in their native states. This has implications for drug design and targeted therapeutic interventions.

Biosensors and imaging probes illuminate cellular events with extraordinary sensitivity. These technologies, rooted in fluorescence and bioluminescence principles, provide dynamic insights into molecular processes within living organisms, promising non-invasive diagnostics and therapeutic monitoring.

Mass spectrometry stands as a cornerstone for analyzing the molecular landscape, facilitating comprehensive proteomic and metabolomic analyses. High-throughput screening, driven by automation and artificial intelligence, accelerates drug discovery, while computational biology extracts meaningful insights from vast biological datasets.

As the chapter concludes, a glimpse into the future hints at the convergence of multiple imaging modalities, the extension of single-cell analysis to multi-omics profiling, and continuous evolution in biosensors and imaging probes. The interplay of artificial intelligence, long-read sequencing technologies, and refined CRISPR-based techniques promises to propel biological research into uncharted territories, unveiling the secrets of life with unprecedented precision.

In summary, this abstract encapsulates the transformative journey through recent instrumentation advancements, emphasizing the pivotal role these technologies play in advancing our understanding of the complex and intricate mechanisms that underlie life processes.

Introduction: Unveiling the Mysteries of Life

The relentless march of technological progress has become the catalyst for a profound metamorphosis within the field of biological sciences. In this era of relentless innovation, cutting-edge instrumentation stands as the vanguard, propelling our understanding of life to unprecedented heights. This introductory chapter embarks on a compelling journey through the latest frontiers of biological instrumentation, acting as a guide to the revolutionary tools that have emerged as beacons, illuminating the intricate complexities of life processes.

The chapter navigate landscape of scientific transformation, the fusion of biology with technology has birthed a new paradigm where our ability to observe, analyze, and comprehend the living world has reached unparalleled levels. The evolution of instruments has become synonymous with the evolution of our understanding of life itself. From the intricate dance of molecules to the orchestration of cellular events, each instrument serves as a key to unlocking the secrets that have eluded us for centuries.

In this pursuit of knowledge, imaging technologies have emerged as pioneers, defying traditional limits and providing us with unprecedented access to the microscopic wonders that orchestrate life. Super-resolution microscopy and cryo-electron microscopy, among others, have become our lenses into the intricate choreography of cellular structures and molecular interactions. The chapter find ourselves standing at the edge of a realm where the invisible becomes visible, and the once blurry boundaries of observation dissipate into clarity.

Omics technologies, the architects of the genomic revolution, have empowered us to decode the very blueprint of life. Next-generation sequencing platforms, epitomized by Illumina and Oxford Nanopore technologies, have democratized genomics, allowing us to unravel the intricate tapestry of genetic information. The CRISPR-Cas9 system, a revolutionary molecular scalpel, has provided us with the ability to edit the very code of life with unprecedented precision. Proteomics, metabolomics, and lipidomics complete this symphony, offering harmonious melodies of data that contribute to our holistic understanding of biological systems.

The microscope, once a symbol of scientific inquiry, has evolved into an array of instruments that peer not only into the spatial dimensions of life but also into the temporal dynamics that govern it. Single-cell analysis, a discipline at the intersection of innovation and necessity, allows us to unravel the microcosmic intricacies that lie within seemingly homogeneous cell populations. From the symphony of gene expression revealed by single-cell RNA sequencing to the proteomic and metabolomic solos played by individual cells, we find ourselves in a position to appreciate the nuanced melodies that shape life's orchestra.

Structural biology, with its emphasis on unveiling the molecular architecture, takes us deeper into the heart of life. X-ray crystallography, NMR spectroscopy, and the revolutionary cryo-electron microscopy become our tools for dissecting the three-dimensional intricacies of biological macromolecules. The resulting structural revelations not only elucidate fundamental biological processes but also lay the foundation for targeted therapeutic interventions and drug design.

Biosensors and imaging probes, akin to artists wielding brushes, paint vivid portraits of molecular events within living organisms. Rooted in principles of fluorescence and bioluminescence, these technologies offer a dynamic canvas upon which the narratives of cellular dynamics unfold. The real-time detection of specific molecules, coupled with the visualization of cellular processes, transforms these instruments into storytellers, narrating the tales of life at a molecular level.

Mass spectrometry, an analytical juggernaut, enables us to analyze the molecular symphony within biological systems. From the intricate world of proteomics to the nuanced exploration of metabolomics, mass spectrometry becomes our guide through the rich tapestry of biological molecules. High-throughput screening, fuelled by automation and artificial intelligence, accelerates the pace of drug discovery, offering us glimpses into potential therapeutic interventions that might shape the future of medicine.

The chapter navigatelabyrinth of discovery, computational biology emerges as our compass, guiding us through the vast seas of data generated by advanced instrumentation. Bioinformatics becomes the bridge between raw data and meaningful insights, unraveling patterns, identifying biomarkers, and shaping predictive models that deepen our understanding of life processes.

In this era where instruments have become the conduits to unraveling life's intricacies, we stand on the cusp of a new frontier. The journey through the latest frontiers of biological instrumentation is not just a technological pilgrimage; it is an odyssey of understanding, a relentless pursuit of the answers to questions that have echoed through the corridors of scientific inquiry for generations. This introductory chapter serves as an invitation to join this quest, where each instrument is a beacon illuminating the path toward the secrets that have remained tantalizingly out of reach. This book chapter embark on journey through the latest frontiers of biological instrumentation, also highlighted bound not only by the tools in our hands but also by the insatiable curiosity that drives us to unveil the mysteries of life.

Imaging Technologies: Peering Into the Microscopic Realm

In the grand tapestry of biological sciences, imaging technologies serve as the visionary architects, revealing the intricacies of life at scales once unimaginable. This exploration into the microscopic realm is a journey that transcends the limits of human vision, unlocking the mysteries hidden within the minuscule landscapes of cells and molecular structures.

Super-Resolution Microscopy: The Renaissance of Precision

At the forefront of imaging technologies, super-resolution microscopy has revolutionized our ability to peer into the microscopic realm with unprecedented clarity. This technique defies the traditional diffraction limits of light microscopy, providing resolutions beyond the wavelength of light. Through techniques such as stimulated emission depletion (STED) and structured illumination microscopy (SIM), scientists can now visualize cellular structures and molecular interactions at resolutions nearing the nanoscale. The images produced by super-resolution microscopy are akin to finely detailed paintings, offering a level of precision that is reshaping our understanding of cellular dynamics.

Cryo-Electron Microscopy: Flash-Freezing the Essence of Life

The evolution of cryo-electron microscopy marks a paradigm shift in structural biology, allowing researchers to capture snapshots of biological specimens in their native states. By flash-freezing samples at extremely low temperatures, this technique minimizes artifacts and preserves the delicate structures of biomolecules. The resulting images, akin to intricate sculptures, offer near-atomic resolution and reveal the molecular architecture of proteins, viruses, and cellular organelles. Cryo-electron microscopy has become an invaluable tool, unveiling the beauty and complexity of life's building blocks.

Light-Sheet Microscopy: Illuminating Life's Dynamics

Light-sheet microscopy emerges as a dynamic storyteller, capturing the real-time dynamics of living organisms with minimal phototoxicity. This technique employs a thin sheet of laser light to selectively illuminate the imaging plane, reducing the exposure of surrounding tissues to light. This not only enhances imaging speed but also minimizes cellular damage. Light-sheet microscopy has become instrumental in studying developmental processes, organoids, and even entire organisms, providing insights into the dynamic choreography of life unfolding at the microscopic level.

Integrated Imaging Modalities: Bridging Dimensions

The future of imaging technologies lies in the integration of multiple modalities, fusing the strengths of different techniques to create a comprehensive picture of biological processes. Combining fluorescence microscopy with spectroscopy, for example, allows researchers to not only visualize structures but also understand the chemical composition of cellular components. The synergy of various imaging modalities provides a holistic view, akin to a multidimensional artwork that captures the essence of life's complexity.

As we traverse the landscape of imaging technologies, the microscopic realm unfolds before our eyes in unprecedented detail. Super-resolution microscopy, cryo-electron microscopy, light-sheet microscopy, and the integration of diverse modalities are the brushstrokes that paint a vivid picture of life at its smallest scales. These images, more than mere representations, are windows into a world where the dance of molecules and the orchestration of cellular events become tangible. The journey into the microscopic realm continues to shape our perceptions, challenge our preconceptions, and beckon us to explore the beauty concealed within the unseen dimensions of life.

Omics Technologies: Decoding the Blueprint of Life

In the symphony of biological sciences, omics technologies emerge as the virtuosos, playing an instrumental role in deciphering the complex and harmonious melody of life's blueprint. These transformative tools, ranging from genomics to proteomics and metabolomics, are the keys to unraveling the intricacies of genetic information, protein expression, and metabolic pathways.

Genomics: The Architectural Draft of Life

At the core of omics technologies lies genomics, the study of an organism's entire DNA sequence. Illumina and Oxford Nanopore technologies have revolutionized DNA sequencing, making it faster, more accurate, and cost-effective. The resulting genomic blueprints serve as the architectural drafts of life, detailing the genetic code that determines an organism's traits, functions, and evolutionary history.

CRISPR-Cas9: Precision Editing of Life's Script

The advent of CRISPR-Cas9 technology has granted scientists the ability to edit the genomic script of life with unparalleled precision. This revolutionary molecular tool acts as a molecular scalpel, allowing for targeted modifications to specific genes. CRISPR-Cas9 has far-reaching implications, from understanding gene functions to potential therapies for genetic disorders, reshaping the narrative of genetic manipulation.

Proteomics: Unraveling the Protein Symphony

Omics extends its reach to proteomics, the comprehensive study of an organism's entire set of proteins. Mass spectrometry, a cornerstone in proteomic analysis, enables researchers to identify, quantify, and characterize proteins with unparalleled precision. These protein symphonies reveal intricate signaling networks, molecular interactions, and the dynamic orchestration of cellular processes.

Metabolomics: Harmonizing Life's Chemical Composition

In the realm of metabolomics, scientists delve into the small molecules that orchestrate life's chemical composition. Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry unveil the metabolic signatures within cells, tissues, and biological fluids. Metabolomics paints a harmonious picture of cellular metabolism, offering insights into physiological states, disease mechanisms, and potential therapeutic targets.

Lipidomics: Composing Life's Lipid Symphony

Lipidomics, the study of lipids, adds another layer to the omics repertoire. Mass spectrometry, coupled with advanced chromatographic techniques, allows for the identification and quantification of lipid species. This lipid symphony plays a crucial role in cellular structure, signaling, and energy storage, providing a nuanced understanding of the lipid landscape within biological systems.

The landscape of omics technologies, witness the decoding of life's grand composition. Genomics, CRISPR-Cas9, proteomics, metabolomics, and lipidomics collectively form a symphony of data that enriches our comprehension of the intricacies of living organisms. These images and insights are not merely snapshots but portals into the molecular realms, where the orchestra of life unfolds with exquisite precision. Omics technologies stand as the conductors guiding us through this intricate symphony, allowing us to explore, interpret, and appreciate the blueprint of life in its entirety.

Single-Cell Analysis: Unraveling Heterogeneity at the Cellular Level

In the intricate tapestry of biology, the exploration of single-cell analysis marks a revolutionary paradigm shift, allowing scientists to unravel the hidden nuances within seemingly uniform cell populations. This transformative discipline transcends traditional bulk analyses, providing unprecedented insights into the remarkable heterogeneity that defines cellular communities.

Single-Cell RNA Sequencing: Decoding the Transcriptomic Symphony

At the forefront of single-cell analysis is the technique of single-cell RNA sequencing (scRNA-seq), which serves as a powerful tool to decode the transcriptomic symphony of individual cells. Unlike traditional RNA sequencing, scRNA-seq captures the gene expression profiles of single cells, unveiling the dynamic landscape of transcriptomic diversity within a population. This method has proven instrumental in understanding cellular differentiation, developmental processes, and disease progression.

Single-Cell Proteomics and Metabolomics: Profiling Cellular Components

Expanding the scope, single-cell proteomics and metabolomics delve into the intricate world of proteins and metabolites at the single-cell level. Mass spectrometry, combined with microfluidic technologies, enables the comprehensive profiling of cellular components. Unraveling the proteomic and metabolomic landscapes within individual cells provides a granular understanding of cellular heterogeneity, shedding light on functional variations that are obscured in bulk analyses.

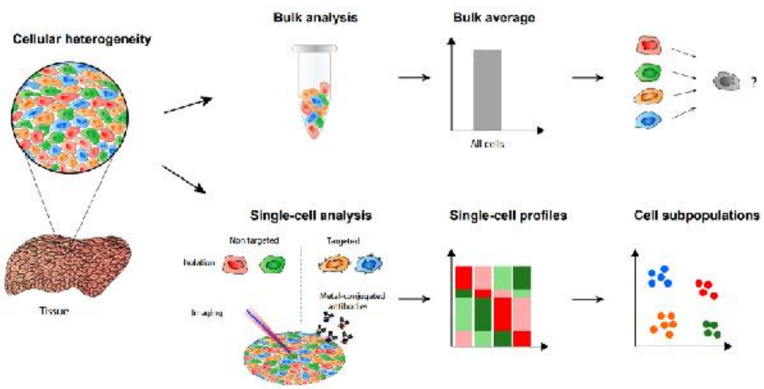


Image Source: Lee *et al*. (2023)

Microfluidic Technologies: Enabling High-Throughput Single-Cell Analyses

Microfluidic technologies play a pivotal role in facilitating high-throughput single-cell analyses. These microscale devices enable the manipulation and analysis of individual cells with precision and efficiency. By compartmentalizing single cells, researchers can study thousands of individual cells in parallel, unveiling the diversity that exists within complex biological systems. Microfluidics has become the engine driving the scalability of single-cell analysis.

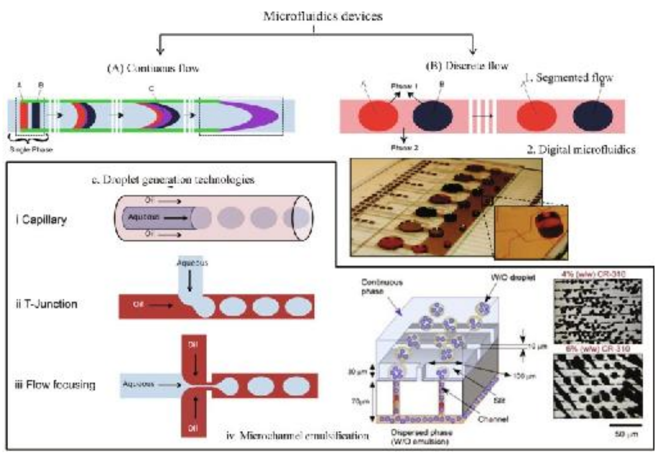


Image Source: Rabiee *et al.* (2021)

Lab-on-a-Chip Platforms: Integrating Multiple Analyses

Lab-on-a-chip platforms represent an integrated approach to single-cell analysis, combining various analytical techniques on a single chip. These platforms seamlessly integrate processes such as cell capture, lysis, and analysis, allowing for a comprehensive exploration of cellular characteristics. Lab-on-a-chip technologies amplify the efficiency and throughput of single-cell analyses, bringing us closer to a holistic understanding of cellular heterogeneity.

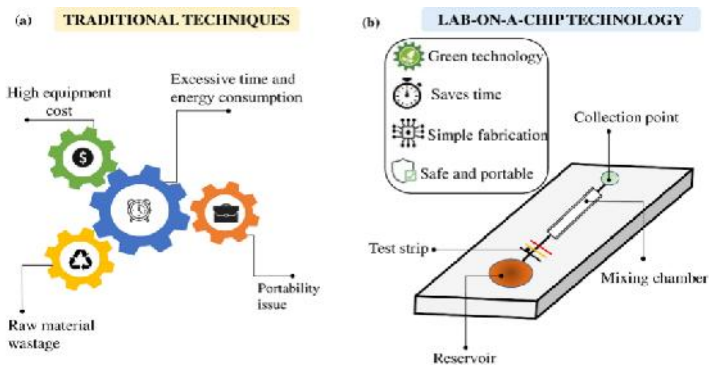


Image Source: Sridhar *et al*. (2022)

Applications in Developmental Biology and Disease Research

The applications of single-cell analysis are far-reaching. In developmental biology, the technique has unveiled the intricacies of cell fate determination, differentiation trajectories, and lineage commitment. In disease research, single-cell analyses have provided a deeper understanding of cellular heterogeneity within tumors, aiding in the identification of rare cell populations and potential therapeutic targets. The ability to dissect complex biological processes at the single-cell level has transformative implications across diverse fields.

The realm of single-cell analysis, we witness the unravelling of cellular heterogeneity with unprecedented precision. From the decoding of transcriptomic symphonies to the profiling of proteomic and metabolomic landscapes, single-cell analysis stands as a cornerstone in our quest to understand the intricacies of life at its most fundamental level. These images and insights are not merely glimpses into cellular diversity but gateways to a deeper comprehension of the dynamic and heterogeneous nature that underlies the unity of cellular life. Single-cell analysis, with its ever-expanding toolkit, invites us to explore the cellular cosmos, where every cell tells a unique story in the grand narrative of biology.

Structural Biology: Illuminating the Molecular Architecture

In the relentless pursuit of understanding life at its most fundamental level, structural biology has emerged as a cornerstone discipline, peeling back the layers of complexity to reveal the intricate molecular architecture that underpins biological processes. Recent advancements in X-ray crystallography, NMR spectroscopy, and cryo-electron microscopy have propelled our ability to visualize the three-dimensional structures of biological macromolecules, ushering in a new era of insight into the molecular world.

X-ray Crystallography: A Historic Lens into Atomic Structures

X-ray crystallography, with its rich history, stands as a venerable technique that continues to provide atomic-level insights into the architecture of proteins. This method involves the crystallization of biomolecules, followed by the diffraction of X-rays through the crystal lattice. The resulting diffraction patterns are then transformed into intricate molecular structures, allowing scientists to decipher the precise arrangement of atoms within proteins. X-ray crystallography has been instrumental in shaping our understanding of enzyme mechanisms, receptor-ligand interactions, and the spatial organization of biological macromolecules.

Image Source: Wikipedia: Freezed XRD - X-ray crystallography - Wikipedia

NMR Spectroscopy: Unveiling Molecular Dynamics in Solution

In contrast, NMR spectroscopy excels in elucidating the dynamic behavior of biomolecules in solution, providing a complementary perspective to the static images obtained through crystallography. This technique relies on the interaction of atomic nuclei with magnetic fields, allowing researchers to probe the structural and dynamic properties of proteins and nucleic acids in their native environments. NMR spectroscopy provides invaluable insights into the flexibility, motions, and interactions that define the functional landscape of biomolecules.

Image Source: NMR Spectroscopy/ChemTalk (chemistrytalk.org)

Cryo-Electron Microscopy: A Paradigm Shift in Structural Insight

The advent of cryo-electron microscopy (cryo-EM) represents a paradigm shift in structural biology, offering a revolutionary approach to imaging biological macromolecules. By flash-freezing samples in vitreous ice, cryo-EM preserves biomolecules in their native states, circumventing the need for crystallization. This technique captures high-resolution images of individual particles, allowing for the reconstruction of three-dimensional structures. Cryo-EM has democratized structural biology, making it applicable to a broader range of macromolecules, including large complexes and flexible structures that were once challenging to study.

Synergy of Experimental Data and Computational Modeling

The marriage of experimental data from structural techniques with computational modeling has become a hallmark of modern structural biology. Computational methods, such as molecular dynamics simulations and homology modeling, complement experimental findings, providing a dynamic understanding of molecular interactions. This synergy has propelled the field forward, enabling the determination of complex structures and the exploration of conformational changes that underlie biological function.

Enriching Drug Design and Therapeutic Interventions

The insights gained from structural biology play a pivotal role in drug discovery and targeted therapeutic interventions. Understanding the three-dimensional architecture of biological macromolecules allows for the identification of potential drug binding sites, the rational design of pharmaceutical compounds, and the optimization of therapeutic efficacy. The precision afforded by structural insights enhances the development of targeted therapies, opening new avenues for combating diseases at the molecular level.

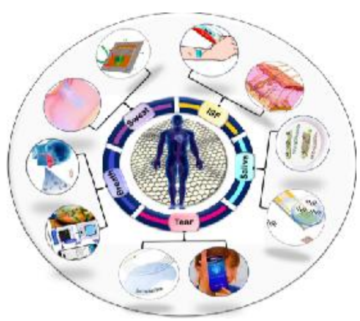
Structural biology stands as a beacon, illuminating the molecular architecture that orchestrates life's processes. From the historical lens of X-ray crystallography to the dynamic insights provided by NMR spectroscopy and the revolutionary capabilities of cryo-electron microscopy, each technique contributes a unique dimension to our understanding. The marriage of experimental and computational approaches not only enriches our comprehension of molecular interactions but also paves the way for innovative drug design strategies and targeted therapeutic interventions, propelling us into a future where the intricacies of life are unveiled with unprecedented clarity.

Biosensors and Imaging Probes: Illuminating Cellular Signatures

In the intricate dance of cellular life, biosensors and imaging probes have emerged as indispensable choreographers, casting light on the subtle nuances of molecular events with unparalleled sensitivity. Rooted in principles of fluorescence and bioluminescence, these technologies provide a dynamic window into the molecular processes that govern cellular function, offering not just snapshots but vivid narratives of life at the cellular level.

Advanced Biosensors: Real-Time Insights into Cellular Dynamics

Advanced biosensors stand as sentinel guardians, enabling the real-time detection of specific molecules, signaling pathways, and cellular responses. These technologies have revolutionized our ability to monitor the dynamic nature of biological systems with exquisite precision. From calcium ions and neurotransmitters to enzyme activities and cellular metabolites, biosensors act as molecular reporters, allowing researchers to witness the orchestrated symphony of molecular interactions within living cells.



Schematic overview of graphene-based wearable biosensors for the detection ofdifferent biomarkers

Image Source: Santoshi *et al*. (2022)

Fluorescent and Bioluminescent Imaging Probes: Visualizing Molecular Processes

Fluorescent and bioluminescent imaging probes, crafted with precision and specificity, serve as artistic brushes that paint vivid portraits of molecular processes within living organisms. These probes, designed to selectively bind to specific targets, emit light that can be captured and visualized. Fluorescence microscopy and bioluminescence imaging techniques, often coupled with advanced imaging platforms, unveil the spatial and temporal dynamics of cellular events. Whether tracking gene expression, protein localization, or metabolic activities, these imaging probes illuminate the intricacies of life in living color.

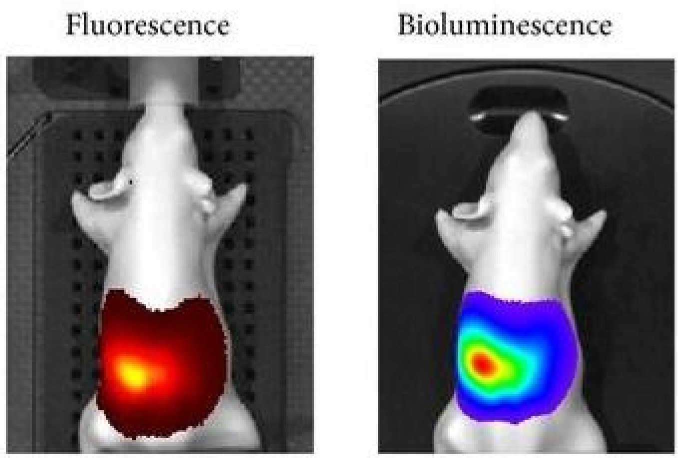
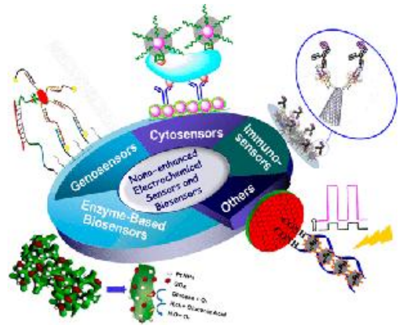


Image Source: Patterson *et al*. (2014)

Nanotechnology's Role: Enhancing Sensitivity and Selectivity

Nanotechnology emerges as a silent maestro, playing a pivotal role in enhancing the sensitivity and selectivity of biosensors. Engineered nanomaterials, such as quantum dots and nanoscale polymers, have empowered biosensors to detect molecular events at the nanoscale. The marriage of nanotechnology with biosensors has opened new frontiers, enabling their deployment in diverse biological contexts and facilitating the exploration of cellular landscapes with unprecedented clarity.



Schematic illustration of electrochemical sensors and biosensors based on nanomaterials and nanostructures

Image Source: Zhu *et al*. (2015)

Applications in Diagnostics and Therapeutic Monitoring

Beyond the realm of research, biosensors and imaging probes have paved the way for transformative applications in diagnostics and therapeutic monitoring. The non-invasive nature of these technologies makes them ideal candidates for tracking disease biomarkers, assessing treatment responses, and monitoring the progression of therapeutic interventions. This marriage of precision and non-invasiveness holds promise for personalized medicine, where cellular signatures become the guiding lights in the journey towards health.

Biosensors and imaging probes stand as luminary instruments, casting light on the cellular signatures that define life's processes. From advanced biosensors providing real-time insights to fluorescent and bioluminescent imaging probes visualizing molecular ballets, these technologies weave a narrative that transcends the boundaries of observation. Enhanced by the symphony of nanotechnology, the integration of biosensors and imaging probes not only deepens our understanding of cellular dynamics but also opens new avenues for diagnostic techniques and therapeutic monitoring, heralding a future where the language of cellular signatures is fluently spoken for the betterment of human health.

Mass Spectrometry in Biological Research: Analyzing the Molecular Landscape

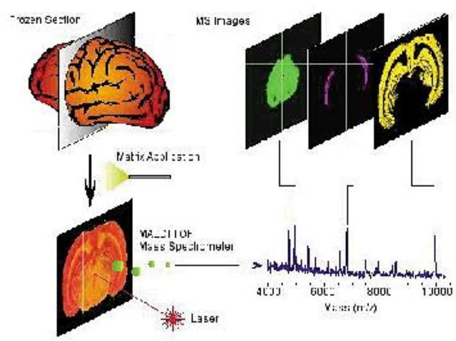
In the intricate tapestry of biological research, mass spectrometry stands as an unrivaled workhorse, wielding unparalleled capabilities to dissect the molecular landscape of complex biological samples. From proteomics to spatially resolved imaging, mass spectrometry has become a cornerstone technology, unraveling the intricate web of molecules that define cellular function and pathology.

Proteomics Unveiled: Identification, Quantification, and Characterization

At the heart of mass spectrometry's prowess in biological research lies its pivotal role in proteomics. This technology enables the identification, quantification, and characterization of proteins with a precision that transcends traditional methodologies. High-resolution mass spectrometry platforms have evolved to become the stalwarts of proteomic analyses, allowing researchers to unravel intricate signaling networks, delineate protein interactions, and elucidate the dynamic orchestration of cellular processes.

Imaging Mass Spectrometry: A Spatial Revolution

The advent of imaging mass spectrometry represents a paradigm shift, extending the capabilities of traditional mass spectrometry into the spatial domain. This powerful approach allows for the spatially resolved molecular analysis within biological tissues, providing a unique lens to understand the distribution of lipids, metabolites, and proteins. The spatial context offered by imaging mass spectrometry is invaluable, offering critical insights into tissue function, disease progression, and the molecular intricacies of cellular microenvironments.

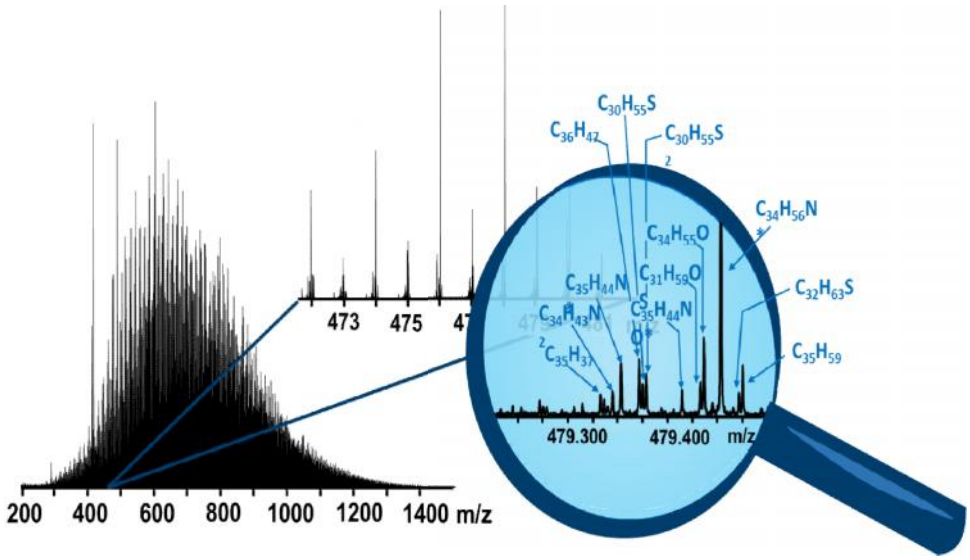


Mass Spectrometry Imaging (MSI)

Image source: Stoeckli *et al*. (2001)

High-Resolution Mass Spectrometry: Precision Redefined

Advances in high-resolution mass spectrometry further elevate the accuracy and depth of molecular identification. This refinement enables researchers to discern subtle differences in molecular masses, facilitating the discrimination of closely related species and the identification of post-translational modifications. High-resolution instruments, coupled with sophisticated data analysis techniques, empower scientists to navigate the intricate landscape of biological molecules with unprecedented precision.



High resolved mass spectrum of a heavy oil

Image Source: https://www.zimmermann.chemie.uni-rostock.de/en/research/advanced-mass-spectrometry/high-resolution-mass-spectrometry/

Indispensable Tools for Unraveling Complexity

Mass spectrometry, in its various forms, has become an indispensable tool for unraveling the complexities of the biological landscape. From elucidating the intricate details of protein structures to mapping the spatial distribution of molecules in tissues, mass spectrometry offers a multidimensional perspective that enriches our understanding of the molecular underpinnings of life. Its versatility spans applications in biomarker discovery, drug development, and the exploration of disease mechanisms.

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Future Horizons: Driving Innovations in Biological Inquiry

As technology continues to evolve, mass spectrometry remains at the forefront of innovation in biological research. Ongoing advancements promise to expand the frontiers of what is possible, pushing the boundaries of sensitivity, speed, and spatial resolution. The integration of mass spectrometry with other cutting-edge technologies, such as artificial intelligence and automation, heralds a future where biological inquiries are driven by unprecedented insights and efficiency.

Mass spectrometry stands as a beacon in the realm of biological research, illuminating the molecular landscape with precision and depth. From unraveling the intricacies of the proteome to spatially mapping the molecules within tissues, mass spectrometry has become an indispensable ally in the quest to decipher the language of biological molecules. As we navigate the complexities of life, mass spectrometry continues to evolve, propelling us into a future where the secrets of the biological landscape are unveiled with unprecedented clarity and sophistication.

High-Throughput Screening: Accelerating Drug Discovery

In the relentless pursuit of novel therapeutics, high-throughput screening (HTS) technologies have emerged as transformative engines, propelling drug discovery into a new era of speed, efficiency, and precision. With automated screening platforms, robotics, and cutting-edge instrumentation, HTS has revolutionized the evaluation of vast compound libraries against biological targets, significantly expediting the identification of potential drug candidates for a myriad of diseases.

Automated Platforms: The Accelerators of Drug Discovery

The linchpin of high-throughput screening lies in automated platforms, equipped with robotics that tirelessly execute complex experimental protocols with unparalleled speed and precision. These platforms enable the rapid evaluation of large compound libraries against biological targets, a process that once demanded extensive time and resources. With high-throughput capabilities, researchers can swiftly sift through immense chemical spaces, identifying compounds with therapeutic potential across various diseases, including cancer, infectious diseases, and neurological disorders.

Artificial Intelligence and Machine Learning: Refining the Screening Process

The integration of artificial intelligence (AI) and machine learning (ML) algorithms marks a paradigm shift in high-throughput screening. These intelligent systems analyze vast datasets, identify patterns, and predict potential drug candidates with unprecedented efficiency. By learning from experimental outcomes, AI and ML contribute to the optimization of experimental designs, reducing the need for exhaustive trial-and-error approaches. This synergy between human ingenuity and computational prowess enhances the precision and success rates of drug discovery endeavors.

CRISPR-Based Functional Genomics Screening: Unleashing Systematic Exploration

A transformative approach within high-throughput screening is the utilization of CRISPR-based functional genomics screening. Leveraging the revolutionary CRISPR-Cas9 gene-editing system, this technique enables the systematic exploration of gene function and its implications in disease pathways. By selectively perturbing genes in a high-throughput manner, researchers can uncover the roles of specific genes in cellular processes and identify potential therapeutic targets. CRISPR-based functional genomics screening adds a layer of depth to HTS, revealing the molecular intricacies that underlie diseases.

Efficiency and Precision in Targeted Therapies

The collective advancements in high-throughput screening, bolstered by automation, artificial intelligence, and functional genomics, have redefined the landscape of drug discovery. The efficiency and precision achieved through HTS have streamlined the identification of targeted therapies, allowing researchers to focus on promising candidates with higher confidence. This paradigm shift not only accelerates the drug discovery timeline but also increases the likelihood of bringing effective therapies to patients in need.

Future Horizons: Innovation in Drug Discovery

As high-throughput screening continues to evolve, the future of drug discovery holds the promise of even greater innovation. Integrating emerging technologies, such as advanced robotics, microfluidics, and organ-on-a-chip systems, will further enhance the capabilities of HTS. The marriage of technological advancements with biological insights positions drug discovery at the forefront of innovation, ushering in a new era where targeted therapies for a wide range of diseases are identified and developed with unprecedented speed and precision.

High-throughput screening stands as a cornerstone in the quest for novel therapeutics, revolutionizing drug discovery through automation, artificial intelligence, and functional genomics. The accelerated pace, increased efficiency, and precision brought about by HTS reshape the landscape of drug development, offering hope for the rapid identification of targeted therapies that address unmet medical needs. As we navigate the future of drug discovery, high-throughput screening continues to be a beacon, guiding researchers towards innovative solutions and breakthroughs in the realm of medicine.

Bioinformatics and Computational Biology: Deciphering the Data Deluge

In the era of big data, the exponential growth of biological information has ushered in an age where the management, analysis, and interpretation of vast datasets are paramount. Bioinformatics and computational biology emerge as indispensable disciplines, providing the tools and methodologies essential for unraveling the complexities hidden within genomics, proteomics, metabolomics, and imaging data.

The Data Deluge: Necessitating Computational Solutions

The deluge of biological data, generated through high-throughput technologies, necessitates robust computational approaches to distill meaningful insights. Bioinformatics acts as the digital bridge, facilitating the storage, retrieval, and analysis of biological information. Whether deciphering the intricacies of DNA sequences, unraveling the proteomic landscape, or mining metabolomic profiles, bioinformatics becomes the linchpin for transforming raw data into actionable knowledge.

Machine Learning and Artificial Intelligence: Game-Changers in Computational Biology

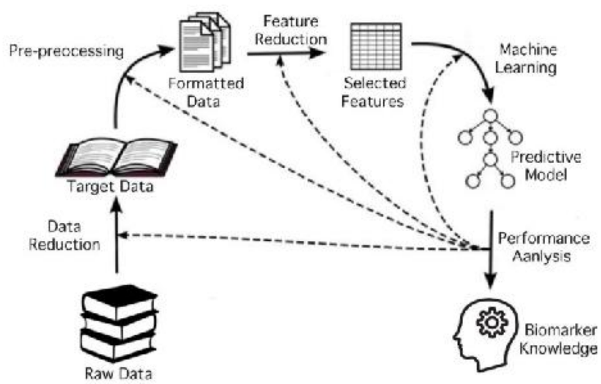
The advent of machine learning (ML) and artificial intelligence (AI) marks a paradigm shift in computational biology, where these technologies evolve from tools into game-changers. ML algorithms, armed with predictive models and pattern recognition capabilities, empower researchers to extract hidden patterns from vast datasets. AI, with its capacity for autonomous learning, contributes to the development of sophisticated models for disease diagnosis, prognosis, and the identification of novel biomarkers. The integration of ML and AI transcends traditional analytical boundaries, offering unprecedented insights into the molecular tapestry of life.

Synergy between Experimental Data and Computational Approaches

The symbiotic relationship between experimental data and computational methodologies is the driving force behind the advancement of biological understanding. As biological datasets evolve in complexity and scale, the synergy between experimental insights and computational prowess becomes increasingly crucial. Computational models not only validate experimental findings but also provide a framework for extrapolating knowledge, making sense of intricate relationships within biological systems.

Predictive Models and Biomarker Discovery

One of the crowning achievements of bioinformatics and computational biology lies in their ability to construct predictive models and unearth novel biomarkers. These models, often fueled by machine learning algorithms, empower researchers to forecast disease outcomes, identify potential therapeutic targets, and optimize treatment strategies. The integration of computational approaches in biomarker discovery accelerates the translation of research findings into tangible clinical applications.



Biomarker discovery process.

Image source: Bazazeh, *et al*., 2016

Challenges and Opportunities on the Horizon

While bioinformatics and computational biology have transformed the landscape of biological research, challenges persist. The integration of multi-omics data, addressing data heterogeneity, and ensuring the reproducibility of computational analyses remain areas of ongoing refinement. However, with challenges come opportunities for innovation. The future of computational biology holds promises of advanced algorithms, personalized medicine tailored to individual genomic profiles, and the integration of AI-driven insights into routine clinical practice.

Bioinformatics and computational biology stand as linchpins in the face of the biological data deluge. From managing and interpreting large-scale datasets to harnessing the power of machine learning and artificial intelligence, these disciplines shape the trajectory of biological research. The synergy between experimental data and computational methodologies is a testament to the transformative potential of combining biological insights with computational prowess. As we navigate the complexities of biological information, bioinformatics and computational biology remain at the forefront, deciphering the data deluge and paving the way for a future where the intricacies of life are decoded with unprecedented precision.

Future Perspectives: Charting the Course Ahead

At the intersection of technological innovation and biological discovery, the future holds boundless possibilities. Anticipating the trajectory of instrumentation in biological sciences requires a holistic perspective on emerging trends and potential breakthroughs.

In the realm of imaging technologies, the integration of artificial intelligence for real-time image analysis and interpretation is poised to enhance the efficiency of experiments and accelerate discoveries. Furthermore, the convergence of multiple imaging modalities, such as combining microscopy with spectroscopy, promises a more comprehensive understanding of cellular dynamics.

Omics technologies will continue to evolve, with advancements in long-read sequencing technologies addressing the limitations of short-read platforms. The refinement of CRISPR-based techniques and the development of novel gene-editing tools will further empower precision genomics.

Single-cell analysis is on the cusp of broader applications, extending beyond RNA sequencing to encompass multi-omics profiling at the single-cell level. Microfluidic technologies will play a pivotal role in enabling high-throughput single-cell analyses, contributing to our understanding of cellular heterogeneity in health and disease.

Structural biology is set to benefit from advancements in cryo-electron microscopy, particularly in sample preparation techniques and data analysis algorithms. The integration of artificial intelligence in structural biology workflows holds the potential to expedite the determination of complex structures.

Biosensors and imaging probes will continue to evolve with the refinement of nanomaterials and the development of new imaging modalities. The application of biosensors for real-time monitoring within living organisms is an exciting frontier, offering dynamic insights into physiological processes.

Mass spectrometry will witness advancements in sensitivity and resolution, enabling more comprehensive and accurate analyses of complex biological samples. Innovations in ambient ionization techniques will further broaden the applicability of mass spectrometry in various research fields.

High-throughput screening will benefit from the integration of organ-on-a-chip technologies, allowing for more physiologically relevant drug testing. The combination of high-throughput screening with CRISPR-based approaches will deepen our understanding of gene function and its implications for drug discovery.

Bioinformatics and computational biology will grapple with the challenges posed by big data, prompting the development of scalable and efficient algorithms. Explainable artificial intelligence models will gain prominence in biological research, fostering greater trust in computational predictions.

In conclusion, the chapter on "Advances in Instrumentation for Biological Sciences: Unveiling the Secrets of Life" serves as a testament to the transformative impact of cutting-edge technologies on our understanding of life processes. From the microscopic world revealed by advanced imaging to the intricate molecular interactions unveiled by omics technologies, single-cell analysis, structural biology, biosensors, mass spectrometry, high-throughput screening, and computational biology, each instrument represents a powerful tool in our scientific arsenal. As we navigate the complex landscape of biological sciences, the continuous evolution of instrumentation promises to unravel new layers of biological complexity, paving the way for groundbreaking discoveries and transformative applications in medicine, biotechnology, and beyond.

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Reptilia - Squamata: General organization and Affinities

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Introduction

Squamata (scaled reptiles) is the most diverse order of extant reptiles, comprised of the lizards and snakes and characterized a flexible jaw structure (movable quadrate bones) and having scales or shields rather than shells or secondary palates. Of the four surviving orders-the others being Crocodilia, Rhynchocephalia, and Testudines—the squamates represent more than 95 percent of the known living species. Despite their diverse forms—such as the lack of legs in snakes, presence of legs in lizards, and resemblance of the amphisbaenians to worms-squamates share many of the same traits. Some of these characters are not shared with any other reptiles, and in the case of paired penes, with any other vertebrates.

This reflects that all squamates belong to the same lineage, as descendants of a common ancestor. Squamates first appeared in the fossil record during the mid-Jurassic and probably existed before that time. The fossil record for squamates is rather sparse. Modern squamates arose about 160 million years ago, during the late Jurassic. The earliest lizard fossils are between 185 and 165 million years old. The closest living relatives of the squamates are the tuatara, followed by the crocodiles and birds. Of all living reptiles, turtles are the most distant relatives of the squamates. Like crocodilians, squamates are diapsids, a group of reptiles that possess two holes (or temporal fenestra) on each Squamates (Squamata) are the most diverse of all the reptile groups, with approximately 7400 living species. Squamates include lizards, snakes, and worm lizards.

Characters

There are two characteristics that unite the squamates. The first is that they shed their skin periodically. Some squamates, such as snakes, shed their skin in one piece. Other squamates, such as many lizards, shed their skin in patches. In contrast, non-squamate reptiles regenerate their scales by other means—for example, crocodiles shed a single scale at a time while turtles do not shed the scales that cover their carapace and instead add new layers from beneath.

The second characteristic shared by squamates is their uniquely jointed skulls and jaws, which are both strong and flexible. The extraordinary jaw mobility of squamates enables them to open their mouths very wide and in doing so, consume large prey. Additionally, the strength of their skull and jaws provides squamates with a powerful bite grip.

The 7,200 species of snakes, lizards, and wormlizards all fall under the order Squamata and are therefore known as squamates (SKWAH-mates). Perhaps the most noticeable difference between the snakes and the lizards are the legs, or the lack of them. Most lizards, except for a few species, have working legs. Snakes are legless. The most noticeable feature of the worm lizards is their earthworm-like body. While they have scales and earthworms do not, worm lizards' scales are arranged in rings and separated with grooves to give them the appearance of an earthworm's ringed body. Most of the worm lizards are legless, although a few have two front legs just behind the head.

Besides smelling with their noses and tasting with their tongues, most squamates also smell with a special organ on the roof of their mouths. They use it by first flicking or otherwise picking up chemicals on the tongue. They then place the tongue on the roof of the mouth at what is called the Jacobson's organ, which smells the chemicals. For hearing, many lizards have ears that are visible as a hole on either side of the head. Neither the snakes nor the wormlizards have the openings for their ears. Scientists believe that snakes can probably only hear very low-pitched sounds, including ground vibrations that they sense in the jaw and send to the ear.

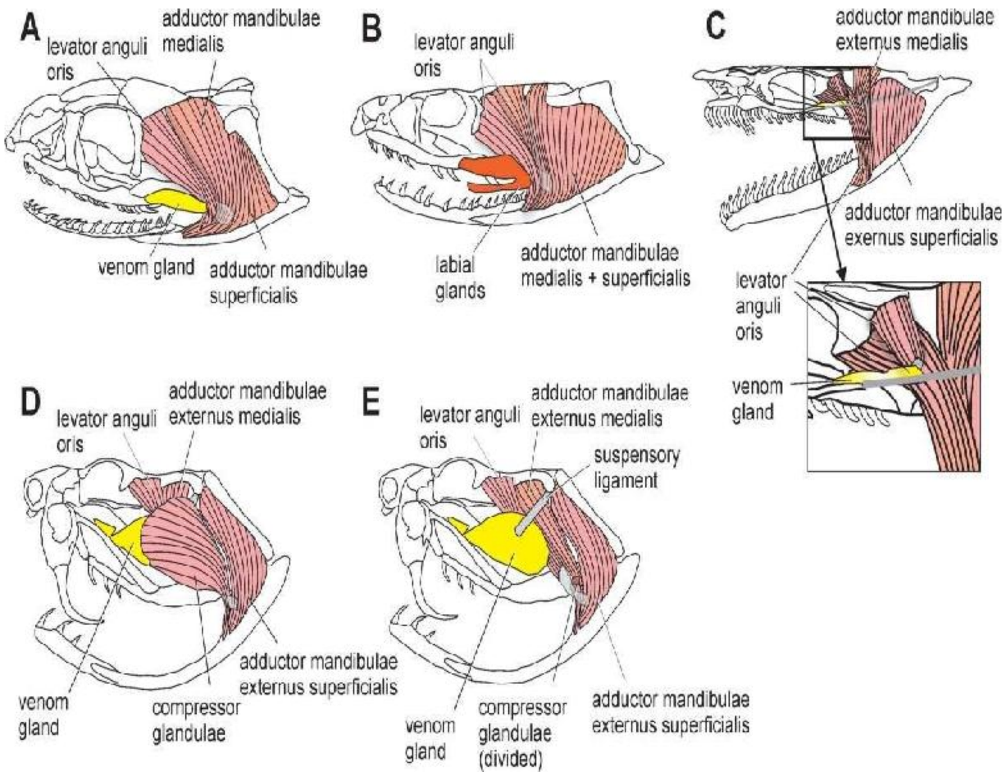
In addition to the presence or absence of ear holes, known as external ears, snakes and lizards have another obvious difference. The majority of lizards have eyelids that close and open. Snakes, on the other hand, have a spectacle over their eyes. A spectacle is a clear scale, which looks much like a contact lens. In other words, a lizard noticeably blinks, but a snake always appears to have its eyes open. Although most squamates have obvious eyes, those species that spend most of their lives underground often have very small eyes, which are sometimes invisible under their scales.

Size

The size of the squamate depends on the species. Among the lizards, the heaviest is the Indonesian Komodo dragon, which can grow to be at least 9.9 feet (3 meters) long and 330 pounds (150 kilograms). Many people consider the crocodile monitor to be the world's longest lizard. It can reach 12 feet (nearly 3.7 meters) long, although some reports claim that the lizards can reach 15 to 19 feet (4.6 to 5.8 meters) long. The smallest lizard, on the other hand, is the jaragua lizard, also known as the dwarf gecko. From one end to the other, adults of this recently discovered species only reach about 1.2 inches (3.2 centimeters) long. Snakes also come in different sizes. Some of the smallest are in the blind snake and slender blind snake families, which include species that only grow to 4 inches (10 centimeters) long and weigh just 0.05 ounces (1.4 grams). This compares to the reticulated python, which often reaches 20 feet (6.1 meters) or more. The largest reticulated python ever discovered was killed in 1912 in Indonesia. This beast measured 33 feet (10.1 meters) in length. The South American green anaconda is another enormous species, often reaching 25 feet (7.7 meters) long and 300 pounds (136 kilograms). Wormlizard adults range from 3.1 inches (8 centimeters) to more than 32 inches (81 centimeters) long.

Skull and jaws

The skull is derived from the primitive diapsid condition, but the lower bar leading back to the quadrate bone is absent, however, giving greater flexibility to the jaw. In some burrowers (such as Anniella and the worm lizards) as well as some surface-living forms (such as the geckos), the upper and lower temporal bars have been lost. Small burrowing lizards have thick, tightly bound skulls with braincases that are well protected by bony walls.



In most lizards, the front of the braincase is made up of thin cartilage and membrane, and the eyes are separated by a thin, vertical interorbital septum. In burrowing forms with degenerate eyes, the septum is reduced and adds to the compactness of the skull. Most lizard skulls, particularly in the Scleroglossa, are kinetic (that is, the upper jaw can move in relation to the rest of the cranium). Since the anterior part of the braincase is cartilaginous and elastic, the entire front end of the skull can move as a single segment on the back part, which is solidly ossified. This increases the gape of the jaws and probably assists in pulling struggling prey into the mouth.

Scales and Colour Change

Lizards come in a wide variety of colors, including red, orange, yellow, green, blue, indigo, and violet. Most match the color of substrates on which they live, offering camouflage, which confers some degree of protection from predators. Snakes are equally colorful, with some, such as coral snakes (Micrurus), being warningly colored with bright bands of red, yellow, and black.

Except for openings of nostrils, mouth, eyes, and cloaca, most lizards are completely covered in scales. Scales may be smooth and overlapping, form a mosaic of flat plates, or have keels or tubercles. The arrangement varies among species and by body part. The outer parts of the scales are composed of dead horny tissue made up largely of the protein keratin. The dead layer is shed at intervals and is replaced by proliferating cells in the deep part of the epidermis. In some lizards, osteoderms, which are bony plates that develop in the dermis, underlie head and body scales. In addition, certain lizards have scale organs, with a stiff projecting seta emerging from the serrated edge of the scale. Presumably, these setae are responsive to touch.

Many lizards can change colour. The most notable groups in this regard are the chameleons and the anoles. Some species can change from bright green to deep, chocolate brown, and patterns such as lines and bars may appear and disappear along their bodies. Melanophores are the pigment cells that permit colour change, and the concentration of pigment granules within these cells determine the type of colour that is produced. In general, the animal appears lighter coloured when pigment is concentrated and dark when pigment is dispersed throughout the cells. The animal's colour state at any given time is controlled by a complex interaction of hormones, temperature, and the nervous system.

Behavior

Because they are ectothermic, which means that their body temperature changes based on the outside temperature, many squamates sunbathe, or bask, to warm up. Others, however, stay out of sight during the day. Some of the fossorial species rarely come out of the ground at all. These species will sometimes increase their body temperatures by moving to a warmer underground spot. For hunting, many of the squamates actively walk or slither about looking for prey. Others, however, hunt by ambush, which means that they sit still, wait for a prey animal to come along, then spring out to grab and eat it. Some snakes, including the pit vipers and the boas, have a special method of hunting. They can sense heat through small holes, called pit organs, on the face. Using these pit organs, they are able actually to see the heat given off by an animal in 3-D. These pit organs come in especially handy when hunting for food at night or in places where the snake has a limited view.

Some important features

1. Advance, small, to medium, elongated
2. Limbs clawed, absent in snake and few lizards
3. Exoskeleton of horny epidermal scales, shield and spines
4. Skull diapsid, Quadrate movable
5. Jaws designed for applying crushing or gripping force to prey
6. Vertebrae procoelous, ribs single headed
7. Teeth acrodont or pleurodont
8. Ectothermic

9. Heart incompletely 4 chambered
10. Respiration by Lungs
11. Cloacal aperture is transverse
12. Male with eversible double copulatory organ (hemipenes)
13. Egg covered in leathery shells

Distribution and Habitat

Snakes and lizards are found everywhere in the world, except at very high latitudes, on cold mountaintops, and in the Arctic and Antarctic. At high latitudes and elevations, temperature becomes a limiting factor for animals that rely on external heat sources (ectotherms). Nevertheless, many lizards and snakes have evolved adaptations, such as viviparity (bearing live young), that facilitate living in cold environments.

Besides the Arctic, Antarctic, and other very cold places, squamates live almost the world over. Squamates can live in many habitats, from the dry conditions in the desert to the wet and warm rainforests. Many of them, including numerous lizards and snakes, live above ground on land. Some, such as the wormlizards, are fossorial, which means that they remain underground most of the time. Others, including many snakes, are arboreal, which means that they often live above the ground among tree branches. Some, like the water snakes, rarely leave their freshwater streams or ponds, while the sea kraits are snakes that spend their lives in salt water.

Sense organs

Eyes

In general construction the eyes of reptiles are like those of other vertebrates. Accommodation for near vision in all living reptiles except snakes is accomplished by pressure being exerted on the lens by the surrounding muscular ring (ciliary body), which thus makes the lens more spherical. In snakes the same end is achieved by the lens being brought forward. The lens moves as a result of the pressure built up on the vitreous humour by contractions of muscles located at the base of the iris. The pupil shape varies remarkably among living reptiles, from the round opening characteristic of all turtles and many diurnal lizards and snakes to the vertical slit of crocodiles and nocturnal snakes and the horizontal slits of a few tree snakes. Undoubtedly the most bizarre pupil shape is that of some geckos, in which the pupil contracts to form a series of pinholes, one above the other. The lower eyelid has the greater range of movement in most reptiles. In crocodiles the upper lid is more mobile. Snakes have no movable eyelids, their eyes being covered by a fixed transparent scale. tuatara and all crocodiles have a third eyelid, the nictitating membrane, a transparent sheet that moves sideways across the eye from the inner corner, cleansing and moistening the cornea without shutting out the light.

Visual acuity varies greatly among living reptiles, being poorest in the burrowing lizards and snakes (which often have very small eyes) and greatest in active diurnal species (which usually have large eyes). Judging by the size of the skull opening in which the eye is situated, similar variation existed among the extinct reptiles. Extinct forms, such as the ichthyosaurs, that hunted active prey had large eyes and presumably excellent vision; many herbivorous types, such as the horned dinosaur Triceratops, had relatively small eyes and weak vision. Colour vision has been demonstrated in few living reptiles.

Hearing

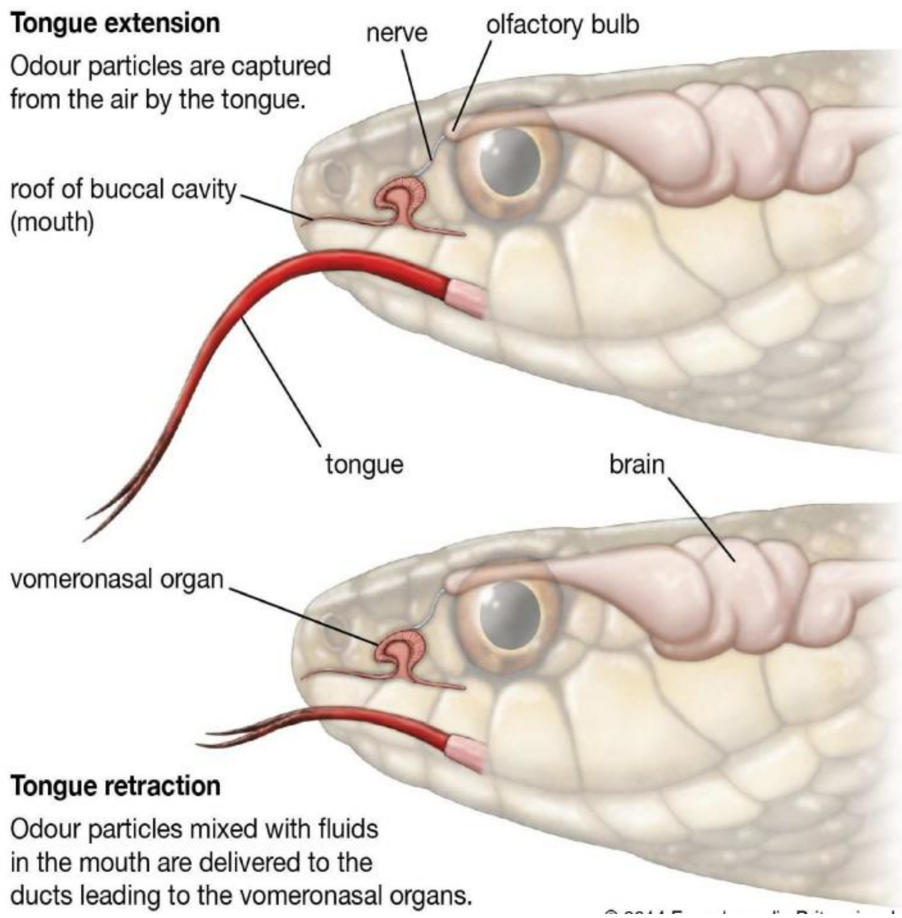
The power of hearing is variously developed among living reptiles. Crocodiles and most lizards hear reasonably well. Snakes and turtles are sensitive to low-frequency vibrations, thus they "hear" mostly earth-borne, rather than aerial, sound waves. The reptilian auditory apparatus is typically made up of a tympanum, a thin membrane located at the rear of the head; the stapes, a small bone running between the tympanum and the skull in the tympanic cavity (the middle ear); the inner ear; and a eustachian tube connecting the middle ear with the mouth cavity. In reptiles that can hear, the tympanum vibrates in response to sound waves and transmits the vibrations to the stapes. The inner end of the stapes abuts against a small opening (the foramen ovale) to the cavity in the skull containing the inner ear. The inner ear consists of a series of hollow interconnected parts: the semicircular canals; the ovoidal or spheroidal chambers called the utriculus and sacculus; and the lagena, a small outgrowth of the sacculus. The tubes of the inner ear, suspended in a fluid called perilymph, contain another fluid, the endolymph. When the stapes is set in motion by the tympanum, it develops vibrations in the fluid of the inner ear; these vibrations activate cells in the lagena, the seat of the sense of hearing. The semicircular canals are concerned with equilibrium.

Most lizards can hear. The majority have their best hearing in the range of 400 to 1,500 hertz and possess a tympanum, a tympanic cavity, and a eustachian tube. The tympanum, usually exposed at the surface of the head or at the end of a short open tube, may be covered by scales or may be absent. In general the last two conditions are characteristic of lizards that lead a more or less completely subterranean life. For subterranean lizards airborne sounds are less important than the low-frequency sounds passing through the ground. The middle ear of these burrowers is usually degenerate as well, often lacking the tympanic cavity and eustachian tube.

Snakes have neither tympanum nor eustachian tube, and the stapes is attached to the quadrate bone on which the lower jaw swings. Snakes are obviously more sensitive to vibrations in the ground than to airborne sounds. A loud sound above a snake does not elicit any response, provided that the object making the sound does not move or, if it does, the movements are not seen by the snake. On the other hand, the same snake will raise its head slightly and flick its tongue in and out rapidly if the ground behind it is tapped or scratched. Snakes undoubtedly “hear” these vibrations by means of bone conduction. Sound waves travel more rapidly and strongly in solids than in the air and are probably transmitted first to the inner ear of snakes through the lower jaw, which is normally touching the ground, thence to the quadrate bone, and finally to the stapes. Burrowing lizards presumably hear ground vibrations in the same way.

Chemoreception

Chemically sensitive organs, used by many reptiles to find their prey, are located in the nose and in the roof of the mouth. Part of the lining of the nose is made up of cells subserving the function of smell and corresponding to similar cells in other vertebrates. The second chemoreceptor is the Jacobson’s organ, which originated as an outpocketing of the nasal sac in amphibians; it remained as such in tuatara and crocodiles. The Jacobson’s organ is most developed in lizards and snakes, in which its connection with the nasal cavity has been closed and is replaced by an opening into the mouth. The nerve connecting Jacobson’s organ to the brain is a branch of the olfactory nerve. In turtles the Jacobson’s organ has been lost.



The use of the Jacobson's organ is most obvious in snakes. If a strong odour or vibration stimulates a snake, its tongue is flicked in and out rapidly. With each retraction, the forked tip touches the roof of the mouth near the opening of the Jacobson's organ, transferring any odour particles adhering to the tongue. In effect, the Jacobson's organ is a short-range chemoreceptor of nonairborne odours, as contrasted to the detection of airborne odours, smelling in the usual sense, by olfactory sensory patches in the nasal tube. Some snakes (notably the large vipers) and scleroglossan lizards (such as skinks, monitors, and burrowing species of other families) rely upon the olfactory tissue and the Jacobson's organ to locate food, almost to the exclusion of other senses. Other reptiles, such as certain diurnal lizards and crocodiles, appear not to use scent in searching for prey, though they may use their sense of smell for locating a mate.

The pit vipers (family Viperidae), boas and pythons (family Boidae), and a few other snakes have special heat-sensitive organs (infrared receptors) on their heads as part of their fooddetecting apparatus. Just below and behind the nostril of a pit viper is the pit that gives the group its common name. The lip scales of many pythons and boas have depressions (labial pits) that are analogous to the viper's pit. The labial pits of pythons and boas are lined with skin thinner than that covering the rest of the head and are supplied with dense networks of blood capillaries and nerve fibres. The facial pit of the viper is relatively deeper than the boa's labial pits and consists of two chambers separated by a thin membrane bearing a rich supply of fine blood vessels and nerves. In experiments using warm and cold covered electric light bulbs, pit vipers and pitted boas have been shown to detect temperature differences of less than 0.6 °C (1.1 °F).

Many pit vipers, pythons, and boas are nocturnal and feed largely on mammals and birds. Infrared receptors, located on the face, enable these reptiles to direct their strikes accurately in the dark, once their warm-blooded prey arrives within range. The approach of prey is likely identified by the vibrations they make on the ground; however, the sense of sight and perhaps even the sense of smell are also used. The pit organs simply confirm the identity of the prey and aim the strike.

Locomotion and Limb adaptations

Most lizards are quadrupedal and have a powerful limb musculature. They are capable of rapid acceleration and can rapidly change direction. The racerunners or whiptails (Aspidoscelis) can attain speeds of 29 km (18 miles) per hour, which, in terms of their own body length (less than 50 cm [20 inches] long), puts them in a class with fast terrestrial mammals. A tendency toward elongation of the body is found in some families, and a reduction of limb length or a complete loss of limbs often accompanies such elongation. Such lizards propel themselves entirely by lateral undulations emanating from highly complicated ventral abdominal muscles. Limbless lizards that move quickly on the surface or through sand (such as glass snakes [Ophisaurus]) tend to have elongate tails, whereas the burrowers have extremely reduced tails. Some burrowers (such as the amphisbaenians) dig by ramming the head into the substrate. This is followed by the rotation of the head around the head joint to compress the substrate. Others, like the California legless lizards (Anniella), literally "swim" through the sand.

Many modifications of the toes occur in lizards. Some desert geckos, the iguanid Uma, and the lacertidAcanthodactylus have fringes on the toes that provide increased surface area, preventing the lizard from sinking into loose desert sand. Arboreal geckos and anoles (Anolis) have lamellae (fine plates) on the undersides of the toes. Each lamella is made up of brushlike setae. The tips of each seta divide hundreds of times into tiny spatulae (spoon-shaped strands); the final strand is less than 0.25 micrometre (0.00001 inch) in diameter. (A tokay gecko [Gekko gecko], for example, has about half a million setae on each foot.) These fine hairlike processes greatly enhance the clinging ability of the lizards, allowing some to easily climb vertical panes of glass.

Intermolecular forces between spatulae on the gecko’s setae and the surface provide the adhesion.

