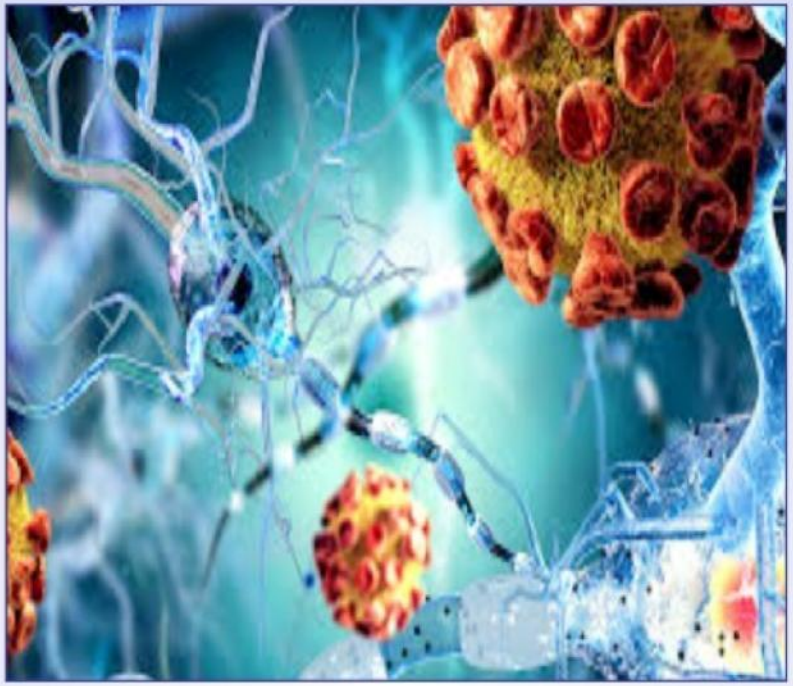

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CURRENT TRENDS IN BIOLOGICAL SCIENCES

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First Edition

Dr. R. B. Tripathi
Dr. Kena P.Anshuman
Dr. P. Vijaya
Dr. Anjani Kumar Shukla

THANUJ INTERNATIONAL PUBLISHERS, TAMIL NADU, INDIA

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Preface

Current Trends in Biological Sciences are more inclined toward interdisciplinary studies.The present book provides a balanced approach to higher levels of biological organization. It also serves in the emerging disciplines of conservation biology and natural resource management. Recent developments in the technologies have led to a better understanding of the living system and this has removed the demarcations between various disciplines of biological sciences. This book discusses and interprets major issues in environmental science, environmental technology, the effect of climate and weather on sericulture and aquaculture, toxicology, ecotoxicology, oncology, epidemiology, public health, biology and control of insect pests, haloarchaea, antimicrobials, transgenic plant development, ethnobotany, food and nutrition, pharmaceutical, soil science, biofertilizers this is all used to understand the challenges found in biological sciences. We attempted to provide up-to-current knowledge based on a basic concept in biological research involving a merger of diverse disciplines. Moreover, it takes a futuristic look at such important topics as sustainability, environmental problems and the relationship between toxicology, ecotoxicology and environmental science.

We are very much thankful to Thanuj International Publishers who readily accept and publish this subject. Also, the author is very much thankful to Prof. Indu Singh, Dr Ashok Kumar, Nandani Solanki, Hetal Khandla, Avantika Gohil, Devangi Patel, Munira Kapasi, Heervaba Gohil, Dr. Manisha N Shah, Dr C.Shobana, Dr B.Usharani, Ayushi Pandey, Ram Pravesh, Dr.Sanjai Kumar Gupta, Dr.G.Durai MuthuMani, Dr.R .Manikandan, N.Sreevalli, H.R. Sudha, Dr.Dinesh Kumar Maurya, Dr.Kamalesh Kumar, Dr.Ajay Kumar Kushwaha, Mr.D.E.Nirman Kanna, Ms.Subbulakshmi Packirisamy, Dr.Deepa Rajendiran, Rohit Kumar, Dr.KamleshYadav, Vipin Kumar Pandey, Rakesh Kumar Pandey, Rahul Kumar, Shiv Mahendra Singh, Mohammad Akmal, Zaheen Hasan, Prof.D.D.Tewari, Mansi Patel, Amar Santosh Dhalla, Rashida Khatoon, Dr.Karunesh Singh, Nirali Thakkar, Bhavisha Gajjar, Dr. Akanksha Tripathi, Dr.H.D. Dwivedi, Dr. Deepti Sharma, Dr. P. Karthika, Mrs. S. Priyanka, Dr.T.Poongodi Vijaya Kumar, Mrs. S. Jothimangalam, Dr. M. Deepa, Dr.R.Gandhimathi, Dr.A.Saravana Kumar and Dr. M.Vijey Aanandhi for contributing their help and support for this work.

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Dr. R. B. Tripathi
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Dr. Anjani Kumar Shukla

About Editors

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Dr. P. Vijaya is working in Department of Zoology, Bangalore University, Bangalore (Karnataka). She obtained her M.Sc., M.Phil and Ph.D in Zoology from Punjabi University, Patiala (Punjab), India. She has major specialization in Physiology, Toxicology, Biochemistry and Pathology. She has published 16 research publications in national and international journals. She also has 4 book chapters and 1 book to her credit. She has 4 years of P.G teaching experience and 3 years of U.G teaching experience. She carried out her research on toxicity and various natural antioxidants for treating various diseases related to different organs in mice.

Dr.Anjani Kumar Shukla MSc. PhD. (Assistant Professor) Department of Zoology Kisan Post Graduate College (University of Dr. Ram Manohar Lohiya Ayodhya) Bahraich Uttar Pradesh has more than 14 years of teaching experience to undergraduate and post graduate students. He has more than 12 research publications on histomorphology and affect of xenobiotics in gonadial cycle of some specific fresh water fishes and few liminological and pest control in reputed International and National journals. He published three chapters in Recent Advancements and Research in Biological Sciences (ISBN: 978-81-952529-1-6), Thanuj international Publishers Tamilnadu, India. Presently he is actively involved in research in the field of toxicological studies of metals in food and drinking water in ruler and urban civilized regions and its impact on health and environment. He is also active in both academics and social activities.

Current Trends in Biological Sciences

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Contents

Biology and Control of Oil Seeds Insect-Pests

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Introduction

Among the principal commercial crops growing in India,the oil seeds rank first in area and second in production.They occupy about a tenth of total cropped area in the country and about 8 % of the value of the total agricultural production.They play an important role in the economy of country because of their value in import substitution.Sesamum and Castor have their separate identity as oil of these is generally consumed for medicinal purposes as well as perfumery.While compared to other seed producing countries India is second largest producer of Sesamum and Castor seeds.But at present the yield of these crops per unit area is very low because of manifold reasons and the ignorance of plant protection technology.The losses caused by insects to oil seeds are alarming and can be reduced to the level of economic importance by adopting suitable plant protection measures.

The some insect-pests of Oil Seeds recorded in India as under-

A: Mustard Aphid

B: Mustard Saw-fly

C: Painted bug

A: MustardAphid

Local name: Mahon, Mahun, Chenpa, Tela, Mawa and Lahi

Scientific name: Lipaphis erysimi Kalt **(*Siphocoryne indobrassicae*)**

Classification

Phylum-Arthropoda

Class -Insecta

Subclass- Pterygota

Division-Exopterygota

 ISBN: 978-93-94638-00-6

Order-Hemiptera

Suborder-Homoptera

Family-Aphididae

Genus- Lipaphis

Species- erysimi

Food plants: Mustard, Cauliflower, Cabbage, Radish and Turnip etc.

Distribution: In India, it is found in all the states speciallyin Uttar Pradesh, M.P., Punjab, Maharashtra, Bihar, Gujrat, Mysore and Rajasthan.

Nature of damage: It appears in the last week of November or early December in U.P. and reaches to its peak in the month of February.Both nymphs and adults suck the sap from the tender portion of the plants. Hundreds and thousands of these may be seen on leaves or tender shoot.In mild case, the shoot wilts and in severe cases it completely gets killed. The honey dew produced by such a large number of individuals covers practically the whole surface of the tender leaves and shoot, a kind of black mould develops which interferes the photosynthetic activities plant. Honey-dew secreted by Aphid is liked by number of other insects, specially by ants who for the sake of ensuring the supply of this wet liquid, protect the Aphids and also provide them transport, this type of association is known as commensalism. The cloudy and humid atmosphere is most suitable for its multiplication and attack.

Life cycle of Lipaphis erysimi

A different type of life cycle has been found in plain and hill regions.In plains, the insect does not lay eggs hence only nymphs and adults are found.

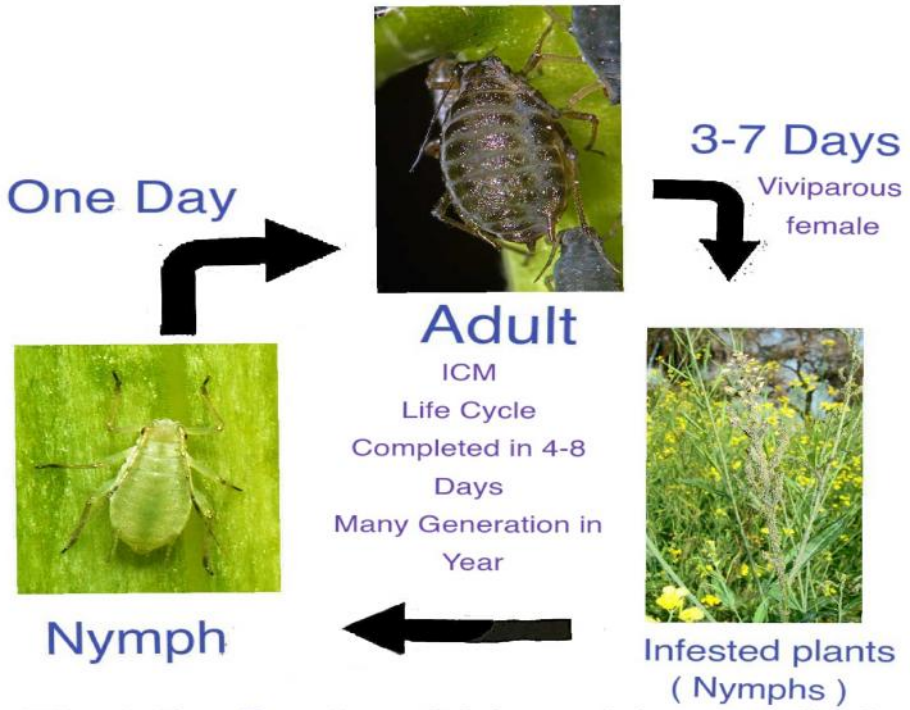

Fig.Life Cycle of Lipaphis erysimi

In Plains: They appear in November-December on mustard plants among them females are more in number than males. The females are viviparous and they do not lay eggs but produce many nymphs.The nymphs are produced by following ways-

i- After mating the males.
ii- Without mating the males but parthenogenetically.

The nymphs become adult with in 3-7 days and start producing apterous offsprings.In this way they increase great in number and spread over a big area in a very short time.There are several generations during the cold season.

In hills: In hills the life cycle of Aphids is just like in European country.Aphids are remarkable on account of their peculiar mode of development and the polymorphism exhibited in different generation of the same species.The following types of individuals are present in the life cycle of migratory Aphids.

1-Fundatrices: These are apterous**,** viviparous parthenogenetic females which emerge in spring from the over winter and eggs. Only one generation is found of this group.

2-Fundatrigeniae: Theseare also apterous, parthenogenetic, viviparous females and are the progeny of fundatrices.They live on the primary host and complete three generations.

3-Migratory: These usually develop in the IInd, IIIrd or later generations of fundatrigeniae and consist of winged parthenogenetic, viviparous females.They develop on the primary host and subsequently fly to the secondary host.

4-Alienicolae: Parthenogenetic, viviparous females developing for the most part on the secondary host.They often markedly from the fundatrices and migrants. They are similar to fundatrigeniae but differ from them by living on secondary host while former on primary host.Many generations may be produced comprising both apterous and winged forms.

5-Sexuparae: Parthenogenetic, viviparous females which develop on the secondary host and migrate to the primary host at the end of summer.

6: Sexuales: These are the progeny of sexuparae produced on the primary host. They consist of sexually reproducing males and females. The females lay eggs on the primary host after copulating the males which passes winter as such and hatch out in following spring and fundatrices females are produced.

Adult- TheAphids are small generally 2 mm in size,green sucking insects.They are provided with a pair of small tubular structure projecting out from dorsal surface of the posterior region of the body known as cornicles or siphons or honey tubes. Two pairs of transparent wings are found in which costa and subcosta veins are absent.

Prevention and Control:

1: Early sowing or early maturing varieties should be grownto escape the damage.

2: Resistant varieties viz.AGH-4and T-101 should be preferred to grow.

3: The crop may be dusted with any one of the following insecticides

a: Malathion 5% dust @25kg/ha.

b:Carbaryl 10% dust @25kg/ha.

C: Fenetrothion 5% dust @20kg/ha.

4: The spraying of any one of the following insecticides has been found effective to control the Aphids-

a: Phosphomidon 100 Ec @ 0.250 lit/ha.

b:Dimethoate 30 Ec @1.00 lit/ha.

C:Endosulfan 35 Ec@1.25 lit/ha.

d: Formothion 25 Ec @0.650 lit/ha.

e: Methyl demeton 25 Ec @ 1.00 lit/ha.

f: Malathion 50 Ec @ 0.600lit/ha.

g: Chlorpyrifos 20 Ec @ 0.600 lit/ha.

Natural enemies: The following insects feed on Aphids

i: Coccinellaseptempunctata (Lady bird beetle).

ii: Chrysopa sp.(Aphid lion).

iii**:** Syrphidsp.(Maggot of Syrphid).

B: Mustard Saw-fly

Local name- Sarson ki illi

Scientific name- *Athalia proxima* Klug

Classification

Phylum- Arthropoda

Class- Insecta

Subclass-Pterygota

Division-Endopterygota

Order-Hymenoptera

Family-Tenthredinidae

Genus- Athalia

Species- proxima

Food plants: Mustard, radish, Turnip, Cabbage, Cauliflower and other Cruciferous plants.

Distribution: The pest is distributed practically throughout India.Besides India, it is widely found in Indonesia, Formosa and Burma.

Nature of damage: In most parts of the country it is pest of cold weather and its activity confined generally to the period from October to March although in certain western regions it is reported to be prevalent round about August.The larval stage damages crop by voractiously feeding on the young crop. Its feeding activities are generally confined to mornings and evenings. As a result of feeding the leaves become full of holes and the plants either dry up or remain stunted. In case of severe infestation all the leaves are eaten and the plants die.

Life cycle of Athalia proxima:

Generally it belongs order-Hymenoptera and it is clear that in Hymenopteran four development life stage will be reported such as eggs, larva, pupa and adult.

Eggs: The female lays eggs singly in to the tissues of the food plants.The egg laying organ (Ovipositor) is highly specialized for sliting open the margin of the leaves with in which eggs are laid.Initially they are milky in colour which turn in to dark brown or black at the time of hatching. An egg is 2 mm long and many eggs may be laid in to one leaf.Each female is capable of laying usually 30-61eggs which in some cases may reach up to 150 eggs. Hatching period of eggs varies from 4-27 days depending upon the environmental conditions.

Larva (Grub): The young grub is greenish grey in colour and its body surface is hairless measuring 2 mm long. It begins to feed on the margin of the leaf and as it grows, its colour getting darker.Fully grown grub is 15 to 20 mm long having 3 pairs thoracic and 8 pairs abdominal legs and provided with 5 stripes on the back. The larval period lasts for 12 to 18 days.

Pupa: For pupation larva or grub goes in to soil wherein it prepares cocoon made of silk butteressed by soil particles. The cocoon is 7-11 mm long and 4-6 mm wide. The pupal period is of about 10-12 days. During severe cold it hibernates in pupa stage.

Adult: The adult Saw-fly has a sort thick-set body with a mixture of yellow-brown marking on it and a dark reddish brown colour on the wings. It has two pairs of wings and its activities are diurnal. The fly is also capable to lay eggs parthenogenetically but only male emerges out from these eggs.

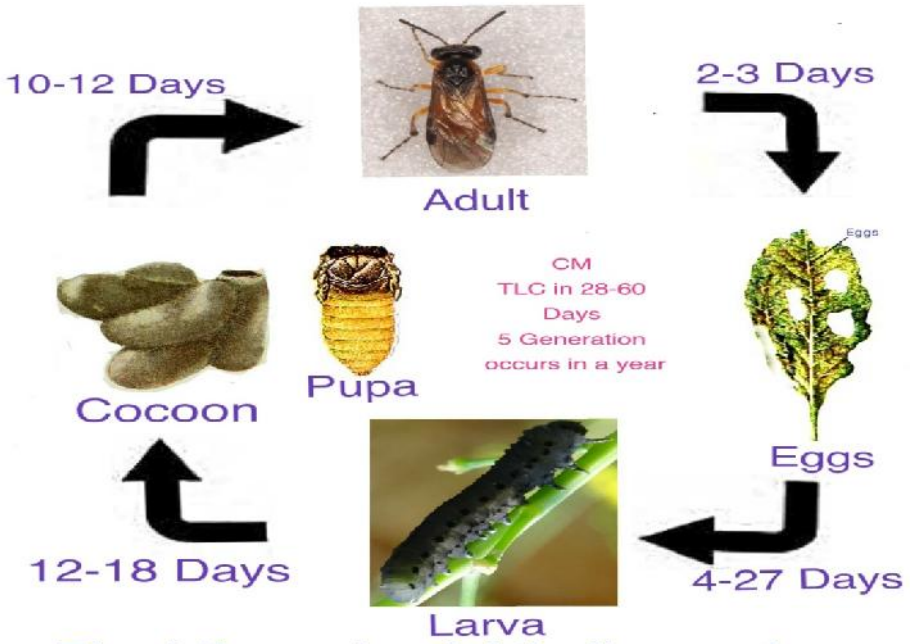

Fig.-Life cycle of Athalia proxima

Prevention and Control:

1: Hand picking of grubs early in the morning is found useful to check the population of the pest.

2: Dusting the crop with any one of the following insecticides has been found effective-

i- Malathion 5% dust @ 25 kg./ha.

ii- Thiodon 4% dust @ 30 kg/ha.

iii- Carbaryl 5%dust @ 25 kg/ha.

3: The crop may also be sprayed by any one of the following insecticide to destroy the insect-pests-

i- Dimecron 85% @ 0.250 lit/ha

ii- Thiodon 35 Ec @ 1.0 lit/ha.

iii- Nuvan 100 @ 0.25 lit/ha.

4: 0.1 % spray of brestnol 45 WP protectthe crop from grubs. It acts as antifeedant due to which the larvae are unable to locate its host and finally die.

Natural enemies

Larval parasites: Exacrodus populence.

C: Paintedbug

Local name: Sundar**,** Jhanga, Dagila Keet

Scientific name: *Bagrada cruciferarum* Krik

Classification

Phylum-Arthropoda

Class-Insecta

Subclass-Pterygota

Division-Exopterygota

Order-Hemiptera

Suborder-Heteroptera

Family-Pentatomidae

Genus-Bagrada

Species-cruciferarum

Food plants: Mustard, Cabbage, Cauliflower, Knolkhol, Radish, Turnip and other Cruciferous plants.

Distribution: It is found throughout India besides India, it is distributed in Ceylon, Burma, Iron and East Africa.

Nature of damage: Both nymph and adult cause the damage to the crop by sucking the sap from the leaves, stems and tender parts of the plants. The attack of pest is noted after few days of sowing the crop, if they are in great number may destroy the whole crop and necessitate resowing. The attacked plants look sickly and dry up or may get stuned in growth. The black fungus is also attracted at the feeding point due to which brown or black spots are seen on the leaves.In the later stage of the crop they suck the sap from the flowers and pods resulting in the poor formation of pods and yield. After harvesting pest may be seen in greater number on threshing floor.

Life cycle of Bagrada cruciferarum

Generally it belongs order-Hemiptera and it is clear that in Hemipteran three developmental life stages will be reported such as eggs, nymph and adult.

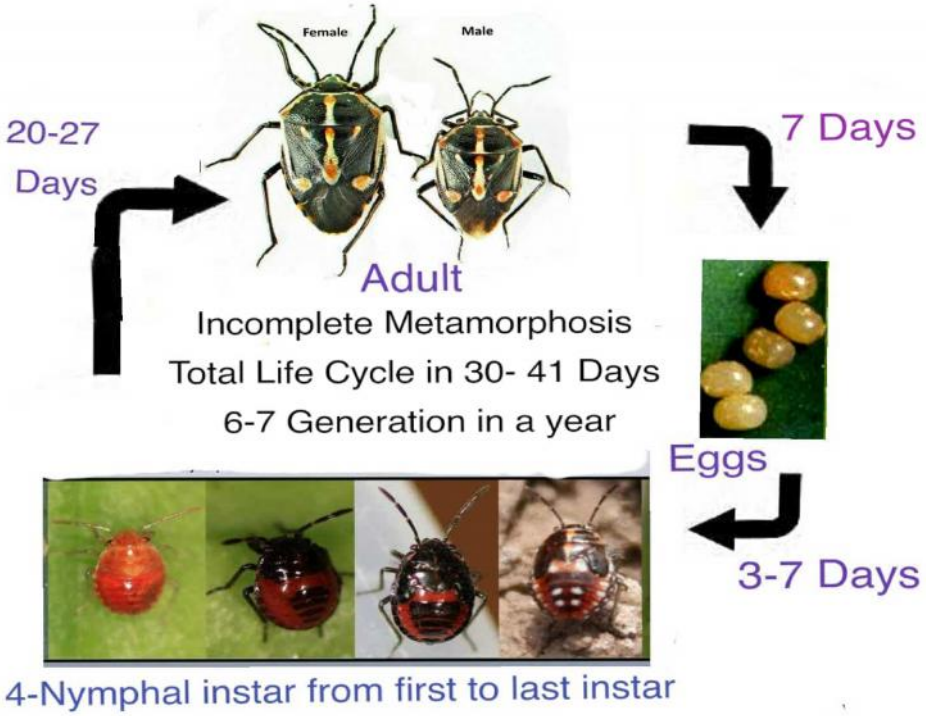

Fig.-Life cycle of Bagrada cruciferarum

Eggs-The eggs are laid by female but usually singly on the leaves, leaf stalks and stems of the host plants.Some times female also lays eggs in soil near the roots in cluster. The number of eggs laid by each female varies from 90-250. Freshly laid eggs are pale yellow in colour which turn in to pinkish at the time of hatching.The individual egg measures 1mm × ½ mm. Hatching period of eggs varies 3 to 7 days.

Nymph- The newly hatched nymph is small, bright orange in colour and with dark red eyes. They are 1.3 mm in length and 1 mm in breadth. They start feeding on the plants and develop rapidly, nymph moults 5 times (in figure seen 4 nyphal instars) and attained the winged in adult stage. Fully grown nymph measures 4.5 mm × 3.2 mm.The nymphal period varies from 20-27 days.

Adult- The painted bug is flat, bright black in colour with reddish yellow spots and dots, measures 5 mm long. The head is small, triangular and provided with black eyes, two ocelli and setaceous antennae. The scutellum is very large.

The life cycle is completed in 30–41 days and 5 to 7 generation are found in a year. It is most abundant from February to April in the plains of Uttar Pradesh. After that most of them die due to heat and only few survive in moist places.

Prevention and Control:

1: Since bug congregate on the leaves and stems,they may be collected and destroyed.

2: Irrigate the field by mixing 5 kg of crude oil emulsion per hectare to destroy the hiding bugs in cracks and crevices.

3: Dusting of crop with following insecticides have been found quite effective-

i-Malathion 5% dust @ 25 kg/ha.

ii-Carbaryl 10% dust @ 25 kg/ha.

4: Spraying of any one of the following insecticides is also effective to control the bug-

i-Malathion 50 Ec @ 1.25 lit/ha.

ii-Dimecron 85% @ 0.250 lit/ha.

iii-Rogor 30 Ec @ 1.00 lit/ha.

Natural enemies: The following parasites have been found on different stages of the pests-

Egg parasite- Typhodytes sp.

Nymph and adult parasite- Alphora sp.

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Halophilic Archaea: An Over view

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Abstract

Archaea are extremophiles, found in extreme environments like salty, acidic, alkaline, hot, cold or anaerobic. Halophiles are domain of Archaea, salt loving organisms belong to family: Halobacteriaceae. They require high salt concentration (4.5M). Generally they are found worldwide in aquatic of high and low salinity, marshy places, hypersaline environments like salt pans and ponds, deserts, hypersaline soil, salted foods, Great salt lake, Dead Sea etc. They are pleomorphic, gram positive or gram negative rods or cocci. They have unique osmo regulation by novel lipids, pigments, compatible solutes, enzymes etc. Laboratory cultivation can be done in extreme and moderate salt containing medium. As halophilic archaea has unique metabolic versatility, used in industries, pharmaceuticals, medicine, agriculture etc. It also plays important role in cosmetic, fermented food product, preservatives, bioplastic production, photoelectric divice, halogram, as biosensor etc. Due to its simple nutrition requirements and wide applications, it is promising candidate for research.

Key words: Archaea, Halophiles, Extremophiles, Hypersaline environment, Biotechnological applications.

Introduction

The word “Archaea” means “Ancient thing” (from Greek). It refers to a group of prokaryotic, single-cell microorganisms (Woese and Fox, 1977). Archaea represent one of the three primary lineages of modern cellular organisms. Three domain classification given by Woose in 1977 i.e: Bacteria, Archaea and Eukarya. The classification based on 16s rRNA sequence analysis. Archaea exhibit certain common characteristics with bacteria and eukarya (Grogan, 2013). Archaea generally detected in nearly all environments. However, well- characterized archaea have been cultured from extreme environment like salty, acidic, alkaline, hot, cold or anaerobic (DasSarma et al, 2009).

Many archaea are chemoautotrophs and can grow on simple inorganic chemicals, other are heterotrophs require complex organic material. Few are phototrophs, can use light energy for their growth (DasSarma et al, 2009).Archaea are not bacteria. At molecular level archaea are more closely related to eukarya than bacteria, because they have similar gene for transcription and translation to eukarya. They have unique membrane lipid contain branched chain isoprenoid units in fatty chains linked to a glycerol-1-Phosphate head groups via ether linkages (Dodds and Whiles, 2010).

Classification of Archaea:

Based on their phylogenetic relationship archaea are classified as following (Barns et al, 1994, 1996) (Fig.1):

1) Crenarcheaota:

They are basically thermophilic, heat-tolerant form, having special protein can function at high temperature as 230^{o}F. They can survive in very acidic environment too.e.g. Deep sea vents.

2) Euryarchaeota:

They include methanogens, produce methane. For respiration they use carbon as their electron acceptor. They found in Marshes and wetland, deep sea sediments.

3) Koraarchaeota:

They are Halophiles, oldest survival organism on earth. They found in hydrothermal environment much like crenarcheaota.

4) Thaumarchaeota:

They were considered as crenarcheaota.

5) Nanoarchaeota:

They are small coccoids, live as parasites or symbionts.

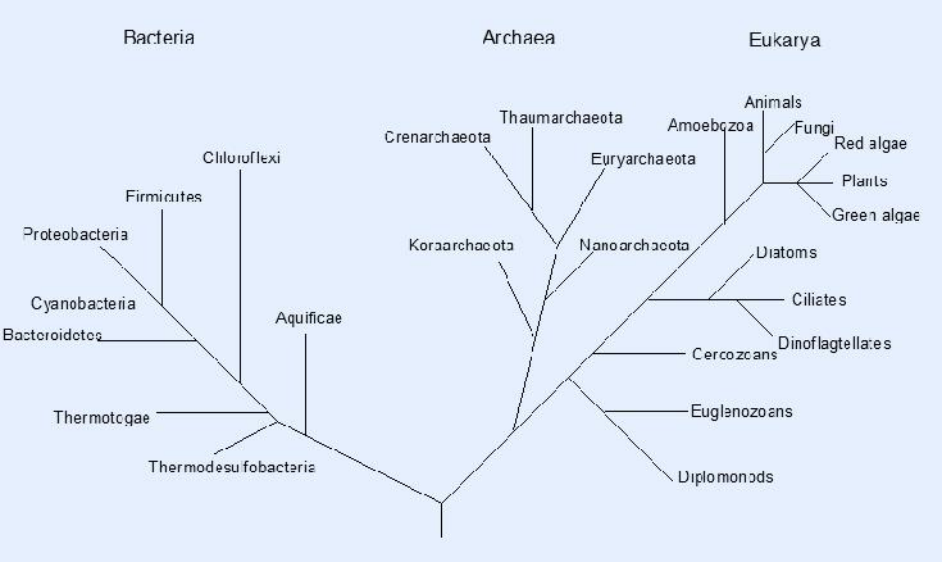

Fig.1 - Phylogenetic Tree (The photo © to author)

Halophilic archaea:

Halophilic archaea are salt-loving organisms, belong to family: Halobacteriaceae (Irwin, 2020).Currently it has 50 genera with 213 species (Gupta et al, 2015, 2016).

Habitate:

Generally halophilesgrow at high salt concentrations (4.5M NaCl). They are distributed worldwide, like India, USA, Spain, Austria, Australia, Egypt, Kenya, France, Taiwan, Korea etc. (Corral et al, 2020). They can be found in aquatic of high and low salinity, marshy places, hypersaline environments like salt pans and ponds, deserts, hypersaline soil, salted foods, Great salt lake, Dead Sea where pH around 7 and also found with high pH (9-11) like Soda lakes (Samylina et al, 2019). Some well known hypersaline ecosystems investigated for the diversity of halophiles are: The Great Salt Lake, Utah, USA, the Dead Sea, the extremely alkaline brines of the Wadi Natrun, Egypt and lake Magadi, Kenya (Oren, 1994; Kamekura, 1998).

Hypersaline lakes, Salterns evaporator andCrystallizer ponds produce salt from seawater. And form habitat for halophilic archaea.Hyper saline aquaties classified in “Thalassohaline” and “Athalassohaline”. Thalasohalines, are marine origin and has salt concentration equivalent to marine water, e.g. Artificial solar salterns. While, Athlassohalines, has salt concentration differ from sea water, e.g. Natural terminal lakes (Singh et al, 2019).

Classification of Halophiles:

Based on salt concentrations halophiles are classified in four categories (Kushner, 1978; Kushner and Kamekura, 1988, Kanekar et al, 2012):

(a) Slight halophiles, grow at 2-5% NaCl concentrations

(b) Moderate halophiles, grow optimally at 5-20% salt concentrations

(c) Extreme halophiles, grow at 20-30% salt concentrations, depending on temperature and in which the compatible solutes are K+ ions and Cl–ions (Grant and Larson, 1989).

(d) Halotolerant: Grow in the absence as well as in presence of high salt concentrations, and in which the compatible solute is neither K+ nor Cl–ions, but organic compounds.

Novel lipids such as Phospholipids and glycolipids also use as chemotaxonomic marker for classification (Anshuman, 2008). Moreover, 16s rRNA analysis and PCR survey further classifies the halophiles.

Physiology:

Halophilic archaea are physiologically diverse group mostly aerobic as well as anaerobic, heterotrophic, phototrophic and chemoautotrophic. They have unique osmoadaptation strategies to withstand in such high salt concentration. These are: (1) Salt-in-strategy: Accumulation of K+ ion in cytoplasm and export Na+ ions to extracellular space. (2) Synthesis of compatible solute: Betaine and Ectoine in cytoplasm to achieve osmotic regulation (Oren, 2008; Abaramak et al, 2020).Ectoine can protect many unstable enzymes and nucleic acids against high salinity, thermal denaturation, desiccation and freezing, thereby increasing shelf life and activity of enzyme preparations (Galinski et al, 1989; Lippert and Galinski, 1992 and Kolp et al, 2006).

Stress protection by Unique light driven proton pump “Bacteriorhodopsin”, to generate an electrochemical proton gradient across the membrane (Ihara, 1999). Halophiles produce pigments like bacteriorhodopsin, halorhodopsin, sensoryrhodopsin and photorhodopsin protect halophiles in high salt environment (Anshuman, 2021). Because the K+ concentration inside the cell is 100 times higher than in the surrounding environment, a part of the proton motive force (PMF) must be used to maintain the ion gradient. Proton motive force is generated by Respiration and Photosynthesis.

The discovery of numerous halophilic enzymes further signifies the importance of harsh environment condition. These enzymes not only remain active and stable in hypersaline environments but are alsothermo tolerant. These properties made halophilic enzymes attractive for various biotechnological applications (Moreno et al, 2009 and Shirazian et al, 2016)

Cell shape:

They are generally pleomorphic rods and cocci. Other one unusual shape is the "Square haloarchaeon of Walsby (Walsby, 1980). They are gram positive or gram negative, motile or nonmotile.

Flagella of archaea are known as "Archaella", it operate like bacterial flagella. The subunit composition, assembly mechanism of archaeal flagella is very different from bacterial flagella (Thomas et al. 2001). The presence of gas vesicles, allow halophiles to float to the brine surface. Gas vesicles may also be effective as a shield against harmful UV radiation was not confirmed in laboratory experiments (Simon R. D., 1980).

Exopolysaccharides (EPS):

Exopolysaccharides produced extracellularly by microorganisms, composed of simple sugar. Halophiles also produced them. It resists extreme pH and temperature. It has numerous applications in food, pharmaceutical, petroleum, oil recovery and other industries (Sutherland 1990,1998; Tombs and Harding,1998).

Cultivation:

To cultivate halophilic archaea in laboratory different moderate and high salt containing media such as Tryptone Yeast Extract Salt medium (TYES), Mullakhan and Larsen medium (M&L) and Larson media are used. Different halophilic colonies are in different colors such as red, pink, orange, white etc (Mullakhan and Larsen, 1975; Larsen, 1981 and Krieg and Holt, 1984).

Importance:

Halophilic archaea plays important role in industries like cosmetic, fermented food product, preservatives, bioplastic production, photoelectric divice, halogram, as biosensor etc.The pigment β -carotene is in high demand as an antioxidant, as a source of pro-vitamin A (retinol) and as a food colouring agent (Kanekar et al,2012; Yin et al, 2015 and Giami et al,2019). It's antioxidant activities make it popular for use in health food. Carotenoids, has anticancer activity or other bioactive molecules produced by halophiles can be

used for cancer treatment. Among extremophiles, halophilic and halotolerant microorganisms found in hypersaline environment are considered as source of antitumor metabolites (Corral et al, 2020). But with few side effect several study focus on halophilic metabolites for cancer treatment.

Antimicrobial compound i.e, Halocin, was reported by Rodriguez-Valera et al, 1982 in genus Halobacterium. They cause lysis of surrounding microbiota. Ecological point of view, they are also used in wastewater treatment system to degrade solidsi.e. Sewage treatment. Halophilic archaea have also been used for bioremediation in harsh environment or oil polluted sites to degrade organic pollutants and also in the treatment of concentrated textile waste waters as well as azo dye decoloration (Anshuman, 2007, Kanekar et al, 2012 and Kiadehi eta al, 2018).

They also used as PGPR, phosphate solubalizing agent (Bonete et al, 2015) and recently some genera produce siderophores, which solubilize and transport iron into microbial cell (Dave et al, 2006).

As halophilic archaea has unique metabolic versatility, novel lipids, low nutritional requirements, adaptation to harsh conditions and due to wide applications, it is promising candidate for research.

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Potential role of Lycopene against cadmium induced Toxicity in mice following chronic exposure

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Abstract

The present study has been undertaken to evaluate the protective efficacy of lycopene on cadmium induced toxicity in testis of albino mice. Albino mice were divided into four groups. Group I mice were kept as control. Group II animals were administered a daily dose of cadmium chloride (0.32mg/kg bw) intraperitoneally. Group III animals were injected with 20mg/kg bw of olive oil (positive control). Group IV animals were injected a daily dose of $CdCl_2$ followed by a chronic dose of lycopene (20mg/kg bw). Autopsies were done at 15 days post treatment. Cadmium leads to decrease in weight of testis as compared to control. Alterations in testis were in the form of degenerated germinal layer, almost complete loss of spermatogenic activity and leydig cell lysis. Lycopene administration to mice showed increase in weight of organ and showed significant protection in the alleviation of cadmium induced injury.

Keywords: Cadmium (Cd), lycopene and histopathology.

Introduction

Heavy metals are highly persistent and can bioaccumulate and biomagnify in the food chain, thus becoming toxic to living organisms (Deepmala *et al*. 2013). Cadmium is an environmental pollutant that is used in manufacturing accessories such as batteries, paints and plastics. According to WHO reports, approximately 60-70 μ*m/day* is tolerable for a person weighing 70 *kg*. In addition, the uptake andaccumulation of cadmium in smokers and nonsmokers is different and the concentration of cadmium in smokers is higher (Thomson and Bannigan, 2008).

Cadmium is a cytotoxic, carcinogenic, and mutagenic industrial product or by product (Cui *et al*., 2021). Chronic exposure to cadmium is associated with increased incidence of various neoplastic and non-neoplastic diseases of

kidney, liver, lungs, bone, brain, thyroid gland and other organs (Zhou *et al*., 2012) but the report on structural changes in the myocardium under the influence of cadmium are rarely found in literature (Lei *et al*., 2011). Cadmium was also found to generate reactive oxygen species (ROS) that cause apoptosis (Yuan *et al*., 2016). Some studies have also shown that cadmium can cause cell death by necrosis in testicular tissue (El-Shahat *et al*., 2009). Cadmium causes necrosis and atrophy in testicular tissue by suppression of glutathione peroxidase activity (Patra *et al*., 2011).

Carotenoids are present at considerable amounts in plasma and human tissues and may have specific functions in relation to their high antioxidant capacity (Viuda-Martos *et al*., 2014). However lycopene, as the main carotenoid in tomato products, possesses the greatest ability to quench singlet oxygen compared to the other carotenoids (Di Mascio *et al*., 1989). *In vitro* studies have shown that lycopene, a polyunsaturated hydrocarbon with a molecular formula of $C_{40}H_{56}$, has the highest antioxidant capacity to quench singlet oxygen and trap peroxyl radicals (Srinivasan *et al*., 2007). Although lycopene is chemically a carotene it has no vitamin A activity. Tomato products have higher levels of antioxidant activity and therefore are more potent than tomatoes in reducing the risk of oxidation-related diseases (Sandhir *et al*., 2010). Eleven conjugated double bonds of lycopene give it a deep red color and are responsible for its antioxidant activity. Increased ingestion of tomatoes and tomato products that contain lycopene is associated with decreased risk of chronic diseases including cancer (Franceschi *et al*., 1994).

The present work aims to explore the possible ameliorative effect of lycopene on cadmium chloride induced toxicity on testis of albino rats.

Materials and Methods

Animals: Swiss albino mice weighing 20-25g were procured from GADVASU, Ludhiana. They were kept and acclimatized to the laboratory conditions for 15 days under optimal conditions of light and temperature. They had *adlibitum* access to tap water. The animals were handled with humane care in accordance with the guidelines of the Institutional Animal Ethical Committee.

Chemicals: Cadmium chloride ($CdCl_2$) was bought from S.D FINE CHEM LIMITED, Mumbai. It was dissolved in double glass distilled water and administered intraperitoneally (i.p.) to mice. Lycopene was obtained from PASSIM Pharmaceuticals Limited, Baddi (HP). It was dissolved in olive oil and administered intraperitoneally to mice.

Experimental Design: The mice were divided into four groups of six mice each. Group I – Control animals were given distilled water. Group II – Animals were administered a daily dose of 0.32 mg/kg bw of cadmium (i.p.). Group III – Animals were kept as positive control and were injected (i.p.) 20 mg/kg bw of olive oil daily. Group IV – Animals were injected a chronic dose of 0.32 mg/kg bw of cadmium (i.p.) followed by a daily dose of 20 mg/kg bw of lycopene for 15 days. Autopsies were done on 15 days post treatment.

Testis was removed, blotted dry and weighted separately.

Histopathological studies: Testes were fixed in Bouin's fixative, embedded in paraffin wax (58-60^{o}C) and 5-7mμ thick sections were stained with haematoxylin and eosin stains.

Statistical analysis: The data was analysed by using Student's *t*-test.

Results and Discussion

Cd administration does not produce any discernible signs and symptoms of sickness in mice. Also, there was observed no mortality during the entire period of experiment. The testis weight of Cd treated mice with controls are summarized in Fig. 1, which showed that Cdtreatment caused significant ($p<0.001$) reduction intesticular weight on15 days post treatment as compared to control group.

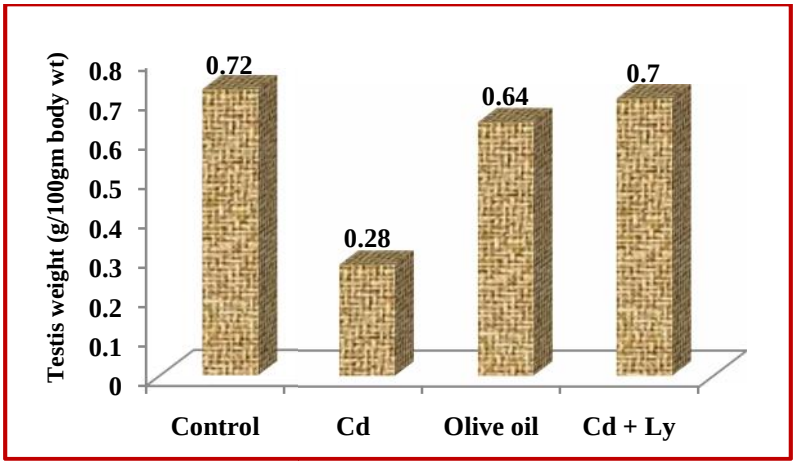


Fig.1 Comparison of testes weight of control and treated group.

Organ toxicity can be revealed by considering the weight of organs after exposure in animal toxicity studies (Anderson *et al*., 1999). The regression in weight of testis is attributed mainly to the necrosis of seminiferous tubules induced by Cd cytotoxicity. Also, Cd gets accumulated in testes and hence continuously keeps on damaging its cell population (Bench *et al.,* 1999). According to Salman *et al*. (2010) weight of reproductive organ is an essential benchmark for risk assessment in toxicological studies and testicular size is the best primary tool for assessment of spermatogenesis, since the tubules and germinal elements account for approximately 98% of the whole testicular mass.

It is considered that the toxicity of cadmium, among other things, comes from cadmium reaction with sulfhydryl groups, thus changing the activity of many enzymes. Although cadmium is not a redox-active metal, it indirectly leads to oxidative stress and tissue damage (Zhou *et al*., 2012). Membrane lipids are highly susceptible to free radical damage. Lipids when reacted with free radicals can undergo the highly damaging chain reaction of lipid peroxidation. Moreover, the heavy metal binds to other relevant biomolecules present in subcellular membrane, endoplasmic reticulum, mitochondria or within the nucleus causing their damage (Mitra *et al*., 2012). Primary injury appears to be caused by the binding of Cd to sulphydryl groups on critical molecules in mitochondria resulting in thiol group inactivation which causes oxidative stress, the mitochondrial permeability transition and mitochondrial dysfunction.

Normal testis depicted organized germ cells in seminiferous tubules, normal leydig cells in interstitial mass and cuboidal germinal epithelium (Fig.2). Histology of testis of olive oil treated group showed normal structure in the experiment (Fig.3). The testes of cadmium treated group showed atrophy of seminiferous tubules (Figs.4,5). The germinal layer was degenerated. The seminiferous tubule epithelium shows areas of destruction and edema in between cells. Abnormal small oval cells with darkly stained condensed chromatin appeared separated from the basal lamina. The interstitial spaces are widened with areas of tissue destruction and edema. The interstitial blood vessels were congested and lined by thick fenestrated endothe-lium.

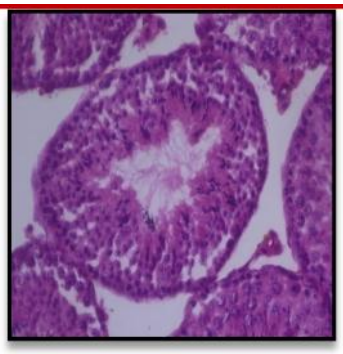
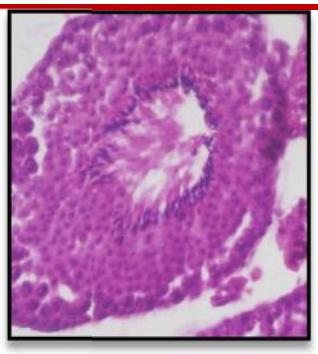


Fig.2



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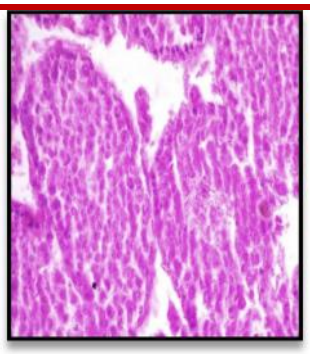

Fig.4

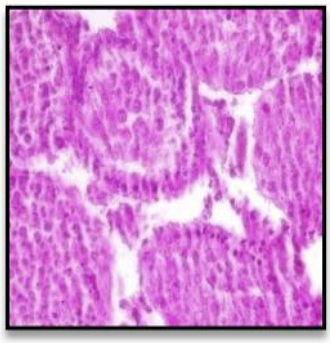

Fig.5

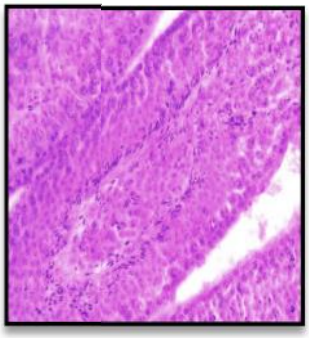

Fig.6

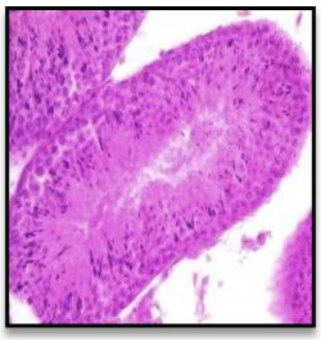

Fig.7

Fig.2 - Testes of control mice showed many seminiferous tubules and spermatogonia in different stages of development. **Fig.3 -** Testes of olive oil treated group showed almost normal structure. **Fig.4 and 5 -** Testes of Cd treated group showed hyperaemia, clumping of numerous seminiferous tubules along with atrophied testicular tissue and dead cells. **Fig.6 and 7 -** Testes of Cd + Ly treated group showed intact structure of seminiferous tubule with numerous sperms alongside with all the stages of spermatogenesis. Further, plenty of sperms with heads and tails are also visible.**X400**.

Though, in some of the seminiferous tubules, the tunica albuginea was found to be fibrous and spermatids were almost absent in seminiferous tubules. This was in accordance with that reported by Benoff *et al*., (2000); Biswas *et al*., (2001); Yang *et al*., (2006); Siu *et al*., (2009); Sharma and Kaur, 2012; 2013. Siu *et al*., (2009) reported that in vivo acute exposure to Cd caused germ cell loss, testicular edema, hemorrhage, necrosis, and sterility in several

mammalian species. They reported that the underlying mechanism(s) was not known at the time.

During present study, the damages observed in testes tissue indicate that maturation of spermatogonia through the process of meiosis was completely disrupted following Cd exposure. Lee and Dixon (1973) postulated two possibilities for the primary mechanism of Cd induced toxicity in testes (i) circulatory failure due to vascular damage. (ii) decreased utilization of zinc by spermatogenic cells due to competitive action of cadmium.

Cadmium and other heavy metals can target the vascular system in variety of ways. According to Cheng and Mruk (2012) the unusual sensitivity of the testes to Cd is related to unique feature of its vasculature namely the pulseless, semistagnant flow of blood in the intratesticular course of the testicular artery, which permit cadmium to alter capillary endothelium permeability, resulting in oedema and pressure effects leading to anoxia. Leydig cell degeneration observed in the treated groups during present work would have resulted in direct destruction to the hypothalamus-pitutary gonadal axis resulting the decreased synthesis of testosterone, which in turn disturbs the process of spermatogenesis (Stohs *et al.,* 2001). Many studies have revealed that leydig cells play an important role in the structural and functional integrity of seminiferous tubules and synthesis of testosterone which is the one of the main components for regulation of post meiotic stages of spermatogenesis (Stohs *et al.,* 2001).

Many studies proved that most important mechanism responsible for tissue damages by heavy metals is oxidative stress (Garnier *et al.,* 2006). Cadmium induced oxidative stress has been associated with production of reactive oxygen species (ROS) comprising mainly superoxide radical anion, hydrogen peroxide and hydroxyl radicals which lead to lipid peroxidation, membrane protein and DNA damage and can also results in carcinogenesis, apoptosis, necrosis and cell proliferation (Habeebu *et al.,* 1998).

Histologically we noticed that the damaging effect of Cd was ameliorated by concomitant administration of lycopene. Still there was little testiculardamage but less than that of Cd-received animals alone. No abnormal spermatogenic cells observed within the semi-niferous tubular epithelium. Lycopene could normalize the damaging effect of oxidative stress induced by free radicals in rat heart, liver and testes by improving male fertility (Kutlubay *et al*., 2007). Lycopene also showed protection against cadmium induced testis (Fig. 6, 7).

Conclusion

In conclusion, in the present study we showed that Cd treatment markedly impaired organ functions as well as caused oxidative stress in Swiss albino mice. Importantly, treatment with lycopene prevented such toxic effects. Our results also indicate that lycopene could represent a preventive approach to minimize the deleterious effects associated with Cd exposure. Thus, we recommend consumption of lycopene wherever the toxicity with cadmium is the matter of concern.

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Cancer therapy and Treatment ongoing and upcoming

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Introduction

The Term Cancer, which means (crab) in Latin, which was coined by Hippocrates in the Fifth century B.C. to describe diseases in which tissues grow and spread unrestrained throughout the body, eventually choking off life (Becker et.al., 2007) Hippocrates used, the words carcinus, carcinoma and cancer to describe the tumours (Cooper and Housman).

Prior to 1900, most human deaths were due to infectious diseases such as pneumonia and tuberculosis, and life expectancy was less than 50 years. Cancer was rare disease that accounted, for only a small percentage of deaths.

According to recent survey Conducted by American cancer society in 2005, more than 5 lac Americans die of cancer each year. The four most common cancers accounting for more than half of all Cancer cases are those of the Prostate, breast, lungs, and colon /rectum . Lung cancer, by far the most lethal is responsible for nearly 30% of all cancer death therefore, How can we apply our growing understanding of the biology of the cancer to combat the disease? Prevention is always better than cure. Although the primary goal of cancer treatment is to eliminate the cancer. If this Primary goal Can not be accomplished the goal of cancer treatment shifts to palliation, the improvement of symptoms , and preservation of quality of life while striving to extend life. The Conventional method of treatment through.

Surgery, chemotherapy and radiation does not successfully cure once the cancer is metastasized. Chemotherapy and radiation affect normal Cells and have various adverse side effects.

(i) Surgery:- The most common treatment of cancer involves surgery to remove the primary tumour followed (if necessary) by radiation therapy and/or chemotherapy to destroy any remaining cancer cells.

(ii) Radiation therapy:-. This approach employs high energy X-rays or other forms of ionizing radiation to kill cancer cells. We already know that DNA damage created by ionizing radiation can cause cancer, but paradoxically, the same type of radiation is also used in higher doses to destroy cancer cells in

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people who already have the disease, Ionizing radiation kills cells in two different ways. First, DNA damage caused by radiation activates the p53 signaling pathway, which then triggers cell death. However, many cancers have mutations that disable the p53 pathway, so p53-induced apoptosis plays only a modest role in the response of most cancers to radiation treatment In the second mechanism, radiation kills cells by causing chromosomal damage that is so severe that it prevents cells from progressing through mitosis, and the cells therefore die while trying to divide.

(iii) Chemotherapy:- Most forms of chemotherapy use drugs that, like radiation, are intended to kill dividing cells. Such drugs can be subdividing into four major categories:

(a) Antimetabolites.:- They inhibit metabolic pathways required for DNA synthesis by acting as competitive inhibitors that bind to enzyme active sites in place of normal substrate molecules. Examples of such antimetabolites include methotrexate, fluorouracil and mercaptopurine.

(b) Alkylating agents. :- They inhibit DNA function by chemically cross-linking the DNA double helix, Examples of such drugs include cyclophosphamide, chlorambucil and cisplatin.

(c) Antibiotics. :-These are substances made by microorganisms that inhibit DNA function by either binding to DNA or inhibiting topoisomerases required for DNA replication. Examples of such antibiotics include bleomycin and doxorubicin.

(d) Plant-derived drugs:- These drugs either inhibit topoisomerases or disrupt the microtubules of the mitotic spindle. Examples of such drugs include the topoisomeraseinhibitoretoposide and the microtubule disrupting drug, taxol.

One problem with such drugs (and radiation therapy) is that they are toxic to normal dividing cells as well as to cancer cells. When cancer arises in a tissue whose growth requires a specific hormone, it may be treated in a less toxic manner using drugs that block the action of that particular hormone. For example, many breast cancers require estrogen for their growth. Estrogens tend to exert their effects by binding to nuclear receptor proteins that activate the expression of specific genes. The drug tamoxifen, a common treatment for breast cancer, binds to estrogen receptors in place of estrogen and prevents the receptors from being activated.

Newer treatment approaches include immunotherapies that exploit the ability of the immune system to attack cancer cells, molecular targeting drugs aimed at proteins that are critical to the cancer cells, and antiangiogenic agents that attacks a blood supply or the tumours.

Chemotherapeutic agents can act by one of the following mechanisms:-

Direct DNA interacting agent. Eg. Melphalan, Cisplatin

Antitumor Antibiotics and Topoisomerase Poisons. Eg. Topotecan, Mitoxantrone

Indirectly DNA-Interacting Agents. Eg. 6 Mercaptopurine (6-MP), 5-fluorouracil

Antimitotic Agents. Eg. Vinblastine, Paclitaxel

Diagnostically Guided Protein Kinase Antagonists. Eg. Imatinib, Nilotinib

Multikinase Inhibitors. Eg. Sorafenib, Axitinib

Proteasome Inhibitors. Eg. Carfilzomib

Histone Deacetylase Inhibitors. Eg.Vorinostat

Current Therapies Exploit the Loss of Cell-Cycle Control and the GeneticInstability of Cancer Cells:-

Anticancer therapies need to take advantage of some property of cancer cells that distinguishes them from normal cells. One such property is the genetic instability that results from loss of chromosome maintenance or DNA repair mechanisms. Remarkably, it seems that most existing cancer therapies work because, unknown to the people who developed them, they exploit these molecular defects. Traditional anticancer therapies mostly rely on agents-drugs and ionizing radiation-that damage DNA and the machinery that maintains chromosomal integrity. Such treatments preferentially kill certain kinds of cancer cells because these mutants have a diminished ability to survive the damage. Normal cells, when treated with radiation, for example, will suffer damage to their DNA, but will then arrest their cell cycle until they have repaired it. Tumor cells that have defects in various cell-cycle checkpoints, on the other hand, lose the ability to arrest the cell cycle in these circumstances, and so continue to multiply immediately after irradiation. Almost all of these cells will therefore die after a few days as a result of the catastrophic DNA damage they sustain when they attempt to divide with defective chromosomes.

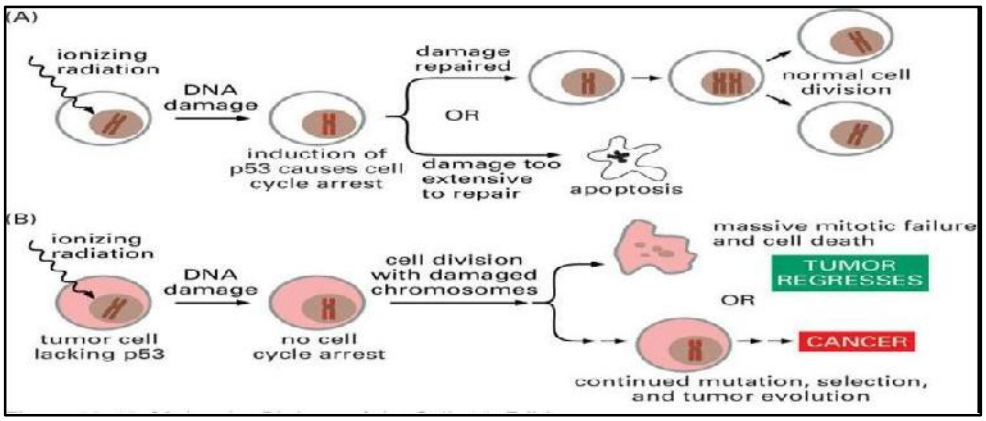

Figure :- Effects of ionizing radiation on normal cells(A) and cancer cells (B).Cancer cells tend to be more susceptible than normal cells to the damaging effects of ionizing radiation because they lack an ability to arrest the cell cycle and make the necessary repairs. Unfortunately, the same genetic defects may render some cancer cells resistant to radiation treatment, as they may also be less adept at activating apoptosis in the face of DNA damage.

New Therapies May Emerge From Our Knowledge of Cancer Biology:-

Our growing understanding of cancer cell biology and tumor progression is gradually leading to better methods for treating the disease, and not only by targeting defects in cell cycle arrest and DNA repair processes. As an example, estrogen antagonists (such as tamoxifen) and drugs that block estrogen synthesis are now widely used in patients to prevent or delay recurrence of breast cancer (and they are even being tested as agents to prevent new cancers from arising). Such antiestrogen compounds do not directly kill off the tumour cells.

Nevertheless, they improve the patient's prospect of survival, presumably because estrogens are necessary for the growth of normal mammary epithelium and a proportion of breast cancers retain this hormone dependence.

The greatest hopes lie, however, in finding more powerful and selective ways to directly exterminate cancer cells. Now that we can pinpoint their genetic lesions, can we use our knowledge of cell biology to kill them off? In recent years, a wide variety of adventurous new ways to attack tumor cells have been suggested, many of which have been shown to work in model systems-typically reducing or preventing tumor growth in mice. Many of these protocols will turn out to be of no medical use, because they do not work in

humans, have bad side effects, or are simply too difficult to implement. But some seem likely to succeed. For example, some tumor cells are heavily dependent on a particular protein that they overproduce (although it may not be unique to them). Blocking the activity of this protein may be an effective means of treating cancer if it does not unduly damage normal tissues. For example, about 25% of breast tumors express unusually high levels of the Her2 protein, a receptor tyrosine kinase, related to the EGF receptor, that normally plays a part in the development of the mammary epithelium. Thus, shutting off Her2 function might be expected to slow or halt the growth of breast tumors in humans; in fact, this approach is cur rently being tested with some success in clinical trials, using as the blocking agent a monoclonal antibody that recognizes Her2.

Another approach to destroying tumors targets the delivery of a toxic com pound directly to the cancer cells by exploiting proteins like Her2 that are abundant on their surface. Antibodies against such proteins can be armed with a toxin, or made to carry an enzyme that cleaves a harmless 'prodrug' into a toxic molecule. In the latter case, one molecule of enzyme can then generate a large number of toxic molecules. A virtue of this strategy is thatthe toxic drug generated enzymatically can then diffuse to neighboring tumor cells, increasing the odds that they too will be killed, even if the antibody did not bind to them directly. Immunotherapy/ Biologic therapy: -

Two treatment strategies have been devised: Passive and active immunotherapy. Passive therapy involved the administration of antibodies, which binds to target tumor cells and evoke an immune response. Humanized monoclonal antibodies are developed, which are more effective. Antibodies work as tumor regulatory or immunoregulatory antibodies. A tumor regulatory antibody such as Herceptin is directed against Her2, a cell-surface receptor, for a growth factor in breast cancer. Immunoregulatory antibodies producetumordirected cytotoxicity such as Atezolizumab against PD-L1 for the treatment of non-small cell lung cancer.

The normal immune system may not respond to inappropriate or modified proteins expressed on tumor cells or tumor cells may develop a mechanism to hide from immune response. Active antibodies are modified antibodies which are used to enhance or mount a strong immune response againstthe cancerous cells. The development of chimeric CAR T cells is one of the strategies to treat cancer which has evaded the immune system.

Other strategies to treat cancer are the use of anti-tumor vaccines, oncolytic viruses, inhibiting cancer promoting proteins, inhibiting angiogenesis, etc. However, relapse of cancer and resistance to therapy are still the major hurdles in the treatment of cancer.

Specialized Cells in tumour microenvironment:-

Tumors are generally highly heterogeneous and complex in genetics. It is hypothesized that the crosstalk between cancer cells and their surrounding TME factors plays a pivotal role in tumor development. TME is dominated by the tumor, which orchestrates molecular and cellular signaling events taking place in surrounding cells. Diverse types of cells in TME, including fibroblasts, endothelial cells, immune cells, Mesenchymal stem cells (MSCs)and neuroendocrine (NE) cells, have special functions in cancer progression as summarized in Table 1.

Cancer-associated fibroblasts:- In response to tissue injury , residential fibroblasts differentiate into myofibroblasts also known as cancer-associated fibroblasts (CAFs). CAFs further cause organ fibrosis, hence enhancing the risk of cancer and constitute a major part of TME. CAF's secrete various growth factors, such as the EGF family members hepatocyte growth factor (HGF), fibroblast growth factor (FGFs), insulin-like growth factor 1 (IGF1), and transformation factor-beta (TGF b) which are mitogenicandsome contribute to the immune suppressive microenvironment. Fibroblast-produced chemokines have chemoattractant properties that can stimulate the migration of other stromal cell types and their progenitors into the TME.

Immune cells:- Evading the immune system is one of the hallmarks of cancer cells. During the early stages of the cancer, the response of immune cells in TME has antitumoral properties. Natural Killer (NK) cells, CD8+cytotoxic T cells, M1 macrophages, T helper-1 cells, and antigen-presenting cells

Table 1 :- Cells in the tumor microenvironment with their specialized function and classification -

Component	Classification.	Function.
Neutrophils	Tumor promoting (N2); tumorinhibiting (N1); increased levels in the colon, stomach, and lung cancer patients.	Enhancement of angiogenesis and metastasis; associated with poor prognosis.
Tumor-asso-ciated macro phages(TAMs)	Tumor promoting (M2); tumorinhibiting (M1); the major protumoral component in TME; infiltrates the tumor; attracted by chemokines secreted by both malig. Nant and stromal cells.	Promoting degradation of the extracellular matrix; aiding the expansion of inflammatory cytokines, such as TNF- β enhancement of angiogenesis, and remodeling.
CD8+ cytotoxic T cells (CTL)	Tumor inhibiting; the major antitumoral com-ponent in TME.	Induce apoptosis, necrosis, and growth arrest by releasing IFN-γ and other cyto toxic cytokines; establishing an antitumor environment
Regulatory T cells (Tregs)	Tumor promoting; promotes tumor maintenance.	Secreting cytokines such as IL-10, TGF-β establishing an immunosuppressive environment; associated with poor prognosis.
Myeloid-derived suppressor cells (MDSCs)	Tumor promoting Increased in almost all individuals with cancer; includes premature granulocytes, macro-phages, dendritic cells, and myeloid precursors.	Associated with tumor progression and neo angiogenesis; suppressing T cells and NK cells; differentiating into TAMs under hypoxic conditions

Mesenchymal stem cells (MSCs)	Tumor promoting; the major component of stromal cells in TME.	Vasculogenic mimicry; forming the premeta static niche; promoting cancer initiation and malignancy.
Neuroendocrine cells (NE cells)	Tumor promoting.	Promoting prolifera- tive signaling; secre- ting neurotransmitters, including CgA, and vasoactive polypeptide; regulating NK cell migration and toxicity ability
Cancer-asso-ciatedfibrob-lasts (CAFS)	Tumor promoting; abundant in TME; commonly used markers including α-SMA, FAPα, S100A4, and PDGFR β; CAFS can differentiate stimulation by ROS and TGFβ1-dependent and independent mechanisms	Sustaining prolifera- tive signaling; acti- vating angiogenesis and metastasis; tumor -promoting inflammation; evading immune destruction; reprogramming cellular metabolism; promoting genome instability and mutations
Adipocytes	Tumor promoting.	Regulating the bala- nce of systematic energyandmetabolism; secreting cyto- kines, chemokines, and hormones;promoting cancer progression.

(APCs) act as tumor opponentsand suppress tumor growth. TME also consists of a plethora of tumor-promoting immune cells, such as neutrophils, tumor-associated macrophages (TAMs), CD4+ T helper-2 cells, and regulatory T cells (Tregs), which are key components of the immune suppression environment, enabling, promoting the survival, metastasis and immune evasion.

Mesenchymal Stem Cells (MSCs):-

Stromal cells of mesenchymal tissues such as the bone marrow, cartilage, and fat tissues consist of MSCs which can further differentiate into multiple cell types. These stem cells can migrate towards the inflammatory site

and form the premetastatic niche to promote cancer cell quiescence and drug resistance. Cancer cells communicate with MSCs at multiple stages of cancer progression to promote metastasis and epithelial-to mesenchymal transition Neuroendocrine cells (NE cells):-

NE cells are vastly spread throughout the body tissues including the hypothalamus, anterior pituitary gland, thymus, thyroid gland (calcitonin secreting cells), breast, and pancreatic islets. These cells can secrete multiple neurotransmitters, such as chromogranin A (CgA), chromophilic polypeptide, and vasoactive polypeptide, that can exert tumorpromoting functions and enhances proliferation. NE cells are also reported to influence the immune cell functions such as NK cell viability. Adipocytes:-

Adipose tissue can secrete various cytokines, hormone-like factors, and chemokines that actively aid the recruitment of malignant cells. These cells provide fatty acids as fuel for cancer cells and participates in cancer growth and progression. Recent evidence highlights adipocytes as a key component of breast cancer and intra-abdominal tumors that metastasize to the omentum.

Summary

Our growing understanding of cancer cell biology should lead to better ways of diagnosing and treating this disease. Anticancer therapies can be designed to destroy cancer cells preferentially by exploiting the properties that distinguish them from normal cells, including the defects they harbor in their DNA repair mechanisms, cell-cycle checkpoints, and apoptosis pathways. Tumors can also be attacked through their dependence on their blood supply. By understanding the normal control mechanisms and exactly how they are subverted in specific cancers, it becomes possible to devise drugs to target cancers more precisely. As we become better able to determine which genes are amplified, which are deleted, and which are mutated in the cells of any given tumor, we can begin to tailor treatments more accurately to each idividual patient.

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Impacts of heavy metals on aquatic life, soil, plants, and Human health

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Introduction

Composting can be defined as the process in which the organic waste treatment by aerobic micro-organisms,assuch,it comprises three major phases: mesophilic and thermophilic stages and cooling (the compost stabilization stage). It can reduce the solid waste volume by 40-50%, pathogens are destroy by the metabolic heat generated by the thermophilicphase, degrade a big number of hazardous organic pollutants and make available a final product that can be used as a soil improvement or fertilizer. If the final product contains high heavy metals concentration it may be noxious to soil, plants and human health. Heavy metals uptake by plants and successive accumulation in human tissues and biomagnificationthrough the food chain causes both human health and environment concerns.