The true chameleons (family Chamaeleonidae), a predominantly arboreal group, have a different type of highly specialized limb. The digits on each foot are divided into two groups by webs of skin. On each hind limb, three of the toes face away from the body, whereas two face toward the body; on each forelimb, the pattern is reversed. Each foot can thus be divided into an outer and an inner portion, which can be opposed as the branch is gripped. Chameleons and some other lizards have prehensile tails, which also aid in grasping branches.

Several terrestrial lizards are able to run bipedally. Basilisk lizards (Basiliscus) are actually able to run across water for short distances. During bipedal locomotion the tail is held out backward and upward and acts as a counterweight. The frilled lizard (Chlamydosauruskingii) can also run bipedally.

Some lizards are able to parachute or glide through the air and make soft landings. The most highly adapted of these are the flying lizards (Draco), a group of agamids from Southeast Asia. The “wings” that enable this lizard to glide are extensible lateral folds of skin that are supported by elongate ribs.

Diet and Digestive system

Dentition

Most lizards eat a variety of arthropods, with sharp, tricuspid teeth adapted for grabbing and holding. In most lizards, teeth are present along the jaw margin (on the maxilla, premaxilla, and dentary bones). However, in some forms, teeth may also be found on the palate. In the embryo, an egg tooth develops on the premaxilla bone and projects forward from the snout. Although it aids in piercing the shell, it is lost soon after hatching. This is a true tooth, unlike the horny epidermal point in turtles and crocodilians. The teeth of some large predators are conical and slightly recurved. The Komodo dragon (Varanuskomodoensis), for example, has serrated teeth that are curved like a scalpel blade; these teeth can cut through the leg muscle of a full-grown water buffalo (Bubalusbubalis) and cause it to bleed to death. In contrast, mollusk and crustacean feeders, such as the caiman lizard (Dracaena), have blunt, rounded teeth in the back of the jaw designed for crushing. Some herbivorous species (such as iguanas) have leaf-shaped tooth crowns with serrated cutting edges. The venomous lizards (Heloderma) have a longitudinal groove or fold on the inner side of each mandibular tooth; these grooves conduct the venom from the lizard to its victim.

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The common mode of tooth implantation is pleurodonty, in which the teeth are fused to the inner side of the labial wall. In the other mode, acrodonty, teeth are fused to the tooth-bearing bone, often to the crest of the bone. Acrodont teeth are rarely replaced once a certain growth stage is reached. The dentition of the Agamidae is usually described as acrodont, but most species have several pleurodont teeth at the front of the upper and lower jaws.

Diet

Most of the squamates eat other animals. Many of the lizards and the smaller snake species eat insects or other invertebrates, which are animals without backbones. Even some of the mediumsized snakes eat invertebrates. Eastern garter snakes, for example, like to dine on earthworms. A large number of the medium- to large-sized snakes, however, eat other snakes, lizards, frogs and tadpoles, mammals and other vertebrates, which are animals with backbones. Boa constrictors, pythons, and other very large snakes sometimes eat calves, deer, and other big mammals. Monitor lizards, which can grow to 12 feet (3.7 meters) or longer, can also capture, kill, and eat large mammals, such as deer, monkeys, wild pigs, and even buffalo. They are also known to eat dead animals, or carrion, that they come across. Some species of squamates eat plants either in addition to or instead of meat. Many of the iguanas, for instance, eat flowers, fruits, and leaves.

The digestive system of modern reptiles is similar in general plan to that of all higher vertebrates. It includes the mouth and its salivary glands, the esophagus, the stomach, and the intestine and ends in a cloaca. Of the few specializations of the reptilian digestive system, the evolution of one pair of salivary glands into poison glands in the venomous snakes is the most remarkable.

Compared to mammals and birds, squamates must have meals much less frequently. Because they are ectothermic and do not have to use their energy to keep up a constant body temperature, as the mammals and birds do, they can get by on much less food. Some of the large snakes can survive many months -even a full year-on one big meal.

Urinogenital system

In snakes and lizards, must conserve body water, and they convert their nitrogenous wastes to insoluble, harmless uric acid, which forms a more or less solid mass in the cloaca. In snakes and lizards, these wastes are eliminated from the cloaca together with wastes from the digestive system.

Prior to the evolution of the metanephric kidney, the products of the male gonad, the testis, traveled through the same duct with the nitrogenous wastes from the kidney. But with the appearance of the metanephros, the two systems became separated. The female reproductive system never shared a common tube with the kidney. Oviducts in all female vertebrates arise as separate tubes with openings usually near, but not connected to, the ovaries. The oviducts, like the Wolffian ducts of the testes, open to the cloaca. Both ovaries and testes lie in the body cavity near the kidneys.

Courtship and territoriality

Observe an anole change its skin colour for camouflage and fan its dewlap to mark possession or attract a mate. Social interactions among lizards are best understood for the species that respond to visual stimuli. Many lizards defend certain areas against intruders of the same or closely related species. Territorial defense does not always involve actual combat. Presumably to avoid physical harm, elaborate, ritualized displays have evolved in many species. These presentations often involve the erection of crests along the back and neck and the sudden increase in the apparent size of an individual through puffing and posturing. Many species display bright colours by extending a throat fan or exposing a coloured patch of skin and engage in stereotyped movements such as push-ups, head bobbing, and tail waving. Large, colourful horns and other forms of conspicuous head and body ornamentation are often restricted to males, but females of many species defend their territories by employing stereotyped movements similar to those of males. A displaying male that stands out against his surroundings is vulnerable to predation.

However, territoriality is evidently advantageous and has evolved through natural selection. Territories are usually associated with limited resources (such as nest sites, food, and refuges from predators), and a male that possesses a territory will likely attract females. Thus, he will have a higher probability of reproductive success than one living in a marginal area. The displays used by males in establishing territories may also function to “advertise” their presence to females; in species that breed seasonally, territoriality typically diminishes during the nonbreeding season. In iguanids, actual courtship displays differ from territorial displays in that males approach females with pulsating, jerky movements.

In addition to the visual cues used for bringing the sexes together, chemical stimuli play a role in some species of iguanian lizards. For example, desert iguanas (Dipsosaurus dorsalis) can discriminate between their own odours and those of other desert iguanas. In addition, numerous lizard species have femoral pores, which are small blind tubes along the inner surface of the thighs, whose function may be the secretion of chemical attractants and territorial markers.

The social systems of autarchoglossan lizards are fundamentally different. Rather than visual displays, chemical communication between individuals is used. Autarchoglossan males that rely heavily on vomerolfaction can distinguish species, sex, and sexual receptivity using chemical cues alone. Some lizards (such as those of families Teiidae, Varanidae, and Helodermatidae) have deeply forked tongues and may be able to use them to determine the direction of chemical signals in a manner similar to snakes.

Geckos use auditory cues in social interactions, but they also have the ability to discriminate between chemical signals using olfaction.

Copulation follows a common pattern. The male grasps the female by the skin, often on the neck or side of the head, and places his forelegs and hind legs over her body. He then pushes his tail beneath hers and twists his body to bring the cloacae together. One hemipenis is then everted and inserted into the cloaca of the female. Depending upon the species, copulation may last from a few seconds to 15 minutes or more.

Parental care

Parental care among lizards tends to be minimal following egg deposition, but there are striking exceptions. Many species dig holes in which the eggs are placed, whereas others bury them under leaf litter or deposit them in crannies of trees or caves. In contrast, females of some species, notably the five-lined skink (Eumecesfasciatus) of the United States and many of its relatives, remain with their eggs throughout the incubation time (about six weeks); they leave the clutch infrequently to feed. These skinks turn their eggs regularly and, if the eggs are experimentally scattered, will return them to the nest cavity. As soon as the young disperse, family ties are severed. Glass lizards (Ophisaurus, family Anguidae) appear to do the same thing. In addition, a number of viviparous lizards remove and eat the placental membranes from young when they are born.

In Australia, juvenile sleepy lizards (Tiliquarugosa) remain in their mother’s home range for an extended period, and this behaviour suggests that they gain a survival advantage by doing so. Female sleepy lizards and those of the Baudin Island spiny-tailed skink (Egerniastokesiiaethiops) recognize their own offspring on the basis of chemical signals. Consequently, parental care in lizards may be more widespread than previously thought. Nevertheless, since recognition systems are subtle, they are difficult to study.

Certain lizards, particularly some species of Gekkonidae, are known to be communal egg layers, with many females depositing their eggs at the same site. In addition, it appears that the same individual female may return to a particular site throughout her lifetime to deposit clutches of eggs. In Tropidurussemitaeniatus and T. hispidus, two species of South American ground lizards, females nest communally under slabs of rock situated on top of large boulders. In this specialized habitat, only a few appropriate nest sites are available, and thus they are limited resources. Males appear to take advantage of this situation, especially if nesting sites are located within their territories. It is likely that if a male defends a good nesting site, he should have access to more females than males who govern areas without high-quality nesting sites.

Parthenogenesis

Parthenogenesis (reproduction without fertilization) has evolved many times among lizards and in at least one snake. In such all-female unisexual "species," a female lays eggs that develop into exact genetic clones of herself. Many such parthenoforms have arisen via hybridization of sexual parental species. Because no energy is invested in making males, parthenogenetic forms have a much faster rate of increase than do sexual species. Without sexual reproduction, however, they cannot evolve. If heterozygosity itself confers fitness, asexual reproduction maintains existing heterozygosity (acquired in hybridization), whereas in sexual reproduction recombination will disrupt heterozygosity. Depending on the species, a squamate female may lay eggs or give birth to live young. Many species lay their eggs in nests, which are little more than holes dug in moist ground. A few, like the wormlizards, lay their eggs inside ant or termite nests. Most squamate mothers provide no care for their young and leave almost immediately after they lay their eggs or give birth. Some lizards and snakes are exceptions. Many female skinks, for example, stay with the eggs until they hatch.

While most species reproduce only after the male and female mate, some species are parthenogenic, which means that a female can produce young by herself. In many of these species, such as the lizard known as the desert grassland whiptail, only females exist. The female's young are all identical copies of herself. Besides this species in the whiptail family of lizards, seven other families of lizards and snakes have some all-female species.

Classification

Squamates are classified within the following taxonomic hierarchy:

Animals > Chordates > Vertebrates >Tetrapods> Reptiles >Squamates

Classically, the Squamata order is divided into three suborders

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Lizards (**Lacertilia**): There are more than 4,500 species of lizards alive today, making them the most diverse group of all squamates. Members of this group include iguanas, chameleons, geckos, night lizards, blind lizards, skinks, anguids, beaded lizards and many others.

Snakes (**Serpentes**): There are about 2,900 species of snakes alive today. Members of this group include boas, colubrids, pythons, vipers, blind snakes, mole vipers, and sunbeam snakes. Snakes have no limbs but their legless nature doesn't stop them from being among the world's most formidable reptilian predators.

Worm lizards (**Amphisbaenia**): There are about 130 species of worm lizards alive today. Members of this group are burrowing reptiles that spend most of their life underground. Worm lizards have sturdy skulls that are well suited for digging tunnels.

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Beekeeping for Maintaining Biodiversity and Livelihood Enhancement

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Abstract

The present study has been undertaken to assess the entrepreneurial potentials, scopes, and opportunities of beekeeping in Kavassery village, Erattakulm Taluk in Palakkad district fromJuly 2022 toMarch 2023. Thus, a sample of 40 respondents from Palakkad district was selected using a pretested interview schedule. This paper mainly focused on beekeeping entrepreneurship, entrepreneurship skills, and the needfor beekeeping entrepreneurship development in Palakkad along with the major reason for promoting beekeeping entrepreneurship development in the district. This study gave a real existing scenario of problems and prospects of beekeeping entrepreneurship in the Alathur region. Beyond improving beekeepers' pragmatic knowledge through training, and establishing beekeeper associations, entrepreneurship, and institutional, policy measures are needed to enhance this sector's performance. Finally, the ultimate issue underlying the study is to provide some initiatives to building beekeeping entrepreneurship for expanding the honey market, conserving biodiversity, and finally getting livelihood enhancement among the rural masses.

Index Terms – Potentials, Beekeeping, Entrepreneurship, Biodiversity.

Introduction

The history of honeybee and honey finds a special mention in the Indian epics for its medicinal and therapeutic values and dates to about 2000 to 2500 years.The beekeeping method was the oldest known method in food production. Beekeeping in India has great scope for self-employment of rural and tribal families as they can make an annual income by producing tons of honey. Honeybees are a main group of beneficial insects that provide not only honey and other valuable hive products but also enhance crop productivity through pollination. The bees have a vital role in pollination of crops in sustainable agriculture resulting in food safety. Among the different states in the country, Kerala and Tamil Nadu were traditionally the leading states in beekeeping with Kerala contributing 70 percent of the annual production of honey in India.

The honeybees belonging to the genus *Apis* and the stingless bees belonging to the genera *Trigona* and *Melipona* are known to be useful. Beekeeping is a profitable enterprise, of the maintenance of honeybee colonies, commonly in hives, by humans (Syngkon W, 2017). There is no negative impact of beekeeping on the environment (Verma S and Attri PK, 2008). Many organizations have already undertaken beekeeping programs as a good weapon for self-employment and poverty reduction for rural people (Saha GC, 1990). Apiculture has a prime significance in sustaining an ever-growing population's health care and nutrition. Traditionally, apiculture is seen as a low-tech honey-collecting, processing, and marketing industry with limited dynamics dominated by a few small family farms, which are mostly focused on making things better rather than doing new things (Bairwa et al., 2014). Beekeeping plays a major role in the socio-economic development of rural livelihoods (Islam etal., 2016).To give a positive trend in beekeeping and commercial honey production, beekeeping management practices need to be improved depending on the changes in environmental conditions. Extension activities at the local level, result in better agricultural productivity, increase farmer incomes, enhance food quality and security, reduce the use of pesticides, and improve management of ecosystem services. The field demonstrations increase awareness among the farming community and the local people which results in the wider use of pollinators in agriculture that causes an increase in the yield of crops. Beekeeping plays an important role in creating employment opportunities among the rural masses. This industry can be promoted largely by various programs implemented by the Government to generate more income for farmers.

Beekeeping

The process of keeping bee colonies in hives for the goal of producing honey, pollinating crops, and producing other hive products like beeswax, propolis, and royal jelly is called beekeeping, often referred to as apiculture. Beekeepers provide appropriate living conditions, control pests and illnesses, and gather the products produced by the bees to maintain the health and productivity of the colonies (Crane Eva, 2009). Engaging in beekeeping serves as an additional income stream for farmers, beyond economic benefits, the establishment of beehives contributes to the overall biodiversity and health of the farm ecosystem. This not only supports the flourishing of additional plant varieties but also fosters the well-being of diverse animal species within the agricultural environment (Gene Kritsky, 2017).

Beekeeping for increased income

The integration of beekeeping in rubber plantation areas has an additional income stream for rubber smallholders subsequently boosting their socioeconomic standards. Rubber tree with their extrafloral nectaries provides an essential source and favourable environment for bees to flourish. The rubber tree's flower is a good source of pollen and nectar. Honey qualities are primarily affected by species, surrounding vegetation, foraging behaviour, resources, etc. Honey, propolis, and beebread could also be used in developing cosmetic products such as soap, cream, lotion, etc. The beekeepers in the rubber environment are advised to develop a beekeeping calendar that integrates floral phenology with local weather, human activities, bee activities like swarming, migrations, absconding, and bee-related chores. Favourable weather conditions, with dominant vegetation of Rubber trees, nectar, and pollen-producing plants, made the farmers think about the potentiality of beekeeping. Honey is a highly-priced natural product in the market with medicinal value. Honeybees are harmless potential pollinators to beekeepers; they visit a wide range of crops and have a vital role in pollination of crops in sustainable agriculture resulting in food safety. They are tolerant to high temperatures, active throughout the year, and can be transported easily. There is no high technology involved in beekeeping so an illiterate person can start this enterprise by taking training on all aspects of beekeeping. It is an environment-friendly enterprise with no extra land needed and acts as an additional income source thereby improving the standard of living.

The potential of beekeeping

The study was conducted fromJuly 2022 to March 2023 at Kavassery village, Erattakulm Taluk in Palakkad.To carry out this work and to find the objectives, a detailed structured interview schedule was used as a tool for data collection, consisting of open-ended questions for collecting data from the respondents. For the study, an entrepreneurial potential scale with 30 statements combined with five components; viz: innovativeness, economic motivation, need for achievement, risk-taking ability, and self-confidence (measuring instrument) was developed and analyzed exclusively for beekeepers to know beekeeping potentials. The main objective of this study is to identify key areas of research required to effectively evaluate the potential integration of bees in rubber smallholdings. The farmers with limited education have to accept low-level employment and low wages from their employers in the neighbouring localities. Some of them work as house helpers, farmers, coolies, etc., and the integration of beekeeping in rubber plantation areascan provide additional revenue streams to smallholders to boost socioeconomic standards.The bee species used in the study is the Indian honeybee (*Apis cerana indica*),which produces honey with potential health benefits and plays a key role in balancing the ecosystem. For the practical implementation of beekeeping in rubber plantations farmers and unprivileged women from the rural areas of Palakkad District and students at Sree Narayana College, Alathur were given training on Honeybee farming techniques for attaining higher income with less investment.

Before starting a honey beekeeping project, the entrepreneurs/farmers are generally advised to undergo training. The awareness of beekeeping and the availability of training facilities and resources are inadequate in and near the Sree Narayana College, Erattakulam, Alathur. Hence to provide training on Honey bee-keeping to farmers and students both onsite and off-site giving special thrust on scientific production technology and marketing was planned to be given. The 2-Day Workshop was successfully concluded with a field exposure visit to the cage installed at the Sree Narayana College Campus.The trainer discussed the different types of honeybees, different types of bee hives such as Langstroth, Top Bar, and Warre hives used in beekeeping, the seasonal management of honeybee colonies, and practical training on various aspects of beekeeping. Understanding their lifecycle, the queen rearing, its introduction to a new hive, and maintaining healthy colonies free from pests and diseases, are the major aspects to be aware of successful in beekeeping. He described thetypes of equipment used for Beekeeping such as knives, veils, caps, smokers, etc. He demonstrated the installation of a new beehive and its requirements. He explained the role of honeybees in the pollination of various fruits and vegetable crops.

Honey, propolis, and beebread, which are the main product of beekeeping, are primarily affected by species, surrounding vegetation, foraging behaviour, resources, etc. Rubber trees with their extrafloral nectaries and flowers provide an essential source and environment for bees to thrive. Products at a very high price (stingless bee honey could fetch a price of RM400/kg) could provide an excellent source of supplementary income for rubber smallholders. Developing cosmetic products such as soap, cream, lotion, etc will give additional value to beekeeping activities.

Future Outcomes of the Training Program

- To develop entrepreneurship skills among students and to make students aware of opportunities in the apiculture industry.
- The farmers of the nearby locality will be educated on scientific lines regarding various aspects of Honey beekeeping. It will help them to improve their knowledge and skills regarding scientific practices to enable them to adopt the same.
- Necessary assistance will be given for the setting ofa model Honey beekeeping unit, bee boxes with bee Colonies, Tool Kits, and other accessories for the farmers.
- Field visits to apiaries will be arranged for progressive farmers and to research stations which will motivate them to adopt good scientific practices.
- Capability among Honey beekeeping growers and processors will be built to produce quality products.
- Visits of farmers will be arranged to exhibitions/fields with the prime objective of exposing them the technological innovations. The information available on various websites on Honey beekeeping which provides useful content will be shared with farmers.
- Installation of 500 bee boxes in the neighbouring localities of the college.

Impact of the Beekeeping Training Program

After conducting training, the impact of the training was analyzed by the evaluation sheet filled by the participants and most of the participants (100%) were satisfied that the training was need-based and helped to sort out the problems and questions that were in their minds before starting the training. Many of the participants were satisfied with the theoretical and practical sessions, moreover, they became a part of biodiversity conservation and management. Efforts were made for the handling of honeybees by each participant during exposure and practical sessions so that they may be comfortable with the handling of bees and should not fear the bee stings while handling bees in the future. The trainees gained knowledge on the importance of bees for the pollination of crops and conservation of biodiversity and hence showed their eagerness to adopt beekeeping for pollination services as well as in honey production and its sale.

Conclusion

The beekeeping activity was found very promising due to the continuous requirement of nectar and pollen as a source of secondary income to uplift their socio-economic conditions. The people were made aware of the scientific ways of beekeeping, their management and handling, and the making of Farmers Producer Organizations (FPO), especially for Honey and other bee products. Whilst the above recommendations are general in nature – they provide a framework for future research in the integration of stingless bee keeping in rubber-growing areas. It should be noted that other challenges need to be addressed to promote the new farming system, Subbey (2009), found that the greatest challenges to the development of the honey industry were lack of access to financial resources, weak organizational structures, unresolved land usage rights, inadequate infrastructure and modern technologies for post-harvest storage and processing of primary agricultural products.

For honey and beeswax production inadequate equipment, lack of technical assistance, absconding of bees, and lack of capital havebecome the crucial obstacles. Extension activities and regulatory programs including technical support, training of beekeepers to improve skills and information in quality assurance for marketing, effective management programs for pest and disease control, and provision of appropriate harvesting, processing, and packaging facilities help the beekeepers to succeed. In the study area beekeeping has been practiced sideways with other agricultural activities and there were no respondents who base their livelihood only on this sector. Beekeeping plays a sustainable role in promoting biodiversity is an additional source of income for farmers and supports the state’s agricultural ecosystem.

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Micro- and Nano- Plastics: Respiratory Complications Is Micro and Nano Plastic Showing any Impact on Respiratory System?

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Abstract

Microplastics are a pervasive environmental contaminant that can be found both indoors and outdoors. Every area of daily life, including technology, health care, and household appliances, is significantly impacted by plastic. Consumers just after single uses typically discarded plastics anywhere, which has become a significant environmental problem because the materials wind up in junkyards, oceans, and other waterways causing pollution in the atmosphere. The fact that these plastics are broken down into micro- and nano-sized particles and thrown in such large quantities every day has raised concerns about how harmful they are to both the environment and people. Human lung tissue and sputum have both been discovered to contain microplastics. An increase in oxidative stress caused by the effect of transcriptome sequencing and gene alteration may be the main mechanism behind nano plastic's harmful effects on cells. This study offers details on the toxicity of nano and microplastics in human lung cells at environmental quantities thatmakes a better understanding of the risks associated with the respiratory system. Nevertheless, more research is required to determine the early health impacts of low-dose nano plastics on the respiratory system, which are anticipated to represent the risk of atmospheric nano plastics. In this review we explored the potential pathways for exposure of micro and nanoplasticspresent in air and their influences in lung illness while also outlining the shortcomings of the available research.

Keywords: Microplastics, respiratory system, nano plastics, lung diseases, epithelial barrier dysfunction, oxidative stress

Introduction

Plastic is one of the most manufactured materials across the world. It has enormous impacts to our daily lifestyle from technology to tools. Maximum of these used plastics are not properly disposed and discarded anywhere in the environment causes a huge problem in environment ultimately leading to the environmental pollution. These discarded plastics are further broken down into the microplastics (<5mm) and nano plastics (<1000nm) which has led worries about its toxic effect to human health and environment [Gigault et al., 2018]. Year by year the production and uses of plastic is increasing worldwide.

Plastics are made up from the natural resources via several physical and chemical methods. Polymerization and polycondensation are the two primary procedures that structurally change the basic components and convert them into polymer chains [Shrivastava, 2018]. These polymer chains further undergo various chemical procedures to be changed into plastic materials. Due to its chemically stable nature the accumulation of plastics is increasing day by day [Liu, 2017]. When plastic waste is discarded, it comes in contact with several environmental factors and breaks down into large quantities of microplastics which have less than 5 mm of diameter and nano plastics having diameter of less than 0.1 mm [Yee, 2021]. Community behaviour, human activities, and weather conditions all have an impact on the type of airborne microplastics and its concentrations [Chen et al., 2020; Mbachu et al., 2020]. These microplastics are present in internal and external environment and can be inhaled by human due to its small size [Lu, 2022]. Studies have shown that the polymers used in food and beverages have much lesser effect on human health than the polymers present in indoor objects, materials, plastic debris [Rist et al, 2018]. Inhaled small particles of plastic materials frequently cause respiratory problems due to inflammatory reactions in the interstitium and airways, especially in exposed workers [Prata, 2018]. Although the potential effect of nano-particles is still under-studied. Here in this study the source of micro and nano particles, their breakdown, toxicity level, potential impacts and possibilities Mechanisms on respiratory health is discussed along with the suggestions for additional research.

Research Methodology:

For the study an electronic search has been undertaken utilizing the databases viz. Google, Google Scholar, Science Direct, and PubMed. The timeframe used in this search was restricted from 2006 to 2021. Keywords used while searching microplastics, respiratory system, pulmonary toxicity, nano plastics, respiratory diseases, lung diseases.

Sources of Microplastic and Nano Plastics:

Microplastics or nano plastics mainly derives from primary plastic junks and its derivatives. Primary plastic wastes are dumped into the earth in micro- or nanoscale form, coming from sources like personal care items, nanomedicine, nanoimaging, and nanosensors. Secondary derivatives are produced when plastics break down physically or chemically. [Lai, 2022]. Among these microplastics more than 80% is produced in soil where rest 20% is originated from the sea. Majority of the plastics that pollutes marine environments mainly comes from terrestrial sources, fishing, various types of aquaculture activities etc. however, through natural erosion micro and nano plastics come into river this way. A study had been conducted on the role of a municipal wastewater treatment plant (WWTP) sewage and coastal landfill to evaluate the discharge of microplastics (MPs) input into the marine environment. The study found that the daily discharge of microparticles into the marine environment is approximately 227 million. As reported by the study blue mussels are the responsible species for MPs entrance (<200 μm) [Maria, 2019]. According to statisticsfrom the United Nations Environment Programme (UNEP), total 4.8 to 12.7 million tonnes of plastic garbage were produced in 2010, with an estimated 1.5 million tonnes ending up in landfills (UNEP) [Mattsson, 2018].

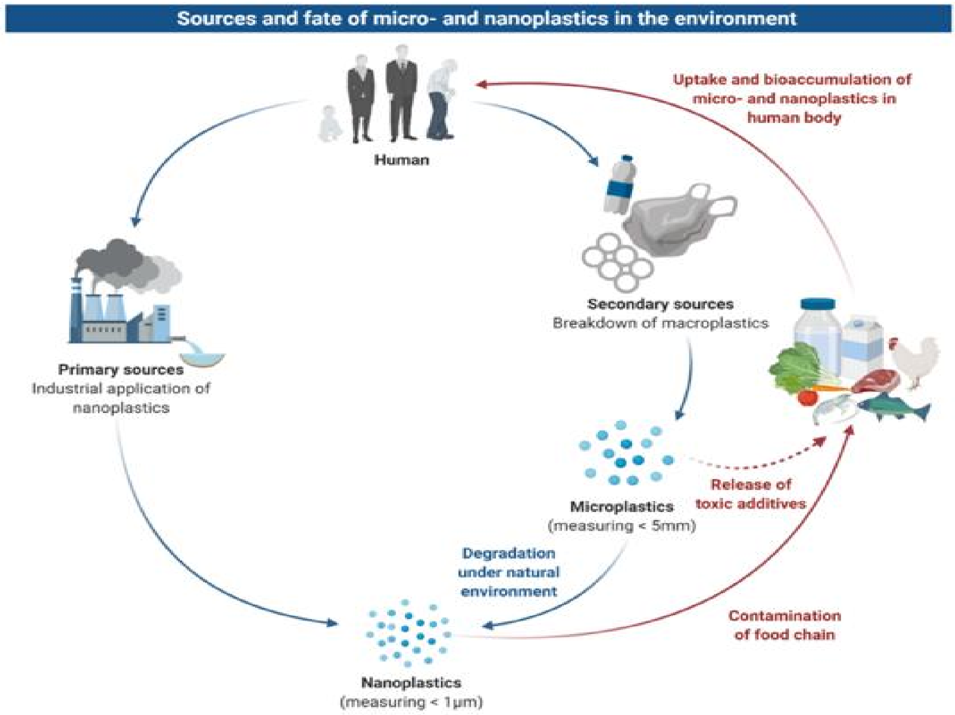
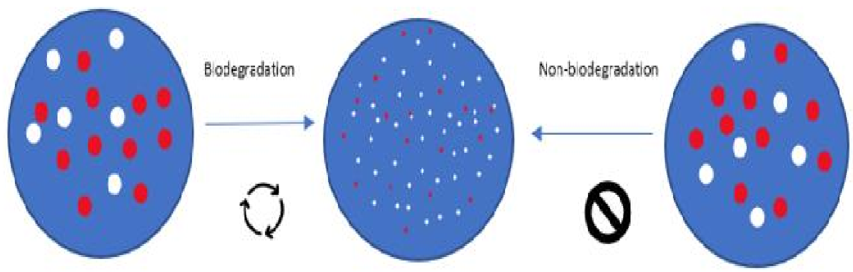


Figure 1: Micro and Nano Plastic Sources in the Environment

Fate of Micro and Nano Plastics:

Two pathways that exist for breaking down of plastics into microplastics and nano plastics are biodegradation and non-biodegradation. In non-biodegradation processes the chemical bonds between plastics are broken down and converts into its monomeric form by the use of water or UV rays [Lucas, 2008]. The non-biodegradation process includes thermal and physical decay, photodegradation (by the action of light) and thermo-oxidative degradation of plastics. As the name suggests biodegradation this method mainly mediated by environmental bacteria and microorganisms [Lambert, 2016]. Extracellular enzymes also helped in this biodegradation process of breaking down the chemical bonds of plastics [Yuan, 2020].



Extracellular enzymes, Microorganisms
Physical and Thermal decay, Degradation by light, Hydrolysis

Figure 2: Degradation mechanism: micro and nanoplastics under natural phenomena

Entry Routes of Micro and Nano Plastics in Human Body:

Micro and nanoplastic particles enter into our body through three main routes: inhalation, ingestion and skin contact [Rahman, A, 2021]. Among all these microplastics which are inhaled through the air are the synthetic fabrics and rubber that mainly comes from urban dust [Prata,2018]. Ingestion of the microplastics mainly happens through the food and water supplies[Carbery, 2018]. While they enter into human body by skin membranes through its hair follicle, sweat gland or open wounds [Schneider, 2009].

Pulmonary Exposure and Lung Injury:

A complex layer of lipids and proteins called surface-active substances is present on the alveolar surface. These pulmonary surfactants create a complicated structural air-water interface in the alveoli and reduces surface tension, thus prevents alveolar collapse during exhale. Hence low amount of energy is required for respiration [Lu,2022]. After providing nasal microplastic drops (1-5 m, 300 g/20 l)to mice, it has been demonstrated that microplastics can pass through the alveolar epithelial barrier of the respiratory system. Also, the presence of microplastics were found in the alveoli, airlanes and interstitium of the mice [Lu et al., 2021]. When microplastics were injected into rats' tracheas at a maximum dosage of 2 mg/200 µl, the alveolar structure was disrupted, and the bronchial epithelium was arranged in a disordered manner [Fan et al., 2022].

Lung Injury via Oxidative Stress:

Nano plastics are the most common pollutants, which has caused a lot of people to worry about their toxicity. Research into lung injury and the underlying mechanisms brought on by nano plastics is therefore urgently needed [Lai, 2022]. Pulmonary epithelial cell that is located between the host and the environment is in direct contact with airborne microplastics. A recent study has been done on the effect of polystyrene nano plasticsinducedlung injury via activating Oxidative Stress. A transcriptome analysis was conducted to fully understand the relevant genes and probable pathways that might be involved in the cytotoxicity of BEAS-2B cells brought on bypolystyrenenano plastics exposure. Additionally, the study identified the primary TFs (transcription factors) controlling DEGs and created a network of TF-mRNA regulatory factors connected to oxidative stress. To conduct the study a control group, a low-dose group, and a high-dose group was considered. These three groupswere taken for transcriptome sequencing to check the dynamic alterations of genes in BEAS-2B cells caused by polystyrene nanoplastics exposure. In the two treatment groups, 1812 genes in total saw consistent trend changes. Notably, almost 700 genes underwent significant alterations in cells exposed to low doses but not high doses [Zhang, 2022]; these genes may be essential at sublethal stages.The cells might be capable of returning to their normal state if the irritating environment is eliminated. According to GO analysis, one-fourth of the intersecting genes between the low-dose group and the high-dose group, oxido-reduction process, oxidoreductase activity, oxidoreduction coenzyme metabolic process and others oxidative stress was associated with around. In one wordthe studyshowed that nano plastics reduce the survival ofepithelial cells of human lung through an oxidative stress-moderated mechanism [Zhang, 2022].

Effect on Cell Viability:

Polystyrene microplastics (PS-MPs) with diameter of 1 µm and 10 µm, acts as an environmental pollutant, was introduced to the cultured human alveolar A549 cells for the sake of evaluation of the possible toxicological side effects of microplastics on human cells. According to the preservation of cell viabilities asconducted by trypan blue staining and Calcein-AM staining, it has been observed that the both sizes of microplastics significantly reduced cell growth but showed insignificant cytotoxicity.Even at 100 µg/mL concentration, the cell viabilities did not fall below 93%(figure 3) [Goodman, 2021]. Despite these high viabilities, additional experiments showed that PS-MP exposed cells had dramatically lower rates of proliferation and a decline in its metabolic activity. Additionally, the result of phase contrast imaging of living cells at 72 h indicated that the uptake of numerous1µm polystyrene-microplastics into the cells causes significant morphological alterations in cells when exposed to microplastics. This study finds that microplastic pollution to human exposure has many serious side effects and poses a great risk factor to their health[Goodman, 2021].

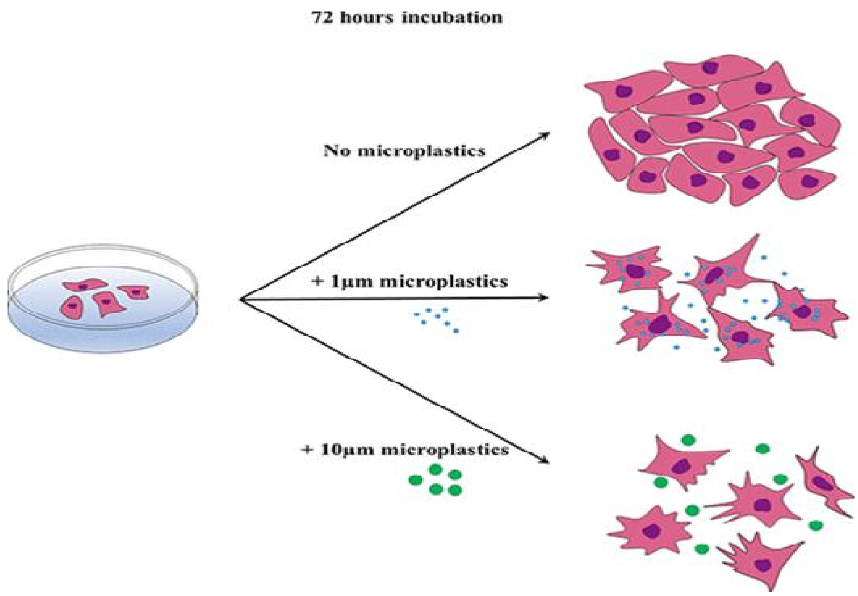


Figure 3: Effect of Microplastics on Cell Viability

Conclusion

Researchers have investigated microplastics with chemical compositions, particle sizes, and concentrations to study its negative impacts on the environment. The most popular among all these are polystyrene microplastics. The real world does not contain just them only but organic or metallic ions adsorbed on their surfaces. In terms of adverse impacts on people, microplastics and these chemicals work in concert. Therefore, in-depth research is required to determine how exposure to numerous microplastics in combination affects lung health. Despite the little researches on the negative effects of microplastics on respiratory health, it is undeniable that these materials can really have harmful impacts.This review outlines the potential risks and processes of airborne microplastics' adverse impacts on respiratory health. Meanwhile, we urge greater focus on microplastics and the risks they bring to respiratory health.

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Applications of Artificial Intelligence in Microbiology

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Introduction

People frequently associate the term "artificial intelligence" with killer robots and supercomputer overlords. Artificial intelligence undoubtedly carries significant concerns, including the possibility of autonomous weaponry and certain social media functions. However, artificial intelligence is capable of more. The internet, self-driving cars, and healthcare are just a few of the areas in which artificial intelligence has already impacted our lives. Artificial intelligence has also been successfully used in microbiology for a number of purposes, such as drug development, microbe imaging, and diagnostics. Artificial intelligence, like many other inventions, can be used for good or harm. It can relieve us of tiresome and repetitive duties, raise the standard of healthcare, and positively impact our lives.

A wide range of tools and applications are used to create artificial intelligence, which is used to make films like The Matrix and Terminator, in which an intelligent supercomputer hunts and enslaves humanity. Alternatively, they recall films such as 2001: A Space Odyssey and Alien, in which a spaceship's robot turns rogue and chooses to prioritize the mission over the lives of its human crew members.

The general people should be aware of the possible advantages and difficulties of artificial intelligence. Since the last Industrial Revolution, research and technological advancements have not been expected to have a greater positive impact on our lives than artificial intelligence.

Artificial intelligence

The Industrial Revolution has so far taken place three times.

Technological advancements like the steam engine, which significantly reduced the need for human arm strength and replaced it with machine power, served as the catalyst for the First Industrial Revolution. This had a profound effect on people's daily lives and on society.

The telephone, telegraph, and electrical power were among the innovations that enhanced the Second Industrial Revolution. Similar to earlier industrial revolutions, this one brought about significant changes in how people worked, lived, interacted with one another, and passed their leisure time.

The Third Industrial Revolution powered by inventions such as computers and digital cellphones, which became widely used by the general public. Internet, which most of us cannot imagine live without nowadays, was also invented during this period.

We live in the time of the Fourth Industrial Revolution. It is expected thatit will lead to equally profound, if not greater, changes to our lives as theprevious three Industrial Revolutions. And this time it is artificial intelligence, which is among the key research and technology advances fueling the fire of change.

Long gone are times owning the Commodore 64 with a whopping 64 kilobytes (KB) of operational memory (RAM) and later the first Pentium-powered computer. Personal computers and mobile devices nowadays pack more computing power than the fastest supercomputers of that era.

In essence, neural networks are programs, which consist of a set of nodeswired together and resembling neurons in the human brain. Figures 1 and 2 show simplified views of a single artificial neuron and of the wholeneural network, respectively.

Artificial neuron

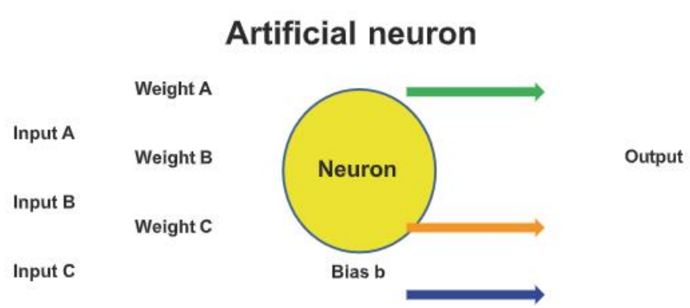

Fig. 1 Artificial neuron. Schematic view of artificial neuron. Output is calculatedfrom the weights of each input and bias of the artificial neuron Neural Network

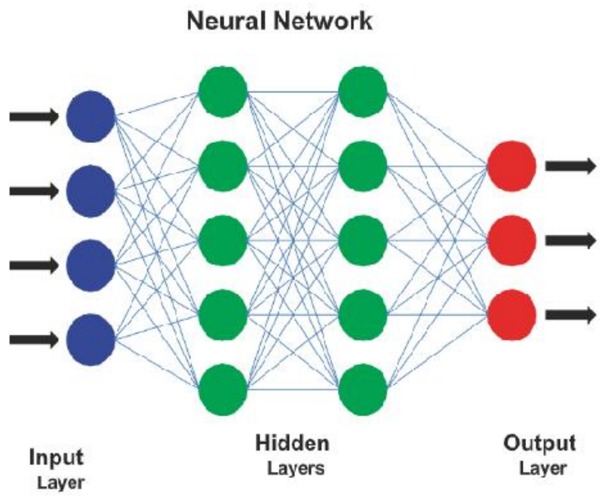

Fig. 2 Neural network. Schematic view of a neural network composed of input, hid- den, and output layers of artificial neurons

Let’s look first at individual artificial neurons. Into one end of thissingle- neuron system is fed the input information and each input has itsown weight. Artificial neuron processes this information and based on theweights of each input and bias of the artificial neuron calculates theoutput (Fig.1).

These artificial neurons are arranged in layers to form neural networks: an input layer, one or more hidden layers, and an output layer. The final output of a neural network is determined by the weights of connections between artificial neurons (Fig. 2).

To complete complex tasks, including making predictions, neural networks must learn from the large amounts of data that are provided to them. Machine learning and deep learning are two subfields of artificial intelligence that concentrate on learning elements of AI.

Machine learning

Artificial intelligence has an area called machine learning. Supervised, unsupervised, and semi-supervised learning are among the machine learning techniques (Botvinick et al. 2020; Choi et al. 2020a; Lo Vercio et al. 2020; Glielmo et al. 2021; Roscow et al. 2021; Eckardt et al. 2022). 2022). As the name implies, supervised learning necessitates close supervision and involvement. To train for certain tasks, annotated data is also required. Because it is frequently difficult to collect annotated data, supervised learning necessitates the use of skilled humans to annotate data.

Not a lot of input or data annotation is needed for unsupervised learning. It enables computers to find patterns in datasets and resolve issues even in cases where they are not trained for.

The characteristics of both supervised and unsupervised learning are combined in semi-supervised learning. It works best with datasets that have both annotated and unannotated data. Each of these machine learning techniques has benefits and drawbacks, making them appropriate for various jobs. Combining several learning methodologies may consequently be necessary to build better artificial intelligence systems.

Deep learning

Deep learning is a subfield of machine learning that is especially useful for finding patterns in big, high-dimensional datasets. Following the groundbreaking research on neural networks conducted in the middle of the 20th century, a number of noteworthy developments have been made (Fukushima 1980; Hopfield 1982; Fodor and Pylyshyn 1988; Eberhart and Dobbins 1990; McCulloch and Pitts 1990; Hinton et al. 1995; Hochreiter and Schmidhuber 1997; Wang and Terman 1995, 1997; Wang and Brown 1999; Hinton et al. 2006; Soman et al. 2018).

However, this area has not seen much progress. Neural network research was even on the verge of being discontinued for a while. The fact that computers at the time lagged behind theoretical research on neural networks was one of the causes of it.

Artificial Intelligence in Microbiology

Healthcare artificial intelligence

Artificial intelligence has advanced at an exponential rate due to increased processing power. Artificial intelligence in healthcare saves doctors time by relieving them of time-consuming and repetitive chores, giving them more time for making decisions and interacting personally with patients. Additionally, by treating more patients, this increases the effectiveness and efficiency of healthcare. Personalized medicine is an excellent illustration of how artificial intelligence is being used in healthcare. Training artificial intelligence can be done with big information, like genetic profiles of past patients, X-ray pictures, and different test findings. It can then forecast and diagnose a variety of ailments based on learnt information, and it can suggest a course of treatment that will probably be effective for each patient. Artificial intelligence can recommend personalized treatment options foravariety of disorders, such as high blood pressure, diabetes, and cancer (Chaikijurajai et al. 2020; Subramanian et al. 2020).

Conventional cancer treatment may not always be the most effective because it frequently employs a similar strategy for each patient. Based on each patient's unique genetic profile, artificial intelligence may suggest a personalized cancer treatment plan (Rafique et al. 2021; Esteva et al. 2022; Hwang et al. 2022). The diagnosis of breast cancer and the prediction of the most effective course of treatment have both been successfully accomplished by artificial intelligence (Corti et al. 2022; Dang et al. 2022; Fong et al. 2022; Marinovich et al. 2022; Shamai et al. 2022; Taylor-Phillips et al. 2022; Wu et al. 2022). Lung cancer has also been diagnosed, treated, and prognosed with the aid of artificial intelligence. (Choi et al. 2022; Churchill et al. 2022;Hosny et al. 2022; Kim et al. 2022; Pei et al. 2022; Wang et al. 2022).

Since diseases are the result of the combination of numerous genes and risk factors, artificial intelligence may also be used to discover the genes and risk factors that are involved. Artificial intelligence may have a significant impact on several ailments, such as Alzheimer's disease, heart issues, schizophrenia, aging, and various metabolic disorders. (Wang et al. 2018; Wu et al. 2019; Li et al. 2020b; Ghazal et al.2022; Rahman et al. 2022).

Drug discovery

However, this is only a small portion of the uses of AI in the medical field. Numerous additional biomedical applications have also effectively incorporated artificial intelligence (Cao et al. 2018; Levine et al. 2019; Liu et al. 2019). Artificial intelligence has also been used in microbiology for a number of purposes, such as drug development and microbe imaging and diagnostics. (Riordon et al. 2019; Draz et al. 2020; Stokes et al. 2020).

Conventional methods for drug development are labor-intensive and time-consuming. As a result, they frequently are unable to keep up with the harmful microbes and their rapidly evolving new mutations. Artificial intelligence can navigate through enormous databases of high-through put test findings and enormous libraries of possible antibacterial agents and their biological and chemical properties more quickly than traditional methods can. Artificial intelligence has the ability to identify medications that are most likely to be successful against particular diseases and bacteria based on data gleaned from these datasets. (Bender et al.2007; Stephenson et al. 2019; Neves et al. 2020; Stokes et al. 2020;Zahradník et al. 2021).

Artificial intelligence has been used for the successful identification ofdrugs against a broad spectrum of microorganisms. Example of a drug against bacteria identified by artificial intelligence is Halicin. It has been selected by artificial intelligence from the database, which contains more than 200 million compounds, as a promising drug against antibiotic-resistant bacteria. Laboratory tests confirmed antibacterial activity of Halicin against many antibiotic-resistant bacteria (Stokes et al. 2020; Zhang et al. 2021b).

Artificial intelligence used screening of a second database containing almost two million chemicals to identify many promising anti-malarial medicines with minimal cytotoxicity and strong activity predictions. The chosen compounds do, in fact, show potent antimalarial action and low cytotoxicity, according to experiments conducted later (Neves et al. 2020).

Artificial intelligence has discovered several possible antiviral medications from millions of substances in databases, in addition to germs and parasites. These comprise, for example, HIV-fighting potential medications and antiviral peptides. (Andrianov et al. 2022).

It has also been effective to use artificial intelligence to find prospective medications that combat SARS-CoV-2. Artificial intelligence screening of large compound databases has identified several compounds that may be useful in the fight against SARS-CoV-2. It has also emphasized a number of medications that have been shown to have antiviral qualities against other viruses and that may be modified for use against SARS-CoV-2.

Artificial intelligence either chose these substances and medications based on their shown ability to suppress viral reproduction, reduce inflammation, or serve as an antiviral against other viruses. Some, like the ACE2 receptor and TMPRSS2, were also chosen because of their propensity to interact with SARS-CoV-2 proteins and host cell proteins that are necessary for cell entrance. (Beck et al. 2020;Choi et al. 2020b; Nand et al. 2020; Ton et al. 2020; Zeng et al. 2020; Baiet al. 2021; Zahradník et al. 2021).

Even with artificial intelligence's numerous achievements in drug discovery, certain obstacles still need to be overcome. Artificial intelligence-driven searches, for example, frequently target a single category of data. They just sort through chemo-informatics data; they don't combine it with information from microscopic imaging, clinical, molecular biology, or cellular biology (Zhang et al. 2021b). Therefore, it may be able to incorporate these various types of data in future AI-based drug development searches to identify the most promising compounds against particular pathogenic microbes.

The black box aspect of artificial intelligence-powered systems presents another difficulty. Although we are aware of the input and output that were supplied into the system, it is not always evident what transpired in between. Thus, creating more understandable artificial intelligence-powered systems is one of the difficulties for this field's future study. Enhancing the comprehensibility of the system's reasoning would be advantageous not only for broad research but also for creating more dependable and effective drug discovery searches and simplifying the evaluation of the possible medications chosen by artificial intelligence (Zhang et al., 2021b).

Artificial intelligence can assist curb the spread of dangerous microbes by expediting the medicine research process.

Imaging and diagnostics

Numerous microscopic images are produced by microbiology research. These are employed in the identification of pathogenic bacteria during clinical diagnostic procedures. However, it takes a lot of time and skilled workers to produce high-quality microscopic images and evaluate them. It is an expensive and difficult task, especially in developing nations where there are frequently neither microscopes nor skilled personnel to operate them. Even with conventional computing tools, it is challenging to interpret the vast amount of acquired microscopic pictures. (Goldsmith and Miller 2009, Das et al. 2015, Stévenin andEnninga 2019, Li et al. 2020a, c, Zhang et al. 2021a).

The speed and accuracy of diagnosing various microorganisms can be significantly increased with the use of artificial intelligence-powered microscopes. The identification of viruses in transmission electron microscopy pictures has been accomplished with the use of artificial intelligence. In these investigations, artificial intelligence out performed professional microbiologists in the detection of human cytomegalovirus, feline calicivirus, adeno-associated virus, and human enterovirus 71 (Devan et al. 2019; Matuszewski and Sintorn 2019; Xiao et al. 2020; Shaga Devan et al. 2021).

Additionally, artificial intelligence performed well when it came to identifying various germs in tiny photos. Numerous bacterial species, including *Escherichia coli*, *Vibrio*, *Pseudomonas*, *Bacillus subtilis*, *Klebsiella aerogenes*, and *Klebsiella pneumoniae*, have been successfully discovered in these experiments. Notably, in these investigations, artificial intelligence was employed to examine not just typical microscopic pictures but also blood culture gram stains and 3D microscopic images (Zieliński et al. 2017; Hay and Parthasarathy2018; Smith et al. 2018; Lugagne et al. 2020; Zhang et al. 2021a).

Artificial intelligence has demonstrated strong performance in identifying minute fungus, including yeast, in microscopic images, in addition to viruses and bacteria. It's interesting to note that many fungal spore types, such as Aspergillus and Cladosporium, were also identified in these investigations by artificial intelligence in addition to fungal cells (Tahir et al. 2018; Zieliński et al. 2020).

Additionally, the successful detection of several parasites in microscopic images has been attributed to artificial intelligence. These research have identified Plasmodium, Toxoplasma, and Babesia parasites. Numerous identification investigations using artificial intelligence have focused on the malaria causative agent, Plasmodium.

In blood smear microscopy images, including those evaluated by smartphones, Plasmodium was successfully recognized with artificial intelligence-controlled application (Quan et al. 2020; Yang et al. 2020; Abdurahman et al. 2021; Kassim et al. 2021). Plasmodium detection via a smartphone could potentially address the shortage of skilled microbiologists in developing nations. In other investigations, artificial intelligence systems were able to identify these parasites in microscopic images by using transferred knowledge about their unique morphologies and geometric shapes. For example, under a microscope, Plasmodium is known to resemble a ring, whereas Toxoplasma and Babesia are known to resemble bananas and pears, respectively. In the future, a similar method based on geometrical properties might be used to other kinds of microbes, such fungi, bacteria, and viruses (Jiang et al. 2020, Li et al. 2020a, c, Zhang et al. 2021a, 2022a, b).

Even with the effective use of artificial intelligence in imaging and microbe identification, there are still certain obstacles to overcome. For example, artificial intelligence might be used to identify microorganisms in fluorescence microscopy photos, rather than the normal microscopy and transmission electron microscopy images utilized in the experiments mentioned above.

Future microscopes and systems driven by artificial intelligence may be able to diagnose different kinds of germs from different kinds of microscopic images and tell medical professionals about the course of a disease.

However, perils often accompany artificial intelligence's quick advancements. The most evident dangers stem from their military applications.

While certain military systems already employ artificial intelligence, fully autonomous weapons pose the true threat in this field. The artificial intelligence-controlled drone's operator still makes the option to fire a weapon or drop a bomb. Furthermore, we have to make sure that in the future, artificial intelligence is not allowed to make this choice.

The incorrect application of artificial intelligence in social media poses a less serious risk but has far-reaching effects. Artificial intelligence in social media, when misused, can push people into dangerous social circles and cause them to act and behave in ways they might not have otherwise.

Even with current technology, artificial intelligence still makes mistakes from time to time. It may, for example, misidentify items in a photograph. Therefore, it is preferable to involve humans in the decision-making process whenever possible for safety considerations, especially in situations where artificial intelligence is unsure of the optimal course of action.

Artificial intelligence can swiftly sift through enormous databases of chemical compounds to find the most promising candidates in the process of developing new medications to treat microbiological diseases. However, human beings conduct safety evaluations and grant ultimate permission for the drug's use.

Artificial intelligence in diagnostics can swiftly sift through enormous volumes of microscopic images and identify those that need physicians' careful attention. Cooperation between humans and artificial intelligence therefore seems to be the best way forward. By cooperation, humans and artificial intelligence can augment each other and leveragetheir strengths to achieve the best overall outcome.

Presently seen in many systems around us, artificial intelligence is a relatively specific and limited field. It is proficient in speech and language recognition, illness diagnosis, prediction, and treatment, and it can even outplay us at our hardest video games. It can compete with us or perhaps outperform us in some activities.

We anticipate that artificial general intelligence will develop and proliferate in the upcoming stage. Artificial general intelligence systems have several benefits. For example, narrow artificial intelligence systems that are already in use require specialized development and substantial training for particular jobs. Moreover, at that point, their only usefulness is limited to the task for which they were taught.

Conversely, artificial general intelligence is capable of generalization and the pursuit of novel ideas. As a result, it may be adjusted to a wide range of jobs. Artificial general intelligence is capable of simultaneously processing a wide range of inputs, including text, pictures, voice, and video. Even non-experts without a background in computer science can utilize it. By entering data into the system, non-specialists may simply train and modify it for certain purposes. Artificial general intelligence would probably learn from human interactions and start to take more of our values into account when making judgments.

We might witness the advent of artificial superintelligence in the next stage. Many scientists and technology experts are confident that artificial super intelligence is on the horizon, even if we are still a long way off. Without biological constraints, artificial intelligence is likely to outperform humans quickly once it reaches our level. It is therefore imperative that we ensure that it is consistent with our values.

Artificial intelligence can be used for good or harm, much like many other inventions. It can free us from time-consuming, monotonous jobs, giving us more time for creative endeavors. Robots with deadly intent and supercomputer overlords are not the same as artificial intelligence. It has the power to enrich our lives and raise the standard of healthcare.

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Advance Research Trends in Biofertilizers

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Introduction

Biological resources are the most important primary source of material basis for human living and sustainable development. They are the most significant treasure reserve of Mother Nature to whole human beings as well as strategic resources for a country. Biological resources embody natural resources of plants, animals, and microorganisms. One of the major challenges facing humanity in the 21st century is how to resolve the paradox of the increasing demand on biological resources vs. our sustainability in future. The confluence of our knowledge intending to solve this paradox is managed hopefully to quickly develop revolutionary biotechnologies in order to develop new supper varieties to pace up with the fast and continual economic and social development.

New technology has accelerated the process, transforming native genetic resources into commercial products, mainly developed by companies from countries at a different scale of industrialization. They include the production of certified seeds for agriculture and forestry, production of dyes, medicines, bio-fertilizers and bio-pesticides, as well as high quality fibers and fuels.

Sustainable agriculture for profitable crop production and food security is becoming an essential part of global civilisation under ever-changing climatic conditions. Use of chemical fertilizers in this scenario is mostly preferred to enrich soil nutrient status for rapid production and productivity of crop plants. Over the period of time, application of chemical fertilizers above threshold limit leads to decreased soil fertility, lesser crop yield, emergence of pests and disease, loss of biodiversity and environmental pollution. Imbalanced application of chemical fertilizers also causes low nutrient use efficiency, high mineral leaching, low nutrient assimilation potency, depletion of organic matter content and deficiency of secondary and micronutrients in soil and plant. Too much dependence on chemical fertilizer like ammonia, urea, nitrate or phosphate compounds in agriculture leads to severe damage to both environmental ecosystem as well as animal and human health. This has made the environmentalists and agricultural scientists to search for a nontoxic eco-friendly alternative to achieve the desired goal, to increase the agriculture productivity without associated side problems.

Microbiome:

Etymology

The word microbiome was from the Greek word, micro meaning "small" and bíos meaning "life". It was first used by J.L. Mohr in 1952.

Definition:

"The microbiome is the community of microorganisms (such as fungi, bacteria and viruses) that exists in a particular environment".

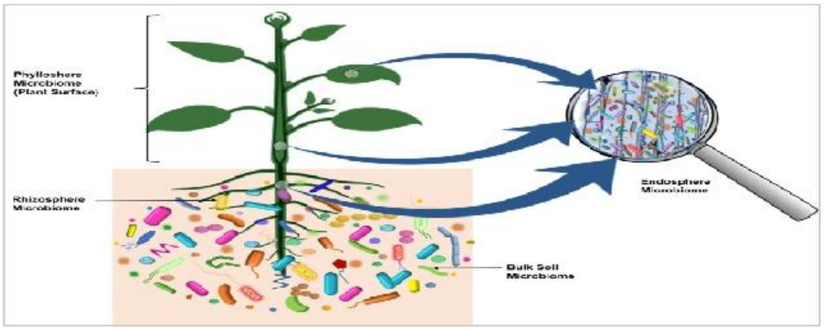
Microbiome may be of various types namely, Plant microbiome, animal microbiome, ocean microbiome etc. The microbiome has been defined as "a characteristic microbial community occupying a reasonably well-defined habitat which has distinct physio-chemical properties. The term thus not only refers to the microorganisms involved but also encompasses their theatre of activity".

Plant microbiome:

Plant microbiome also known as the phytomicrobiome, plays roles in plant health and productivity in agriculture and has received significant attention in recent years. Plants live in association with diverse microbial consortia. These microbes, referred to as the plant's microbiota, live both inside (the endosphere) and outside (the episphere) of plant tissues, and play important roles in the ecology and physiology of plants. "The core plant microbiome is thought to comprise keystone microbial taxa that are important for plant fitness.

The study of the association of plants with microorganisms precedes that of the animal and human microbiomes, notably the roles of microbes in nitrogen and phosphorus uptake. The most notable examples are plant root-arbuscular mycorrhizal (AM) and legume-rhizobial symbioses, both of which greatly influence the ability of roots to uptake various nutrients from the soil. Some of these microbes cannot survive in the absence of the plant host (obligate symbionts include viruses and some bacteria and fungi), which provides space, oxygen, proteins, and carbohydrates to the microorganisms. The association of AM fungi with plants has been known since 1842, and over

80% of land plants are found associated with them. It is thought AM fungi helped in the domestication of plants.



Diverse microbial communities of characteristic microbiota are part of plant microbiomes, and are found on the outside surfaces and in the internal tissues of the host plant, as well as in the surrounding soil.

Traditionally, plant-microbe interaction studies have been confined to culturable microbes. The numerous microbes that could not be cultured have remained uninvestigated, so knowledge of their roles is largely unknown. The possibilities of unraveling the types and outcomes of these plant-microbe interactions has generated considerable interest among ecologists, evolutionary biologists, plant biologists, and agronomists. Recent developments in multiomics and the establishment of large collections of microorganisms have dramatically increased knowledge of the plant microbiome composition and diversity.

Green Agriculture:

Green agriculture is farming practices that uphold and enhance farm productivity and profitability on a sustainable basis, decrease adverse externalities and reconstruct lost ecological resources with greater efficiency by minimizing pollution. In general, green agriculture is locally adaptable agricultural procedures and authentic market-driven certifications such as Good Agricultural Practices (GAP), organic farming and conservation agriculture and related techniques in which bioinoculants play a very crucial role by maintaining soil health. A change of mind-set and paradigm shift towards green agriculture and green economy may save the agroecosystem from further degradation.

Role of Bioinoculants in Green Agriculture:

Bioinoculants are formulations comprised of microbes used as a tool in green agriculture. In phyto-microbiomes, microbes are abundant and they form holobiont in association with plant and plants flourish with microorganisms association. The microbes of rhizo-microbiomes play a significant role in the growth and development of plants. Microbes have the capacity to assimilate and acquire essential nutrients of plants, improve soil physicochemical properties, and modulate secondary metabolites, antibiotics, plant hormones and various signal compounds. Additionally, microbes secrete different biostimulants, which play important roles in influencing physiological and metabolic activities. Different beneficial microorganisms have been considered for green agriculture to improve nutrient availability in the rhizosphere and uptake, tolerance of abiotic and biotic stress.

Plants require inorganic minerals for their nutrition, growth and development, of which phosphorous (P), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) are known as macronutrients, while micronutrients are copper (Cu), manganese (Mn), zinc (Zn), boron (B), molybdenum (Mo), iron (Fe) and chlorine (Cl). The micronutrients are found in plant tissues in a very less concentration ($<0.01\%$ of the dry weight of plant tissues). Some plants like legumes, wheat, barley, citrus, peach also require nickel (Ni) and the urease enzyme consists of Ni that takes part in the hydrolysis of urea in tissues. There are some more minerals in the list which are found as important to some plants grown in a specific environment and these are aluminium (Al), cobalt (Co), selenium (Se), silicon (Si) and sodium (Na), but the essentiality of these elements has not been recognized. These nutrients are present in nature in different forms and microbial inoculants assimilate and ensure bioavailability to plants. Microorganisms get involved in composite processes in nutrient assimilation in plants and facilitating the growth of the plants. Bioinoculants flourishing in the neighbourhood of roots enhances plant health by increasing uptake and availability of nutrients through the process of N fixation, P solubilization and mobilization, K mobilization and other micronutrient mobilization

Biofertilizers:

Definition:

"Biofertilizers are substances that contain microorganisms like bacteria, fungi, algae etc., which when added to the soil increase its fertility and promotes plant growth." These biofertilizers can also be applied to seeds and plant surface which promote plant growth by increasing the supply of essential nutrients to the plants.

The most commonly used biofertilizers are:

1. **Nitrogen fixing biofertilizers**

Example: a. Free living: Azotobacter

b. Symbiotic: Rhizobium

Example: Associative symbiotic: Azospirillum

2. **Phosphorous solubilizing biofertilizers**

Example: a. Bacteria: Pseudomonas striata

b. Fungi: Penicillium spp. Aspergillus spp.

3. **Phosphorus mobilizing biofertilizers**

Example: a.Arbuscular Mycorrhiza: Glomus spp.

b.Ectomycorrhiza: Amanita spp.

c.Endomycorrhiza

4. **Plant growth promoting rhizobacteria**

5. **Biofertilizers for micronutrient**

1. Nitrogen fixing biofertilizers

N is required in the desired quantity to plants because it is constituent of amino acids (used in forming protoplasm), enzymes, chlorophyll and several vitamins and is indispensable for physiological and developmental mechanisms of plants. In absence of enough N in unfertile soil, plants are unable to perform all necessary processes. To fulfil the requirement of N exogenous application of manures and fertilizers are common in crop production. However, N is abundant in the atmosphere and capturing of atmospheric N biologically is possible, in which different microorganisms play a pivotal role and the process is known as biological nitrogen fixation (BNF). Nitrogenase is the most important enzyme involved in nitrogen fixation. Bacteria like Azotobacter species have several types of nitrogenase enzymes. Rhizobium and Azospirillum are the majorly used nitrogen fixing bacteria. Rhizobium has the ability to develop symbiotic association with the roots of legumes, due to which it is used as biofertilizer in the cultivation of leguminous crops.

2. Phosphorous solubilizing biofertilizers

Phosphorus is one of the essential plant nutrients. It is second only to nitrogen and is considered one of the most growth-limiting macronutrients of plants. Soil is rich in insoluble phosphates. But it is deficient in soluble phosphates, which can be absorbed by plants. Plants absorb phosphorus in the form of orthophosphate. Phosphorus deficiency severely restricts plant growth, development, and yield. In order to overcome P scarcity in the agricultural soil, phosphorus fertilizers are added. In fact, phosphorus is the second most applied nutrient in agriculture. Soil microorganisms play an essential role in the P cycling and phosphorus nutrition of plants. Some microorganisms participate in mineral phosphate solubilization and facilitate phosphate mobilization in the soil.

Phosphate solubilizing microorganisms are the microbes that have mineralization and solubilization potential for organic and inorganic phosphorus, respectively. Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, through which their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms. Phosphate solubilization takes place through various microbial processes/mechanisms, including organic acid production and proton extrusion. A wide range of microbial P solubilization mechanisms exist in nature, and much of the global cycling of insoluble organic and inorganic soil phosphates is attributed to bacteria and fungi. Phosphorus solubilization is carried out by a large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates. Among different microbes, bacterial species from genera *Bacillus*, *Pseudomonas*, and *Rhizobium*, fungal species from genera *Penicillium* and *Aspergillus*, actinomycetes, and arbuscular mycorrhizae are popular phosphate solubilizing microbes inhabiting the soil.

3. Phosphorus mobilizing biofertilizers

Phosphate mobilizing microorganisms are the microbes that participate in the mobilization of phosphorus in the soil. The majority of phosphate mobilizing microbes are phosphate solubilizing microorganisms. They release phosphorus from the insoluble and fixed forms of phosphorus in the soil. As a result, soil P availability increases and the plants are able to absorb phosphorus in a sustainable manner. Phosphate mobilizing microbes mobilize phosphorus by changing pH and also by producing chelating substances. The terms phosphate solubilizing and phosphate mobilizing are interchangeably used to refer to phosphate solubilizing microorganisms. Phosphate mobilizing microbes are involved in the transformation processes of soil P.

4. Plant growth promoting rhizobacteria

Plant-growth-promoting rhizobacteria (PGPR) are a promising potential tool to sustainable agricultural production. PGPR are a group of bacteria that can improve growth by different mechanisms. Many species of PGPR enhance the availability of essential nutrients and improve the efficiency of the applied nutrients, provide growth hormones to the plants, and improve plant resistance against pathogens and abiotic stress. Kosakonia radicincitans (formerly Enterobacter radicincitans) is a bacterium belonging to the PGPR, which is able to colonize plant surfaces and tissues. K. radicincitans can provide many advantages to plants due to its ability to fix atmospheric N, solubilizing P, producing growth hormones, and inhibiting pathogenic fungi. Other examples for PGPR are as follows:Flavobacterium, Streptomyces, Xanthomonas, Agrobacterium, Achromobacter, Alcaligenes and Arthrobacter.

5. Biofertilizers for micronutrient

Micronutrients are important for plant growth, as plants require a proper balance of all the essential nutrients for normal growth and optimum yield. The essential nutrients derived from the soil are referred to as micronutrients, because they are needed in small amounts. They are boron (B), chloride (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn).

Examples: a. Potassium solubilizers: Bacillus edaphicus, B. mucilaginosus, and Paenibacillus glucanolyticus.

b.Silicate and zinc solubilizers: Bacillus subtilis, Thiobacillus thioxidans, and Saccharomyces sp

Composite inoculants

The combination of strains of Plant Growth Promoting Rhizobacteria (PGPR) has been shown to benefit rice and barley. The main benefit from dual inoculation is increased plant nutrient uptake from both soil and fertilizer. Multiple strains of inoculant have also been demonstrated to increase total nitrogenase activity compared to single strains of inoculants, even when only one strain is diazotrophic.

Example: PGPR and arbuscular mycorrhizae in combination can be useful in increasing wheat growth in nutrient poor soil and improving nitrogen-extraction from fertilized soils.

Effective Microorganisms

Effective microorganisms (EM) are microbial inoculants that stimulate plant growth and soil fertility in agriculture. Generally, EM suspension contains a group of microorganisms such as lactic acid bacteria, yeast, and photosynthetic bacteria. These microorganisms have unique activity by virtue of which they help in improving plant growth. For example, lactic acid bacteria produce lactic acid and suppress many pathogens. The application of EM has been found to improve soil health and soil biodiversity. It has also been reported to improve plant growth, yield and nutritional quality of crops. The application of EMs has also been found for composting and water quality improvement

Disadvantages of Biofertilizers:

Biofertilizers in agriculture comprises some major problems such as,

1. Poor shelf-life
2. Specific biofertilizer for specific crop
3. Instability in field due lack of prescribed soil and environmental conditions (temperature, radiation, pH sensitive),
4. Require specific storage conditions,
5. Limited availability of beneficial microflora, susceptible to desiccation and large dosage etc.

To overcome all these constraints nanoparticle-based formulations of biofertilizers have been developed to get more efficient and enhanced productivity of crops.

Nanobiofertilizers:

Nanotechnology presents a solution through nano biofertilizers which have a promising future in the field of sustainable agriculture management. Nano biofertilizers will act as a potential “nutrient booster” allowing the slow and sustained release of nutrients to plants throughout the growth period. Wherever possible, organic manures and other organic materials should be used in an integrated fashion with nano-based fertilizers to ensure efficient and effective nutrient use as well as better soil health. Nano-biofertilizer could promote several benefits to plants, i.e., slow-release characteristics, enhanced stability of functional ingredients, use of small dose, limited nutrients loss by degradation and leaching, masking soil nutrient depletion and improving crop yield attributes. Hence, nano biofertilizers are considered as future of sustainable agriculture as they are intrinsically environmentally friendly as well as economical. Nowadays, bio-fertilizers being eco-friendly microbial amendments (bacteria, fungi, algae), highly proclaims sustainability in recharging soil nutrients without any disruption of soil-microbiome interaction.

"Nano-biofertilizer" is defined as a hybrid combination of nano and biofertilizer developed by the formulation of organic fertilizer (biofertilizer) to nanosize (1–100 nm) with the help of certain nanomaterial coating. The materials used to make nano particles are metal oxides, ceramics, silicates, magnetic materials, semi-conductor quantum dots, lipids, polymers, dendrimers and emulsions. The nano materials (chitosan, zeolite and polymers) used for coating help in the slow and constant release of nutrients to plants. The nanoparticles act as versatile tool to protect biofertilizer components containing plant growth promoting rhizobacteria (PGPR) thereby enhancing their shelf-life.

Advantages of Nano-biofertilizers:

- Nano formulations may enhance the stability of bio-fertilizers and bio-stimulators with respect to desiccation, heat, and UV inactivation.
- Application of nano-biofertilizer can increase plant yield and quality by improving photosynthesis, nutrients absorption efficiency, photosynthate accumulation and nutrients translocation to the economic parts.
- Nano-biofertilizer augment the soil nutrient status by various mechanisms like nitrogen fixation, solubilization and mobilization of phosphates, production of siderophores and synthesis of plant hormones.
- The PGPR in nano-biofertilizer (bioorganic component) help in nitrogen-fixation, phosphate-solubilizing and help in restoring soil fertility while nanomaterials help in the slow and steady release of nutrients as per crop demand in a synchronized mode.
- It also increases resistance against pest and disease pathogens in plant by acting as resistance inducing agent. The nano claycoated biological agent like Trichoderma sp. and Pseudomonas sp. are used to control fungal or nematode disease in rabi crops. It also provides crop resistance against abiotic stresses.

Biofilm:

Biofilm is the community of micro-organisms, developed in vitro by accumulating altogether with adhesive forces embedded in a matrix containing polymers that can secrete extra cellular polymeric substances (EPS) for self-protection from various environmental stresses. It enhances nutrient cycling and availability, bio-control of pests and diseases, and improves soil fertility as well as productivity. Microorganisms that can easily adhere on a surface of any substrate, soil particles and plant roots are preferably selected for biofilm production. Both gram positive and gram negative bacteria having antibiotic-resistance properties are used. Combination of Bacterial species along with mycorrhizal fungal and other microorganisms are also used to form biofilms.

The use of biofilm in recent era admirably reduces harmful effects of chemical fertilizers and improves crop productivity by maintaining sustainability of ecosystem. But, the tripartite interaction between fungal hyphae, colonized plant parts and bacteria in rhizosphere as well as regulatory mechanisms of the organisms involved in biofilm community needs much specific investigation using some culture-independent approaches such as metagenomics.

Bacterial strain used in biofilm:

1. Pseudomonas sp. can be used for bioremediation of phenanthrene in rice crop.
2. Azospirillum brasilense can be used for nitrogen fixation in wheat crop.
3. Paenibacillus polymyxa can be used as biocontrol agent against crown rot disease in pea nut.

Bacterial biofilm associated with mycorrhizal fungi:

1. Pseudomonas sp. combined with Rhizophagus irregularis for Phosphorus solubilization.
2. Bacillus sp, Bacillus thuringiensis, Paenibacillus rhizosphaerae combined with Gigaspora margarita helps in Increasing hyphal growth, ethylene production, growth inhibitor of fungal pathogens around root of the plants.

Advantages of Biofilm:

Co-migration of bacteria with fungal hyphae in the tripartite association, called biofilm being eco-friendly and environmentally viable has so many positive impacts on modern agriculture as follows:

- Increases yield of rice grains by improving soil health as well as soil-microbial interaction.
- Applicable in non-leguminous crops like maize, vegetables, and plantation crops too.
- Increases availability of phosphorus and suppresses basal rot disease incidence of red onion.
- Improves the soil organic matter content and availability of nutrients (N, P and K).
- Reduces leaching loses of nutrients and increases plant dry matter accumulation.

Conclusion

Microorganisms, because of their vast diversity, the multiplicity of roles, wider range of ecological amplitude and host preference, require thorough examination before suggesting their use as bioinoculants. Location and crop-specific studies will help in understanding their mode of action, possible beneficial use and their effective utilization as sustainability enhancers. Microorganisms can be beneficially utilized in agriculture for improving soil fertility. The soil fertility enhancement can be brought about by nutrient addition (example: BNF), nutrient solubilization (Ex: PSB) or through nutrient mobilization (ex: VAM). In addition to these, many microorganisms secrete plant growth-promoting substances in the rhizospheric region that can enhance plant growth. Many microorganisms also help in suppressing pathogenic microorganisms and thus help in suppressing or minimizing disease incidence. Many microorganisms have also been found to impart stress tolerance through different mechanisms. Considering the wide range of benefits the microorganisms offer in an agroecosystem, their isolation and culture and their use as bioinoculants helps in attaining a sustainable and climate-smart agriculture production system.

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Urban Ecosystem

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Introduction

Urban Ecology:

"To truly advance the discipline of urban ecology requires the creation of note hypotheses and the identification to conformed generalizations McDonell & Niemela.Urban ecology is a multifaceted research traditions and methods from a wide range of backgrounds and disciplines. Over the past century , it has been adopted and expanded by research from fields as diverse as the social sciences, natural sciences and engineering . while urban ecology used to be underrepresented in textbooks and journals of ecology.it is now recognised as an important research filed for ecologists, evolutionary biologists and others . with urban systems beings responsible for 60 to 80% of naturals resource consumption and substantially impacting every other ecosystem on the globe, urban ecology has become key research filed in tacking the sustainability crisis.Urban ecology has different meanings to different researchers and stakeholders a circumstance that is rooted in the history of the field the unstandardized use of the term "urban"(De Deyn G. *et al*. 2011)

Ways to define Urban Ecology

The aim of urban ecology is to study these effects. According to scer&wris ,the term 'urban ecology can be defined in two ways .within the natural sciences ,urban ecology can be addresses biological patterns and associated environmental processes in urban areas as a subdiscipline of biology and ecology . in this sense, urban ecology endeavours to analyses the relationships between plants and animal populations and their communities as Well as their relationships and environmental factors including human influences . from this person in urban, the research is unconstrained by anthropocentric evaluations (Lange M. *et al.* 2015). However the second, complementary, definition implies the anthropocentric perspective .here, urban ecology is understood as approach to improvement living conditions for the human population in cities, referring to the ecology functions.

Urban ecology in an interdisciplinary field that supports societies attempts to be come mote sustainable. It has deep to in many disciplines including program, sociology, urban planning, landscape architecture,engineering, anthropology, public health, andecology. As interprimary nature and unique focus on humans and naturals systems within areas ,urban ecology has been sold variously to describe the study of humans in cities ,nature in cities and the coupled relationship of humans and nature (Giovannetti M *et al.,* 1980)

Conceptual history of research in urben ecology

Urban ecology has many disciplinary roots. In recent decades, the conceptual approach of the “Berlin school of urban Ecology “promoted mainly by HERBERT SUKOPP since the 1970s, was influential. By this approach,urban habitats and associated environmental prosses were analysed at local and regions scales by different disciplines of natural sciences. This includes biodiversity patterns as well as characteristics of urban soils and climate and their variation in time and space due to changing urban land uses. While the contemporary ecosystem approach of DUVIGNEAUD mainly addressed fluxes of energy and matters at the city level, the Berlin approach focussed on the explicit spatial variation of ecological components within urban environment. This also led to the first model of a city characterised by idealised variation in climate, soils,terrain, vegetation and fauna along a transect from the densely built up city centre to the outskirts. SUKOPP distinguishes a core surrounded by three rings the densely built up sub centres may be found and finally the interior and exterior border zones. Concentric models of the spatial organisation of land uses have existed since VON THONEN and BURGESS developed a model of the concentric structure of cities from the perspective of social sciences. SUKOPP was the first to qualify such a model with a broad array of ecological factors(De Brito A. M.*et al*., 1995)

Perhaps the most often reproduced diagram in urban ecology shows a transect through the concentric rings and its consequences for climate, soil and water, topography, vegetation and animal life in the different urban zones. Many studies of urban ecology follow Sukopp’s transect approach, comparing the specific ecological situation of each zone with the others, and the whole city with its surrounding environment. The urban heat island of densely built up environments is an important factor of additional stress in summer months. Lower work efficiency, enhanced morbidity, hydrosphere, and the biosphere .Urbanised areas may serve as subject of field experiments in order to investigate plant responses to climate change, since temperature and co2 concentrations are already increased in cities. Impacts are highly variable, but include an increased burden of diseases, increased morbidity and mortality from more frequent and intense heat waves superimposed on the urban heat island coastal megacities are particularly at risk from floods, storms and droughts (Lal R. 2020)

Demographic change : May also exert an influence on the anthroposphere.in highly industrialised countries people are growing older then ever before, while birth rate is simultaneously decreasing in countries like Germany . The proportion of senior citizens has expected to increase, and the pyramid population is likely space to change its shape. This causes modification of behaviour and demand for living space.However ,it is indicative that demographic changes offer potential for improving the ecological conditions of cities ,not only due to a reduced number of improving the ecological conditions of cities , not only due to a reduced number of individuals and therefore demand for water, energy, transport etc, but also in the context of a decreasing pressure on land use and the possibility of alternatives to the context of a decreasing pressure on land use and the possibility of alternatives to the classical growth of urban development conversely to ensure cost efficient technical infrastructures ,building density should not fall below a certain threshold (Chen Q. *et al.* 2022).

Economic change is one of the most important factors for the functions and development of urban agglomerations. A towns role in the interregional and supranational network of cities is affected by its economic structure and, in addition, existing economic activity dominates the urban environment .cities have under gone rapid changes to their economic structures during recent decades. They had became increasingly integrated into global supply and demand systems, which depends on the process of globalisation. Alongside these developments, a key factor in advanced economics for urban agglomeration is the switch from industrial to service based economies; spatial characteristics of this change are the appearance of brownfields on former industrial land growing demand for spaces for high-ranking services (Hernandez-Espinoza L*et al*., 2020)

The four above-mentioned changes are important issues to been taken into account in future urban ecological research and planning processes. Urban ecology is an interdisciplinary science where element of the natural spheres and the anthroposphere with its socio economic aspects must be taken into account(Lekberg Y*et al.*, 2008).

Principles of landscape ecology

Regarding landscape ecology ,author Richard Forman writes ',its large area and long term focus provide an obvious foundation for how we can design and pan the land for a more sustainable future' understanding the language of landscape ecology is therefore essential to making planning decisions that enhance the ecological function of an area.

Ecosystem function:

Processes throughout a landscape interact to define its ecological function. This ability to function is described by Marina Alberti as 'the ability of earth processes to sustain life over a long period of time.(Alberti,M*et al*.,2009).

Resilience:

"The ability of a system to adapt to changing internal and external processes '.resilience in an urban system depends on the city ability to maintain ecological and human functions simultaneously(Lal R.et al., 2015).

Hierarchy and scale:

Scales are linked in a hierarchical manner and actions at one level of biological and social organization influences the patterns and mechanisms operating at lower and higher scales .for example bird abundance and diversity in urban ecosystem varies over time of day, season and among years(Clark R. *et al.* 2013).

Strategies for urbon ecological health:

Indispensable patterns

There are four documented 'indispensable patterns ' that authors claim provide ecological benefits that cannot be substituted by technology alternatives .these patterns include ;large natural vegetation patches ,wide vegetation corridors surrounding waterways ,connectivity among large patches of movement of target species and small patches and corridors 'bits of nature' that provide heterogeneity in developed areas.

Main features : Urban areas acts as population providing goods and services not only for its population but also for populations worldwide . Urban ecosystem can no longer be impacts on the immediate and wider environments as they have direct and indirect impacts on the immediate and wider environments. Many of the environmental problems faced today had traced back to cities and lifestyle choices .With urban population levels expected to reach 60% in the next 30years and themajority of urbanisation to occur in developing countries,urban environmental managements is being increasingly important (Diekmann L.et al., 2020)

Urban areas can not exist in isolation .They can required inputs from and waste assimilation functions of ,other ecosystem .Ecosystem footprint analysis has shown that many cities require a production land sea areas several times cities size in order to support the population .

Multidisciplinary in natural urban ecosystem management requires a composite of social ,environmental ,economic and decision making tools and institutions that are flexible and can adapt quickly to change in one or more systems(Denneman C et al., 1990).

The urban ecosystem approach encourages the alignment of cities to that of natural ecosystems where resources ,processes and products are used more effectively ,creating less waste, requiring less waste requiring less input and viewing by products as resources.

Case studies and explaination:

1) UNEPIETC -The ecosystems approach to urban environmental management

2) United nations university – urban ecosystem management

Target sectors:

Governments, Non-governmental organisations, Research institutions, businessesexport, decision makers, industrial organisations and the community are primary stakeholders in the urban ecosystem concept (Egendorf S. *et al.* 2018).

Scale of operation:

Urban ecosystems cover an urban area.

Urban green infrastructure

Research green infrastructure stared to gain more momentum 2010 by becoming a motor theme of urban sustainability assessment for the first time , which is strongly linked to issues such as climate change adaptation and mitigation,microclimate regulation ,and ecosystem services ,suggesting that urban green infrastructure is essential for achieving sustainability .regarding the “green infrastructure” sub themes ,analysis of the thematic networks shows that more attention has been paid to the health and well being impacts during this period(Gandullo J. *et al*. 2021).

The term “urban green infrastructure” refers to engineered and non-engineered habitat structure in connection with natural and seminatural areas and others environmental features, which are, designed to deliver enormous ecosystem services from nature to people and to protect biodiversity.

Ecosystem Services:

Biodiversity:

Urban development threatens some elements of biodiversity, yet urban areas often contain significant biodiversity, including threatened species.

Biodiversity loss has become a major global issue, and the current rates of species decline are unprecedented. Among the different species, vertebrates, particularly mammals and birds have more attention for scientists and publics, whereas insects had routinely underrepresented in biodiversity and conservation studies in split of their paramount importance to the overall functions and stability of ecosystem worldwide.

Biological Factors

Urban development is a major threat to conservation .animals including mammals, insects and birds are among the urban wildlife that need attention for conservation .thus ,ecological and life history traits cab predict species responses to urbanization .In new environmental which totally differ from their natural habitat, they have to move and disperse in order to maintain their species development through colonization.

Habitat change

Over the past centuries human activities such as industrialization, agricultural intensification for food production, deforestation in tropical countries and urbanization have boosted the susceptibility of specialist pollinators to land use changes, appears to be a determining factor in the decline of many bumblebees and wild bees.

Pollution

Pollution is the second major driver of insects and other species in urban areas. The factors caused environmental pollution include fertilization and synthetic pesticides used (Awasthi M.*et al.*, 2016) agricultural production ,sewage and landfill leachates from urbanized areas and industrial chemicals form factories and mining sites.

Climate change

Urban areas are under the pressures of pollution growth, urbanization and suburbanization processes, which interact with the climate, leading to the established of the urban climate.

Some particular features such as heat islands effects, dryness, urban flooding, cold, humidity waves, and drought generally characterize urban climate, and flooding are the three most important stresses having huge multi lateral impacts.

Urban microbiome

The term microbiomes refers to complex communities of bacteria, fungi, viruses, and micro eukaryotes and are an integral part of human and natural ecosystem. In terms of environment, soil microbial communities are a key factor in biochemical process which support plant growth and others ecosystem services of GI features (Huang Y*et al.*, 2017)

Growing the world population accelerate the increase of population and consequently can jeopardize the people life by being exposure to pollutants. Therefore, it is imperative to adopt sustainable practices and enhance the health of urban environmental, considering the implementation of surveillance programs, discovering the genetic characterization and functional diversity of microbes in the cities

What goods and services do urban ecosystems provide?

The human elements of the city – its man made infrastructure and economy provides goods and services of enormous value(Cerveny C*et al*.,2011). Cities are the heart of human commerce and industry and therefore the primary centres of employment of employment, housing, transportation, and the range of social services from health care to education. However, urban green spaces contribute their own unique and essential services to the urban mix.

Shade and temperature control

Street trees and other green spaces help to battle the urban heat island effect in the summer. Temperatures in the heart of a city may be 0.6 to 1.3 C warmer than in rural areas due to the large heat absorbing surfaces of buildings and asphalt, as well as high-energyuse. Trees provide shade and transpire large amounts of water that, when evaporated, provide a cooling effect. Whencombined, these effects can lower local temperature sometimes by many degrees in the vegetated area. The shading effect of trees translates to energy saving and reduced regional pollution.

Air filtering

City plants are efficient air pollution removing. In a park, the leaf surfaces of trees can filter out as much as 85% of the ambient air pollution mostly particulates. Street trees can also be effective air purifiers, removing up to 75% of particulates on a tree-linkedstreet. Urban forests in the Baltimore Washington region remove some 17000 tons of air pollutants per year worth at least 88million annually.

Noise reduction

Trees and shrubs can help filter out noise pollution too .A 30-meter belt of tall dense trees combined with soft surfaces can reduce local noise levels by 50%

Stormwater control

City watersheds function differently than watersheds that are more rural because they had covered with building and paved areas that water could notpenetrate. Urbanforests,wetlands, and streamside vegetation help to restore some of the natural balance by buffering stormwater runoff, absorbing pollutants, and recharging groundwater reservoirs. Forests in the Washington baltimore corridor had estimated to been saved the region about 1 billion in costs for constructing stormwater control facilities like retention ponds and reinforced in the United States participate in wildlife watching activities within 1 mile of their homes, according to the US park service .one study in Chicago estimated that planting about three more trees per building lot could save air conditions costs per house (Cheng Z. *et al.* 2015)

Recreation, aesthetic, and spiritual values

Parks and green spaces provide city dwellers with invaluable recreational opportunities. Just ask the bikers , joggers, skaters, and dog walkers in any urban park , any day of the week . Also we tend to dote on the patches of nature we surround ourselves with our lawns and yards .urban real estate costs continually bear out the value we place upon landscaping , yard size , and the proximity to parks .

Food production

Although it is not so prevalent in the US, urban agriculture is very important on a global basis .world wide some 800 million city residents grow food in backyards , vacant lots , roadsides , and small suburban farms . in Kenya and Tanzania , 2 out of 3 urban families are engaged in forming . in Taiwan, , more than half of all urban families are members of farming associations . in cuba in 1999 urban agriculture produced 8000000 tons of fresh organic produce and employed 165000 people .

What is threatening the worlds urban ecosystems

Intensive and rapid urban growth is the greatest pressure on urban ecosystems. The prospects for global urban growth population of 2.9 billion to 4.9 billion .in fact, in today world nearly all population growth is urban growth . rural populations will remain steady over the next 30 years , while growth is channelled into cities and suburbs .

To put a more local face on urban growth ,consider that over the next 3 decades , the population of us cities will increase by more than 2 million people per year . today, three quarters of the united states population is urban about 215 million people .

Growth of giant cities megacities with more than 10 million people has spread over a significations larger area than urban areas will be changing natural areas like forests,grasslands, and farms into urban and suburban environments. Within existing cities more people will use parks and other green areas , and development will gradually fill in vacant parcels , increasing the stress on the remaining green areas . the discovery of the ecological value of man made settled areas is connected to earlier words about the positive perception of which was precisely due to the impact of human activity

Challenges for urban ecology and the city

It has emerged that urban locations can be ecologically abundant due to the specific characteristics of each subsystem and their densities . such systems demonstrate higher biodiversity than some of the areas traditionally perceived as near naturals

Amin and thrift emphasised that nature and the city can no larger the considered dichotomic as a matter of contradiction per se, yet the boundaries between them have blurred to a significant extent . this is evident in the diversity of species hosted by modern cities . in turn this may prove to alter human perception of nature . as Amin and Thrift stated , the environmental agency of London sold about 200000 fishing rod licences to people in Landon in 1999 almost a third of the annual total for England and wales . accordingly the recently noted appearance of wild animals in cities and the increasing invasion of non native plants indicate the changes in urban and thus societal relations to nature .

It is widely accepted that urbanisation has a significant effect on existing rural and natural landscapes but the perception of urban industrial has yet to progress from one sided negative evaluation . they may contribute to the stabilisation and improvement of natural living conditions , regardless of whether they are in regular use . urban ecology studies have reintroduced the significance of urbanised areas as ecological relevant .in this context conceptualisation of urban space as an ecological entity as described above represents a paradigm shift in environmental research .

A novel concept of urban ecology arises from the discussion of these and various American concepts, with particular focus the human dimension. Or as Alberti have suggested the actual challenge is integration humans into ecology. There is a profound disconnection between nature and wilderness on one hand and the built up environmental of cities on the other.

Cities are usually so large that city dwellers contact with nature is difficult and often it is only poor industrial agriculture or tree monoculture that can be easily accessed outside the city, whereas biodiversity inside the city is high and different but often not recognised. the ecologic and economic values of the fourth nature or new urban wilderness are not yet broadly appreciated . city dwellers spend most of their time indoors in environments with artificially heated or cooled ambient air ,treated drinking water from pipes ,soil in flower pots with or some way to replacing the outdoors type that city dwellers are disconnected from. However human well-being, work efficiency and health also depend on intact natural elements close to daily life in cities. The colourful,spotted, “harlequin pattern” is not only typical for urban biotopes, but can be found in a multitude of local climates and soil sites.

Human activity must be considered as an essential part of urban ecology, and the integration of geo biosphere and anthroposphere approaches is urgently needed . Good practice example of such integrated actions can be developed at ecological hotspots in cities .

Advandages and disadvandages of urbanization

Trade and commerce

Urbanization advances the country business sectors be providing more jobs and a more diverse economy . a vast network of goods and services has helped develop modern commercial institutions and exchanges that have empowered the growth of urban areas .commercialization and trade offers town and cities better business opportunities and returns compared to rural areas .

Tourism industries

More people in cities means the need for better transportation systems .foreigners are attracted to cities with great transportation for easy mobility as well as unique attractions partially supported by infrastructure .

It provide great foreign current inflows for the cities economics

Culture and sciences

Improvements in culture and science are projected to increase through increased urbanization .as diverse cultures interact ,work , and communication with one another in close proximity ,cultures are integrated more Smoothly.

Growth in industrial productions

The production in various industrial sectors like cement ,iron ,steel , textile, fertilizers etc., Are helping in the economic growth of the county . Export increase and this forest reserve increases.

Labor productivity

The process of urbanization contributes to an increase in labour productivity ,allows solving many social problems of society. People who live in urban area tend to earn due to the availability of more jobs.

Disadvantages

Development of housing issues

The rapid flow of rural populations into urban areas mean there will be a rise in housing scarcity . insufficient space for housing and public building may give rise to housing problems ,poverty , and unemployment . costly building materials may be unaffordable for some individuals , resulting in slums. Pricing of houses is an issue as well . it is one of the main elements affected by the agglomeration in cities .thus , living in a big city is always more expensive small urban center.

Decrease in rural populations

The decrease in rural populations means a shortage of workers who will be able to produce agriculture as a result, there may be an in demand need to growing populations within cities but there will also be a dramatic escalation of solid waste production in cities and on farms trying to grow enough food .

Air quality

Air quality in towns and cities is frequently very poor because of air pollution from many different sources.

Smoke from domestic fires.Poor air quality has significant impact on the health of many urban residents as well as leaving a damaging and unsightly layer of dust on plants, building and other surfaces (Bernal M. *et al.,* 1998).

Pollution

According to a WHO study, more then 80% of people living in urban areas has exposed to levels of pollution that exceed those recommended by the same organization.

The environmental will become hotter and more humid because everyone is crammed in one centrals area.

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Endocrine Glands and Hormones

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Introduction

Human hormones are chemical messengers produced by glands in the endocrine system. They travel through the bloodstream, regulating various physiological processes, including growth, metabolism, mood, and reproductive functions.

The term **Hormone (hormao = to excite)** was first used by William M.Bayliss and his brother-in-law Ernest H.Starling, both of did their profession in London University College, in 1904, who discovered that a chemical substances from the intestine could stimulate the action of a pancreatic secretion and later named it as “Secretin”

Hormones had called as **“**chemicalmessengers**”.** Went and Thimann (1937) defined hormones as “Substances produced in any one part of an organism, is transferred to another part and there influence a specific physiological process”.

Hormones – biologically active substances, which released into the blood by the endocrine glands and humoral way (through blood, lymph, saliva, cerebrospinal fluid). They regulate metabolism and physiological processes. Hormones, as universal regulators of the body functioning, play an important role in the maintenance of homeostasis. They influence all essential life processes, such as growth, metabolism, development, immune defence, reproduction, behaviour and adaptation to the conditions of existence.

The hormonal effects on metabolic processes of target cells are realised through interaction with specific receptors. Depending on the localization of these receptors, different mechanisms of action of hormones had presented.

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In this chapter, we discuss about the structure, classification, mechanisms of action and biological effects of various hormones in human.

Growth and change

Everyone starts out as a baby. A baby's bones and muscles grow. Babies start out crawling but after a while, they can stand up and walk. Soon after that, the child can run and move in all sorts of directions. As the years go by, growthcontinues until the body is mature**.**

Humans go through a predictable sequence of development from infancy, through childhood and adolescence, and into adulthood. As the human body matures, it develops different functions related to the different stages of life. The body as structures called Glands**.** That produce substances that control the processes of bodily change.

Glands produce chemical substances called **"**Hormone**"**. Hormones signal tissues and organs in the body to perform functionsor to change.

The effects of hormones can vary. Hormones can trigger sudden change in the body or sustained gradual change. For example, one hormone in suddenly make a heart beat faster when a person is surprised. Other hormones very gradually make a person grow and mature.

Glands and hormones

To function well and stay alive, the human body tries to maintain a condition called Homeostasis**.** Think of homeostasis as internal balance. The body has much system; all performs the complicated functions at the same time. The body has a system that maintains balance by subjecting all of its processes to the chemical commands by hormones. Hormones, the structures that make them and the pathways they travel make up the endocrinesystem**.**

The human body functions best when its systems are stable – all parts are getting enough of what they need but not too much. When a funcyion becomes unstable, the body responds and starts a change process. For example, when a person needs energy from food, a part of the endocrine system releases a hormone. This hormone travel to the brain and signals "I am hungry". Once the person has enough to eat, another hormone has released that the body "I am full".

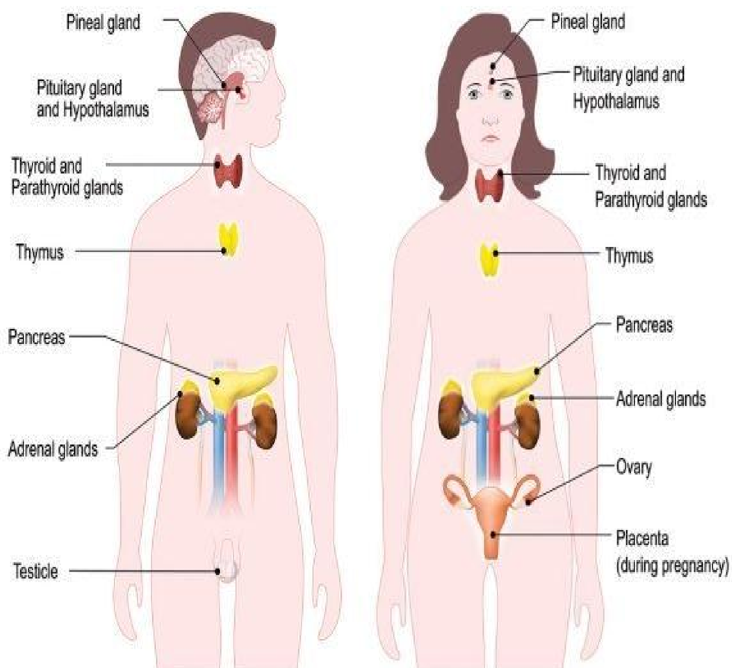
The endocrine system

The endocrine system produces hormones. It also controls when the hormones are released into the bloodstream.

Some of the body processes that hormones control include the following:

- Digestion
- Homeostasis
- Growth of the body
- Repair to damages
- Reproduction

The endocrine system routinely has to start and stop processes, such as hunger, sleep, and growth. Males and females have some difference in their endocrine systems.



Hormones and growth

Hormones also regulate a body's growth. The pituitary gland secretes many different hormones, including growth hormone and a hormone that controls the sex hormones. Early in life, when growth must occur for survival, the body secretes more growth hormone. The amount of growth hormone secreted by the pituitary gland decreases with age.The parathyroid gland controls the levels of calcium and phosphorous in the body. Both minerals are required for bone growth. Too little of this hormone can result in too much calcium and phosphorous in the body, which can be harmful.

Finally, the endocrine system produces gender-specific hormones, known as the sex hormones. Sex hormones play little role in humans until adolescence. Sex hormones control the significant body change that occur during adolescence. Sex hormones has been released by ovaries in females and by testes in males.

We are following detail a list of hormones found in *Homo sapiens.*Although hormones reach all parts of the body, only target cells with compatible receptors are equipped to respond. Over 50 hormones have been identified in humans.

List of human hormones

Adrenaline (epinephrine) :

Type : Amino acid derivative
Tissue : adrenal gland
Cells : Adrenal medulla / Tyrosine
Receptor :adrenergic receptor
Target tissue : nearly all tissues
Effect : blood pressure, glycogenolysis, lipolysis,etc.

Melatonin

Type : Amino acid derivative
Tissue : pineal gland
Cells :Pinealocyte / Tryptophan
Receptor : melatonin receptor
Target tissue : CNS and peripheral tissue
Effect : sleep-wake cycle

Noradrenaline or Norepinephrine

Type : Amino acid derivative
Tissue : adrenal gland
Cells :Adrenal medulla / Tyrosine
Receptor : noradrenergic receptor
Target tissue : nearly all tissues
Effect : Blood pressure , glycogenolysis , lipolysis, etc.

Triiodothyronine

Type : Amino acid derivative
Tissue : Peripheral tissue of thyroid gland
Cells : Thyroid follicular cell
Receptor : thyroid hormone receptor
Target tissue : nearly every cell in the body
Effect : increased metabolism

Thyroxine

Type : Amino acid derivative
Tissue : thyroid gland
Cells : Thyroid follicular cell
Recetor : thyroid hormone receptor
Target tissue : nearly every cell in the body
Effect : similar effect as T3 but much weaker : converted to T3 in target cells.

Dopamine

Type : Amino acid derivative
Tissue : substantia nigra
Cells : Phenylalanine
Receptor : D1 and D2
Target tissue : system wide
Effect : regulation of cellular CAMP levels, prolactin antagonist

Prostaglandins

Type : Eicosanoid
Tissue : All nucleated cells
Receptor : prostaglandin receptor
Effect : Vasodilation

Leukotrienes

Type : Eicosanoid
Tissue : Blood

Cells : white blood cells
Receptor : G protein coupled receptos
Effect : increase vascular permeability

Prostacylin

Type : Eicosanoid
Receptor : prostacyclin receptor
Effect : vasodilation, platelet activation inhibitor

Thromboxane

Type : Eicosanoid
Tissue : Blood
Cell : platelets
Receptor : thromboxane receptor
Effect : vasoconstriction, Platelet Aggregation

Amylin

Type : Peptide
Tissue : pancreas
Cell : pancreatic beta-cells
Receptor : amylin receptor
Effect : slowing down gastric emptying, inhibition of digestive secrestion, in order to reduce food intake

Adiponectin

Type : Peptide
Tissue : adipose tissue
Receptor : Adiponectin receptors
Effect : Regulating glucose level

Anti-Mullerian hormone

Type : Peptide
Tissue : Testes
Cell :Sertoli cell

Receptor : AMHR2
Effect : Inhibit release of prolactin and TRH from anterior pituitary

Adrenocorticotropic hormone

Type : Peptide
Tissue : anterior pituitary
Cell :Corticotrope
Receptor : ACTH receptor
Effect : Synthesis of corticosteroids in adrenocortical cells

Angiotensinogen

Type : Peptide
Cell : liver
Receptor : angiotensin receptor
Effect : vasoconstriction release of aldosterone from adrenal cortex dipsogen

Antidiuretic hormone

Type : Peptide
Tissue : posterior pituitary
Cell : Parvocellular neurosecretory neurons in hypothalamus
Receptor : AVPRs, VACM-1
Effect : retention of water in kidneys moderate vasoconstriction Release ACTH in anterior pituitary

Artrial natriuretic peptide :

Type : Peptide
Tissue : Heart
Receptor : ANP receptor
Effect : Increase sodium and GFR excretion, antagonize venal constriction, inhibit renin secretion

Brain natriuretic peptide

Type : Peptide
Tissue : Heart

Cell : Cardiac myocytes
Receptor : NPR
Effect : Reducing systemic vascular resistance, reducing blood water , sodium and fats

Calcitonin

Type : Peptide
Tissue : thyroid gland
Cell :Parafollicular cell
Receptor : CT receptor
Effect : Construct bone , reduce blood

Cholecystokinin

Type : Peptide
Tissue : Duodenum
Receptor : CCK receptor
Effect : Release of digestive enzyme from pancreas

Corticotropin releasing hormone

Type : Peptide
Tissue : hypothalamus
Receptor : CRF1
Effect : Release ACTH from anterior pituitary

Cortistain

Type : Peptide
Tissue : Cerebral cortex
Cell : inhibitory neurons
Receptor : Somatostatin receptor
Effect : Depression of neuronal activity : induction of slow wave sleep

Erythropoietin

Type : Peptide
Tissue : Kidney

Cell :Extraglomerular mesangial cells
Receptor :EpoR
Effect : Stimulate erythrocyte production

Enkephalin

Type : Peptide
Tissue : Kidney
Cell :Chromaffin cells
Receptors : Opioid receptor
Effect : Smooth muscle contraction of medium sixed vessels

Endothelin

Type : Peptide
Tissue : Vascular endothelium
Cell : ET receptor
Effect : Stimulate erythrocyte production

Follicle – stimulating hormone

Type : Peptide
Tissue : anterior pituitary
Cell :gonadotrope
Receptor : FSH receptor
Effect : In female : Stimulate maturation of Graafian follicles in ovary.
In male : Spermatogenesis.

Galanin

Type : Peptide
Tissue : Central nervous system and gastrointestinal tract
Cell : GALR1, GALR2, GALR3
Effect : Modulation and inhibition of action potentials in neurons

Gastric inhibitory polypeptide

Type : Peptide
Tissue : Mucosa of the duodenum and the jejunum

Cell : K cell
Receptor : GIPR
Effect : Induces insulin secretion

Gastrin

Type :Pepptide
Tiisue : Stomach
Cell : P/D1 cell
Receptor : ghrelin receptor
Effect : Stimulate appetite, secretion of growth hormone

Ghrelin

Type : Peptide
Tissue : Stomach
Cell : G cell
Receptor : CCK2
Effect : Secretion of gastric acid by parietal cells

Glucagon

Type : Peptide
Tissue : pancreas
Cell : alpha cells
Receptor : Glucagon receptor
Effect :Glocogenolysis and gluconeogenesis

Glucagon – like peptide 1

Type : Peptide
Tissue : ileum
Cell : L cell
Receptor : GLP1R, GLP2R
Effect : Stimulates the adenylyl cyclase pathway , release of insulin

Gonadotropin- releasing hormone

Type: Peptide Tissue ; hypothalamus
Receptor :GnRH receptor
Effect : Release of FSH and LH from anterior pituitary

Endocrine system consists of the following glands:

Hypothalamus:

Hypothalamus is a small region of our brain that connects to your pituitary gland through the pituitary stalk.

Pituitary gland :

Pituitary gland is a pea-sized gland at the base of brain , behind the bridge of your nose and directly below your hypothalamus.

Pineal gland :

Pineal gland is a tiny gland in your brain that's located beneath the back part of the corpus callosum.

Thyroid gland :

Thyroid is a small , butterfly shaped gland located at the front of our neck under our skin.

Parathyroid glands :

Most people have four pea-sized parathyroid glands located behind their thyroid gland.

Adrenal glands :

Adrenal glamds also known as suprarenal glands are small triangle shaped glands that are located on top of each of your two kidneys.

Pancreas :

Pancreas is an organ in the back of our abdomen. Its part of our digestive system and endocrine system

Ovaries :

People assigned female at birth have two ovaries. Each located on both sides of their uterus below the opening of the fallopian tubes.

Testes :

People assigned male at birth have two testes that hang in a ouch outside of their body below their penis

Kidneys

Kidneys are two bean shaped organs that filter your blood.

Liver

Liver is an essential organ and gland, performing hundreds of functions necessary to your sustain life.

Gut

It is a gastrointestinal tract is the long , connected tube that starts at our mouth and ends at anus. Its responsible for digestion.

Placenta

Placenta is a temporary organ that develops in your uterus during pregnancy. It provides oxygen and nutrients to the developing fetus.

Hormonal imbalances

Each hormones-related condition can have several different possible causes. In general, the main conditions or situations that cause hormone imbalances include;

- ❖ Tumors , adenomas or other growths.
- ❖ Damage or injury an endocrine gland.
- ❖ Autoimmune conditions.
- ❖ Herediatary gene mutations that cause problems with the structure and function of an endocrine gland.

Doctor treats hormone-related conditions:

Primary healthcare providers can diagnose and help you manage many hormone conditions. However, you may benefit from seeing an endocrinologist.

An endocrinologist is a healthcare provide who specializes in endocrinology, a field of medicine that studies conditions related to your hormones. An endocrinologist can diagnose endocrine conditions, develop treatment and management plans for them and prescribe medication.

Note: (A Note from Cleveland Clinic)

Hormones are an important and essential part of human exixtence. While your body normolly carefully balances its hormones, having too little or too much of a certain hormone can lead to health problems. If you're experiencing any concerning symptoms, it's important to talk to your healthcare provider. They can order tests to see if you have a hormone imbalance or if something else is causing your symptoms.

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Evolution, Classification and Applications of Algae in various industries

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Introduction

Algae are a broad class of photosynthetic organisms that are found in freshwater and marine environments. In damp terrestrial environments with lichens, cyanobacteria can also coexist in symbiotic relationships with fungi. Microorganisms such as phytoplankton and seaweeds are few examples of tiny algae.By using chlorophyll and other pigments in photosynthesis, these organisms transform sunlight into energy and are essential to ecosystem because they produce large amount of oxygen. Diverse pigments in algae allow them to adapt to all harsh environmental conditions. Algae can be green, red, brown, or blue-green in colour in accordance to the pigments present.

Various applications of algaeinclude food (many seaweeds are edible and are eaten by people from different civilizations). They are also useful in biofuel production and waste water treatment. Additionally, they are rich sources of many bioactive compounds that are useful in industries for mass production. Thus, they contribute to the overall food chain and the ecosystem balance.

Classification of Algae

Based on their properties, algae can be categorised into several types, representing a diverse group of photosynthetic organisms. Algal taxonomy continually changes as our knowledge of these species deepens. These are a few of the most common kinds of algae:

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S.NO	Division	Chlorophyll and characteristics	Habitat	Examples
1.	Chlorophyta	Green in colour and contains chlorophyll a and b.	Moist terrestrial areas and aquatic settings	*Ulva sp., Chlamydomonas sp., and Spirogyra sp.*
2.	Phaeophyta	Brown in colour due to fucoxanthin contains chlorophyll a and c	Marine environment	*Laminaria (kelp) and Fucus (rockweed).*
3.	Rhodophyta	Red in colour due to phycobiliproteins contains chlorophyll a and d	Marine environment and in deep waters.	*Porphyra (nori), Corallina (coralline algae).*
4.	Bacillariophyta	Unicellular or colonial,Cell wall made of silica	Both freshwater and marine	*Navicula, Cyclotella.*
5.	Euglenophyta	Unicellular, Flexible outer covering pellicle	Freshwater	*Euglena, Phacus*
6.	Dinophyta	Unicellular, Flagellated amor like plates	Marine but some are found in freshwater.	*Karenia, Alexandrium.*
7.	Blue-Green Algae	Prokaryotic, Chlorophyll a and phycobiliproteins	freshwater and marine	*Anabaena, Spirulina.*

1. Chlorophyta (green algae):

- Characteristics: They are green in colour because they contain chlorophyll a and b.

- Habitat: Found in mostly in moist terrestrial areas and a variety of aquatic settings.

Examples include *Ulvasp., Chlamydomonassp., and Spirogyrasp.*

2. Brown Algae (Phaeophyta): -

Features: It is Brown in colour and is due to fucoxanthinpigment and the presence of chlorophyll a and c.

- Habitat: mostly marine, particularly seen in the colder months.

-Examples include *Laminaria (kelp) and Fucus (rockweed)*.

3. Rhodophyta, or red algae): - Features: Possess phycobiliproteins and chlorophyll a and d, which give them a red colour. They are present in the marine environment and are found in deep waters.

- Examples: *Porphyra (nori), Corallina (coralline algae).*

4. Diatoms (Bacillariophyta):

- Characteristics: Unicellular or colonial, has a unique cell wall made of silica.

- Habitat: Found in both freshwater and marine environments.

- Examples: *Navicula*, *Cyclotella.*

5. Euglenophyta:

- Characteristics: Unicellular, often has a flexible outer covering called a pellicle.

- Habitat: Found predominantly in freshwater environments.

- Examples: *Euglena, Phacus.*

6. Dinoflagellates (Dinophyta):

- Characteristics: Unicellular, typically have two flagella and may possess armor-like plates.

- Habitat: Common in marine environments, but some are found in freshwater.

- Examples: *Karenia, Alexandrium.*

7. Cyanobacteria (Blue-Green Algae):

- Characteristics: Prokaryotic, contain chlorophyll a and phycobiliproteins.

- Habitat: Common in freshwater and marine environments, as well as terrestrial habitats.

- Examples: *Anabaena, Spirulina.*

Current applications of algae

1. Biofuel Production: Microalgae are investigated for their proper usage and as a sustainable source for biofuel production. They can accumulate high amounts of lipids (oils) that can be converted into biodiesel by various biochemical treatments.

2. Nutritional Supplements: Some microalgae, namely spirulina and chlorella, are enriched in proteins, vitamins, and minerals. They are used as nutritional supplements and functional food ingredients.

3. Aquaculture Feed: Microalgae serves as a natural and nutritious feed source for aquaculture, providing essential nutrients for fish and shellfish.

4. Wastewater Treatment: Microalgae are used in wastewater treatment processes to absorb and remove nutrients and pollutants, acting as a natural water purification system.

5. Bioremediation: Some microalgae can help in the bioremediation of contaminated environments by absorbing heavy metals and other pollutants from water and soil.

6. Carbon Capture: Microalgae can capture and convert carbon dioxide (CO2) into biomass through photosynthesis, offering a potential solution for carbon capture and sequestration.

7. Pharmaceuticals and Bioproducts: Microalgae produce a variety of bioactive compounds, some of which have pharmaceutical and biotechnological applications. These include antioxidants, anti-inflammatory agents, and enzymes.

8. Cosmetics: Microalgae extracts are used in cosmetic products for their potential skin benefits, such as moisturization, anti-aging properties, and protection against environmental stress.

9. Research and Education: Microalgae are valuable tools in scientific research and education. They are used in laboratories for studying photosynthesis, algal biology, and various biotechnological applications.

10. Artificial Intelligence and Biotechnology: Advances in biotechnology and artificial intelligence are being used for the cultivation and harvesting of microalgae for various applications.

Applications of Macroalgae and their composition

Out of all the macroalgae species recognised worldwide, just five percent are being used for food and fodder nowadays. The macroalgal biomass is generally described in scientific literature as containing substantial amount of carbohydrates (up to 60%), also indicating medium to high-proteins up to (10–47%), and relatively low lipid contents up to (1-3%), and having a varied mineral content (7–38%). By creating special secondary metabolites including proteins, lipids, polysaccharides, and phenolic substances, macroalgae can adapt according to the swift changes in the marine environment, including variations in sun light and temperature. Carbon dioxide and other free radicals can develop in the biomass of macroalgae exposed to environmental stresses over extended periods of time, such as temperature, sun radiation, and water level variations. Depending upon the macroalgal classification such as green, red, or brown macroalgae, the species, their stage of biomass development and environmental (stressors i.e.) seasonal shifts, can influence their biology and their structural makeup. The structural composition of macroalgae will differ substantially according to these parameters. Outlining these compositional shifts is especially important for researchers aiming to extract valuable chemicals from their biomass as their application in nutraceuticals.[1]

As a method for exploring strain improvement for microalgal production, Adaptive laboratory evolution (ALE) has been applied extensively to produce novel phenotypic and biological amenities. To be more precise, strains have been evolved by ALE to become more suited to certain environments. It has evolved into a fresh approach to enhancing strain performance in microalgae biotechnology applications. To maximise the efficacy of microalgae ALE was developed.It facilitates the treatment of wastewater and helps to raise cell growth rate, product yield, and environmental tolerance. Research is also underway to further develop ALE to provide theoretical support and to produce high-value goods from microalgal production. [2]

Evolution of Algae by endosymbiosis

A key event in the evolution of eukaryotes was the endosymbiotic origin of plastids, or chloroplasts, from cyanobacteria. It contributed to the oxygenation of the biosphere and served as the catalyst for later endosymbiotic occurrences by enabling some eukaryotes to achieve photosynthesis. Through the process of secondary endosymbiosis—the assimilation of a primary plastid-bearing alga by a eukaryotic heterotroph—so-called "primary" plastids have been transferred from one eukaryotic lineage to another on several times.Plasmid biochemistry, molecular biology, and genetics all clearly indicate that they are descended from cyanobacteria. Together with the gene content and genome structure, this also considers their coloration, light-harvesting systems, and protein import apparatus.As a marine and freshwater protist with pellicles—a sequence of proteinaceous strips located beneath the outer membrane—*Euglena gracilis* belongs to the euglenids, a plentiful and well-researched lineage.*Euglenid*cells use their pellicle in conjunction with their flagella to aid in their motility. When viewed under a scanning electron microscope, the pellicle can give the cell strips.Apicomorphae, dinoflagellates, perkinsids, ciliates, and the recently found chromerids are the five sublineages that make up the enormous and complex protist lineage known as alveolates. The term "alveolar" describes the conspicuous cortical alveoli, also known as cavities or sacs, that are located underneath their cell surface.[6]

Bioreactors and cultivation of Algae

Multiple culture techniques, such as batch, staged, and continuous culture, have been effectively employed for artificial intelligence (ALE). Cell densities and nutrient supplies can be continuously maintained in the process conditions through continuous culture. The bioreactor approach can be applied to continuous evolution studies, in a manner like "Bioreactor Batch Cultivations." The idea behind this training is to enable the biomass to expand at a specific rate eternally by introducing new nutrients into the medium at an appropriate pace throughout exponential growth. An environment can be brought to a steady state where the growth rate of the microbial population remains constant. There are, however, several drawbacks as well, like the high cost and challenging management.[2]

Leveraging a specific volume of media in a closed reactor is batch culture and it is a method for cultivating algal strains. The loading of culture medium and the initial bacterial inoculation are the key features of this technique. This approach maintains the culture medium's volume and temperature.An alternative to batch culture is fed-batch culture, in which the substrate is added either constantly or progressively while leaving all the biomass intact. Fed-batch culture has several advantages over standard batch culture, including more productivity, shorter fermentation times, and enough nutrient supply.

It is necessary to consider the feeding strategy since the fed-batch culture operates in a more complex manner. Erythritol was produced by fed-batch culture of Trichosporon sp., yielding great and steady productivity. Batch and fed-batch cultures' gene expression profiles differed sporadically, as shown by general expression patterns, which the fed-batch method is responsible for to boost biomass and lipid output, fed-batch culture has been used extensively.

The method of a two-stage process has been established to overcome the contradiction that criteria for biomass build up are typically different from those for product accumulation. "Chemical modulators based adaptive laboratory evolution" (CM-ALE) is a new ALE approach that utilises this methodology. Applying pressure using acetyl-CoA carboxylase (ACCase) was the first step in increasing the strains' production of lipid and docosahexaenoic acid (DHA) by 50% and 90%, respectively. Next, to boosting cell development and raise fat and DHA output to 100% and130%, respectively, the second stage employed sesamol, which was based on the tensile strength of ACCase. The two-step CM-ALE can boost cell proliferation and desired products simultaneously. For increasing production, the two-stage procedure was thought to be the preferable method with the approach and, pressure selection is essential. Strategies for microalgal production can be derived from an understanding of the connection between the ROS quenching process and carbon metabolism.

When it comes to biotechnological applications, cyanobacteria and thermophilic microalgae have been the subject of several investigations. These include supplements for nutrition (omega-3 and omega-6 polyunsaturated fatty acids, for example), fertilisers, pigment synthesis (carotenoids and phycobiliproteins, for example), and secondary metabolites (exopolysaccharides, vitamins, toxins, and biologically active substances with antiviral, antiseptic, antifungal, and anticancer properties).Additionally, both algae are appropriate for oily wastewater biodegradation and agro-industrial wastewater bioremediation.[3]. Bioreactors called microbial fuel cells (MFCs) convert chemical energy into electrical energy through the action of microorganisms, which function as biocatalysts. An MFC is made up of two basic parts: the anode and cathode chambers are separated by a proton exchange membrane (PEM).

Factors affecting lipid accumulation and their production

Some physical parameters that may affect the quantity and quantity of lipids include P^H, Oxidative stress, Temperature, Light intensity, and Nutrient limitation through all their growth phases. Polar lipids such as (phospholipids and glycolipids) make up a large portion of the lipids in algae cultivated without stress, but TAG level are rather low. Among the most popular and extensively utilised TAGs induction strategies for several phytoplankton species is nutrient deprivation. In both open and closed systems, nitrogen is the most often reported component to boost lipid accumulation in various species. Nitrogen makes up 1–10% of the total dry matter. Each living thing has unique genetic traits that determine whether it will accumulate lipids or carbs in response to nitrogen deprivation. Growth rates and fat content in response to N deficiency. Undertaking a proteomic analysis on a cyanobacterium, it was shown that proteins connected to the synthesis of carbohydrates were upregulated, while breakdown of glycogen was downregulated. Additionally, Carbon fixation pathways are species- specific in microalgae.[4]

Chlorella vulgaris has different amounts of Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Palmitoleic acid, Oleic acid, Linoleic acid, Linolenic acid, γ-linolenic acid, Arachidonic acid, and Eicosapentaenoic acid among its fatty acid composition.[5]

Lipid or biodiesel production from Microalgae

Biodiesel's primary component, fatty acid methyl ester (FAME), is produced through the transesterification of lipids derived from biological sources. The quality of the final product and the method used to produce biodiesel are thus greatly influenced by the content of lipids. Polyphospholipids and glycolipids, which belong to polar lipids and promote emulsification and catalyst depletion, are somewhat undesirable for biodiesel production, whereas TAGs are the ideal compounds. Other lipids that include higher levels of phosphorus and sulphur might decrease the quality of the biodiesel.A temporal profile of growth rate, C:N ratio, and lipid accumulation at different periods is necessary to assess the lipid productivity more precisely in the growth cycle. When a batch culture grows through several stages, changes in its biochemical composition must be carefully monitored by balancing the progressive build-up of metabolites in the medium and the concurrent exhaustion of its nutrients.[4]

Microalgae and biofilm formation

An integrated group of microbes enclosed by an extracellular polymeric substance (EPS) matrix which forms on its own is called a biofilm. The slimy, glue-like substance that makes up this matrix is made up of proteins, polysaccharides, and other organic and inorganic components. Rocks, pipes, medical equipment, biological tissues, and other surfaces can all develop biofilms.In aquatic conditions, microalgae, or microscopic algae, can aid in the development of biofilms. A few phases are usually involved in the biofilm generation process by microalgae:

Attachments: Microalgae use appendages like flagella or pili to connect to surfaces like rocks, sediments, or man-made structures.

Manufacturing of Extracellular Polymeric Substance (EPS): Microalgae release extracellular polymer (EPS) to create an adhesive and protective matrix once they are adhered. This matrix serves as an anchor for the surface for desiccation or predation.

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Microalgae within the biofilm begin to proliferate, resulting in the formation of a denser and more intricate community through cell proliferation.

Maturation: The thicker and betterorganized EPS matrix of the biofilm results in a more robust and stable structure as it ages.
Certain microalgae synchronise the actions of their cells inside the biofilm by use of a communication system called quorum sensing, which involves the release of signalling chemicals.As their surroundings change, the microbes can react as a group.

Commercial uses

Few more applications of algae that are commercially used include: Recycling of paper, Algal Dyes, Algae food thickeners, Algal feed, Algae toothpaste, Cosmetics and Shoe polishes. Brief description about their commercial applications islisted below: -

Algae and paper production:

Algae cell coverings are quite diverse, yet cellulose makes up most of the plant cell walls. Their crusty cell wallstypically consist of calcium carbonate or silica deposits, some algae species contain defensive barriers. Steady sulphated polysaccharides found in marine algae provide structural support for most algae; chitin, cellulose, carrageenan, and alginate are other potential sources. In the industry, all of them are few extremely beneficial products.

Algae as food thickeners and natural colourants:

Due to health and environmental concerns, algae-based dyes and colourants have become more and more significant. Emulsion paints that we apply on our walls include algae derivatives. Glazing clay and varnish polishes also include these compounds. Paper, latex stabilisation, and polishing wax production all depend on the presence of algae and their components.

In addition, a variety of algae-derived pigments can be utilised as natural food colourants in products including orange juice, wasabi, chewing gum, ice sorbets, candies, and soft beverages. Carrageenin is a substance that resembles agar and is derived from red algae is frequently used as a stabiliser in ice cream, paints, and some medications.

Algae as a food thickener:

Derived from the cell walls of red algae, agar is a gelatinous substance having extensive palette of uses. In microbiology, it is used as a culture medium. In the culinary business, it thickens soups, jellies, and candies. In brewing, it serves as a clarifying agent. Paper and cloth sizing are further uses for it. Made from specific red algae, agar is a seaweed product that is used to make soups and jellies, as well as to make cosmetics, drugs and as a culture medium in laboratories.

Algae as feed crops:

Halogens and potash are artificially extracted from marine algae because of their high mineral content. Cattle are with fed either raw or dried marine algae, a practice that is very common in Europe. Choosing it for as an animal feed has grown in prominence. Wide tracts of arable land might be freed up, and at the same time, algae-based feeds could solve food security concerns in a time when the demand for animal proteins is rising. Algae-based feeds have been shown to be as nutritious and digestible as other feedstocks, if not more so. Algae may be grown with significantly less water and land input and is already a very effective feed ingredient for animals, according to research. The production of algae for food could free up millions of acres and currently utilised for feed crops.

The emergence of Algal lineages

History of algae

Algae have an amazing evolutionary history. Throughout the prokaryotic and eukaryotic trees of life, about 350,000 different species of algae have emerged. In the domain Eukaryota, the evolution of certain organelles is intimately linked to the interactions between algae.Organelles that originated in prokaryotes include mitochondria and chloroplasts. The prokaryotic cell was ingested by the eukaryotic host cell in both cases. The eukaryotic host cell eventually became the prokaryotic cell's sole source of support.

Cyanophyta or Blue Green algae

For more than 1.5 billion years, cyanobacteria algae dominated the earth's biological diversity. They produced chlorophyll and other pigments and were the first species to photosynthesize. Chloroplasts in all eukaryotic algae and vascular plants have their evolutionary origins in cyanobacteria.

Cyanobacteria are little and generally unseen. There are two types of them: filamentous and basic, unicellular. Particles like phycocyanin and chlorophyll an are used by most cyanobacteria. They are known by their common name, blue-green algae, due to the pigments that give them this colour.Different pigments, such as the pink-coloured pigment phycoerythrin and orange-coloured pigments known as carotenoids, are present in some cyanobacteria species. Green, blue, purple, brown, or black are some of the colours that cyanobacteria can be. Cyanobacteria *Oscillatoria,* which periodically blooms crimson, is what gives the Red Sea its name. Similarly, cyanobacteria Spirulina gives African flamingos their pink hue.Examples:*Lyngbya* sp.,*Nostoc pruniforme*and *Oscillatoria* sp.

Chlorophyta or Green algae

The green algae are included under the division Chlorophyta. In freshwater and coastline marine environments, most of these species exist. Some 4,000–7,000 different types of green algae are estimated to exist.Examples:*Ulva*sp., *Halimeda*sp.,*Caulerpa lentillifera* (Sea grapes) and *Chlamydomonas nivalis*that thrives in ice giving pinkish tint.

Rhodophyta or Red algae

The red algae or the Rhodophyta are most red algal species, which numbers between 5,000 and 6,000, and are marine. All freshwater red algae species together, nevertheless, number about 150. Macroalgae forms in the intertidal and subtidal zones exhibit a wide variety, spanning from unicellular to multicellular red algae. Calcium carbonate is present in the tissue of certain red algae, which are calcified. Certain calcified structures develop into flat surfaces that encrusts as they enlarge. As a surface for growing new coral, these crusts can aid in stabilising coral reef environments. Due to the photosynthetic pigment phycoerythrin, most red algae have a red or pink colour. Chlorophyll a's green tint is hidden by this crimson pigment.