Heavy metals are considered one of the major sources of soil pollution.Heavy metal pollution of the soil is caused by various metals specially Cu, Ni, Cd, Zn, Cr and Pb. Someheavy metals (like Fe,Zn,Ca and Mg) have been reported to be of bio-importance to man and their daily medicinal and dietary allowances had been recommended. However, some others (like As, Cd, Pb and methylated forms of Hg) have been reported to have no known bio-importance in human biochemistry and physiology and consumption even at very low concentration can be toxic.

Heavy metals exert toxic effect of soil micro-organism hence result in the change of the diversity, population size and overall activity of the soil microbial communities. Elevated Pb in soils may decrease soil productivity and a very low Pb concentration may inhibit some vital plant processes that is photosynthesis, mitosis and water absorption with toxic symptoms of dark green leaves, wilting of older leaves, stunted foliage and brown short leaves, stunted foliage and brown short roots.The metal plant uptake from soils at high concentrations may result in a great health risk considering food chain implications. Uptake of heavy metals by plants and subsequent accumulation along the food chain is a potential threat to human health. The consumption of

heavy metal contaminated food can seriously deplete some essential nutrients in the body that are further responsible for decreasing immunological defenses,intrauterine growth retardation, disabilities associated with malnutrition and high prevalence of upper gastro-intestinal cancer rates.

Heavy metals containing agricultural runoff enter in aquatic environment it may toxic to aquatic plants and animals. If compastable waste such as sewage sludge, municipal solid waste and pig manure contain heavy metals,it may change the composting process by inhibiting bacterial growth.In the vermicomposting process heavy metals affects earth worm life cycle. Present study was to evaluate the impacts of heavy metal containing compost on aquatic life, soil, plants and human health as well as impacts of heavy metal containing compostable material on composting process.

Effects on Aquatic Environment

Heavy metals are highly persistent toxic in trace amounts and can potentially induce severe oxidative stress in aquatic organisms.Thus, these contaminants are highly significant in term of ecotoxicology.Moreover, metals are not subject to bacterial degradation and hence remain permanently in the marine environment. Contamination of a river with heavy metals may cause devastating effects on the ecological balance of the aquatic environment and the diversity of aquatic organisms becomes limited with the extent of contamination.

Heavy metals released in to aquatic systems are generally bound to particulate matter, which eventually settle down and become incorporated in to sediments. Surface sediment therefore is the most important reservoir or sink of metals and other pollutants in aquatic environments. Sediment bound pollutants in aquatic environments. Sediment bound pollutants can be taken up by rooted aquatic macrophytes and other aquatic organisms. Because a major fraction of the trace metals introduced in to the aquatic environment eventually become associated with the bottom sediments, environmental degradation by metals can occur in areas where water quality criteria are not exceeded,yetorganisms in or near the sediments are adversely affected. Diatom community structure can be affected by high levels of micropollutants and in particular by metals,which are often found in rivers. Once heavy metals are accumulated by an aquatic organism, they can be transferred through the upper classes of the food chain. Carnivoresat the top of the food chain including humans,obtain most of their heavy metals burden from the aquatic ecosystem by way of their food, specially where fishes are present so there exist the potential for considerable biomagnification. Contaminants in aquatic system including

heavy metals, stimulate the production of reactive oxygen species (ROS) that can damage fishes and other aquatic organisms. Fish is a commodity of potential public health concern as it can be contaminated with a range of environmentally persistent chemicals, including heavy metals. The consumption of fish containing elevated levels of metals is a concern because chronic exposure to heavy metals can cause health problems. Mercury (Hg) is one of the most important pollutants both because of its effect on marine organisms and it is potentially hazardous to humans.Methylmercury, which is formed in aquatic sediments through the bacterial methylation of organic mercury, is toxic chemicals compound of mercury, infact, nearly all of the mercury in fish muscles occurs as Methyl mercury. Transport of metals in fish occurs through the blood where the ions are usually bound to proteins.The metals are brought in to contact with the organs and tissues of the fish and consequently accumulated to a different extent in different organs or tissues of the fish. There are five potential routes for a pollutant to enter a fish. These routes are through the food, non food particles, gills, oral consumption of water and the skin.Once the pollutants are absorbed, they are transported by the blood to either a storage point (that is bone) or to the liver for transformation and storage.If the pollutants are transformed by the liver, they may be stored there or excreted in the bile or passed back in to the blood for possible excretion by the gills or kidneys or stored in fat, which is an extra hepatic tissue.

Benthic macroinvertebrate assemblages contain species with various sensitivities to contaminants and have been widely used to evaluate the ecological impacts of metal contamination in streams They play vital roles in lotic food webs by forming a major link between primary producers and higher trophic levels and in lotic ecosystems by regulating organic matter decomposition and nutrient cycling. However, the impact of heavy metals on macro-invertebrates has not been evaluated in terms of their food value for fish, even though invertebrates are an important food source for many moving water fish species. It is of particular importance to evaluate the effects of heavy metal pollution on drift-prone macro-invertebrates, on which most commercially or recreationally important salmonid species depend.

Effect on soil

Soil contamination by heavy metal is of most important apprehension throughout the industrialized world. Heavy metal pollution not only result in adverse effects on various parameters relating to plant quality and yield but also cause changes in the size, composition and activity of the microbial community. Therefore, heavy metals are considered as one of the major

sources of soil pollution. Heavy metal pollution of the soil is caused by various metalsspecially Cu, Ni, Cd, Zn, Cr and Pb. The adverse effects of heavy metals on soil biological and biochemical properties are well documented.The soil properties that is organic matter,clay contents and pH have major influences on the extent of the effects of metals on biological and biochemical properties.

Heavy metals indirectly affect soil enzymatic activities by shifting the microbial community which synthesizes enzymes. Heavy metals exhibit toxic effects towards soil biota by affecting key microbial processes and decrease the number and activity of soil micro-organisms.Conversely, long term heavy metals effects can increase bacterial community tolerance as well as the tolerance of fungi such as arbuscular mycorrhizal fungi, which can play an important role in the restoration of contaminated ecosystems.

Diversity and activity of soil microbes play significant role in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals and the control of plant pests and plant growth communities are important indices of soil quality. It is important to investigate the functioning of soil micro-organisms in ecosystems exposed to long term contamination by heavy metals. Chromium is commonly present in soils as Cr (III) and Cr(VI), which are characterized by distinct chemical properties and toxicities. Cr(VI) is a strong oxidizing agent and is highly toxic,whereas Cr(III) is a micronutrient and a non-hazardous species 10 to 100 times less toxic than Cr(VI).Cr(VI) has been reported to cause shifts in the composition of soil microbial populations and known to cause detrimental effects on microbial cell metabolism at high concentration.Ashraf and Ali also reported that the heavy metals exert toxic effects on soil micro-organism hence results in the changes diversity, population size and overall activity of the soil microbial communities observed. The heavy metals (Cr, Zn and Cd) pollution influenced the metabolism of soil microbes in all cases. In general, an increase of metal concentration adversely affects soil microbial properties Ex.respiration rate, enzyme activity, which appears to be very useful indicators of soil pollution.In case of soil contaminated with lead slight change was observed in the soil microbial profile.

Effects on Plants

Some heavy metals that is As, Cd, Hg, Pb or Se are not essential for plant growth, since they do not perform any known physiological function in plants, others that is Co, Cu, Fe, Mn, Mo, Ni and Zn are essential elements required for normal growth and metabolism of plants but these elements can easily lead to poisoning when their concentration greater than optimal values.

The use of compost to improve agricultural yield without caring with possible negative effects might be a problem since the waste composts are most applied to improve soils used to grow vegetables. Considering the edible part of the plant in most vegetable species,the risk of transference of heavy metals from soil to human should be a matter of concern.

Uptake of heavy metals by plants and subsequent accumulation along the food chain is a potential threat to animal and human health. The absorption by plant roots is one of the main routes of entrance of heavy metals in food chain.Absorption and accumulation of heavy metals in plant tissue depend upon many factors which include temperature, moisture, organic matter, pH and nutrient availability. The uptake and accumulation of Cd, Zn, Cr and Mn in Beta vulgaris were higher during the summer season, whereas Cu, Ni and Pb accumulated more during the winter season.It may be expected that during the summer season the relatively high decomposition rate of organic matter is likely to release heavy metals in soil solution for possible uptake by plants. The higher uptake of heavy metals that is Cd, Zn, Cr and Mn during the summer season may be due to high transpiration rates as compared to the winter season due to high ambient temperature and low humidity.

Heavy metal accumulation in plants depends upon plant species and the efficiency of different plants in absorbing metal is evaluated by either plant uptake or soil to plant transfer factors of metals. Elevated Pb in soils may decrease soil productivity and a very low Pb concentration may inhibit some vital plant processes such as photosynthesis, mitosis and water absorption with toxic symptoms of dark green leaves, wilting of older leaves, stunted foliage and brown short roots. Heavy metals are potentially toxic and phytotoxicity for plants resulting in chlorosis, weak plant growth, yield depression and may even be accompanied by reduced nutrient uptake, disorders in plant metabolism and reduced ability to fixate molecular nitrogen in leguminous plants. Seed germination was gradually delayed in the presence of increasing concentration of lead (Pb), it may be due to prolong incubation of the seeds that must have resulted in the neutralization Ex.-leaching, chelation, metal binding or accumulation by micro-organisms.

Effects on human health

The plant uptake of heavy metals from soil at high concentration may result in a great health risk taking in to consideration food chain implications. Utilization of food crops contaminated with heavy metals is a major food chain route for human exposure. The food plants whose examination system is based on exhaustive and continuous cultivation have great capacity of extracting

elements from soils. The cultivation of such plants in contaminated soil represent a potential risk since the vegetable tissues can accumulate heavy metals. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Chronic level ingestion of toxic metals has undesirable impacts on humans and the associated harmful impacts become perceptible only after several years of exposure.

Cadmium is a well known heavy metal toxicant with a specific gravity 8.65 times greater than water. The target organs for Cd toxicity have been identified as liver, placenta, kidney, lungs, brain and bones. Depending on the severity of exposure, the symptoms of effects include nausea, vomiting, abdominal pains, dyspnea and muscular weakness.Severe exposure may result in pulmonary odema and death. Pulmonary effects (emphysema, bronchiolitis and alveolitis) and renal effects may occur following subchronic inhalation exposure to cadmium and it's compounds. The Itai-itai disease in Japan brought the dangers of environmental Cd to world attention. Cd has been associated to a lesser or greater extent with many clinical condition including anaemia, cardiac failure, cancers, cerebrovascular infraction, emphysema, osteoporosis, proteinuria in the eyes. Zincis considered to be relatively non toxic, specially if taken orally. However, excess amount can cause system dysfunction that result in impairment of growth and reproduction. The clinical sign of Zinc toxicity have been reported as vomiting, diarrhea, bloody urine, icterus (yellow mucus membrane), liver failure, kidney failure and anaemia.

Copper (Cu) is an essential element in mammalian nutrition as a component of metalloenzymes in which it acts as an electron donor or acceptor.Conversely, exposure to high levels of Copper can result in a number of adverse health effects. Exposure of humans to Cu occurs primarily from the consumption of food and drinking water. Acute Cu toxicity is generally associated with accidental ingestion. However, some member of the population may be more susceptible to the adverse effects of high Cu intake due to genetic predisposition or disease. Excessive human intake of Cu may leads to severe mucosal irritation and corrosion, widespread capillary damage, hepatic, renal damage and central nervous systemirritation followed by depression.Severe gastro-intestinal irritation and possible necrotic changes in the liver and kidney can also occur. The effects of Ni exposure vary from skin irritation to damage to the lungs, nervous system and mucous membranes.

Lead is physiological and neurological toxic to humans.Acute Pb poisoning may result in a dysfunction in the kidney, reproductive system, liver and brain resulting in sickness and death.heads and threats even at extremely

low concentrations. A notablyserious effect of lead toxicity is teratogenic effect. Lead poisoning also causes inhibition of the synthesis of haemoglobin, cardiovascular system and acute and chronic damage to the central nervous system (CNS) and peripheral nervous system (PNS). Other chronic effects include anaemia, fatigue, gastro-intestinal problems and anoxia. Lead can causes difficulties in pregnancy, high blood pressure, muscle and joint pain.

Other effects include damage to the gastro-intestinal tract (GIT) and urinary tract resulting in bloody urine, neurological disorder and can cause severe and permanent brain damage. While inorganic forms of lead, typically affect the CNS, PNS, GIT and other biosystems, organic forms presumably affect the CNS. Lead affects children, particularly in the 2-3 years old range by leading to the poor development of the grey matter of the brain, thereby resulting in poor intelligence quotient(IQ).Its absorption in the body is enhanced by Ca and Zn deficiencies.

Chromium (Cr) is the 10^{th} abundant element in the earth's and persists in the environment as either Cr(III) or Cr(VI) is toxic to plants and animals, being a strong oxidizing agent, corrosive, soluble in alkaline and mildy acidic water, toxicand potential carcinogens. The toxicity of Cr (VI) derives from its ability to diffuse through cell membranes and oxidize biological molecules.

Mercury is toxic and has no known function in human biochemistry and physiology.Inorganicforms of mercury cause spontaneous abortion, congenital malformation and gastro-intestinal disorders.poisoning by its organic forms, which include monomethyl and dimethyl mercury presents with erethism (an abnormal irritation or sensitivity ofan organ or body part to stimulation), neurological disorders, total damage to the brain and CNS and are also associated with congenital malformation.

As with Lead and Mercury, Arsenic toxicity symptoms depend on the chemical form ingested. Arsenic acts to coagulate protein forms complexes with co-enzymes and inhibits the production of adenosine triphosphate (ATP) during respiration. It is possibly carcinogenic in compound of all its oxidation states and high level exposure can cause death.Arsenictoxicity also presents a disorder,whichis similar to and often confused with Guillain-Barre syndrome,an anti-immune disorder that occurs when the body's immune system mistakenly attacks part of the PNS, resultingin nerve inflammation that causes muscles weakness.

Effects on Composting Process

Heavy metals effects are not limiting up to soil,plants and human health but also affect composting process by changing microbial diversity.Micro-organisms are helpful in degradation of organic matter,detoxify some organic and inorganic pollutants, change mobility and bioavailability of heavy metals to plants.Since heavy metals can affect the microbial reproduction and cause morphological and physiological changes.So the biodegradation processes might be influenced by toxic heavy metals in environment. Microbial enzymes might be affected by heavy metals due to the potential inhibition to both enzymatic reactions and complex metabolic processes. Heavy metals decrease the phosphate synthesis during the composting process. Micro-organisms have to cope with toxic Pbduring their growth in the Pb-contaminated substrates and the exposure of micro-organism to metals always inhibits microbial growth and activity.

Heavy metals in general are potent inhibitors of enzymatic reactions. Cu and Cd in addition to binding with aromatic amino acids residues in enzyme molecules can also cause oxidative stress associated with the production of reactive oxygen species like hydroxyl or superoxide radicals. The significant impacts of high Cu and Zn on a vermicomposting system including accumulation in worm tissues and reduction in the number of Jiveniles produced. While *Eisenia fetida* earthworm has the potential to be used in the vermicomposting of organic waste, the level of Cu and Zn needs to be monitored to ensure the earthworm population in the system.

Conclusion

Heavy metals containing compost may change the physical, chemical and biological properties of soil.These metals uptake by plants from the soil, it reduces the the crop productivity by inhibiting physiological metabolism. Heavy metals uptake by plants and successive accumulation in human tissues and biomagnification through the food chain causes both human health and environment concerns. Heavy metals containing agricultural runoff enter in aquatic environment and harm to aquatic plants and animals. Therefore, if the compost has to be applied in agriculture it should be free from pathogens and heavy metals.

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Toxicants' impacts on Ecosystems and Ecotoxicology

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Absract

Ecology is the study of interactions that are often steady-state and sustained between groups of species and their environment. These interactions between species and their environment exist in ecosystems. An ecosystem can be seen on a relatively small scale. For instance, the animals that live there and the photosynthetic phytoplankton that serves as the base of the food chain make up the ecology of a small pond. The size may be much enlarged; in fact, the entire world might be seen as one massive ecosystem. Toxicology is the study of how poisons are fatal to living things. Environmental toxins are any dangerous substances that humans produce or release into the environment as a result of human activities. As a result, a wide range of compounds, including inorganic objects like metals and other organic substances like pharmaceutical medications, are regarded as toxicants. Since environmental pollution is believed to contribute to the pathophysiology of many human diseases, it has become a significant health concern across the world.

Keywords: Environment, Toxicants, Environmental Pollution

Introduction

Toxic substances, toxicants, or poisons are compounds that have adverse effects may be defined as the amount of a material to which an organism is exposed and the method of exposure determine whether or not the substance is harmful. At increased exposure levels, certain compounds that are safe or even helpful at low concentrations become poisonous. It should be clear that poisonous compounds have a significant impact on ecosystems and the species that live there. In a tiny pond environment, adding copper sulphate to the water can kill the microscopic algae, disrupting the photosynthetic food source of the ecology and leading to its collapse. The interplay between toxicity and ecology should thus be taken into account. These relationships between the many creatures might be intricate. Complex food webs and food chains may be involved. As an illustration, persistent organohalide substances could grow increasingly concentrated as they go up food chains and exert their most harmful effects on creatures like raptors at the apex of the food chain.

Ecotoxicology, a branch of environmental science that has grown into a significant field, is the study of the interactions between ecology and toxicology, or the impact of toxic compounds on ecosystems.

The first step consists of the introduction of a toxicant or pollutant into thesystem. This may result in biochemical changes at the molecular level. As a result, physiologicalchanges may occur in tissues and organs. These may result in detrimental alterations of organisms.As a result, the organisms affected may undergo population changes, such as that which occurredwith hawks exposed to DDT. Suchchanges can alter communities; for example, decreased numbersof hawks may allow increased numbers of rodents, accompanied by greater destruction of graincrops. Finally, whole ecosystems may be altered significantly.

The first stage involves the introduction of a toxin or pollutant into the system. This could change biochemistry at the molecular level. Consequently, physiological changes in tissues and organs may occur. These might alter organisms negatively. The affected organisms may see population changes as a result, as was the case with hawks exposed to DDT. Such changes may have an impact on communities; for instance, a decrease in hawk numbers may lead to an increase in rat populations and more destruction of grain crops. Last but not least, whole ecosystems may undergo significant alterations.

Toxicants

The kind of organism exposed, the amount of the material, and the method of exposure all play a role in determining whether a substance is toxic. Whether a toxin is consumed, inhaled, or exposed to via the skin may all have a significant impact on how badly it affects a person. Humans, for instance, can tolerate a few parts per million of copper in drinking water. While copper is a necessary ingredient for the growth of algae at a concentration of a few parts per billion, it is fatal to algae at that level in their aqueous habitat. These types of minute variations happen with many different kinds of substances.

Numerous different toxicant types have the potential to affect ecosystems. Some chemicals that are not poisonous initially are transformed into toxic species during metabolism. To create species that are the ultimate carcinogenic agents, the majority of compounds thought to cause cancer need to be metabolically activated. Metabolic mechanisms detoxify toxic species. The effects of hazardous chemicals are offset by metabolic mechanisms. Toxic chemicals in the water and soil habitats are broken down by fungus, bacteria, and protozoa through their metabolic activities. One of the main ways that

hazardous chemicals work is to have negative impacts on metabolic processes. For instance, in the respiration process by which molecular oxygen is utilised, cyanide ion forms a bond with ferricytochrome oxidase, a type of enzyme-containing iron (III), which cycles with ferrous cytochrome oxidase, containing iron(II), preventing the utilisation of O2 and resulting in rapid death.

These are typically thought of as pollutants that originate from manmade sources, although they can also arise from natural sources. Hydrogen sulphide from geothermal sources and heavy metals, including lead, leached from minerals are two examples of these naturally occurring toxicants. The most dangerous metals are heavy metals, including cadmium, lead, and mercury. For purposes of discussing its toxicity, arsenic is frequently categorized as heavy metal even though it is a metalloid possessing traits of both metals and nonmetals.

Arsenic, zinc is plentiful and can occasionally reach dangerous quantities, although not highly harmful. For instance, agricultural output has decreased on land treated with sewage sludge as a result of zinc buildup. Plants could be harmful to copper. Aluminium, a component of soil that is naturally present, may be leached from the soil by acidic rainwater that has been tainted and accumulated to dangerous amounts for plants. Chromium, cobalt, iron, nickel, and vanadium are other metals whose toxicities may be of interest. Even at very low quantities in water or food, the radioactive metal radium, which emits alpha particles, may be extremely hazardous.

Metals are not biodegradable, as opposed to organic substances. The majority of the heavy metals that are most concerning form insoluble compounds with hydroxide, carbonate, or sulphide and hence have a propensity to assemble in sediments. In general, this is advantageous since it reduces the availability of metals to organisms. On the other hand, species that consume organisms that dwell and grow in sediments can accumulate dangerous quantities of heavy metals. In the case of mercury, soluble methylated organometallic species can be produced by sediment-dwelling anaerobic bacteria in the form of monomethyl mercury cation and dimethylmercury. These species have the potential to infiltrate food webs, accumulating deadly amounts of mercury in fish or birds at the top of the food web.

It's possible for a number of nonmetallic inorganic organisms to poison ecosystems. Small streams have been momentarily rendered sterile by cyanide salt spills from mining operations. In ruminant animals' and young children's stomachs, nitrite ions, which are present in polluted well water, can be formed. Blood haemoglobin’s iron (II) is changed to iron (III) by the nitrite, resulting in

methemoglobin, which is ineffective for carrying oxygen in the blood. Animals and newborn humans have both perished in severe circumstances. Carbon dioxide may be harmful or even lethal at high concentrations in both water and the atmosphere.Because of their capacity to bioaccumulate in lipid (fat) tissue and to become progressively concentrated in such tissue as they move through food chains, persistent organic toxicants are perhaps the pollutants that ecotoxicologists are most concerned about.

Organohalides are the most prevalent of these substances. DDT is the typical persistent organic halide substance. It was widely employed as a highly efficient pesticide in the middle of the 20th century. An excellent illustration of a substance that is notable for its ecotoxicological consequences is DDT. It's safe to use near people and other mammals because its acute toxicological effects are minimal. Sadly, it sticks around throughout food chains and builds up to high levels in birds of prey at the top of the food chain, leading them to lay thin-shelled eggs that crack before hatching. Prior to DDT's general usage restriction, populations of eagles, ospreys, hawks, and other related species were all but wiped off. Aldrin and dieldrin were two related organohalides that were employed as pesticides that were similarly involved and prohibited.

Ecological routes for toxicants

The major pathways by which toxicants enter ecosystems are through

(1) Water

(2) Food

(3) Soil and

(4) Air.

These paths heavily overlap one another. Understanding how harmful compounds flow between and within the water, air, soil, and living compartments of the environment is essential, as is taking into account how toxic substances are metabolized, broken down, concentrated, and stored within these compartments. Chemodynamics is the science that examines these elements of chemical release and absorption, transport, degradation, and impacts. Both biotic and abiotic elements, such as temperature, wind, water movement, and sunshine, must be taken into account in order to comprehend the chemodynamics of environmental chemicals.

The majority of the time, surface water in rivers, lakes, ponds, and other bodies of water has an impact on ecosystems. In such bodies, water trapped by sediments may serve as significant harmful chemical repositories. Underground aquifer groundwater may be discharged into surface sources, where it may come into touch with living things. Plant roots may be hydrated by shallow groundwater and water stored in the soil.

A variety of different sources can release toxicants into water. Water toxicants can come from a variety of sources, including anthropogenic releases of water contaminants. Water contamination from sewage, whether treated or not, is possible. High amounts of organic compounds that may break down in the water are found in sewage, and these compounds use up oxygen as they do so. Biologically treated sewage may still include salts, other substances that can enter the water, and residual organic matter. The biological sewage treatment process produces sludge, which is frequently dispersed on land and can release toxins into the water. Heavy metals and organic contaminants in water might come from industrial effluent that has been unlawfully dumped into natural waterways.

Food may be a source of toxicants in organisms. In rare instances, animals or even people may consume pesticides or other harmful organic compound residues that were sprayed on food crops. As species higher on the food chain eat animals lower on the chain, such toxicants may infiltrate food chains in nature. The lethal concentrations of DDT and other toxicants that got excessively concentrated in birds of prey at the apex of food chains were caused by this phenomenon, virtually causing the extinction of several raptors. Due to the close relationship that land-dwelling plants and animals have with the soil, exposure to toxicants from terrestrial sources is highly frequent.

Herbicides and other pesticides must be applied in enormous quantities to plants and soil in modern agricultural methods; leftovers of these chemicals can easily penetrate living things. Several air contaminants, particularly airborne particles that land on the soil, are stored in the soil. Soil exposure is partly due to improper disposal of toxic wastes from hazardous waste sites and toxic compounds that have been washed or blown off of hazardous waste sites. Animals and plants that live and thrive in the air on land surfaces are subject to any hazardous compounds that may be present in the atmosphere. Toxic compounds can enter the atmosphere from a variety of sources. Heavy metals, acidic chemicals, and poisonous organic molecules can all be released directly into the atmosphere via industrial sources and engine emissions.

Secondary pollutants, which are those created by atmospheric chemical reactions, include certain dangerous air pollutants. The atmospheric oxidation of inorganic gases, particularly SO_2 (producing H_2SO_4) and NO and NO_2, results in the formation of strong acids that cause acid rain (forming HNO_3). Reactive hydrocarbons and nitrogen oxides from automobile exhausts produce a variety of noxious organic aldehydes and oxidants, including ozone, which are all toxic to plants and animals. These compounds are formed when the right conditions of stagnant air, low humidity, and strong sunlight are present.

Environmental Spheres Exchanging Toxicants

Toxicants often reach the atmosphere by evaporation, the solids in the geosphere through adsorption, and the hydrosphere through dissolution. The vapour pressures of the chemicals have a significant impact on how they move between the atmosphere and other spheres; those with higher vapour pressures tend to stay in the atmosphere longer. Another crucial factor is temperature. Toxic chemicals are often significantly more mobile in the atmosphere than in the other environmental spheres due to the low viscosity of air and the continual circulation of air masses.

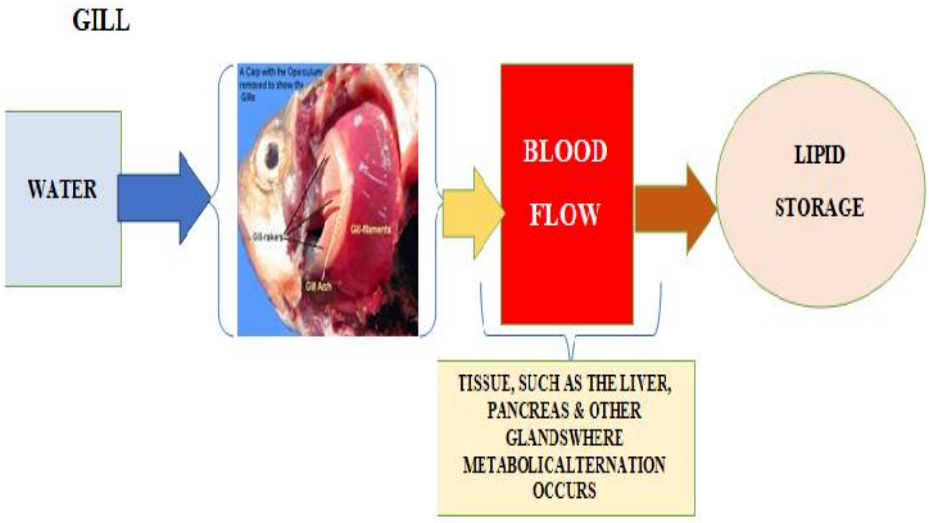
The tendency of some volatile organic compounds to evaporate into the atmosphere in warmer regions and then condense in polar and cold mountainous areas is an intriguing feature. As a result, even though these areas are far from industrial sources, they frequently receive a high dose of organic toxicants as a result. The tendency of compounds to collect in sediments and on soil is correlated with their low water solubility, whereas highly soluble substances may be carried by water easily. In the lipid tissue of animals in the biosphere, chemicals can build up due to high lipid solubility and low water solubility.

Transfers of Toxicants to Organisms

Transferring toxicants to species in the biosphere is a critical component in the exchange of pollutants between environmental spheres. Toxins that organisms pick up from their environment can be categorized into three main groups: those that are ingested with food or water and enter the digestive tract, those that are absorbed from the ambient environment, particularly by fish that live in water, and those that are inhaled in the air and enter the lungs. A trophic level is a collection of species that obtain their energy from the same area of the food chain in a biological community. Persistent, lipophilic (fat-seeking), poorly digested toxicants have a tendency to linger and increase in concentration in higher trophic levels of organisms.

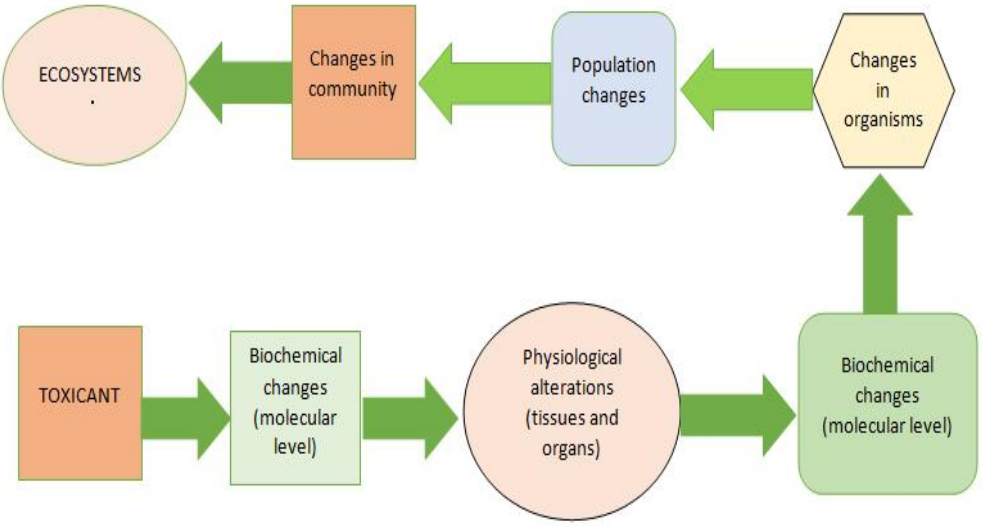
This can lead to biomagnification of toxicants in organisms higher in the food chain. In some birds of prey, which are not only at the top of the food chain but also lack well-developed metabolic systems for coping with such toxicants, lipophilic toxicants may accumulate to exceptionally high levels. Toxic compounds can disperse across considerable distances in food webs due to the movement of creatures, particularly birds.

Fish, aquatic invertebrates, and amphibians that live in water can all be directly impacted by poisonous compounds that are suspended or dissolved in the water. Toxins can be absorbed predominantly through the gills of fish and through the respiratory surfaces of aquatic invertebrates. These creatures and amphibians may also directly absorb some water through their skin. The bioavailability of different chemicals, or more simply the propensity of a substance to enter an organism's system upon exposure, is a crucial factor in the absorption of toxicants by organisms. Lead's bioavailability has been extensively researched and is highly dependent on the chemical form of the metal.



Ingesting inorganic mercury that is water-soluble is extremely hazardous. The lungs may quickly assimilate mercury metal vapours. The skin has the capacity to absorb lethal quantities of lipid-soluble organometallic dimethylmercury. Through their root systems and over the cuticles of their leaves, plants’have two primary routes for ingesting harmful chemicals.

Toxic materials may enter plants through stomata, the specialized apertures in plant leaves that let carbon dioxide needed for photosynthesis to enter the leaves and oxygen and water vapour depart. There has been a great deal of research on the methods by which systemic pesticides and herbicides are absorbed by plants and disseminated throughout the plant. Substances that undergo bioconcentration are hydrophobic and lipophilic and therefore tend to undergo transfer from water media to fish lipid tissue. The simplest model of bioconcentration views the phenomenon on the basis of the physical properties of the contaminant and does not account for physiologic variables (such as variable blood flow) or metabolism of the substance. Such a simple model forms the basis of the hydrophobicity model of bioconcentration, in which bioconcentration is regarded from the viewpoint of a dynamic equilibrium between the substance dissolved in an aqueous solution and the same substance dissolved in lipid tissue.



Responses to toxicants at different organizational levels in life systems

The tendency of a chemical to leave an aqueous solution and enter a food chain is important in determining its environmental effects and is expressed through the concept of bioconcentration. Bioconcentration may be viewed as a special case of bioaccumulation in which dissolved substance is selectively taken up from water solution and concentrated in tissue by non-

dietary routes. Bioconcentration applies specifically to the concentration of materials from water into organisms that live in water, especially fish.

The model of bioconcentration is based on a process by which contaminants in water traverse fish gill epithelium and are transported by the blood through highly vascularized tissues to lipid tissue, which serves as a storage sink for hydrophobic substances. Transport through the blood is affected by several factors, including the rate of blood flow and the degree and strength of binding to blood plasma protein. Prior to reaching the lipid tissue sink, some of the compounds may be metabolized to different forms. The concept of bioconcentration is most applicable under theconditions as the drug is absorbed and expelled via passive transport mechanisms. The drug is either not or just slowly metabolized. The chemical is just slightly soluble in water. The chemical is surprisingly lipid-soluble.

Toxicants' impacts on ecosystems

An ecosystem is made up of several populations of living things that coexist in a largely stable environment. The movement of energy and materials throughout ecosystems is crucial. The impacts of toxicants may disturb the complicated interactions that the creatures in an ecosystem have with one another and with their environment. The distribution and exposure to toxicants may be influenced by the flow of materials natural to ecosystems. Exposure to xenobiotics may change an organism's homeostasis (same-state balance with its environment), which can have an impact on populations of certain organism types, communities of organisms, and entire ecosystems.