Due to the photosynthetic pigment phycoerythrin, most red algae display a red or pink colour. Chlorophyll a's green tint is hidden by this crimson pigment. Light in the blue and green wavelengths is effectively absorbed by phycoerythrin. When a large portion of the red light in subtidal environments has been absorbed or reflected by water, this can be advantageous. From a business perspective, red algae are crucial. Red algae are the source of many industrially vital products. Products like agarose, carrageenan, and agar, for instance, are used in many food and pharmaceuticals, including toothpaste, ice cream, and medicines. Red algae is the usual source of these chemicals because it's hard to manufacture industrially.

Example: *Porphyra* sp.

Phaeophyceae or Brown Algae

A class of brown macroalgae called Phaeophyceae has over 2,000 species. Near subtidal and intertidal shorelines, these algae are often noticed. Kelp is a term used to describe the largest species of brown algae.Certain species can reach heights exceeding 150 feet and grow as much as 2 feet a day. Several marine organisms are supported by giant kelp, which grows in gigantic forests along the shore.Pneumatocysts, or buoyant, gas-filled bladders that allow brown algae to grow upright, are a characteristic feature of these peculiar organisms. A particular type of brown alga, *Sargassum natans*, can float on the ocean's surface is by virtue of some tiny, spherical structures known pneumatocysts.Examples: *Sargassum natans*, *Macrocystis pyrifera*, *Padina* sp.

Red algae's endosymbiotic relationship with brown algae is believed by scientists to have led towards its evolution. This has led to the historical inclusion of brown algae in the varied eukaryotic organism group known as stramenopiles. It is likely that the four membranes found on the chloroplasts of stemmopiles originated when the first brown algae ate the red algae.The majority of stramenopiles at some stage in their life cycle also contain two flagella of varying length. Even while brown algae make up the majority of stramenopiles, not all of them are photosynthetic. For instance, stramenopiles are the organisms responsible for both seed and potato rot.

Diatoms

Diatoms are a ubiquitous kind of phytoplankton that are widely distributed in both freshwater and marine environments. Soils and other moist habitats are also home to diatoms. Diatoms can build colonial chains of organisms, even though they are mostly single-celled organisms.With over 100,000 species, 200 genera, and a vast range of sizes and forms, the diatom category is huge. In the cretaceous epoch, some 100 million years ago, diatoms first show up in the fossil record of marine environments. Diatoms' and red algae's chloroplasts are extremely similar. Example *Naviculastes vicensis*

Diatoms are distinct in most aquatic habitats because of their silica cell wall. Sturdy and very resistant to deterioration is the diatom silica wall. *Dictoptera*, which comprise most phytoplankton and carry out up to half of the ocean's photosynthesis, are these algae's primary contributors to the worldwide ocean. Diatoms have the intriguing ability to use the urea cycle to change ammonia into urea. Due to urea's significantly lower tissue toxicity than ammonia, the conversion is crucial for marine life forms. Urea cycle was only associated with animals until scientists revealed that diatoms could convert ammonia to urea. As indicators of the quality of the water in aquatic situations, diatoms are extremely helpful to scientists.

Dinoflagellates

Within freshwater and marine habitats, dinoflagellates are eukaryotic, single-celled creatures. Some marine dinoflagellates are predatory, meaning they devour creatures, but the majority are photosynthesizers, making food out of light. Dinoflagellates are known to exist in over 1,500 species. In reef environments, they can exist as symbionts or as free-living planktonic organisms. When chemical processes occur inside the cell, some dinoflagellates exhibit bioluminescence, which is the emission of light energy. When the boat motor or the sound of breaking waves disturbs these bioluminescent dinoflagellates, they release a blue-green light. By shocking predators and drawing attention to larger predators, they are believed to employ bioluminescence as a defensive tactic.Examples *Symbiodinium.*

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Recent and Emerging trends in Plant pathology and Plant host defense systems

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Introduction

Our ecosystem is made up of innumerable species ranging from microbes to macrobes. They are interdependent on each other in order to create a niche and function in the ecosystem. Plant species area marvelous macrobes which are quite beneficial to many ecosystems especially the soil ecosystem as well as to the animal ecosystem. The interdependence may lead to many beneficial, mutualistic or symbiotic and parasitic associations. These parasites are called plant pathogens. The study of the pathogens, mechanism of attack, effects of the attack on the plants and surrounding biota, the defense mechanisms portrayed by plants to resist the attacks is called plant pathology.

Ensuring the quality of the plant to prevent pathogen attack and disease control is an essential part of horticulture and agricultural ecosystems. This is also important in the forestry to preserve native and rare plant species which have high economic value.

Plant pathology is an emerging as well as established area of study, due to the evolving ecosystem. Apart from natural defense mechanisms, artificially mediated host defense responses are incorporated with the help of ever emerging biological, molecular and bioinformatics techniques. The omics techniques help us understand the plants and microbes more deeply and help us achieve various insights on crop quality enhancement and plant disease control.

The induction and resistance of plant diseases are controlled by both biotic and abiotic factors. While the abiotic factors include climate, temperature atmosphere, soil conditions, etc., the biotic factors include microbes and other living organisms. The external microbiome of the plants also include algal species along with bacteria, fungi, lichens, etc.

Plant microbiomes:

The plant micro biome is formed due to the association of diverse range of microorganism with plant. Fungi, bacteria, virus, protozoa, archaic, lichens, etc. are some of the species of the plant microbioata. They are spread through phyllosphere(ariel plant habitat like stem and leaf surfaces), episphere like on flower and immediate plant surrounding, carposphere (on fruit surfaces), spermosphere on germinated see coatsand rhizosphere (the root microbiota). The plant microbiomes is not only restricted to terrestrial species. The aquatic phyto-microbiomes is essential for the hydrophytes to function.These association is achieved by both entophytic(microbes which live inside plant tissues) and epiphytic (microbes which live on or outside plant tissues) microbes. These microbiota are essential to determine the physiology and development of the phytosystem. It has a huge influence in the ecology and physiology of the plants.

These microbiomes may positive and negative effects on the plants. For example ,phytoparacytic nematodes like cereal cysts like *Heterodera* spp. and Potato cysts like *Globodera* spp.,nematodes like root-knot and lesion which are *Meloidogyne*spp., *Pratylenchus* spp. cause major damage to the crops while free living nematodes like nitrogen fixing bacteria and certain mycorrhizal species help in improving the plant performance by suppressing the parasitic activity in the rhizobiome via inhibiting other pathogen mechanisms and enhancing the stress tolerance and nutritional value of the plants.

The evolution of the plant microbiome:

The improvement in agricultural, horticultural and forestry practices has a brought a major effect on the evolution of the native microbiota. The application of various natural and chemical fertilizers is one of the prime factors for the altering of plant microbiome. The introduction of biological disease control factors like other microbes will also lead to alteration in the natural population. Introduction of cross breading and other agricultural practices, introduction of disease resistant plants, etc. are also the major factors of microbiome evolution.

Plant disease control:

Plant disease control is of various types. They are broadly divided into biological, chemical, physical, cultural and breeding methods. Over the years these techniques has been modified due to the change in the environmental, climatic conditions and other external factors. Control methods should be thought and applied from a multifaceted view of the nature, society, economy, side effects apart from disease control.

Integrated plant disease control includes the comprehensive use of various types of control methods to achieve effective disease control approach. It helps in overcoming the drawbacks of all the methods included and helps in achieving a sustainable way of disease control.

Biological methods – Now and Then:

Biological methods conventionally include the usage of natural or biological things to alleviate the natural defense mechanism or introduce new defense responsethrough resisting plant diseases, enhancing plant immunity, altering the surroundings with the use of chemicals as well as advantageous microbes, etc.These are achieved bynatural antagonists, which alter relationbetween the environment, plants and the pests. These agents as they trigger off a chain off natural, societal and economic factors, they need to be thoroughly evaluated before use.

The biological method is usually preferred because of specific inhibition mechanisms and long term sustainability in the target site thus being ecological friendly and cost effective. It is important to maintain the durability, effectiveness and sustainability of BCAs to be successful.

Biorational products:

In recent times pheromones are being used. Pheromones are chemical substance that an animal releases into the environment (especially an insect or a mammal and that affects the physiology or behavior of its other species) or living microorganisms that are harmless and have minimal ecological influence. For example, pheromones are used as an effective method for killing ants. An effective approach to keep ants away from insecticide is to employ a mix of pheromones and insecticide to block ants' routes and nests. In order to eradicate the pesticide, it thereby increases ant exposure to it while decreasing contact of the insecticide with the environment.

Insect Growth Regulators:

Insect growth regulators are hormones used as insecticides. They prevent the maturation or inhibit the significant metabolism in injected insects. These can be used against pathogens for effective disease management. Usually IGRs are non toxic to other species like humans and plants.

Endophytic modification:

With little impact on the environment, antagonistic endophytes serve as biocontrol agents and present an alluring substitute for the treatment of several plant diseases. Microbial endophytes are a type of endosymbiotic group that inhabits plant tissues asymptomatically and may provide biocontrol. Numerous bioactive metabolites, including phenolic acids, terpenoids, alkaloids, and hormones, have been identified in endophytes, making them a viable option for managing stress caused by pathogens.

Algae in plant disease resistance:

Algal species are both prokaryotic and eukaryotic organisms present in all varieties of habitats across the world ranging from Tundra in arctic to equatorial and temperate regions. They are classified as,

- Micro (less than 50µm) and macroalgae (more than 2 meters) according to their size,
- as Red Algae (rhodophytes), Blue-green algae (cyanobacteria), Brown Algae (phaeophytes), chlorophytes, euglenophytes, charophytes, diatoms, dinoflagellates, and cryptophytes according to their Primary pigments,
- as Marine algae, Land-based algae, and Algae in extreme environments according to their habitats etc.

The algal species are marvelous organisms. They have rich biochemical constituents, secondary metabolites, etc. that have vast applications across sectors like pharmaceutical, medical, engineering, bioremediation, cosmetics, Research and Analysis, electrical, etc. The algal cell in itself is a natural engineering marvel that portrays various pathways and metabolism.

Its role in Plant disease resistance:

The need to find a natural alternative for inducing and maintaining disease resistance in plants is still under analysis and research. Algae has high antimicrobial resistance due to its cell and cell wall composition. The secondary metabolites or bioactive compounds like alkaloids, flavonoids, phenols, proteins, polysaccharides, lipids, etc. are responsible for the antibacterial, anti-viral activities, etc. the use of algae as a natural pesticide or insecticide helps in inhibition of pests as well as helps in replenishing the soil of its fertility as well as helps in achieving a sustainable agricultural practice. The relation between the algae and the other species in the plant micro biome helps in achieving an effective plant disease resistance. The relation is achieved by mutual exchange of algal metabolites and microbial metabolites. The interchange includes siderophores, algal secondary metabolites, extra polymeric substances like proteins, eDNA, lipids, etc. The interchange helps in thriving of plant friendly bacteria and fungi, thus enhancing the disease resistance in plants.

Algal polysaccharides as a plant disease resistance promoter:

Polysaccharides, the complex sugar molecules present in all living organisms have many applications in up regulating the immunity, gene expression and other metabolisms. Polysaccharides and their simpler forms oligosaccharides, have biochemical activities in many living organisms including plants. Algal polysaccharides are said to have deep influence in triggering and enhancing the immune response as well as defense response by triggering the gene markers responsible for pathways like Salicylic acid pathways and Jasmonic acid pathways. These not only enhance the gene expression, but also upregulate the synthesis of fatty acids, sterols and alkaloids. Certain examples of polysaccharides responsible for plant disease resistance are:

Sulphated polysaccharides are predominant algal polysaccharides obtained from Rhodophyceae family. This is responsible for the upregulation of Salicylic and Jasmonic acid pathways, which are sensory pathways eliciting defense responses. They enhanced the regulation of immune genes like, PR1, PDF 1, PR2, PR5, AOS, etc. which express proteins They effectively enhanced the Systamatic and Induced Acquired resistance in plants. Oligopolysaccharides like oligochitin trigger the metabolites which minimize the damage due ROS system and eventually inhibit pathogen attacks.

According to *Roohallah et al.*, Polysaccharides like Xyloglucans, chitins and chitosans, β- Glucans from algae upregulated the production of phenols, phytoalexins like Gossypol, Casbene, Rishitin, Cell wall proteins, etc. Seaweeds like *laminaria*, *ulva*, and microalgae like *Desmodesmus*, species induce the Microbe and pathogen associated molecular patterns to regulate the immune response. *Roohallahet. al.*Algal Polysaccharides are easily degradable as well as strong and also keep the level of plant growth promoting bacteria in good proportion to keep the plants healthy and disease free.

Algal metabolites, Micro and Macro nutrients in plant disease resistance:

The antimicrobial activity of algal species is due to the micro, macro and other biochemical compounds present and synthesized by them. They are rich in Phosphorus, calcium, iron, vitamins A, B, C, and E, folic acid, biotin, beta-carotene, pantothenic acid, and vitamin B12 , etc. which promote plant immunity. Apart from these they are very rich in Nitrogen, phosphorus, potassium, carbon, etc. The algae thus acts as a nutrient source to both the microbes in soil and the plant. This helps in promoting plant friendly microbes and the growth of plants. The secondary metabolites from algae have pesticidal activity.

D-lactic acid is produced by degradation of D-lactate dehydrogenase present in the mitochondria of algal cells, especially in chlorella. D lactic acid results in the synthesis of mitochondrial anti-oxidant enzyme (mtROS) which triggers COX2 and AOX1 genes, which helps the plant to inhibit the Oxidative stress by pathogen.

Cyanobacteria produce certain kinds of toxins like neurotoxins, hepatotoxins, which inhibit the pathogen metabolism by disrupting essential microbial pathways. Nodularin, anoxin-a, are some of the cyanotoxins produced by species like *Nodulariaspumigena*, which cause muscle contractions and cease mobility of pathogens.

Algae and Cyanobacteria as biofertilisers:

Algal and cyanobacterial species are used as external application like biopesticides. Due to their phytochemical constituents like alkaloids, diterpenoids, carotenoids, flavonoids and macro and micro nutrients, they protect the plants, enhance their innate immunity, inhibit pest attack by acting on the microbes as they are present as a coating on the plant surface.

Algal cells contain fungal cell wall degeneration enzymes like benzoic acids, β-1,4glucanase, etc. Cyanobacteria like Stylepta species help to degenerate bacterial metabolism, but also fix nitrogen in the soil. Follicle application, spray application of such algal and cyanobacterial supernatants helps in achieving good pest inhibition.

Botryococcus species, *Isochrysis*sp.,*Phaeodactylum* species, are highly antibacterial in nature.Algal species like *Sargassum*, *Gracillaria*, *Dunellia* sp., *Chlorella* sps., *Hematococcus* sp., *Muriellopsis* sp., are cultivated commercially to be used as biopesticides and biofertilisers. They help maintain the soil pH between 5.5 to 7 and maintain Carbon-nitrogen content in clay and loamy soils.

Algae and Cyanobacteria as Plant metabolism Mimicking systems:

Micro, macroalgae and cyanobacteria produce Plant growth promoting harmones like IAA, IBA, Gibberllins, etc. These are essential for the development of the plant, but also help in developing plant immunity. Algal and cyanobacterial species also produce enzymes which regulate Salicylic Acid ,Jasmonic acid metabolism which are the main plant defense responses. Therefore application of these algal compositions help in enhancing the plant disease resistance as well as help in achieving a sustainable agricultural practises.

Molecular techniques in biological methods:

Use of CRISPR Gene editing:

Key genes that control an insect's fertility and sex determination can be changed using the CRISPR gene editing technique. A novel, efficient control method that can be safe, self-limiting, and scalable for a particular species' genetic population has been developed by researchers thanks to CRISPR technology. A wide range of insect pests and disease vectors could benefit from its development and application. Target species can be peacefully suppressed or even wiped off in the field with this technology.

Sensors or reporter genes:

Plant pathogens, poisons, or nutrients are reported by genes or proteins known as phytosensors. To protect themselves from poisons, infections, and dietary deficits, plants have an inherent, reversible defense system. Reporter genes, like fluorescent proteins, are fused to synthetic inducible plant defense promoters to generate phytosensors. Plants are able todiagnose infectious organisms even at elemental compositionsand exhibit symptoms or flurescence sooner bybinding the sensors to their stress promotors.The ability to detect infections quickly is made possible by the fact that apparent symptoms may not appear for several days or weeks after the infection. Due to the inherent ability of a plant to perceive physical and biological stresses and modify their physiological characteristics through epigenetic modifications, Phytosensors have great potential as a modular, easily reconfigurable biosensor. This kind of sensor can be used for detection in the field on the ground, or it can be utilized more extensively to use image detection technologies and satellite images to observe fields. The creation of phytosensors might make advantage of a broad categories consisting innate plant responses, but the level specificity as well as sensitivity would differ significantly depending on the promoter and element.Natural pigments modified as phytopsensors are salicylic acid, jasmonic acid, etc.

Plant biotechnology in disease control:

Here molecular and biological tools like restriction enzymes, vectors, genes , enzymes, etc are used to manipulate the genes responsible for expression of resistance or defense mechanisms in plants. The introduction of Bt. Cotton in late 1900s was revolutionary. From then the use of biotechnology for this purpose has improved and evolved.

A plant disease resistance consiststhree phases: pathogen identification, transmission of signals and defense reaction.The secondary metabolite and other metabolic byproducts are responsible for the expression of defense mechanism. These are expressed by particular gene regulations. Molecular biology is used to screen these proteins and genes, manipulate the genes and eventually protein expression and thus creating or alleviating new defense resistance against pathogens.

Molecular basis of plant defense mechanism can be modified by studying the quantitative and qualitative defense resistance(QDR) which are expressed by major and minor gene regulations. In order to achieve effective plant disease resistance, mapping of these gene and their modification during pest attack stress is necessary. quantitative trait loci (QTL) mapping and genome-wide association study (GWAS) are recent mapping techniques used for (QDR) mapping studies.

For example, a proper agent with full potential to rectify faulty crop cultivation which is Flavonoid maintaining gene AtMYB12 because, according to Ding et al., its expression in tobacco increased the quantity of the compound while also boosting defenses inhibiting aphids, like *Colletotrichum nicotianae*, *Ralstonia solanacearum* and *Alternaria alternata*.Understanding of signalling molecules or ellicitors like Oligogalaturonides (OGs)which are a representative DAMPs and PAMPs. Some signaling pathways that trigger immune mechanism and signals in plants include Host damage-associated molecular patterns (DAMPs).

RNAi may be a mechanism for RNA guided regulation of organic phenomenon within which double stranded ribonucleic acid (ds RNA) prevents the complementary gene expression and nucleo-tide orders.Use of these RNAi mechanism for tissue specific disease resistance help in battling crucial pathogens.

Chemical methods – Then and Now:

Chemical methods of plant disease resistance includes use of various pesticides like fungicides, insecticids, etc. These contain many chemicals like atrazine, paraquat, methyl bromide, chloropicrin, chlorpyrifos, abamectin, bifenthrin, oxamyl, tefluthrin, lambda-cyhalothrin and diphacinone, etc, which are successful in killing and inhibiting the pestsnecessary to stop large-scale agricultural losses and the spread of diseases, but it has a number of unwanted effects onnontargetplants, animals and environment and ecotoxicity. It can also bioaccumulate. They alter the metabolism of plants and accumulate in the soil and water thus affecting the soil and aquatic ecosystems. Humans have been reported to develop several malignancies, respiratory conditions, and hormonal abnormalities when exposed synthetic insecticides, fungal insecticides, etc. In addition, statistics from the US Food and Drug Administration and the FAO-WHO indicate some persistent organic pollutants (POPs) resistbreak down readily and accumulate on plant products , eventually finding their way into higher food levels such as milk products.Moreover, the majority of chemical control techniques are becoming less effective due to the persistent rise in disease resistance brought on by the use of chemical pesticides. In order to achieve a sustainable plant defense resistance techniques many natural chemical and biological components are isolated and modified to be used as pathogen inhibitors while being non toxic to the plants and environment.

Essential oils as pesticides:

One of the ecofriendly Plant disease management is the application of Essential oil from plants. Essential lipids from plantsexhibitvarying degrees of antimicrobial efficacies against diverse plant fungal or bacterial pathogens, and they effectively decreased the predominant crop illnesses. Predominantly most of the Essential oils show antimicrobial activity, at their specific concentrations. Essential oils to be used as biopesticides require a lot of modification. They may need to be applied more frequently and at higher rates (active component or combination of actives in tiny proportion in the complex mixture). Few procedures, such as chemical processes like molecular inclusion or interfacial polymerization, physicochemical techniques like coacervation and liposome encapsulation, or physical procedures like spray drying, spray chilling, or cooling, crystallization, extrusion, or fluidized-bed coating, have been described for the formulation of essential oils as biopesticides.

Enzymatic approaches to plant disease resistance:

Numerous naturally occurring substances, including anything like outer layer of cell, cell constituents , proteins responsible for mechanisms, are reported to safeguard plants against pest attacks thus offer targeted host resistance against infections, a phenomenon known as "induced resistance." Inorder for the continuation of molecular and cellular interactions before or after cell elicitation due to pest attack, the process contains multiple essentialmolecules like DNA, RNA, etc. During pathogen infection, predominant modifications in the target comprise the synthesis of ROS components, triggering of defense mechanisms consisting the photoactive componds,proteinaceous and non- proteinaceous mechanisms, expression of proteins related to pathogenesis, phytoalexin production, changes in components of external cell layer and polyamines activity, carotenoid accumulation, etc. Therefore, the altered biochemical component concentration in host plants limits the spread of disease.Peroxidase, Phenyl peroxidases, phenylalanine, ammonia lyase, chitinase, and β-1,3-glucanase are specific proteins that induce defense reactions in plants by being harmful to the pathogen.

For example, According to Ashfaq et al. rice genotypes' biochemical profiles, which display varying degrees of resistance to Bipolarisoryzae-caused rice brown spot. Because they have been shown to respond differently in sensitive and resistant genotypes, the concentration of defense enzymes can be used as natural markers for earlier indication of diseases. These include enzymes such as β-1,3-glucanase, polyphenol oxidase (PPO), peroxidase (POD), phenylalanine ammonia-lyase (PAL), and catalase (CAT). Assembling defense-related enzymes at different stages of the disease and with varying degrees of severity helps to understand the defense mechanisms against rice brown spot.

Other chemical approaches include:

Reduced ROS concentrations have been shown to control systemic responses, redox homeostasis, differentiation, stress signaling, and higher plants. On the other hand, increased ROS concentrations damage contents of cell by lipid peroxidation, peptide damage and loss of membrane.The ROS is produced at a tremendous level by cell organelles like mitochondria, peroxisomes, cell wall and plasma membrane..Directly or indirectly, ROS may cause alteration and oxidation of amino acids. Some of direct modifications in organelles are carboxylation, formation of disulphide bond, etc. Additionally, indirect alterations may result from the relation between protein and lipid peroxidase compound. Strongly vulnerable to ROS assault are certain amino acids, including proline, lysine, threonine, arginine, methionine, and cysteine. They have to do with modifications in defensive/antioxidative enzymes like Ascorbate peroxidase (APX), tyrosine ammonia-lyase (TAL), SOD, CAT, PAL, PPO, and secondary metabolites such phenols and condensed tannins, as well as by the use of H_2O_2 and malondialdehyde (MDA). For example according to Torun et.al, 2019 to prevent barley loss due to ROS, SOD provides the primary immunity.

Use of Nanotechnology in plant disease control and management:

Nanotechnology is the study of a broad class of substances made up of materials with at least one dimension smaller than 100 nm that are particulate. Nanoparticles due to their wondrous size have different physiochemical characteristics from their precusors. This is significantly manipulated to achieve various applications across industries. Because to their special characteristics, nanoparticles have been proposed to be appropriate carriers for enhancing crop protection, enabling controlled nutrient transfer, and stabilizing herbicides and fertilizers.The emergence of green nanomolecules, i.e. nanoparticles from natural substances like plant, bacterial and fungal metabolites has alleviated its use as effective and sustainable antimicrobial substance.

Lipososomes and phytosomes are sustainably synthesized nanoparticles made of lipid and other phytocomponds. They are non toxic and easily degradable. They are used as carrier molecules and drug delivery systems in molecular biology. Usage of these lionanosomes to encapsulate natural antimicrobial pigments like terpenoids, tocopherols, alkaloids, flavonoids etc. help in alleviating natural plant defense mechanisms. It has been reported that these nanosomes have helped in wound healing and tissue regeneration in plants. For example, quercetin has high anti microbial activity is elevated by being encapsulated in liposomes.Nbhsp70er-1 and Nbhsp70c-A are Quercetin gene targets for biotic and abiotic stress. The gene and protein activity were alleviated by quercetinnanoliposomes.

Advantages of using nanoparticles in plant disease control:

Nano sized biopesticides can cause inhibition of the infection with small amounts of pesticide quantity. So nanoencapsulated pesticide molecules are preferred for their ability of controlled release of insecticides with specificity.Numerous biological, physicochemical, or electrochemical sensors are used in the fabrication of nanobiosensors, such as enzyme biosensors, Geno sensors, and antibody sensors. The rapid, precise, and sensitive way in which these sensors recognize contaminants and harmful pests has drawn a lot of interest to their application.

Other natural Plant defense regulators :

Extracellular Vesicles as plant defense regulators:

Extracellular vesicles are membrane-enclosed phospholipid bilayer subcellular components that carry a variety of cargos, such as miRNA, mRNA, DNA, proteins, etc. Extracellular vesicles are released by both bacterial and eukaryotic cells. Ectosomes (size: 100–500 nm) and exosomes (size: 30–150 nm) are the two main types of extracellular vesicles. Plant extracellular vesicles, or PELNs, are a type of vesicle that can range in size from 50 to 500 nm. PELNs include a composite mixture of tiny metabolic and organelle collection like RNA, amino acids, etc.in addition to animal vesicles. PELNs from a variety of fruits and plants, including coconut, blueberries, and ginger, have demonstrated anti-inflammatory qualities and antimicrobial components.

Plant exosomes:

In recent times the role of exosomes in plant defense mechanisms is being heavily studied. A pathogen enters a plant host by a convoluted procedure that takes several steps. Numerous molecular interactions arise during the operation as a result of the pathogen's survival strategies and the plant's protection mechanisms. Extracellular vesicles found in plants, particularly exosomes with a diameter of 50–150 nm, are essential to plant defense. They carry bioactive lipids, proteins, RNA, and metabolites between the pathogen and the host as signalosomes. These extracellular vesicles include short RNA, stress response proteins, and antimicrobial chemicals, according to recent studies. According to recent research, extracellular vesicles secreted by pathogens and plants alike are essential for cross-kingdom communication, which controls the host response and boosts plant immunity. Presently, the development of sustainable plant-protection methods requires an in-depth comprehension of the process by which the EVs coordinate this cross-kingdom and inter-species regulation.

For example, RPM1-INTERACTING PROTEIN4 (RIN4), a signal transmission protein that interacts with bacterial endothelial cells to initiate immunization, is one of the proteins included in the proteome. Immune signaling-related proteins are enriched in exosomes, which implies that exosomes may be involved in delivering signals to cells that trigger pathogen identification. The EV proteome identified additional defense-related proteins implicated in the myrosinase-glycosinolate system, including PEN3, NRT1, the myrosinaseepithiospecfic modifier 1 and ROS signaling proteins.Additionally, it was evident that the EVs had an anti-fungal effect since they decreased the spores' viability and also prevented hyphal development in the spores that were germinating.

Summary and Future:

The plant defense mechanisms play a crucial role the survival of plants and the functioning of the ecosystem. The ever evolving community of pathogen microbiota has lead to continuous evolution in the plant defense strategies. But it is important to maintain these stratergies in a sustainable manner for the maintenance of a healthy ecosystem. The use algae and plant exosomes as a suitable plant disease resistance promoter is still under discussion and research. Further developments may include the collaboration of nanotechnology, algal technology, and plant physiology to achieve enhanced results to resist and manipulate the ever evolving microbial world.

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Aflatoxin – An Overview of Human health management.

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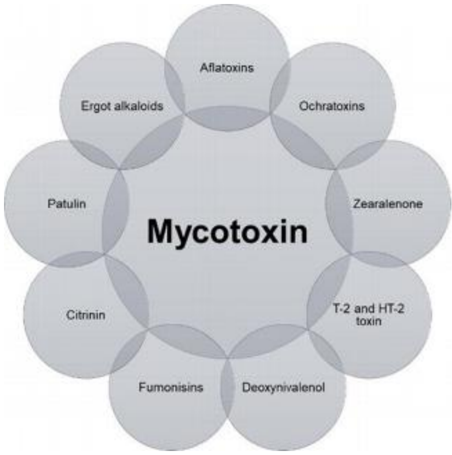
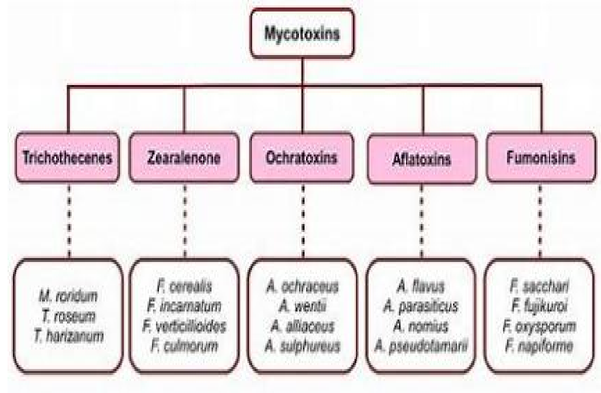
Introduction

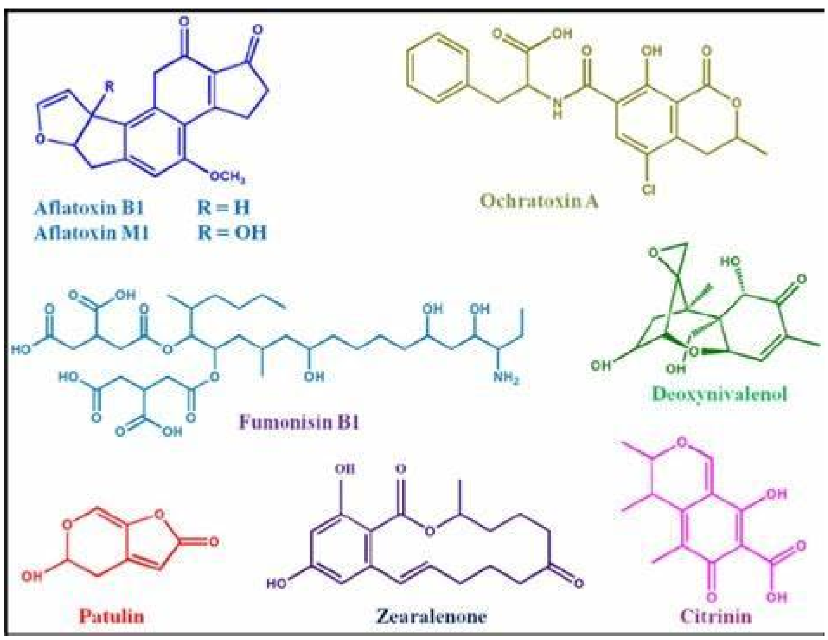
Mycotoxins are secondary metabolites produced by many fungal species and molds. They are produced by various fungal species like *Aspergillus, Penicillium, Fusarium*. The toxicities of these mycotoxins pose a variety of health problems, including carcinogenic, mutagenic, hepatotoxic, nephrotoxic, genotoxic, and/or biotoxicological components. When ingested by humans, mycotoxins have both acute and chronic effects that results in mycotoxicosis. Some of the mycotoxins are Aflatoxin, Patulin, Fumonisins, Ochratoxin A, Deoxynivalenol, Zearalenone (ZEA), Ergot Alkaloids, Citrinin. Owing to their increased frequency and incidence in foods and feeds ingested by humans and animals Aflatoxins, Deoxynivalenol, Fumonisins, Ochratoxin A, and Citrinin are the most hazardous mycotoxins. This chapter focusses on aflatoxin, its toxicity, detection and management.

Aflatoxins are a group of approximately 20 fungal metabolites. They are potent genotoxin produced by various species of fungi such as *Aspergillus niger, Aspergillus flavus, Aspergillus parasiticus*.Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), Aflatoxin G2 (AFG2), Aflatoxin M1 (AFM1), and Aflatoxin M2 (AFM2) are the six primary forms of aflatoxins (Quadri et al. 2012). Of these, M1 (Metabolite of B1) and M2 are found in animal byproducts like dairy products, whereas B1, B2, and G1 are found in food crops or their products.Out of all these Aflatoxin B 1 is a potent carcinogen. A long-term exposure to aflatoxin B1 causes formation of DNA adducts, genetic alterations which further leads to hepatocellular carcinoma.





Structure of Mycotoxins

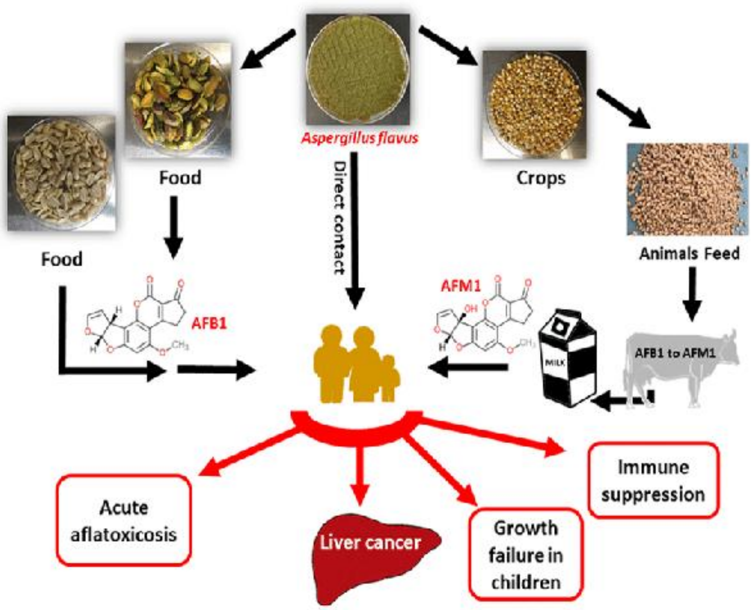


Origin and distribution of aflatoxin

The term "aflatoxin" was derived from *Aspergillus flavus*.After being determined to be the cause of the turkey X disease, it was given the name in 1960. It has also been identified as the cause of cancer in rainbow trout fed peanut and cottonseed meals.(Aayush Dhakal1; Muhammad F. Hashmi2;Evelyn Sbar3 NIH). Additionally, aflatoxin was the cause of many outbreaks in India and other African nations. Fungal toxins are produced at multiple stages of food production including harvesting, processing, and storage.Crops become infected with *Aspergillus species* during growth and development. When products are stored or transported in warm, humid environments or during a prolonged dry spell, contamination may develop.

Crops affected by aflatoxin:

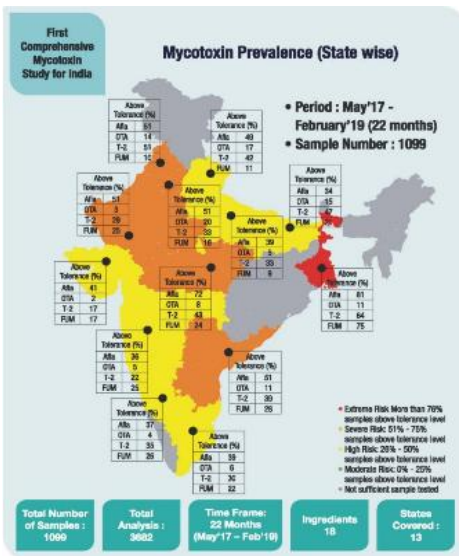
The commonly affected crops by aflatoxin are cassava, cottonseed, chili, pepper, maize, wheat, corn, millet, peanut, rice, sesame, sunflower seed, and many spices.



Aflatoxins are produced in crops by fungi which enters into the crop by various factors like wound, through insects etc. It gets transmitted to humans and animal feed there by causing health issues.

Outbreaks

In 1974, one of the earliest known outbreaks of aflatoxicosis was reported in western India, killing 106 native people who relied primarily on maize for sustenance. In India in 1974, there was another case of toxic hepatitis that affected both humans and canines. Aflatoxin-related mass mortality in chicks was documented in the Chittoor area of Andhra Pradesh in 1982 (Reddy and Chinnam, 2007). There was an outbreak in Tanzania in 2016, which resulted in over 53 illnesses and eight deaths linked to aflatoxin poisoning. This outbreak was caused in part by poorly harvested maize that was prone to mold and drought damage.Aflatoxins are a evident group of mycotoxins. A severe outbreak in 2004, 125 people died in Kenya, and approximately 200 were treated after consuming aflatoxin-contaminated maize [28,34,35]. Aflatoxin's hazardous and carcinogenic properties have led the Food and Drug Administration (FDA) and the US Department of Agriculture (USDA) to impose a 20 ppb limitations presence in food. The United States Food and Drug Administration (FDA) considers it as an unavoidable food contamination.According to the Food Safety and Standards Regulations, 2011, all food commodities in **India** are subject to a maximum limit of 30 µg/kg. The crop's growth, yield, and market value are all impacted by the fungal infestations.



Factors influencing aflatoxin production

There are various factors influencing the growth of fungi which are listed below. A range of physical, chemical, and biological techniques, as well as breeding and genetic engineering approaches, have been employed to reduce the quantity of aflatoxin below the acceptable threshold and minimize its toxicity in crop plants.

Physical factors

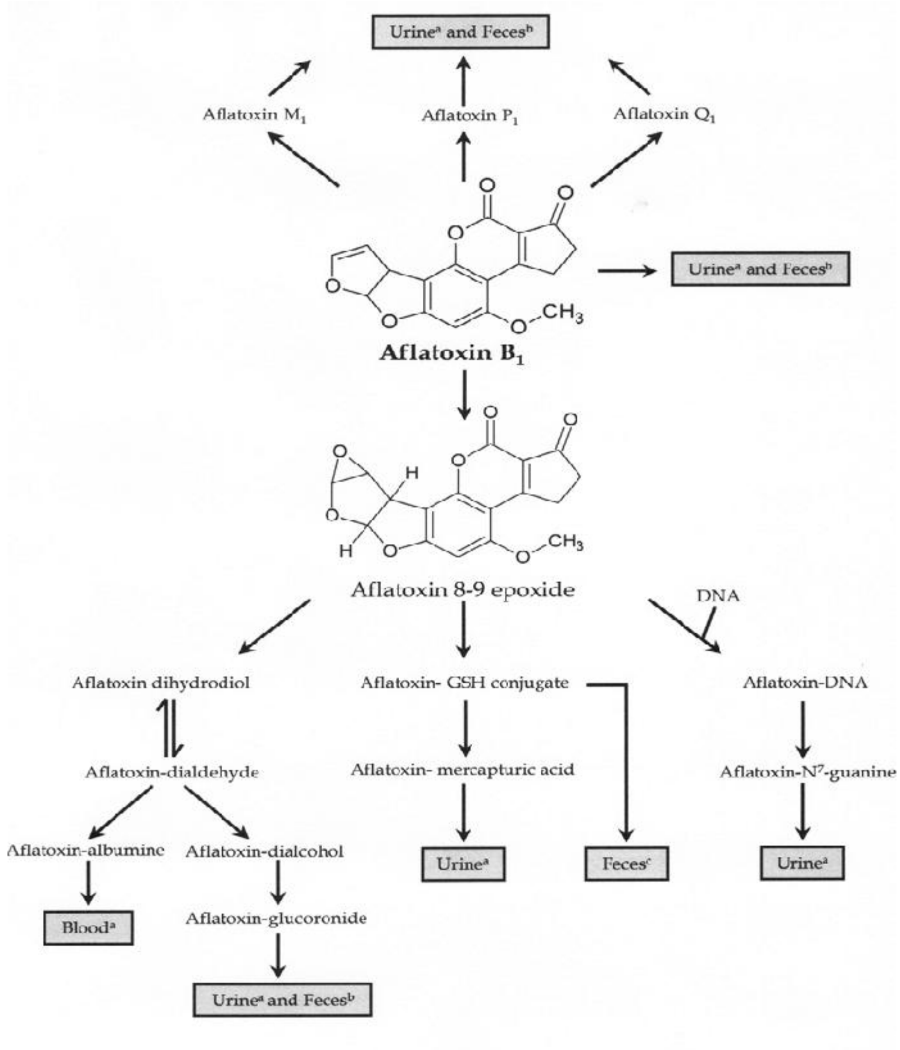
These fungi were also able to enter via wounds caused by nematodes and insects (Jeyaramraja et al. 2018). In addition to impairing crop plants' ability to defend themselves against fungal attacks, the introduction of fungi into crops contaminates crop seeds, which in turn causes the production of aflatoxin. Although molds and fungi that produce aflatoxin can develop in a wide pH range (1.7–9.3), a pH range of 3–7 is optimum (Yoshinari et al. 2010). A slightly higher pH (6) encourages the production of aflatoxins as well as fungal development, while a lower pH (1-3) reduces fungal growth (Eshelli et al. 2015). Although *Aspergillus flavus* can survive and thrive in a wide range of temperatures, from 12 °C to 48 °C, its ideal growing temperature range is between 28 °C to 37°C (Hawkins et al. 2005). Dark conditions and moisture enhance the growth of fungi which in order increases the aflatoxin production.

Biological factors

Weeds, insects, and fungal species are examples of biological factors. Aflatoxin production is linked to plant stress caused by weeds, which primarily flourish as competitors. The primary determinant of aflatoxin formation is the type of fungi present; insects cut into the plant induce stress and provide as a breeding ground for aflatoxigenic fungi (Kinyungu 2019).Because *A. flavus* naturally occurs in soil, pre-harvest contaminations of field crops are prevalent; post-harvest contaminations also occur because *A. flavus* ruins food grains during storage.

Impact of aflatoxin in human health

There are various health effects post exposure to aflatoxin depending upon the stage. The primary or acute state of consuming aspergillus contaminated food leads to aspergillosis. The acute phase leads to aflatoxicosis which further develops in hepatocellular carcinoma. Acute effects appear within 72 hours of exposure, whereas chronic effects develop longer and may last months, years, or even decades.Aflatoxin exposure can produce acute nausea, vomiting, abdominal discomfort, and convulsions, as well as long-term consequences such as hepatotoxicity, immunotoxicity, and teratogenicity.



Aflatoxin is capable of damaging DNA by oxidation or lipid peroxidation, which is therefore carcinogenic (Zhang et al. 2015). Aflatoxin is metabolized by specific P450 enzymes in the liver to form a reactive oxygen species (aflatoxin-8,9-epoxide), which can subsequently bind to proteins to induce acute toxicity (aflatoxicosis) or to DNA to cause lesions that over time increase the risk of HCC (Groopman et al 1988).Several carcinogenic effects are caused by the chemical AFB1 8,9-epoxide (Denissenko et al. 1999). Aflatoxin is carcinogenic, but it also damages the kidney, heart, liver, testicles, and brain.

Detection

The detection of aflatoxin done by following techniques. The preliminary method involves culture-based techniques where different medias are used to cultivate the toxigenic fungi followed by molecular techniques.

- Polymerase chain reaction (PCR)
- Thin-layer chromatography (TLC)
- High-performance liquid chromatography (HPLC)
- Liquid chromatography-mass spectroscopy (LCMS)
- Enzyme-linked immune-sorbent assay (ELISA)

The PCR approach is mostly utilized to detect Aflatoxins-producing fungi *A. flavus* at the molecular level (Tao et al. 2018). As a result, genes identified during molecular screening of *A. flavus* that are important for Aflatoxin production were employed as a target gene to identify Aflatoxin using multiplex PCR.

AFB1-lysine, a metabolite of AFB1, can be used in ELISA testing to measure aflatoxin levels in patient blood sample. HPLC-fluorescence has been used to determine the amount of residual aflatoxin and its metabolites, AFM1, AFB1, and AFQ1, in urine (Tang et al., 2008; Polychronaki et al., 2008; Romero et al., 2010). However, AFB1, AFB2, AFG1, AFG2, AFM1 have recently been successfully determined in urine using HPLC-MS/MS (Everley et al., 2007).

The levels of aflatoxin can be determined using several methods. Measuring the AFB-guanine adduct in patients' urine is one approach.Only recent exposure during the last 24 hours is measured using this method. This substance has a variable half-life, so depending on diet, its level may fluctuate over time. As such, it is not the best method for evaluating prolonged exposure. Another technique to identify prolonged aflatoxin exposure is to measure the quantity of AFB-albumin adduct in the blood serum.

Prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), albumin, bilirubin, aspartate transaminase, and other tests are also used to monitor liver function.The biosensors enable quick and rapid detection of aflatoxins with lower costs, minimal sample, portability, and on-the-spot identification using an electrochemical enzyme-linked oligonucleotide array (Rotariu et al. 2016; Selvolini et al. 2019).The use of aptamer-based molecular assays and the notable use of nanoparticles in biosensors are recent developments in the detection of aflatoxins.

Control strategies for aflatoxin contamination

Aflatoxin infection in vegetation prompted a severe hazard to production, market, health, and economics. Several ways have been developed to minimize aflatoxin contamination in crops, including physical, chemical, and biological strategies.

Physical methods

Physical methods, inclusive of steam below pressure, dry roasting, and different cooking methods, are determined to be powerful withinside the manipulate or to lessen the aflatoxin infection in lots of crops (Peng et al. 2018). Heating the seed samples at 180 °C also resulted in a 40–73% reduction in aflatoxin levels (Opoku 2013). When groundnut and corn seed was roasted with 30% moisture at 100 °C temperature for 2 hours, there is an 85% reduction in aflatoxin (Leong et al. 2010).

AFB1, AFB2, and AFM1 aflatoxins are also degraded by UV irradiation (220–400 nm), with a degradation potential ranging from 77 to 99.12% (Diao et al. 2015). As a result, gamma radiation exposure was also found to reduce the aflatoxin level (Aquino 2011).

Chemical methods

According to Udomkun et al. (2017), a number of substances, including acids, alkalis, oxidizing agents, aldehydes, and various gases, have also been shown to reduce the growth of aflatoxigenic fungus and the generation of aflatoxin when employed in the proper amounts. According to research, ozone is the gas that most effectively maximizes the breakdown of aflatoxin on grains and legumes through an electrophilic attack on the carbon bonds in the furan ring (Jalili 2016). Ozone treatment is less useful for post-harvest crop treatment, nevertheless, because of its high cost. The ammonization procedure in corn and other food commodities can also prevent the development of aflatoxin (Karlovsky et al. 2016).High-pressure ammonization (0.25, 0.5, 1.5, and 2%) reduces the amount of time needed to lower the formation of aflatoxin in a number of crops and food commodities, which is a benefit of the ammonization method (Temba et al. 2016). Aflatoxin in numerous food items is also significantly reduced by a number of substances, including sodium bisulfite, calcium hydroxide, formaldehyde, sodium hypochlorite, sodium borate, and sorbents (Carvajal & Castillo 2009).

Management

A wide range of experts, including farmers, agriculture engineers, food technicians, veterinary physicians, and other health professionals, are involved in the management and mitigation of aflatoxin toxicity. One of the most important steps in reducing aflatoxin's negative effects on health is to protect food from it. Health practitioners ought to be informed about local food practices and the prevalence of aflatoxin poisoning. Mycotoxin contamination of food grains can be prevented by following good farming techniques, storing food properly, as well as maintaining hygiene practices.

The majority of aflatoxin poisoning patients manifest at a late stage following persistent exposure. In developing countries, aflatoxin poisoning is a primary cause of hepatocellular cancer and liver cirrhosis.The financial consequences resulting from animal and crop losses caused by aflatoxin contamination are highly significant. Economic effects result from the expense of the regulatory procedures intended to lower risks to the health of people and animals.

Conclusion

A major cause of disease outbreaks globally, aflatoxins are consumed in contaminated food and feed due to a lack of awareness about them. Aflatoxin levels in non-industrialized food that are excessive are a serious problem. A number of efficient methods involving physical, chemical, biological, and genetic engineering have been used to reduce, manage, and effectively regulate aflatoxins in food. To counter pre-harvest infections and their consequences, it is crucial to produce hybrids and crops that are resistant to fungi and insects. Farmers rarely use post-harvest processes such alkalization, ammonization, heat, or gamma radiation to eliminate aflatoxins. On the other hand, a few naturally occurring soil bacteria can break down and lessen the aflatoxin contamination in many agricultural products. Many researches are focusing on early detection of aflatoxin using biosensors and other molecular methods. Methods using microorganisms to eradicate aflatoxins are also carried out in huge numbers. Therefore, aflatoxin being a carcinogenic compound its early detection and management needs to be focused.

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Biopesticides and their role in sustainable agriculture

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Abstract

Biopesticides are naturally occurring compounds or agents that are obtained from animals, plants, and microorganisms such as bacteria, cyanobacteria, microalgae, plant and animal sources and are used to control agricultural pests and pathogens. According to the US Environmental Protection Agency, 'biopesticides are derived from natural materials such as animals, plants, bacteria, and certain minerals'. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides. As of August 31, 2020, there were 390 registered biopesticide active ingredients. The use of biopesticides is, by far, more advantageous than the use of their counterparts, traditional chemical pesticides, as they are eco-friendly and host-specific. The use and application of agro-based chemicals in the agricultural sector to protect crop plants from invading and infecting pests can be greatly improved by employing biopesticides

Introduction

Agricultural development has a long history in many places around the world. The agricultural practice began about 10,000 years ago in the Fertile Crescent of Mesopotamia, corresponding roughly to most of today's Iraq, Turkey, Syria, and Jordan. People who lived in these areas collected edible seeds through means such as fire-stick farming, and forest gardening. When the population became more settled and lived on farms, large amounts of wheat, barley, peas, lentils, chickpeas, bitter vetch, and flax were cultivated. Rice and sorghum were farmed in the Sahel region of Africa about 7500 years ago. Furthermore, indicates that some local crops were also domesticated independently in West Africa as well as in New Guinea and Ethiopia about 7500 years ago. Rice and millet were domesticated in China. America independently domesticated corn, squashes, potato, and sunflowers

The history of agriculture in India dates back to the neolothic. India ranks second worldwide in farm outputs. As per Indian economic survey 2022-23, agriculture employed more than 58% of the Indian work force and contributed 18.3% to country's GDP

India ranks first in the world with highest net cropped area followed by US and China and India ranks second globally in agriculture production. The economic contribution of agriculture to India's GDP is steadily declining with the country's broad-based economic growth. Still, agriculture is demographically the broadest economic sector and plays a significant role in the overall socio-economic fabric of India.

India making it the ninth largest agricultural exporter worldwide and the sixth largest net exporter. Most of its agriculture exports serve developing and least developed nations. Indian agricultural/horticultural and processed foods are exported to more than 120 countries, primarily to the Japan, Southeast Asia, SAARC countries, the European Union and the United States.

As per the 2022 FAO world agriculture statistics India is the world's largest producer of many fresh fruits like banana, mango, guava, papaya, lemon and vegetables like chickpea, okra and milk, major spices like chili pepper, ginger, fibrous crops such as jute, staples such as millets and castor oil seed. India is the second largest producer of wheat and rice, and the world's major food staples. India is ranked under the world's five largest producers of over 80% of agricultural produce items, including many cash crops such as coffee and cotton, in 2022.

"Slow agricultural growth is a concern for policymakers as some two-thirds of India's people depend on rural employment for a living. Current agricultural practices are neither economically nor environmentally sustainable and India's yields for many agricultural commodities are low. Poorly maintained irrigation systems and almost universal lack of good extension services are among the factors responsible. Farmers' access to markets is hampered by poor roads, rudimentary market infrastructure, and excessive regulation."

One of the biggest issues facing the agricultural sector in India is low yield: India’s farm yield is 30-50% lower than that of developed nations. Average farm size, poor infrastructure, low use of farm technologies and best farming techniques, decrease of soil fertility due to over fertilization and sustained pesticide use, are leading contributors to low agricultural productivity. Indian farms are small (70% are less than 1 hectare, the national average is less than 2 hectares) and therefore have limited access to resources such as financial services, credit (or lenders), support expertise, educational services or irrigation solutions. In the short-term yield directly impacts a farmer's cash flow and the ability to respond to fluctuations in the market. Long-term yield limits a farmer's ability to invest into their farm's future to increase productivity and decrease risks associated with their crops (via inputs such as seeds, fertilizer, crop insurance, market/weather info, livestock health support, etc.) but also to invest into their families in areas such as education, healthcare, training,etc.

Challenges of Indian Agriculture

Instability: Agriculture in India is largely depending on monsoon. As a result, production of food-grains fluctuates year after year. A year of abundant output of cereals is often followed by a year of acute shortage.

Cropping Pattern: The crops that are grown in India are divided into two broad categories: food crops and non-food crops. While the former comprise food-grains, sugarcane and other beverages, the latter includes different kinds of fibres and oilseeds.

Land Ownership: Although the ownership of agricultural land in India is fairly widely distributed, there is some degree of concentration of land holding. Inequality in land distribution is also due to the fact that there are frequent changes in land ownership in India. It is believed that large parcels of land in India are owned by a- relatively small section of the rich farmers, landlords and money-lenders, while the vast majority of farmers own very little amount of land, or no land at all.

Sub-Division and Fragmentation of Holding: Due to the growth of population and breakdown of the joint family system, there has occurred continuous sub-division of agricultural land into smaller and smaller plots. At times small farmers are forced to sell a portion of their land to repay their debt. This creates further sub-division of land.

Land Tenure: The land tenure system of India is also far from perfect. In the pre-independence period, most tenants suffered from insecurity of tenancy. They could be evicted at any time. However, various steps have been taken after independence to provide security of tenancy.

Conditions of Agricultural Labourers: The conditions of most agricultural labourers in India are far from satisfactory. There is also the problem of surplus labour or disguised unemployment. This pushes the wage rates below the subsistence levels.

Manures, Fertilizers, and Biocides: Indian soils have been used for growing crops for thousands of years without caring much for replenishing. This has led to depletion and exhaustion of soils resulting in their low productivity. The average yields of almost all the crops are among the lowest in the world. This is a serious problem which can be solved by using more manures and fertilizers.

Irrigation: Although India is the second largest irrigated country of the world after China, only one-third of the cropped area is under irrigation. Irrigation is the most important agricultural input in a tropical monsoon country like India, where rainfall is uncertain, unreliable and erratic India cannot achieve sustained progress in agriculture unless and until more than half of the cropped area is brought under assured irrigation.

Lack of mechanization: In spite of the large-scale mechanization of agriculture in some parts of the country, most of the agricultural operations in larger parts are carried on by human hand using simple and conventional tools and implements like wooden plough, sickle, etc. Little or no use of machines is made in ploughing, sowing, irrigating, thinning and pruning, weeding, harvesting threshing and transporting the crops.

Agricultural Marketing: Agricultural marketing still continues to be in a bad shape in rural India. In the absence of sound marketing facilities, the farmers have to depend upon local traders and middlemen for the disposal of their farm produce which is sold at throw-away price.

Inadequate transport: One of the main handicaps with Indian agriculture is the lack of cheap and efficient means of transportation. Even at present there are lakhs of villages which are not well connected with main roads or with market centres.

Historical Perspectives of Pesticide Usage

The history of pesticide use can be divided into three periods of time.

During the first period before the 1870s, pests were controlled by using various natural compounds. The first recorded use of insecticides was about 4500 years ago by Sumerians. They used sulfur compounds to control insects and mites. About 3200 years ago, the Chinese used mercury and arsenical compounds to control body lice. There was no chemical industry, so all products used were derived directly from readily available animal, plant, or mineral sources. For example, volatile substances were often applied by "smoking". The principle was to burn straw, chaff, hedge clippings, crabs, fish, dung, or other animal products, so that the smoke, preferably malodorous, could spread throughout the orchard, crop, or vineyard. It was generally assumed that such smoke would eliminate blight or mildew. Smoke was also used against insects. People controlled weeds mainly by hand weeding, while various chemical methods were also reported. Pyrethrum is obtained from the dried flowers of the chrysanthemum CINERARIA FOLIUM, "pyrethrum daisies", and has been used as an insecticide for over 2000 years.

During the second period, between 1870 and 1945, people began to use inorganic synthetic materials. At the end of the 1800s, people in Sweden used copper and sulfur compounds against fungal attack in fruit and potatoes. Since then, people have been using many inorganic chemicals, including the Bordeaux mixture, based on copper sulfate and lime arsenic, as pesticides, and they are still being used to prevent numerous fungal diseases.

The third period started after 1945, represented by the use of synthetic pesticides with the discovery of the effects of Dichlorodiphenyltrichloroethane (DDT), β-Hexachlorocyclohexane (BHC), aldrin, dieldrin, endrin, chlordane, parathion, captan, and 2,4-D. The disadvantages of many of these products were at their high rates of application, lack of selectivity, and high toxicity. For example, DDT was widely used all over the world since it had low toxicity to mammals, and it reduced insect-borne diseases, such as malaria, yellow fever, and typhus. The book "Silent Spring" indicated the negative impacts of pesticides on the environment and human health. The book aroused great attention among scholars and the public. DDT was banned in 1972 in the US because of its harm to non-target plants and animals, as well as problems with its significant ability to accumulate in tissues and persist, causing long-term damage. Between the 1970s and 1990s, new families of chemicals, such as triazolopyrimidine, triketone and isoxazole herbicides, strobilurin and azolone fungicides, chloronicotinyl, spinosyn, fiprole diacylhydrazine, and organophosphate insecticides, have been introduced to the market and most of the new chemicals can be used in grams rather than kilograms per hectare.

In modern agriculture, scholars are trying to develop genetically engineered crops designed to produce their own insecticides or exhibit resistance to broad-spectrum herbicide products or pests. This new pest management could reduce chemical use and its negative impacts on the environment.

Types of Pesticide in Use

Pesticides are classified by different classification terms such as chemical classes, functional groups, modes of action, and toxicity.

Firstly, pesticides are classified by different targets of pests, including fungicides, insecticides, herbicides, and rodenticides.

For example, fungicides are used to kill fungi, insecticides are used to kill insects, while herbicides are used to kill weeds.

In terms of chemical classes, pesticides are classified into organic and inorganic ingredients.

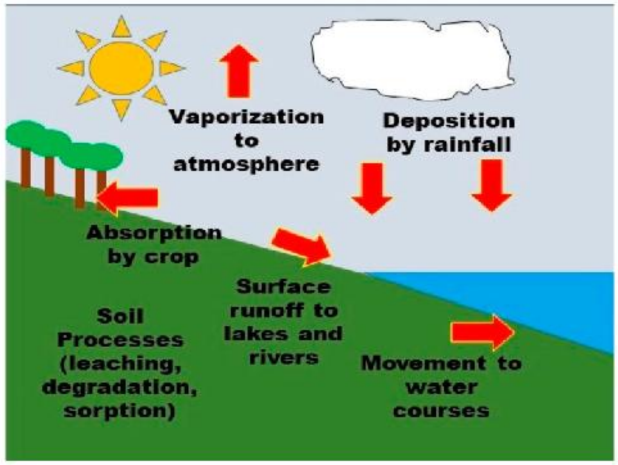
Inorganic pesticides include copper sulfate, ferrous sulfate, copper, lime, and sulfur. The ingredients of organic pesticides are more complicated.

Organic pesticides can be classified according to their chemical structure, such as

Chlorohydrocarbon insecticides, Organophosphorus insecticides, Carbamate insecticides, Synthetic Pyrethroid insecticides, Metabolite and Hormone analog herbicides, Synthetic urea herbicides, Triazine herbicides, Benzimidazole nematocides, Metaldehyde molluscicides, Metal phosphide rodenticides, and D group vitamin-based rodenticides.

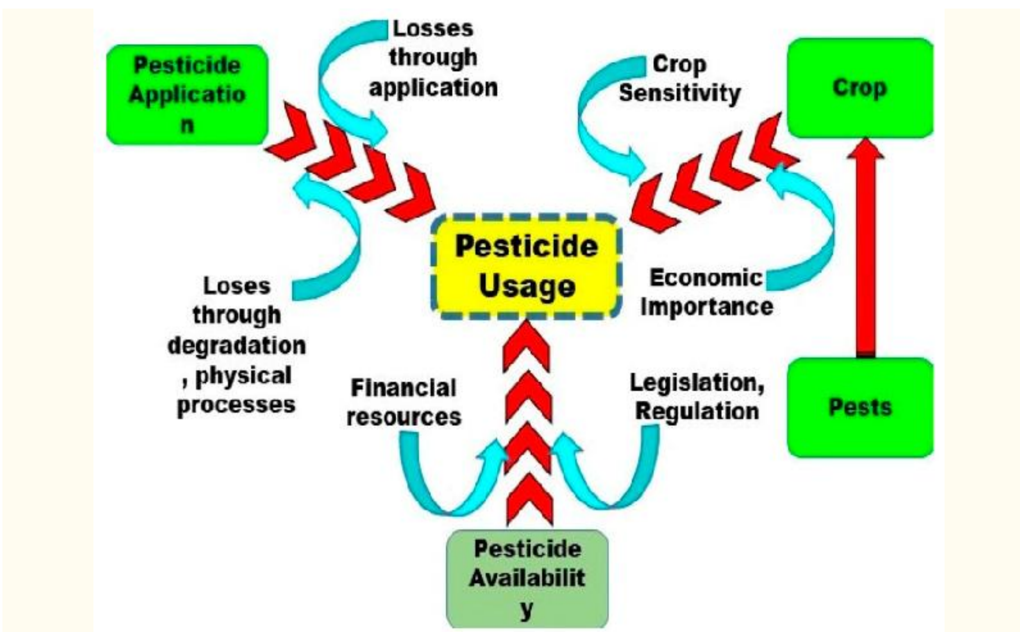
Pesticide Behavior in the Environment

When pesticides are applied to a target plant or disposed of, they have the potential to enter the environment. On entering the environment, pesticides can undergo processes such as transfer (or movement) and degradation. Pesticide degradation in the environment produces new chemicals. Pesticides relocate from the target site to other environmental media or non-target plants by transfer processes including adsorption, leaching, volatilization, spray drift, and runoff. The different types of chemicals indicate their differences in environmental behavior. For example, organochlorine compounds such as DDT have low acute toxicity but show a significant ability to accumulate in tissues and persist in causing long-term damage. They have been banned from sale in most countries, but their residues remain in the environment for a long time because of their nature. While organophosphate pesticides are of low persistence, they have appreciable acute toxicity in mammals.



Impact of Climate Change-Related Factors on Pesticide Use

The use of synthetic pesticides has increased rapidly since World War II (1939–1945) to prevent, mitigate, or destroy pests, reduce agricultural production losses, and improve affordable yields and the quality of food. Pesticide application is influenced by many factors, such as socioeconomic factors, environmental factors including soil condition, crop growth, and the occurrence of insect pests, weeds and diseases, and pesticide behavior in the environment. These factors are mostly influenced by climate change.



Limitations and Challenges in the Use of Conventional Pesticides

There are harmful effects associated with the use of synthetic pesticides such as toxicity and poisoning. Synthetic pesticides also lead to environmental pollution due to the unbiodegradable nature of their constituent compounds. According to Parliman, degradation of metham sodium and other fumigants was reported to last up to over six months after application. In a report by PAN, metham sodium pollutes the air and soil thereby affecting the population of natural enemies in the soil. Methyl bromide has been banned from agricultural use due to its negative impact on the environment. It is associated with depletion of ozone layer which contributes significantly to climate change. The constituent compounds of chemical pesticides contaminate soils rendering them unsuitable for crop production. They also pollute surface and ground water, killing aqua life after inhalation and consumption. Use of dichloro diphenyl trichloroethane (DDT) for instance led to poisoning of birds, marine species and humans. It has been reported to have carcinogenic properties leading to its ban from agricultural use.

Continuous use of synthetic pesticides leads to development of resistant plant pathogen strains leading to their resurgence. Farmers apply more chemicals in an effort to eradicate such pests. In the process of managing target pests, synthetic pesticides kill non-target beneficial organisms such as pollinators, predators and antagonists thereby disrupting biodiversity. After application, the active compounds of the synthetic pesticides are taken up and retained by crops. Consumption of such crops poses chronic health problems to humans due to the accumulated toxic chemical residues. Exposure to pesticides adversely affects the human population, directly or indirectly. For example, pesticides containing Malathion and Trichlorfon have been reported to cause reproductive complications in humans. Exposure to some pesticides have also been reported to retard growth, induce chemical and structural changes in body organs as well as disturb immune responses. They also reduce resistance of animals to disease-causing pathogen infections. Continuous exposure to pesticides such as chloropyrifos cause gene mutations, genetic damages, reproductive health problems and chronic diseases such as asthma, hypertension and cancer.

In a study by Xavier et al., application of Fenpyroximate on chilli peppers (Capsicum annum L) resulted in retention of its residues even after sun drying and processing. Similarly, spinosad (spinosyn A and spinosyn D), Indoxacarb and Deltamethrin containing insecticides used to control

Rhizoctonia dominica, Sitophilus oryzae and Trogoderma granarium were found to be persistent for up to 120 days after application.

The horticulture sector in many developing countries has been particularly adversely affected by the use of synthetic pesticides. The European Union (EU) set out strict regulations regarding levels of pesticide residues and safety of agricultural produce exported to their markets. The use of pesticides containing Dimethoate on vegetables was banned by EU. Failure to comply with this regulation led to rejection and destruction of fresh vegetable consignments containing chemical residues above the required limits. Residues of the restricted chemicals should not exceed 0.02 parts per million (ppm) in a sample of vegetables. The percentage of inspection was increased to 10% on fresh produce at ports of entry into the European Union. According to European Commission, Maximum Residue Levels (MRLs) of unknown pesticides should not exceed 0.01mg/Kg and there was imposed a 10% sampling per consignment in fresh beans and pods. Interceptions of fresh produce almost ruined Kenya's export market reputation due to presence of traces of banned pesticides. Following the guidelines made by the EU and the losses incurred due to rejection and destruction of fresh vegetable consignments, there was a reduction in volumes of horticultural exports. This negatively affected the livelihoods of small holder farmers who are the major producers of vegetable crops. This led to introduction of a cloud-based traceability system which uses a quick reference (QR) code and GPS coordinates to pinpoint the individual farmer whose consignment fails to comply with regulations. This has resulted in increase of the cost of production and several farmers opted out of the export business.

Biofertilizers and Biopesticides are alternative for chemical fertilizers and pesticides

The use of chemical pesticides and fertilisers in Indian agriculture has seen a sharp increase in recent years and in some areas has reached alarming levels with grave implications for human health, the ecosystem and ground water. It is therefore urgent that environmentally friendly methods of improving soil fertility, pests and disease control are used. Biofertilisers are considered as an important alternative source of plant nutrition. Biopesticides and Biofertilizers have emerged as a potential environment friendly input that are supplemented for proper plant growth. They hold vast potential in meeting plant nutrient requirements while minimizing the use of chemical fertilizers. These, bioinputs on supply to plants improve their growth and yield. A biofertilizer is an organic product containing a specific micro-organism in concentrated form which is derived either from the plant roots or from the soil of root zone. Due to heavy usage of chemical fertilizers and harmful pesticides on the crops, sustainability of the agriculture systems collapsed, cost of cultivation soared at a high rate, income of farmers stagnated, food security and safety became a scary challenge. Indiscriminate and imbalanced use of chemical fertilizers, especially urea along with chemical pesticides and unavailability of organic manures has led to considerable reduction in soil health. Biopesticides are target specific and does not leave harmful residues. They are the living organisms that can destroy agricultural pests. The adequate use of Bioinputs helps in restoring soil health and thus provides a cost-effective way to manage crop yield along with balancing the environment.

Biopesticides

Biopesticides are naturally occurring compounds or agents that are obtained from animals, plants, and microorganisms such as bacteria, cyanobacteria, microalgae, plant and animal sources and are used to control agricultural pests and pathogens. According to the US Environmental Protection Agency, 'biopesticides are derived from natural materials such as animals, plants, bacteria and certain minerals'. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides. As of August 31, 2020, there were 390 registered biopesticide active ingredients.

Classes of Biopesticides

Biopesticides fall into three major classes:

1. Biochemical pesticides

Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances that interfere with mating, such as insect sex pheromones, as well as various scented plant extracts that attract insect pests to traps. Because it is sometimes difficult to determine whether a substance meets the criteria for classification as a biochemical pesticide, EPA has established a special committee to make such decisions.

2. Microbial pesticides

Microbial pesticides consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest[s]. For example, there are fungi that control certain weeds and other fungi that kill specific insects.

The most widely used microbial pesticides are subspecies and strains of Bacillus thuringiensis, or Bt. Each strain of this bacterium produces a different mix of proteins and specifically kills one or a few related species of insect larvae. While some Bt ingredients control moth larvae found on plants, other Bt ingredients are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular Bt produces a protein that can bind to a larval gut receptor, thereby causing the insect larvae to starve.

3. Plant-Incorporated-Protectants

Plant-Incorporated-Protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the Bt pesticidal protein and introduce the gene into the plant's own genetic material. Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest. The protein and its genetic material, but not the plant itself, are regulated by EPA.

Advantages of using biopesticides

Biopesticides are usually inherently less toxic than conventional pesticides.

Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects and mammals.

Biopesticides often are effective in very small quantities and often decompose quickly, resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.

When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly reduce the use of conventional pesticides, while crop yields remain high.

Biopesticides in Sustainable Agricultural Production

Biopesticides are as effective as synthetic pesticides in management of crop pests. Natural products are also eco-friendly since they are easily biodegradable and therefore do not pollute the environment. Consumer tastes and preferences fluctuate over time and following the demand for organically produced food, this makes biopesticides suitable alternatives to synthetic pesticides. Biopesticides have very short pre-harvest intervals and are therefore safe to use on fresh fruits and vegetables. They are also target specific and hence do not affect the beneficial organisms such as the natural enemies. They are effective in small quantities and their use promotes sustainable pest management and hence contribute towards sustainable agriculture.

Natural pesticides do not cause resistance build up among pests. Availability of their source materials makes them inexpensive to attain since they are found within the natural environment and some of them are used for other purposes like food and feed. Biopesticides are safe products both for the applicant and the consumer since they have no toxicity. Therefore, biopesticides can suitably be incorporated in integrated pest management (IPM) which helps reduce the amounts of chemical pesticides used in management of crop pests. Natural products decompose quickly which makes them safer for use in the environment. Pesticides from natural sources have very short re-entry intervals which guarantee safety for the applicant. Biopesticides are also used in decontamination of agricultural soils through introduction of important microbial species.

Types of Biopesticides

There are many types of biopesticides, and they are classified according to their extraction sources and the type of molecule/compound used for their preparation. The categories are listed below.

Microbial Pesticides

These are derived from microorganisms including bacteria, fungi, and viruses. The active molecules/compounds isolated from these organisms attack specific pest species or entomopathogenic nematodes. Those known as bioinsecticides, target insects that harm crops, while those that control weeds via microorganisms, such as fungi are referred to as bioherbicides. Over the last decade, extensive research activities on microbial biopesticides have led to the discovery and development of a good number of biopesticides and have paved the way for their marketability. The successful use of BACILLUS THURINGIENSIS (Bt) and some other microbial species led to the discovery of many new microbial species and strains, and their valuable toxins and virulence factors that could be a boon for the biopesticide industry, and some of these have been translated into commercial products as well. Major groups of bacterial entomopathogens include species of Pseudomonas, Yersinia, Chromobacterium, etc., while fungi comprise species of Beauveria, Metarhizium, Verticillium, Lecanicillium, Hirsutella, Paecilomyces, etc. Other important microbial pesticide producers are baculoviruses that are species specific and their infectivity is associated with the crystalline occlusion bodies that are active against chewing insects (Lepidopteran caterpillars). The baculoviral occlusion body is basically a virion that is combined with the Bt toxin to produce recombinant baculovirus (ColorBtrus), producing occlusion bodies that incorporate the Bt insecticidal Cry1Ac toxin protein for enhancing the speed of action and pathogenicity with respect to its wild-type counterpart. Entomopathogenic nematodes (EPNs) used as biocontrol agents belong primarily to species in the genera Heterorhabditis and Steinernema, associated with mutualistic symbiotic bacteria of the genera Photorhabdus and Xenorhabdus and are safe to mammals, environment, and nontarget organisms. Their commercial development as biocontrol agents has been convenient because of their ease in mass production, using in vivo or in vitro techniques, and exemption from registration.

Biochemical Pesticides

Biochemical pesticides are naturally occurring products that are used to control pests through nontoxic mechanisms, whereas chemical pesticides use synthetic molecules that directly kill pests. Biochemical pesticides are further classified into different types depending upon whether they function in controlling infestations of insect pests by exploiting pheromones (semiochemicals), plant extracts/oils, or natural insect growth regulators.

A. Insect Pheromones

These are chemicals produced by insects which are mimicked for use in controlling insects in integrated pest management programs. These chemicals are effective in disrupting insect mating to prevent the success of mating, thus reducing the number of insect progeny. The insects exploited in this process act as dispensers of pheromones that become confused due to the presence of pheromone flumes diffused in the surroundings. Insect pheromones are not true ‘insecticides’ since they do not kill insects but influence their olfactory system to affect behavior. In summary, the antennae of the perceiving insect adsorb pheromones, which then diffuse into the interior of the sensilla through microscopic pores in the cuticle. Once inside, these are transferred through the hydrophilic sensillum to the chemosensory membranes by pheromone-binding proteins (PBPs). Subsequently, the pheromone or pheromone–PBP complex interacts with a specific receptor protein, which transduces the chemical signal into an amplified electric signal by a second messenger system connected with neuronal machinery.

B. Plant-Based Extracts and Essential Oils

Over the last several years, plant-based extracts and essential oils have emerged as attractive alternatives to synthetic insecticides for insect pest management. These insecticides are naturally occurring insecticides as they are derived from plants and contain a range of bioactive chemicals. Depending on physiological characteristics of insect species as well as the type of plant, plant extracts and essential oils (EOs) exhibit a wide range of action against insects: they can act as repellents, attractants, or antifeedants; they also may inhibit respiration, hamper the identification of host plants by insects, inhibit oviposition and decrease adult emergence by ovicidal and larvicidal effects. Their composition varies greatly. Well-known examples in this regard are neem and lemongrass oil, which are very common in global herbal markets. A comprehensive study by Halder et al. showed that a combination of neem oil with entomopathogenic microorganisms, including *Beauveria bassiana*, was very successful against vegetable sucking pests. However, it is very important to determine the dose of azadirachtin content in neem oil so as not to kill the nontarget organisms. A similar strategy has to be established for the entomopathogenic fungi that need to be supported by complementary laboratory bioassays, station, and/or field experiments for effective management of the target pests without affecting nontarget insects. As regards the marketability of essential oils, they in fact, represent a market estimated at USD 700.00 million and a total world production of 45,000 tons, and industries in the US are able to bring essential oil-based pesticides to market in a shortened time period, as compared to the time taken in conventional pesticide launch.

C. Insect Growth Regulators

Insect growth regulators (IGRs) inhibit certain fundamental processes required for the survival of insects, thereby killing them. Furthermore, these compounds are highly selective and less toxic to nontarget organisms. Depending on the mode of action, IGRs had been recently grouped in chitin synthesis inhibitors (CSIs) and substances that interfere with the action of insect hormones (i.e., juvenile hormone analogues and ecdysteroids). IGRs can control many types of insects including fleas, cockroaches, and mosquitos even though they are not so fatal for adult insects. Although low in toxicity to humans, they prevent reproduction, egg-hatch, and molting from one stage to the next in the young insects, while mixing them with other insecticides can kill even the adult insects.

Biopesticides from Algal and Cyanobacterial Sources

Microalgae can be used as an alternative technology to increase productivity in sustainable agricultural systems. Several microalgae strains produce biologically active compounds that include antimicrobial compounds with the potential to act as biopesticides. The biomass (extracts) can be applied as an alternative to chemical pesticides, since it can enhance plant growth and protect crops. The filamentous cyanobacterium NOSTOC PISCINALE and two single-celled green algae, CHLAMYDOPODIUM FUSIFORME and CHLORELLA VULGARIS are reported to have biopesticide activity against certain pathogens. Some important microalgae have been exploited for their beneficial biopesticide activity in the cultivation of spices.

Biopesticide Activity from RNAi-Based Treatments

RNA interference technology is being used in the production of biopesticides due to the increased sensitivity towards pests and pathogens. Many transgenic crops (maize, soybean, and cotton) have been developed for resistance against particular pests. Due to the limited consumption of genetically modified crops, RNA interference (RNAi) can be used as an alternative to overcome this problem. Studies carried out by Ratcliff et al. and Ruiz et al. demonstrated that transgenes had a significant impact on the functioning of plants upon viral infection through an RNAi mechanism. Similarly, Wang et al. produced a barley crop completely resistant to barley yellow dwarf virus.

The mechanism of RNAi includes the expression of transgene dsRNA, which induces virus resistance and gene silencing in plants. Guide RNAs are formed as intermediaries; these are around 25 nt long and guide target RNAs for their degradation. Dalmay et al. reported that the process involves the use of RNA-dependent RNA polymerase RDR6 to generate double-stranded RNA (dsRNA) from target transcripts in plants, leading to the formation of small interfering RNA (siRNA) which, in turn, has silencing potential. The RNase III domain-containing enzyme responsible for dsRNA cleavage, as observed in DROSOPHILA, is called Dicer (also seen in plants and fungi). Following this, RNA-induced silencing complex (RISC)—a member of the conserved Argonaute family—is recruited, which mediates the cleavage of the target transcript, thus conferring resistance to the host.

RNAi technology has been used as a promising tool to overcome the ill effects of pests and pathogens. An RNAi method for oral application was developed by Baum et al. using an artificial diet or transgenic maize against western corn rootworm (DIABROTICA VIRGIFERA) to target V-ATPase subunits and alpha-tubulin. Similarly, research conducted by Mao et al. showed the induction of growth defects in HELICOVERPA ARMIGERA, the cotton bollworm, when given plant leaf material expressing a dsRNA specific to a cytochrome P450 gene. The first commercial, genetically modified variety showing the expression of dsRNA against an insect pest was developed in 2017 when Monsanto and Dow approved SmartStax PRO maize containing dsRNA against the western corn rootworm Snf7 gene. Similarly, apple and potato expressing dsRNAs were approved for regulation of endogenous gene expression for quality enhancement. Apart from insects and viruses, the mechanism of RNAi-mediated silencing has been used to control other plant pests and pathogens, including bacteria such as AGROBACTERIUM, fungi such as powdery mildew, and root-knot nematodes. The US environmental protection agency (EPA) approved the first PIP called SmartStax Pro in June 2017 that will help US farmers control corn rootworm, a devastating corn pest that has developed resistance to several other pesticides.

Nanobiopesticides

The concept of ‘nano’ in biopesticides has revolutionized the field due to the size, structure, and nature of substances, which are formed in a size range of 1–100 nm. These small biologically active particles can prevent the growth of pathogens by either destroying or repelling them. Nanoencapsulation, nanocontainers, and nanocages, because of their property of degradability, increase the stability and efficacy of pest control, and lower amounts are used when delivering nanobiopesticides. The damages caused by the phytopathogens can also be overcome by the application of nanobiopesticides, primarily the metallic nanoparticles (NPs) of zinc, gold, silver, nickel, and titanium owing to their inherent antimicrobial properties. These have some added advantages over other biopesticides because of their increased solubilisation abilities and target-oriented delivery of the compound with enhanced efficiency. Bacterial, fungal, and plant extracts are used for the synthesis of NPs. It has been shown that silver nanobiopesticides (AgNPs) can be synthesised using marine organisms such as *Sargassum muticum*, *Mesocyclops longisetus*, and *Caulerpa scalpelliformis*. The benefit of the use of microorganisms in the preparations of NPs is that microorganisms can withstand high concentrations of metals over plants and also their rate of production and management is much easier, as compared to the plants. Needless to stress here that microorganisms being very tiny, have better

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penetration ability than plants. Narware et al. have mentioned a number of microorganism-derived NPs that are very useful in pest control.

Bioherbicides have also been used in the formulations of nanobioherbicides. The efficacy of metabolites of PHOTORHABDUS LUMINESCENCE, an endosymbiotic bacterium of the HETERORHABDITIS INDICA, entomopathogenic and parasitic nematodes, are controlled. Similarly, nanofungicides have also been prepared to control various pathogenic fungi which include BIPOLARIS SOROKINIANA, FUSARIUM sp., ALTERNARIA ALTERNATA, and many others through AgNPs and MAGNAPORTHE GRISEA and B. SOROKINIANA using metal nanoparticles. Apart from their ability of being readily soluble, the nanofungicides are very economical, eco-friendly, and safe.

Biopesticides from Aquatic Plants

Duckweed (*Lemna minor*), muskgrass (*Chara* spp.), water hyacinth (*Eichhornia crassipes*), hydrilla (*Hydrilla verticillate*), water lettuce (*Pistia stratiotes*), and filamentous algae (*Lyngbya wollei*) are some common aquatic plants. It is observed that some plants produce allelopathic compounds which have the potential to prevent the growth, germination, survival, and reproduction of surrounding organisms. Neem (*Azadirachta indica*) extract kills many insects, while *Eichhornia crassipes* has the ability to inhibit the growth of *Spodoptera litura*, a lepidopteran pest. Similarly, *Chenopodium album* is inhibited by the presence of duckweed and water lettuce. These examples illustrate that similar plants (or weeds) and their allelopathic chemicals have highly potent inhibitory properties against the pathogens and hence can be substituted for conventional chemical pesticides.

Merits of Biopesticides over Chemical Pesticides

Biopesticides have several merits over conventional chemical pesticides. They are environmentally friendly, target specific, and not deleterious to nontarget organisms and hence potent enough to replace synthetic pesticides for pest management provides an overview of the disadvantages of using conventional chemical pesticides instead of biopesticides.

In recent years, the use of biopesticides is gaining momentum because they can be efficiently used in sustainable agricultural practices. Biopesticides are highly effective in small amounts and decompose quickly without leaving problematic residues and hence can reduce the use of conventional pesticides as an integral component of IPM programs. However, despite the merits of using biopesticides, their use has not been as widespread as expected, for the following reasons:

1. High cost of pesticide production due to the costs involved in screening, developing, and getting regulatory clearance for new biological agents;
2. Short shelf life due to the sensitivity of biopesticides to fluctuations in temperature and humidity;
3. Limited field efficacy due to climatic/regional variations in temperature, humidity, soil conditions, etc.;
4. Due to the high specificity of the biopesticides, i.e., they are only effective against target pathogens and pests, farmers are disinterested in them. They need to use multiple biological agents to control different pathogens and pests in the field. These agents are confusing, costly, and cumbersome, and are also not available for every pest or pathogen.

Limitations Facing Use of Biopesticides

While biopesticides provide such advantages as safe environment and healthy food for human consumption, there are factors that limit their full adoption as pest and disease management options. High doses of the constituent compounds are needed for efficacy under field conditions. The concentration of the bioactive compounds in plants is dictated by the environment under which they grow. The constituent active compounds are also dictated by the diversity of plants and their varieties resulting in differences in the responses to pathogens. The quality of botanical extracts is also dependednt on the method of extraction used. During formulation, it is sometimes challenging to get the right proportions of the active and inert ingredients needed. There are also no standard preparation methods and guidelines for efficacy testing especially under field conditions. While the in vitro tests produce excellent results, there are always inconsistencies at the field due to low shelf life and sometimes poor quality of source materials or preparation methods.

Adoption of biopesticides of predatory nature need a lot of consideration such as host crops and dispersal capability. Crop coverage and exposure time are essential and for a small acreage this could prove expensive since application may be manual. Registration of the products requires data on chemistry, toxicity, packaging and formulation which is not always readily available. The cost of producing a new pesticide product is usually high and has a lot of resource limitations. Lack of a readily available market makes it hard to invest in biopesticides. There are insufficient facilities and capital for production of biopesticides especially in the slowly developing countries. The shelf life of natural products is dependent on many factors such as temeperatures and moisture which are sometimes difficult to control. Biopesticides also face high competition from synthetic pesticides and if the former were produced for a small agricultural activity, the costs may be relatively high and therefore not feasible. There is insufficient awareness about biopesticides especially among the small-scale growers, stake holders and policy makers. In the case of microbial pesticides, there is usually no trust in the value and use chain between producers, buyers and users and considering the risk of importation, synthetic pesticides appear reliable.

Conclusion

Biopesticides emerge as indispensable allies in fostering sustainable agriculture. Their origin from natural sources, minimal environmental impact, and compatibility with integrated pest management practices make them pivotal for maintaining ecological balance. By reducing reliance on synthetic alternatives, biopesticides contribute significantly to sustainable farming, safeguarding both crop health and the long-term well-being of our ecosystems. Embracing these eco-friendly solutions represents a crucial step towards a resilient and environmentally conscious agricultural future.

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Endophytic Microbes: Current Scenario and Future aspects in Agriculture and Environmental Sustainability

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1. Introduction

Living in a variety of habitats, plants interact with a wide range of microorganisms. The notion of "holobiont" was developed as a result of the understanding that bacteria and plants work together as a dynamic entity to provide an integrated response to environmental challenges (Vandenkoornhuyse *et al*., 2015).One of the most significant species that can form advantageous relationships with plants are microorganisms.These bacteria can reside inside their host plant or outside of it. According to Compant*et al*. (2010), bacteria that residing in the soil around plant roots, are classified as rhizospheric, or dwelling on the leaf surfaces of the plants are epiphytic. Endophytic bacteria, on the other hand, are bacteria that reside and flourish inside their host plant.All known plant species contain endophytes, which can live in a variety of plant compartments, including the leaf, stem, root, kernel, and flower (Reinhold-Hurek and Hurek, 2011; Hardoim *et al*., 2015).

Plant growth promoting rhizobacteria (PGPR) are a subclass of rhizospheric bacteria that include endophytic bacteria. Actually, these are a unique class of rhizobacteria that have developed the capacity to infiltrate their plant host. They have all the essential characteristics that rhizobacteria use to promote the growth of host plants. Nonetheless, many rhizospheric bacteria typically do not have the same beneficial impacts on host plants as do endophytic bacteria (Chanway *et al*., 2000; Hardoim *et al*., 2008). The majority of plant endophytes are bacteria and fungi; however, they can also be algae, protozoa, nematodes, and archaebacteria. Despite their rarity, these organisms have a major impact on plants (Agler *et al*., 2016; Berendsen *et al*., 2018).

Based on their biology, genetics, and modes of transmission, endophytes can be divided into two groups: systemic/true endophytes and non-systemic/transient endophytes.Systemic endophytes are vertically transmitted by seeds, frequently exhibit mutualism, and colonise the plant's intercellular spaces in a systemic fashion. On the other hand, a taxonomically varied group of microorganisms known as non-systemic endophytes do not colonise hosts systemically and do not spread vertically (Wani *et al*., 2015).This new area of study on endophytes may offer both a platform to exploit the benefits offered by endophytes and unique systems for studying the interactions between plants and endophytes. The use of endophytes and their secondary metabolites in biomedicine, biotechnology, and other sectors is still in its infancy, despite early successes demonstrating the benefits of endophytes in agriculture.

2. Overview of bacterial and fungal endophytes

2.1. Bacterial Endophytes

According to Ibą́nez *et al*. (2017), bacterial endophytes are extremely varied and polyphyletic in nature. They are more densely arranged in the roots and can be segregated from both the above and below ground portions of the plant. According to reports, the rhizosphere is the most frequent site of bacterial endophyte colonisation (Papik *et al*., 2020). A study by Hardoim*et al*. used high-throughput 16S rRNA curated data to demonstrate a wide variety of endophytic bacteria. Only four phyla—54% Proteobacteria, 20% Actinobacteria, 16% Firmicutes, and 6% Bacteroidetes—accounted for 96% of the variety among the 21 bacterial phyla found in this study (Hardoim *et al*., 2015).*Pseudomonas*, *Bacillus*, *Burkholderia*, *Stenotrophomonas*, *Micrococcus*, *Pantoea*, and*Microbacterium* are the most frequently found bacterial genera (Romero *et al*., 2014; Papik *et al*., 2020). The most significant class of bacterial endophytes in terms of practical uses is represented by endophytic Actinobacteria, which are predominantly found in roots, somewhat in stems, and infrequently in leaves (Ganapathy and Natesan, 2018).

2.2. Fungal Endophytes:

Fungal endophytes are a significant part of the endophytic community associated with plants. According to Nisa *et al*. (2015), the phyla Ascomycota, Basidiomycota, and Mucoromycota comprise the majority of endophytic fungi that have been documented. Many plants, including rice, maize, wheat, tomato, chick pea, and pigeon pea, have been shown to have a diverse and abundant population of endophytic fungus that can withstand harsh temperatures, droughts, pH levels, salinities, and other environmental conditions (Rana *et al*., 2019). Based on factors such as taxonomy, host range, transmission, and plant fitness benefits, fungi endophytes can be broadly categorised into two groups, which are then further split into four classes: clavicipitaceous (Class I) and non-clavicipitaceous (Class II, III, and IV). (Rodriguez *et al*., 2009).

Prime example of intriguing and significant interactions with plants are the clavicipitaceous endophytes of grasses (Pérez *et al*., 2020). Specifically, the USA, Australia, and New Zealand have successfully generated and commercialised Epichlo¨e sp. (Class I) endophytes for agricultural use (Hume *et al*., 2016; Kauppinen *et al*., 2016). Despite the fact that clavicipitaceous endophytes have been the subject of a great deal of study, their particular biologywhich includes their obligatory nature and co-evolution with their host plantsmakes it impossible to draw generalisations about them from other endophyte families (Wang *et al*., 2020).

3. Mechanisms of Host plant growth promotion

Endophytic bacteria have been shown to impart several beneficial effects on their plant host directly or indirectly. Direct benefits to plants include helping them absorb nutrients and enhancing their growth by regulating growth-related hormones, which can make plants grow more robustly in both normal and stressful environments (Ma *et al*., 2016). Endophytic bacteria indirectly promote plant growth by inhibiting phytopathogens through the production of antibiotics and lytic enzymes, depriving the pathogens of nutrients, enhancing plant defence mechanisms, and shielding the plants from subsequent pathogen attacks **(Figure 1)** (Miliute *et al*., 2015).

3.1. Phosphate Solubilization

Since phosphorous is the second most important nutrient after nitrogen, phosphate solubilization is a crucial feature of endophytes that promotes plant growth (Mehta *et al*., 2019). In the soil, phosphorus is found naturally as the mineral's apatite, flourapatite, and hydroxyapatite. But the plants cannot use them because of their insolubility (Mahanty*et al*., 2017). Endophytic microorganisms use mechanisms like chelation and exchange to solubilize both organic and inorganic forms of phosphorus, reducing pH and causing acidification through the release of organic acids. According to Delvasto*et al*. (2008), the primary process involved is the production of organic acids such lactic and acetic acid, which chelate the cations linked to phosphate and ultimately transform insoluble phosphorous into soluble form.Phosphatases are secreted by certain endophytic bacteria, which solubilize organic phosphorus by mineralization. Phosphate solubilizers have been identified as bacterial endophytes belonging to the genera Rhizobium, Bacillus, Klebsiella, Pseudomonas, Pantoea, Methylobacterium, Acinetobacter, Microccocus, and Burkholderia (Rana *et al*., 2020).

3.2. Siderophore production

Iron is a crucial microelement needed for healthy plant growth. By generating siderophores, which are water soluble, low molecular weight iron chelators with a strong affinity for the Fe3+ ion and are involved in preserving iron homeostasis, endophytes aid in the uptake of iron (Ahmed and Holmstrom, 2014). Endophytes generate the hydroxamate and catecholate types of siderophores, which have a variety of functions include biocontrollingphytopathogens by restricting the pathogens' ability to absorb iron, lowering the toxicity of heavy metals, and inducing induced systemic resistance (ISR) (Aznar *et al*., 2015). Siderophores have been reported to be produced by endophytic bacteria, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pantoea ananatis,* and *Pantoea agglomerans* (Yang *et al*., 2008). There have also been reports of siderophores being produced by Bacillus and Streptomyces bacterial endophytes. An endophytic fungus, *Piriformosporaindica*was reported to be involved in iron scavenging by producing siderophores (Bajaj *et al*., 2018).

3.3. Phytohormone production and modulation:

Endophytic bacteria can produce growth-regulating phytohormones, which can improve the host plant's metabolism and nutrient uptake.Endophytic colonisation increased plant biomass and nutrient intake, according to recent research exploring the potential involvement of plant hormones secreted by endophytic bacteria (Shi *et al*., 2014). Plant hormones can be broadly classified into five categories: abscisic acid, cytokinins, ethylene, gibberellins, and indole-3-acetic acid (IAA). Of these, ethylene and IAA are the most significant hormones in plant-bacteria. Furthermore, fungal endophytes such as *Piriformospora indica*, *Pestalotiopsis aff. neglecta*, *Aspergillus fumigatus*TS1 and *Fusarium proliferatum* BRL1, also produce IAA, GA and/or cytokinins (Hoffman *et al*., 2013; Khan *et al*., 2015). Last but not least, ethylene is a plant hormone that increases under stress

3.4. Induced systematic resistance:

According to Bastías *et al*. (2018), plant hormones coordinate immune responses that are highly targeted and efficient against a variety of infections. In order to invade plants and evade the host's defence mechanism, endophytes are known to induce systemic resistance (ISR) in plants, making them resistant to diseases (Pieterse *et al*., 2014). In contrast to infections or chemicals that cause SAR (systemic acquired resistance), microorganisms typically cause ISR in plants by triggering the ethylene and/or jasmonic acid signalling pathways. In particular, according to Kloepper and Ryu (2006), bacterial endophytes are thought to be the most effective ISR inducers in plants. The bacterial components that cause ISR include antibiotics, siderophores, flagella, salicylic acid, jasmonic acid, N-acylhomoserine lactones, volatile chemicals, and lipopolysaccharides.Endophytic Bacillus and Pseudomonas species are the common ISR inducers. For example, *Burkholderia phytofirmans* PsJN provided resistance against *Botrytis cinerea* in grapevine by inducing ISR (Miotto-Vilanova *et al*., 2016).

3.5. ACC deaminase production

An essential plant hormone that regulates how a plant reacts to both biotic and abiotic stressors is ethylene. According to Sun *et al*. (2016), it has the ability to regulate a variety of physiological and developmental processes, including fruit ripening, root initiation, leaf senescence, root nodulation, abscission, cell elongation, and auxin transport. Plants exposed to biotic and abiotic stressors produce more ethylene, which inhibits the growth of lateral roots, root hair creation, and root elongation.1-Aminocyclopropane-1-carboxylate (ACC) deaminase is an enzyme produced by endophytic bacteria that has the ability to hydrolyze ACC, a precursor to the plant hormone. According to Sun *et al*. (2009), bacteria that break down ACC can attach to plant roots, cleave the ACC that is secreted into α-ketobutyrate and ammonia, and utilise it as a source of nitrogen. Accordingly, plant stress can be reduced by ACC hydrolysis, which will enhance plant growth in stressful environments (Santoyo *et al*., 2016). ACC deaminase activity has been observed in several plant growth-promoting endophytic bacteria (Rashid *et al*., 2012).

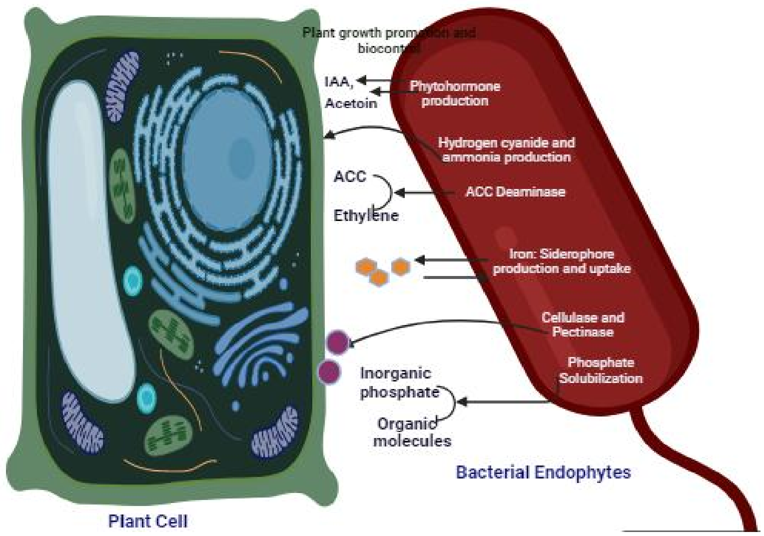

Figure 1: Mechanism of Plant Growth Promotion by Endophytic Microbes

3.6. Biocontrol agents

In order to protect crops from phytopathogens, endophytic microorganisms have been used as biocontrol agents (Ahmed *et al*., 2020). Endophytes have been shown to have the ability to suppress disease or significantly lessen the intensity of disease symptoms. It has been demonstrated that bacterial endophytes use several strategies to inhibit phytopathogens.The development of induced systemic resistance (ISR) (Kloepper and Ryu, 2006), competition for nutrients and niches with phytopathogens (niche exclusion), antibiosis (Berg and Hallmann, 2006), siderophore-mediated competition for Fe3+ (Rajkumar *et al*., 2010), and predation and parasitism (P&P) (Chernin and Chet, 2002) are among the mechanisms that are employed.

The biocontrol characteristics displayed by fungal endophytes may be attributed to many factors such as the release of chitinolytic enzymes, competition for nutrients and space, mycoparasitism, and the synthesis of inhibitory chemicals.According to Kari Dolatabadi*et al*. (2012), and others, *Piriformospora indica*, *Sebacina vermifera*, and *Trichoderma* species aid in the biocontrol of phytopathogens like *Gaeumannomyces graminis*, which causes take-all disease of wheat, *Fusarium oxysporum*, which causes Fusarium wilt of lentils, and *Rhizoctoniasolani*, a powerful phytopathogen. *Sarocladium zeae*, a systemic wheat endophyte, was linked to biocontrol action against the Fusarium head blight disease (Kemp *et al*., 2020). A number of methods for screening novel biocontrol drugs that exhibit direct or indirect biocontrol activity have recently been developed (Raymaekers *et al*., 2020).

3.7. Protection against abiotic stress

By shielding the host plants from abiotic challenges including salinity, dryness, and high temperatures, endophytes can also offer a unique line of defence. One well-known method of plant stress alleviation induced by bacterial endophytes is the synthesis of the enzyme 1-aminocyclopropane-1-carboxylase deaminase, which decreases the ethylene levels in plants (Glick *et al*., 1998). *Burkholderia phytofirmans* strain PsJN, for instance, has been shown to improve grapevine cold tolerance and reduce drought stress in maize and wheat (Barka *et al*., 2006). It has been demonstrated that Oryza sativa L. cv. KDML105 exhibits salt resistance due to the production of 1-aminocyclopropane-1-carboxylate deaminase (ACCD) by Streptomyces sp (Jaemsaeng *et al*., 2018).While *Piriformospora indica* was able to induce salt tolerance in barley (Baltruschat *et al*., 2008) and drought tolerance in Chinese cabbage plants (Sun *et al*., 2010), fungal endophytes like *Neotyphodium spp*. and *Trichoderma spp*. have been shown to increase drought tolerance in grass and cocoa plants (Bae *et al*., 2009).

4. Endophytic microbes: a new source of bioactive compounds

The extranutritional components known as "bioactive" or "biologically active" substances are found in trace amounts in lipid-rich meals and plant-based products (Cammack *et al*., 2006). Actinomycetes, bacteria, and endophytic fungi are important producers of bioactive substances **(Figure 2)**. Bioactive compounds like alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols and phenols, and the natural insecticide azadirachtin produced by endophytic bacteria have agricultural, industrial and medical applications. Plants produce bioactive chemicals that have robust physiological actions. Nevertheless, their output is plagued by a number of issues, such as uneven quality and low productivity. Under carefully regulated environmental conditions, microorganisms produce primary and secondary metabolites with excellent quality and uniformity at maximum efficiency (Sato and Kumagai, 2013).

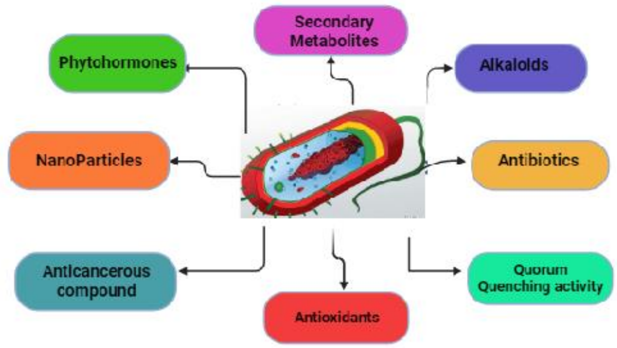

Figure 2: Bioactive compounds synthesized by endophytic microbes

Compared to plants alone, the combination of plants and endophytic microbes has been shown to be a source of materials and products with significant therapeutic potential.Secondary metabolites and enzymes produced by microorganisms are examples of bioactive compounds that are widely used in pharmaceuticals, biofuels, biopesticides, detergents, and in the production process of these industrial products (Merino and Cherry, 2007).

4.1. Antimicrobial compounds

The bioactive natural substances that endophytes create are antimicrobial metabolites (Guo *et al*., 2008). By creating secondary metabolites, endophytes have evolved a defence mechanism against pathogenic invasion (Tan and Zou, 2001). Numerous structural groups, including peptides, alkaloids, steroids, quinines, terpenoids, phenols, and flavonoids, are comprised of the antimicrobial chemicals generated by endophytes (Yu *et al*., 2010). Endophyte new antimicrobial metabolites are emerging as a viable alternative to combat the rising drug resistance (Ferlay *et al*., 2010).As antibacterial agents, nanoparticles may have a big role. The therapy of some cancer types has been significantly impacted by nanoparticles. Endophyticbacteria synthesize various nanoparticles whichemerges as a novel field in the research area of pharmaceuticalengineering

4.2. Anti cancerous compound

Numerous bioactive substances generated by endophytes have been found to have anti-cancer properties (Firakova*et al*., 2007). Strong antiangiogenic activity was demonstrated by the endophytic bacterial strain EML-CAP3, which was isolated from C. annuum L. (red pepper) leaves. According to Jung *et al*. (2015), this endophytic bacterial strain produced lipophilic peptides that prevented human umbilical vein endothelial cells from proliferating and also showed anti-angiogenic potential in the development of tumours. The anti-cancerous properties of ginsenosides are what make Panax ginseng, or ginseng, famous. High ginsenoside content was observed in the modified endophytic bacterium *Paenibacilluspolymyxa* from ginseng leaves.

4.3. Quorum Quenching compound– Anti-quorum sensing inhibitor

A new target- a novel strategy to combat and lessen bacterial pathogenicity and has been made apparent by the identification of the QS system and its crucial role in bacterial virulence and survival. The QS system can be interrupted in a variety of ways. One of the most promising approaches to treating microbial diseases is the synthesis of anti-quorum sensing (anti-QS) chemicals (Rice *et al*., 2005). The applications of QS inhibition in biotechnology, industry, and medicine have led to a surge in research activity in recent years. It would be feasible to suppress the expression of QS-regulated phenotypes by interfering with bacteria's language (March and Bentley, 2004).

Recent research has revealed that many eukaryotes, especially plants and even bacteria, create quorum quenchers in their search for QS inhibitors. Otto (2004) claims that anti-QS chemicals regulate bacterial virulence factors and impede the emergence of resistant strains rather than killing or stopping the growth of bacteria. The powerful effect of enzyme-based AHL degradation has been observed in a broad variety of soil bacteria as one of the quorum quenching techniques **(Figure 3)**.Huma *et al*. (2011) reported that interfering with quorum sensing is a powerful strategy to overcome bacterial infections

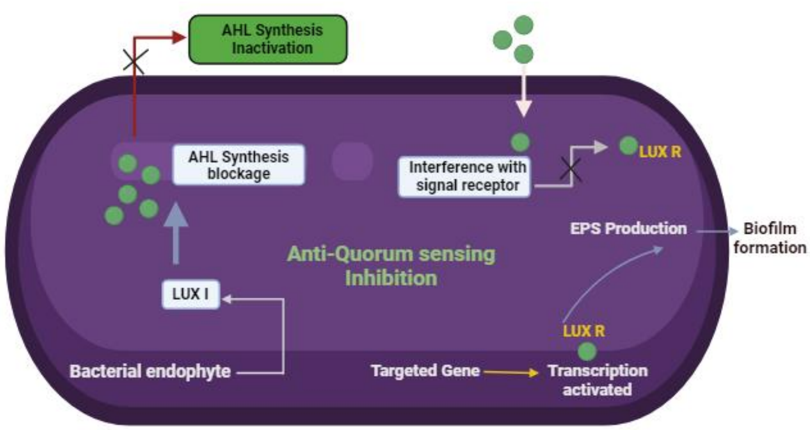

Figure 3: Anti-Quorum sensing inhibition and Anti-Biofilm activity of Bacterial Endophytes

5. Application of endophytes in various aspects

5.1. Application of endophytes to pharmaceuticals

To meet the need for new therapeutic agents to treat human diseases, both bacterial and fungal endophytes isolated from medicinal plants have the potential to produce novel bioactive compounds with various functional roles and pharmaceutically important effects (e.g., antioxidants, immunosuppressants, antiarthritic, antimicrobial, antidiabetic, anticancer, and anti-inflammatory activities) (Gouda *et al*., 2016).The endophyte *Metarhizium anisopliae*, which colonises the bark of the Taxusbrevifolia tree, produces the diterpenoidtaxol (C47H51NO14). Its distinct mode of action, which stops tubulin molecules from depolymerizing during cell division, has drawn a lot of interest as a possible anticancer medication.According to recent reports, two endophytic fungi have been found to create taxol: *Epicoccum nigrum* TXB502 from *Taxus baccata* and *Hippocrea lixii* from *Cajanus cajan*, which is known to produce cajanol, an anticancer drug (El-Sayed *et al*., 2020). *Camptotheca acuminata*, an endophytic fungus isolated from a deciduous tree, yields camptothecin ($C_{20}H_{16}N_2O_4$), a strong anticancer agent that is the precursor of topotecan and irinotecan (Kusari *et al*., 2009).

Antioxidant chemicals defend cells against reactive oxygen species and free radicals, both of which can cause oxidative damage, cellular degeneration and cancer. Concentration-dependent anti-inflammatory, antibacterial, antiatherosclerotic, anticarcinogenic, and antiviral activities are exhibited by the majority of antioxidant substances. According to Huang *et al*. (2007), the use of antioxidants is thought to be an effective therapy for the treatment of diseases linked to reactive oxygen species (ROS), such as cancer, cardiovascular disease, atherosclerosis, hypertension, ischemia/reperfusion injury, neurodegenerative diseases (like Parkinson's and Alzheimer's), rheumatoid arthritis, and ageing. Antioxidant metabolites are frequently produced by endophytic bacteria and fungi. For instance, *Pestalotiopsismicrospora*, which was isolated from *Terminalia morobensis*, is said to produce the powerful antioxidants pestacin ($C_{15}H_{14}O_4$) and isopestacin (Strobel *et al*., 2002).

5.2. Nanoparticle Bio-synthesizers

Since these particles have a wide range of applications in medicine, their potential bioactivity, non-pathogenic nature, and biosynthesis, the use of endophytic bacteria to create nanoparticles has become an emerging frontier in technology (Saravanan *et al*., 2020). These biosynthesized nanoparticles offer a wide range of potential uses in nanomedicine because of their antibacterial, antifungal, antioxidant, antimicrobial, antidiabetic, anticancer, and photocatalytic degradation capabilities (Rahman *et al*., 2019). In particular, silver nanoparticles are frequently utilised in bio-labeling, antibacterial agents, catalysts and sensors due to their unique optical, electrical and magnetic properties.It has been reported that silver nanoparticles produced from endophytic bacteria that were isolated from the host plants Adhatodabeddomei, Piper nigrum, Annona squamosa, and Bacillus cereus exhibit antibacterial activity (Baker *et al*., 2015). XRD, SEM with EDX, TEM, and FTIR are typically used to validate the presence of silver nanoparticles (AgNPs) (Zhao *et al*., 2018). A thorough analysis of the production of silver nanoparticles from endophytes and their possible uses in therapies has been conducted by Rahman *et al*. (2019).

5.3. Bioremediation

The technique known as "bioremediation" involves treating contaminated surroundings with microbes, plants, or their enzymes.Endophytes stimulate this biodegra-dation process which is diagrammatically represented in Figure 4.Research (Pietro-Souza *et al*., 2020; Prakash, 2021) has demonstrated the ability of endophytes to degrade hazardous xenobiotic contaminants**(Figure 4)**. Bioremediation of hydrocarbons, toxic herbicides, organic pollutants, xenobiotics, BTEX (benzene, toluene, ethylbenzene, and xylene), mercury, and heavy metals has been effectively accomplished with endophytes like *Burkholderia cepacia*, *Enterobacter sp.*, *Pantoea sp.*, and *Pseudomonas sp.*It has been demonstrated that two halotolerant and endophytic *Bacillus megaterium* species isolated from mangroves can convert poisonous selenite to elemental selenium, which is non-toxic (Mishra *et al*., 2011).

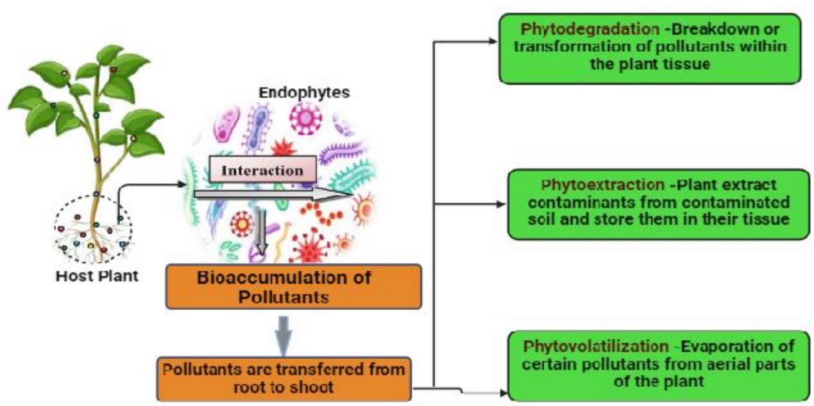

Figure 4: Plant-Endophyte synergistic interaction in Phytoremediation

According to Shahzad *et al*. (2019), an endophytic*Bacillus amyloliquefaciens* RWL-1 from rice was found to reduce the accumulation of heavy metals and encourage plant development in Cu2+-contaminated soil. It was recently discovered for the first time that endophytic *Stenotrophomonas s*p. S20, *Pseudomonas sp.* P21, and *Sphingobium sp.* S42 from the wetland plant Tamarixchinensis had the ability to remediate nickel (Chen *et al*., 2020).A viable approach to bioremediation may involve the use of modified plant endophytes with perhaps high degrading capacity (Kumari and Chaudhary, 2020).

5.4. Endophyte Genomics

The mysteries of endophytism and plant-endophyte interactions may be solved by endophyte studies utilising metagenomics in conjunction with metatranscriptomics, metaproteomics, and metabolomics (Bosamia*et al*., 2020). Individual endophyte sequencing and comparative genomic investigations would provide insight into their functional potential in addition to revealing their genome architecture. The characteristic genes and genetic factors that support endophytism have been found, and studies focused on endophyte genomics have been successful in illuminating the subtleties of plant-endophyte interactions. Notably, these studies identified the genes that confer in plants the ability to withstand biotic and/or abiotic stress, stimulate immune responses, initiate the production of phytohormones, biocontrol, tolerance to heavy metals, acquire nutrients, fix N_2, phosphate solubilize, and produce siderophores.Notably, research on the molecular interactions and functional characteristics of endophytes has made it possible to predict new bioactive secondary metabolites, possible drugs, biofertilizers, and agrochemical fungicides. This has made the commercialization and realisation of endophytes a feasible alternative for advancing both novel pharmaceuticals and sustainable, climate-resilient agriculture. (Subudhi *et al*., 2019).

Several endophytic microbial genomes have been sequenced and published. Among the sequenced endophytic bacterial genomes are two from poplar trees (Taghavi *et al*., 2009), eleven from Poison Ivy (*Toxicodendronradiatans*) (Tran *et al*., 2015), twenty-one from rice seed endophytes (Chaudhry *et al*., 2017), six from sugarcane (Parthasarathy *et al*., 2018), and Burkholderia sp. strain KJ006 (Kwak *et al*., 2012).

Table 1: Selected endophytic genes involved in endosphericcolonization, host interaction and promotion of plant growth (Afzal *et al*., 2019)

Gene	Functions	Techniques	Endophytes
acds (ACC deaminase)	Stress reduction	Gene deletion, complementation, gene disruption	*Burkholderia phytofirmansPsJN*
Iac C N-acyl-homoserine lactone synthase	IAA Degradation Quorum Sensing	Gene disruption	*Burkholderia phytofirmansPsJN*
nifH (nitrogenase)	Nitrogen fixation	Pnif:gusA fusion reporter system; Rt-PCR	*Herbaspirillum seropedicae;Bradyrhizobium, pelomonas, Bacillus sp., Cyanobacteria*
eglA:eglS (endoglucanase)	Systemic colonization	Transposon mutagenesis, Genedisruption	*Azoarcussp strain BH72: Bacillus amyloliquefaciens*
Pectinase	Systemic colonization	Gene overexpression	*Bacillus sp*
alkB (alkane monooxygenase)	Diesel degradation	Real –time PCR	*Pseudomonas sp., Rhodococcussp*
carAB	Pathogen cell-cell signalling disruption	Gene disruption, complementation	*Pseudomonas sp, strain G*
pilT *(type IV pili)*	Endophytic colonization by Twitching Mobility	Deletion, Mutation	*Azoaracus sp Strain BH72*
Multiple genes	Stress response, Chemotaxis, metabolism and global regulation	*dapB*-Based In Vivo Expression Technology System	*Pseudomonas sp A15*
Ferritin	Iron Storage	Real-time PCR	*Burkholderia phytofirmansPsJN*
TanB dependent siderophore receptor	Siderophore mediated iron uptake	Real-time PCR	*Burkholderia phytofirmansPsJN*
L-ornithine 5-monooxygense	Siderophore synthesis	Real-time PCR	*Burkholderia phytofirmansPsJN*

6. Conclusion and Perspective

Endophytes engage in a wide range of activities to support sustainable agriculture and the environment.Without having an adverse influence on the environment, they hasten the growth and productivity of plants. During the last decade, the number of research on endophyte biology and uses has increased dramatically. This is because of advancements in technology like metagenomics methods, which have made it possible for scientists to learn more about the characteristics and diversity of endophytes. It is clear that even with this advancement, what we now know is only the beginning. Given that different organs of a same host have varied endophyte compositions, we must first develop a comprehensive inventory of endophytes encompassing a diverse range of plant species, including aquatic ones, in order to fully utilise the benefits and/or qualities of endophytes. Practical applications cannot be directly achieved by using metagenomics methods alone to uncover the hidden diversity and functional implications of endophytes.

Endophytes are now a significant biological resource repository. Endophytes are a source of resources for bioprospecting as well as bioformulations. For serious problems like Endophytes have the potential to offer at least partial, if not whole, solutions to the growing number of antibiotic-resistant illnesses, environmental pollution, climate change, and increasing strain on the world's food supply. Therefore, in order to reap the benefits of endophytes through application, their biology needs to be thoroughly investigated in future research.

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Types and Recent Advances in Chromatography

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Introduction

Chromatography operates on the principle wherein molecules within a mixture are applied onto a surface or into a solid, fluid stationary phase. The separation of these molecules occurs as they move with the assistance of a mobile phase (Hostettmann *et al*..., 2011). Various factors influence this separation process, including molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and differences in affinity or molecular weights. Due to these distinctions, certain components of the mixture linger longer in the stationary phase, causing them to move more slowly in the chromatography system. Conversely, other components swiftly transition into the mobile phase, exiting the system at a faster rate (Harris DC, 2004).

This chromatographic technique is based on three key components:

1. **Stationary Phase:** This phase always consists of either a "solid" phase or "a layer of a liquid adsorbed on the surface of a solid support."(Donald PL,2005)
2. **Mobile Phase:** This phase is invariably composed of either a "liquid" or a "gaseous component."
3. **Separated Molecules:** The separation of molecules within the mixture is achieved through the interplay of the stationary and mobile phases, where differences in adsorption, partition, and molecular characteristics lead to distinct migration rates, ultimately resulting in the separation of components.

The fundamental interaction among the stationary phase, mobile phase, and substances within the mixture is a crucial determinant in the separation of molecules. Chromatography methods that rely on partition are highly effective for separating and identifying small molecules such as amino acids, carbohydrates, and fatty acids. On the other hand, affinity chromatographies, such as ion-exchange chromatography, excel in the separation of macromolecules like nucleic acids and proteins.

Various chromatographic techniques serve specific purposes:

1. **Paper Chromatography:** Used for the separation of proteins and in studies related to protein synthesis.
2. **Gas-Liquid Chromatography:** Employed for the separation of alcohol, esters, lipids, amino groups, and the observation of enzymatic interactions.
3. **Molecular-Sieve Chromatography:** Particularly useful for determining the molecular weights of proteins.
4. **Agarose-Gel Chromatography:** Utilized for the purification of RNA, DNA particles, and viruses.

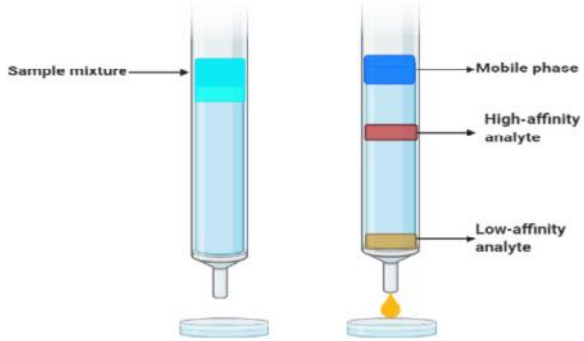
The stationary phase in chromatography can be either a solid phase or a liquid phase coated on the surface of a solid phase. Depending on the nature of the mobile phase, chromatography is categorized into liquid chromatography (LC) when the mobile phase is liquid, and gas chromatography (GC) when the mobile phase is gas. Gas chromatography is suitable for gases, mixtures of volatile liquids, and solid materials, while liquid chromatography is especially useful for thermal unstable and non-volatile samples.

Chromatography, beyond its separation capabilities, is applied for quantitative analysis. The objective is to achieve satisfactory separation within a suitable time interval, leading to the development of various chromatography methods. These include column chromatography, thin-layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high-pressure liquid chromatography, and affinity chromatography.

The types of chromatography encompass a diverse range:

1. Column chromatography
2. Ion-exchange chromatography
3. Gel-permeation (molecular sieve) chromatography
4. Affinity chromatography
5. Paper chromatography
6. Thin-layer chromatography
7. High-pressure liquid chromatography (HPLC)

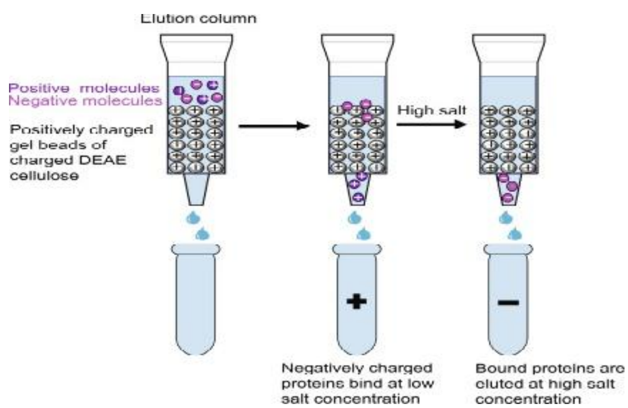
1. Column chromatography



Column chromatography is a commonly used purification technique in labs of organic chemistry. Done right it can simply and quickly isolate desired compounds from a mixture. But like many aspects of practical chemistry, the quick and efficient setting up and running of a column is something that can take time to master. Here are some instructions to help you set up the column.

In column chromatography, a stationary phase is packed into a column, and the sample to be separated is introduced onto the column, followed by the application of a wash buffer as the mobile phase. The flow of these components through the column material, typically supported on a fiberglass support, is carefully regulated. As a result, the samples move through the column and accumulate at the bottom of the device in a time- and volume-dependent manner. This method allows for the efficient separation and purification of proteins based on their distinct characteristics.

2. Ion-exchange chromatography



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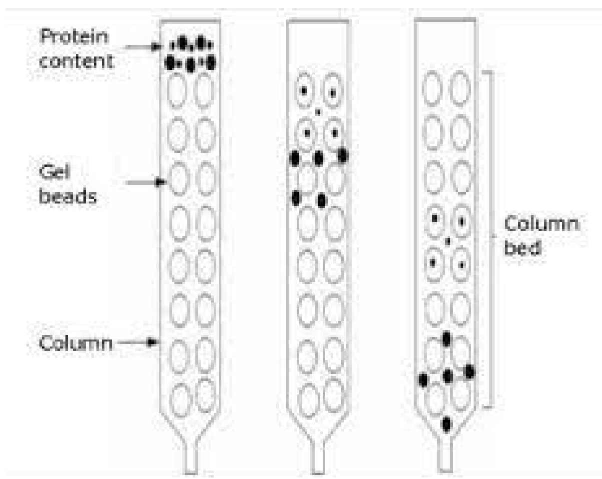
Ion-exchange chromatography operates on the basis of electrostatic interactions between charged protein groups and the solid support material (matrix). The matrix possesses an ion load opposite to that of the protein to be separated, and the affinity of the protein to the column is established through ionic ties. Proteins are then separated from the column by altering the pH, concentration of ion salts, or ionic strength of the buffer solution (Konieczka, 2009).

There are two types of ion-exchange matrices, each with distinct charge characteristics:

1. **Anion-Exchange Matrices:** Positively charged matrices that adsorb negatively charged proteins.
2. **Cation-Exchange Matrices:** Matrices bound with negatively charged groups that adsorb positively charged proteins **(Amercham Pharmacia, 2004).**

3.Gel-permeation (molecular sieve) chromatography



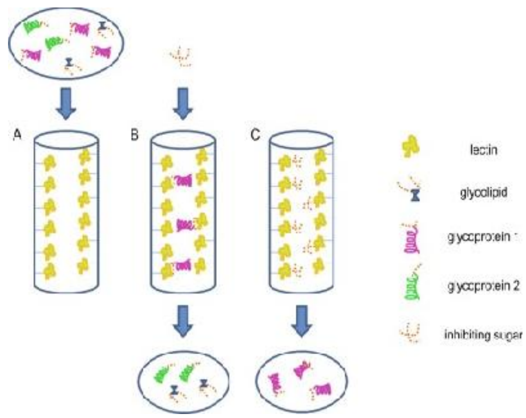
The fundamental principle of gel-permeation chromatography involves the utilization of dextran-containing materials to separate macromolecules based on differences in their molecular sizes. This method is primarily employed for determining the molecular weights of proteins and reducing salt concentrations in protein solutions (Walls *et al.*,2011).

In a gel-permeation column, the stationary phase comprises inert molecules with small pores. The solution, containing molecules of varying dimensions, is continuously passed through the column at a constant flow rate.

Molecules larger than the pores are unable to permeate the gel particles and are retained between the particles in a confined area. On the other hand, larger molecules pass through the spaces between porous particles, swiftly traversing the column. As molecules decrease in size, they diffuse into the pores, and smaller molecules leave the column with proportionally longer retention times (Determann,2011).

The most commonly used column material for gel-permeation chromatography is Sephadeks G type. Additionally, materials such as dextran, agarose, and polyacrylamide are also employed as column materials .This technique provides an effective means for separating macromolecules based on their molecular sizes, contributing to the accurate determination of molecular weights and the purification of protein solutions.

4. Affinity chromatography



Affinity chromatography is a highly specialized technique employed for the purification of enzymes, hormones, antibodies, nucleic acids, and specific proteins (**my Sci. blog, 2013**). The distinguishing feature of this chromatography method is the use of a ligand, which can form a complex with a specific protein (such as dextran, polyacrylamide, cellulose, etc.), bound to the column's filling material. This ligand specifically interacts with the protein of interest.

Here's an overview of the process:

1. **Column Filling Material:** The ligand-bound filling material is introduced into the column.

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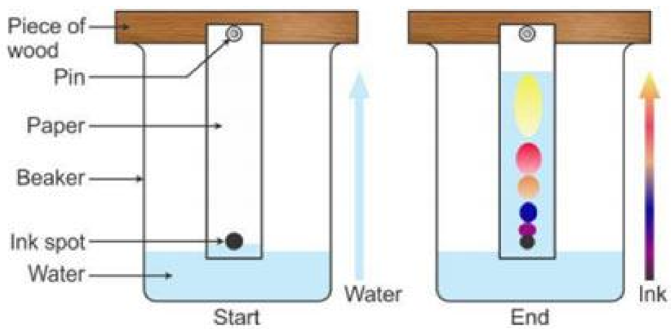
2. **Matrix Attachment:** The specific protein that forms a complex with the ligand is attached to the solid support (matrix) and retained within the column.
3. **Separation:** As a result, the specific protein is retained in the column, while free proteins that do not interact with the ligand leave the column.
4. **Elution:** The bound protein is then eluted from the column by altering the ionic strength through changes in pH or the addition of a salt solution.

Affinity chromatography is particularly effective for isolating and purifying specific biomolecules due to the specificity of the ligand-protein interaction. This targeted approach enables the selective capture and subsequent release of the protein of interest.

5. Paper chromatography



Paper Chromatography

Paper chromatography employs a support material that comprises a layer of cellulose highly saturated with water. In this method, a thick filter paper serves as the support, and water drops settled in its pores constitute the stationary "liquid phase." The mobile phase consists of an appropriate fluid placed in a developing tank. It is important to note that paper chromatography falls under the category of "liquid-liquid" chromatography.