Even though they are obvious and unmistakable, the observation of organism fatalities and the ensuing population losses frequently falls short of providing a comprehensive explanation for how hazardous compounds affect ecosystems. Sublethal impacts, which may have negative consequences on populations without actually killing people, should also be taken into account. The rates of juveniles surviving to maturity and the reproduction rates of organisms can both be impacted by toxicant exposure. Predator avoidance skills might be restricted, foraging abilities could be diminished, and homing and migratory instincts could be negatively impacted.

Conclusion

Any harmful material created by people or released into the environment as a result of human activity is considered an environmental toxicant. As a result, a wide variety of molecules, from various organic substances like pharmaceutical drugs to inorganic things like metals, are

considered toxicants. Around the world, environmental pollution in any form has been a major health issue since it is thought to play a role in the pathophysiology of various human diseases.

The human body can be exposed to toxins by inhalation, the skin, food, or water consumption. For instance, the prevalence of lead (Pb) in the environment or arsenic (As) in drinking water has elevated to a significant public health problem globally. Pesticides also represent a serious hazard to both the environment and human life. Pesticides are heavily used in agriculture, which has led to the contamination of drinking water supplies, vegetables, livestock feed, milk, and seafood. Pesticide exposure has been linked to a number of chronic illnesses, much like heavy metal exposure has. In addition, there are a number of chemical toxicants in our environment, such as chemical or pharmaceutical industry waste that act as endocrine disruptor and is to blame for a number of human disorders.

The science of ecotoxicology focuses on how toxic substances affect living things, particularly at the population, community, ecosystem, and biosphere levels. Ecology and toxicology are combined in the interdisciplinary discipline of ecotoxicology**.**Ecotoxicology's overarching objective is to identify and foresee the consequences of pollution in the context of all other environmental conditions. Based on this information, the best course of action to stop or reverse any negative effects may be determined. Ecotoxicological research can help decision-makers repair ecosystem services, structures, and functions efficiently and effectively in ecosystems that have already been harmed by pollution.Environmental toxicology involves toxicity to people and frequently focuses on impacts at the organism level and below, whereas ecotoxicology incorporates the effects of stressors across all levels of biological organization, from the molecular to whole communities and ecosystems.

Relationships between predators and their prey: either the toxin affects the predator, causing a decline in the predator population and an increase in the prey population, or the toxin affects the prey population, causing a decline in the prey population that, in turn, will cause a decline in the predator population because of a lack of food resources.The field of community ecotoxicology investigates the impacts of all pollutants on patterns of species diversity, abundance, and interactions. Communities that rely largely on rivalry and predatory behaviour will find it challenging to adapt to and thrive in the presence of pollutants. A community with a high species diversity will fare better in the aftermath of an exotoxin disruption than a population with a low

species diversity. By being contaminated by foreign substances, a species might be quickly wiped off. For the maintenance of a robust, well-balanced ecosystem, it is crucial to protect different community levels, such as species richness and variety.

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Antimicrobial Activity of Citrus Lemon Juice on Different Microorganisms

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Abstract

Plants are use as valuable source of natural products. Their different parts such as leave, stem, root, peels, flowers, seed, etc. Citrus fruit is one among the main farming crops worldwide. Many citrus fruit are found in environment such as Lemon, lime, orange, grapefruits, angserine etc. Each of this fruits has its own unique properties. Citrus fruit contain carbohydrates such as cellulose, hemicelluloses, pectin, sugar and various organic acids. About 30% citrus fruits are found in various products and specially, juices which provide energy to human being and increase immunity by defence mechanism. Citrus fruit has type of acid which is also helpful in digestive system and for many diseases like heart disease, blood pressure, cancer, diabetes etc. Here we have used lemon (citrus lemon) as a citrus fruits to check its antimicrobial activity against different organism such *as Staphylococcus aureus*, *E. coli, Enterobactor andproteus vulgaris.* These are human normal microbial flora too. But are pathogenic and cause disease as well. To check it's antimicrobial effect, zone of inhibition was measured. And the result showed antimicrobial activity ofconcentrated lemon juice more in*proteus vulgaris*while, less in *Enterobactor*.It suggests that lemon juice has strong antimicrobial activity.And having such citrus fruits in diet increase our immunity by killing pathogenic microorganisms.Nowadays it is very much in use to fight against corona virus too and provide defence.

Key Words: Citrus fruits, lemon, antimicrobial activity, immunity

Introduction

Plants are used as valuable sources of natural products such as food, beverages, cosmetics and pharmaceutical industries like textile and electroplating. Crude extract of different parts of plant such as leaves, stems, roots, juices, peel, flowers, seeds, fruits etc are used for such products. Citrus fruit is one of the largest horticultural crops in the world. There are many types of citrus fruits i.e. Lemons, limes, oranges, grapefruits, pomelos etc. It is very much in demand to produce and to consume because it has wide range of nutritional values.China, Brazil and Unite states account for about two third of the total citrus fruit production (Matheyambath et al, 2016). Citrus fruit pre dominant with citric acid of total organic acid and also contain large amount of Vitamin C.It can be extracted from peel and pith waste, while malic, tartaric, benzoic, oxalic and succinic acid found in trace amount and could not be extracted from citrus waste (Karadeniz, 2004; Kundu et al,2020).It is rich source of secondary metabolites (Ghasemi at a1, 2007) and produce many Compound like protopine, caryclaline, alkaloids, lactons, polyactylene, lypericin, pseudo hypericla etc. are effective against many microorganisms(Keles at a1, 2001 ; Maruti at a1, 2011).

Plants have been used as medicine since age. About 80% of individuals from developed countries use traditional medicine derived from medicinal plants (Hindi& Chabuck, 2013). Citrus plant also has many beneficial effects on human health. It work as antimicrobial compound against many pathogenic microorganism and provide immunity.

Lemon is one of importantcitrus fruit of family Rutaceae. Main raw material is essential oil and juice obtained from it.It is most cultivated species. It has been cultivate insouthern Italy since 3re century AD and in Iraq and Egypt since 700AD, and in many more countries it is cultivated sine age. Nowadays USA is the largest producer of citrus limon (Klimeu – Szczykutowicz et al, 2020). Lemon has many types like bush lemon, eureka, Lisbon, verna etc. It contain large amount of nutrition. (Mohanapriya et a1, 2013). It can be used in breverages, refreshing, pickle, jam, body oil, cosmetic product etc.

It contain flavonoid, has great medicinal use as antibacterial, antifungal, antiviral, antidiabetic, anticancer, etc. It also contains alkaloid which has anticancer and antibacterial activities(Burt, 2004: Ortuno etal, 2006: Ma et al,2020).Table-1 showed nutritional value of raw lemon without peel.Citrus peel also rich in nutrients and Phytochemicals, like flavonoids, glycosides, phenolic compounds, ascorbic acid, volatile olis etc(Ali et al, 2017). Johann et

a1,2007 and Giuseppe et a1, 2007 also suggested that presence of limonoids in citrus species has antibacterial & antifungal activity. Traditionally its juice has been used to cure scurvy before discovery of vitamin C (Mabberley, 2004). It also use for common cold, high blood pressure, irregular menstruation and its essential oil is a good remedy for cough (Papp et al, 2011;Clement et al, 2015 and Bhatia et al, 2015).

Table.1 - Nutritional value of 100grams of raw lemon extract without peel (Mohanapriya et al, 2013)

No.	Nutritional value
1.	Energy - 121 kj (29Kcal)
2.	Carbohydrates - 9.32g
3.	Sugar - 2.50g
4.	Fat - 0.30g
5.	Protein – 1.10g
6.	Vitamin B2 - 0.020mg (1%)
7.	Vitamin C - 53.0mg (88%)
8.	Calcium - 26mg (3%)
9.	Iron - 0.60mg (5%)
10.	Magnesium - 8mg (2%)
11.	Phosphorus - 16mg (2%)
12.	Potassium - 138mg (3%)
13.	Zinc - 0.06mg (1%)
14.	Dietary fibre – 2.8g

Due to its high energy level & rich of nutrition it has greatuse in skin care, digestion, surgery in peptic ulcer in piles, eye care constipation. it is widely used as appetizer too It can also very effective in diseaseslike arthritis, rheumatism,prostrate, colon cancer, diabetes, heart disease etc (Mohanapriya et al, 2013; Zhang et al,2020). Nowadays it is used to fight against corona virus & boost up immunity.

Citrus fruit has great commercial value too, 30% of citrus fruits are use for various products specially Juice (Izquierda et al, 2003). In our research we have taken Lemon as citrus fruit and check its antimicrobial activity against one gram positive and three gram negative pathogenic microorganisms i.e. *Staphylococcus aureus*,*E. coli, Enterobactor and proteus vulgaris*byagar - well diffusion method. We have taken concentrated juice of lemon in our research.

Materal and Method:

Preparation of Extracts: The fresh lemons were washed in running tap water in laboratory. Surface of lemon sterilized with 70% alcohol and rinsed with sterile distilled water. Cut open with sterile knife and the juice collected in sterile container.

Young culture: Prepare 24hrs young culture of *Staphylococcus aureus*, *E. coli, Enterobactor and proteus vulgaris*

Antimicrobial activity using agar well diffusion method:

1. Inoculate 0.2ml of young test cultures in meltednutrient agar previously cooled to 50^{o}C.
2. Mix it well and pour it in to sterile empty petriplates.
3. Allowed it to solidify.
4. Make well with the help of cup borer in the center of the petriplates.
5. Add fresh concentratedlemon juice to the well directly with sterile pipette. Take precautions not to overflow cups.
6. The plates kept in the refrigerator at 4^{O}C for 1 h.
7. Then incubate the plates in upright position at 37 ^{o}C for 24 hour.
8. Next day observe the plate for the zone of inhibition and measure the zone size.

Table.2 - Zone of inhibition of lemon juice with different Microorganisms

Sr No.	Microorganism	Zone of inhibition
1	*Proteus vulgaris*	**31mm**
2	*E. coli*	27mm
3	*Staphylococcus aureus*	26mm
4	*Enterobactor*	**21mm**

Fig.1: Zone of inhibition of lemon juice.

Results and Discussions:

Result showed that antimicrobial activity ofconcentrated lemon juice was more in *Proteous vulgaris* followed by *E coli* and *S. aureus,* i.e., 31mm, 27mm and 26mm respectively. While, less zone size was observed with *Enterobacter*, i.e., 21mm (Table.2 and Fig.1). Lemon juice showed killing effect against all organisms. But, more effective to Gram negative *proteus spp.* and less effective to *Enterobacter.*

But here we have taken concentrated lemon juicewhile, Okeke *et al*, 2015 has taken fruit juice extract was re- constituted in distilled water to a concentration of 100 mg / ml of citrus lemon fruits juice & check its effective against gram positive and gram negative bacteria. He found maximum zone of inhibition with *Staphylococcus aureus* (ATCC 12600), 21mm and lowest with *E. coli* (clinical) 14mm. So, direct concentrated juice of lemon is more effective then re- constituted in D/W.

Oikeh et al, 2016 found that gram positive showed higher susceptibility then gram negative strain. i.e. *Staphylococcus* has 20mm while, *E.coli* has 12mm zone of inhibition for lemon. He observed lower zone of inhibition in gram negative compare to gram positive strain. This is because gram negative has outer membrane act as barrier and prevent outer environment substrate to

enter in cell. Moreover, periplasmic place also contain enzyme which break foreign molecule attempting to gain enter in to cell (Holetz et al, 2002; Cheruiyot et al, 2009).

Deshwal & Kaur, (2018) also tested antimicrobial activity of citrus fruits lemon on various pathogenic microorganisms. And found maximum effectiveness with *Staphylococcus aureus* & lowest with *Pseudomonas aerogenosa.* Presence of secondary metabolites like organic acids, vitamins,flavonoids etc compound in lemon fruits to inhibited growth of microorganism. Thus, lemon juice control many pathogenic microorganisms and it provide immunity.

Ali at al., (2017) studied antimicrobial activity of lemon peel extratct against Gram positive *S.aureus* and Gram negative *E.coli* and with two fungi. He also found significant antimicrobial activity with different concentrations.

Today in corona virus infection, lemon juice was very effective to fight against such virus infection. Citrus fruits boost up our immunity by killing many pathogenic microorganisms and provide defence.

Still more investigation and research required for different concentrations and MIC (Minimum inhibition concentration) determination of various juices either combination with other juicesor with/without dilution.

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Antimicrobial activity of routine spices against pathogenic microorganisms

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Abstract

The use of spices and herbs found since prehistoric times and it is very important in our routine diet. Generally they are used for flavor, seasoning, aroma etc. Clove, ginger and garlic are very much common spices of our routine diet. It contain many Phytochemicals and bioactive compounds, which helps as antibacterial, antifungal, antiviral, antioxidant etc and cure many acute and chronic diseases like fever, arthritis, inflammation, cancer, diabetes, rheumatism etc. In our study we have checked antibacterial activity of three spices like clove, ginger and garlic against Gram positive *S. aureus* and Gram negative *E.coli*. All spices show antibacterial activity. Maximum zone of inhibition observed with garlic and less with ginger. So, garlic has more antibacterial activity. Thus spices and herbs are very much effective to boost up our immunity. It is very much necessary to include such natural spices and its product in our routine diet to increase immunity.

Keywords: Spices, Clove, Garlic, Ginger, Antimicrobial effect.

Introduction

Since ancient times spices and herbs have been widely uses in many countries including India. In recent years use of spices/herbs is increases in many developing countries (Aleem et al, 2020b).

The use of herbs and spices found since prehistoric times and it is very important in routine diet. The term herbs and spices are often use interchange but actually, they are 2 distinct types made from different section of plant and proceed in different ways. Herbs are leaves derived from herbaceous oregano plants (Lackwoody stem) spices like cinnamon made from aromatic seeds,

barks, flowers and root of plants that have been dried and crushed. Most of herbs and spices used in kitchen daily.

Natural compounds extracted from herbs and spices are used for flavour, seasoning, aroma etc since age. Use of chemicals is adversely affects on human health. So, nowadays use of natural medicinal plant, spices, herbs or it's extract increase (Srinivasan, 2014).It improves sensory characteristics of food, preserve it for long time and also work as antimicrobial agent (Martinez-Gracia et al,2015).

Accordig to Monte et al, 2014 spices and herbs are rich source of phytochemicals, bioactive compound derived from plants that inhibit microbial growth. Bioactive compound consist of Vitamin C, E, carotenoids, phenolic compounds, flavonoids, tannins and flavones (Sokamte et al, 2019). It has medicinal effects and act as antioxidant, antiinflammation, antihypertension, anti cardiovascular disease etc (Li et al, 2019).

In our study we have selected routine spices such as Clove, Ginger and Garlic to check its's antibacterial activity against pathogenic organisms like gram positive *Staphylococcus spp.*andgram negative *E. coli.*

Clove (*Syzygium aromaticum*):

Clove (*Syzygium aromaticum*) is a tree native to Indonesia. It's dry flower bud are popular spice and it is very much used in ayurvedic medicine. Clove oil, dried flower buds, leaves stem used to make medicine. It has also antimicrobial and antioxidant activity higher than many fruits, vegetables and other spices. But also show the antifungal, antiviral and anticarcinogenic properties (Cortes-Rojas, et al 2014). It is also used in food, and food preservatives. Many countries like Indonesia, India, Malaysia, Shrilanka, Tanzania are largest producer of clove (kamatou et al, 2012).

Clove represent major source of phenolic compound as flavonoids, hidroxibenzoic acid, hydroxicinamic and hidroxiphenyl propens. Eugenol is the main bioactive compound of clove. Phenolic acid and other phenolic acid like caffeic, ferulic, elogic and salicylic acid found in clove. Gallic acid also found in higher concentration.

It also work as antioxidant, antimicrobial, antiviral etc. Clove oil also works as insecticide (Cortes – Rojas et al, 2014).

Ginger (*Zingiber officinale*):

Ginger belongs to Zingiberaceae family (Sharma et al, 2010). Because of easy avaibility it is inexpensive. In many countries it is widely used in food. It is tropical plant grow well in hot and humid climate (Aleem et al, 2020a).

Ginger is a flowering plant whose rhizome root is a widely used as a spice and for medicine. It has fat, protein, thiamine, riboflavin and some other materials. It is widely used worldwide for varieties of food item such as vegetables, pickles, alcoholic beverages etc. It is fragrant kitchen spice and it can be steeped in boiling water to make ginger herb tea.

Mature ginger rhizomes are fibrous and nearly dry. Ginger rhizome contains some constituents which has antibacterial and antifungal effects (Beristain – Bauza et al, 2019).The juice from ginger root is often used in Indian recipes and it is a common ingredient of Chinese, Korean, Japanese and many South Asian cuisines for flavorings dishes. It has several medicinal uses such as nausea, vomiting, digestion etc. Antioxidant and nutrients in ginger roots beneficial in arthritis, inflammation, cancer, diabetes, cold, asthma, blood pressure (Ardekani et al, 2012 and Zargari, 1999).

It has nutritional, medicinal and ethnomedical value so, it is commonly used as spice.The characteristic order and taste of ginger is due to mixture of non- volatile pungent compound like zingerone, shogaols and gingerols (Mbaveng and Kuete, 20017). It contain monoterpenoids, sesquiter penoids, phenolic compound and it's derivatives, aldehydes, ketones, alcohol, esters which provide antimicrobial effect (Beristain-Bauza et al, 2019).Further it is widely used for pain, cramps, rheumatism, sore throats, muscular aches, constipation, vomiting, hypertension indigestion, fever, infectious diseases etc (Ali et al, 2008).

Garlic (*Allium sativum*):

Garlic (*Allium sativum* L.) also one of important spice in Indian food. In the earliest Olympics in Greece, garlic was fed to the athletes for stamina (Lowson and Bauer, 1998). It is easy to grow in mild climates. Fresh garlic, garlic powder and garlic oil are used in flavour foods and in herbal medicines. Allicin is most important biological compound of garlic. It shows antimicrobial activity (Uzun et al, 2019).

Ancient India and Chinese recommended garlic to aid respiration and digestion, to treat leprosy and parasitic infection (Rivlrn, 1998). Further, it is also used in treatment of arthritis, toothache, chronic cough, constipation, parasitic infection etc (Bayan et al, 2014).

The bulb of the garlic are the most commonly used part of the plants for medicinal purpose it has a typical pungent odor and antibacterial activity. Garlic is found to have antihypertensive (Silagy and Neil, 1994 and Ernst, 1987), antioxidant (Phelps and Haris, 1993), antiplatelet effect (Kiesewetter, et al 1991). Garlic is a useful compound in treatment of arthritis, toothache, chronic cough, snake and insect bites as well as in infectious diseases.

Extraction Method of Spices:

Approximately 1gm of spices were grinded with mortar, pestle and they were dissolved in 9ml of sterile distilled water to make 10ml of aqueous extract (1.1% W/V). This mixture was filtered and extract used directly to check it's activity by well- diffusion agar method.

Young culture: Prepare 24 hrs young culture of *Staphylococcus aureus* and *E. coli*

Antimicrobial activity using agar well diffusion method:

1. Inoculate 0.2ml of young test cultures *(Staphylococcus aureus* and *E. coli)* inmelted nutrient agar previously cooled to 50^{o}C.

2. Mix it well and pour it in to sterile empty petriplates.

3. Allowed it to solidify.

4. Make well with the help of cup borer in the center of the petriplates.

5. Add fresh concentrated Clove, ginger and garlic extract to the well directly with sterile pipette. Take precautions not to overflow cups.

6. The plates kept in the refrigerator at 4^{O}C for 1 h.

7. Then incubate the plates in upright position at 37 ^{o}C for 24 hour.

8. Next day observe the plates for the zone of inhibition and measure the zone size.

Table.1 - Zone of inhibitions of spices/Herbs extract with different Microorganisms

No.	Test Organism	Zone of inhibition		
		Clove	Ginger	Garlic
1.	*S. aureus*	17mm	15 mm	30 mm
2.	*E.coli*	19 mm	14 mm	34 mm

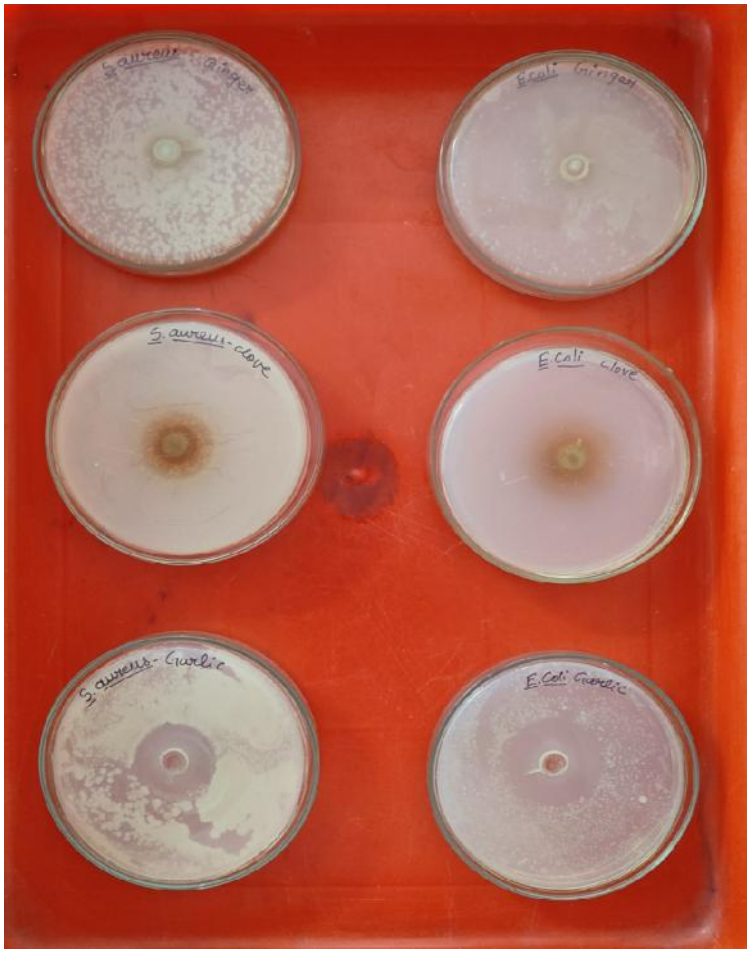

Fig.1: Zone of inhibition of Clove, Ginger and Garlic extracts.

Results and Discussion

Result suggested that both *S. aureus* and *E.coli* were susceptible to clove, ginger and garlic. Garlic showed more killing effect for both organisms compare to clove and ginger. Maximum zone of inhibition observed with garlic in *S. aureus* was 30mm and with *E.coli* 34mm. While least with ginger, i.e 15mm with *S. aureus* and 14mm with *E. coli*. So, we can conclude that spices having antibacterial effect and protect our body with such pathogens.

Gupta and Prakash (2021), checked antimicrobial activity of clove oil and clove extract on oral pathogens and he found good antimicrobial activity with clove oil and less with clove extract. He found highest inhibition zone with *Pseudomonas sp.* (14mm), while lowest with *Staphylococcus, Bacillus* and *Veilonella sp.* (13mm) with clove extract. But with clove oil he found highest zone with *Lactobacillus sp.* and *Halobaterium sp* (19mm) and lowest with *Streptococcus Salivrium* (9mm). Thus, clove oil is more effective because it contain eugenol, oleic acid and lipid.

Accouding to Tshabalala et al, 2021, clove and cumin have excellent antibacterial activity compare to other spices such as rosemary, parsley, ginger, cayenne pepper and white pepper.

Islam et al, 2014 tested antimicrobial activity of ginger extract against food-borne pathogeinic bacteria like *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio cholera, Salmonella spp.* and *Klebsiella sp.* And he found excellent antimicrobial activity with all pathogens. He found highest antimicrobial activity against *Salminella spp.* while lowest with *E. coli*. In our study we also found lowest with *E.coli* But highest with *Staphylococcus aureus*against ginger extract.

Gunda et al, 2018 tested garlic leaf and bulb crude extract against bacteria (*B.subtilis*) and fungi *(A. niger*). He found marked antimicrobial properties with it. He also found garlic bulb extract was moreeffective than garlic leaf extract. Yadav et al, 2015 also studied antimicrobial activity of five pathogenic microorganisms (i.e *E.coli, K. Pneumonia, P. mirabilis. P.aeruginosa* and *S. aureus)* against varing concentrations of fresh garlic. And found good inhibition effect with all five test organisms.

Our results also match to them and show good antibacterial effect with higher zone sizes. But still Minimum Inhibition Concentration (MIC), combination with other herbs and spices, require. Thus herbs and spices show excellent antimicrobial effect and including such herbs and spices in our routine diet increases our immunity.

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Non-Transferrin-Bound Iron: Iron Overload Biomarker!

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Introduction

It is well known that Iron is an essential micronutrient for almost all living organisms and also plays a crucial role in cellular processes like DNA synthesis, epigenetic regulation, or cellular respiration [1]. Its presence in hemoglobin, make possible the transportation of oxygen. Hemoglobin-bound iron, thus present in circulating erythrocytes and developing erythroid precursors, represents about two-thirds of the total iron present in the body [2]. As only 1 to 2 mg of iron are daily absorbed from the diet, recycling mechanism is very important that recycling senescent red cells promoted by macrophages of the reticuloendothelial system. Extra iron may remained as stored iron (in hepatocytes and macrophages), or it is being used for cellular metabolic processes.

What is Tf?

Transferrin (Tf), a glycoprotein, produced in the hepatocytes is very important for iron transportation. Tf has high affinity for the Fe3+ ion [3]. Tf saturation levels in healthy individuals is usually around 30% that guarantee large blood plasma unsaturated iron-binding capacity [4]. Moreover, transferrin receptor (TfR) by its specifications, prevent undesired tissue iron deposition. Thus Free, unbound iron is incompatible with either plasma iron transport (it would precipitate) or with intracytosolic iron trafficking (it would damage the cellular environment). Therefore, iron and ligands need to bind appropriately.

NTBI!

NTBI, a Non transferrin bound iron, the free form of iron that present in blood plasma. It includes various forms of iron that present but only those which are not bound to Tf, plus the haem, apoferritin, hemosiderin etc (traditional iron binding proteins). Depending on final location of its deposition, unbound iron known to cause an immense amount of damage to various organs of the body including heart, liver, pancreas, endocrine glands

and erythroid cells etc.Different terms like “NTBI”, “free iron”, “catalytic iron pool”, “labile iron” etc. were used by various research workers [5].

In any case, high iron concentration is prevail in the circulation, the binding capacity of transferrin is need to exceeded.If not, iron is released into the bloodstream, where it binds to several small circulating molecules, such as citrate, acetate and albumin [6] [7] [8]. This leads to produce NTBI, a chemically heterogeneous group of iron complexes [9]. The form of NTBI present in the individual patient may be dependent on the type of iron overload disease.

NTBI in normal healthy individual do not exceed 1 μmol/L, as in normal physiological condition the level of Tf is adequate enough for binding free iron and ensuring its absence in internal surroundings [10]. However, NTBI has been observed under several pathological conditions in blood serum and proposed as a main toxicity mediator that leading to increased oxidative stress and tissue iron deposition.

As shown in Table 1, NTBI or other relevant subfractions are associated with various iron overload conditions: (Non Transferrin Bound Iron: Nature, Manifestations and Analytical Approaches for Estimation, [11]

Table 1NTBI or its subfraction in various clinical conditions

Various Clinical Conditions	NTBI or its subfraction	Concentration (μmol/l)
Genetic hemochromatosis with iron overload [12].	NTBI	0.761 ± 0.504
Genetic hemochromatosis with iron overload [12].	LPI0	250 ± 0.289
Heredity hemochromatosis [13].	NTBI	4.0–16.3
β Thalassemia major [14].	NTBI	0.375 ± 0.028
Diabetes mellitus [15].	NTBI	0.62 ± 0.43
Cancer patients undergoing chemotherapy [16].	NPBI	10.6 ± 6.6
End stage renal disease [13].	NTBI	0.1–13.5

Determination of NTBI:

Accurate measurement of NTBI is challenging as other iron sources such as transferrin bound iron (TBI) and drug bound iron (DI) are present together. Many analytical methods are available in the literature for the determination of iron [17] [18] [19], no suitable analytical method was available for accurately measuring all iron forms. NTBI estimation methods are based on three approaches and based on these approaches various methods have been developed [11].

1st approach is to indirectly estimate NTBI with bleomycin., an anti-tumor antibiotic.

2nd approach is to determine Chelation of NTBI with chelator

3rd approach is of direct estimation of NTBI with iron sensitive fluorescent probe.

The 1st approach, is the oldest method, firstly suggested by Gutteridge et al. [20]. As Bleomycin have ability to degrade DNA in the presence of ferrous iron (Fe2+), it generate extremely reactive oxygen radicals [20] [21] [22]. Thusall the ferric ions were converted to ferrous form by using reducing agent like ascorbate and reflect the degradation of the DNA present in the reaction mixture, in the form of malonaldehyde (MDA). This degraded DNA product molecules are than reacting with thiobarbituric acid that lead to generate colored compounds. Intensity of this coloris measured at 532 nm. NTBI detected by this approach is known as Bleomycin detectable iron (BDI).

The 2nd approach manifests in two steps; (I) Chrlator will chelate iron and than separated from the biological fluid. (ii) Chelated iron in first step in now estimated with various techniques. Ethylenediamine tetraacetic acid (EDTA), Nitrilotriacetic acid (NTA) and oxalate etc.usedby various researcher as chelators for this assay [13] [23] [24].

Limitation of this approach is under estimation of NTBI, principally when Tf saturation is normal. The leading trouble with this approach is to the selection of an appropriate type and concentration of the chelator.

In 3rd approach is Fluorescent based technique, was introduced by Breuer el al. [25]. Here, NTBI is not determined by the mobilizer or chelator rather it measure the quenching or dequenching of fluorescence and thus it required the fluorimeter as detection system. As per this approach, fluorescent labeled apo transferrin (Fl-aTf) is used that on binding with iron, undergoes for fluorescent

quenching.In this technique, mobilizer (10 mM oxalate) and blocking agent (0.1 mM gallium (III)) is used.

NTBI in various clinical conditions:

In recent years, NTBI has received a lot of attention as the NTBI complexes are therefore considered to be potentially toxic and seen as the pathophysiological substrate of oxidative organ damage. This damage is contributed by intracellular uptake of NTBI that may be mediated by several, organ-specific, receptors and transporters, e.g.transporter 'Zrt/Irt-like protein 14' (Zip14), the receptor TfR2 etc. [26].

β-thalassemia major and intermedia are well known hereditary hemochromatosis or hematological iron overload disorders that identified as iron deposition in tissues that lead to iron overload diseases [27] [28].

In cases of diabetes mellitus (DM)and type 2 DM patients also observed to have hematological iron overload disorders as reflecting high level of hereditary hemochromatosis (HH) [29]. However, the exact correlation in terms of pathogenesis is not clear but it is suggested that it may play role by adding oxidative stress, particularly to pancreatic cells [30].

Sullivan in 1981, postulated the possible role of iron with cardio vascular disease (CVD) [31][32].

Several pathways/studies have been suggested association between ironoverload and heart diseases. They explained the tissue cell loss and atherosclerosis through ROS production [33] and ROS well known to be produced by NTBI and thus its indirectly the cause of inflammation and contribute to cell damage [34][35]. Such ROS are also observed cause oxidation of LDL that results in to induction of plaque formation [36].

Even platelet aggregation influence suggested by Pratico et al. [37]. Oxidative damage of Iron also observed to cause apoptosis that lead to cardiomyocyte loss [38].

As NTBI known to produce ROS and these molecules suggested as the cause of chronic kidney disease. This may be by glomerular basement membrane (GBM) damage, by affecting glomerular filtration rate (GFR) and by causing morphological changes in kidney [39].

In conclusion, the discovery of NTBI, and its presence in excess and/or present in free form in various iron overload disorders, make it most significant clinically. The major organs influenced by iron overload are pancreas, heart, kidney etc. Major iron mediated pathogenesis is thought to have due to

increased oxidative stress through generation of free oxygen radicals. A range of analytical approaches have been used for estimation of non transferrin bound iron. But the universally accepted standard method that can operate in clinical laboratories is needed to be developed.

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Vitamin D in Physiological and Pathological Conditions

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Introduction

In spite of the fact that vitamin D is characterized under fat solvent nutrient,it should be viewed as more precisely as a supportive of chemical, andits metabolite 1,25(OH)2 cholecalciferol as the significant steroidchemical associated with mineral particle homeostasis in the body. It issince that its system of activity is like that of thesteroid chemical and in typical physiologic setting it very well may be combined in the body and in the event that the body gets sufficient openness todaylight, then necessity of vitamin D through diet is pretty much nothing ornot by any stretch of the imagination. Aside from its part in calcium homeostasis, it is tooassociated with cell separation and multiplication. Its inadequacy may prompt rickets and osteomalacia, especially without any satisfactory day light exposer. Other than this, it has likewise been accounted for to diminish the gamble of insulin obstruction, metabolic condition, heftiness and various tumors [1].

Physiology of vitamin D

Types and sources

Vitamin D got from the creature sources is asnutrient D3 (cholecalciferol) and that from plant sources is nutrient D2 (ergocalciferol). These two are the chief types of vitamin D. The wellsprings of previous incorporate fish oil (saltwater fish esespecially salmon, sardines, and herring), liver and egg yolk andthat of the last option incorporates essentially hay, agaricus etc. Over the most recent quite a few years, expanding utilization of sunblock and diminishing openness to daylight found in North America and Western Europe, individuals' reliance on food as the wellspring of nutrientD has expanded. For this, milk, spread and other dairy items, oats are being invigorated by nutrient D2 ready by lightof ergosterol from yeast. Vitamin D power is estimated by themicrogram of cholecalciferol (1 microgram of cholecalciferol =40 IU). Biologic action and intensity are comparable for the

two structures, however as of late, some contention have emerged with respect to. Yet, both of these are utilized in human incomparative style [1].