Here's a brief explanation of the process:

1. **Support Material:** The support material is a layer of cellulose-saturated paper.
2. **Stationary Phase:** Water drops settled in the pores of the paper act as the stationary "liquid phase."

3. **Mobile Phase:** An appropriate fluid serves as the mobile phase and is placed in a developing tank.

Paper chromatography is particularly useful for separating and analyzing mixtures of substances based on their different affinities for the stationary and mobile phases. The relative movement of components through the paper allows for visualizing the separation of compounds, making it a widely used technique, especially in educational and research settings.

6. Thin layer chromatography

Thin layer chromatography is a kind of chromatography used to separate and isolate mixtures that are non-volatile in nature. Just like other chromatography processes, this one consists of a mobile phase and a stationary phase.

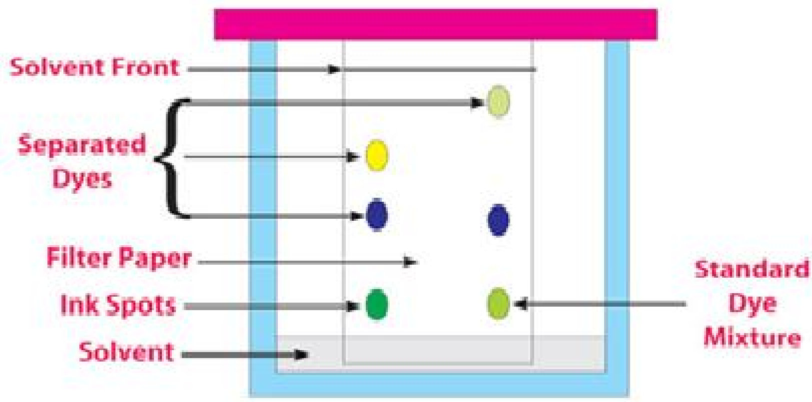
The latter one here is a thin layer of absorbent material, such as aluminium oxide, silica gel, or cellulose. This layer is applied to plastic, glass, or aluminium foil sheets called an inert substrate. The mobile phase in the TLC procedure is a solvent or a mixture of it.

Principle of thin layer chromatography

The separation principle of the TLC procedure is based on the given compound's relative affinity towards the mobile and the stationary phase. The process begins here by moving the mobile phase over the stationary phase's surface. During this movement, the higher affinity compounds gain less speed as compared to the lower affinity compounds. This results in their separation.

Once the procedure gets completed, different spots can be found on the stationary surface at distinct levels, reflecting various elements of the mixture. Basically, the compounds that are more attracted towards the stationary phase secure their position at lower levels while others move towards the higher levels of the surface. So their spots can be seen accordingly.This upward travelling rate depends on the polarity of the material, solid phase, and of the solvent.

Thin layer chromatography procedure



TLC Plates: These are used for applying the thin layer of stationary phase. They are inert or stable in nature. The layer of stationary phase is kept even throughout these plates for better analysis. Usually, ready-to-use plates are preferred by the people conducting experiments.

Mobile Phase: This comprises a solvent (or solvent mixture). The taken solvent needs to be chemically inert, of the highest possible purity, and particulate-free. Only then can the TLC spots be able to develop.

TLC Chamber: This is where the thin layer chromatography procedure takes place. It keeps the dust particles away from the process and does not let the solvent evaporate. In order to develop the spots appropriately, a uniform environment is maintained inside this chamber.

Filter Paper: This gets placed inside the chamber after being moistened with the mobile phase solution. It ensures that the mobile phase rises uniformly throughout the TLC plate's length.

After collecting all these components, the process begins. Here are the steps followed in it:

- The process starts by making a thin mark on the TLC plate's bottom with a pencil. It helps in the application of sample spots. These spots are kept at equal distances.
- The sample is then applied to these spots made on the line.
- Then the TLC chamber is filled with the mobile phase up to a few centimetres of its bottom.

- After pouring the mobile phase, the moistened filter paper is placed along with the inside of the chamber wall. This helps to avoid the edge effect by maintaining equal humidity.
- Finally, the prepared stationary phase plate is put inside the chamber. At this point, the sample spots are kept on the mobile phase's side.
- The chamber is then closed after placing the plate into it.
- Once enough time has elapsed for the process, the plate is taken out and allowed to dry.
- At last, the sample spots get analyzed through a suitable method for the sample, such as UV light, KMnO4 stain, and iodine staining.

This way, the TLC procedure gets completed. After analyzing the compound, it gets described in its relative mobility's terms, i.e., its Rf value is calculated. This value changes for each compound, even under the same circumstances.

Usually, relative Rf comes into use here because keeping all the TLC factors constant may not be possible. These aspects include adsorbent, temperature, adsorbent thickness, spotted material's amount, and solvent system. The formula used for Rf value calculation is:

Rf = (distance covered by the sample) / (distance covered by the solvent)

Thin layer chromatography applications

- Being a separation process, TLC proves to be highly effective for separating pharmaceutical formulations that consist of multiple components.
- The process can be used to examine a given product's purity.
- Medicines like local anaesthetics, analgesics, sedatives, hypnotics, anticonvulsant tranquilizers, and steroids go through the TLC procedure for their qualitative testing.
- The cosmetic industry also uses TLC for checking the presence of preservatives in the products.
- A given compound can be purified using TLC and then compared with a standard sample.
- TLC also finds its use in Biochemical analysis. Here, it can be used for biochemical metabolites' separation from urine, blood plasma, serum, and body fluids.

- Just like the cosmetic industry, the food industry also utilizes TLC for the detection of preservatives, artificial colours, and sweetening agents.
- A reaction's progress can also get tracked with TLC to see whether it is complete or not.

Advantages of TLC

- The separated spots of TLC can be further visualized without any trouble.
- This chromatography process is cost-effective as compared to other methods.
- It can be used for a number of compounds, and it does not take much time because it is quicker.
- The process is much more straightforward than other methods.
- TLC makes it simple to analyze any given compound's purity standards.
- Several compounds can easily get isolated through TLC.

Disadvantages of TLC

The drawbacks of the process are:

- The TLC procedure cannot be used for lower detection limit experiments because it has a high detection limit.
- The plates used in TLC do not possess a more extended stationary phase.
- Result reproduction is challenging in TLC.
- TLC is limited to qualitative analysis, and it cannot be used for quantitative analysis.
- The separation length is also restricted as compared to other chromatography methods.
- The process here does not take place in a closed system. Therefore, aspects like temperature and humidity can affect the results, making them inaccurate.

7. High – Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography or commonly known as HPLC, is an analytical technique used to separate, identify or quantify each component in a mixture.

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The mixture is separated using the basic principle of column **chromatography** and then identified and quantified by spectroscopy.

In the 1960s, the column chromatography LC with its low-pressure suitable glass columns was further developed to the HPLC with its high-pressure adapted metal columns.

HPLC is thus basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres.The stationary phase of RP-HPLC is non-polar, and the mobile phase is polar or moderately polar. The notion of hydrophobic interaction underpins RP-HPLC.**(Yadav MK, 2021)**.

Principle of High – Performance Liquid Chromatography (HPLC)

- The purification takes place in a separation column between a stationary and a mobile phase.
- The stationary phase is a granular material with very small porous particles in a separation column.
- The mobile phase, on the other hand, is a solvent or solvent mixture which is forced at high pressure through the separation column.
- Via a valve with a connected sample loop, i.e. a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe.
- Subsequently, the individual components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions with the stationary phase.
- After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer.
- At the end of this operation/run, a chromatogram in the HPLC software on the computer is obtained.
- The chromatogram allows the identification and quantification of the different substances.

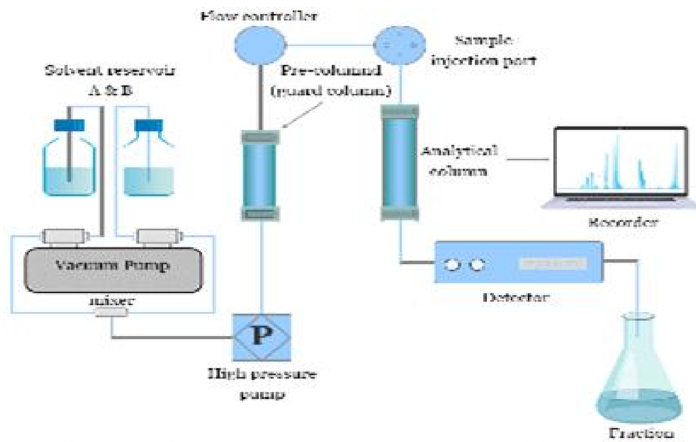

Figure 6.High – Performance Liquid Chromatography

The Pump

- The development of HPLC led to the development of the pump system.
- The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from the solvent reservoir into the system.
- High-pressure generation is a “standard” requirement of pumps besides which, it should also to be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate.
- Most pumps used in current LC systems generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces “pulses”.

Injector

- An injector is placed next to the pump.
- The simplest method is to use a syringe, and the sample is introduced to the flow of eluent.
- The most widely used injection method is based on sampling loops.
- The use of the autosampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing.

Column

- The separation is performed inside the column.
- The recent columns are often prepared in a stainless steel housing, instead of glass columns.
- The packing material generally used is silica or polymer gels compared to calcium carbonate.
- The eluent used for LC varies from acidic to basic solvents.
- Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents.

Detector

- Separation of analytes is performed inside the column, whereas a detector is used to observe the obtained separation.
- The composition of the eluent is consistent when no analyte is present. While the presence of analyte changes the composition of the eluent. What detector does is to measure these differences.
- This difference is monitored as a form of an electronic signal. There are different types of detectors available.

Recorder

- The change in eluent detected by a detector is in the form of an electronic signal, and thus it is still not visible to our eyes.
- In older days, the pen (paper)-chart recorder was popularly used. Nowadays, a computer-based data processor (integrator) is more common.
- There are various types of data processors; from a simple system consisting of the in-built printer and word processor while those with software that are specifically designed for an LC system which not only data acquisition but features like peak-fitting, baseline correction, automatic concentration calculation, molecular weight determination, etc.

Degasser

The eluent used for LC analysis may contain gases such as oxygen that are non-visible to our eyes.

- When gas is present in the eluent, this is detected as noise and causes an unstable baseline.
- Degasser uses special polymer membrane tubing to remove gases.
- The numerous very small pores on the surface of the polymer tube allow the air to go through while preventing any liquid to go through the pore.

Column Heater

The LC separation is often largely influenced by the column temperature.

- In order to obtain repeatable results, it is important to keep consistent temperature conditions.
- Also, for some analysis, such as sugar and organic acid, better resolutions can be obtained at elevated temperatures (50 to 80°C).
- Thus, columns are generally kept inside the column oven (column heater).

Types of High – Performance Liquid Chromatography (HPLC)

1. **Normal phase:**

   Column packing is polar (e.g silica) and the mobile phase is non-polar. It is used for water-sensitive compounds, geometric isomers, cis-trans isomers, and chiral compounds.

2. **Reverse phase:**

   The column packing is non-polar (e.g C18), the mobile phase is water+ miscible solvent (e.g methanol). It can be used for polar, non-polar, ionizable, and ionic samples.

3. **Ion exchange:**

   Column packing contains ionic groups and the mobile phase is buffer. It is used to separate anions and cations.

4. **Size exclusion:**

   Molecules diffuse into pores of a porous medium and are separated according to their relative size to the pore size. Large molecules elute first and smaller molecules elute later.

Applications of High – Performance Liquid Chromatography (HPLC)

The HPLC has developed into a universally applicable method so that it finds its use in almost all areas of chemistry, biochemistry, and pharmacy.

- Analysis of drugs
- Analysis of synthetic polymers
- Analysis of pollutants in environmental analytics
- Determination of drugs in biological matrices
- Isolation of valuable products
- Product purity and quality control of industrial products and fine chemicals
- Separation and purification of biopolymers such as enzymes or nucleic acids
- Water purification
- Pre-concentration of trace components
- Ligand-exchange chromatography
- Ion-exchange chromatography of proteins
- High-pH anion-exchange chromatography of carbohydrates and oligosaccharides

Advantages ofHigh – Performance Liquid Chromatography (HPLC)

1. Speed
2. Efficiency
3. Accuracy
4. Versatile and extremely precise when it comes to identifying and quantifying chemical components.

Limitations

1. **Cost:** Despite its advantages, HPLC can be costly, requiring large quantities of expensive organics.
2. **Complexity**

3. HPLC does have **low sensitivity** for certain compounds, and some cannot be detected as they are irreversibly adsorbed.
4. Volatile substances are better separated by gas chromatography.

Recent Advances in HPLC-MS for Pharmaceutical Analysis

High-Performance Liquid Chromatography-Mass Spectrometry, often abbreviated as HPLC-MS, is a powerful analytical technique that combines the separation capabilities of liquid chromatography (LC) with the detection specificity of mass spectrometry (MS). This hybrid approach has emerged as an indispensable tool in the pharmaceutical industry, driven by its high throughput, sensitivity, and selectivity.

1. **Separation Power of LC:** HPLC provides high-resolution separation of complex mixtures, allowing for the precise isolation of individual components.
2. **Detection Specificity of MS:** Mass spectrometry enhances detection specificity by providing information about the mass-to-charge ratio of ions, aiding in the identification of compounds.
3. **Indispensable Analytical Tool:** HPLC-MS has become an essential analytical tool in pharmaceutical research and development, as well as in quality control processes.
4. **High Throughput:** The combination of HPLC and MS enables rapid and efficient analysis, contributing to high throughput in pharmaceutical laboratories.
5. **Sensitivity:** HPLC-MS is highly sensitive, capable of detecting and quantifying compounds even at low concentrations.
6. **Selectivity:** The technique offers high selectivity, allowing for the discrimination of closely related compounds based on their mass spectra.
7. The recent research has delved into the exploration of multidimensional separation methods, aiming to enhance separation performance for complex proteomics samples. The focus has been on improving sensitivity, peak capacity, and throughput, ultimately enabling the profiling of low-microgram samples. These advancements are anticipated to have a profound impact on pharmaceutical analysis in the years to come. (Gang L., (2023)
8. It provides valuable insights into how these cutting-edge techniques and technologies will shape the landscape of pharmaceutical analysis in the

coming decades. The emphasis on multidimensional separation methods signifies a commitment to addressing the challenges posed by complex samples in proteomics, contributing to advancements in sensitivity and overall analytical efficiency. This forward-looking approach is crucial for staying at the forefront of pharmaceutical research and development.

Future Development in Chromatography

Chromatography, a well-established laboratory technique for several decades, continues to undergo significant advancements, driven by the evolving demands of key industries like pharmaceuticals, forensics, and metabolomics. (Rathore *et al.*..., 2018)

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Interpretation of Biology with Computer applications- The Bioinformatics

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Introduction

As an interdisciplinary field of science, bioinformatics combines computer science, statistics, mathematics engineering and biology. Bioinformatics is at the heart of modern biological research, especially so, in whole genome studies and second generation sequencing (2GS) data analysis.

Now a day's Bioinformatics uses apart from analysis of genome sequence data Bioinformatics is now being used for a vast array of other important tasks,including analysis of gene variation and expression analysis and prediction of gene and protein structure and function ,prediction and detection of gene regulation networks ,simulation environments.

Professor Margaret Dayhoff is known as the founder of Bioinformatics.This she did by pioneering the application of mathematics and computational techniques to the sequencing of proteins and nucleic acids and establishing the first publicly avaliable database for research in the area.

There are three main branches in Bioinformatics

1) Genomics

2) Proteomics and

3) Systems

- Genomics includes DNA sequence data
- proteomics specifically deals with the function ,shapes,interactions and abdudance of proteins.
- Systems biology is an approach in biomedical research to understanding the larger picture—be it at the level of the organism, tissue, or cell—by putting its pieces together.

Types of biological database

Biological databases

- These are the databases consisting of biological data like protein sequencing, molecular structure, DNA sequences, etc in an organized form.
- Several computer tools are there to manipulate the biological data like an update, delete, insert, etc. Scientists, and researchers from all over the world enter their experiment data and results in a biological database so that it is available to a wider audience.
- Biological databases are free to use and contain a huge collection of a variety of biological data.

Uses of biological databases

- It helps the researchers to study the available data and form a new thesis, anti-virus, helpful bacteria, medicines, etc.
- It helps scientists to understand the concepts of biological phenomena.
- The database acts as a storage of information.
- It helps remove the redundancy of data.

Types of biological databases

There arethree types of biological databases are as follows.

Primary databases:

- It might also called an archival database since it archives the experimental results submitted by the scientists. The primary database populated with experimentally derived data like genome sequence, macromolecular structure, etc. The data entered here remains uncurated(no modifications are performed over the data).
- It contains unique data obtained from the laboratory and these data are made accessible to normal users without any change.
- The data had given accession numbers when they had entered into the database. The same data might later retrieved using the accession number. Accession number identifies each data uniquely and it never changes.

Examples of primary database-

Nucleic Acid Databases are GenBank and DDBJ

Protein Databases are PDB,SwissProt,PIR,TrEMBL,Metacyc, etc.

Secondary database

- The data stored in these types of databases are the analyzed result of the primary database. Computational algorithms are applied to the primary database and meaningful and informative data is stored inside the secondary database.
- The data here are highly curated(processing the data before it is presented in the database). A secondary database is better and contains more valuable knowledge compared to the primary database.

Examples of Secondary databases are as follows

- InterPro (protein families, motifs, and domains)
- UniProt Knowledgebase (sequence and functional information on proteins)

Composite Databases

- The data entered in these types of databases had first compared and then filtered based on desired criteria.
- The initial data taken from the primary database, and then they are merged together based on certain conditions.
- It helps in searching sequences rapidly. Composite Databases contain non-redundant data.

Examples of Composite Databases are as follows.

- Composite Databases -OWL,NRD and Swissport +TREMBL

  Genbank

  Nucleotide

  Blast

  Chemical database

  DNA Data Bank of Japan

  EMBL

Genome

Macromolecular 3D structure

Metabolic pathways

Important database	Important tools
NCBI (Integrated database)	BLAST(Search and homology tools)
EMBL(Nucleotide databse)	FASTA (Search and homology tools)
DDBJ (Nucleotide databse)	Bankit(Submission tool)
Genbank (Nucleotide databse)	Seavin (Submission tool)
SWISSPORT (Protein database)	ORF Finder (Analysis tool)
OMIM (Disease database)	TX search (Retrieval tool for taxonomy database)
KEGG (Metabolic database)	SAKURA(Submission tool in DDBJ)
Pubmed(Literature database)	Clustal w(Multiple sequence alignment)
Enzymes(Enzyme database)	MSD Fold (Protein secondary structure comparison tool)
PANDIT (Taxonomy database)	
Array expressm(Microarray database)	

Database

Database are simply the repositories in which all the biological data is stored as computer language.Database are variously classified on varying basis like data type,datasource,organism etc.

Tools

Tools are software developed to perform various tasks over the stored data such as searches, analysis, submission, annotation etc.

Genbank

Genetic sequence databank is one of the fastest growing repositories of known genetic sequences. Genbank the primary biological database managed by the National Center for Biotechnology Information(NCBI).Genbank files contains information like accession numbers abd gene names, phylogenetic classification and references to published literature.It has an annotated collection of publicly available sequences which includes information about genes,proteins and other genetic elements.Genbank is part of international nucleotide sequence database collaboration (INSDC),which is a joint effort between three primar databases,genbank,DDBJ and EMBL.These organization were collaboratively to share sequence data from around the world on a daily basis and ensure thet the data in database up-to- date and accurate.

Genbank accepts mRNA (or) genomic sequence data with proper source organism information and annotation provided by the submitter. However the database does not accept noncontiguoussequences, primary sequences, protein sequences without underlying nucleotide submission mixed genomic and mRNA sequence, consensus sequences or sequence with lengths of less than 200 nucleotides.To submit sequences to this database,there are several web based tools available,including bankit,sequin and tblasn

Bankit is web based submission tools is used for more complex submission such as those containing long sequences,multiple annotations (or)gapped sequences.

For even larger submissions, the blast submission tool had used.

Primary databases respective websites are following

NCBI	EMBL	PDB	DDBJ	Array express

EMBL

The European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database (http://www.ebi.ac. uk/embl/index.html) is maintained at the European Bioinformatics Institute (EBI) in an international collaboration with the DNA Data Bank of Japan (DDBJ) and GenBank (USA). Data has exchanged amongst the collaborative databases on a daily basis. The major contributors to the EMBL database are individual authors and genome project groups. WEBIN is the preferred web-based submission system for individual submitters, whilst automatic procedures allow incorporation of sequence data from large-scale genome sequencing centres and from the European Patent Office (EPO).

Network services allow free access to the most up-to-date data collection via Internet and WWW interfaces. EBI's Sequence Retrieval System (SRS) is a network browser for databanks in molecular biology, integrating and linking the main nucleotide and protein databases plus many specialized databases. For sequence similarity searching a variety of tools (e.g., BLITZ, FASTA, BLAST) are available which allow external users to compare their own sequences against the most currently available data in the EMBL Nucleotide Sequence Database and SWISS-PROT.

Uses of EMBL

EMBL-EBI web services allow you to query our large biological data resources programmatically, so that you can develop data analysis pipelines or integrate public data with your own applications.

BLAST is an acronym for Basic Local Alignment Search Tool and refers to a suite of programs used to generate alignments between a nucleotide or protein sequence, referred to as a "query" and nucleotide or protein sequences within a database, referred to as "subject" sequences.

BLAST Type	Query Sequence	Database
Blastn	nucleotide	nucleotide
Blastx	nucleotide (translated to protein)	protein
Blastp	protein	protein
Tblastx	nucleotide (translated to protein)	nucleotide (translated to protein)

The program compares nucleotide or protein sequences and calculates the statistical significance of matches. BLAST used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

DDBJ

The DNA Data Bank of Japan (DDBJ, http://www.ddbj.nig.ac.jp) (1) is a public database of nucleotide sequences established at the National Institute of Genetics (NIG). Since 1987, the DDBJ has been collecting annotated nucleotide sequences as its traditional database service.

Purpose of DDBJ

DDBJ serves as the only nucleotide sequence archive database in Asia. The database mainly collects original DNA data from Japanese researchers, with the remainder from China, Korea, Taiwan, and other countries. These sequences then annotated and subsequently disseminated to the public.

Resource of DDBJ

The central DDBJ resource consists of public, open-access nucleotide sequence databases including raw sequence reads, assembly information and functional annotation. Database content exchanged with EBI and NCBI within the framework of the International Nucleotide Sequence Database Collaboration (INSDC).

Metabolic pathways

A metabolic pathway is a linked series of chemical reactions that occur within a cell. These reactions catalyzed by enzymes, where the product of one enzyme acts as the substrate for the next. The reactants, products, and intermediates of an enzymatic reaction known as metabolites.

Uses of metabolic pathways

A pathway database is an effort to handle the current knowledge of biochemical pathways and in addition can used for interpretation of sequence data. Some of the existing pathway databases interpreted as detailed functional annotations of genomes because they tightly integrated with genomic information.KEGG (Kyoto Encyclopedia of Genes and Genomes) is one of the most complete and widely used databases containing metabolic pathways (372 reference pathwasy) from a wide variety of species (>700).

MetaCyc is a database of nonredundant, experimentally elucidated metabolic pathways. MetaCyc contains more than 1,100 pathways from more than 1,500 different species. MetaCyc is curated from the scientific experimental literature and contains pathways involved in both primary and secondary metabolism, as well as associated compounds, enzymes, and genes.

HumanCyc is a bioinformatics database that describes the human metabolic pathways and the human genome. The current version of HumanCyc constructed using Build 31 of the human genome. The resulting pathway/genome database (PGDB) includes information on 28,783 genes, their products and the metabolic reactions and pathways they catalyze.

BioCyc is a collection of 371 Pathway/Genome Databases. Each database in the BioCyc collection describes the genome and metabolic pathways of a single species. The databases within the BioCyc collection organized into tiers according to the amount of manual review and updating they have received. Tier 1 DBs had created through intensive manual efforts and include EcoCyc, MetaCyc and the BioCyc Open Compounds Database (BOCD). BOCD includes metabolites, enzyme activators, inhibitors, and cofactors derived from hundreds of species. Tier 2 and Tier 3 databases contain computationally predicted metabolic pathways, as well as predictions as to which genes code for missing enzymes in metabolic pathways, and predicted operons.

Reactome is a curated, peer-reviewed knowledgbase of biological pathways, including metabolic pathways as well as protein_complex trafficking and signaling pathways. Reactome includes several types of reactions in its pathway diagram collection including experimentally confirmed, manually inferred and electronically inferred reactions. Reactome has pathway data on more than 20 different species but the primary species of interest is Homo sapiens. Reactome has data and pathway diagrams for >2700 protein_complexes, 2800 reactions and 860 pathways for humans.

WikiPathways is an open, collaborative platform dedicated to the curation of biological pathways. It is based on the MediaWiki open source software used by Wikipedia, coupled to a custom graphical pathway editing tool and integrated databases covering major gene, protein_complex, and small-molecule systems. WikiPathways currently contains 544 species-specific pathways for human, mouse, rat, zebrafish, fruit fly, worm, and yeast.

The Medical Biochemistry Page focuses on human pathways, with highly detailed descriptions of human processes, hormones, and metabolite/protein_complex interactions. Additionally, the website contains clinical information on disease states and inborn errors of metabolism.

Pathway Commons is a collection of publicly available pathways for many species from multiple sources, including other pathway databases. Pathway information available from Pathway Commons includes biochemical reactions, complex assembly, transport and catalysis events, and physical interactions involving protein_complexes, DNA, RNA, small molecules and complexes. It is a central repository of pathway information which uses the standardized Biological Pathway Exchange (BioPAX) format to consolidate pathway information.

Biocarta's pathway maps focus on protein_complex interactions in the field of proteomics, the study of protein_complex expression and function. Biocarta's focus is on enhancing genomic information or as an alternative route of basic science investigation and drug discovery. Biocarta is an open source database of pathways highlighting molecular relationships from areas of active research as well as classical pathway maps. It also catalogs and summarizes important resources providing information for over 120,000 genes from multiple species.

Cell Signaling Technology is dedicated to providing innovative research tools that used to help define mechanisms underlying cell function and disease, and has a database of pathway maps focusing on protein complexsignaling. It also provides genetic information for disease states associated with dysfunction in protein complex based regulation of cellular processes.

Sigma Aldrich life sciences pathway slides focus on cell signaling and neuroscience. This resource includes 13 apoptosis and cell cycle pathways, 25 cytokine, growth factor and hormone pathways, 5 cytoskeleton and extracellular matrix pathways, 20 gene regulation and expression pathways, 3 pathways depicting G-protein_complex and nucleotide interactions, 2 pathways involved with immune signaling and blood, 12 ion channel slides, 5 lipid cell signaling pathways, 4 pathways involved in multi-drug resistance, 12 neurobiology and neurotransmission pathways, 3 protein_complex phosphorylation pathways, and 9 nitric oxide and cell stress pathways, comprising a valuable resource of 115 pathways relating to human signalling processes.

Macromolecular 3D structure

Structural bioinformatics is the branch of bioinformatics that is related to the analysis and prediction of the three-dimensional structure of biological macromolecules such as proteins, RNA, and DNA. It deals with generalizations about macromolecular 3D structures such as comparisons of overall folds and local motifs, principles of molecular folding, evolution, binding interactions, and structure/function relationships, working both from experimentally solved structures and from computational models. The term *structural* has the same meaning as in structural biology, and structural bioinformatics can see as a part of computational structural biology. The main objective of structural bioinformatics is the creation of new methods of

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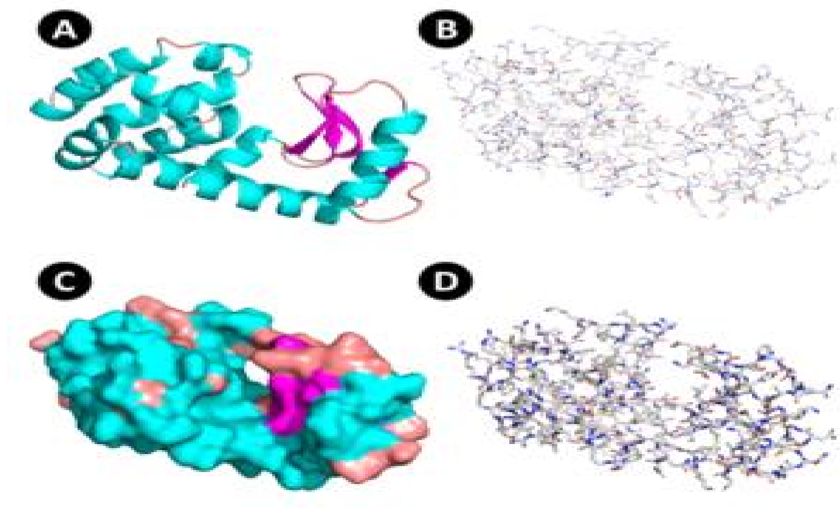
analyzing and manipulating biological macromolecular data in order to solve problems in biology and generate new knowledge.

Structure visualization



(A) Cartoon; (B) Lines; (C) Surface; (D) Sticks.

Protein structure visualization is an important issue for structural bioinformatics.[4] It allows users to observe static or dynamic representations of the molecules, also allowing the detection of interactions that used to make inferences about molecular mechanisms. The most common types of visualization are:

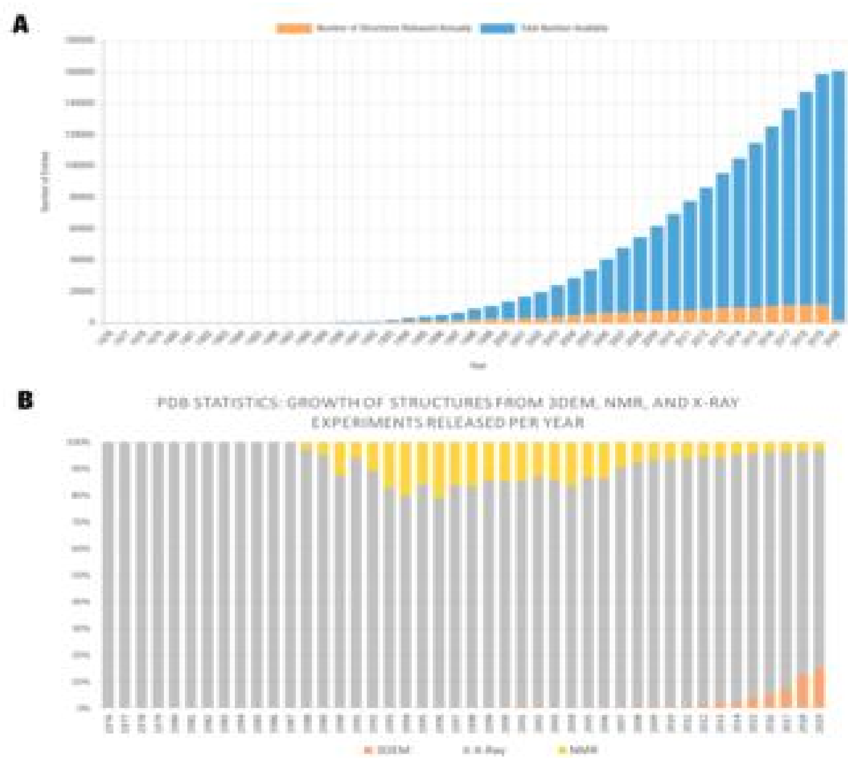
- Cartoon: this type of protein visualization highlights the secondary structure differences. In general, α-helix has represented as a type of screw, β-strands as arrows, and loops as lines.
- Lines: each amino acid residue has represented by thin lines, which allows a low cost for graphic rendering.
- Surface: in this visualization, the external shape of the molecule has shown.
- Sticks: each covalent bond between amino acid atoms has represented as a stick. This type of visualization has most used to visualize interactions between amino acids.

Structural alignment

Structural alignment is a method for comparison between 3D structures based on their shape and conformation. It used to infer the evolutionary relationship among a set of proteins even with low sequence similarity. Structural alignment implies superimposing a 3D structure over a second one, rotating and translating atoms in corresponding positions (in general, using the C_α atoms or even the backbone heavy atoms C, N, O, and C_α). Usually, the alignment quality has evaluated based on the root-mean-square deviation (RMSD) of atomic positions, *i.e.*, the average distance between atoms after superimposition:

Where δ_i is the distance between atom i and either a reference atom corresponding in the other structure or the mean coordinate of the N equivalent atoms. In general, the RMSD outcome measured in Ångström (Å) unit, which is equivalent to 10^{-10} m. The nearer to zero the RMSD value, the more similar are the structures.

Protein Data Bank (PDB)



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The number of structures from PDB. (A) The overall growth of released structures in Protein DataBank per year. (B) Growth of structures deposited in PDB from X-ray crystallography, NMR spectroscopy, and 3D electron microscopy experiments per year. Source: https://www.rcsb.org/stats/growth

The Protein Data Bank (PDB) is a database of 3D structure data for large biological molecules, such as proteins, DNA, and RNA. PDB is managed by an international organization called the Worldwide Protein Data Bank (wwPDB), which is composed of several local organizations, as. PDBe, PDBj, RCSB, and BMRB. They are responsible for keeping copies of PDB data available on the internet at no charge. The number of structure data available at PDB has increased each year, being obtained typically by X-ray crystallography, NMR spectroscopy, or cryo-electron microscopy.

Genome

Bioinformatics, as related to genetics and genomics, is a scientific subdiscipline that involves using computer technology to collect, store, analyze and disseminate biological data and information, such as DNA and amino acid sequences or annotations about those sequences. Scientists and clinicians use databases that organize and index such biological information to increase our understanding of health and disease and, in certain cases, as part of medical care.

Application of genome

Bioinformatics helps to give meaning to the data, which can used to make a diagnosis for a patient with a rare condition, to track and monitor infectious organisms as they move through a population, or to identify the best treatment for a patient with cancer.

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Renewable Energy Solutions: Harnessing Sustainable Power for a Greener Future

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Introduction

In the delicate tapestry of our planet's ecosystems, the threads of biodiversity weave a rich and intricate story. From the smallest microorganisms to the towering giants of the forest, the diverse array of life forms the foundation of our natural world. As we embark on a journey through the pages of this chapter, "Harmony in Nature," we delve into the captivating realms of Biodiversity and Ecosystem Dynamics, guided by the imperative to explore Conservation Strategies and Sustainable Practices. Biodiversity, the dazzling mosaic of life, is the result of billions of years of evolution, culminating in an awe-inspiring array of species. Each organism, from the microscopic to the majestic, contributes to the symphony of life on Earth. Within this chapter, we unravel the complexities of biodiversity, examining not only the sheer variety of species but also their interactions, adaptations, and vital roles in maintaining the equilibrium of ecosystems. Ecosystems, dynamic and ever-changing, are the theatres where the drama of life unfolds. From the vibrant coral reefs to the dense rainforests and expansive deserts, these habitats are the stages upon which biodiversity manifests itself. As we navigate through the intricacies of ecosystem dynamics, we will discover the delicate balance that sustains life and the factors that influence its ebb and flow. Yet, this intricate dance of life faces unprecedented challenges. Human activities, climate change, and habitat degradation threaten the delicate balance of ecosystems and endanger the very biodiversity that gives life its vibrancy. It is in recognizing these challenges that the importance of Conservation Strategies and Sustainable Practices becomes paramount. Conservation is not merely an endeavour to protect individual species; it is a commitment to safeguard the intricate tapestry of life for future generations. In this chapter, we explore the innovative strategies employed by scientists, conservationists, and communities worldwide to preserve biodiversity and promote sustainability. From wildlife corridors to community-based conservation initiatives, we uncover the diverse approaches that harmonize human needs with the preservation of our planet's natural treasures. As stewards of this remarkable planet, it is our collective responsibility to understand, appreciate, and actively participate in the conservation of biodiversity and the sustainable management of ecosystems. "Harmony in Nature" invites you to embark on a journey of discovery, appreciation, and advocacy, as we explore the interconnected web of life and strive to achieve a harmonious coexistence with the natural world.

In the intricate dance of life on Earth, the concept of "Harmony in Nature" emerges as a guiding principle for understanding and safeguarding our planet's biodiversity and ecosystems. This chapter embarks on a profound exploration, weaving together the threads of Biodiversity, Ecosystem Dynamics, and Sustainable Conservation Practices. As we delve into the richness of our natural world, we uncover the symphony that arises when diverse life forms and ecosystems coexist in balance. Life on Earth is a mesmerizing tapestry woven by the myriad forms of biodiversity. In this chapter, we embark on a captivating exploration of Biodiversity and Ecosystem Dynamics, delving into the essence of life's diversity and the intricate dance of ecosystems. Our journey begins with a profound understanding of the role biodiversity plays in shaping the vibrant landscapes we call home.

1. Biodiversity Exploration:

1.1 The Rich Tapestry of Biodiversity:

Biodiversity often referred to as the "web of life," encompasses the variety of genes, species, and ecosystems on Earth. We navigate through the staggering diversity of life, from the microscopic world of bacteria to the charismatic mega fauna, highlighting the interconnectedness of all living organisms.We begin our journey by immersing ourselves in the sheer diversity of life. From microscopic organisms to majestic creatures, we explore the intricate patterns woven into the tapestry of biodiversity that spans across ecosystems. The chapter highlights the interconnectedness and interdependence of species, emphasizing the pivotal role each organism plays in maintaining ecological harmony.

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1.2 Species Interactions and Adaptations:

As we venture deeper, we uncover the dynamic relationships among species. The intricate web of interactions, from mutualistic partnerships to competitive struggles, shapes the resilience and stability of ecosystems. An exploration of adaptations reveals the ingenious mechanisms that enable organisms to thrive in diverse environments, showcasing the evolutionary marvels of life. Venturing deeper into biodiversity, we unravel the complex web of interactions among species. Mutualistic relationships, predator-prey dynamics, and symbiotic alliances create a dynamic ecosystem. The exploration extends to the incredible adaptations that have evolved over eons, showcasing nature's ingenious solutions to the challenges of survival.

1.3 Biodiversity as a Symphony:

Biodiversity is portrayed as a symphony, where each species is akin to a musical note, contributing its unique sound to the harmonious composition of nature. The metaphor extends beyond a mere collection of species; it encapsulates the dynamic interactions, adaptations, and evolutionary processes that shape the ever-evolving melody of life.

1.4 Ecological Resilience and Stability:

Examining the rich tapestry of biodiversity, the chapter highlights the ecological resilience that arises from diversity. Diverse ecosystems exhibit a remarkable ability to adapt to environmental changes, ensuring their stability and sustainability over time. The interconnectedness of species serves as a natural buffer against disturbances, fostering resilience in the face of challenges.

1.5 The Human Connection:

As we navigate through the tapestry of biodiversity, it becomes evident that humans, too, are integral participants in this grand symphony. The chapter explores the ways in which human actions impact biodiversity, both positively and negatively, emphasizing the responsibility we bear as stewards of this extraordinary diversity.

2. Ecosystem Dynamics- The Rhythm of Nature

2.1 Dynamic Nature of Ecosystems:

Ecosystems, the theatres of life's drama, are in a constant state of flux. We unravel the complex dynamics governing these ecological communities, from succession patterns to cyclical processes, and gain insights into the delicate balance that sustains life. Ecosystems, the living stages where biodiversity unfolds, are dynamic entities. We delve into the rhythmic patterns of life, from the seasonal changes to the cyclical processes that govern ecosystems. Understanding these dynamics becomes crucial in appreciating the delicate equilibrium that sustains life on Earth (Odum, 1969; Leibold *et al.*, 2004). The journey begins with an exploration into the dynamic nature of ecosystems. These living stages are ever-changing, shaped by the interplay of abiotic and biotic factors. Seasonal transitions, life cycles, and nutrient cycles contribute to the rhythmic heartbeat that governs the flow of life within ecosystems. The chapter sheds light on the inherent dynamism that defines these intricate landscapes.

2.2 Factors Influencing Stability and Change:

Ecosystem stability is influenced by various factors, both natural and anthropogenic. Climate, geological processes, and human activities can tip this balance, leading to shifts in biodiversity and ecosystem structure. We examine the forces shaping ecosystems and the consequences of disruptions.

2.3 Forces Shaping Ecosystems:

As we navigate through the chapters of ecological narratives, we explore the various forces influencing the stability and change in ecosystems. Climate variations, geological processes, and anthropogenic activities are examined to grasp the intricate forces that shape the destiny of ecosystems.The exploration deepens as we unravel the forces that shape ecosystems. From climatic influences and geological processes to anthropogenic impacts, each force contributes to the intricate tapestry of ecological dynamics. The chapter examines how these external factors influence the stability, composition, and resilience of ecosystems, emphasizing the delicate equilibrium that sustains life (Chapin *et al.*, 2011; Sala *et al.*, 2000).

2.3 Resilience and Adaptation:

Ecosystems, akin to living organisms, exhibit resilience in the face of disturbances. The chapter delves into the mechanisms that allow ecosystems to adapt and recover from environmental changes. Concepts such as ecological succession and the ability of ecosystems to absorb shocks are explored, showcasing the inherent ability of nature to find equilibrium (Folke*et al.*, 2002; Walker *et al.*., 2004).

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2.4 Case Studies in Ecosystem Dynamics:

The chapter comes to life through compelling case studies that showcase the real-world application of ecosystem dynamics. From the intricate coral reefs to the sprawling Amazon rainforest, these case studies provide tangible examples of the rhythmic patterns, adaptive strategies, and forces at play in different ecosystems (Hughes *et al.*, 2000; Malhi *et al.*, 2008).

3. Sustainable Conservation Practices - Nurturing the Symphony

As we navigate the intricate tapestry of nature, the chapter on Sustainable Conservation Practices emerges as a beacon of hope and action. This section not only acknowledges the challenges posed to biodiversity and ecosystems but also unveils the innovative and responsible strategies employed to nurture the symphony of life on our planet.

3.1 Challenges to Harmony:

Acknowledging the challenges faced by biodiversity and ecosystems, we confront issues such as habitat loss, climate change, and over-exploitation. This section emphasizes the urgency of addressing these challenges and protecting the delicate harmony that sustains life.This segment initiates by acknowledging the challenges faced by biodiversity and ecosystems. From habitat loss and climate change to pollution and over-exploitation, the chapter provides an honest exploration of the threats that compromise the delicate balance of our natural world. It draws attention to the urgency of addressing these challenges to maintain the harmonious coexistence of species and ecosystems (Sala *et al.*, 2000; Millennium Ecosystem Assessment, 2005).

3.2 Strategies for Conservation:

The chapter then unfolds a panorama of innovative conservation strategies, revealing a tapestry of hope for the future. From the establishment of protected areas and wildlife corridors to the empowering initiatives led by local communities, this section showcases diverse and dynamic approaches to preserve biodiversity and sustain ecosystems. The emphasis is on interdisciplinary collaboration and adaptive management to address the complex challenges faced by our planet (Margules and Pressey, 2000; Terborgh and Peres, 2002). In the pursuit of harmony, we turn our attention to innovative conservation strategies. From protected areas and wildlife corridors to community-based initiatives, we explore the diverse approaches that aim to preserve biodiversity while promoting sustainable coexistence between humans and nature.

3.3 Building a Sustainable Future:

The chapter concludes with a focus on fostering a sustainable future. Advocacy, education, and global collaboration are presented as essential tools in ensuring that the harmony in nature endures for generations to come. The chapter unfolds with a focus on building a sustainable future. It explores the interconnectedness of human activities and the environment, emphasizing the need for responsible resource management. Sustainable practices, ranging from renewable energy adoption to eco-friendly agricultural methods, are spotlighted as essential tools in achieving a balanced and sustainable coexistence between humanity and nature (Foley *et al.*, 2005; Rockström *et al.*, 2009)

3.4 Community-Based Conservation Initiatives:

A spotlight is cast on community-based conservation initiatives, recognizing the pivotal role that local communities play in the preservation of biodiversity. By actively involving communities in conservation efforts, this approach fosters a sense of stewardship and empowers individuals to take ownership of their natural surroundings (Berkes, 2004; Mascia and Claus, 2009).

4. Preserving Ecosystem Equilibrium:

Within the delicate balance of ecosystems lies the critical chapter on "Preserving Ecosystem Equilibrium." This section delves into the symbiotic relationship between human societies and the environment, exploring the interconnectedness of our well-being with the health of ecosystems.

4.1 Understanding the Interconnectedness:

The journey commences by unraveling the intricate web connecting human activities and the environment. Acknowledging the dependence of societies on ecosystems for essential services, the chapter underscores the imperative of understanding and respecting the delicate interconnectedness that shapes our collective well-being (Daily *et al.*, 1997; Millennium Ecosystem Assessment, 2005).

4.2 Balancing Human Needs with Conservation:

The chapter explores the intricate task of balancing human needs with the imperative of conservation. It acknowledges that sustainable development requires a nuanced approach—one that aligns human activities with the resilience and vitality of ecosystems. By examining case studies and successful models, the section provides insights into how communities can thrive while safeguarding the ecosystems they depend upon (Sachs *et al*., 2010; Kareiva *et al.*, 2010).

4.3 Eco-friendly Resource Management:

Highlighting the role of responsible resource management, the chapter examines eco-friendly practices that mitigate the impact of human activities on ecosystems. Concepts such as sustainable agriculture, efficient water use, and renewable energy adoption are presented as pivotal strategies in preserving ecosystem equilibrium (Liu *et al.*, 2007; Pretty, 2008).

4.4 The Role of Conservation Education:

Recognizing the power of knowledge, the chapter emphasizes the role of conservation education. It explores how increasing awareness and fostering an understanding of ecosystems can empower individuals and communities to become stewards of the environment (Chawla, 1999; Krasny and Tidball, 2009).

5. Harmonizing Human Needs with Environmental Protection:

In the intricate interplay between human societies and the environment, the chapter on "Harmonizing Human Needs with Environmental Protection" stands as a testament to the delicate balance required for sustainable coexistence. This section delves into the nuanced task of meeting the diverse needs of humanity while safeguarding the integrity of our planet's ecosystems.

5.1 Understanding Human-Ecosystem Interdependence:

The exploration commences with a deep dive into the profound interdependence between human societies and ecosystems. This understanding forms the foundation for the intricate task of harmonization, acknowledging that the well-being of communities is intricately linked to the health and resilience of the environment (Lele *et al.*, 2013).

5.2 Sustainable Development Models:

This section explores various sustainable development models that seek to strike a balance between human needs and environmental protection. Case studies and examples are presented to illustrate successful instances where communities have embraced practices that promote economic growth while respecting ecological boundaries (Reid *et al.*, 2005; Sachs *et al.*, 2010).

5.3 Conservation Psychology and Behaviour Change:

An exploration into conservation psychology offers insights into understanding human behavior in the context of environmental protection. This chapter examines how behavioral change and the adoption of pro-environmental practices can contribute to harmonizing human activities with ecological conservation (Schultz *et al.*, 2007; Clayton and Myers, 2009).

5.4 Corporate Social Responsibility and Green Practices:

The chapter further explores the role of corporations in harmonizing human needs with environmental protection. By examining corporate social responsibility initiatives and the adoption of green practices, it highlights how businesses can contribute to ecological sustainability while meeting societal demands (Carroll, 1999; Schaltegger and Burritt, 2006).

6. Stewardship Ethic: Nurturing a Covenant with Nature

The concept of the Stewardship Ethic emerges as a guiding principle in the intricate relationship between humanity and the environment. This chapter delves into the philosophy of stewardship — a commitment to responsible caretaking, fostering sustainability, and preserving the natural world for present and future generations.

6.1 Foundations of Stewardship Ethic:

The exploration begins with an examination of the foundations that underpin the Stewardship Ethic. Drawing on ethical frameworks and cultural perspectives, this section aims to unveil the moral imperatives and philosophical underpinnings that guide individuals and communities toward responsible environmental stewardship (Leopold, 1949; Callicott, 1989).

6.2 Covenant with Nature:

At the heart of the Stewardship Ethic lies the idea of a covenant with nature - a reciprocal relationship where humans acknowledge their responsibility as stewards of the Earth. This section explores the metaphor of a covenant, emphasizing the mutual respect and commitment between humanity and the natural world (Norton, 1984; Rolston, 1988).

6.3 Indigenous Wisdom and Stewardship:

Examining indigenous perspectives on stewardship enriches the narrative, highlighting the wisdom embedded in traditional ecological knowledge. By drawing on indigenous philosophies, this section demonstrates how different cultures have long embraced stewardship as a way of life, fostering a harmonious coexistence with the environment (Cajete, 1994; Kimmerer, 2013).

6.4 Practical Applications of Stewardship:

The chapter then transitions into practical applications of the Stewardship Ethic. It explores how individuals, communities, and organizations can embody stewardship in everyday practices, ranging from sustainable agriculture and resource management to community engagement and conservation initiatives (Meine and Knight, 1999; Dietz *et al.*, 2003).

6.5 Educational Initiatives:

Recognizing the role of education in fostering a Stewardship Ethic, this section explores initiatives that promote environmental awareness, ethics, and a sense of responsibility. It showcases educational programs and initiatives aimed at cultivating a new generation of stewards committed to the well-being of the planet (Orr, 1994; Sobel, 2008).

Global Collaboration for a Sustainable Future: Bridging Borders for Environmental Harmony

This chapter focuses on the imperative of global collaboration to address environmental challenges and pave the way for a sustainable future. It explores how nations, organizations, and individuals can unite across borders, pooling resources and expertise to tackle shared environmental issues.

7.1 The Urgency of Global Collaboration:

The chapter begins by emphasizing the urgency of global collaboration in the face of pressing environmental challenges. It delves into the interconnected nature of environmental issues, recognizing that pollution, climate change, and biodiversity loss are global phenomena that require collective action on an international scale (United Nations, 2015).

7.2 International Environmental Agreements:

The chapter explores the role of international agreements in fostering global collaboration for environmental sustainability. It examines key treaties and conventions such as the Paris Agreement, the Convention on Biological Diversity, and the Kyoto Protocol, shedding light on the successes, challenges, and on-going efforts in international environmental governanceConvention on Biological Diversity (1992) and Intergovernmental Panel on Climate Change (2014).

7.3 Cross-Sectoral Partnerships:

The chapter delves into the power of cross-sectoral partnerships, exploring collaborations between governments, non-governmental organizations (NGOs), businesses, and research institutions. Case studies and examples illustrate how diverse stakeholders can pool resources, share knowledge, and work collectively toward sustainable solutions (World Economic Forum, 2019); UN Global Compact, 2019).

7.4 Innovative Technologies for Collaboration:

The chapter explores how innovative technologies facilitate global collaboration for sustainability. It examines the role of data sharing, remote sensing, and digital platforms in monitoring environmental changes, fostering real-time communication, and enabling collaborative efforts across geographical boundaries (NASA, 2020; Google Earth Engine, 2021).

7.5 Grassroots Movements and Citizen Engagement:

Recognizing the power of grassroots movements, the chapter sheds light on how individuals and communities can contribute to global sustainability efforts. It explores the role of citizen science, activism, and community engagement in creating bottom-up momentum for environmental change (Bonney*et al.*, 2009; Extinction Rebellion, 2021).

Conclusion:

Harmony in Nature

As we draw the final chords of our exploration into "Harmony in Nature: Exploring Biodiversity, Ecosystem Dynamics, and Sustainable Conservation Practices," we find ourselves immersed in the symphony of life on Earth. This chapter has been a journey through the interconnected realms of biodiversity, the rhythmic dance of ecosystems, and the conscientious practices essential for preserving the delicate balance of our planet.

Reflecting on Biodiversity:

In unravelling the rich tapestry of biodiversity, we discovered the sheer beauty and complexity of life. From microscopic organisms to majestic creatures, each species contributes a unique note to the grand symphony of existence. The interconnectedness of all living organisms emerged as a central theme, emphasizing the delicate harmony that relies on the diversity of life forms.

Unveiling Ecosystem Dynamics:

The exploration of ecosystem dynamics has been a journey into the heartbeat of nature. Ecosystems, like living stages, pulsate with the rhythm of life. Forces shaping these ecosystems, from climatic influences to human activities, were revealed as pivotal elements in the ever-evolving narrative of nature. The resilience and adaptability of ecosystems showcased the intricate dance of balance and change.

Embracing Sustainable Conservation Practices:

Our quest led us to the heart of conservation practices, where we encountered innovative strategies designed to nurture the symphony of nature sustainably. From protected areas and community-based initiatives to eco-friendly practices, these strategies became the notes that resonate with the planet's well-being. The chapter highlighted the challenges faced by biodiversity and ecosystems, emphasizing the urgency of adopting responsible practices.

Harmony as a Guiding Principle:

"Harmony in Nature" is not merely a descriptive phrase but a guiding principle. It encapsulates the interconnected exploration of biodiversity, the rhythmic dance of ecosystems, and the responsible practices essential for maintaining the delicate balance. It invites us to view nature as a symphony, where every species, ecosystem, and conservation effort plays a unique role in the grand composition of life.

The Symphony Continues:

As we conclude this chapter, the journey does not end; it transforms. The symphony of nature continues to evolve, and our role as stewards becomes even more critical. The chapters ahead beckon us to delve deeper into the challenges and solutions that shape our environmental narrative. The call to action is clear — to embrace the principles of harmony in our relationship with nature, fostering a sustainable and balanced future for generations to come.In this exploration, we've glimpsed the intricate threads that weave together the harmony of nature. Let this understanding guide our actions and decisions as we move forward, harmonizing with the planet and contributing to the flourishing melody of life.

As we conclude this chapter, readers will have gained a profound appreciation for the intricate patterns of biodiversity and the dynamic nature of ecosystems. Recognizing the significance of each species and understanding the delicate equilibrium of ecosystems sets the stage for our exploration of Conservation Strategies and Sustainable Practices in subsequent chapters, ensuring the preservation of this intricate tapestry for generations to come.

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Culminating Efficacy: Exploring the Smoke Toxicity and repellent properties of *Gloriosa superba* L extract against *Aedes aegypti* and *Culex quinquefasciatus* Mosquitoes

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Abstract

This study investigates the repellent activities and smoke toxicity effects of the petroleum ether seed extract of *Gloriosa superba* against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes. Repellency tests were conducted at different concentrations (10ppm, 15ppm, 20ppm, 25ppm, and 30ppm) and time intervals (5.00-6.00 pm to 9.00-10.00 pm). Results demonstrated a concentration-dependent reduction in mosquito bites, with the highest concentration (30ppm) consistently exhibiting the most significant repellent effect. *A. aegypti* showed a range of protection from 10.26% to 70.84%, while *C. quinquefasciatus* exhibited protection from 16.17% to 95.53%. Smoke toxicity effects were evaluated using petroleum ether seed extract amended mosquito coils against adult *A. aegypti* and *C. quinquefasciatus*. The coils demonstrated varying degrees of fed, alive, dead and unfed mosquitoes, with CD (5%) values indicating significant differences in toxicity levels. Additionally, the study assessed the smoke toxicity effects of *G. superba* extracts on the reproduction and survival of both mosquito species, revealing varied impacts among different coils. These findings provide comprehensive insights into the potential of *G. superba* as a natural mosquito repellent and highlight its multifaceted effects on mosquito behavior and survival.

Keywords: *Gloriosa superba*, mosquito repellent, smoke toxicity, Coil assay, survival rate

Introduction

Dengue fever, Zika, and chikungunya are among the many mosquito-borne diseases that continue to pose significant threats to public health, particularly in tropical and subtropical regions (Lim *et al.*, 2023). The global burden of mosquito-borne diseases continues to underscore the critical need for innovative and sustainable mosquito control strategies. Among the myriad approaches, plant-derived compounds have gained prominence for their potential as eco-friendly alternatives to conventional insecticides. In this study, we delve into the multifaceted effects of *Gloriosa superba* petroleum ether seed extract, specifically examining the smoke toxicity and repellent properties against two significant mosquito species *Aedes aegypti* and *Culex quinquefasciatus.* These mosquitoes are notorious vectors of diseases such as dengue, Zika, and filariasis, posing substantial threats to public health.

Our investigation encompasses a comprehensive analysis of the smoke toxicity effects of *G. superba* extracts on the reproduction and survival of both *A. aegypti* and *C. quinquefasciatus*. We explore distinct plant parts, including seeds, tubers and leaves, utilizing various coils to release the plant-derived smoke. The study evaluates the number of eggs laid, larvae hatched, and the resultant percentage reduction in comparison to negative controls. Additionally, we investigate the repellent activities of *G. superba* extracts against *A. aegypti* and *C. quinquefasciatus*, focusing on different concentrations and time intervals.

This research seeks not only to contribute valuable insights into the potential of *G. superba* as an effective mosquito control agent but also to address the dual facets of smoke toxicity and repellency. The integration of these properties in a single plant extract could offer a holistic approach to combat mosquito-borne diseases while aligning with the growing demand for environmentally sustainable vector control methods. As the world grapples with emerging health challenges, this study paves the way for novel, nature-inspired solutions with the potential to mitigate the impact of mosquito-borne illnesses.

Materials and methods

Rearing of *A. aegypti* and *C. quinquefasciatus*

Eggs of *A.aegypti* and *C. quinquefasciatus* were collected from the standardized colony at National Institute of Communicable Diseases (NICD), Mettupalayam, in Tamilnadu (India) and it was maintained in sterilized containers with unchlorinated tap water under laboratory conditions. Freshly hatched larvae were collected and maintained in separate containers with unchlorinated tap water, dog biscuit with yeast (2:1 ratio) was given as the source of food. Water was changed on alternate days. The culture medium was regularly checked and dead larvae were removed daily. The culture troughs were kept closed with mosquito net for preventing interference of foreign mosquitoes.

Pupae were isolated from the culture troughs and allowed to emerge into adults in the mosquito cages (32cm X 32 cm X 30 cm). The freshly emerged adults were maintained at 27 ± 2^0C, 75-85% RH, under 14L: 10D photoperiod cycles. The adult males were fed with 10% sucrose solution in cotton wick and female mosquitoes were fed with blood of chick (One week old chick). Different batches of adults were maintained in the cage by introducing sufficient number of pupae. An oviposition trap was kept in cage to facilitate the female to lay eggs. The egg rafts in the case of *C. quinquefasciatus* and individual eggs in the case of *A. aegypti* in the container were removed carefully and allowed to hatch. The hatched larvae were maintained for many generations in the laboratory and the eggs and larvae obtained from this culture were used for the experiments.Insome occurrence egg rafts and eggs of *C. quinquefasciatus* and *A. aegypti* were collected in the early morning and brought to the laboratory in containers and allowed to hatch separately. The matured larvae were maintained until adult emergence.

Collection and preparation of plant extract using solvents

Seeds of *G. superba* were collected from the fields (Coimbatore district) and brought to the laboratory. The completely dried seeds were powdered with an electrical blender and sieved to get fine powder. The powders were stored separately in airtight containers for further analysis. The plant powder wasextracted with petroleum ether by using Soxhlet apparatus for 8 hours (Vogal, 1978). The extracts were concentrated using a vacuum evaporator at 45^0C under low pressure. After complete evaporation of the solvent, the concentrated extract was collected and stored in separate glass vials at 4^0C in refrigerator for further experiments. The yield (%) value was calculated by the following formula

$$\text{Extractive value (\%)} = \frac{\text{Yield}}{\text{Weight of raw material}} \times 100$$

Repellent bioassay

The repellent bioassay was studied by adopting the standard procedure of Fradin and Day, (2002). The repellent bioassay was carried out with petroleum ether seed extract of *Gloriosa superba* against *A. aegypti* and *C. quinquefasciatus* with human volunteers. For each test, 10 disease free, laboratory-reared female mosquitoes were placed into separate laboratory cages (32 x 32 x 30 cm). Before each test, the volunteer's skin was washed with unscented soap and the test extract was applied from the elbow to the finger tips. After air drying the arm, 25cm^2 of the dorsal side of the skin was exposed and the remaining area was covered with rubber gloves. The exposed area was treated with plant extract of varying concentrations i.e. 10, 15, 20, 25 and 30ppm. The treated and control arms were interchanged regularly to eliminate bias and each test concentration was repeated five times. Volunteers were made to involve in the test of each concentration by inserting the treated and control arms alternatively into the same cage for one full minute for every fifteen minutes, until the first bite occurred or until the landing of two mosquitoes. The percentage of repellency was calculated at the end of every test by using the following formula

$$\% \text{ of protection} = \frac{C - T}{C} \times 100$$

Where,

C – No. of bites received by control arm

T – No. of bites received by treated arm

Skin-irritant potential

Skin-irritant potential of petroleum ether seed extract of *Gloriosa superba* was evaluated in 25 male and female healthy volunteers, aged between 18 and 30 years, who showed no signs of dermatological diseases. A piece of filter paper was embedded with petroleum ether seed extract (1%) was applied on the skin of dorsal arm and tightly covered by a surgical tape. Following a 5-hr period of exposure, the occlusive patches were removed, and the sites of application were gently washed with water. The skin was examined and scored at 24, 48 and 72 hr after patch removal. Skin reactions were scored, as suggested by Basketter *et al*. (1997), by using a four point scale: (0) absence of reaction; (+) weakly positive reaction, i.e. mild redness or dryness across most of the treatment site; (++) moderately positive reaction, i.e. a more intense redness generally spreading beyond the treatment site; and (+++) strongly positive reaction, i.e. strong redness generally accompanied by edema. A volunteer with a '+' or greater reaction at any of the assessments was considered to have shown a positive skin-irritant reaction.

Smoke Toxicity Test

Petroleum ether seed extract of *G. superba*were used for smoke toxicity assay. The mosquito coil was prepared by following the method of Saini *et al*. (1986) with suitable modifications. One gram of active ingredient, two grams of saw-dust as binding material and two grams of coconut shell charcoal powder as burning material was used in the preparation of the coil. All the three sample sources were thoroughly mixed with distilled water and a semisolid paste was prepared. Mosquito coils (0.6 cm thickness) were prepared manually from the semisolid paste and were shade dried. The negative control (without plant ingredient) and positive control (Good night) was used to compare the effectiveness of plant coils.