Metabolism of vitamin D

In the Skin

In the skin, a photochemical response on openness to Ultraviolet (UV) part of daylight causes the development of cholecalciferol from 7-dehydrocholesterol. The last option is a middle in union of the cholesterol. It gets amassed in theskin. UV beams cause non enzymatic change of 7-dehydrocholesterol to previtamin D which, on warm ionization for period of hours, yields cholecalciferol or calciol or nutrient D3, what's more, thisgets assimilated in the blood. The creation of cholecalciferol in the skin is impacted by accessibility of daylight. It is diminished by high measure of melanin in the skin and further rmore by the utilization of high assurance sun-bar, which hinders the infiltration of skin by UV beams. The plasma convergence of vitamin D is most elevated atthe finish of summer and least toward the finish of winter. The accessibility of UV part of daylight declines past 400 scopes. [1].

In the liver

Cholecalciferol, whether created in the skin or consumed from the digestive tract, arrives at the flow where it ties to vitamin D restricting protein. Then it arrives at the liver where it is hydroxylated at 25-position by acytochrome P450 like chemical,calciol-25-hydroxylase, in mitochondria and microsomes. Ityields 25-hydroxy subordinate 25-hydroxycholecalciferol or calcidiol. The last option is delivered into the flow. This is the primary flowing and stock piling type of vitamin D. In blood, 88% of itties to vitamin D restricting protein, 0.03% is free and the restis bound to egg whites. Its half-life is 2-3 weeks, which can be decreased in the condition like nephrotic disorder due to urinaryloss of vitamin D restricting protein [1].

In the kidney

Following stage in the digestion of vitamin D happens in the kidney. Here, 25-hydroxyvitamin D-1α hydroxylase makes the subsequent hydroxylation produce 1,25-dihydroxycholecalciferol orcalcitriol, the organically dynamic type of vitamin D. This response happens in the proximal tangled tubules in the kidney. This protein is cytochrome P450 like blended capability oxidase, whichis under close guideline not at all like the calciol 25-hydroxylase in the liver. The previous is initiated by parathormone (PTH) and hypophosphatemia and down regulated by calcium, fibroblast development

factor (FGF23) and 1,25-dihydroxycholecalciferol. This proteinis additionally present in various tissues like mind, colon, bosom, prostate, beta cells of pancreas, vascular smooth muscle, macrophage and epidermal keratinocyte. Be that as it may, this doesn't add to the flowing degree of dynamic type of vitamin D, rather ithas paracrine activity in those tissues [1].

In kidney there is likewise another catalyst, vitamin D-24-hydroxylase which hydroxylates at 24th place of 25-hydroxycholecalciferol yielding 24,25-dihydroxycholecalciferol, an idle metabolite. This is the foremost pathway of inactivationof vitamin D, which is available in pretty much every tissue. Strangely, 1,25-dihydroxycholecalciferol builds the activity of vitamin D-24-hydroxylase prompting inactivation and lessenedorganic action of vitamin D. Other than these, there are different polar metabolites of vitamin D, which are emitted in bilewhat's more, reabsorbed by enterohepatic dissemination. Thus, if the last option ishampered because of any explanation like infections of terminal ileum, itcauses the expanded loss of vitamin D metabolites [1].

Metabolic role of vitamin D in body

How vitamin D exerts its effects at molecular level

Vitamin D demonstrations through Vitamin D Receptor (VDR), a partof atomic receptor superfamily. This receptor has a place with a subfamily that like wise incorporates the thyroid receptors, retinoid receptors and the peroxisome proliferator-initiated receptor. Among these, VDR has only one isoform. From past conversations, vitamin D behaves like a steroid chemical and applies its belongings by restricting to VDR. The last option ties to RetinoidX Receptors (RXR-which can tie to 9-cis-retinoic corrosive) andframing a heterodimer, which ties to DNA succession, invigorates enrollment of essential particles and consequently starts the articulation of the objective quality. The RXRs additionally structure hetero dimer with thyroid and other atomic acting receptors. Lack what's more, overabundance of vitamin A can diminish the activity of vitamin D. In lack, there is diminished degree of 9-cis retinoic corrosive tostructure heterodimers, while in overabundance, there is arrangement of RXRhomodimers what make the RXR receptors inaccessible to frameheterodimer with VDR. For some objective qualities, VDR applies its inhibitory activity by meddling the actual activity of the record factors or by enrolling novel proteins to VDR complex. 1,25-dihydroxycholecalciferol has multiple times more liking than othervitamin D metabolites thus in physiologic condition the last optiontry not to straightforwardly interface with the receptors. However, in the event of vitamin D poisonousness, 25-hydroxycholecalciferol can straight forwardly cooperate with VDR, bringing

about hypercalcemia and furthermore can dislodge 1,25-dihydroxycholecalciferol from vitamin D restricting protein driving to ascent of its bioavailability [1].

Functions of vitamin D

Role in mineral ion homeostasis

Vitamin D assumes a significant part in calcium homeostasis. It applies its belongings in three ways:

a) Vitamin D prompts the union of calcium restricting protein calbindin 9K which is available in the gastrointestinal mucosal cells and is engaged with dynamic vehicle of calcium. Aside from this, there are two significant calcium carrier, TRPV5 and TRPV6 (transient receptor potential vanilloid), in the digestive mucosa, which are additionally actuated by 1,25-dihydroxycholecalciferol. By inciting the statement of these proteins in the gastrointestinal epithelial cells, the last option builds the gastrointestinal ingestion of calcium [1].

b) This nutrient is likewise significant for preparation of calcium from bone. Here, it acts synergistically with PTH. Nutrient D influences osteoblast through its receptor, VDR and builds the statement of various qualities encoding bone grid proteins, osteocalcin and osteopontin and diminishes that of type I collagen. Other than this, 1,25-dihydroxycholecalciferol and PTH both prompt the union of Receptor Activator of Nuclear Factor k B (RANK) ligand, which ties toRANK present on osteoclast ancestors and mature osteoclasts. This is the means by which they increment the osteoclast separation and their movement and accordingly bone resorption.

However, it was recently believed that 24,25-dihydroxycholecalciferol was an idle metabolite yet late examinations on take out mouse have showed its poorly characterized job inbone digestion [1].

c) 1,25-dihydroxycholecalciferol and PTH together incrementthe reabsorption of calcium from distal tangled tubules of kidney and accordingly diminishing the renal discharge of kidney [1].

In these three ways, vitamin D assists with keeping up with the serumcalcium levels in the body [1].

In cell expansion

Vitamin D is remembered to regulatorily affect cell expansion. Having hostile to proliferative effect has been shown on various cells like keratinocyte, bosom malignant growth cells, prostatedisease cells [1].

Impact on endocrine framework

1,25-dihydroxycholecalciferol acts through VDR receptors communicated on parathyroid organ and applies an enemy of proliferative impact and diminishes the record of PTH, subsequently diminishing its activity. This is the foundation information for methodology to forestall and treat the hyperparathyroidism in renal deficiency [1]. It might play part in discharge of insulin from pancreatic betacells and furthermore in union and emission of thyroid chemical[1].

Different capabilities relegated to vitamin D incorporate

a) Regulation of ordinary working of natural and versatile framework It has impact in restraint of creation of interleukins by actuated T lymphocytes, and of immunoglobulins by enacted B lymphocytes and furthermore in separationof monocyte forerunner cells [1].

b) Normal working of neuromuscular activity [1].

c) Regulation of pulse [1].

Pathology related with vitamin D digestion

Adjustment of vitamin D digestion should be visible in differentneurotic circumstances like rickets, osteomalacia, renal dystrophy, fundamental hypertension, various sclerosis, rheumatoid joint inflammation, various diseases and so on. Out of them, scarcely any worth focusing on messes have been portrayed here.

Lack of vitamin D

Lack of vitamin D is major worldwide general medical condition revealed from both daylight insufficient and day light adequatenations. Disregarding this, it is the most under diagnosed and undertreated nourishing lack on the planet. Just about 1 billion individuals are accounted for to experience the ill effects of lack of vitamin Dwhat's more, half of the populace from vitamin D inadequacy [2]. The pervasiveness of lack of vitamin D is more in older and fat patients and furthermore in hospitalized patients and nursing homeoccupants. It is 35% higher in fat individual regardless ofscope and age [3]. In United States (US), half to 60% of hospitalized and nursing home

occupant patients were accounted for tohave lack of vitamin D [4,5]. The condition might be connected withdiminished sun openness coming about because of higher skin content of melanin and furthermore the act of higher skin inclusion particularly in Middle Eastern nations. In United States, 47% of African American babies and 56% of Caucasian newborn children, while 90% of newborn children in Iran, Turkey and India have lack of vitamin D. Among the grown-ups, it has been seen that 35% in US have nutrientD lack, then again, in India, Pakistan and Bangladesh over 80% are vitamin D lacking. With regards toolder populace (>60 years), 61% in US, 90% in Turkey, 96%in India, 72% in Pakistan and 67% in Iran are vitamin D lacking[6].

Reasons for lack of vitamin D

The lack of vitamin D primarily results from diminishedcreation in skin, absence of dietary admission, expanded misfortune, weakened vitamin D enactment and protection from biologic activity of 1,25-dihydroxycholecalciferol.

a) Vitamin D lack because of diminished creation or admission:

1) In the event of matured and hospitalized patients, there is diminished openness to daylight as well as diminished retention in digestive tract with age, prompting lack.
2) Reduced consumption through diet might be a reason in different populaces.
3) Intestinal malabsorption, especially of dietary fats, uniquely in terminal illeal sickness where there is diminished enterohepatic flow of vitamin D metabolites bringing about expanded loss of those mixtures.

b) Increased loss of vitamin D:

1) Due to expanded catabolism coming about because of acceptance ofhepatic cytochrome P-450 by different medications like barbiturates, phenytoin, rifampin.
2) Decreased enterohepatic course prompting expandedloss of vitamin D metabolites.
3) Nephrotic disorder

c) Decreased 25-hydroxylation:

1) In extreme liver sickness
2) Use of isoniazid

d) Reduced 1α hydroxylation:

1) Renal disappointment
2) 1α hydroxylase change like in hereditary confusion pseudovitamin D lack.
3) Hypoparathyroidism
4) Oncogenic osteomalacia
5) X-connected hypophosphatemic rickets
6) Use of ketoconazole

e) Resistance in the objective organ:

Change in the vitamin D receptor like genetic vitamin Dsafe rickets which is more hard to treat [7].

Clinical highlights

The clinical elements for the most part result from the disabled calciumassimilation. Gentle to direct inadequacy might be asymptomatic yet, long standing one might be appeared with auxiliary hyperparathyroidism, debilitated mineralization of bone portrayedby osteopenia on X-beam and proximal myopathy. The last once in a long while may show the highlights of intense hypocalcemia as deadness shivering seizures [7].

Rickets and osteomalacia

Vitamin D is appeared as rickets in youngsters before epiphyseal combination. There is proceeded with arrangement of osteoid frameworkalso, ligament yet there is ill-advised mineralization of it bringing about delicate, malleable bones, development hindrance with extensionof development plate. In ordinary cases, three layers of chondrocytesare available in development plate-the hold zone, the proliferativezone, and hypertrophic zone. Rickets is portrayed by development of hypertrophic layer as the after effect of impeded apoptosis of late hypertrophic chondrocytes. Additionally, in murine models, itis seen that hypophosphatemia is significant etiologic variable forthe advancement of rachitic development plate. [8] Clinical elementsinclude:

1) Craniotabes: In this component, tension over delicate membranous skull bone will give sensation of ping-pong ball beingpacked.
2) Bossing of the skull: Bossing of front facing and parietal bone,mostly seen following a half year old enough.
3) Broadening of the finish of the long bones, mostly aroundwrists and knees, seen around 6-9 months old enough.
4) Delayed teeth ejection.

5) Harrison's sulcus: Horizontal discouragement along the lower some portion of chest relating to addition of the stomach.
6) Pigeon chest: Sternum is unmistakable.
7) Rachitic rosary: The costo-chondral intersections of foremostchest wall become unmistakable.
8) Muscular hypotonia: The youngster's mid-region is protruberant(pot midsection), and visceroptosis and lumbar lordosis are moreoverseen.
9) Deformities: different disfigurements like thump knees orbowed legs which become clear as the youngster starts towalk [9].

Osteomalacia happens because of lack of vitamin D in grown-ups bringing about demineralization of prior bone prompting moredefenselessness to crack. In contrast to osteoporosis, osteoid frameworkstays in salvageable shape here. It could be a component of well established hypophosphatemia because of either renal phosphate squandering or ongoingutilization of etidronate or phosphate-restricting acid neutralizer. [8] Clinical highlights include:

1) Bone torments: It might go from skeletal distress to diffuse agony, even delicacy is normal.
2) Muscular shortcoming: Difficulty in scaling and downsteps. Waddling walk might be seen. Tetany might appear atcarpopedal fit and facial jerking.
3) Spontaneous crack: Mainly in spine prompting kyphosis[9].

Finding of lack of vitamin D, rickets and osteomalacia

1) The most unambiguous evaluating test for lack of nutrient isassessment of serum 25-hydroxycholecalciferol. The various standards are expressed in Table 1 [10].

Table 1: Recommended serum levels of 25-(OH) vitamin D.

Condition	Serum levels of 25-(OH) vitamin D
Sufficiency	More than or equal to 30 ng/mL
Relative Insufficiency	21-29 ng/Ml
Deficiency	Less than or equal to 20 ng/mL
Toxicity	>150 ng/mL

2) There may be decreased serum total and ionized calcium[8].
3) Due to PTH induced bone turn over there may be increased alkaline phosphatase. It is also associated withphosphaturia and hypophosphatemia as PTH induces theurinary calcium retention and phosphate excretion [8].
4) As PTH is an important stimulus for renal 1α hydroxylase,paradoxically level of 1,25-dihydroxycholecalciferol maybe increased sometimes. This is the reason why the latter does not reflect the status of vitamin D in body andshould not be used to diagnose the vitamin D deficiency[8].
5) Radiologic findings:
   For rickets: Delayed appearances of epiphysis, wideningof epiphyseal plate, cupping and splaying of metaphysis,bone deformities and in late cases rarefaction of diaphyseal cortex [9].
   For osteomalacia: Diffuse rarefaction of bones, looser'szone or pseudofracture (radiolucent zone at sites of stress;common sites include pubic rami, axillary border of scapula, ribs, the medial cortex of the neck of femur; it is dueto rapid resorption and slow mineralization and the zonemay be surrounded by collar of callus), triradiate pelvis infemales, protrusio-acetabuli (protruding acetabulum intopelvis) [9].

Treatment of vitamin D deficiency

The condition is treated with vitamin D supplementation. Based on observation that 400IU supplementation is often insufficient to prevent deficiency, and 800IU along with calcium supplementation reduces the risk of hip fractures in elderly women, the high doses are preferred. Vitamin D should always be supplemented along with calcium because most of the features of deficiency are due to hypocalcemia. Toxicity occurs with the dose 40000IU daily. Patients having impaired1α-hydroxylation, are treated by metabolites not requiring this activation step like 1,25-dihydroxy vitamin D3 (calcitriol,0.25-0.5µg/d) and 1α-hydroxy vitamin D2 (hectotrol,2.5-5µg/d). Severe deficiency is treated by initially 50000IU weekly for 3-12 weeks, followed by 800IU daily. Calcium supplementation should be1.5-2 g/d of elemental calcium [8].

In response to treatment, normokalaemia occurs within oneweek, though, increased PTH and alkaline phosphatase levels persist for 3-6 months. Treatment monitoring is done by measuring serum and urinary calcium. If the treatment is adequate then, 24-hour urinary calcium excretion will be in the range of100-250mg/24-hours, if less, it means any problem regardingpatient's

compliance to treatment regimen or absorption of calcium or vitamin D supplement. The levels of >250mg/24-hours are associated with nephrolithiasis requiring a reductionof doses [8].

Vitamin D harmfulness

The expanded degree of plasma fixation prompts harmfulness portrayed by withdrawal of veins, coming about inexpanded circulatory strain and calcinosis for example affidavit of calcium in the delicate tissues. With expanded degree of vitamin D, therewill likewise be expanded bone resorption and calcium ingestion bringing about hypercalcemia and hypercalciuria, and the last option willincrement the inclination of renal stone arrangement. A few newborn children might be delicate to portion of vitamin D as low as 50µg/day.Poisonousness is seen in extreme dietary admission however not in unnecessary openness to daylight. It is on the grounds that that our body has restricted ability to combine antecedent, 7-dehydrocholesterol and unnecessary openness brings about arrangement of latent metabolites [10].

Vitamin D and different infections

Other than assuming a significant part in calcium homeostasis, nutrient D applies various capabilities in the body. By virtue of this truth, modified degrees of vitamin D have been viewed as related with various clinical issues. A couple of them have been examined here in a piece detail:

Renal osteodystrophy

One of the entanglements of the ongoing renal disappointment is renal osteodystrophy. There is failure of body to create 1,25-dihydroxy cholecalciferol thus the bone calcium turns into thesource to keep up with the serum level. Furthermore, the condition is related with renal maintenance of phosphate and hyperphosphataemia. This prompts arrangement of calcium phosphate coming aboutin affidavit of the last option in delicate tissues, metastatic calcification, diminishing the degree of calcium further. Consequently the blend ofhypocalcemia and hypophosphatemia builds the emissionof parathyroid chemicals bringing about bone resorption. Subsequently, for this situation treatment with just vitamin D or its metabolites won't help since it will rather increment metastatic calcification because of hypercalcemia coming about because of the treatment and previously existing hypophosphatemia. In this way, significant is to decreasethe phosphate level close by the high calcium slims down/nutrientD enhancements. As it is challenging to diminish phosphate part in diet, vegetable proteins are liked over creature ones, for, in them phosphates are as phytates which arenot promptly

retained. The patients are likewise approached to keep away from handled food as phosphates are added to them. For all the above-mentioned reasons, phosphate fasteners are utilized. They are calcium acetic acid derivationfurthermore, cationic polymer called sevalamer hydrochloride. If orallyregulated 1,25-dihydroxyvitamin D isn't adequate to raiseserum calcium level, as in serious hyperparathyroidism, intravenous structure might be given. Parcel of exploration is proceeding to createcalcimimetic specialists which can tie to calcium sensor on theparathyroid organ, along these lines diminishing its discharge [11].

Cystic fibrosis

This is the illness brought about by the change of the quality encoding Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), a cAMP managed chloride channel. It is the mostnormal deadly acquired infection among caucassians. This deformity prompts issue in the action of exocrine organs influencing for the most part the lungs and the pancreas. In the lungs, there is viscid emission in bronchi and bronchioles hindering the lumen furthermore, it additionally brings about continuous contaminations. Then again,brokenness of exocrine cells of pancreas brings about inadequacyof pancreatic catalysts and furthermore fractional block of normalbile pipe. Thusly, lack of pancreatic amylase, proteases and lipase will prompt extreme malabsorption. Because of fatmalabsorption coming about because of lipase and bile inadequacy, therewill be related fat solvent lack of nutrient. Calcium tieswith long chain unsaturated fats to shape insoluble salts. To some degree processed food bolus also frustrates the ingestion of starch and proteins by actual entanglement of those particles. Other than these, expanded bodily fluid discharge in the gastrointestinal lumen additionally lessensthe retention of a few micronutrients like iron [11].

Patients are treated with planning of pancreatic chemicals. Even though, with this, the ingestion of protein and starches approximates to ordinary, that of fat doesn't. It is because of the realitythat there stays the industrious lack of bile corrosive and exorbitant bodily fluid emission. Besides, for certain patients calorierequest is expanded because of persistent contamination. The suggestion is high energy, high protein diet without limitation of dietary fats (half carb, 15% protein, 35% fat). Assuming eating routine fizzles to supply the need of the body, then strengthening feed or enteral taking care of comes into the image. Now and then, medium chainunsaturated fats might be exhorted on the grounds that they get ingested straightforwardlyin the digestive mucosa. Offspring old enough 2-8 years are

dealt withwith multivitamin arrangement containing 400IU of vitamin Dwhat's more, 5000IU of vitamin A, however, more seasoned kids, juvenile andgrown-ups need 1-2 portion each day. Vitamin K is just suggestedat the point when patient is on anti-microbials or experiencing cholestatic liverillness. Disregarding the way that lack of iron is regularly connected with this condition, it isn't suggested in light ofchance of elevated degree of iron to improve likelihood of fundamentalbacterial disease. Even though calcium level is ordinary in thesepatients, keeping up with its suggested level is vitalin diet [11].

Vitamin D and malignant growth

Change of vitamin D level has been ensnared in the gamblealso, movement of different malignant growths. Different exploration workshave upheld this reality. It goes long back to 1915 when Hoffman previously saw the relationship of sun openness and scopewith disease mortality. Peller and Stephenson tracked down that individualwho were presented to daylight adequate to incite non-deadly, non-melanoma skin disease, had lower occurrence of additional threatening cancers. In 1980s to 90s, Garland et al and Gorham et alled various epidemiologic examinations and closedthat there were areas of strength for a relationship between scope,sun openness and unfortunate vitamin D status and the gamble of creating numerous lethal malignant growths like that of colon, bosom, ovarian furthermore, melanoma. Age changed passing rates among the patients of colon malignant growth have been viewed as high in regions with lowlevels of winter daylight. A review showed that the people with 25-hydroxyvitamin D level <30ng/mL had higher rate of colonic adenomas. The relationship of 25-hydroxyvitamin Dwith chance of colon disease was available both early and late infollow-up, recommending that vitamin D metabolites might have impacts at all phases of carcinogenesis. There are 7 epidemiological studies demonstrating the higher gamble of colon disease in peopletaking lower measures of vitamin D including Western Electric Associate Study, the Nurses' Health Study, the Male Health Professionals' Follow-Up Study, the Iowa Women's Health Study, andthe American Cancer Society Cancer Prevention Study II (CPS II) Associate Study and 2 case control study. There are additionally studies detailing that bosom disease passing rates are higher in regions withlow winter daylight levels. Ladies who are presented to adequate daylight on ordinary premise, appear to have essentially loweroccurrence of bosom disease. Low degrees of 25-hydroxy vitamin Dmight be ascribed to quicker movement of bosom disease. Besides, death paces of perimenopausal ovarian disease have likewise been tracked down lower in bright districts. A similar impact can be found if there

should arise an occurrence of prostate malignant growth. In an investigation of 19000 men, thosewith levels of 25-hydroxyvitamin D <16ng/mL had a higher occurrence of prostate malignant growth than those having more than thisesteem. In spite of the fact that, there are not many examinations showing no affiliation between vitamin D and malignant growth, making the point questionable[12,13].

We realize that vitamin D is engaged with guideline of cellmultiplication and development. In any case, the specific component isn't completely perceived. Various qualities controlling multiplication, separation, apoptosis and angiogenesis, are straight forwardly or by implication impacted by vitamin D. The dynamic type of the last option increments inhibitors and diminished activators of cyclin subordinate kinase buildings as well as expanding levels of cyclin subordinate kinase inhibitors Cip/Kip proteinsP21 and P27. These proteins are known to keep the cell cyclein the G1/S stage, forestalling DNA combination thus, the cellmultiplication [12].

Vitamin D and immune system infections

Vitamin D is known to have significant immunomodulatory impacts in body. Dendritic cells are essential focuses for this movement of 1,25-dihydroxycholecalciferol, the dynamic type of nutrientD. It is demonstrated by restrained dendritic cells separation and development prompting down managed articulation of MHC-II, co-stimulatory particles like CD40, CD80, CD86 and diminishedcreation of IL-12. Other than these, 1,25-dihydroxycholecalciferol increments IL-10 creation and consequently, advances dendriticcell apoptosis. Along these lines, vitamin D restrains dendritic cell subordinate T-cell actuation. Aside from these, vitamin D receptor agonists appear to restrain pathogenic supportive of provocative T-cellslike Th1 and Th17 and under fitting circumstances they tend to lean toward a deviation to Th2 pathway [14].These kinds of immunomodulatory and calming impacts are especially productive for the rheumatoid joint pain patients to help a restorative job of 1,25-dihydroxycholecalciferol in this illness. From various investigations, it is additionally seen that vitamin D might assume a significant part in support of B-cell homeostasis and revision of vitamin D insufficient state might influence the treatment of B-cell intervened immune system problems for example, fundamental lupus erythematosus (SLE) [14].

Conclusion

It is clear from the above conversation that vitamin D is significant in the body to keep up with physiologic homeostasis. Other than this, adjustment of vitamin D status in the body, underlies different pathologic circumstances. Subsequently, vitamin D is acquiring extraordinary interest in ongoing biomedical examination works.

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Biology and Control of cereal crops Insect – Pests

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Introduction

Rice is the most important cereal crops in the world occupying 113.6 million hectares and producing about 150 million tons of rice. It is extensively grown throughout India in about 30 million hectares which is more than one third of the world area under rice cultivation. The rice crop is ravaged by a much larger number of insect-pests than any other cereal crops. As many as 90 insect species have been recorded to feed on different parts of the plant seedlings stage till harvest of the crop.About one-fifth of them are considered to be important or major pests causingenormouslosses to rice crops.It has been estimated that rice crop alone suffers damage due to insect-pests and diseases to the extent of Rs 100 crores annually.Out of which the loss caused by the rice stem borer alone is about Rs.10 Crores. This crop therefore requires more attention on protection technology in order to obtain maximum production per unit area. The following insect-pests have been observed attacking rice fields in principal rice growing areas of India specially Uttar Pradesh-

(1) Rice Hispa

(2) Rice bug or Rice Gundhi bug

(1): Rice Hispa

Hindi name: Dhan ka Hispa

Scientific name: *Hispa armigera* or *Dicladispa armigera*

Classification:

Phylum-Arthropoda

Class - Insecta,

Subclass- Pterygota,

Division - Endopterygota

Order- Coleoptera

Family - Chrysomelidae,

Genus - Hispa

Species - armigera

Distribution: *Hispa armigera* is distributed throughout India viz. Punjab, Himachal Pradesh, Jammu & Kasmir, West Bengal, Assam, Tamil Nadu, Andhra Pradesh, Karnataka, Orissa and U.P. Apart from India it is also reported from Nepal, China, Pakistan, Srilanka, Malaysia, Indonesia, Bhutan, Thailand.

Host plants: Rice is the main host plant in its absence it sustains itself on the sugarcane, sorghum and wild grasses.

Nature of damage: This is another pest of minor importance. The beetle feeds on matter of leaves by scrapping .The grubs of this beetle mine the rice leaves generally from tip downwards and feed on tissues in between the two epidermal layers, white parallel lines are made on rice leaves which in turn becomes membranous and finally die as a result of its attack. The crop remains stunted and tips dry up. It prefers young plants partly submerged in water. The attack is severe when hot and humid climate prevails.

Life cycle of *Hispa armigera*: Generally it belongs order coleoptera and it is clear that in coleopterans four developmental life stage will be reported such as eggs, larva, pupa and adult.

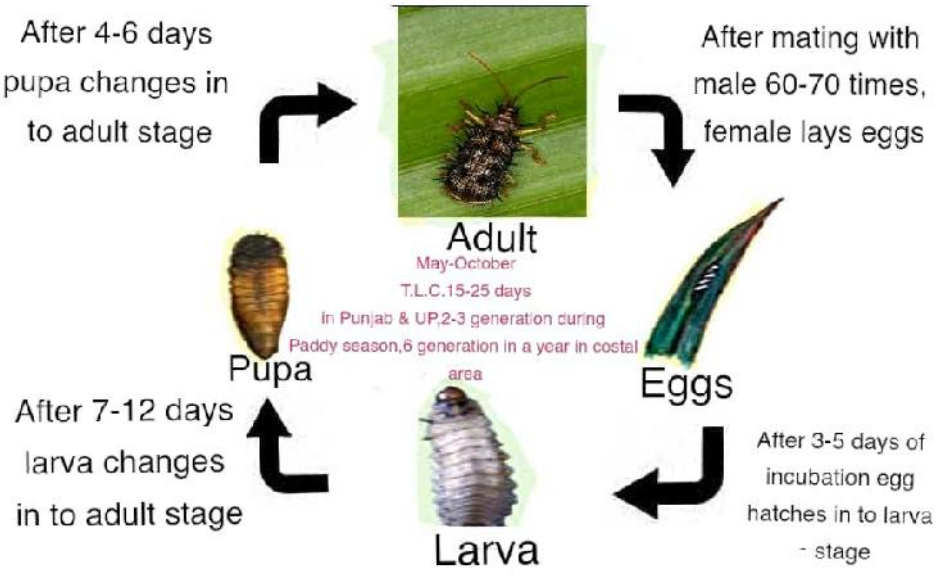

Fig.-Life cycle of Hispa armigera

1: Eggs: The female lays eggs after 3-4 days of emergence and continues up to a month. A single female may deposit 30-300 eggs. The egg which are oval and about 1mm long, each egg being inserted in the epidermal tissue in the upper part of the leaves. The incubation period ranges from 3-5 days.

2: Larva (Grub): The newly hatched larva is pale yellow, dorsoventrally flattened and about 2-4 mm long. The larva feed on the mesophyll of the leaf, eating its way and producing a yellow spot. The larva may easily be seen if a spotted leaf is held up to the light. A single larva may consume about 125 mm square of leaf area per day.

Larval stage lasts for 7-12 days passing through four instars.

3: Pupa: Pupation takes place with in the larval mines in a period of 4-6 days. The pupa is flat and exarate. Upon emergence from pupal case, the adult beetle cuts its way out of the rice leaf and become external feeder. Female survive for 30-50 days. Total life cycle is completed in 15-25 days. There are six generations in a year in coastal area, however in Punjab and U.P. it completes 2-3 generations during paddy season.

4: Adult: The adult beetle is 5 cm long, shiny and bluish black in colour and several shorter spines over body.

Prevention and control:

1: Manual collection and killing of beetles with hand nets may help in reducing the population of pests.

2: the crops with10 % BHC dust @ 30kg / ha. atleast two times at an interval of 40 days proves effective.

3: Spraying of the crop with 0.07 % endosulphan or 0.03% phosphamidon @700 litre/ha. used for control of the pests .iv) Control operation in nursery should necessarily be done.

(2): Gundhi bug

Local name: Gundhi

Scientific name: *Leptocorisa varicornis, L. costalis, L.acuta*

Classification:

Phylum-Arthropoda

Class-Insecta

Subclass-Pterygota

Division-Exopterygota

Order- Hemiptera

Suborder-Heteroptera

Family-Coreidae,

Genus- Leptocorisa

species - varicornis

Food plants: It mainly feeds on paddy but also feeding on maize, bajara, jowar and some grasses.

Distribution: This pest is widely distributed in India, tropical and subtropical parts of oriental region. In Uttar pradesh, Gundhi bug causes severe damage to paddy crops.

Nature of damage: This is a major pest of rice and attacks the paddy field at the milky stage. The nymphs and adults both suck the juice from developing grains in month of August to October as a result of which the ears become

white and do not form the maturegrains.The attacks seeds are marked by black spots of fungus which appears around the puntured point.

Life cycle of *Leptocorisa varicornis:*

Generally it belongs order hemiptera and it is clear that in hemipterans three developmental life stage will be reported such as eggs, nymph and adult.

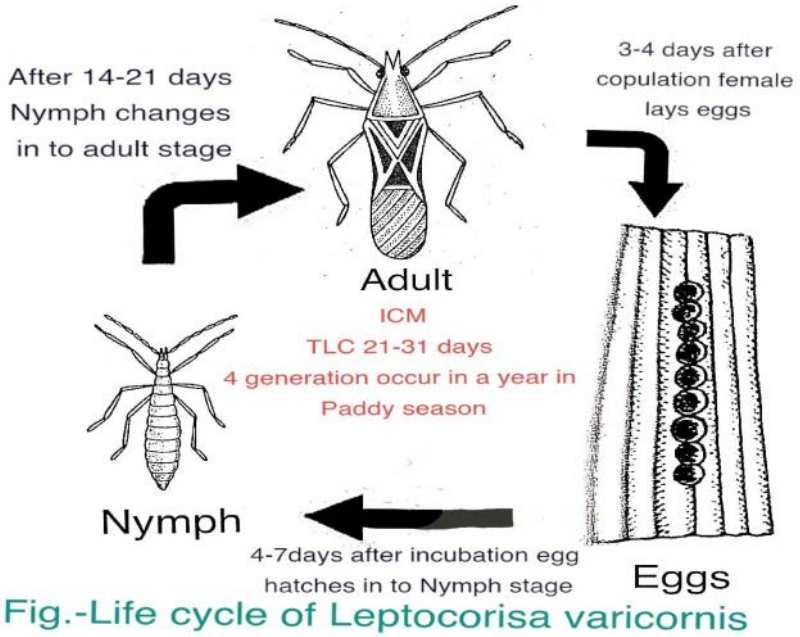

Fig.-Life cycle of Leptocorisa varicornis

Eggs: The eggs are laid by the female bug in the month of July and August. They are deposited in three stripes on the lower side of the leaves. Each strip contain 10 to 20 eggs and about 40 eggs are laid at the place. The eggs are blackish -brown small bead like in structure. Depending on the prevailing temperature, the eggs hatch in about 4-7 days. At the time of hatching their colour changes in to black.

Nymph: The slender greenish nymphs hatch out from the eggs, start immediately sucking the plant juice. At the time of hatching it is about 1.8 mm long which grows up to 2.0 mm with in 6 hours. There are five moults after which the nymph becomes adult with wings. The wings pad may seen in the second nymphal instar. The nymphal period is of 14 to 20 days. The fully grown nymph measure 15 mm long.

Adult: The adult bug is about 15-16 mm long slender and greenish yellow in colour, head is small and provided with 4 segmented rostrum. The antennae are larger than the body and red in colour. Metathorax provided with glands emiting unpleasant odour. The forewings are hemelytrate. The life cycle is completed in 21 to31 days and several overlapping generations are found but on paddy 4 to 5 generations are completed. The bugs copulate after 12 to 14 days of emergence and female survive up to 55 days while male only for 33 days. The pest passes its winter in adult stage and migrate to grasses and other plants to continue its life cycle.

Prevention and control:

1. Insects should be picked up by hand net and killed by strong crude oil emulsion.
2. In irrigated fields the crude oil emulsion should be sprayed which forms thin film of oil resulting in to the killing of Gundhi bugs and nymphs.
3. Pest should be attracted by light trap and destroyed.
4. The variety of rice used should be such in which grains come out at a late stage from the sheath.
5. Wild grasses should be removed from the paddy growing areas.
6. 0.25 BHC dust should be sprayed before flowering.
7. The dusting of paddy fields by 2.5 % Aldrin, 5 % Chlordane and 5 - 10 % BHC has been found to be useful for the control of the pests.

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Biology of Lac Insect and Lac culture

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Introduction

Common Name: Lac insect

Scientific Name: *Laccifer lacca* or *Kerria lacca*

Classification:

Phylum- Arthropoda

Class- Insecta

Subclass - Pterygota

Division - Exopterygota

Order- Hemiptera

Suborder- Homoptera

Family- Lacciferidae

Genus- Tachardia or Kerria or Laccifer

Species- lacca

Introduction

Lac has been used in India from time immemorial for several purposes, from the epic of Mahabharat it has been recorded that Kauravas built a place of Lac for the destruction of Pandavas. We come across references of Lac in the Atharvaveda and Mahabharat, so it can be presumed that ancient Hindus were quite familiar with lac and its uses.

Scientific study: Scientific study of lac started much later. Father of Lac insect: Tachard (1709) discovered the insect that produced Lac. First of all Kerr (1782) gave the name Coccus lacca which was also agreed by Ratzeburi (1833) and Carter (1861). Later Green (1922) and Chatterjee (1915) called the

insect as *Tachardia lacca*. Finally the name was given as Laccifer lacca and recently it was renamed as Kerria lacca.

Distribution in India and Abroad: India held virtual monopoly of Lac until 1950 and accounted for about 85% of the world's production of sticklac. After 1950, Thailand became the main competitor of India.Other countries like Africa, Australia, Brazil, Burma, Srilanka, China, France, Japan and West Germany also produce Lac.

In India major Lac producing regions are Assam, Bengal, Bihar, Delhi, Gujarat, Hyderabad, Kashmir, Madhya Pradesh, Chennai, Coimbatore, Mysore, Rajasthan and Uttar Pradesh. Over 90%of Indian Lac produced comes from the states of Bihar, Jharkhand, West Bengal, Madhya Pradesh, Chhattisgarh, Eastern Maharashtra and Northern Orissa. Some pockets of Lac cultivation also exist in Andhra Pradesh, Punjab, Rajasthan, Mysore, Gujarat and Mirzapur and Sonebhdra district of Uttar Pradesh.

Host plants: The Lac insect is domesticated on the following plants -

1: Palas (*Butea monosperma ,B.frondosa*)

2: Ber (*Ziziphus mauritiana*)

3: Kusum (*Schleichera oleosa*)

4: Arhar (*Cajanus cajan*)

5: Pippal (*Ficus religiosa*)

6: Babul (*Acacia nilotica*)

7: Khair (*Acacia catechu*)

Life cycle of Lac insect

Copulation and oviposition: The adult males

After emerging from the cells through its posterior opening, walk over the female cells mate with several females in succession for 3-4 days. Fertilization takes place in the ovarioles of female where the embryo is developed and nourished. The Lac insects are ovo-viviparous in nature in which the nymphs are enclosed in thin egg shell. The mated females continue to grow and secrete lac till it begins to lay eggs .The female lays 300-1000 eggs inside the incubating chamber formed with in the cell by shrinking the body anteriorly. In India, Lac insect also reproduce by deuterotokous parthenogenesis that is unmated females give rise to both male and female progenies .There are no difference between sexually and asexually reproducing

female regarding the amount of Lac produced , fertility and the progeny sex ratio.

Three developmental stages are found in its life cycle *viz*.eggs, nymph and adult

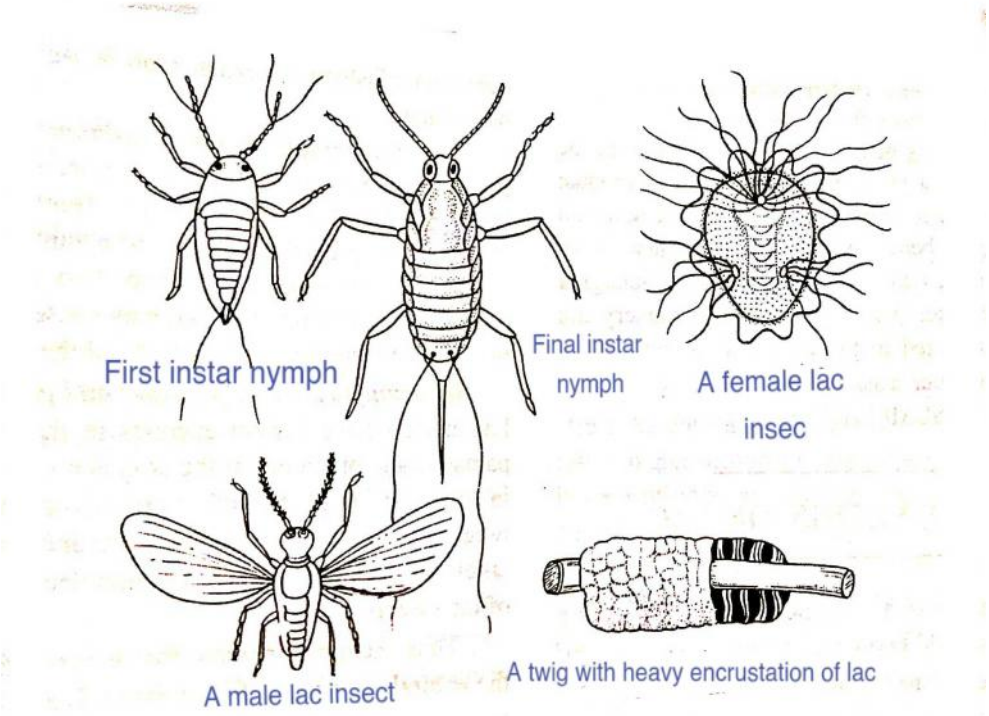


Fig.-Life history of Tachardia lacca

Eggs: The eggs are laid by the female with in the Lac cell (chamber).It lays about 300-1000 eggs which may be fertilized or unfertilized .Both types of eggs give birth to male and female nymphs.

The female lays three types of eggs -

1. The eggs from which both males and females hatch out in equal number.
2. From which males are less than females.
3. Males are more than females.

An individual egg is 0.4mm×0.2mm long and pinkish in colour which changes in to brown after sometimes .The female generally lays well developed eggs which hatch out within a few hours that's why some scientist call them viviparous females .

Nymphs: The newly hatched nymph is 0.6mm×0.25mm long and pinkish in colour .It possesses a pair of antennae, three pair of legs, a pair of compound eye and six anal setae. The nymphs emerge in large number and crawl over the surface of twigs and branches of plants in search of suitable pieces for settlement. They mostly settle on shoots gregariously. Once settled, the nymph thrusts its proboscis in to the bark of the twig and does not move from its place.After one or two days of settelling they start seceting resin from glands distributed under the cuticle all over the body except near the mouth parts, two breathing pores and anus. Thus the nymphs gets in cased in a cell of its secretion which increases in size with the growth of the insect. They moult thrice before reaching maturity and after Ist moult the nymph loses its eyes, legs and antennae but anal setae become 10 instead of 06. The sex is readily recognized by the shape of Lac cells even in the early stage of development. In the case of the male cell, the growth is more along the longitude while in the female cell it is more along the vertical axis. The differentiation between sexes is more pronounced after the first moult. The male cell is elongated with a pair of holes at the anterior end from which thin whitish hairs come out, while a female cell is some what globular with an irregular margin and with three holes on the top, one at the hind end and two at the anterior end .The male nymphs develops the organs after the second moult, both the males and females continue to develop. From the male cells both winged and wingless males emerge after about two months. These males fertilize the females in their cells and after fertilization female develop very fast, swell up and secretes the Lac at a faster rate. The size of the female as well as that of the enveloping Lac cell increases rapidly and reaches a size several times that of the male Lac cell. Thus the females are the chief source of Lac secretion .As the time for egg laying approaches, the posterior region of the female develops a yellow spot which is indication of egg laying. The Lac insect usually passes through two generation in a year.

Adult: The adult male and female are different from each other .Female is about 3 times longer than male.

Male: They are pinkish-red in colour, about 1.2 - 1.5 mm in length and two types winged and wingless. Winged male possesses only one pair of translucent membranous forewings. They are mostly found during the dry

season (Baisakhi and Jethwai). They survive only 3-4 days and die after copulation. The following of the main characters -

1. The head is a large, prominent mouth parts but vestigeal.
2. Ocelli are 2 pairs with seven segmented antennae which have hairs on them.
3. Tarsi 3 segmented.
4. The abdomen is 8 segmented, broader anteriorly and narrower posteriorly .The last abdominal segment bears pointed penis.

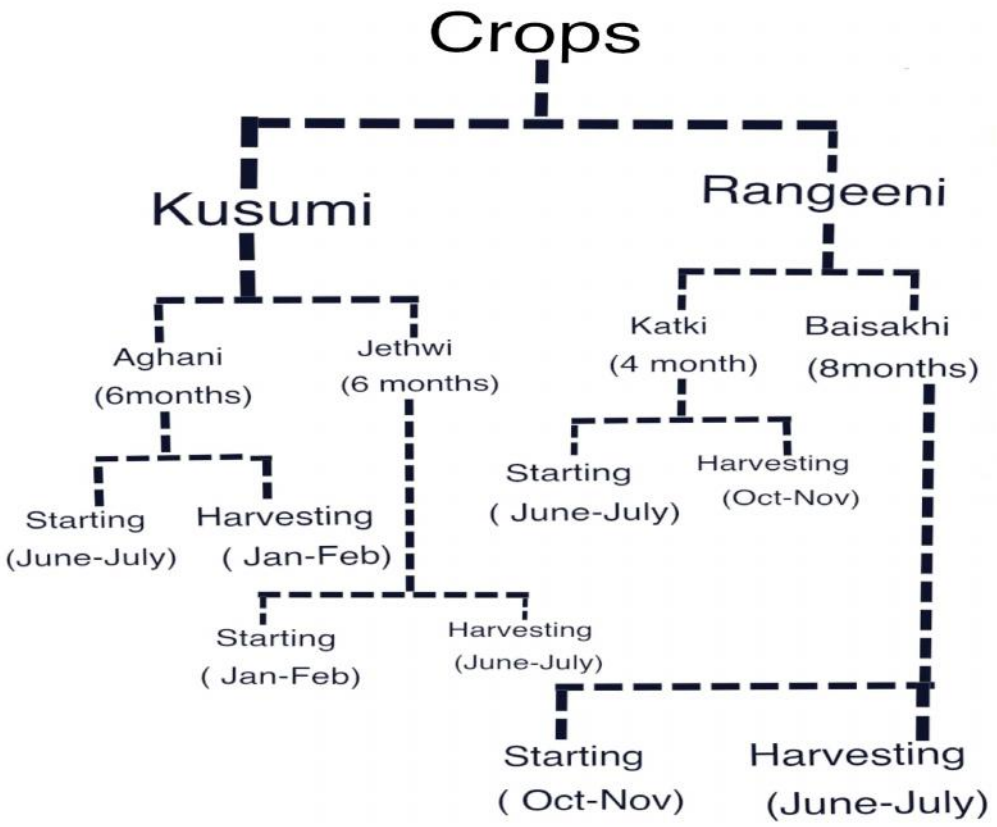
Female: The female is pinkish in colour and about 1.5 mm in length. The ventral surface of the body is flat while dorsal surface is convex. The main characters are -

1. The female deprives of eyes, legs and wings.
2. Antennae are vestigeal, small and 3-4segmented.
3. The mouth parts are piercing and sucking type and rostrum is two segmented.
4. The female remains attached at a point and sucks the juice from the host.
5. Mesothorax is provided with an appendage on which spiracles are open.
6. The abdomen is round on the dorsal surface & a spine is provided. The last abdominal segment is two segmented and anus is friged.

Lac culture

Lac culture is the scientific management of Lac insects to obtain a high amount of quality Lac. This involves selection and maintenance of host plants with healthy Lac insects collection and processing of Lac and protection against enemies. The Lac industry is well developed in India and at present it account to about 70-80% of the total world production. The Lac produced by different states of the country is given here -1: Bihar -41.3% 2: Madhya Pradesh -26.5% 3: West Bengal -20.6% 4: Maharastra 5.9% 5: Uttar Pradesh, Orissa and Assam -Little.

There are two district strains of the Lac insect in India, called Kusumi (Kusum trees) and Rangeeni (it grows on trees other than Kusum, inoculated in OctoberNovember and harvested in May-June).The former is raised mainly on the Kusum trees or on other hosts with Kusum and the later on all other Lac hosts such as Palas, Ber and Arhar etc. Each of the strains produces two crops in a year thus there are four crops which has been shown in the chart -



Of the countries total production of Lac 90%are obtained from the Rangeeni crops and only 10% from the Kusum crops. Though Kusum crop is produced in smaller quantities but superior in quality. The production of all those crops is given here -

1: Baisakhi crop - 2,91600 qntls, 2: Katki crop -1,12200 qntls, 3:Aghani crop - 33,400 qntls ,4: Jethwi crop -15,400 qntls

The two methods of Lac cultivation are popular 1: Local cultivation 2: Modern method

1: Local cultivation: This is the crude and unscientific method of Lac cultivation in which Lac is collected from the trees growing in jungles. Lac is left for continuous self inoculation on the same tree and every time a partial

crop is taken. The method of Lac cultivation has generally failed to provide sustained supplies of brood lac for crop inoculation and optimum yield of Lac.

2: Modern method: This method was developed by Indian Lac Research Institute Ranchi and is known se coupe system. In this system as against the local practice of keeping all the tree under continuous cropping a certain number of trees in a coupe or compartment are inoculated fully and when Lac matures it is fully reaped and another set of trees from another coupe is taken up for subsequent inoculation. The coupe from which the crop was reaped not only got sufficient time to put forth new shoots and foliage but also get rest in regaining the vigour. To get good crop, following points should be kept in mind -

(A): Selection of suitable site: The site should be selected on the basis of host plants, where environmental conditions suitable for host trees should be preferred.

(B): Pruning of host plants: This is an important and essential operation carried out with the object of inducing the tree to put forth maximum number of shoots, suitable in condition for successful colonisation by the nymphs(larvae) of the lac insect.Before starting Lac cultivation,care should be taken that the trees have sufficient tender branches to enable the Lac insect to establish itself . It is not not so the trees should be pruned. Pruning should always be carried out lightly and branches more than 2.5 cm in diameter should not be cut. The pruning is dependent upon the type of the host plant. Example- In case of Ber trees, a large number of succulent shoots are produced after pruning while Palas and Kusum do not ordinarily require pruning.

(C): Inoculation of brood Lac: Lac sticks bearing a live mother cell which produce Lac nymphs (larvae) are known as brood lac. The brood lac sticks should be cut in to convenient length of 15-30cm and tied in succulent shoots of the host tree. The tying may be done either longitudinally or laterally .The correct amount of brood lac (covered 15 to 20 times of its length) should be used for inoculation .The nymphs (larvae) after hatching will transfer themselves in to succulent shoots. The brood used for inoculation should not be left on the trees for more than 3 weeks because keeping beyond this time, there is a danger of enemies to spread. When the brood lac is removed from the trees, it is known as Phunkhi lac . There are following types of Lac -

1: Ari Lac: This is the immature lac and cutting of ari lac should be avoided.

2: Stick Lac: Mature lac harvested in the form of stick is called as stick lac.

3: Seed Lac: The lac obtained after removing and washing from the stick is known as seed lac.

4: Dust Lac: Dust lac is obtained after grinding the seed lac.

5: Shel Lac: Shel lac is prepared after heating the seed lac and dust lac.

(D) Scrapping of brood Lac: When the brood lac sticks are removed from the tree (Phunki lac) all other, either dead or rejected as unfit for brood purposes is scraped as early as possible. The possible date of crop maturity may be known by the following points -

(i) : Appearance of cracks in the lac before 15-20 days.

(ii): Granular appearance of the body contents of the female lac insect.

(iii): The eggs become separated from the masses.

(iv): The eggs seem to be granular.

(V): The lac becomes dry and yellow in colour.

(E): Washing of Lac: The lac obtained after scrapping is washed with water and dried under shadow .The colour (lac dye) is obtained due to the washing which is used for various purposes .

(F): Preparation of Shel lac: The following are the methods for preparing the shel lac - (a): Indian method (b): Hydraulic method (c): Solvent method (d): Autoclave method

: Indian method: It is the oldest method in which the Seed lac is put in to long cloth bags of 6.5 cm width. The bag is tide with a pivot of wheel and circulated .The heat is also provided side by side .The melted lac due to the pressure comes out which is collected and made in the form of tablets . This is an expansive method, it is estimated that to wash one Quantal of lac required one metre cloth, one Quantal coal and 6 men in a day.

: Solvent method: This process has been developed by Indian Lac Research Institute Ranchi in which the seed lac is put in the bags of Iron nets and heated in a special autoclave. After some time it is removed from here and heated again .The melted Shel lac is spread in to thin sheets and dried.

Enemies of Lac insect:

The enemies of lac insect may be broadly grouped in to two –

a: Insect parasites and predators

b: Non insect enemies

a: **Insect parasites and predators**

i- **Parasites**: The parasites of lac belong to the super family - Chalcidoidea which lay their eggs inside lac cell either in or on the body of the Lac insect. The grubs after hatching feed on the Lac insect and the adult come out by biting a hole in the Lac cell. 5-10% damage is caused by these parasites .The important species of parasites are -

(a): *Tetrastichus purpureus*

(b): *Mariettas javensis*

(c): *Eupelmus tachardiae*

ii-Predators: The two predators namely Eublema amabilis and Holcocera pulverea are more important. The larvae of these moths feed on the Lac insect and the larvae of which suck the body fluid of the host.

b: Non-insect enemies: Some birds and Squirrels also destroy the Lac insect , Lac encrustations and caused considerable loss.

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Impact of air Pollution on Sericulture Industry

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Introduction

More than 35 centuries countless generation of silkworm are continuously breeding, feeding on mulberry leaves, spinning their cocoons and dying, an everlasting sacrifice to the demond of human beings for decoration.

For the first time in 2697 B.C. Lotzu Empress of kwang- Ti discovered the foncy origin of beautifull silk in the form of thread.

Thus, technique of cloth preparation from the cultivated silk was known to Chinese peple for more than 2000 years ago.

Now a day sericulture has become one of the most important cottage industries in a number of countries like Japan, China, India, Brazil, Russia and France.

The experimental and systematic study on sericulture was started in Japan in 1911 after establishment of the sericulture experiment staion and in 1979 the national sericulture experimental station was set up in Tokyo.

In India first of all Lefroy (1905-1906) started investigation on the silkworm and sericulture of Pusa Institute New Delhi.

“All India Exhibition” organised by the Government of UP at Prayagraj in 1901- 1910 to draw the attertion of scientists and general Public towords the sericulture Inddustry.

Silk

The silk is a pasty secretion of the silkmoth produced by the silkgland (a modified salivary gland). As this pasty secretion comes in contact with air, it become hard and form strong and pliable silk thread. This secretion forms two core of fibroin i.e – a tough elastic insoluble Protein sericin and a gelatinous Protein which is easily soluble in warm water.

Some quantity of waz and carotenoid pigment are also detected.

Sericulture Industry

The production of silk from silkwarm by rearing practices on commercial scale is called sericulture. Although silk is very beautiful and fancy gift of nature but its commercial production is very much complicated and a tough job which requires heavy man power and favourable environmental condition.

Before going into the details of the sericulture industry it is essential to have an idea about the major steps and requirement of sericulture. Improved race of Bombyxmori and good nutritive type of mulberry plantation is the chief need for this industry. In sericulture mainly two species of mulberry plant are used i.e *Morus alba* Linn. (Toot, Tootri)- it is widely used for rearing the silkworm and MorusIndica Linn.(Shahtoot).

Economic Importance of silk

The raw silk is used in the manufacture of woven materials and the knitted fabrics for the preparation of garments, parachutes, fishing lines, insulation coil for telephone and wireless receivers etc.

Fabrics for garments in various weaves plain, twill, stain georgette, velvet, gloves, socks, dyed and printed ornamented fabrics for saries, jackets, shawls and wrappers are made out of this material.

Status of Silk Production in India

India has the unique distination of being the only country producing all the five commercial silks namely mulberry, Tropical tasar, oak tasar, eri and muga silk, of which muga with its golden yellow glitter is unique and prerogative of India. Mulberry sericulture is mainly practised in states such as Karnataka, Andhra Pradesh, Assam and Bodoland (Kokrajhar, Chirang and Baksa districts of Assam) West Bengal, Jharkhand and Tamilnadu. Who arethe major silk producing state in th country.

India is the second largest producer of silk in the world, first is the China. Amoung the four varieties of silk produced is 2020-21, Mulberry accounted for 70.72%, Tasar 8.02%, Eri 20.55% and Muga 0.71% of the total raw silk production of 33739 mitric ton.

The silk production is reduced in the country during 2020-21 due to coused by the Covid-19 pandemic. The total raw silk production the country

during 2020-21 was 33739 M.T., which was 5.8% lesser then the production achieved during previous year 2019-20.

The export earning during 2020-21 were 1418.97 crores. The estimated employment Generation under sericulture in the country was 8.7 million person during 2020-21 compared 9.4 million person in 2019-20 indicating a reduction of 7.4%.

The demand for superior quality bivoltine silk is increasing in india for domestic consumption as well as value added silk products for the export market.

The ministry of textiles Govt. of India and department sericulture in various state provide technical and financial assistance for enhancing the bivoltine silk production.

Air Pollution:

Air Pollution is defined as the addition of particles, gases and chemicals into the atmosphere that have potential to adversely affect human health, health of animal, vegetation, natural resoureces as well as interferes with environmental processes.

Cause of Air Pollution:

Pollen, spores, volcanic gas, marsh gas and harmful chemicals synthesized by electric storms are natural air pollutants, Anthropogenic sources of air pollution are combustion of natural gas petroleum, coal and wood in industries, automobiles, air crafts, railway, thermal plants etc.

Chemical industries including pesticides, fertilizers, weedicides and fungicides, processing industries like cotton textiles, wheat flour mills asbestos.

Primary Air Pollutants:

Primary Air Pollutants include the directly emitted substancs from identifiable sources like CO_2, CO, NO_X, SO_X, Fluorides, Hydrocarbon and CFC.

Secondary Air Pollutants:

They are produced photochemically from primary air pollutants the enhanced effect of secondary air pollutants is called synergism. Some of the secondary air pollutants and their effects are –

Smog-

It is opaque or dark fog having condensed water vapour, dust, smoke and gases like SO_2, H_2S, NO_2 etc. It produces necrosis in plant asthma and bronchitis in human.

PAN (Peroxyacyl Nitrate):

It is part of photochemical smog and produced due to reaction between nitrogendioxides and hydrocarbons under effect of UV Radiation of sun light.

PAN causes eye irritation, respiratory tract disease in human. Several enzyme are deactivated by PAN. PAN block Hill reaction in Plant and causes injury in several plant like primose, lettuce, tobacco etc.

Ozone:

Being strong oxidant, ozone destroys, chlorenchyma, produces necrosis, harden rubber, damages textiles, corrodes surface of marble statues and heritage buildings, injures mucous membranes, dries throat, causes haemorrhages and eye irritation.

Pollen and microbes:

They are normal constituents of air, in certain season, pollens are produced in excess and cause allergic reaction such as asthma, bronchitis which are collectively called as hay fever. Microbes causes diseases and damage vegetation, food articles etc.

Acid Rain:

The term Acid Rain was coined by Robert August 1872. It is a consequence of air pollution. It includes the rainfall and other form of Precipitation with a PH of less then 5, of narmal rain is 5.6 - 6.5. it is, infact a mixtures of H_2SO_4 and HNO_3. The ratio of the two acids vary depending on the relative quantities of sulphur oxides and Nitogen oxides in the atmosphere. These oxides are mainly produced by combustion of fossil fuels, industries, power plant, automobiles etc.

$$2\,SO_2 + O_2 \rightarrow 2\,SO_3$$

$$SO_3 + H_2O \rightarrow H_2SO_4$$

$$2\,NO + 3(O) \rightarrow N_2O_5$$

$$N_2O_5 + H_2O \rightarrow 2\,HNO_3 \quad \text{(Nitric Acid)}$$

Acids from atmosphere are deposited over earth in two form-

Wet deposition- it occurs in the form of rain, snow or fog.

Dry deposition- it refers to setting down of wind blown acidic gases (Nitigen oxides and sulphuroxides) and other particles in dry state.

Effect of Acid Rain:

Acid Rain damages plants by direct effect on foliage and growing point. Cholrosis, necrosis, defoliation, die-back etc.

Acid rain causes reduced rate of photosynthesis and growth, and increased sensitivity to drought and diseases.

It causes leaching of essential minerals of soil etc.

Effect of Air Pollution on Sericulture:

The special feature of air pollution is that damage to mulberry tree leaves appears as damage to silkworm cocoons.

To exhausted pollutants from factories go from air in to soil and are taken up by the mulberry tree. Healthy silkworm suffer injury in a very short time. Due to feeding this leaves. The atmospheric pollutants play an important role in determining the quality and quantity of natural silk production (Ghosh et.al 2013), and it necessary to know the sources and concentration of pollutants having direct impact on silk production for a quantitative assessment of the impacts.

Silk production depends on healthy cocoons, and if there are produced in an area located close to an oil field, the emissions arising from exploration production and transport of crude oil including controlled flaring and venting of the associated natural gas are most likely to have adverse impacts on the cocoons (Madduri and Reddy- 2003, Rao et.al 2006).

A gradually declining trend in muga production in the oil field area has been reported for quite some time showing the muga worm to be very sensitive to changing environmental condition.

The effects are also extended to the M. bombycina leaves on which the worms feeds. The combined effect of the contaminated environment (air and soil) might have been appreciable affecting the larvae (Ghosh et.al 2013).

The mulberry plant is the important part of the sericulture industry, hence it also affecting through the air pollution like acid rain and other harmful gases, that affect the leaf of the plant. Therefore, the leaves are yellowing and

its tissues are dead, after some time the abscission also occur in the leaf. So creat a problems of food in sericulture industry for silkmoth. Some important gases in air like NO_2, SO_2 and CO_2, that are very harmful in air and cause pollution. They move in to atmosphere and form harmful acid and return as a acid rain and affect the vegetation.

Conclusion

In this chapter, described the history of silk, about sericulture indurtry briefly and status of silk production in India and impact of air pollution on this indurtry and impact on silkmoth.

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Acute toxicity of the ethanolic leaf extract of *Euphorbia thymofilia*

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Abstract

Aim: *Euphorbia thymifolia* L. is one of the vital herbal plants in the *Euphorbiaceae* family. The leaves, seeds and fresh juice of whole plant are used on bone dislocation of animals, snake bite, ring worm and laxative for children and many more diseases therapeutically. The present study aims to determine the toxicity profile of the ethanolic extract of *E. thymifolia*. **Materials and Methods:** The acute toxicity study of the ethanolic extract of *E. thymifolia* leaves are carried out by using the well defined methods. In the present study, the single dose (150, 300, 600, 1200 and 2500 mg/kg body weight) of plant extract is treated to the adult rats. After 24 hours the physical activity and some biochemical parameters such as aspartate aminotransferase, alanine aminetransferase, alkaline phosphatase, urea and creatinine levels are measured. **Results:** Some physical changes and mortality was identified in the higher comcentration of dose of 2500 mg/kg body weight. The levels of biochemical parameters are also changed in the higher doses. Based on these results, we predict that the dose of upto 1200 mg/kg b.w is does not cause any adverse effect in the experimental rats. **Conclusion**: The results of this study confirm that the ethanolic extract of *E. thymofilia* leaves is safer upto 1200 mg/kg b.w is the effective dose.

Keywords: *Euphorbia thymofilia*, acute toxicity, lethal dose, ethanolic extract.

Introduction

Plants are considered not only as dietary supplement to living organism but also traditionally used for treating many health problems. Medicinal values of many plants still remain unexplored investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents (Anushia *et al*., 2009; Murugan *et al*., 2013; Albino Wins *et al*., 2013). The antioxidant compounds from medicinal plants can prevent free radical mediated oxidative damage to cellular components (Amat *et al*., 2010). There are many medicinal plants possess hepatoprotective properties by improving the antioxidant components (Kamisan *et al*., 2014), they are important in reducing the prevalence of diverse liver disorders and other oxidative stress related diseases (Vladimir-Knežević *et al*., 2015). Atmaca *et al*. (2011) showed that hepatoprotective effect of coumarins against oxidative stress and liver damage induced by CCl_4 in male rats. In addition, role in neutralizing free radical, quenching singlet and triplet oxygen, decomposing peroxides, stabilizing lipid peroxidation and protecting the cells against oxidative damage (Mossa *et al*., 2015).

Medicinal herbs and extracts prepared from the plants are widely used in the treatment of the liver disease like hepatitis, cirrhosis and loss of appetite. Many plants have been used for the development of new therapeutic agents for treating the liver disease (Recknagel, 1983; Murugan *et al*., 2013).

The expanding use of plant extricates in food, cosmetic agents and pharmaceutical industry demands poisonous quality appraisal. No medication is utilized clinically without its clinical trials and poisonous quality examinations (Anisuzzaman *et al*., 2001). The determining of the acute toxicity of the plant extract is important for its safe, it empowers the meaning of the natural harmfulness of the plant and the impacts of intense overdose is identified by the different factors in natural conditions (Parra *et al*., 2001). The present study aims to determine the toxicity profile of the ethanolic leaf extract of *E. thymifolia*.

Materials and Methods

Collection and authentication of plant materials

The fresh leaves of *E. thymofilia* are collected from Kanchipuram district, Tamilnadu. The species were identified and a voucher specimen was deposited at the Rapinet Herbarium (GDMM 001) St. Joseph's College, Tiruchirappalli, Tamilnadu. The shade dried *E. thymofilia* leaves are powdered

mechanically and stored in a container. The extraction is carried out by the successive solvent extraction method using a Soxhlet apparatus.

Materials: The samples used in the present investigation are the leaves of *E. thymofilia.* The solvent is ethanol and extraction is done using soxhlet apparatus.

Procedure: The powdered leaf is taken and subjected to successive solvent extraction. The extraction is carried out for 18 hours with the ethanol as a solvent with a ratio 1:4 w/v. The filtered extracts were evaporated on a water bath and finally dried in vaccum.The extracts were preserved in refrigerator till further use.

Acute Toxicity Study

Acute oral toxicity (Ecobichon, 1997) study is performed as per Organisation for Economic Co-operation and Development (OECD)-423 guideline (Acute toxic class method). Healthy male albino rats are selected by random sampling technique and divided into 6 groups of 4 animals in each group. The animals are kept fasting for overnight providing only water, after which the extract of *E. thymofilia* leaves are administered orally in an increasing dose (150, 300, 600, 1200 and 2500 mg/kg b.w) by intra gastric tube for determining the safe dose of up and down staircase method (Ghosh, 1984). The animals are observed continuously for 1 hour, then frequently for 4 hours and later at the end of 24 h for general behavioral and autonomic profile. If mortality is observed in 2-3 animals, then the dose administered is assigned as toxic dose. If mortality is observed in one animal, then the same dose is repeated again to confirm the toxic dose.

Animal grouping

Animal groups were categorized as control (basal diet, G1) were given saline water (2 ml/kg b.w), G1 supplemented with 150, 300, 600, 1200 and 2500 mg/kg of ethanol extract of *E. thymifolia* are categorized as G2, G3, G4, G5 and G6 respectively. All the chemicals and reagents used were of higher analytical grade required for biochemical assays were obtained from Sigma-Aldrich, Banglore, India.

Hepatic and renal parameters

After 24 hours the samples are collected the aspartate transferase (AST) (Reitman and Frankel, 1957), alanine transferase (ALT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Kind and King’s method, 1954), urea

(Fawcett and Scott, 1960) and creatinine (Jaffe, 1986) levels are measured and the physical activity and hair fall rate are identified.

Statistical analysis

All data obtained in the biochemical parameters were measured for statistical significance using one-way analysis of variance by Duncan's Multiple Range Test (SPSS® for Windows,V.17.0, Chicago, USA) and table values presented as mean and standard deviation. A probability value of $p \leq 0.05$ was considered to be statistically significant in all test.

Result and Discussion

The physical activity and hair fall is noted in the rats treated with the various concentrations of *E. thymofilia* leaves (**Table 1**). The results proves the dose ranges from 150 to 1200 mg/kg b.w does not cause any toxic effect in the rats, but 2500 mg/kg b.w of the leaves extract cause some alteration in the experimental rats physical activity. Based on the results, the concentration of 1200 mg/kg b.w is taken as a safer dose.