After the experiment was over, the fed and unfed (alive and dead) mosquitoes were counted. The protection given by the smoke from plant samples against the biting of *A. aegypti* and *C. quinquefasciatus* was calculated in terms of percentage of unfed mosquitoes due to treatment (Thangam and Kathiresan, 1992).

$$= \frac{\text{Number of unfed mosquitoes in treatment} - \text{Number of unfed mosquito in controll}}{\text{Number of mosquitoes treated}} \times 100$$

Fecundity test

The fecundity test was carried out as detailed by Vineetha and Murugan (2009). After the smoke exposure, the fed mosquitoes (25 female and male mosquitoes of *A. aegypti* and *C. quinquefasciatus*) were transferred to separate cages. The alive mosquitoes fed with blood meal were reared in mosquito cage, (measuring 32 x 32 x 30 cm). The eggs from the cage were collected daily till all the mosquitoes died. 50 to 100 eggs were allowed to hatch in each plastic tray containing about 5 liters of unchlorinated tap water. The larvae hatched from the eggs were fed with a mixture of dog biscuits and yeast powder in the ratio of 2:1 and water in the tray was changed daily and hatched larvae were counted at second instar stage. The reduction in the population from the smoke treated mosquitoes was calculated by using the formula.

$$\text{Population reduction (\%)} = \frac{\text{Number of larvae hatched in control 1 - Number of larvae hatched in treated}}{\text{Number of larvae hatched in control 1}} \times 100$$

Results and discussion

In the present investigation the extractive value for the petroleum ether seed extract of *G. superba* was calculated to be 2.5 grams. The extractive value, a measure of the quantity of constituents extracted from a plant material, holds paramount importance in various aspects. This metric serves as a quantitative indicator of the efficiency of the extraction process, providing insights into the concentration of bioactive compounds obtained from the raw material. A higher extractive value often suggests a more potent and concentrated extract, which can be of significant relevance in industries such as pharmaceuticals, where the efficacy of natural products is closely tied to their concentration. Researchers and scientists utilize extractive values to assess the success of different extraction methods, allowing for the optimization of processes to achieve higher yields and improved purity. Furthermore, in fields like phytochemistry and pharmacognosy, understanding the extractive value contributes to the characterization of plant extracts, aiding in the identification of potential therapeutic agents. This metric also plays a pivotal role in quality control, ensuring consistency and reproducibility in the extraction process, which is crucial for both research endeavors and industrial applications. In essence, the extractive value serves as a valuable parameter guiding the extraction of bioactive compounds, facilitating advancements in diverse areas ranging from medicine to agriculture (Smith, 2023).

Repellent activity

Repellent activity against particular mosquito species may be due to the synergistic effects of a combination of phytochemicals present in each plant extracts and essential oils. These differential responses are influenced by the differences in distribution of toxic chemicals in different parts of the plants. Repellent protection time in laboratory bioassays however can change depending on the biological characteristics of the mosquito test population (Singh *et al*., 2009). Differences in sensitivity to repellents between species have been widely documented (Cantrell *et al.*, 2005). The quality of repellent may vary due to the quality of extract such as chemical constituents and physical properties. Factors affecting the quality of essential oils include plant species (variety), cultivating conditions, maturation of harvested plant, plant storage, plant preparation and method of extraction (Tawatsin *et al.*, 2001).

The repellent activities of the petroleum ether seed extract of *G. superba* against *A. aegypti* and *C. quinquefasciatus* mosquitoes were investigated across different concentrations (10ppm, 15ppm, 20ppm, 25ppm, and 30ppm) and time intervals (5.00-6.00 pm, 6.00-7.00 pm, 7.00-8.00 pm, 8.00-9.00 pm, and 9.00-10.00 pm) (Table.1 & 2.). The mean number of mosquito bites, along with the standard deviation, was recorded for each concentration and time segment. During the 5.00-6.00 pm interval, the control group experienced 34.2 ± 1.64 bites, while the 30ppm concentration demonstrated a notable reduction to 2.00 ± 1.87 bites, indicating a potential repellent effect. This trend continued throughout subsequent time intervals, with increasing concentrations leading to a significant decrease in mosquito bites compared to the control group. The highest concentration (30ppm) consistently exhibited the strongest repellent activity, suggesting a dose-dependent effect. The percentage of protection ranged from 10.26% to an impressive 70.84%, showcasing the efficacy of *G. superba* seed extract in repelling *A. aegypti* mosquitoes. *C. quinquefasciatus* mosquitoes the control group experienced 21.0 ± 2.55 bites, while the concentrations of 10ppm, 15ppm, and 20ppm showed a notable reduction in bites, highlighting a repellent effect. However, observations at 25ppm and 30ppm were not feasible. Similar trends persisted across subsequent time intervals, with higher concentrations consistently demonstrating greater repellent activities. The percentage of protection values ranged from 16.17% to an impressive 95.53%, emphasizing the efficacy of Gloriosa superba seed extract in safeguarding against mosquito bites. These findings underscore the potential of *G. superba* as a natural repellent against *C. quinquefasciatus* mosquitoes, with concentration-dependent efficacy throughout different hours of the evening.Additionally, the percentage of protection indicates a substantial reduction in mosquito bites, highlighting the extract's effectiveness as a mosquito repellent. Differences in body size, sugar water availability, adult density in test cages and mosquito age can affect test results. Generally, the effectiveness and duration of repellency depend on multiple factors including the type of repellent, the mode of application, local conditions (temperature, humidity and wind), the attractiveness of the individual to insects, loss due to removal by perspiration and abrasion, the sensitivity of the insect to the repellent and biting density (Barnard, 2000).Both *A. aegypti* and *C. quinquefasciatus* exhibited distinct responses to the *G. superba* seed extract. The repellent activity is comparable with previously reported studies with varying doses. Singh *et al.* (2009) have reported the repellent activity of hexane tuber extract of *Cyperus rotundus* against adult mosquitoes of *Anopheles* *culicifacies, A. stephensi* and *C. quinquefasciatus.* During the study period no skin irritation was found in volunteers.

Table. 1. Repellent activities of petroleum ether seed extract of *Gloriosa superba* against *A. aegypti.*

Repellent activity observed time (Hrs)	Mean number of bites ± SD						CD (5%)
	Control	10ppm	15ppm	20ppm	25ppm	30ppm	
5.00-6.00pm	34.2 ± 1.6432[a]	9.4 ± 2.4083[b]	6.2 ± 1.7889[c]	4.4 ± 1.1402[cd]	2.00 ± 1.870[d]	NB	2.652
6.00-7.00pm	25.6 ± 2.0736[a]	14.8 ± 0.8367[b]	12.2 ± 0.8367[c]	9.4 ± 1.6733[d]	4.20 ± 0.836[e]	3.6 ± 1.3416[e]	1.961
7.00-8.00pm	19.4 ± 3.9749[a]	19.4 ± 1.1402[a]	16.0 ± 1.2247[b]	14.8 ± 1.4832[c]	7.40 ± 1.673[d]	5.4 ± 1.1402[e]	2.590
8.00-9.00pm	11.8 ± 1.3038[c]	21.0 ± 0.7071[a]	18.4 ± 1.6733[b]	17.4 ± .8944[b]	11.6 ± 0.894[c]	9.4 ± 1.8166[d]	1.426
9.00-10.00pm	6.40 ± 1.5166[e]	28.8 ± 5.6747[a]	26.2 ± 2.1679[a]	21.4 ± 2.1679[b]	16.6 ± 1.140[c]	10.0 ± 1.2247[d]	3.371
Fed mosquitoes	97.4	87.4	83.6	69.6	42.2	28.4	
Unfed mosquitoes	5.8	12.6	16.4	30.4	57.8	71.6	
% of protection		10.26	14.16	28.5	56.67	70.84	

NB-No biting; Values given in each cell is the mean ± SD of four replicates.
[a-e] Mean values within a column with no common superscript differ significantly ($p<0.05$).

Table. 2. Repellent activities of petroleum ether seed extract of *Gloriosa superba* against *C. quinquefasciatus.*

Repellent activity observed time (Hrs)	Mean number of bites ± SD						CD (5%)
	Control	10ppm	15ppm	20ppm	25ppm	30ppm	
5.00-6.00pm	21.0 ± 2.5495[a]	7.0 ± 2.1213[b]	5.4 ± 0.8944[bc]	3.4 ± 1.1402[c]	NB	NB	2.816
6.00-7.00pm	23.2 ± 1.0954[a]	12.4 ± 0.5477[b]	10.4 ± 1.5166[c]	7.2 ± 1.0954[d]	3.8 ± 0.8367[e]	NB	1.402
7.00-8.00pm	19.2 ± 4.0866[a]	17.0 ± 3.4641[a]	13.0 ± 3.8730[b]	9.2 ± 3.0332[c]	4.8 ± 1.6432[d]	NB	3.662
8.00-9.00pm	16.6 ± 4.2190[a]	19.2 ± 4.2661[a]	17.4 ± 1.9494[a]	12.4 ± 1.5166[b]	8.2 ± 3.1145[c]	NB	3.946
9.00-10.00pm	14.0 ± 1.8708[b]	23.2 ± 3.4928[a]	22.8 ±1.7889[a]	16.6 ± 1.3416[b]	11.6 ± 2.1909[c]	4.2 ± 2.280[d]	2.758
Fed mosquitoes	94	78.8	69	48.8	28.2	4.2	
Unfed mosquitoes	6	21.2	31	51.2	71.6	95.8	
% of protection		16.17	26.60	48.08	70	95.53	

NB-No biting; Values given in each cell is the mean ± SD of four replicates. [a-e] Mean values within a column with no common superscript differ significantly ($p<0.05$).

Smoke toxicity test

Plant-derived smoke contains an array of chemicals with different mode of action, which kills mosquitoes. The smokes from plants are cheap, target specific, self sustained and highly toxic to the adult mosquitoes, even at very low doses (Vineetha and Murugan, 2009).

In the present study adult female *Aedes aegypti* and *Culex quinquefasciatus* were repelled by the smoke from the petroleum ether seed (coil-I), tuber (Coil-II) and leaf (coil-III) extracts of *Gloriosa superba.* Afterexposure of the mosquito species to coil I, coil II, and coil III, the fed and unfed mosquitoes were counted. In *Aedes aegypti* the fed mosquito percentage was 24%, 22.6% and 26.2% when exposed to coil I, II and coil III respectively. The unfed percentage calculated was 53.8%, 58.8% and 48.8% for coil I, II and coil III respectively. In *Culex quinquefasciatus* the fed mosquito percentage was 17.0% in coil I, 21.6% in coil II and 22.6% in coil III. The unfed mosquito were 61.6%, 59.2% and 56.0% for coil I, coil II and coil III respectively. Although these chemicals are not volatile, they may be used as repellents by burning plant material; either on a fire or in a mosquito coil to create an insecticidal smoke, which repels the insects through direct toxicity.

Table .3. Smoke toxicity effect of *G. superba* seed, tuber and leaf extracts against the adult mosquitoes of *A. aegypti*.

Plant parts used	No. of mosquitoes tested	Fed mosquitoes	Alive mosquitoes (%)	Dead mosquitoes (%)	% unfed over negative control
Coil I	100	24.0 ± 2.4495^{bc}	34.6 ± 1.6733^{c}	46.2 ± 3.0332^{a}	53.4 ± 2.4083^{c}
Coil-II	100	22.6 ± 1.9494^{c}	33.8 ± 7.3959^{c}	43.6 ± 9.0167^{b}	58.8 ± 7.7910^{b}
Coil III	100	26.2 ± 2.3875^{b}	46.6 ± 3.9115^{a}	29.0 ± 3.9370^{c}	48.8 ± 0.8367^{d}
Negative control	100	74.6 ± 3.5071^{a}	92.4 ± 2.5100^{d}	0	-
Positive coil	100	14.2 ± 2.4900^{d}	40.4 ± 3. 5757^{b}	45.4 ± 1.9080^{ab}	63.6 ± 2.0736^{a}
CD(5%)		3.06	5.18	2.84	5.36

Values given in each cell is the mean ± SD of four replicates.
$^{a-d}$ Mean values within a column with no common superscript differ significantly ($p<0.05$).

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Table.4. Smoke toxicity effect of *G. superba* seed, tuber and leaf extracts **against the adult mosquito *C. quinquefasciatus.***

Plant parts used	No. of mosquitoes tested	Fed mosquitoes	Alive mosquitoes (%)	Dead mosquitoes (%)	% unfed over negative control
Coil I	100	17.0 ± 2.1213^{c}	31.2 ± 3.9623^{c}	51.8 ± 4.6583^{b}	61.6 ± 2.8810^{b}
Coil-II	100	21.6 ± 4.3932^{b}	34.6 ± 1.6733^{b}	46.2 ± 3.0332^{c}	59.2 ± 7.3280^{bc}
Coil III	100	22.6 ± 1.9494^{b}	45.8 ± 2.7749^{a}	31.6 ± 2.7019^{d}	56.0 ± 3.2404^{c}
Negative control	100	78.6 ± 2.302^{a}	21.4 ± 2.3022^{d}	0	-
Positive coil	100	8.8 ± 4.086^{d}	30.0 ± 4.5826^{c}	61.0 ± 3.3912^{a}	71.4 ± 5.5946^{a}
CD (5%)		3.55	2.96	3.79	6.58

Values given in each cell is the mean ± SD of four replicates.

$^{a-d}$ Mean values within a column with no common superscript differ significantly ($p<0.05$).

Thangam and Kathiresan (1996) reported that the smoke toxicity effect of ten mangrove plants *Acanthus ilicifolius*, *Aegiceras cornicufatum*, *Avicennia marina*, *Avicennia officinalis*, *Bruguiera cylindrical*, *Ceriops decandra*, *Exocoecaria agallocha*, *Lumnitzera racemosa*, *Rhizophora apiculat* and *Rhizophora lamarchii* against the adult female mosquitoes of *Aedes aegypti* exhibit 50% protection. Murugan *et al.* (2007) reported the smoke toxicity effect of leaves of *Albizzia amara* and *Ocimum basilicum* against the dengue vector *Aedes aegypti*. The combination of two powders increased the toxicity of the smoke compared to individual plant powders. Fed and unfed mosquitoes counted after the individual treatment of *Albizzia amara* and *Ocimum basilicum* were 18 and 20 (fed) and 53 and 51 (unfed) respectively. After combined treatment of *Albizzia amara* and *Ocimum basilicum*, there were 16 fed and 55 unfed mosquitoes.

Kamalakannan *et al.* (2009) have reported that the smoke toxicity effect of leaves of *Tridax procumbans* with 80% unfed and 20% fed adult mosquitoes after treatment in *Anopheles stephensi.* Smoke toxicity effect of *Aegle marmelos* and *Toddalia asiatica* were reported by Vineetha and Murugan

(2009). The combination of two powders increased the toxicity of the smoke compared to individual plant powders. Fed and unfed mosquitoes were counted after the individual treatment of *Aegel marmelos, Toddalia asiatica* and *Aegel marmelos* +*Toddalia asiatica* were 22, 18 and 16 as fed; 50, 51 and 55 as unfed respectively against the adult female mosquitoes of *Aedes aegypti*.

It has been suggested that smoke may mask human kairomones, particularly carbon dioxide. Smoke production also lowers humidity by reducing the moisture carrying capacity of the air. This makes mosquito susceptible to desiccation and reduces sensory input because mosquito chemoreceptors are more responsive in the presence of moisture (Davis and Bowen, 1994).

Fecundity studies

Smoke not only affects adult mosquitoes but also affect their egg laying capacity. This smoke toxicity may be due to the volatile compounds which might affect the target organ and reduce the fecundity (Kamalakannan *et al*., 2009). In the present study, the mosquito adults exposed to smoke oviposited fewer eggs possibly due to the vapour of plant active compounds. After the smoke exposure of *Gloriosa superba* the mosquito egg laying capacity was reduced to 70.34% in coil I, 54.26% in coil II and 33.27% in coil III against adult *Aedes aegypti*. The percentage of egg hatchability was noticed in coil I, coil II and coil III and found to be 29.6%, 45.78% and 66.74% respectively in *A. aegypti*. The egg laying capacity was reduced to 69.48%, 52.27% and 40.03% and egg hatchability was reduced 30.51%, 48.66% and 59.95% in coil I, coil II and coil III respectively in *C. quinquefasciatus*. Based on the results it is suggested that the chemical factors from herbal coils may cause reduction in egg laying capacity and egg hatchability in *A. aegypti* and *C. quinquefasciatus*.

Table.5. Smoke toxicity effects of *Gloriosa superba* plant extracts **on reproduction andsurvival of *Aedes aegypti.***

Plant parts used	No. mosquitoes tested	Total no. eggs	Total no. of larvae hatched from the eggs	% reduction over negative control
Coil I	25	726.4 ± 40.0787[d]	434.6 ± 34.4282[d]	70.344 ± 40.0787[a]
Coil-II	25	938.2 ± 61.6417[c]	667.6 ± 25.4814[c]	54.264 ± 5.7940[b]
Coil III	25	1078.6 ± 32.5853[b]	973.0 ± 16.1864[b]	33.256 ± 8.9907[c]
Negative control	25	1828.0 ± 60.9893[a]	1478.0 ± 187.535[a]	-
Positive coil	25	312.4 ± 17.5869[e]	173.4 ± 24.9560[e]	88.0 ± 2.8093[a]
CD (5%)		49.63	86.51	23.19

Values given in each cell is the mean ± SD of four replicates.
[a-e] Mean values within a column with no common superscript differ significantly ($p<0.05$).

Table.6. Smoke toxicity effects of G*loriosa superba* plant extracts **on reproduction and survival of *Culex quinquefasciatus.***

Plant parts used	No. mosquitoes tested	Total no. egg rafts	Total no. (%) larvae hatched from the eggs	% reduction over negative control
Coil I	25	8.4 ± 1.5166[d]	571.2 ± 1.3038[d]	69.476 ± 0.087[b]
Coil-II	25	10.6 ± 1.3416[c]	910.8 ± 2.3875[c]	52.272 ± 1.4256[c]
Coil III	25	13.8 ± 1.0954[b]	1122.2 ± 2.2804[a]	40.03 ± 0.1717[d]
Negative control	25	18.6 ± 1.1402[a]	1871.6 ± 2.3022[b*]	-
Positive coil	25	5.8 ± 1.9235[e]	414.2 ± 2.1659[e]	77.9 ± 0.1150[a]
CD (5%)		1.67	2.27	0.85

Values given in each cell is the mean ± SD of four replicates.
[a-e] Mean values within a column with no common superscript differ significantly ($p<0.05$).

Similarly, reports have also been observed in egg laying capacity in *Anopheles stephensi* after exposure to smoke of *Tridax procumbans* (Kamalakanna *et al*., 2009). Vineetha and Murugan (2009) reported reduction of egg hatchability in *Aedes aegypti* when exposed tocoils prepared from leaves of *Aegle marmelos* and *Toddalia asiatica* against *Aedes aegypti.* Some chemical components present in the petroleum seed extract of *Gloriosa superba* are not volatile, they may be used as a repellent by burning the mosquito coil to create an insecticidal property.

This comprehensive study delved into the larvicidal, ovicidal, and smoke toxicity effects of *Gloriosa superba* extracts, particularly the petroleum ether seed extract, against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes. The petroleum ether seed extract exhibited significant larvicidal and ovicidal activities, demonstrating its potential in controlling mosquito larvae and reducing egg hatching rates. Smoke toxicity assessments using different coils further revealed nuanced impacts on mosquito behavior, including feeding, mortality, and repellency. The calculated CD (5%) values emphasized statistically significant differences in toxicity levels among the coils. Overall, these findings illuminate the multifaceted bioactivity of Gloriosa superba extracts, presenting a promising avenue for the development of eco-friendly and plant-derived insecticides with potential applications in effective mosquito control strategies.

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Polychaetes and Microplastic Pollution

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Introduction

Polychaetes are segmented worms with parapodia and bristle; they belong to the phylum Annelida and class Polychaeta. Polychaetes (chaetopods) are the dominant macrofaunal taxa in all marine sediments from the intertidal area down to the deep sea. They constitute more of the total macrobenthic animals in terms of several species and individuals. The majority of the species is quite small and short-lived exhibiting a high secondary production. Hence, they are an important link in marine food webs and feature significantly in the diets of many bottom-feeding fishes. Being small-sized organisms, they play a crucial role in ecology and EIA (Environmental Impact Assessment) studies. (Khan and Murugesan, 2005)

It is well known that polychaetes are used as pollution indicators worldwide to investigate the coastal environment (Jesudoss Ruben Prabakaran et al., 2019). In the environment, microplastic debris (<5 mm) proliferates, migrates and accumulates in natural habitats from pole to pole and from the ocean surface to the seabed; the debris is also deposited on urban beaches and pristine sediments. This type of pollution is ubiquitous and persistent in the world's oceans and openly threatens marine biota. Currently, the most widely used synthetic plastics are low- and high-density polyethene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polystyrene (PS) and polyethene terephthalate (PET). Altogether, these plastics represent ~90% of the total world production. The majority of the items polluting coastal and marine environments are comprised of microplastics (Ivar do Sul, J. A and

Costa M. F., 2014). Microplastics, especially in fibre form, pose threats to organisms that consume them as they can cause blockages in the digestive tract, become translocated to different tissues within the organism, and undergo accumulation (Alysee Mathalon and Paul Hill., 2014). The presence of microplastics in sediment-dwelling polychaetes from deep-sea sediments has not been investigated. Microplastics are classified into primary and secondary MPs according to their source (Cole et al., 2011). Primary MPs are manufactured as small plastic particles for specific use in domestic and industrial sectors. These MPs are present in cleaning products, synthetic clothes, and in cosmetic products such as shampoos, facial creams, deodorants, baby products, lotions, makeup, and sunscreen. On the other hand, secondary MPs are small plastic particles derived from the breakdown of larger plastic debris. Over time, when disposed of in the environment, plastic debris goes through physical, biological and chemical processes that can reduce its structural integrity resulting in its fragmentation into smaller particles. Secondary MP is mostly formed in the beaches and intertidal zones due to a greater incidence of ultraviolet light (UV), which causes oxidation of plastic material, weakening its structure, and physical degradation by wave turbulence (GESAMP, 2014).

What are polychaetes?

Globally 12,530 were recorded and out of it, 1142 were reported from Indian coastlines (WoRMS, 2020; Nilesh A et al., 2022). Polychaetes (bristle worms) is an economically important organism used as fish bait and even humans consume it in some regions. They are cosmopolitan and found in different habitats from semi-terrestrial, and freshwater to marine habitats. They are usually in white or cream colour even bright coloured polychaetes are also present. They have segmented bodies, each segment bears parapodia (projections on parapodia called chaetae) which act as locomotory organs, head or prostomium containing mouth, eyes (complex in few), antennae (palps in some), statocyst and nuchal organs. Some polychaetes produce toxins to catch prey and a few exhibits bioluminescence. These organisms live in burrows in mud or tubes they made. Many polychaetes are sessile and tube-dwelling forms, often gregarious, which create primary biogenic structures or modify the abiotic characteristics of the pre-existing substrate. They act as real builders of forest-like structures that offer new substrates for other benthic species, affect habitat conditions and regulate ecosystem functioning (Giangrande et al., 2020). Polychaetes carry certain features that help in the adaptation for survival. They are known to secrete mucus protecting themselves within peculiar habitats (Kokkadan et al., 2023). As per the feeding guild scheme

(Fauchald and Jumars., 1979) polychaetes are omnivores, herbivores, carnivores, filter feeders, surface deposit feeders and burrowers. They are hermaphroditic, but also many species exhibit in-between sexual characteristics. Both the males and females show multiple sexual phenotypes. In the protandric polychaete *Ophryotrocha puerile puerilis*, in which an individual may repeatedly change sex, both members of a reproducing pair may simultaneously change sex after several spawning events (Berglund. 1986). Sexual reproduction (eggs hatch into trochophore larvae and metamorphosis into an adult worm) is common; also, asexual reproduction (budding) is reported in some species. Polychaetes show a great diversity in reproductive traits, but some patterns can be found, and the families can be grouped within long-living iteroparous forms, semelparous forms, and short-living iteroparous forms, the first being the most common strategy. Adults can range in length from a fraction of a millimetre to well over 6 metres. (Rouse et al., 2001). They have symbiotic and commensalic interactions with several organisms including sponges, gastropods, bivalves and other polychaetes. Polychaete can regenerate posteriorly. Anterior regeneration is present in varying degrees (Hyman et al., 1940).

Importance of polychaetes in marine ecosystem.

Polychaetes constitute the major population of macro invertebrates in marine ecosystems. Thus, stress or pollution affects the abundance of the organisms in that habitat. They are used as indicators of marine health. They respond to disturbances induced by different kinds of pollution and exhibit quantitative changes in assemblage distributions (Díaz-Castañeda et al., 1989). They can tolerate different environmental parameters such as salinity, temperature, and dissolved oxygen. Polychaetes play a major role in benthic communities, in terms of recycling and reworking benthic sediments, bioturbating sediments and in the burial of organic matter (Diaz et al., 2009). Polychaetes are major controllers of sediment ecosystems and their mixing of sediment particles is an important driving force behind chemical reactions and transport of organic matter in marine sediments (Levinton, 1995). They are used as bioindicators in detecting and monitoring microbial pollution, as well as potential bioremediation by reducing pollution in the water column (Diaz et al, 2009). Polychaetes have an important role in the food web; it is food for other invertebrates, fishes and birds. Filter-feeding polychaetes are biofilters, which filter water and in turn, provide clean water. Polychaetes are less sensitive to toxic chemicals and several species were used in toxicological tests.

Microplastic pollution in the marine environment and the threat posed by it on the biota and to the functioning of the ecosystem forms a prime area of research in recent decades worldwide. Currently, almost all parts of the ocean basins face the threat of plastic pollution irrespective of developed or underdeveloped regions in the world (Thushari and Senevirathna, 2020). Plastic litter in aquatic ecosystems are often categorized as megaplastic (>1 m), macroplastics (2.5 cm–1 m), mesoplastics (5 mm–2.5 cm), microplastics (1 µm –5 mm), and nanoplastics (< 0.1 µm) (Kershaw et al., 2019, Thushari and Senevirathna, 2020). Among the varied size categories, plastics that greatly impact the marine ecosystem and its biota are microplastics of size less than 5 mm. They are often transported to the marine environment as primary and secondary microplastics. Several studies have demonstrated the ingestion of microplastics by both vertebrates including sharks, turtles, seabirds and invertebrates such as squid, clams, mussels, abarnacle, lobster, shrimps, oysters have already been reported from many parts of the world ocean.

Microplastic ingestion and its consequences on benthic invertebrates have been investigated under laboratory conditions with lethal to sub-lethal consequences in various species (Murray and Cowie, 2011). The difference in toxicity between microplastic beads and fibres in *Hediste diversicolor* (marine benthic dwelling polychaete worm) was investigated. *Ophryotrocha veronica* (marine benthic dwelling polychaete worm) exposed to plastics produced fewer offspring and significantly smaller eggs than unexposed mating pairs, which ultimately could lead to deleterious impacts at the population level. Polychaetes that were able to burrow in abandoned expanded polystyrene (EPS) buoys on the coast of South Korea as their habitat, where they would both consume and produce a significant amount of microplastics. It was estimated that a single EPS-dwelling polychaete can produce hundreds of thousands of microparticles per year, up to 50% for polychaetes maintained in sediments spiked with microplastics (unplasticized polyvinylchloride, UPVC) in laboratory tests. The results indicate that depleted energy reserves arise from a combination of reduced feeding activity, longer gut residence times of ingested material and inflammation (Wright et al., 2013). In the commercially important polychaete, *Perinereis aibuhitensis*, the presence of microplastics increased mortality and reduced the rate of posterior segment regeneration. The impact of the micro-polystyrene beads was size-dependent with smaller beads (8-12µm in diameter) being more detrimental than those bigger (32-38µm). Microplastics reduced the posterior segment regeneration rate of the polychaete *Perinereis aibuhitensis* (Marine Pollution Bulletin, 129(2), 782–786.). The effects of polystyrene (PS) microplastic on survival, activity, and body weight, as well as the transfer of 19 polychlorinated biphenyls (PCBs), were assessed in bioassays with *Arenicola marina* (L.). PS was pre-equilibrated in natively contaminated sediment. A positive relation was observed between microplastic concentration in the sediment and both the uptake of plastic particles and weight loss by *A. marina.* Furthermore, a reduction in feeding activity was observed in Leung, J., & Chan, K. Y. K. (2018). In general, information on the occurrence of microplastic in benthic fauna is relatively scarce compared to pelagic species.

Microplastics of size less than 5 mm are often transported to the marine environment as primary and secondary microplastics (Cole et al., 2011). Microplastic ingestion was evident in the polychaete community during both dry and wet periods of 2019 and 2021. The study indicating more microplastic ingestion by the polychaete community signifies the increasing intensity of plastic pollution. The experimental studies on the effects of elevated water temperatures on life-history variation in shell-boring polychaetes are rare, studies investigating the effects of CO_2-induced acidification are virtually non-existent. Several revealed acidification on free-living and benthic polychaetes over the last decade, probably because of their role as important bioindicators. These studies have found mixed results concerning tolerance, but key findings include elevated metabolic rate increased oxidative stress, and reduced sperm motility in polychaetes that were subjected to hypercapnic conditions. In one of these studies, Calosi et al. (2013), using in situ transplant studies, found that *Platynereis dumerilii* (family Phyllodocidae) populations living in CO_2 vents were more physiologically tolerant to elevated pCO_2 levels than nearby populations adapted to significantly lower pCO_2 conditions. In another study with three different species, *Arenicola marina* (family Arenicolidae), *Pomatocercos* (=*Spirobranchus*) *lamarcki* (family Serpulidae), and *Galeolaria caespitosa* (family Serpulidae), sperm motility was significantly reduced during short-term elevated pCO_2 exposure. This is concerning because sperm motility in marine invertebrates is an important factor influencing fertilization success and, should it be compromised because of climate change, would result in lower recruitment success. Finally, Díaz-Castaneda et al. (2019) found that the calcareous tubes of two serpulid polychaetes, *Spirorbis sp*. and *Spirobranchus triqueter*, were more fragile from habitats that had lower pH (~0.3 units below), as a result of dissolution of the calcite and aragonite foundation (similar to what occurs in bivalves and shelled gastropods), providing the first evidence that the structural integrity of polychaete tubes can be compromised in a more acidic ocean. Until similar studies on spionids are carried out, the data on serpulids, which are more evolutionarily related to spionids than any other polychaete family (Struck et al., 2011), provide the best insights into the responses of polydorids to acidification.

Interactive effects of climate change on polychaete-molluscan symbiosis.

As ocean temperatures rise, faster developmental rates and shorter PLD times (resulting in higher probability of local recruitment) of polydorid larvae, coupled with increases in asexually reproducing propagules, means that shellfish infestation will potentially increase. It was found that water temperature was one of two major factors affecting population growth, recruitment, and infestation intensity of shell-boring polychaetes on farmed abalone (*Haliotis midae*) in South Africa. This problem is exacerbated by the fact that generational times are much shorter for polydorids, which can reach sexual maturity from egg to adult in fewer than six weeks for planktrophic brooders (David et al., 2014) compared to *Crassostrea gigas,* whose initial maturation time has been reported as being from four to six months, although this can vary based on population and latitude. Increased ocean acidification is expected to negatively affect the reproductive capacity (e.g., fecundity and sperm motility) of both annelids and molluscs (Lucey et al., 2015), who showed that brooding polychaetes are more tolerant of acidifying conditions than their broadcast-spawning cohorts). While the calcareous tubes of free-living polychaetes (e.g., serpulids) exhibit increased fragility under acidified conditions, polydorids utilize the calcareous matrix of their shellfish hosts to form their tubes, meaning that the condition of their tubes is directly intertwined with shell condition. All previous studies that have explicitly explored the boring mechanism of polydorids have found that worms never burrow through the nacreous layer into the inner layer of the shell. This is due to a rapid calcification response by the mollusc, which adds an extra layer of the shell. If shell dissolution overwhelms carbonate deposition, as has been reported in several studies, adding extra layers as a defence against infestation will become more difficult, allowing worms to burrow closer to the mantle than was previously possible. The overall result is increased physiological stress on shellfish, ultimately resulting in lower growth rates and higher mortalities in both farmed and wild stock. When oyster shells were subjected to extreme drops in pH, they showed a reduced susceptibility to infection by *P. websteri.* The researchers hypothesized either that acidic conditions may have dissolved the softest parts of the shells, with the hardest layers being inaccessible to the worms, or that the worms preferentially avoided more brittle shells—the latter of which is very possible, considering that shell-boring polydorids do exhibit substrate preferences.

Conclusion

Polychaetes play an important role in marine ecosystems. Its burrowing, tube-making habit and feeding behaviour accelerates nutrient cycling, water filtration and organic waste decomposing. Overall, polychaetes help in maintaining the health of marine ecosystems. It is also food for several invertebrates and vertebrates including humans. Microplastic contamination is increasing day by day and become a threat to every organism. Polychaetes are the dominant group of macrobenthic organisms. That's why; they become indicators of marine health. Many polychaetes are tolerated to temperature, salinity, dissolved oxygen amount and even for heavy metals. Even though, many of them died of microplastic ingestion. Polychaetes are a less investigated group of organisms. As this, microplastic contamination persists, polychaetes, nature's friend become extinct before discovering much about them. So, further environmental and conservation-oriented studies are needed as much as possible to help these organisms thrive and also to maintain the marine ecosystem balanced.

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Understanding Chemical Oceanography: Current Perspectives and Emerging Trends

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Abstract

Chemical oceanography is a branch of oceanography that explores the chemical composition of seawater and investigates the processes that govern the distribution and transformation of chemical elements and compounds in the ocean. The discipline encompasses a wide range of topics, including the study of major and trace elements, nutrients, gases, and the interactions between the ocean and the atmosphere. Researchers in chemical oceanography aim to understand the sources and sinks of various chemical species, the impact of biological activities on ocean chemistry, and the role of the ocean in global biogeochemical cycles.

Key areas of study within chemical oceanography include the examination of seawater's salinity, pH, and dissolved gas concentrations. Investigations into nutrient cycling, such as the distribution of nitrogen, phosphorus, and silicon, are crucial for understanding the factors influencing marine productivity and ecosystem dynamics. Additionally, researchers explore the impact of anthropogenic activities on ocean chemistry, including issues such as ocean acidification resulting from increased carbon dioxide levels in the atmosphere.

Chemical oceanography plays a pivotal role in advancing our understanding of the complex interactions between the ocean, atmosphere, and marine life. The knowledge gained from these studies contributes not only to scientific understanding but also to environmental management and policy development, particularly in the context of climate change and sustainable

resource utilization. Through interdisciplinary collaborations, chemical oceanographers strive to unravel the intricacies of the ocean's chemical makeup, providing valuable insights into the Earth's interconnected systems.

Introduction

Chemical oceanography is a branch of oceanography that focuses on the chemical composition and processes occurring within the Earth's oceans. It encompasses the study of various chemical elements, compounds, and cycles that influence the properties and dynamics of seawater. Understanding chemical oceanography is crucial for gaining insights into the complex interactions between the ocean, atmosphere, and marine life, as well as the impact of human activities on marine ecosystems.

Key aspects of chemical oceanography include the distribution of elements and compounds in seawater, the factors influencing their concentrations, and the biogeochemical cycles that govern their movement within the ocean. This field explores the sources and sinks of different chemical species in the ocean, as well as the physical and biological processes that drive these distributions (Smith and Johnson, 2019).

Several fundamental concepts are integral to chemical oceanography:

Salinity: The concentration of dissolved salts in seawater is a critical parameter. Salinity affects the density and properties of seawater, influencing ocean circulation patterns.

Nutrient Cycling: The cycling of essential elements like nitrogen, phosphorus, and carbon is vital for the growth of marine organisms. Understanding nutrient dynamics is crucial for predicting changes in marine ecosystems.

Trace Elements: Studying trace elements provides insights into processes such as mineral weathering, sedimentation, and biological activity. Some trace elements are essential for marine life, while others can be pollutants.

pH and Ocean Acidification: The pH of seawater influences the availability of carbonate ions, which is essential for marine organisms that build shells and skeletons. Ocean acidification, resulting from increased carbon dioxide absorption, poses a threat to marine life(Smith, 2020).

Redox Chemistry: The distribution of oxygen and other redox-sensitive species in seawater is crucial for understanding the microbial processes occurring in different marine environments.

Chemical oceanographers employ various techniques, including sampling, laboratory analyses, and remote sensing, to collect data on the composition of seawater. They conduct research in diverse marine environments, from coastal zones to deep ocean trenches, to unravel the complexities of the chemical processes occurring in the ocean.

As global environmental changes continue, chemical oceanography plays a vital role in monitoring and predicting alterations in ocean chemistry and understanding the implications for marine ecosystems and the planet as a whole(Anderson,2020).

Chemical Composition of Seawater

The chemical composition of seawater is complex and consists of a variety of dissolved substances. Here are some of the major components found in seawater:

Water (H_2O): Water is the primary component of seawater, constituting about 96.5% of its mass.

Salts (mainly NaCl): Salts are the most abundant solutes in seawater. Sodium chloride (NaCl) is the dominant salt, making up approximately 85% of the total salts by weight.

Other Ions: Seawater contains various other ions, including magnesium (Mg^{2}), sulfate (SO_4^{2-}), calcium (Ca^{2}), and potassium (K). These ions contribute to the overall salinity of seawater (Johnson,2018)

Gases: Dissolved gases in seawater include oxygen (O_2), carbon dioxide (CO_2), nitrogen (N_2), and trace amounts of other gases. The concentration of gases can vary with depth and location.

Trace Elements: Seawater contains trace amounts of numerous elements, including iron, manganese, copper, zinc, and others. These trace elements play essential roles in biological processes.

Organic Compounds: Dissolved organic matter, including carbon-based compounds, is present in seawater. This includes substances like dissolved organic carbon (DOC) and various organic molecules produced by marine organisms.

Nutrients: Seawater contains essential nutrients for marine life, including nitrogen compounds (nitrate, nitrite, and ammonia), phosphorus compounds, and silicon. These nutrients are critical for the growth of phytoplankton and other marine organisms.

pH: The pH of seawater is typically around 8.1, making it slightly alkaline. However, this can vary depending on factors such as location and depth.

It's important to note that the chemical composition of seawater can vary in different oceanic regions and at different depths. Oceanographers use various methods, including sampling and laboratory analyses, to measure and understand the chemical properties of seawater. Ongoing research is conducted to monitor changes in seawater chemistry due to natural processes and human activities, such as climate change and pollution.

Major and Minor Constituents

Ionic Constituents:

Seawater is a solution of various ions, which are electrically charged atoms or molecules. The primary ions in seawater include:

Sodium (Na^+) and Chloride (Cl^-): These ions are the dominant components of seawater, making up approximately 85% of the total ions by weight. The main salt in seawater is sodium chloride (NaCl). (Millero *et al*., 2008)

Magnesium (Mg^2) and Sulfate (SO_4^{2-}): These ions are also significant constituents of seawater, contributing to its overall salinity.

Calcium (Ca^2) and Potassium (K): These ions are present in smaller concentrations but still play important roles in the chemistry of seawater Elderfield Schultz (1996).

Major Constituents:

The major constituents of seawater are the components that are present in relatively high concentrations. In addition to the ionic constituents mentioned above, major constituents include:

Water (H_2O): Water is the most abundant component, making up about 96.5% of the mass of seawater.

Dissolved Gases: Oxygen (O_2) and carbon dioxide (CO_2) are the most important dissolved gases in seawater. The concentration of gases can vary with depth and location. (Millero, 2005).

Minor Constituents:

Minor constituents are present in seawater in trace amounts, yet they are essential for various biological and chemical processes. These include:

Trace Elements: Elements like iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and others are present in trace amounts. Despite their low concentrations, these elements are crucial for the growth and functioning of marine organisms. (Bruland *et al*., 2014).

Nutrients: Nitrogen compounds (nitrate, nitrite, and ammonia), phosphorus compounds, and silicon are considered nutrients. They are essential for the growth of phytoplankton and other marine organisms(Karl *et al*., 2002).

Dissolved Organic Matter: Seawater contains dissolved organic compounds, including dissolved organic carbon (DOC) and various organic molecules produced by marine life(Carlson and Hansell, 2015).

Understanding the distribution and variations of these constituents in seawater is fundamental to the field of chemical oceanography. Researchers use sophisticated techniques to measure and analyze the concentrations of different components, helping to unravel the complexities of marine chemistry and its impact on the broader Earth system.

Constancy of Constituents

Constancy of Ionic Compositions: The constancy of ionic compositions in seawater refers to the relatively stable concentrations of major ions over large spatial and temporal scales. This constancy is a result of dynamic equilibrium processes that maintain a balance between input and output fluxes of ions (Broecker, 2003).

Factors Affecting Constancy:

Conservative Behavior: Some ions exhibit conservative behavior, meaning their concentrations are relatively stable and less influenced by biological processes or precipitation/dissolution reactions. Sodium (Na) and chloride (Cl^{-}) are classic examples of conservative ions.

Biological Processes: Biological activities, such as photosynthesis and respiration by marine organisms, can influence the concentrations of ions, particularly nutrients like nitrate (NO_3^{-}), nitrite (NO_2^{-}), and phosphate (PO_4^{3-}). These processes are essential in the marine nutrient cycle.

Ionic Interactions: Ionic interactions and chemical reactions between different ions can impact their concentrations. For example, the carbonate system involves interactions between carbonate (CO_3^{2-}), bicarbonate (HCO_3^{-}), and hydrogen ions (H), affecting the pH of seawater.(Hansell and Carlson, 2015)

Redox Processes: Redox reactions, involving the gain or loss of electrons, can influence the concentrations of certain ions. For instance, the redox cycling of elements like iron (Fe) can affect their distribution and concentration (Luther, 2010).

Ocean Circulation: The movement of water masses within the ocean, including horizontal currents and vertical mixing, helps distribute ions and maintain their relative proportions. This ocean circulation plays a key role in the constancy of ionic compositions.

Climate and Weathering: Climate-related factors, such as temperature and precipitation patterns, influence weathering processes on land. Weathering contributes to the input of ions into the ocean, affecting their concentrations.

Anthropogenic Influences: Human activities, such as industrial discharges and pollution, can introduce additional elements and compounds into seawater, potentially disrupting the natural constancy of ionic compositions in localized areas.

Natural Geological Processes: Geological processes, including seafloor spreading, volcanic activity, and sedimentation, contribute to the overall mineral composition of seawater.

Distribution and Importance of Elements

Major Elements: Major elements in seawater are those that occur in relatively high concentrations. The primary major elements include:(Bruland and Lohan, 2003).

Sodium (Na): The most abundant cation in seawater.

Chloride (Cl): The most abundant anion in seawater, forming common salt (NaCl).

Magnesium (Mg): Occurs in significant quantities, influencing seawater density.

Sulfate (SO_4): A major anion in seawater.

Calcium (Ca): Important for the formation of calcium carbonate ($CaCO_3$) structures, such as shells and corals.

Potassium (K): Present in seawater, but in lower concentrations compared to sodium.

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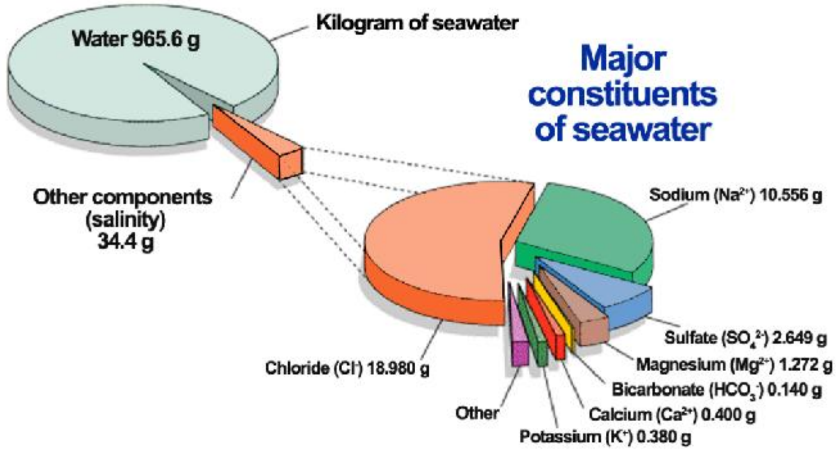

Image source: https://salinity.oceansciences.org/learn-more.htm?id=54

Minor Elements: Minor elements are present in seawater in lower concentrations than major elements but are still essential for various biological and chemical processes:

Strontium (Sr): Plays a role in the formation of marine organisms' skeletons and shells.

Bromine (Br): Occurs in trace amounts and is involved in biological processes.

Boron (B): Essential for the growth of marine plants.

Fluoride (F): Present in minor amounts.

Trace Elements: Trace elements are present in very low concentrations, but they are crucial for biological activity and serve as indicators of various oceanic processes:

Iron (Fe): Essential for phytoplankton growth; its distribution influences primary productivity.

Zinc (Zn): Important for enzymatic processes in marine organisms.

Copper (Cu): Essential for photosynthesis in algae.

Manganese (Mn): Involved in redox reactions and nutrient cycling.

Cobalt (Co): Critical for nitrogen fixation in certain marine bacteria.

Importance of Major, Minor, and Trace Elements

Biological Processes: Major, minor, and trace elements are essential for the growth and development of marine organisms. For example, calcium and carbonate ions are crucial for the formation of shells and skeletons.

Nutrient Cycling: Elements like nitrogen, phosphorus, and silicon, though present in smaller amounts, are vital for nutrient cycling, influencing primary productivity in the ocean.

Redox Reactions: Some trace elements participate in redox reactions, influencing the chemistry of seawater and the availability of nutrients.

Distribution of Elements:

Spatial Variation: The distribution of elements in seawater can vary spatially based on factors such as proximity to coastlines, upwelling zones, and hydrothermal vent areas.

Vertical Variation: The concentration of elements can also vary with depth, with certain elements showing gradients in the water column.

Global Ocean Circulation: Ocean currents play a crucial role in distributing elements throughout the global ocean, influencing their concentrations in different regions.

Biological Uptake: The uptake of elements by marine organisms, especially phytoplankton, influences their distribution. For example, iron is a limiting factor for primary productivity in certain regions.

Chemistry of Seawater Constituents

The chemistry of seawater is complex and involves a variety of constituents, including major ions, minor elements, and trace elements. Here is a brief overview of some key constituents and their roles in seawater:

Major Ions: Sodium (Na) and Chloride (Cl^{-}): These ions are the most abundant and constitute about 85% of the total ions in seawater. The combination of Na^{+} and Cl^{-} forms common salt (NaCl), the predominant salt in seawater.

Magnesium (Mg^{2}), Sulfate (SO_4^{2-}), Calcium (Ca^{2}), and Potassium (K): These ions also contribute significantly to seawater salinity and play important roles in various marine processes.

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Minor Elements:

Strontium (Sr): Plays a role in the formation of marine organisms' skeletons and shells.

Bromine (Br): Occurs in trace amounts and is involved in biological processes.

Boron (B): Essential for the growth of marine plants.

Fluoride (F): Present in minor amounts.

Trace Elements:

Iron (Fe): Despite being present in trace amounts, iron is a crucial micronutrient for marine phytoplankton and influences primary productivity.

Zinc (Zn), Copper (Cu), Manganese (Mn), Cobalt (Co), etc.: Essential trace elements for various biological processes in marine organisms.

Nutrients:

Nitrate (NO_3^-), Nitrite (NO_2^-), Ammonium (NH_4^+), Phosphate (PO_4^{3-}): These are essential nutrients for marine plants, particularly phytoplankton, and play a key role in the marine nitrogen and phosphorus cycles.

Dissolved Gases:

Oxygen (O_2) and Carbon Dioxide (CO_2): These gases are crucial for the respiration and photosynthesis of marine organisms. The solubility and concentration of these gases vary with temperature and pressure.

Dissolved Organic Matter:

Dissolved Organic Carbon (DOC): Represents the organic compounds dissolved in seawater, including complex molecules derived from the decay of marine organisms.

pH and Alkalinity:

Hydrogen Ion Concentration (H): Determines the acidity or alkalinity of seawater. Alkalinity is often maintained by the carbonate buffer system.

Redox-Sensitive Species:

Oxygen Minimum Zones (OMZs): Certain areas in the ocean exhibit low oxygen concentrations due to microbial respiration, leading to the presence of redox-sensitive species.

The distribution of these constituents varies spatially and temporally in response to factors such as ocean currents, biological activity, and geological processes. Ongoing research helps deepen our understanding of the intricate chemistry of seawater and its vital role in global biogeochemicalcycles.

Compound	Concentration,g/L
NaCl	24.53
MgCl	5.2
NaSO	4.09
CaCl	1.16
KCl	0.695
NaHCO	0.201
KBr	0.101
HBO	0.027
SrCl	0.025
NaF	0.003
Ba(NO	9.94E-05
Mn(NO	3.40E-05
Cu(NO	3.08E-05
Zn(NO	9.60E-06
Pb(NO	6.60E-06
AgNO	4.90E-07

Chemical composition of sea water reported by Uy and Dungca (2018)

Concept of Chlorinity and Salinity

Chlorinity: Chlorinity is a concept in oceanography that represents the concentration of chloride ions (Cl^-) in seawater. It is expressed in parts per thousand (ppt) or practical salinity units (psu). Chlorinity was initially introduced as a measure of the chloride content in seawater. The determination of chlorinity involves analyzing the concentration of chloride ions through various methods, including titration with silver nitrate(Riley and Skirrow, 1975).

Chlorinity is related to the salinity of seawater, but it specifically focuses on the chloride ion. The relationship between chlorinity and salinity is given by the chlorinity-salinity relationship, which takes into account the contributions of other major ions (sodium, sulfate, magnesium, and calcium) to the total salinity. Today, salinity is more commonly used as a measure of the total dissolved salts in seawater, but chlorinity remains a valuable parameter in oceanographic studies.

Salinity: Salinity is a measure of the total dissolved salts in seawater, expressed in parts per thousand (ppt) or practical salinity units (psu). It provides an indication of the ocean's saltiness and is a fundamental parameter in oceanography. Salinity is influenced by the major ions present in seawater, primarily chloride, sodium, sulfate, magnesium, and calcium(Millero, 2005).

The practical salinity scale, adopted by the oceanographic community, is based on conductivity measurements, providing a standardized measure of salinity. Modern instruments like conductivity-temperature-depth (CTD) sensors are commonly used for in-situ measurements of salinity.

Salinity is a crucial parameter in understanding ocean circulation, water mass formation, and the global distribution of heat and freshwater. It affects the density of seawater, influencing its behavior in the ocean's vertical and horizontal movements.

In summary, chlorinity is a specific measure of chloride ions, while salinity is a broader measure of the total dissolved salts in seawater. The relationship between chlorinity and salinity is established through the chlorinity-salinity relationship, considering the contributions of various major ions to the overall salinity of seawater. Both chlorinity and salinity play essential roles in oceanographic research and the study of marine environments.

Methods of Measurement

Mohr's Method: A titration method involving the use of silver nitrate to precipitate chloride ions as silver chloride(McDougall and Barker, 2011).

Ion-Selective Electrodes: Modern instruments, such as ion-selective electrodes, measure the potential difference between a reference electrode and a chloride-selective electrode (Grasshoff*et al*., 2007).

Conductivity and Temperature: Salinity can be estimated indirectly by measuring the electrical conductivity of seawater, which is influenced by the concentration of dissolved salts. This method is often used in conductivity-temperature-depth (CTD) probes (Cullen and MacIntyre,2016).

Gravimetric Methods: Involves evaporating a known volume of seawater to dryness and weighing the remaining salts.

Nutrients: Nutrients in seawater refer to essential elements and compounds necessary for the growth and survival of marine organisms. The main nutrients include:

Nitrate (NO_3^-), Nitrite (NO_2^-), and Ammonium (NH_4^+): Critical for the growth of phytoplankton and other marine plants.

Phosphate (PO_4^{3-}):Essential for the synthesis of DNA, RNA, and ATP in marine organisms.

Silicate (SiO_4^{4-}):Required for the formation of diatom skeletons, a type of phytoplankton.

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Dissolved Inorganic Carbon (DIC): Includes carbon dioxide, bicarbonate, and carbonate ions, vital for photosynthesis and calcification (Johnson*et al*., 2017).

Methods of Measurement for Nutrients:

Colorimetric Methods: Nutrients react with specific reagents to produce colour changes, and the intensity of the color is proportional to the nutrient concentration.

Fluorometric Methods: Nutrients can be measured based on their fluorescence properties when exposed to specific wavelengths of light(Murphy and Riley, 1962).

Autoanalyzers: Automated systems that allow for the continuous and simultaneous measurement of multiple nutrient concentrations in seawaterParsons (Maita an Lalli, 1984).

Gas Chromatography/Mass Spectrometry (GC/MS): Used for measuring dissolved gases like nitrous oxide and methane, which are relevant in the nitrogen cycle (Weiss, 1974).

Conclusion

In conclusion, chemical oceanography is a dynamic and interdisciplinary field that plays a crucial role in understanding the complex interactions between the ocean and various chemical processes. Through extensive research and advancements in analytical techniques, scientists have gained valuable insights into the composition, distribution, and cycling of elements and compounds in the marine environment.

Chemical oceanography not only contributes to our understanding of basic oceanic processes but also has significant implications for broader issues such as climate change, nutrient cycling, and marine ecosystem health. The study of ocean chemistry is essential for predicting and mitigating the impact of human activities on marine ecosystems, including pollution, acidification, and the depletion of essential nutrients.

As we continue to face environmental challenges and seek sustainable solutions, the insights gained from chemical oceanography will be crucial for developing informed policies and management strategies to protect and preserve the health of our oceans. Continued collaboration and advancements in technology will further enhance our ability to unravel the complexities of chemical processes in the ocean, contributing to a more comprehensive and holistic understanding of the marine environment.

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An introduction to Sea grass

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Introduction

Sea grasses the word itself determines that it belongs to a coastal environment where the grasses grows in the seanear the submerged photic zone. Photic zone is nothing but the sea grass grows in a region where light penetration occurs to particular level of the ocean. Sea grasses mostly found in shallow salty and brackish water zones in India and across all parts of the world from tropic to arctic regions. These sea grasses are mainly found in bays, coastal water, estuaries, and shallow inshore areas upto 60 to70 meters in length. Sea grasses grow widely from a region of few square meters to hundreds of square kilometers.The peculiarity of sea grass is that the appearance of sea grass was similar to terrestial grasses which are green in color and bear root, leaves and produce flowers and seeds. Sea grasses can form underwater meadows which are densely submerged in oceanic region.The underwater meadows are greatly highlighted from satellite point of view is an addition viewpoint. The great benefit of the sea grass is that it improves the quality of sea water by binding the sediments for its vital growth, absorbing nutrients with the help of its anchorage system, filtering the marine water system and provides a strong protection against coastal erosion.

Sea grasses are an important organic biomass resource that exhibits wide interannual variation in tropical and temperate zones and stability is good at the equator. Other primary producers, such as macro algae and epiphytic algae, typically increase the primary productivity of these environments. Many food webs are based on the copious detritus plant debris found in sea grass meadows. Furthermore, the intricate three-dimensional structure of the sea

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grass bed changes the direction and intensity of water currents, binds sediments, and offers cover and shelter (Short *et al*., 2016).

Layers of the ocean

There are aboutfive different layers of ocean based on the intensity of light penetration (Fig.1). Sea grasses grow most widely in epipelagic or Photic or sunlight zone of ocean where sunlight is abundant for photosynthesis to be performed. In this zone, heat from the sunlight shows wide variations in temperature due to seasonal effects and latitude of the region. The surface temperature of sea in India is as high as 24 to 28° C (U.S Department of Commerce).

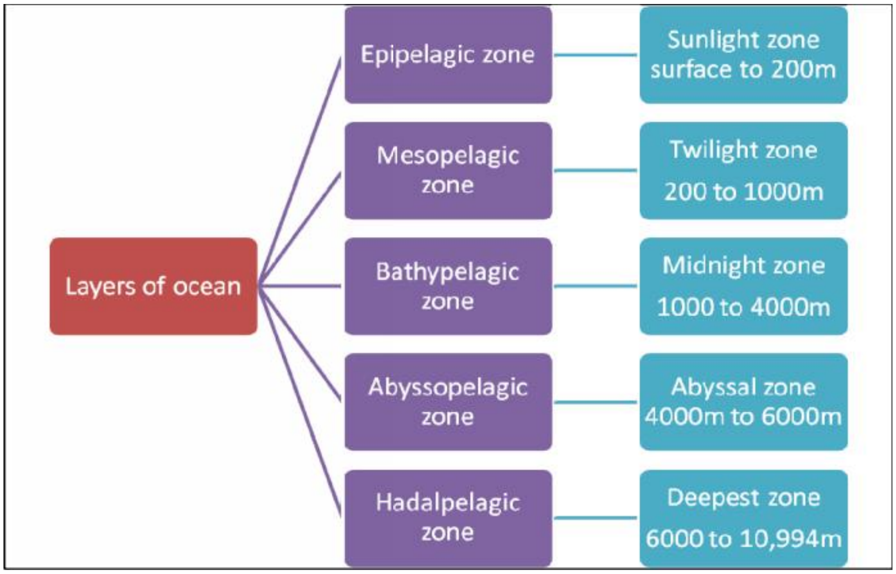

Fig.1. Layers of Ocean

History of Sea grass

The only successful angiosperm organism present in the sea was sea grasses. First person to use the term sea grass was Ascherson in 1871. The evolution of terrestrial plants is from marine algae to Bryophytes to Pteridophytes to Gymnosperms and finally angiosperms the flowering plants whereas the sea grass is evolved from the angiosperm plants that recolonized the ocean 100 million years ago (Fig. 2). The early monocots which have the ability to adapt in marine environment conquered as sea grass. Three independent sea grasses has direct ancestor from monocotyledonous flowering plants. The sea grasses have occupied the continental shelves of the all 7 continents and few scientists illustrate that the Antarctic region is devoid of Sea grass. In this evolutionary context different genes has been lost from angiosperm plant and moreover many genes in sea grasses either reduced or regained depending upon the environmental sustainability.

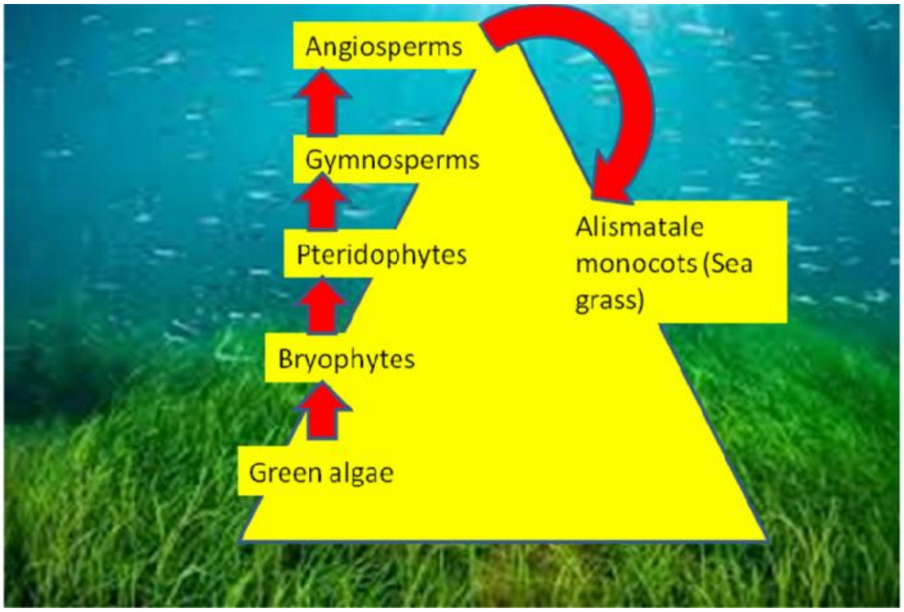

Fig.2. Evolutionary History of Sea grass

Distribution of Sea grass

Six sea grass bioregions are found throughout the world: two tropical and four temperate. The Mediterranean, the Temperate Southern Ocean, the Temperate North Atlantic, and the Temperate North Pacific are among the temperate bioregions. The Tropical Atlantic and Tropical Indo-Pacific areas are the two tropical bioregions (Fig.3).The temperate North Atlantic has low diversity sea grass mostly present in estuaries and lagoons. Tropical Atlantic and Temperate North pacific has high diversity sea grass which grows mainly on shallow banks and coastal surf zones which possess clear water. Mediterranean Sea has deep meadows of moderate diversity of sea grass which is a mix of temperate and tropical region. In tropical Indo specific region is the highest diverse sea grass rich area which predominantly grows on reef flats and deep waters. Finally temperate southern ocean has meadows from low to high diverse regions which grow under extreme environment conditions (Green *et al*., 2003).

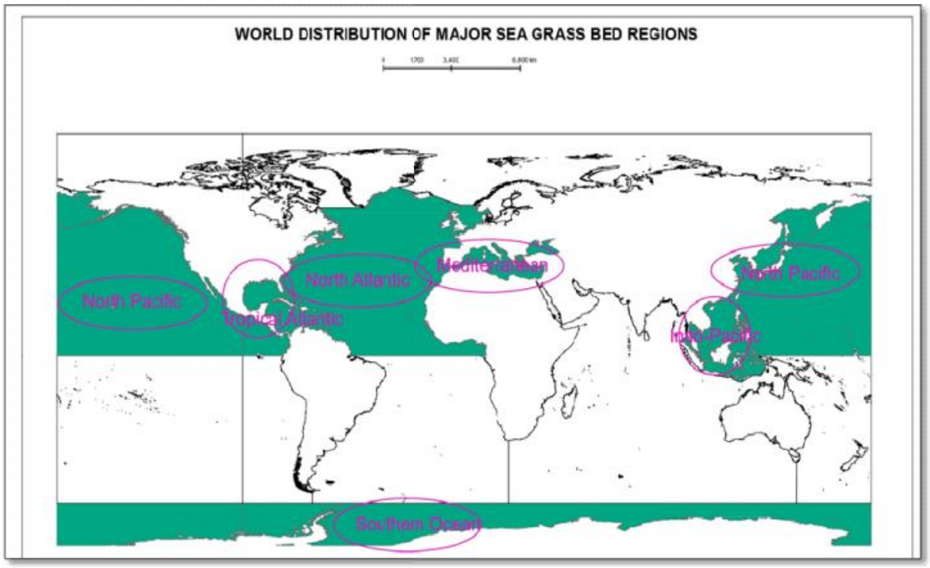

Fig.3: Distribution of Sea grass bed major regions in the world

Major seagrass beds can be found in India's coastline in the Andaman and Nicobar Islands in the Bay of Bengal, the lagoons of islands in Lakshadweep in the Arabian Sea, the Gulf of Kachchh region on the west coast, and the Gulf of Mannar and Palk Bay regions on the east coast (MOEFC, 2022), Odisha, Tamil Nadu and Gujarat (Thangaradjou, 2018) (Fig.4).

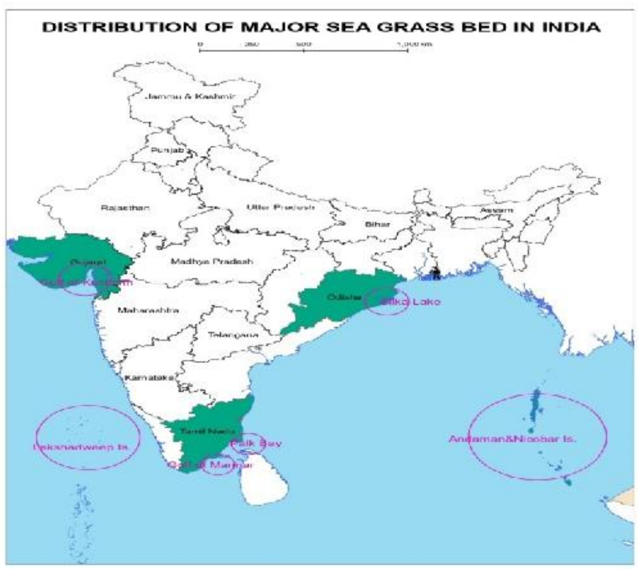

Fig.4: Distribution of Sea grass bed major regions in India

In Tamil Nadu the abundance of sea grass was found to be high in the coastal region of Adirampattinam, Mallipattinam, Rajamadam, Manora, Kattumavadi, Nambytha zhi, Thondi, tirupalakudi, Devipattinam, Pamban, Rameswaram, Dhanushkodi, Pudumadam, Keelakarai and valinokkam(Fig.5) (Newmaster *et al*., 2011).