Table 1: Physical activity Hair fall in the rats treated with various concentration of *E. thymofilia* leaves

Groups	Dose (mg/kg b.w)	Physical activity	Hair fall seen	Death rate
Group I	Control	No change	No	Nil
Group II	150	No change	No	Nil
Group III	300	No change	No	Nil
Group IV	600	No change	No	Nil
Group V	1200	No change	No	Nil
Group VI	2500	Lower activity	Hair fall seen	Nil

Table 2 shows the results of the liver parameters such as AST, ALT and ALP are seen in the rats treated with various concentrations of *E. thymofilia* leaves. This result clearly proves that the dose of 150 mg/kg, 300 mg/kg, 600 mg/kg and 1200 mg/kg of plant extracts does not cause any adverse effect on the rats, but 2500 mg/kg of leaves extracts causes the alterations in biochemical parameters and it elevates the levels of AST, ALT and ALP. Based on the results, the concentration of 2500 mg/kg considered as overdose and up to 1200 mg/kg is taken as a safer dose.

Table 2: Liver and Kidney parameter levels in the various concentration of *E. thymofilia* leaves treated rats

Particulars	AST (IU/L)	ALT (IU/L)	ALP IU/L)	Urea (mg/dl)	Creatinine (mg/dl)
Group I	31.38 ± 1.08[a]	24.49 ± 1.01[a]	101.64 ± 2.12[a]	29.1 ± 0.87[a]	0.7 ± 0.01[a]
Group II	32.12 ± 1.10[a]	25.10 ± 1.68[a]	102.19 ± 2.68[a]	30.1± 0.91[a]	0.7 ± 0.02[a]
Group III	32.84± 1.24[a]	25.39 ± 1.71[a]	103.74 ± 2.61[a]	30.8 ± 0.13[a]	0.8 ± 0.01[a]
Group IV	33.14 ± 1.29[a]	26.12 ± 1.09[a]	104.49 ± 1.98[a]	31.0 ± 0.17[a]	0.8 ± 0.09[a]
Group V	33.71 ± 1.23[a]	26.71± 1.91[a]	105.56 ± 1.61[a]	31.3 ± 0.64[a]	0.9 ± 0.11[a]
Group VI	58.14 ± 2.31[b]	41.71 ± 1.37[b]	148.14 ± 2.19[b]	51.5 ± 0.26[b]	1.6 ± 0.26[b]

Values are expressed as means ± SD for four rats in each group
Values not sharing a common marking (a,b,c……) differ significantly at $p \leq 0.05$ (DMRT)

Toxicology is important to find the safer drug. It is used to determine the side effect nature of the physical agents or chemical agents on the biological system. The number of preclinical study was carried out to find the safety of the drug to the human beings. In human beings, all the detoxification reactions are takes place in the liver and the waste products from the toxicological reactions are eliminated through kidney. In the damage of liver it significantly alters the some biochemical enzymes level which are synthesized from liver and these enzymes may alters the various metabolic reactions in our body. The kidney is a vital organ in our body, it eliminates the metabolic wastes from our body and it has a chance to damage easily by the action of toxins. Therefore, the present study the kidney functions are measure by the urea and creatinine elimination level. Urea and creatinine concentrations are often used as an index of renal glomerular function and will be increased in renal injuries (Moshi *et al.*, 2001; Hughes and Jefferson, 2008).

Because of this the liver and kidney parameters are used to determine the toxicology of the drugs. If there are no alterations are noticed in the liver and kidney parameters, the drug is safer to use. In the present study, the dose up to 1200 mg/kg b.w. dose not cause any significant changes in the liver and kidney parameters, Hence, this may consider as safer dose **(Table 2)**.

Conclusion

The non toxic nature of ethanolic extract prepared from *E. thymofilia* was confirmed by acute oral toxicity test conducted as per the OECD guide lines. The normal behavior of animals during the observation, it suggests the safety and harmless nature of ethanolic extract even up to 1200 mg/kg b.w of experimental rats. Further studies are warranted including sub acute and chronic toxicological evaluations to confirm the safety of this plant extract.

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Total protein, glycogen and cholesterol content in the ovary and liver during prespawning and spawning season in *Mystus vittatus* (Bloch)

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Abstract

The present study has been undertaken to correlate the seasonal variations in biochemical composition of protein, glycogen and cholesterol content of the ovary and liver to its reproductive seasons in a freshwater catfish *Mystus vittatus.*The study on the salinity has been undertaken to find out its effect on the fish protein, glycogen and cholesterol on the ovary and liver. The Gonadosomatic index of the ovary increased during the prespawning season (April, May and June) and spawning season (July, August and September) and Hepatosomatic index of the liver decreased during prespawning season (April, May and June) and spawning season (July, August and September). The protein, glycogen and cholesterol content of the ovary increased and in liver it decreased during the prespawning and spawning season. In fishes subjected to 20% salinity, the Gonadosomatic and Hepatosomatic index of the ovary and liver and the value of the total protein, glycogen and cholesterol content of the ovary and liver increased when compared to that of the controls during the prespawning and spawning season.

Keywords: Gonadosomatic index, Hepatosomatic index, protein, glycogen, cholesterol, prespawning and spawning.

Introduction

Biochemical studies on fish tissues such as ovary and liver have drawn the attention of several researchers, because tissues are the major source of protein, carbohydrate and lipid and have a high calorific value (Joshi *et al*., 1979). Biochemical changes may occur due to the various physiological factors such as maturation and spawning. Biochemical composition of the fish is subjected to seasonal variations were reported in *Sillago sihama* (Shamsan and Ansari, 2010).

Total protein content was reported by Minimary (1996) in tissues such as gonads and liver of *Garra Gotvla gotvla, G.annandali and G.lissorhynchus.* The inter relationship between the liver and gonads were also reported in the fish *Cyprinus carpio* (Sivakami et al., 1986).Seasonal variations in the carbohydrate contents of ovary and liver in relation to the Gonadosomatic index were studied in *Clarias batrachus* by Sinha and Pal 1990. Seasonal changes and annual variation in the protein, glycogen and cholesterol content in the ovary in relation to reproductive cycle were reported in *Mugil cephalus.* (Das, 1978).

Salinity is one of the most abiotic factor in aquaculture and its optimal levels are species specific for growth, survival and production efficiency (Ruscoe et al., 2004).The effect of salinity on gonadal maturation were reported in *Mystus montanus* (Arockiaraj and Haniffa, 2001).The effect of salinity on the larval rearing of freshwater species were reported in *Piaractus mesopatamicus* (Rosangele *et al*., 2012).

Not much work has been done on the freshwater catfish, *Mystus vittatus* (Bloch), a commercial food fish of Karnataka. Hence the present studies were undertaken on the seasonal variation and effect of salinity on the ovary and liver during the prespawning and spawning season. This attempt has been done to find out whether the biochemical constituents i.e. the total protein, glycogen and cholesterol content of the ovary and liver at different seasons could be related to the reproductive cycle of the catfish *Mystus vittatus* (Bloch).

Materials and Methods

Live female fishes of *Mystus vittatus* were collected monthly from April to June 2010 during the pre pawning season and during the spawning season July to September from Ramanayakkan lake in Hosur, 35 kms from Bangalore city situated at an latitude of 12º43'0"N and Longitude 77º49'0"E with an altitude of 878mts. They were brought to the laboratory in plastic containers. They were acclimatized to the laboratory conditions and to the natural photoperiod and temperature of 25º ± 3°C. The fish were fed daily ´adlibitum´ with commercial fish feed. The water in the aquaria was changed every alternate day. The fishes were individually weighed and their body weights were recorded. The ovary and liver were removed and the Gonadosomatic and Hepatosomatic index were determined by using the formula.

$$\textbf{GSI} = \frac{\text{Wet weight of the ovary}}{\text{Live body weight of the fish}} \times 100$$

$$\text{HSI} = \frac{\text{Wet weight of the liver}}{\text{Live body weight of the fish}} \times 100$$

The ovary and liver were estimated for the total protein, glycogen and cholesterol content by using the standard methods mentioned below.

Effect of 20% salinity on the ovary and liver of *Mystus vittatus.*

Live female fishes of *Mystus vittatus* were subjected to 20% salinity for 30 days. On the 31 st day the fishes were autopsied, the ovary and liver were removed; the GSI and HSI were calculated as mentioned above. The fishes were grouped into 2 groups viz., Control and Experimental group. The ovary and liver were analysed for the total protein, glycogen and cholesterol content by using the following standard methods.

- Estimation of total protein content - by Lowry *et al.* Method (1951)
- Estimation of total glycogen content- by Dubois *et al.* Method (1954)
- Estimation of total cholesterol content- by Schoennerimer Sperry and Webb Method (1950)

The values are expressed as mg/gm wet weight of the ovary and liver.

Results and Discussion

During the prespawning season April, May and June in *Mystus vittatus,* there was an increase in the Gonadosomatic index of the ovary and a decrease in the Hepatosomatic index of the liver. In the prespawning season April, May and June, the protein content of the ovary increased and in liver the protein content decreased .The glycogen content of the ovary increased and in liver the glycogen content decreased. The cholesterol content of the ovary increased and in liver the cholesterol content decreased during the prespawning season April, May and June. In the spawning season July, August and September there was marked increase in the Gonadosomatic index of the ovary and decrease in the Hepatosomatic index of the liver. During the spawning season July, August and September, the protein, glycogen and cholesterol content of the ovary increased and in liver the cholesterol content decreased.

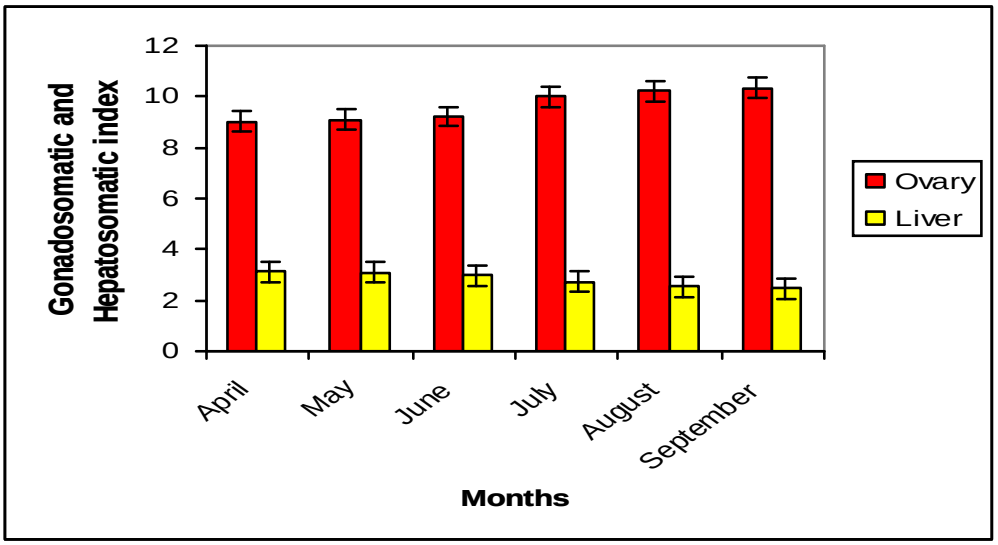

Fig: 1 Seasonal variation in the Gonadosomatic and Hepatosomatic index of the ovary and liver during the pre-spawning and spawning season in *Mystus vittatus* (Bloch). Values represents Mean±S.E.

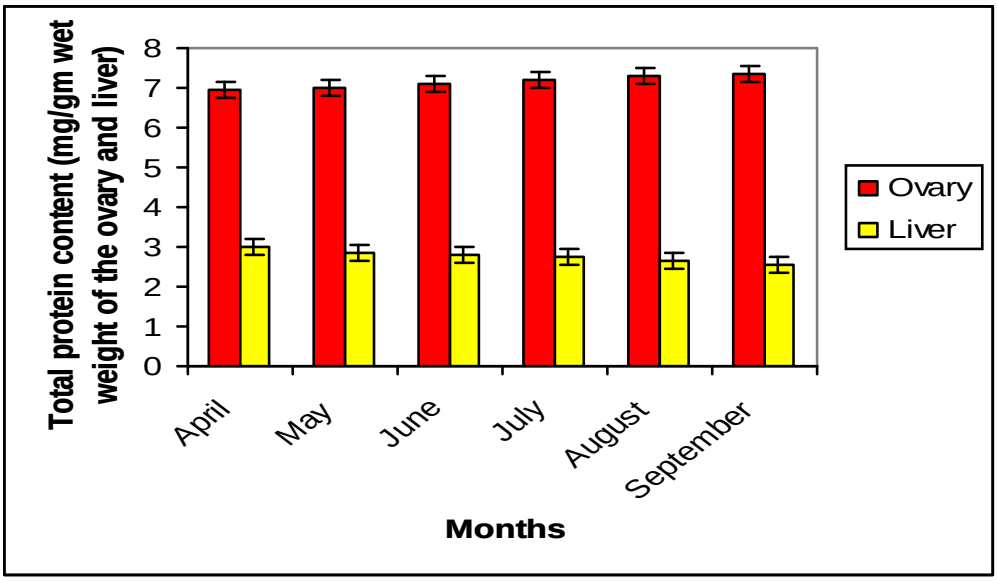

Fig: 2 Seasonal variation in the total protein content of the ovary and liver during the pre- spawning and spawning season in *Mystus vittatus* (Bloch).Values represent Mean±S.E.

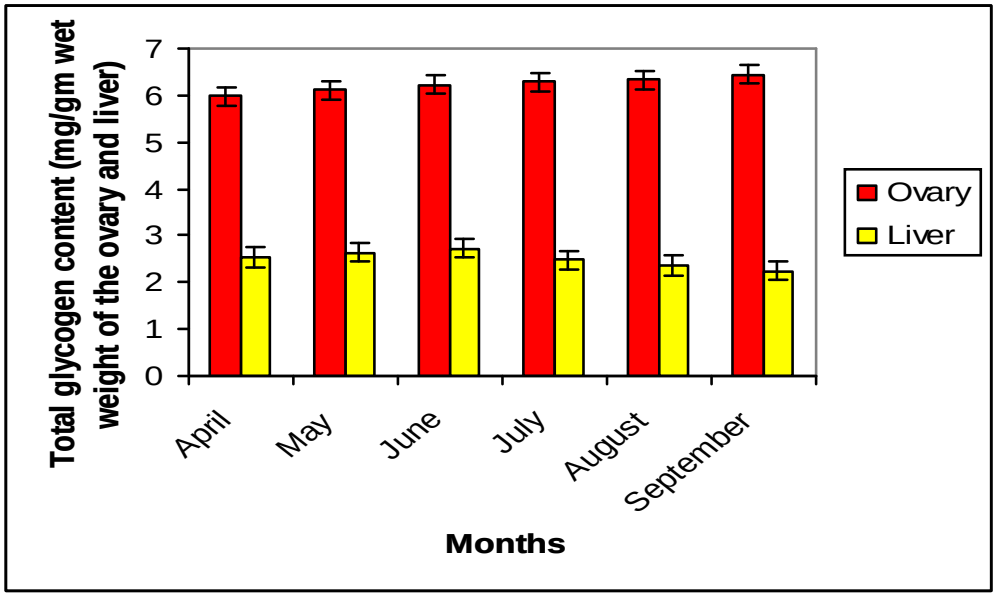

Fig:3 Seasonal variation in the total glycogen content of the ovary and liver during the pre- spawning and spawning season in *Mystus vittatus* (Bloch).Values represent Mean±S.E.

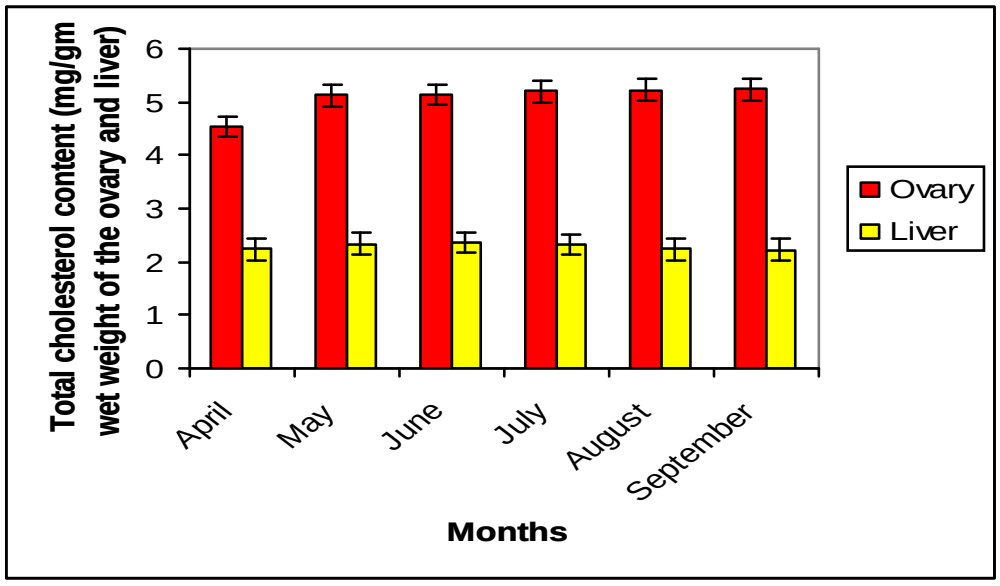

Fig: 4 Seasonal variations in the total cholesterol content of the ovary and liver during the pre-spawning and spawning season in *Mystus vittatus* (Bloch).Values represent Mean±S.E.

It was evident that in the fish subjected to 20% salinity during the prespawning season April, May and June 2010 and spawning season July, August and September, the Gonadosomatic and Hepatosomatic index of the ovary and liver increased when compared to that of the controls in *Mystus vittatus*. The protein, glycogen and cholesterol content of the ovary and liver during the prespawning and spawning season increased in the fish subjected to 20‰ salinity when compared to that of the controls.

The increase in the Gonadosomatic index and the decrease in the Hepatosomatic index during the prespawning and spawning season in *Mystus vittatus* are due to the active somatic energy accumulation in ovary and somatic energy depletion in the liver. Similar results were reported in *Barbus scalateri* (Encina and Lorencio, 1997) and in *Mystus montanus* (Arockiaraj et al., 2004).

The highest protein content of the ovary in the prespawning season in *Mystus vittatus* is due to the change in the endocrine system that monitors supply of nutrients to gonads from all parts of the body including liver. Similar results were reported by Sinha and Pal (1990) in *Clarias batrachus.*

Liver is the main tissue that regulates the glucose supply to cover the increased needs in the female fish. This account for its highest glycogen content in the ovary during the preparatory phase (Prespawning season).Similar results were reported in *Oreochromis mossambuicus* (Soengas et al., 1993).

The ovarian cholesterol increased and hepatic cholesterol decreased which is needed for ovarian growth and vitellogenesis during spawning season. The Similar findings were reported in the females of the pearl spot *Etroplus suratensis*.

The increase in the Gonadosomatic index indicated maturation and hypertrophy of oocytes in salinity as reported in *Mystus montanus*.(Arockiaraj et al., 2001) and grey mullets (Brusle,1981). From these results it is inferred that the salinity has a marked effect on the ovarian and liver maturity. Salinity induces maturation of gonads. Similar findings have been reported on the influence of water salinity on the fish development, growth and reproduction (Bouef and Payan, 2001).

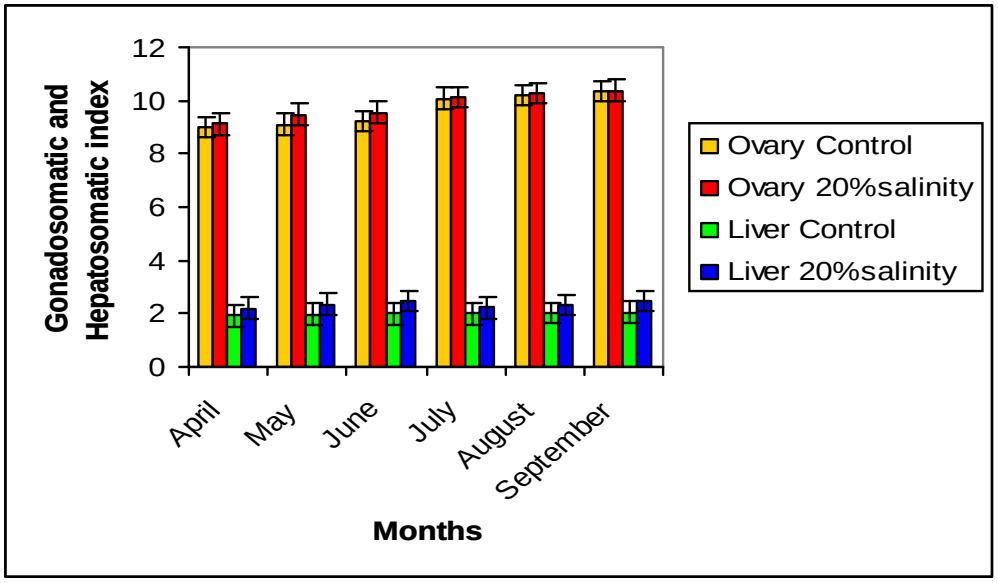

Fig:5 Effect of 20% salinity in the Gonadosomatic and Hepatosomatic index of the ovary and liver during the pre-spawning and spawning season in *Mystus vittatus* (Bloch).Values represent Mean±S.E.

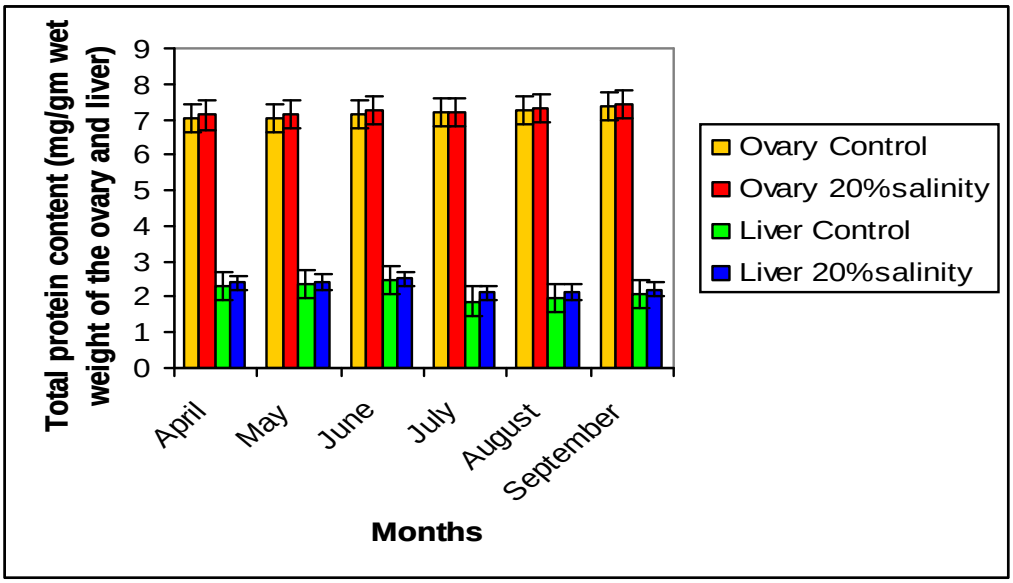

Fig:6 Effect of 20% salinity in the total protein content of the ovary and liver during the pre-spawning and spawning season in *Mystus vittatus* (Bloch).Values represent Mean±S.E.

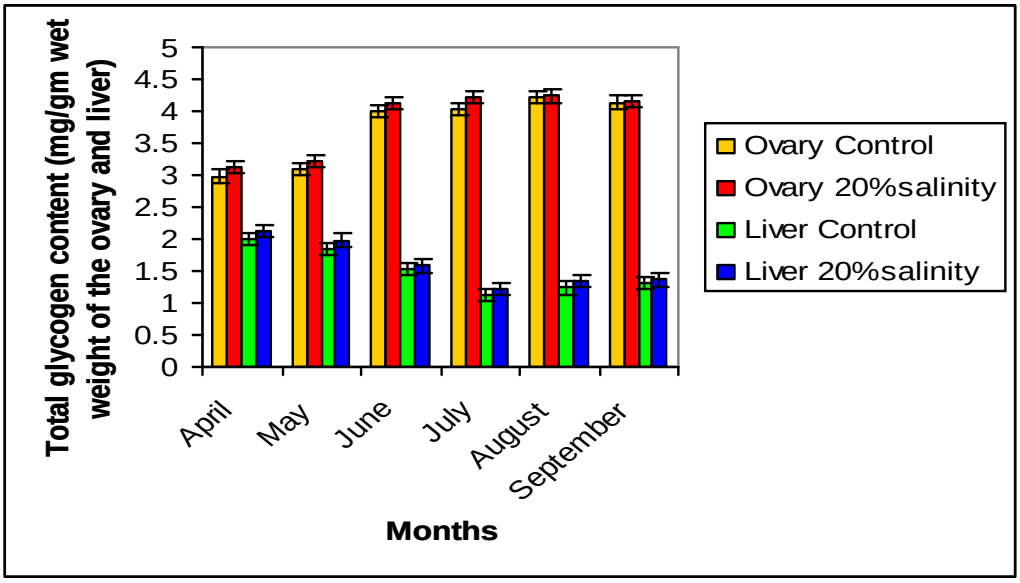

Fig:7 Effect of 20% salinity in the total glycogen content of the ovary and liver during the pre spawning and spawning season in *Mystus vittatus* (Bloch).Values represent Mean±S.E.

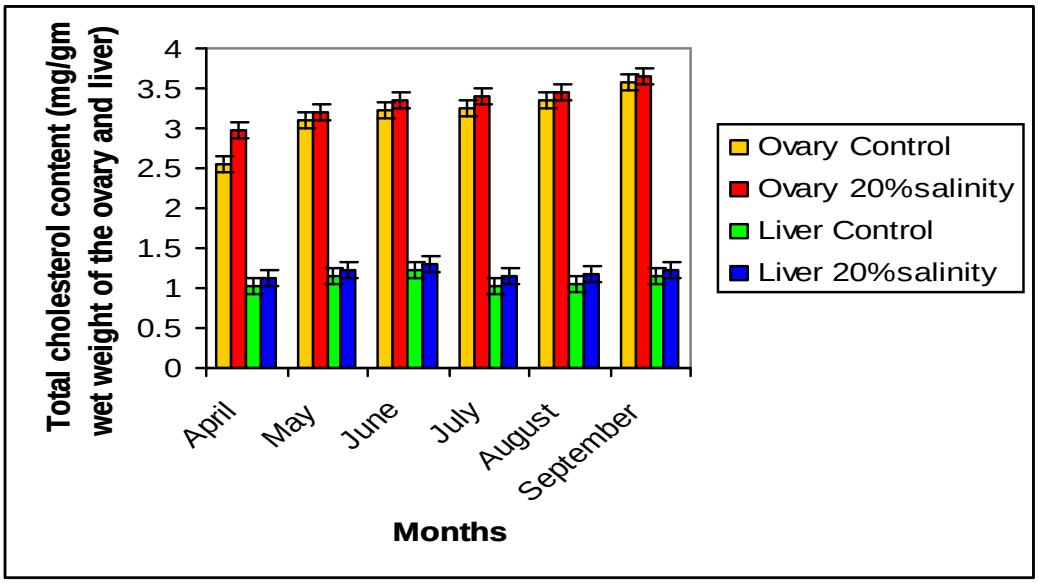

Fig:8 Effect of 20% salinity in the total cholesterol content of the ovary and liver during the pre-spawning and spawning season in *Mystus vittatus* (Bloch).Values represent Mean±S.E.

Conclusion

The GSI and HSI is particularly helpful in identifying days and seasons of spawning, as the ovary of gravid fish swiftly increase in size just prior to breeding season. This information can be helpful for better management of freshwater fish capture in breeding season to conserve the diversity of the fish. Liver is the sensitive tissue where several metabolism takes place during reproduction. Knowledge of biochemical composition of liver helps to elucidate seasonal changes in the contents of liver, found in several fish species which is related to the growth of gonads and other processes associated with spawning. Studies on effect of salinity on the gonads and liver leads to a clear understanding of the reproductive rhythms and biochemical variations in the ovary and liver of *Mystus vittatus* (Bloch), thereby providing the information for the selection process of hatchery for the artificial propagation of this fish.

From the present investigation, exposure of the fish *Mystus vittatus* to salinity effects growth and biochemical composition of tissues. The impact of higher salinity on different freshwater species across the life stages would throw more light on the extent of vulnerability of different fish to the situations linked to climate change and provide necessary guidelines in the selection of suitable fishes

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The challenges of education in rural areas of district Balrampur (U.P), India

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Introduction

The contribution of education to social and economic advancement is well acknowledged. For people to take advantage of new possibilities that support economic growth, access to education is essential. Given this generally acknowledged truth, since the nation gained its freedom, education has received the majority of attention. But providing high-quality education in rural India has proven to be one of the biggest obstacles for the administration. India believed that the greatest method to advance social change is via education.

Soon after gaining independence in 1947, making education available to all has become a priority for the government. The education sector has received considerableattention in the budget for 2011-2012, which has broadcasted a significant increase of 24 percent in the total allocation for the education sector. The operational reforms have been reviewed to implement the right of children to free and compulsory education, which has come into effect on 1 April 2010 (Rural Education, 2011).

A few things are affecting how well the educational system is doing in rural areas. For kids and families in rural regions, there is a rise in demand for excellent pre-primary education. They are aware of the circumstances in the country's developing regions, which is the key factor in their recognition of the value of education and their indicated readiness to provide for their children's education. It is necessary for the educational system in rural regions to corresponding with that in metropolitan ones. In remote regions, schools and training facilities have been established, raising the level of living for the local populace.

These standards have enabled the rural communities to be at par with the urban communities. Allocations to achieve the objectives of the Right to Education, which has been aligned with the Sarva Shiksha Abhiyan, increased from Rs 15,000 crore to Rs 21,000 crore. It targeted at strengthening elementary education. The centre is now focusing on the vocationalisation of secondary education, which will enable students to pursue job-oriented courses at the plus two levels (Rural Education, 2011).

Objectives of Rural Education

The objectives of rural education have been stated as follows: (Sreekanthachari, & Nagaraja, 2013).

1. To Provide Free Standard Education to Children belonging to rural communities.
2. The children should be supported for higher education.
3. Guiding and supporting research scholars in educational development.
4. Implementation of new teaching methodologies and the system of assessment.
5. Promoting a stress-free and amiable atmosphere in all schools.

Scenario of Rural India's Education Sector

In the present existence, the main aspects that highlight the scenario of India's education sector have been specified in the following points:96.5% of children within the age group of six to 14 age group in rural India are enrolled in schools; 71.1% of these children are enrolled in government schools, 24.3% are enrolled in private schools; girls within the age group of 11 to 14, who are still out of school have dropped from 6.8% in 2009 to 5.9% in2010; in the states of Rajasthan, it is 12.1% and Uttar Pradesh, it is 9.7%. The enrolment in private schools in rural India increased from 21.8% in 2009 to 24.3% in 2010. There was an increase in the percentage of five-year-olds enrolled in schools from 54.6% in 2009 to 62.8% in 2010. The largest increase was visible in Karnataka, where the proportion of five-year-oldsenrolled in school increased from 17.1% in 2009 to 67.6% in 2010(Rural Education, 2011).

The changes in the reading levels of students were not much recognized. Only 53.4% of children in class V can read a class II level text. This suggests that even after five years in school, close to half of the students are not even at the level, expected of them after two years in school. On average, there has been a decrease in their numerical abilities. The proportion of class Istudents, who could recognize numbers from one to nine,decreased

from 69.3% in 2009 to 65.8% in 2010. Similarly, the proportion of students in class III, who could solve two-digit subtraction problems decreased from 39% to 36.5% in the same period. Students in class V, who could perform simple division problems also dropped from 38% in 2009 to 35.9% in 2010. Improvements are required to be made in infrastructure and also there is a need to establish more classrooms (Rural Education, 2011).

Differentiation between Urban and the Rural System of Education

The differences in the urban and rural systems of education have been stated with regard to the following aspects: (Sreekanthachari, &Nagaraja, 2013).

Number of Schools – There are many schools, colleges, universities, and other training facilities in metropolitan regions. On the other side, there are fewer schools in rural regions. Individuals from rural villages must move to metropolitan regions to attend higher education since there are no higher education institutions in rural areas. Aiming to improve people's skills and knowledge in a variety of fields, including technology, agriculture, farming methods, handicrafts, child development, health care, and so on, training centres have recently been established in rural areas..

Transportation Facilities – The pupils have access to suitable transit options in metropolitan regions. The children are provided with bus transportation by the schools, and after school hours, they are picked up and dropped off at bus stations that are closer to their houses. Since there are transportation options available, kids can commute to school more easily. The only options available to kids in remote locations are to walk or use their own vehicles to get to school. Bicycles are typically used in situations when walking to school is impractical due to distance.

Provision of Civic Amenities and Facilities – Civic amenities and facilities are offered in urban schools, which are crucial for enabling instruction. These include the accessibility of bathrooms, potable water, classrooms, infrastructure, furniture, tools, and technology. These factors have made a big difference in the way education is provided. On the other hand, the same conveniences and services are not present in rural schools. This is having detrimental repercussions on the pursuit of education. The government has created laws and rules, and actions are being taken to provide remote schools with lavatories, midday meals, technology, infrastructure, machinery, and equipment, as well as adequate classrooms.

Level of Education – Urban schools provide an elevated level of instruction. In addition to academic ideas, students participate in a variety of assignments and activities. These include extracurricular and creative pursuits including athletics, physical exercise, dancing, music, singing, visual arts, handicrafts, and other similar pursuits. Additionally, groups for picnics, contests, and events exist that provide students with the chance to showcase their abilities and advance their knowledge. However, in rural schools, the standard of instruction is low and the availability of extracurricular activities is not in a well-developed condition.

Computer Education– In the present existence, technology has gained prominence. The use of computers has facilitated the acquisition of education to a major extent. In urban areas, schools are providing computer education to the students and also making use of technology in the implementation of tasks and functions. Students make use of the internet to a major extent to acquire knowledge in terms of various areas and also when they are required to prepare their assignments and projects. In rural schools, there are few schools that provide computer education to the students. Whereas in most cases, schools do not provide computer education.