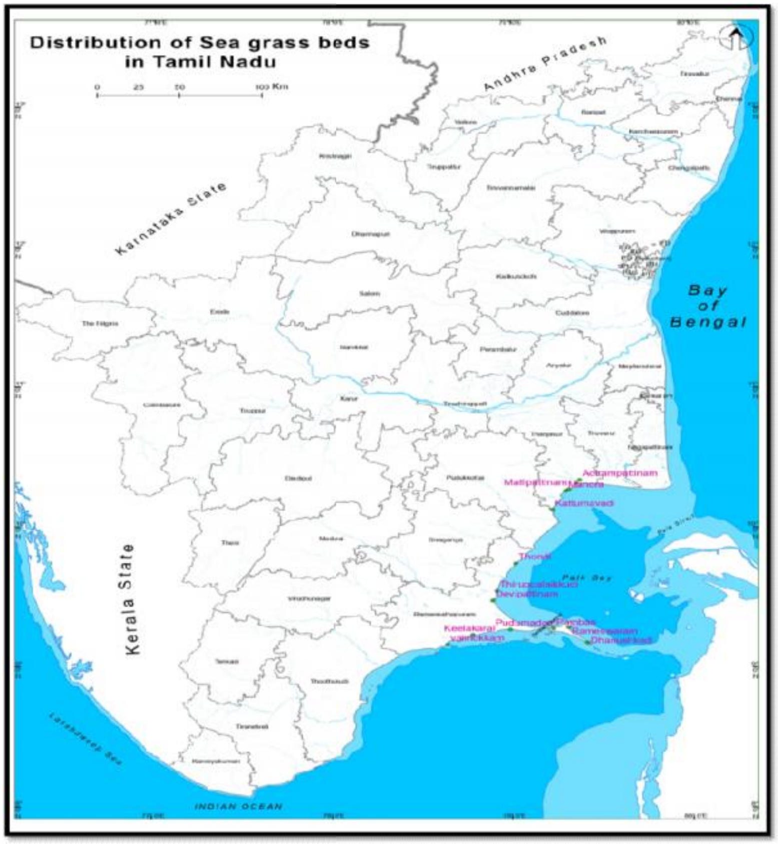

Fig.5: Distribution of Sea grass bed major regions in Tamil Nadu

General Classification of Sea grass

Sea grass classification was exclusively put forward by Cornelis Den Hartog, 2016 which has 12 different genera of angiosperms belongs to four families which are Zosteraceae, Posidoniaceae, Cymodoceaceae, and Hydrocharitaceae (Fig.6).

Fig.6. General classification of Sea grass

Morphology of Sea grass

Sea grass are herbaceous plant found exclusively in marine habitats and they are in three different forms of morphology one exhibit a pair of petiolate leaves at the rhizome and strap shape leaves are absent e.g. Halophila, next type of seagrass possess shoot with erect stem and strap shape leaves are present e.g. Thalassia and Cymodoceaceae family members and finally the sea grass without visible erect stem but rhizome possess strap shaped leaves e.g. Enhalus, Posidonia and the family of Zosteraceae.

The roots of sea grass arise from the nodes of rhizomes Depending upon the family the roots may be branched, unbranched with or without root hairs and the specificity of anchorage system substrate arrangement greatly differs from muddy, sandy substrata, rock surfaces to coral sands (Kuo, and Hartog, 2007). Rhizomes are modified stems which pass horizontally below the surface of sediment. They are leptomorphic and lacunae are present in outer cortical tissues which controls the pressure of gases in the sea to develop the sea grasses (Hemminga & Duarte, 2000).

In stem basal sheaths are present and chlorophyll is absent in epidermis which helps in protection of meristem and newly formed leaves. Leaves of sea grass has long flat blades that look like ribbons to fern or paddle-shaped leaves, cylindrical or spaghetti blades, or branching shoots (Fig. 7) chloroplast present in the leaves trap sunlight and perform photosynthesis, veins in the leaf absorb nutrients and water with small air like gaps called lacunae which help in gas exchange. The stomata are absent in sea grass but cuticle plays a dominant role in direct exchange of gases throughout the plants (Robert Orth, 2006).

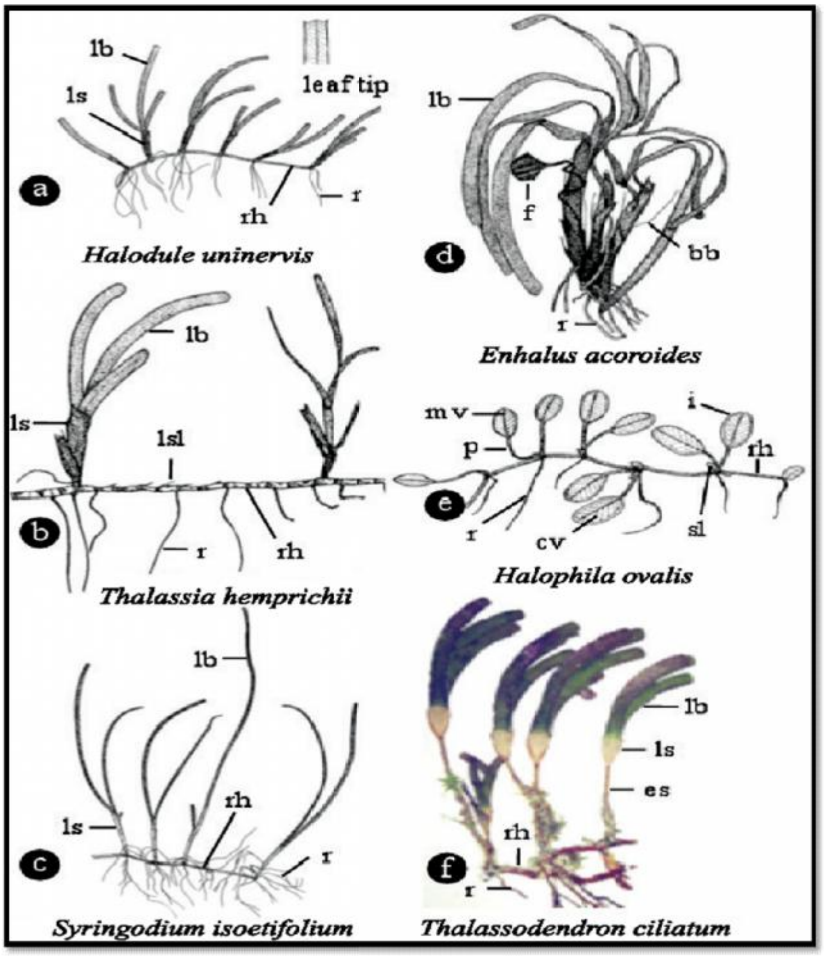

Fig.7. An overview of morphological features of Sea grass lb-leaf blade, ls-leaf sheath, rh-rhizome, r-root, lsl-leaf scale, bb-black bristle, f-fruit, mv-mid vein, p-petiole, cv-cross vein, i-intra marginal vein and es-erect stem (Pic. Courtesy: Bujang, 2012).

Reproduction of sea grass

Sea grasses are the only flowering plants that live in ocean. It reproduces unlike a terrestrial angiospermic grass by two different mechanism of reproduction. They are Vegetativeand sexual method of reproduction. The rhizome breaks into numerous fragments due to strong ocean currents or storms and each fragment moves to different places by the flow of water and gets deposited. After a week's time each fragment develops into a new oceanic plant. By this dispersal and development a mechanism of spreading genes are developed and occupies a new area (Fig. 8).

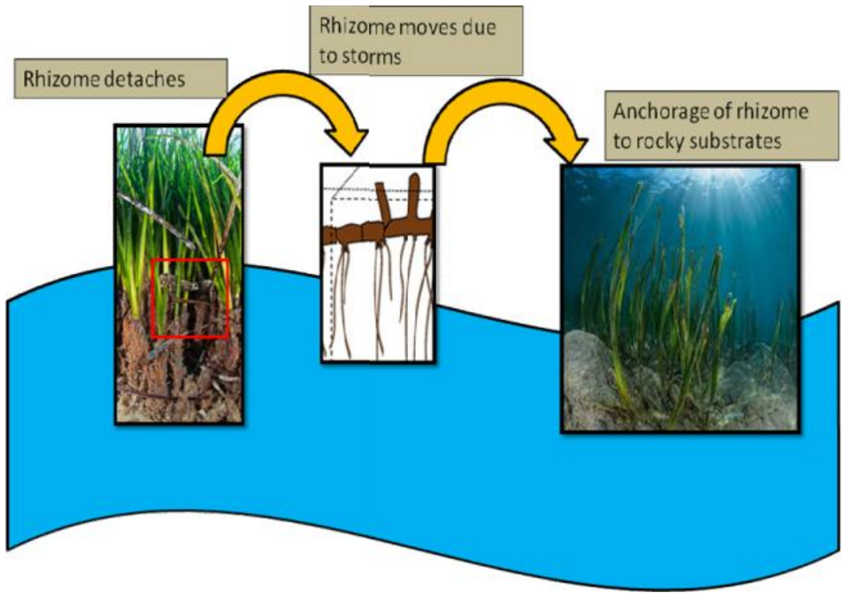

Fig.8. Vegetative method of reproduction

In sexual method of reproduction occurs due to pollination. The pollen grains are transferred via under water currents, waves and tides. The pollen released from the flower is mucilaginous in nature with thick, sticky substance. By the presence of the abiotic drives the pollen is transferred to the female reproductive organ. Most commonly these sea grasses have separate male and female flowers which are dioecious in nature. And another sexual method of reproduction is by the dispersal of seeds and formation of seed banks(Fig.9) (Inglis *et al*., 2000).

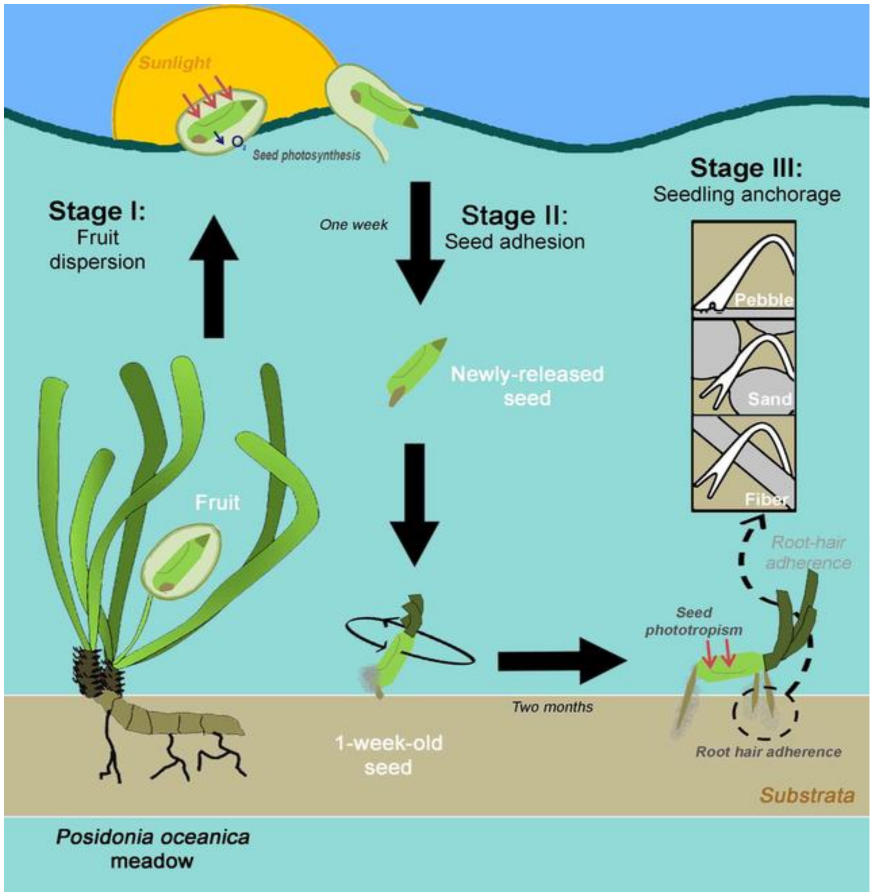

Fig. 9: Dispersion and development in sea grass genera (Laura Guerrero-Meseguer, Carlos Sanz-Lázaro and Arnaldo Marín for PLoS ONE).

Significance of Sea grass

Sea grasses are the lungs of the ocean where they provide strong ecosystem services to release oxygen to perform photosynthesis and it is estimated that the carbon di oxide present in the ocean was greatly absorbed by the underwater meadows. The quality of sea water was greatly influenced by trapping the decomposers and sediments to increase the clarity of water. Eutrophication is greatly absorbed by the meadows to prevent other aquatic animals and other micro organisms in the ocean. The rhizome development has great set back in preventing the oceans soil erosion. For many Pisces and Arthropods these sea grass act as food and plays a dominant role in food chain. Sea grasses protect the organism from large predators and strong ocean currents. It also acts as nursery sites for squids and cuttle fishes.

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Food Microbiology

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Abstract

This chapter addresses the summary of many microbial communities that live in food and then moves through the basic ideas that govern how bacteria and the food matrix interact. The key microbial activities that affect food's sensory qualities, nutritional value, and shelf life are highlighted. the critical role that microorganisms play in the manufacture, preservation, and safety of a variety of food items, delving into the complex field of food microbiology. In the context of food production, bacteria may be both advantageous and harmful. Among the subjects covered is how bacteria play a part in fermentation processes, which give fermented foods their distinct aromas and textures. On the other hand, the possible threats presented by pathogenic microorganisms in foodborne diseases are discussed in detail, as well as themethods used to reduce these threats through regulation, cleanliness, and preservation. Furthermore, the improvement of food safety and quality assurance is examined concerning developmentsin food microbiology technology, such asmolecular methods and bioinformatics. To assure the continuous safety and sustainability of food, the chapter finishes by discussing new trends and problems in food microbiology and highlighting the significance of continual research and innovation. To assure the global food supply's future sustainability and safety.

1. Introduction

Microbiome of foods

Investigating the complex interactions between microorganisms and food items, nutritional microbiology is a dynamic and diverse science. Foods' flavour characteristics, dietary profiles, and safety are profoundly affected by the microbial populations that live within them. Understanding the intricacies of food microbiology is crucial for both industry workers and researchers, as consumers want more varied, convenient, and nutrient-dense food alternatives. In the environment, microorganisms such as bacteria, yeasts, molds, and viruses are common and have the ability to occupy different niches within the food matrix. There are ways to use their activity to your advantage, such as fermenting foods to produce a wide range of creative and traditional foods. Conversely, several bacteria possess the capability to jeopardize food safety by employing infection resulting in foodborne infections that have a major impact on public health.

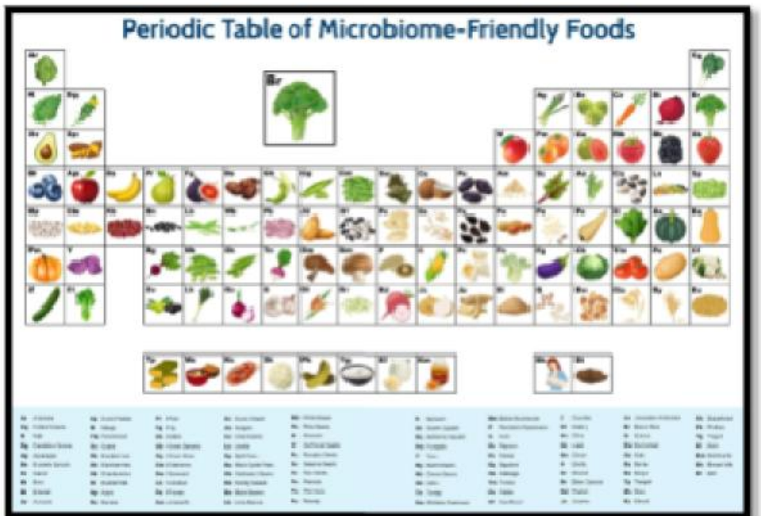

Figure 1: periodic table of microbiome foods

Microbial agents in food processing

Food fermentation has been a long-standing activity across civilizations worldwide in the field of helpful microbes. Microbial activity plays an important role in the formation of unique tastes, textures, and nutritional benefits in food products, ranging from the manufacture of yogurt and cheese to the fermentation of sauerkraut and kimchi. The probiotic potential of fermented foods is highlighted by research by (Parvez *et al*. 2006), highlighting their significance in enhancing gut health and general well-being.

Pathogens and Foodborne Diseases

Nonetheless, there is always a problem since harmful microbes cohabit in the food chain. Foodborne illness outbreaks can result from microbial contamination at several phases of food production, processing, and distribution. *Salmonella, Escherichia coli* (E. coli), and *Listeria monocytogenes* are a few notable examples. Following investigations by (Scallan *et al.* 2011), millions of people globally contract foodborne diseases each year,highlighting the necessity of strict regulation to guarantee food safety.

Advanced strategies and prospects of food microbiology

Technological developments in food microbiology have proven crucial in addressing these issues. Next-generation sequencing and polymerase chain reaction (PCR) are twomolecular methods that allow for the quick and accurate identification of microbes in food samples. Utilizing bioinformatics technologies facilitates the analysis of intricate microbial communities and the comprehension of their functional functions within various food webs. The study of (Quigley *et al.* 2012) discusses notable advances in this field and emphasizes the revolutionary effect of molecular methods in revealing the microbial diversity inside food systems.This chapter seeks to offer a thorough examination of the fundamental ideas, practical applications, and difficulties in the ever-evolving subject of food microbiology as we traverse its terrain. From the helpful roles that microbes play in fermentation to the constant dangers posed by dangerous pathogens, we will examine the complex fabric that describes the interaction between microbes and the food we eat. By comprehending these intricacies, we may create avenues for innovation that improve the quality and safety of the world's food supply.

2. Role of microbes in food industries:

Food production and preservation are intricately linked to the pivotal role played by microbes, encompassing a range of processes vital for the creation and safeguarding of various food items. Microbes actively contribute to the fermentation of diverse foods, spanning categories such as dairy, vegetables, meats, and beverages (Mazhar *et al.*, 2022). Beyond their involvement in fermentation, microbes in agriculture serve multifaceted roles, including nitrogen fixation, phosphate solubilization, and the control of plant diseases and weeds, thereby supporting plant development and fostering soil health (Gaur *et al.*, 1999). Moreover, microbes are integral to the preservation of foods, particularly in the creation of fermented foods and alcoholic beverages (Gondal *et al.*, 2021). This dual role underscores the critical importance of bacteria in ensuring the sustainability, safety, and quality of our global food supply. Additionally, bacteria contribute to broader agricultural benefits, such as enhancing nutrient absorption, managing pests, and mitigating plant stress responses, ultimately bolstering food crop yields (Sharma *et al.*, 2020). The impact of bacteria is pervasive in the food industry, influencing everything from crop development to preservation and manufacturing. Recognizing the significance of microorganisms in the food we consume daily, research highlights the microbial richness present in the USDA-recommended diet (Lang *et al.*, 2014). However, it is crucial to note the potential health risks associated with bacteria found in both conventional and fast food, especially when introduced into the human system through the handling of raw food, contaminated water, or exposure to human waste (Easa *et al.*, 2011).

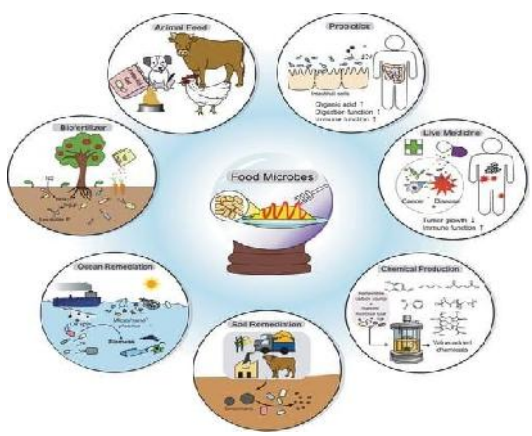

Figure 3: microbes in the food industry

To mitigate these risks and minimize the incidence of foodborne diseases, meticulous kitchen hygiene practices, encompassing proper food preparation and storage, become imperative (Beumer *et al.*, 2003). Despite these potential health risks, harnessing microorganisms for sustainable food production offers advantages such as promoting plant development and reducing the presence of agricultural pests (Noor-Hassim *et al.*, 2023). Thus, the intricate relationship between microbes and the food industry underscores the need for a nuanced understanding of their dual role in both enhancing food production processes and posing potential health challenges. Through a comprehensive exploration of these dynamics, we aim to navigate the complexities of leveraging microorganisms to ensure the safety, sustainability, and quality of our global food supply.

Fermenting agents

In the food and beverage, pharmaceutical, and biofuel sectors, among others, microorganisms—such as bacteria and fungi—are essential to several fermentation processes (Behera. S. S. *et al.*, 2019). They are employed in the food and beverage sector to produce fermented goods like dairy, vegetables, and alcoholic drinks (Nain. N *et al.*, 2020). In these processes, interactions between individual microbes play a significant role in determining the properties of the finished product (Smid. E. J. *et al.*, 2013). In the food fermentation sector, lactic acid bacteria in particular are employed as functional starter cultures, enhancing the safety and quality of the final product (Leroy. F *et al.*, 2004).

Food preservation

Microbes are essential for food preservation because they may ferment food, increase its safety, and lengthen its shelf life (Gondal. A. H. *et al.*, 2021, Pérez-Díaz. I. M. *et al.*, 2017). But there's also a risk associated with their existence since some microorganisms can cause food products to deteriorate and perish (Goresline. H *et al.*, 2010). To guarantee food safety and quality, it is crucial to comprehend and manage the activities of these bacteria during foodpreservation (Sofos. J. N. *et al.*, 1993).

Leavening agents

Microbes, which are leavening agents, are essential for food production because they improve the volume, texture, and digestion of the final product (Neeharika *et al.*, 2020). Microbes are also used in the economical and ecologically benign process of recovering heavy metals from solid industrial wastes, especially acidophilic bacteria and fungi (Mishra. D *et al.*, 2014).

Moreover, uncultivated microorganisms may include beneficial chemicals, including those employed as leavening agents (Newman. D. J. *et al.,* 2016). Last but not least, using microorganisms in bioremediation procedures—which includes heavy metal removal—is a successful and long-term strategy (Dave. D *et al*., 2020).

3. Microbiota in advanced food processing

Microbiota plays a vital role in the production, preservation, and sustainability of foodin modern agriculture. They may be used to make chocolate, food coloring, probiotics, and a range of other food items, such as dairy and fermented meals and drinks (Mazhar *et al*., 2022, Noor-Hassim, 2023), Microbes can improve crop yield, act as substitute food sources, and manage agricultural pests and illnesses in the context of sustainable food production (Noor- Hassim *et al.,* 2023). Additionally, efforts are being undertaken to enhance microbial biofertilizers and biopesticides, since plant-associated microbiomes have the potential to increase plant resistance and yields (Trivedi *et al.,* 2017). Even with these developments, microbial technologies still require improvement in terms of their consistency and usefulness in practical settings (Trivedi *et al.*, 2017).

4. Pathogenic and scavenging microbes in food spoilage:

Nevertheless, they also present serious risks; food contamination is caused by a variety of bacteria, molds, and other microbes (Elshafei *et al.,* 2017). The necessity for efficient control methods is further highlighted by the introduction of novel food-borne diseases. Other newly discovered food-borne infections include *Listeria monocytogenes, Arcobacter butzleri, Helicobacter pylori, Cryptosporidium parvum, and Cyclospora*, which produce the Shiga toxin (Meng. J *et al.,* 1997). Managing these microbiological dangers requires the implementation of the hazard analysis critical control point (HACCP) approach (Untermann. F *et al.*, 1998).

Microorganisms largely cause food deterioration, and in many processed foods, spore-forming bacteria like Bacillus and Clostridium are the primary offenders. These bacteria can withstand heat treatment and storage and are frequently discovered in low-acid canned foods, chilled vacuum-packed meats, and milk products (André *et al.,* 2017). According to Blackburn (2006), other spoilage microorganisms comprise molds, bacteria, and yeasts. Storage circumstances impact the microbial populations linked to the rotting of meat and seafood; the primary source of spoilage bacteria is an environmental core microbiota (Chaillou *et al.,* 2015).

The kind and characteristics of the food, the starting microbial flora, and the processing and storage circumstances all need to be taken into account to prevent microbial spoiling (Abdel-Aziz *et al.*, 2016). Salmonella enteritidis from eggs and *Campylobacter* are the major culprits behind the rise in food-borne illnesses brought on by bacterial contamination in the UK (Lacey. R. W.et al., 1993).

Common in food animals, *Salmonella* is a disease that may cause salmonellosis in humans and is a source of strains that are resistant to antibiotics (Ekperigin. H. E. *et al* 1998). These infections pose serious threats to food safety, as do molds that produce mycotoxin, viruses, protozoa, and prions (Untermann. F *et al.*, 1998). To manage these dangers, it is essential to employ hygiene measures and the Hazard Analysis Critical Control Point (HACCP) system.

5. Microbial community in processed food

The impact of microbes on how perishable and shelf-stable food goods are processed and spoiled. It highlights how different food products' microbial flora are particular to them, depending on the raw ingredients used, how they are processed, and how they are stored. The microflora that survives in shelf-stable items is unique to the product, yet spoiling can still happen if the stabilizing circumstances are disrupted. Despite a diverse flora, a certain set of microbes frequently predominates during storage for perishable goods, leading to recognizable spoiling patterns.

While canned cured meats have a prevalence of mesophilic aerobic and anaerobic spore-forming bacteria, the usual flora of shelf-stable canned foods is composed of thermophilic spore-forming bacteria. There are trace amounts of spore-forming bacteria, yeasts, and lactic acid bacteria While dried spices and vegetables include aerobic spore-forming bacteria, mayonnaise and salad dressing contain modest amounts of yeasts, lactic acid bacteria, and spore-forming bacteria. Yeasts and molds are present in dried fruits, and yeasts are present in carbonated drinks.Shelf-stable items may get spoilt if certain conditions arise, such as exposure to high temperatures or an overabundance of particular microbes. Spoilage is linked to the specific microflora in the product.

Even though the flora of perishable goods is diverse, some microbes, such as pseudomonas, predominate during storage and produce distinctive spoiling patterns. The typical pattern of spoiling acts as an indicator that the item is past its edible point. Modifications in the techniques employed for processing can impact the product's sensory alterations by changing the typical patterns of deterioration (Kautter et al., 1964).

The text emphasizes how crucial it is to take into account how processing modifications may affect patterns of spoiling, particularly concerning perishable goods. It gives the example of smoked whitefish, where alterations in packing caused the flora responsible for spoiling to change, which in turn caused an outbreak of botulism (Kautter *et al.*, 1964).

Food Microbiology: Present Status

The correlation with the significance of microorganisms, particularly noxious ones in food, were still being studied in the beginning of the 20th century. For their identification and separation, particular techniques were created. It has long been understood that maintaining cleanliness is crucial while handling food in order to prevent microbial infection. Particular techniques for eliminating harmful germs and spoiling were researched in addition to growth prevention. Studying the properties of the beneficial bacteria linked to food fermentation, particularly dairy fermentation, was also of interest. But food microbiology started a new chapter after the 1950s. Accessibility of fundamental data regarding the physiological, biochemical, and biological properties of various food kinds, microbiological interactions in food settings, and microbiological physiology, biochemistry, genetics, and immunology.

Food fermentation/prebiotic

Generation of strains by conducting genetic transfer throughout strains with predefined activity in metabolism. The development of lactic acid bacteria resistant to antimicrobial agents strains that have been physiologically engineered to produce more desired compounds invention of techniques for distributing immunity proteins using lactic acid bacteria sequencing the genomes of significant bacteriophages and lactic acid bacteria to learn more regarding their properties. Using beneficial microbial species and their antimicrobial metabolites to preserve food Being aware of the key features of probiotic bacteria and creating the appropriate strains. Reliable strategies for creating initial cultures that can be used instantaneously in food processing.

Food Spoilage

Recognising and handling emerging microorganisms that contribute to spoilage attributed to recent advancements in the preservation and processing of food methodologies Foods having greater shelf life that have been kept frozen or refrigerated can deteriorate due to bacterial enzymes. Building of molecular techniques (nanotechnology) to recognise the metabolites of foodstuff-spoiling bacteria and estimate the possible shelf life of food. The impact of stresses from the environment on spoilage bacteria's resistance to antimicrobial preservatives. Furthermore, Food production, processing, and preservation make use of the utilisation of hazard assessment for critical control points (HACCP). cutting-edge technology for food processing. Microbiology of ready-to-eat foods that were either partially handled or left unprocessed Food microbiological health control (which is additionally referred to as quality assurance or management) from farm to table Food safety legislation.

Characteristics of predominant microorganisms in food

Widely distributed in plants and in places where alcohol fermentation occurs. Important species: Acetobacter aceti.

- **Gluconobacter**: Many characteristics of this group similar to those of Acetobacter. Gluconobacter oxydans causes spoilage of pineapples, apples, and pears (rot). Acinetobacter. Rods (1 x2 um); occur in pairs or small chains: show twitching motility because of the presence of polar fimbriae; strictly aerobic and grow between 20 and 35°C. Found in soil, water, and sewage. Important species: Acinetobacter calcoaceticus.

- **Morexella**: Very short rods, frequently approaching coccoid shape (1 x 1.5 um); 0ccur singly, in pairs, or short chains; may be capsulated; twitching motility may be present in some cells; optimum growth at 30 to 35°C. Found in the mucous membranes of animals and humans. Important species: Morexella lacunata.

- **Alteromonas**: Most currently assigned Alteromonas species are of marine origin and might be present in foods of marine origin. Need 100 mM NaCl for optimum growth (unlike Pseudomonas). Because Alteromonas putrefacience (species recently reclassified as Shewanella putrifacience) has many characters similar to those of Pseudomonas was previously designated as Pseudomonas putrefa cience. Strains are important in fish and meat spoilage. Psychrotrophs.

- **Flavobacteriun:** Rods with parallel sides (0.5 x 3 um); nonmotile; colonies coloured; some species psychrotrophic. Cause spoilage of milk, meat, and other protein foods. Species: Flavobacterium aquatile.

- **Brucella**: Coccobacilli (0.5 x 1.0 um); mostly single; nonmotile. Different species cause disease in animals, including cattle, pigs, and sheep. They are

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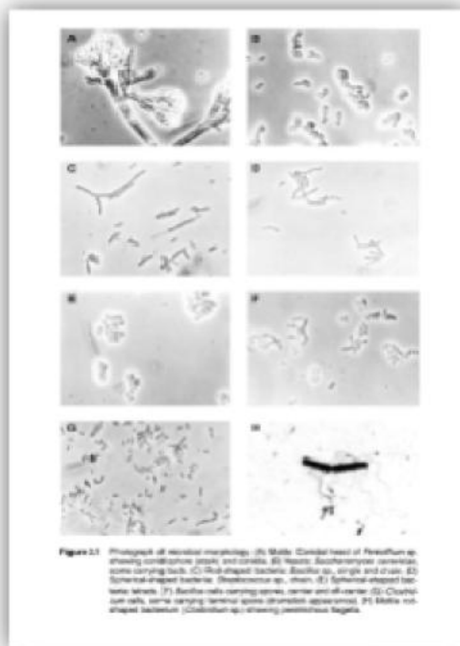
also human pathogens and have been implicated in foodborne brucellosis. Brucella abortus causes abortion in cows.

- **Psychrobacter**: The genus was created in 1986 and contains one species - Psychrobacter inmobilis. Coccobacilli (1 x 1.5 um) and nonmotile. It can grow at 5°C or below, show optimum growth at 20°C, and is unable to grow at 35°C. Found in fish, meat, and poultry products.



Conclusion

In conclusion, microbes and food production have complex interactions that involve critical roles in fermentation, agriculture, and food preservation. Microbes play a major role in many processes, such as the production of fermented foods and alcoholic drinks, which improves the quality, safety, and sustainability of the world's food supply. Although everyday food intake contains a wide variety of microorganisms, it is important to be aware of the possible health hazards connected with these microorganisms. This calls for strict kitchen cleanliness procedures to prevent foodborne diseases. There are benefits to using microbes for sustainable food production, including increased plant growth and decreased agricultural pests. Additionally, microbes are used as leavening and fermenting agents in modern food processing, which includes the creation of probiotics, cocoa, food colouring, and other fermented goods.

The risk of spoiling and the existence of harmful microorganisms, however, underscore the necessity of efficient control measures that prioritize cleanliness and the Hazard Analysis Critical Control Point (HACCP) system. The unique ways that perishable and shelf-stable food items deteriorate emphasize how crucial it is to comprehend processing technique changes and make necessary adjustments to preserve food safety and quality. To put it simply, managing the challenges of using microbes in food production calls for a sophisticated comprehension of their dual function, guaranteeing a careful balance between the advantages and hazards they present.

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Studies on the effects of Insect Growth Regulators on Haemolymph Proteins in Relation to Morbidity in Silkworm of *Bombyx mori.* L.

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Introduction

A new approach to insect pest control is the use of substances that adversely affect insect growth and development. These substances are classified as insect growth regulators (IGRs) owing to their effects on certain physiological regulatory processes essential to the normal development of insects. The IGRs are ecdysone or juvenile hormone mimics or chitin synthesis inhibitors. They are quite selective in their mode of action and potentially act only on target species. The action of IGRs, however are different from other synthetic insecticide such as organophosphate and carbamates as those chemicals interfere with other physiological processes but do not regulate the development of normal insects. IGRs, therefore, do not necessarily have to be toxic to its target, but may lead to various abnormalities that impair insects survival (Siddal, 1976).

IGRs exert their effects by regulating or inhibiting specific biochemical pathways or processes essential to insect growth and development. Some insects exposed to such compounds may die due to abnormal regulation of hormonal mediated cell or organ development. Other insects may die either from a prolonged exposure to other mortality factors like susceptibility to natural enemies; environmental conditions etc. or from an abnormal termination of developmental stage itself (Tunaz and Uygun, 2004).

The use of non-steroidal ecdysteroid agonist in insect pest control had its origin from the studies of Wing (1988) who studied the effects of RH-5849 on *Drosophilla* cell lines. But RH-5849 was not developed commercially. Tebufenozide (RH-5992) was the first commercial example of a highly selective insecticide used in caterpillars pest control. They are marketed under name MIMIC®, CONFIRM® (Rohm and Haas Company, Spring House, PA, USA). It disrupts the molting process by antagonizing ecdysone the molting hormone. They initiate precocious but incomplete molt to lepidopteran larvae (Wing *et al*., 1988, Retnakaran *et al.*, 1995). Lepidopteran pests in vineyard, orchards, vegetable field and forest are controlled while maintaining natural populations of beneficial insect predators and parasites (Dhadialla *et al*., 1998).

Methoxyfenozide (Interpid®), like tubefenozide, is both a stomach and contact insecticide. It is a second-generation ecdysone agonist insecticide developed by Rohm and Haas Company, Philadelphia, U. S. A. This compound controls a broad range of lepidopterous larvae at low use rates primarily *via* ingestion. RH-2485 also exhibits; selective contact, ovicidal and root systemic activity. They bind to the insect's ecdysone receptor within a few minutes of ingestion, arrests larvae feeding and induces a premature lethal molt within a few hours and kills the insect within a few day (Le *et al*., 1996). Global laboratory and field trial evaluations of RH-2485 have shown excellent control of key caterpillar pests of vine, free fruits, vegetables and row crops. RH-2485 is selective towards pollinators, arthropod predators and insect parasites. It is non-phytotoxic. It is ideal for use in integrated pest management.

The insect haemolymph protein attracted a great deal of attention as biochemical model system (Wyatt and Pan, 1978). The haemolymph of insects perform several physiological functions, such as immunity, transport and storage and are attributed to the appearance of selected proteins such as storage proteins (Levenbook, 1985), immune proteins (Boman and Hultmark, 1987), lipophorins (Shapiro *et al*., 1988) and hormone binding proteins (Koeppe *et al*., 1988). In lepidoptera there are a number of well-defined subclasses of storage proteins. In C*alpodes ethlius* besides a hexameric lipo-glyco arylphorins (MW. 470,000; subunit MW. 82,000), two larval serum glycoproteins which lack lipid and high aromatic amino acids and are usual, being rich in glycine, have been described. They are also large (MW. 580,000 with 86,000 subunits = hexamer and MW. 720,000 with 86,000 and 90,000 subunits = octamer). Their physiological significance is not clear (Barrett and Lai-Fook, 1976). A hexameric, 500 kDa protein has been isolated from *Locusta migratoria* and characterized by Koopmanschap and de Kort (1988). It reaches a high concentration in larva but nearly absent from adult.

The silkworm *Bombyx mori* ‘30 kDa lipoprotein’ is the major components of the haemolyph. These proteins were shown to participate in the activation of prophenoloxidase cascade and to interfere with hyphal growth of the eutomopathogenic fungus. Therefore, the lipoprotein plays a role in the protection of *Bombyx mori* against invading fungi (Ujita *et al*., 2005). In insects the prophenoloxidase activation system is a defense mechanism against parasites and pathogens. This system catalyses the oxidation of phenolic compounds that can polymerise to form melanin -immulectin -2 and prophenoloxidase in haemolymph bound to the immobilized recombinant proteinase-like domain of serine proteinase homologue indicating that a complex containing this protein may exist in haemolymph (Yu *et al*., 2003). 20-E can activate the expression of *Hyalophora cecropia* hemolin in the fat body. Hemolin is a moth hemolymph protein belonging to the immunoglobulin superfamily (Roxström *et al*., 2005).

Previous study of fenoxycarb as a juvenile hormone analogue and methoxyfenozide (RH-2485) as a 20-hydroxyecdysone (20E) agonist are two main insect growth regulators used for pest control (Zang *et al*., 2020). These studies paved the way to look for the relationship between doses of RH- 2485 and duration of treatment and the changes in protein profile. In this study only male larvae were chosen to find the changes in haemolymph protein so as to keep away from the complication with female specific protein (SP1).

Since insect haemolymph perform an array of functions like transports, storage and immunity, it was felt necessary to find the influence of insect’s growth regulator RH- 2485 on native haemolymph protein and their subunits. Therefore, the effects RH-2485 on haemolymph native protein and their subunit fractions and relate the same with morbidity in male V instar larva of *Bombyx mori* L were studied.

1. Experimental Design

1.1 Model animal

The model organism selected for this study is the mulberry silkworm, *Bombyx mori* belonging to the order Lepidoptera, Class Insecta and Family Bombicidae. The race chosen was a hybrid of LXNB4D2 (L-Local multi voltine variety, NB4D2 - New bivoltine with dumbbell shaped cocoon), which feeds, mainly on mulberry leaves. It is a holometabolous insect, whose life cycle has four distinct stages namely the egg, larva, pupa, and adult.

1.2 RH-2485 : A Pure Synthetic Non-Steroidal Ecdysone Agonist

Physical and Chemical Properties

Code Number: RH- 2485, RH-112, 485, Molecular Formula $C_{22}H_{28}N_2O_3$, Common Name is Methoxyfenozide, INTREPID®, Chemical Name is NC- Tert- buty- NC-(3,5di methylbenzoyl) (IUPAC) 3-Methoxy –2- methylbenzohydrazide, soluble in Water <1mg/l, DMSO: 11%, Cyclohexanone: 9.9%, 9%Acetone.

1.3 Rearing silkworm

Disease free layings (eggs) of *B. mori* (L x NB4D2) were obtained from the grainage of Regional Sericulture Center of Tiruchirapalli. The larvae were reared by the tray rearing method (Kasiviswanathan *et al*., 1970**)**.

1.4 Preparation of Methoxyfenozide

5 mg of methoxyfenozide (RH- 2485) was dissolved in 1ml acetone and considered as stock. 2µl from the stock and 998µl acetone was added and considered as 10.0.0µg stock, and required concentrations like 0.1, 1.0 and 10.0.0 µg were prepared.

1.5 Study Design

The study was designed to find the effect of RH-2485 on the haemolymph protein profile during metamorphosis in silkworm *B.mori* L. The experiment consisted of 80 V instar male larva. A group of 20 worms served as control and other 3 group of 20 each as experimental. The control groups were applied with acetone mid dorsally. The experimental group was topically applied with 0.1, 1.0 and 10.0.0 µg/larva of RH-2485 in acetone. Care was taken to ensure that control and treated groups were kept in separate places. The observations were made on the effect after treatment during the V instars development period. Haemolymph samples were collected after 24, 48, 72 and 96 hours after topical application. The protein extract, were subjected to electrophoresis separation both in native and SDS– PAGE.

1.6 Collection of haemolymph protein

Collection of haemolymph protein was done by cutting the abdominal legs of *B.mori* larva and haemolymph was bled from the wound directly into the eppendorf tubes coated with thiourea, an antioxidative agent and equal amount of extraction buffer was added then centrifuged at low speed to remove haemocytes the supernatant was stored in a refrigerator until further analysis.

1.7 Estimation of Protein

Protein content of the sample was estimated by adopting the method of

Lowery *et al*. (1951). The non-dissociating system of electrophoresis was done adopting the method of Hames (1981).

1.8 Protein fractions from native gel and its elution

Elution buffer (PH 7.4)

SDS	-	0.1 g
Tris Hcl	-	0.05 M
EDTA	-	0.1mM
DTT	-	5mM
BSA	-	0.1 mg
β mercaptoethanol	-	390 μl

GDW to 100 ml

The Gel was thoroughly rinsed with double distilled water.

1. Gel was washed in 0.25 M Kcl (7.455 g / 100 ml ice cold + 1M DDT (or) 780 μl/lt. β- mercaptoethonol (78 μl / 100 ml) for 5 minutes.
2. Protein bands were cut individually soaked in 1 ml double distilled water containing 1 mM DTT for 15 minutes.
3. The bands were crushed into pieces in 1 ml of elution buffer and incubated overnight at 37 ^{o} C.
4. The samples were centrifuged at high speed and the supernatant was collected.
5. To the supernatant, 4 volumes of cold acetone were added.
6. It was allowed to stand for 30 minutes in a refrigerator (or) dry ice.
7. The mixture was centrifuged for 10minutes at 10,000 rpm.
8. The pellet was dried for about 10 minutes and dissolved in extraction Buffers for 10 min.

The eluted proteins were separated in 7% SDS-Polyacrylamide gel electrophoresis was carried out following the method of Laemmli (1970) and compared with molecular weight marker proteins (Myosin-205kDa, phosphorylase b- 97.4kDa, bovine serum albumin- 66kDa, ovalbumin –43kDa and carbonic anhydrase-29kDa (Genei, Bangalore, India) were loaded simultaneously for comparison.

2. Insect Growth Regulator (Rh-2485) on major Haemolymph Proteins

2.1 Changes in V instar larval behavior and external morphology

Silkworm *B.mori* L. One day old male V instar larva received 0.1, 1.0 and 10.0µg/larva of RH-2485 showed changes in feeding and general health. The responses were found to vary. The treatment groups did not show variation in feeding till 12 hours. 0.1µg treated larvae were found to feed normally for 48 hours. But 1µg treated larvae showed a reduced appetite and stopped feeding after 48 h. In contrast larvae that received 10.0µg stopped feeding within 24 h of treatment. Most of the larvae within this group were found to sway their heads and lose their grasping power. These worms showed changes on the body surface, there were patches of brown colour all over. As time passed there was oozing of haemolymph from their body surface at one or two places and excreted semisolid faeces.

Similar changes were observed in other groups after 72 h of treatment. At the end of 96 h of application all the worms were found to lie upside down or sidewise due to ill health or infection by disease causing microbes. The body colour changed to grayish black with patches of brown in 10.0µg treated worms and they died after 96 h of application. Whereas the worms subjected to topical application of 0.1 and 1.0µg survived without any activity. The 1.0µg treated worms died after 120 h of treatment. Similarly, the larva received 1 g also died after 168 or 192 h of treatment. Irrespective of time all the worms subjected to topical application showed symptoms of microbial infection at varying time. Finally, all the worms of experimental groups (0.1, 1.0 and 10.0 µg /larva) died without any further metamorphic changes (PLATE 1).

To investigate whether these morbid conditions are related to changes in haemolymph proteins an attempt was made to study insect haemolymph protein both in SDS-PAGE and on native PAGE and their elution fractions as well.

2.2 Effect on major haemolymph proteins SDS-PAGE

The V instar male larva received different doses (0.1, 1.0 and 10.0 µg / larva) of RH-2485 showed marked changes in protein profile in 7% SDS-PAGE stained with Coomassie blue. There were differences in intensities as well as number of bands. The molecular weight of these proteins ranged from 200-10 kDa. The staining regions were 80 and30 kDa.There were 2 polypeptides at 80 kDa region (76 and 72 kDa) and 3 polypeptides at 30 kDa region (30, 29 and 26 kDa). The staining intensities of these proteins at 80 kDa and 30 kDa regions were high in all these worms (Fig.1). The protein profile of the larvae of different control and experimental groups after 24 h and 48 h of topical applications are also presented in the Fig.1. The staining intensities at 76 kDa) region was less in control haemolymph (Fig.1, lane-1) when compared to all treated groups (Fig .1, lane -2, 3and 4). The intensities of 30 kDa protein among 10.0 µg treated worms were high (lane-2) compared to control (Fig.1, lane 1 and 2).

The staining intensities of 76 and 72 kDa polypeptides of the larva treated with 0.10µg of RH-2485 and the control were same. But there was a decrease in intensities of 30 kDa protein in the larva treated with 1.0 µg (Fig.1, lane-7) when compared to others (lane 5, 6 and 8).

The protein profile of haemolymph after 72 h and 96 h are presented in Fig .2. There existed a drastic difference in the intensities of various polypeptides in the treated groups (0.1µg, 1.0µg and 10.0µg). In lane-1 and 5 (i.e. control of 72 h group) the polypeptides were with high intensities at 76, 72, 30, 29 and 26 kDa regions (Fig.2, lane -1). New polypeptides appeared at 54 and 20 kDa regions in control but in the treated worms, these polypeptides were absent and there was an appearance of 14.2 kDa protein band at 1.0µg/larva treated groups (lane-3).

The observations on the protein profile after 96 h of treatment revealed the absence 14 kDa polypeptides. In addition, the intensities of polypeptides at 30 kDa region had decreased and 26 kDa was absent (Fig.2, lane-6, 7 and 8) when compared to control (lane-5). Since the 30 kDa protein showed differences at 96 h of treatment it was felt necessary to find the changes in native proteins. The haemolymph of worms were separated in a native PAGE.

2.3 Effect on major haemolymph proteins native PAGE

Fig .3 shows the protein profile of haemolymph in the male V instar larva of *B. mori* in 7% native PAGE. The samples collected from control larvae equivalent to 96 h after treatment revealed 7protein bands ranging in molecular weight from 640- 60 kDa regions. The haemolymph of control worms showed 640, 540 kDa, 500, 260,140, 90 and 60 kDa regions (Fig.3, lane-1). The native protein profile of 0.1 and 1.0 µg treated worms showed a (Fig.3, lane-2 and 3) 500, 260 and 140 kDa regions. 10.0µg treated larva showed only 500 kDa (Fig.3, lane-4) protein as a prominent one. The other 540 kDa, 260, and 140 kDa proteins were not visible in native PAGE.

After finding the protein profile of the haemolymph in 7% native PAGE, the same samples were electrophoretically separated in 5% native gels in order to have good separation to elute the different fractions of native proteins. Fig. 4 shows the subunit fraction of different haemolymph of control eluted from the native proteins separated in 5% native PAGE. Since the proteins at 540, 500, 260,140 and 90 kDa regions were of high intensities they were eluted for further analysis. The first protein at 540 kDa region when eluted and separated in 7% SDS-PAGE showed only one fraction at 30kDa region (Fig.4, lane-1). The second protein at 500kDa region showed 3 fractions at 76, 72 and 29 kDa regions (Fig.4, lane-2). The third protein at 260 kDa got eluted and resolved into 76, 30, 29, 28, 26 and 14 kDa (lane-3). Protein at 140 kDa region when eluted got resolved into 29 kDa only (Fig. 4, lane-4) and the 90 kDa protein also got resolved into 30 kDa subunit(lane-5). Lane-6 of Fig.4 gives the protein profile of whole haemolymph for comparision.

The haemolymph of 0.1 µg treated worm showed 3 proteins in the native gel namely 500, 260 and 140 kDa regions (Fig.5). The subunits of 500 kDa protein showed 4 fractions at 76, 72, 30 and 29 kDa regions in 7% SDS-PAGE (Fig.5, lane-2). The second protein at 260 kDa showed 72and 49 kDa protein fractions (Fig.5, lane -3).The 140 kDa protein got eluted and resolved at 54 kDa subunits. Lane -1 shoes the protein profile of haemolymph of 0.10µg treated larva for comparison.

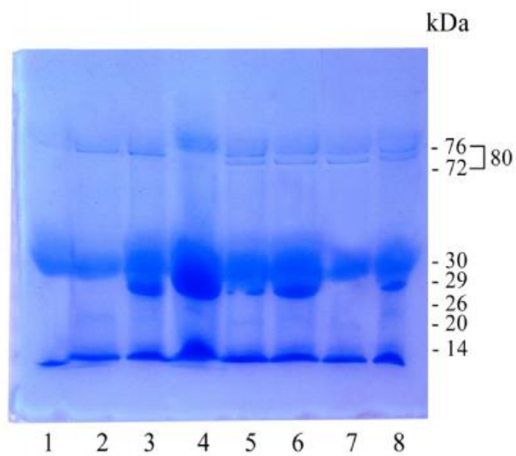
The haemolymph protein of 1.0µg/larva treated worms showed 3 proteins in the native gel at 500, 260 and 140 kDa regions (Fig. 6). The protein at 500 kDa showed 4 fractions with molecular weight of 76, 72, 30 and 29 kDa regions (Fig .6, lane-2). The proteins at 260 kDa got eluted and resolved into 3 bands at 76, 30 and 29 kDa regions (lane -3) and 140 kDa was resolved into 30 kDa polypeptides. (Fig.6, lane – 4).

The polypeptides eluted from the haemolymph native protein of 10.0µg treated worms are presented in Fig.6. Their haemolymph showed only 2 proteins at 500 and 90 kDa regions. 500 kDa protein got eluted and separated into polypeptides with a molecular mass of 76, 72, 30 and 29 kDa (Fig.6, lane-

5). The lane-6 contains the whole haemolymph protein profile of 10.0µg treated larva.

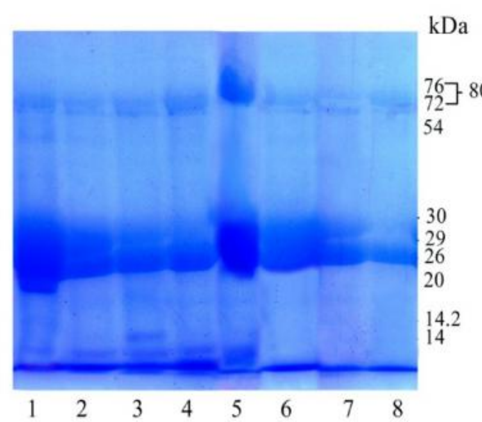
Plate 1. showing the changes among the V instar larva which received 0.1, 1.0 and 10.0µg/larva of RH-2485

Fig - 1



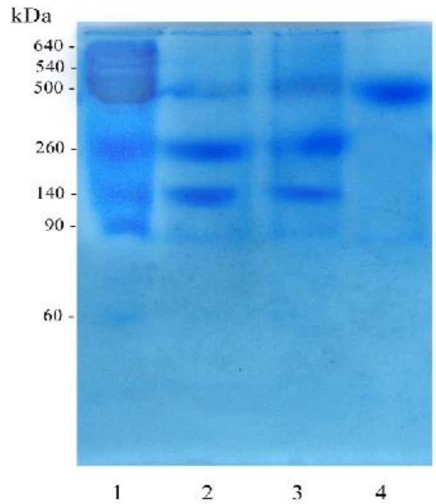
7%SDS-PAGE of haemolymph proteins in the male V instar larvae of *B.mori* after 72 h and 96 h application of RH-2485. Each lane was loaded with 25µl of haemolymph. The gel was stained with Coomassie blue (R-250). Lane 1 to 5- different experimental worms as given in fig.1 after 72 h of treatment. Similarly, lane 5-8 after 96 h of treatment as in fig.1.

Fig - 2



7%SDS-PAGE of haemolymph proteins in the male V instar larvae of *B.mori* after 24 h and 48 h of topical application of RH-2485. Each lane was loaded with 25µl of haemolymph. The gel was stained with Coomassie blue (R-250) lane 1- control, lane 2 treated(0.1 µg/larva), lane 3- treated (1.0 µg/larva), lane 4- treated(10.0 µg/larva). Lane 5, 6, 7 and 8 are control, 0.1, 1.0 and 10.0 µg/larva treated respectively after 48 h of topical application.

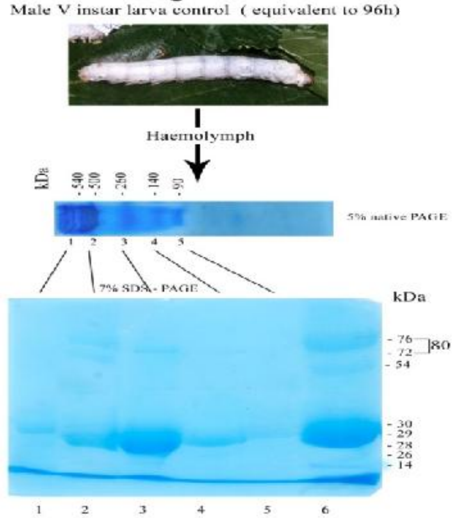
Fig - 3



7% native protein profile of male V instar male larva of *B. mori* after 96 h of RH-2485 treatment. The gel was stained with Coomassie blue (R-250). Each lane was loaded with 25µl of haemolymph. Lane 1-control, lane 2 treated (0.1 µg/larva), lane 3-treated(1.0 µg/larva), lane 4- treated(10.0 µg/larva).

Fig - 4

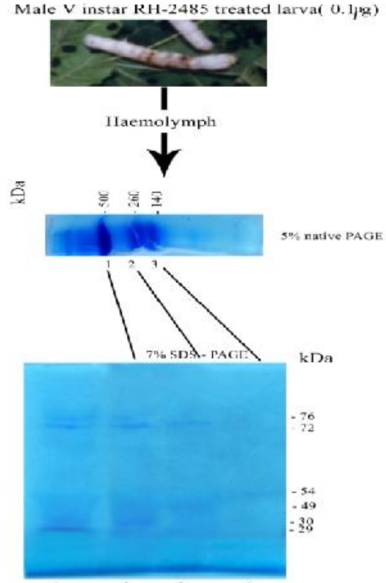
Male V instar larva control (equivalent to 96h)



The control male V instar larva of *B. mori* after 96 h and its haemolymph protein profile in 5% native PAGE and their respective eluted samples in 7% SDS PAGE. The gels were stained with Coomassie blue (R 250). The lane 1-eluted subunits from fraction 1, lane 2- eluted subunits from fraction 2, lane 3-eluted subunits from fraction 3, lane 4- eluted subunits from fraction 4 and lane 5-eluted subunits from fraction 5.Lane 6- the whole haemolymph protein.

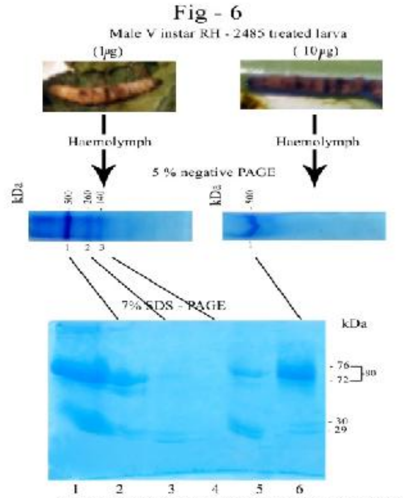
Fig.5

Male V instar RH-2485 treated larva(0.1µg)



The male V instar larva of *B. mori* after 96 h of RH-2485 (0.1 µg/larva) treatment. Its protein profile in 5% native PAGE and eluted subunits of different proteins in 7% SDS-PAGE. The gels were stained with Coomassie blue (R-250). Lane 1-whole haemolymph proteins of (0.1 µg/larva) treated larva. Lane 2-eluted subunits samples from native protein fraction 1, lane 3- eluted subunits from fraction 2 and lane 4 eluted subunits from fraction 3.

Fig - 6



The male V instar larva of *B. mori* after 96 h of topical application with 1.0 and 10.0 µg/larva of RH-2485 and its protein profile in 5% native PAGE and the eluted subunits in 7% SDS-PAGE. The gel was stained with Coomassie blue (R-250). Lane 1- the whole haemolymph protein of 1.0 µg/larva, lane 2- The subunits eluted from fraction 1, lane 3- the eluted subunits from fraction 2 and lane 4 the eluted subunits from fraction 3, lane 5- the eluted subunit from fraction 1 of 10.0 µg treated larva. lane 6-the whole haemolymph protein of 10.0 µg/larva treated larva.

3. Effect of Igr Rh-2485 In Silkworm Larva

3.1 RH-2485 on external morphology and behaviour

Topical application of RH-2485 induced reduction in feeding followed by complete feeding arrest leading to death of the worms. All the treated worms showed symptoms of premature molt, Cessation of feeding, restricted movements, loosening of skin, head capsule slippage are the symptoms of premature molting (Retnakaran *et al.,* 1996; Smagghe *et al.*, 2000; Borchert *et a*l., 2004; Hussein *et al.*, 2005). Termination of feeding is an important preparatory step in molting; it occurs at high ecdysteroid levels in the haemolymph (Kubo *et al*., 1983). But when the feeding stops before the worms are ready with their reserves, the events would not occur in sequence. Further the worms fall as victims to the environmental factors and pathogens as well. In this study all the worms died of morbidity. Therefore, the haemolymph were analysed for the changes in protein to look for morphological and biochemical correlates.

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3.2 RH-2485 on haemolymph proteins

When the worms stopped feeding and forced to molt under the influence of RH-2485 they did not undergo a molt nor able to resist pathogens as a result, may they become easy victim to pathogens. At the time of septic injury humoral responses utilizes various antimicrobial peptides synthesized in the fat body (a functional homology of the mammalian liver some haemocytes secreted into the haemolymph) (Boman and Hultmark,1987).

Increase in polypeptides at 30 and 80 kDa regions observed after 24 and 48 h of topical application of RH-2485 are in accordance with the findings of Smagghe *et al,* (1999) who reported a small increase of total protein content and then a drop in the haemolymph of last instar larva immediately after RH-5992 application. The major haemolymph protein of male V instar control larva showed 6 prominent native proteins with a molecular weight of 640,540, 500, 270, 140 and 90 kDa. But the intensity of 260 kDa at 30 kDa region was very high compared to all other native proteins.

3.3 RH-2485 on storage proteins

Among these native proteins the 500 and 260 kDa proteins had subunits resolved at 80 kDa region (76 and 72 kDa) and 30 kDa region. This 500 kDa protein could be arylphorin a hexameric storage protein with subunits of approximately 72-80 kDa as reported by Tojo *et al*. (1980) in *B. mori*. This arylphorins function as a storage protein for chorion formation and vitellogenesis for growing adult (Ogawa and Tojo, 1981), a component of cuticle formation (Scheller *et al*., 1980) and involved in ligand transport (i.e. arylphorins binds to ecdysteroids) (Enderle *et al*., 1983) and certain insecticides (Haunerland and Bowers, 1986). This 500 kDa protein was present in all the treated worms, even in the worms about to die. Therefore, these proteins do not play a role in the survival but important for reproduction. As early as 1987, it was reported by Roberts that arylphorins null stocks of *Drosophila* were viable. Their absence did not affect the survival but adversely affect fertility. In this study the intensity of 500 kDa protein was found unaffected in RH-2485 induced morbid worms. Hence, these proteins do not contribute to survival of the worms.

3.4 RH-2485 effect on antimicrobial proteins

The presence of 54 and 49 kDa polypeptides in 0.1µg treated worms might be taken as an evidence for the induction of anti-microbial peptides synthesized in the fat body and released into the haemolymph in response to the invading pathogens (Boman *et al*., 1991). Proteins having the molecular weight of 43 and 40 kDa in SDS-PAGE from *B. mori* haemolymph were reported to recognize and bind with *E. coli.* These proteins were named as BmLBP (*B. mori* liposaccharides binding proteins) (Koizumi*et al.*, 1999). The other native proteins namely 540, 270, 140 and 90 kDa protein had 30 kDa subunits in the normal untreated worms. All the 3 proteins namely 540, 270, 140 kDa were found absent in the worms treated with 10.0μg after 96 h of treatment these worms die with symptoms of microbial infection. In all these worms the 30 kDa subunits were found very less. 30 kDa proteins were reported to be highly immunologic.

Lectins in insects are suggested to function in recognizing the pathogens and parasites, synthesized during metamorphosis and function in recognition and clearance of pathogens and disintegrating tissues. The purified lectins composed of subunits with a molecular mass of 30-40 kDa. In addition, 30 kDa proteins were shown to participate in the activation of prophenoloxidase cascade and to interfere with hyphal growth of entomopathogenic and protect against invading fungi (Ujita *et al.*, 2005). The prophenoloxidase activation system is a defense mechanism against pathogens (Yu *et al.*, 2003). 30 kDa proteins also inhibit virus or chemical induced apoptosis in insects and human cells (Kim *et al.*, 2004). 20E activated the expression of *Hyalophora cecropia* hemolin in the fat body. It is a moth immunoglobulin super family (Roxström*et al.*, 2005), and they induce cellular mediated responses in *M. sexta* to bacteria (Eleftherianos *et al.*, 2008).

3.5 Effect of RH-2485 in V instar larva of silkworm *B.mori*

Massive accumulation of haemolymph protein occur during the last larval instars serve as storage reserves of amino acids for further development during non-feeding stages, also act as protective agents in keeping the worms in good health and protect them from pathogens. It appears that there occurs some compartmentalization of reserves, some for future use and others for defense. When normal course of growth was disrupted with Insect growth regulator which caused the worms to stop feeding, it could have induced the worm to synthesize new proteins during early stages but later the fat body cells could have been subjected to apoptosis as a result the worms would not have continued the synthesis. As a result, the worms survived as long as the reserve was available but when exhausted would have become the victims to pathogens and died. The most striking result obtained in this study is the absence of 540, 270 and 140 kDa proteins in the native PAGE and reduction in 80 and 30 kDa proteins in SDS-PAGE. But among these two 30 kDa protein was drastically reduced in morbid worms.

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