Organization of Group Classes – In urban schools, there are organizations of group classes. Through these classes, students interact with each other, share ideas and perspectives and are able to enhance their knowledge. In other words, group discussions are encouraged. In some cases, they even work on projects and assignments jointly. Group classes enable the students to provide solutions to problems and difficulties. On the other hand, in schools in rural areas, there are not any organization of group classes. The students do interact and discuss their matters and concerns with the teachers and fellow students to provide solutions to their problems, but group classes do not take place.

Organization of Picnics- In urban schools, picnics are scheduled primarily with the goal of energising students' mindsets and inspiring them to learn. When picnics are planned, a variety of locations are considered, including parks, museums, historical sites, and places of worship. Picnics are often something that students relish and appreciate. On the other hand, there is no picnic planning in rural schools. Most of the information is given to the pupils in a classroom setting. To put it another way, classroom instruction is promoted. In some circumstances, kids are inspired to study, whereas, in other circumstances, they lose interest and even stop attending school.

Use of Technology – In urban schools, teachers make use of technology such as laptops, computers and printers with the main purpose of providing notes and other learning materials to the students. The use of technology has not only facilitated learning among students, but teachers have also been able to make the teaching processes manageable through the utilization of technology. One of the major benefits of technology is, that it provides extensive knowledge and information in terms of various areas. Whereas, in rural schools, the teachers do not make use of technology and explain the lesson plans to the students by reading from textbooks.

Infrastructure – The provision of infrastructure in urban schools is in a well-developed state as compared to schools in rural areas. Within the classrooms, there are proper seating arrangements, desks, technology and other heating and cooling equipment, which is utilised in accordance with the weather conditions. Whereas, in schools in rural areas, the students are made to sit on the floor and there isno provision of proper infrastructure or furniture or equipment. To facilitate the acquisition of education, it is necessary to make provision proper infrastructure in schools. Lack of infrastructure may impede the learning abilities of the students.

Teachers – The teachers recruited in urban schools are well-qualified and experienced. They are recruited by the school authorities, taking into consideration certain aspects. They are well aware of the performance of their job duties and render an effectual contribution in leading to the growth and development of the students. On the other hand, in schools in rural areas, the teachers usually lack the skills and abilities, they are unaware in terms of the performance of their job duties and do not have much concern regarding effective growth and development of the students. On the whole, teachers in rural schools are not much satisfied with their jobs.

Measures to make Improvements in Rural Education

The measures to bring about improvements in rural education have been stated as follows:

Availability of Infrastructure and Facilities – To promote education among the students in rural schools in a well-organized manner, there is a need to make provision adequate infrastructure and facilities. These mainly include, furniture, proper seating arrangements, blackboards, chalks, heating and cooling equipment, clean drinking water, restrooms, and the overall construction of classrooms and school buildings should have enough room to accommodate the students. When all these facilities would be adequately

available, then the students would be able to concentrate well ontheir studies. The overall classroom and school environmental conditions would get improved due to the availability of infrastructure and facilities.

Provision of Transportation Facilities – In rural areas, schools are mostly located at a distance and students are required to walk miles. Walking mostly is tedious for the students, hence, due to this, they lack interest in studies and do not take pleasure in coming to school. Therefore, when schools make provision transportation facilities for the students, then they would be able to manage their coming and returning from school. It is essential to bring about improvements in roads in rural areas and transportation facilities for the students so that they are able to manage their transfer to schools and back home in an appropriate manner.

ICT-based Education for Students – Information and Communication Technology-based education for students within rural areas would assist in the acquisition of education to a major extent. The students are provided with computer skills, such as Word, PowerPoint, Excel, Access, C++, and other software packages. In the initial stage, students find it hard and feel apprehensive, but within the course of time, they are able to develop efficiency. Generation of efficiency in ICT-based education requires practice. It has also become one of the sources for playing games for students (Roy, 2012). Therefore, ICT-based education has become indispensable for not only improving their skills and abilities but also in making provision recreational activities. In nursery schools, students are shown movies and plays on computers.

Recruitment of Teachers – The recruitment of teachers in rural schools should be done, taking into consideration certain factors, these include, educational qualifications, experience, skills, attitudes and overall personality traits. Teachers are the ones, who render an indispensable contribution in leading to effective growth and development of the students. They make use of effective teaching and learning methods so that they are able to impart the necessary knowledge to the students and generate awareness among them. The other areas that not only facilitate recruitment but also retention of teachers are, the school and the classroom environment should be created in such a manner that teachers should feel satisfied with their jobs; they need to form effectual terms and relationships with their colleagues and other members of the educational institutions; they need to possess proper knowledge and skills in terms of their job duties and need to feel satisfied with the pay and incentives.

Dealing with Students – Teachers must interact with the children in a respectful and pleasant manner. They should develop a thorough grasp of their needs and requirements and then apply the teaching-learning processes and instructional techniques in accordance with their needs and requirements. According to research, teachers harshly discipline pupils who fail to grasp concepts when they do not do their homework or class assignments, perform poorly on tests, or act up in front of the class. As a result, the pupils get uneasy and may even quit school. Therefore, teachers should treat students with respect and kindness and assist them in achieving their academic objectives..

Teaching-Learning Processes – The principals, and the teachers need to work in collaboration and integration with each other and make sure that the teaching-learning processes should be well-organized and adequately implemented. The teaching and the learning processes that are mostly used in rural schools are, the teachers provide explanations of the lesson plans through textbooks. They read the lesson plans and provide explanations to the students, either verbally or in writing on the blackboard. The students are required to bring notebooks and pencils and they take down notes, while the teacher is teaching. It is vital on the part of the students to understand the concepts, so they are able to score good grades on tests and exams.

Promoting Teamwork – Working in teams or groups has proven to be beneficial to students at all levels of education. This is particularly useful when students are working on difficult assignments or are understanding complicated concepts, such as ICT or mathematics. In rural schools, the teachers need to give assignments and projects to the students, so that they are able to work in teams. Team-work enables one to form friendly and effective communication terms. The students are able to bring about solutions to their academic problems and difficulties, they understand each other's viewpoints and perspectives and are able to improve their academic performance.

Promoting Extra-curricular Activities – Extra-curricular activities are important in schools. They stimulate the students and arouse interest and enthusiasm among them towards learning. In some cases, when students do not perform well academically, they excel their skills and abilities in extra-curricular activities. For instance, there are individuals, who depict interest in various sports activities, such as tennis, badminton, wrestling, boxing, swimming etc. Other individuals enhance their skills and abilities in dancing, singing and playing musical instruments. Other extra-curricular activities include artworks and handicrafts. Within rural communities, individuals in some cases, produce handicrafts and artworks in order to meet their livelihoods

requirements. It is essential to promote these activities in schools in order to enrich the education system.

Social and Economic Change – The use of ICT should be implemented in such a manner that it should promote social and economic change. The social and economic change should be brought about within the rural society as well as the rural system of education. The ICT focuses on the use of computer technology. The major change that can influence the overall system of education is, that students should be taught how to make use of technology within the learning methods. They should be taught how to prepare assignments and projects using technology, instead of making use of notebooks, paper and pens. The ICT-based e-learning system contributes a vital role in improving online education in bringing about social and economic change within rural society (Roy, 2012).

Teaching-Learning Materials – One of the elements that is crucial to enabling education is having high-quality teaching and learning resources. These must be in line with the demands of the pupils. In certain circumstances, class V students are unable to read class II textbooks, indicating that neither the methods nor the teaching-learning materials have been created effectively. Therefore, in order for children to get the necessary comprehension, teachers must provide them with practice activities and worksheets. The teachers must utilise handouts, worksheets, notes, and other resources in addition to textbooks. They must also administer tests at the conclusion of each lesson plan.

Fundamental Principles of the High-Quality Rural Education Program

The fundamental principles of the high-quality rural education program have been stated as follows:

Equal Opportunities to All – It should be assumed that all pupils in rural areas are capable of receiving an education. There should be no discrimination based on caste, creed, colour, religion, gender, ethnicity, occupation, or socioeconomic background in schools, and everyone should have access to equal opportunities. Everyone should be given the possibility and opportunity to develop their skills and talents and strive toward the fulfilment of academic goals whenever events, contests, or other activities are organised inside schools. Apart from the availability of equal opportunities, it is necessary to provide students with sufficient educational resources (Malhoit, 2005).

Grievance Redresser Procedures – In schools, students experience a number of problems and challenges. It is necessary to formulate the grievance redresser procedures so that they are able to adequately address their problems and seek solutions. The principals and the teachers are the ones, who listen to the grievances of students. The various problems may arise regarding academics, teaching-learning methods, school and classroom environmental conditions, fellow students, experiencing harsh treatment on the part of the teachers and so forth. In rural schools, girls are the ones, who have experienced severe problems, even sexual harassment in schools. Therefore, it is essential to implement these procedures, so that students may feel at ease in attending school and facilitating the acquisition of education.

Educational Adequacy – The system of education is required to be put into practice in schools on the basis of certain goals and objectives. The teachers have the main objective of performing their job duties well and leading to effective growth and development of the students. On the other hand, students have the main objective of enhancing their educational skills and abilities. Therefore, educational adequacy should not be defined on the basis of the minimum education, but it should be focused on the students meeting their goals and objectives in a suitable manner. The teachers and the students, both are required to work in integration with the purpose of promoting educational adequacy.

Morality and Ethics – Within schools, the aspects that are of utmost significance are the implementation of morality and ethics. The members of the school, principals, teachers, staff members and students are required to be polite, honest and truthful, particularly, when they are working with each other. When the individuals acquire efficient knowledge of morality and ethics, then they are able to not only achieve the goals and objectives of the schools but also would be able to develop into virtuous and principled human beings. It is the job of the teachers to ensure that students learn these traits, besides academic concepts, so that when they graduate from school, they should be able to turn into responsible members and promote the well-being of their families and the community.

Development of Leadership Skills – The principals and the teachers are required to develop leadership skills within them. The leaders have a number of job duties to perform. They need to be aware of various factors that are required to enhance the well-being of other members, particularly the students. The leadership skills possessed by the principals are focused on making effectual decisions, which would be beneficial to all, guiding and directing

othersappropriately, carrying out the administrative functions in an effective manner and depicting generosity and sincerity in their attitude. On the other hand, the leadership skills primarily possessed by the teachers focus on guiding the students, leading them in the right direction, and ensuring that they learn academic concepts and perform well in tests or competitions that are organized.

Implementation of Managerial Functions – Within the schools, the managerial functions of planning, organizing, directing, staffing, controlling and leading should take place in an appropriate manner. The principals and the teachers are required to work in coordination and integration with each other. The major focus of the managerial functions is on the achievement of goals and objectives. In order to implement these functions in a well-organized manner, it is vital to ensure that the individuals possess the required educational qualifications, skills and abilities. The individuals, who are the directors and managers within schools should obtain ideas and suggestions from others, particularly when important decisions are to be made.

Implementing Sound Decision-making – In schools, decisions are regarded as an integral part of the administration of schools. There are two types of decisions, major decisions and minor decisions. Major decisions are regarded as the decisions that are made for a long-term time period. When these decisions are made, then the directors or the principals are required to obtain ideas and suggestions from others. Normally meetings are organized to discuss major decision matters. On the other hand, minor decisions are usually made by the school heads and they may or may not consult others. They need to implement sound decisions, which may be beneficial to all the members of the school, particularly the students.

Provision of Support Services – The students need to be provided with suitable support services. These are the services, which not only facilitate the acquisition of education but also enable them to feel comfortable within the school's environmental conditions. The primary objective of every student is to ensure that they are able to acquire proper education and improve their scores. Therefore, one of the most important support services is making provision of extra classes for the students. Other support services include the availability of technology, innovative methods, strategies, approaches and other methods, which would enrich the skills and abilities of the students.

Financial Resources – The schools are required to plan their finances in accordance with the needs and requirements. When the schools have financial resources, then they plan to bring about innovative techniques and methods. On the other hand, when the budget is limited, then they have to ensure which

areas require improvements and then finances are spent accordingly. Within schools, bringing about improvements in infrastructure, civic amenities, facilities, libraries, computer centres, classroom environment, school environment, bringing in new equipment, machines, technology, books, articles, documents and other materials, all require financial resources. The schools make provision financial assistance and scholarships to the students on the basis of their merit and the availability of financial resources.

School Governance **-** The governance of the school has to be carried out in an efficacious manner. The principals, administrative staff members and teachers are required to work in coordination and integration with each other. It is vital to formulate norms, measures, and policies and individuals should follow them adequately. In school governance, educational planning is also considered an important aspect, the individuals need to seek a more general understanding of educational planning and ensure how it can be productive for the overall national development (Griffiths, 1968). Within schools, the main policies are the availability of equal opportunities to all, anti-discrimination policies, providing effective resolution to the occurrence of conflicts and disputes, treating everyone with respect and courtesy, policies against sexual harassment and criminal and violent acts and leading to effective progress of the students. Apart from understanding the policies, the members are required to implement them in an appropriate manner.

Measures Formulated by the Government

The measures formulated by the Government have been stated as follows: (Sreekanthachari, &Nagaraja, 2013).

Lok Jumbish Project

The LokJumbish (LJ) project has 75 blocks covering approximately 12 million of the population. The functioning of this project is carried out simultaneously with government agencies, teachers, NGOs, elected representatives and the individualsin an interactive group. There have been implementation of efforts with the main purpose of promoting the universalization of primary education. It works on seven guiding principles. These are: a process rather than a product approach, partnerships, decentralized functioning, participatory learning, integration with the mainstream education system, flexibility of management, and creating multiple levels of leadership, committed to the quality and mission mode.

Shiksha Karmi Project

The ShikshaKarmi Project (SKP) is being implemented since 1987, with assistance from the Swedish International Development Cooperation Agency (SIDA). The main objective of this project is universalisation and qualitative improvement of primary education in the backward and remote villages of Rajasthan, with special focus on girls. SKP has set up the Village Education Committees (VECs) in 2000 villages to promote community involvement in primary education and encourage village level planning. SKP also runs non-formal classes, known as PreharPathshalas schools,having suitable timings. To promote education among the girls, AnganPathshalas are being operated in three blocks. The program at present covers over 150,000 students in 1,785 schools and 3,250 PreharPathshalas, involving over 4,271 ShikshaKarmis.

Operation Blackboard

This scheme was initiated in 1987, with the main objective of bringing about improvements within the school environmental conditions. For the purpose of retaining the students and improving their skills, this scheme was initiated. A remarkable progress has been observed in primary education through this scheme. Approximately 5,23,000 schools have been covered since the beginning.

Conclusion

The system of education in rural areas is in a developing state. In rural areas, the problems that are found in the system of education are regarding number of schools, transportation facilities, provision of civic amenities and facilities, level of education, computer education, organization of group classes, organization of picnics, use of technology, infrastructure, and teachers. The measures that are needed to get implemented to make improvements include, availability of infrastructure and facilities, provision of transportation facilities, ICT-based education for students, recruitment of teachers, dealing with students, teaching-learning processes, promoting team-work, promoting extra-curricular activities, social and economic change and teaching-learning materials. The fundamental principles of the high quality rural education program are, equal opportunities to all, grievance redresser procedures, educational adequacy, morality and ethics, development of leadership skills, implementation of managerial functions, implementing sound decision making, provision of support services, financial resources and school governance.

There has been the initiation of programs by the government that aims at bringing about improvements in the system of education in rural areas. These are LJ, SKP and operation blackboard. There are differences between the system of education in urban and rural areas. In urban areas, in schools, there is the provision of all facilities and amenities that would contribute to the enhancement of the system of education. In rural schools, the important aspects that need to be taken into consideration in order to enrich the system of education are, the development of teaching-learning methods and instructional strategies, initiation of infrastructure, technology, equipment, libraries and other materials that would be supportive to learning, the school authorities should possess adequate knowledge regarding the implementation of managerial functions and there should be the provision of equal opportunities to all the students, irrespective of categories and backgrounds. Within rural communities, the system of education should be improved in such a manner that students should feel motivated by it.

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Detrimental effect of Toxic Pesticides

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Introduction

Pesticides are toxic chemical substance or a mixture of substance or biological agentthat is deliberately released into the environment in order to kill, prevent, deters, control, destroy,repel or mitigatethe population of Insect-Pests, weeds, rodents, fungi or other harmful Insect-Pests in an agricultural, domestic and industrial setting. Pesticides serve as regulators or modifiers that work by destroying the Insect-Pests. Pesticides are toxic chemical substance or a mixture of substance orbiological agent that is deliberately released into the environment in order to kill, prevent, deters, control, destroy, repel or mitigate the population of Insect-Pests, weeds, rodents, fungi or other harmful Insect-Pests in an agricultural, domestic and industrial setting. Pesticide serves as a regulator or modifier that work by destroying the pests. In the agricultural field, insecticides are used to increase theproduction of quality by controlling pest and pest-related diseases.The maingroup commonly used pesticides are insecticides, fungicides, fumigants and rodenticides. Insects are the major fountain of crop vandalism.

The use of pesticides has becomea common practice and it increased much fold over the past few decades.It is estimated that about 5.2 billion pounds of pesticides are used worldwide annually (Mahmood et.al. 2015). The majority of pesticides are not particularly targeting thepest.Even they backwash non-targetentities which invoke major problems in society.It has been assessed that only about 0.1% of pesticides stretch out the targetentity and the remaining are traitsof the surrounding environment (Darcinet.al.2017). Major farmers farmer is unaware of pesticide type, level of poisoning, safety precaution and hazards to health and environment (Timbrell 2000). The over and misuse of pesticides has precedence to immense human healthproblems,economic loss and various environmental problems.The resultant healthproblem of a pesticide includes cancer, liver, kidney, neural, birth defect problem and reproductive problem etc. In many developing countries majority of pesticides are associated with adverse effects on human health and the environment due to

the judicial use pesticide side. On the other hand, the overuse of pesticides alsoprecedence to environmental pollution such as water, soil and pollen etc.and cause an imbalance inthe ecosystem.

Misuse of pesticide-inducedtremendous effects on health and the environment.Thevarious effect of pesticides on human health and the environment are as follows-

Effect of pesticides on human health

Human beings are highly vulnerable to the deleterious effect of pesticides due tononspecific nature, hazards application or misuse of pesticides.The pesticide entersthe human body through ingestion, inhalation, and penetration by the skin but the majority of people get affected via intake by pesticide-contaminated food (Mahmood et.al. 2015).

Pesticide shows acute as well as chronic effects: -

Acute effect of Pesticide

Acute effects of pesticides include headache, skin irritation, itching, rash and blisters, diarrhoea, abdominal pain, nausea, vomiting and blindness etc.

Chronic effects of pesticides:

Long term effects of pesticide damage on body organs and diseases are as follows-

a. Carcinogenic: Pesticides associated with brain cancer, ovarian cancer andprostate cancer etc. It is estimated that worldwide chemical exposure isresponsible for 4% of all death from cancer.
b. Immune: Immune effects include hypersensitivity, asthma and allergic reaction.
c. Endocrine disruptors: Pesticides act as endocrine disruptors as it is interfering withthe endocrine system by blocking/mimicking, displaying the hormone in livingorganism.Mainly they confirmed estrogenic action as affecting the reproductivesystem such as stillbirth,miscarriages and abortion etc.The endocrine receptor alsomimics insulin thereby blocking the insulin receptor site and causing diabetes mellitus.
d. Neurological: Pesticides cause neurological health effects to include memory orlearning disability, vision, impairment, signaling disability etc.
e. Other: Long-term exposure to pesticides also damage lungs, liver and kidney etc.

Some pesticides contain an impact on human health and the environment

1. Organophosphorus pesticides

Many different pesticides fall under the category of organophosphates, which were touted as a more environmentally friendly alternative to organochlorines. The most popular of them is glyphosate. This class also contains other well-known pesticides like malathion, parathion, and some others; some of them are recognised for their ability to affect the endocrine system. This class of pesticides has also been linked to genotoxic effects, effects on mitochondrial function, cellular oxidative stress, problems with the nervous and endocrine systems, a reduction in insulin secretion, disruption of normal cellular metabolism of proteins, carbohydrates, and fats, and other effects. Population-based studies have revealed possible relations between exposure to organophosphorus pesticides and serious health effects including cardiovascular diseases, negative effects on the male reproductive system and on the nervous system, dementia and also a possible increased risk for non-Hodgkin & slymphoma. Furthermore, prenatal exposure to organophosphates has been correlated with gestational duration and neurological problems occurring in children.

The most extensively used herbicide in modern agriculture is glyphosphate, whose safety is a matter of continuous scientific debate, particularly in light of the emergence of glyphosate-tolerant genetically modified crops like some varieties of soy and maize. Due to the concurrent exposure to glyphosate and the phytoestrogen "genistein," a prevalent isoflavone found in soybean products, concerns regarding potential synergistic estrogenic effects have been raised by its broad usage in the genetically modified Soybean production process. In vitro effects on human erythrocytes, endocrine disruption, and carcinogenicity in mice skin are all possible effects of glyphosate. Additionally, it is thought to severely disrupt the shikimate pathway, which is a system that is shared by bacteria in human gut bacteria as well as plants and bacteria.This disruption may affect the supplyof human organisms with essential amino acids. Commercial glyphosate formulations are considered to be more toxic than the active substance alone.Glyphosate-based herbicides such as the well-known “Roundup” can cause DNA damage and act as endocrine disruptors in human cell lines and in rattesticular cells,causingdamage to cultured human cutaneous cells and promoting celldeath in the testicular cells of experimental animals.There is evidence also for theirpossible ability to affect cytoskeleton and intracellular transport.

2. Carbamate Pesticides

Carbamate pesticides,such as aldicarb, carbofuran and ziram are another class ofchemical pesticides that have been associated with endocrine-disrupting activity, possible reproductive disorders and effects on cellular metabolic mechanisms andmitochondrial function.Moreover in vitro studies have revealed the ability ofcarbamate pesticides to cause cytotoxic and genotoxic effects in hamster ovariancells and to induce apoptosis and necrosis in human immune cells, natural killercells and also apoptosis in T lymphocytes. Furthermore, it has been confirmed that carbaryl, which belongs to the category of carbamate pesticides can act as a ligand forthe hepatic aryl hydrocarbon receptor,a transcription factor involved in the mechanism of dioxin toxicity.There is also evidence for the ability of carbamatepesticides to cause neurological effects, increased risk for dementia and non-Hodgkin & lymphoma.

3. Organochlorine Pesticides

Chlorinated hydrocarbons, or organochlorine insecticides, were widely used in agricultural and mosquito control from the 1940s until the 1960s. DDT, methoxychlor, dieldrin, chlordane, toxaphene, ketone, lindane, and benzene hexachloride are examples of substances that belong to this category. These pesticides have both short-term and long-term negative health impacts on the nervous system, endocrine systems, and overall health. Pesticides 4-Pyrethrins and Pyrethroids A pyrethroid is an organic substance that is comparable to the naturally occurring pyrethrins produced by pyrethrum flowers (*Chrysanthemum cineraria folium* and *C.coccineum*). Both commercial and domestic pesticides employ pyrethroids.

The majority of the time, pyrethroids are safe for people to consume in little quantities. Pyrethroids are harmful to several other invertebrates, including those that form the foundation of aquatic and terrestrial food webs, such as bees, dragonflies, mayflies, and gadflies. Pyrethroids are poisonous to aquatic life, particularly fish. Through indoor use, they have shown to be a successful malaria outbreak control measure.

Effect of pesticides on the environment

Most farmers and field workers are illiterate or they lose educated and they applied pesticides without screening and proper specific information,due to which various hazardous effects posed on the environment. Myriad use of pesticides without screening on daily basis also affects the non-target organism. Due to irregular screening sometime, they used pesticides

abundantly after damaging the crops. Ultimately those persisting for a long time in the environment cause environmental pollution especially soil pollution. Thunderous use of pesticides also kills helpful micro-organisms as a result of which the self-fertility propertyof soil is reduced.Regarding pesticides, it is important to have a practical understanding of theirphysical and chemical properties, since their solubility determines the transportation of surface runoff and the absorbing capacity of the soil (Bernardes et.al. 2015). In the environment, the pesticides are tempo rated long distance and their deposition to water causes water pollution. In several cases, pests are resistant toparticular pesticides as the effect of natural selection, which causes hazards tonon-target organisms and causes sudden death of that organism. On the contrary, pesticides are demoted through photo-decomposition, micro-organisms orthrough physical or chemical reactions. But the un-demoted pesticides are remaining inthe environment fora long time which greatly causes environmental damage. The long-time persisting pesticides cause hazards to the biodiversity of aquatic or terrestrial organisms. Pesticides entered intothe aquatic ecosystem act as toxic agents and cause hazards to aquatic flora and fauna.

Conclusion

Pests are becoming the main concern for crops everywhere. Numerous pesticides are used without the required information or screening, which has serious negative effects on health. Contrarily, they have a negative impact on the environment, causing ecosystems to be out of balance and causing pollution. Inadequate understanding of pesticide kinds, levels of poisoning, a lack of regular screening, and ignorance of their detrimental effects on the environment and human health are all contributing factors to these pesticide-related issues (Sharma et.al. 2012). Farmers were given particular training about the impacts of pesticides and their screening and monitoring procedures as part of an awareness programme for farmers.

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Studies of medicinal plants for treatment of Joint Pain in rural area of district Kushinagar (U.P.), India

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Abstract

The present paper reports the therapeutic use of medicinal plants for treatment of joint pain by rural communities of Kushinagar district. Total 13 plants contain to 11 families were identified which were being used people of the study area. The information about the plants for treatment of joint pain was gathered from rural peoples, local Vaidyas and Hakims etc.

Key words: Joint pain, Medicinal plants, Treatment and Rural area of Kushinagar

Introduction

Medicinal plants are main ingredients of local medicine and are of vital importance in traditional health care. Atharva-ved is oldest world literature on the plants used against several diseases. A part from the tribal group many other forest dwellers and rural people also possess unique knowledge about plants (Jain, S.K., 1991). Moreover, there is a considerable economic benefits in the development of medicine and in the use of medicinal plants for treatment of various diseases (Azaizeh, H. et. al., 2003).

Joint pain discomfort that arises from any joint point. Joint pain can be mild, short term nuisances or chronic condition that affects quality of life and independence. Joint pain may arise with or without any movement of body part. It can also be caused by injury, affecting any part of ligament, bursae or tendons surrounding the joint.

Several medicinal plants have been used since time immemorial for treatment of joint pain. Some specific plants like Alstonia scholaris, Aloe vera, Bacopa monnieri, Boerhavia diffusa, Curcuma loonga, Datura stramonium, Euphorbia hirta, Linum usitatissum, *Moringa oleifera, Ricinus communis, Solanum nigrum, Thevetia peruviana, Trigonella foenum -graecum, Vitex negundo* and *Zingiber officinale* have used for treatment of joint pain in

rural area of Balrampur district of Uttar Pradesh. The traditional system of medicine is still effective in rural areas of India for treatment of various aliments (Sharma, P.P. and Majumdar, A.M., 2003, Jagtap, S.D. , 2008, Singh, A. and Singh, P.K., 2009). A plant may possesses one or more biologically active compounds that may show positive interaction with body compounds and help us getting rid of specific disorder .

The present work deals with the traditional use of plants as medicines for treatment of joint pain in rural area of district Kushinagar (U.P.), India.

Materials and Methods

The study was conducted (July-2020 to May-2022) in the rural area of Kushinagar district in Uttar Pradesh (Map-1 & 2). A small tribal population and a vast rural mass had a rich traditional knowledge of use of plants as drug for treatment of joint pain.

The information about plant was gathered during field visit by contacting and interviewing traditional healers and other rural people, local Vaidyas, Hakims for treatment of joint pain. Plants have been identified with the helf of books of Indian medicinal plants (Kirtikar, K.R. and Basu, B.D., 1935), Glossary of Indian medicinal plants (Chopra, R.N. et.al., 1956). A detail of plants are mentioned in Table -1.

Map 1: Location of study area in India.

Map 2: Location of study area in Uttar Pradesh

Results and Discussion

A total of 13 medicinal plants distributed in 11 families are documented in Table-1 . The people of studied area still had strong belief in herbal treatment. Herbal treatment is cheap, convenient and easily available with fewer side effects and more popular in rural area in comparison to modern medicines. Herbal plants are used in the treatment of rheumatoid arthritis (Amadeep Kaur et. al., 2012). Seeds of Jatropha curcas were used for arthritis (Khafagy, S.M. et.al., 1997). A study published revealed that both a ginger extract and one containing only gingerols and other non gingerol components were effective in preventing joint inflammation and damage (Funk , J.L. et. al., 2009) .

In the present study (Table -1), it was found that plants commonly used in traditional medicines in rural areas were still found in urban areas of Kushinagar and were frequently used by local inhabitants for various diseases. It is essential that ethnobotanical investigation should persistently be carried on and efforts should be made for proper protection, cultivation and conservation of these precious medicinal plants on large scale.

Table: 1- A list of Medicinal plants reported from district Kushinagar rural communities

S.N.	**Botanical Name**	**Common name**	**Family**	**Plants parts used**
1	Alstonia scholaris	Chhatium	Apocyanacea	Stem barks
2	Aloe vera	Ghritkumari	Xanthorrhoeaceae	Leaf pulp
3	Bacopa monnieri	Brahmi	Scrophulariaceae	Leaf
4	Boerhavia diffusa	Punarnava	Nyctaginaceae	Leaf
5	Curcuma longa	Haldi	Zingiberaceae	Rhizomes
6	Datura stramonium	Dhatura	Solanaceae	Leaf
7	Euphorbia hirta.	Dudhi	Euphorbiaceae	Whole part
8	Linum usitatissium.	Tulshi	Linaceae	Seed
9	Moringa oleifera	Munga Sahjan	Moringaceae	Seed
10	Ricinus communis	Arandi	Euphorbiaceae	Seed
11	Solanum nigrum	Makoi	Solanaceae	Leaf
12	Thevetia peruviana	Kaner	Apocynacea	Leaf
13	Trigonella foenum - graecum	Methi	Fabaceae	Seed

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Cardio protective role of Curcumin

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Absract

Background: According to WHO statistics, cardiovascular disease are the leading causes of death in the world. One of the main factors which is causing heart failure, systolic and diastolic dysfunction, and arrhythmias is a condition named cardiac fibrosis. This condition is defined by the accumulation of fibroblast-produced ECM in myocardium layer of the heart.

The excessive accumulation of ECM elements causes heart stiffness, tissue scarring, electrical conduction disruption and finally cardiac dysfunction and heart failure.

Objective: To describe the role of curcumin in the regulation of different signaling pathways that are involved in cardiac fibrosis.

Results: Curcumin is a natural Indian medicine which currently has been declared to have therapeutic properties such as anti-oxidant and immunomodulatory activities, which prevents oxidative stress, inflammation, and mechanical stress involved in cardiac fibrosis. In this review, we have

gathered several experimental studies in order to represent diverse impacts of this turmeric derivative on pathogenic factors of cardiac fibrosis.

Conclusion: Curcumin will open a new way in the field of cardiovascular treatment.

Keywords: ECM (Extra cellular matrix), curcumin, cardiac fibrosis, cardio protective.

Introduction

Myocardial fibrosis is a significant global health problem associated with nearly all forms of heart disorder, it's the expansion of the cardiac interstitium through sediment of extracellular matrix proteins (EMP), [1,2,3,4]This alteration involves pathological changes that include chamber dilation, cardiomyocyte hypertrophy and apoptosis, and ultimately leads to the progression to heart failure. [1]Despite the critical consequence of fibrosis in cardiovascular disorder, our limited understanding of the cardiac fibroblast impedes the development of potential therapies that effectively target this cell type and its pathological contribution to disease progression. [2] Complicated mechanisms involving oxidative stress, pro-inflammatory cytokines, chemokine families, NLRP3 inflammasomes, growth factors and non-coding RNAs share in cardiac fibrogenesis and make it difficult to designate specific and effective therapies. [3]Fibrosis may reflect activation of reparative or maladaptive processes. [3]Actuated fibroblasts and myofibroblasts are the central cellular effectors in cardiac fibrosis, serving as the main source of matrix proteins. Immune cells, vascular cells and cardiomyocytes may also acquire a fibro genic phenotype under conditions of stress, activating fibroblast populations. Fibro genic growth factors (such as transforming growth factor- β and platelet- derived growth factors), cytokines (including tumor necrosis factor- α, interleukin (IL)- 1, IL- 6, IL- 10, and IL- 4), and neurohumoral pathways trigger fibro genic signaling cascades through binding to surface receptors, and activation of downstream signaling cascades. [3]In addition, matricellular macromolecules are deposited in the remodeling myocardium and regulate matrix assembly, while modulating signal transduction cascades and protease or growth factor activity. [4]Cardiac fibroblasts can also sense mechanical stress through mechanosensitive receptors, ion channels and integrins, activating intracellular fibro genic cascades that contribute to fibrosis in response to pressure overload. [3,4]Although subpopulations of fibroblast-like cells may exert important protective actions in both reparative and interstitial/

perivascular fibrosis, ultimately fibrotic changes perturb systolic and diastolic function, and may play an important role in the pathogenesis of arrhythmias.

[5] Unlike other organs, the heart has limited regenerative capacity after injury, and instead, repair processes involve the removal of necrotic cardiomyocytes followed by fibrotic scar tissue replacement that acts to preserve myocardial structural and functional integrity. [3,4,5]To perform these functions, CFs within the connective tissue convert to their activated form, often known as myofibroblasts, which secrete elevated levels of ECM proteins to promote a profibrotic environment. Cardiac fibrosis provokes pathological changes that crown in chamber dilatation, cardiomyocyte hypertrophy, and apoptosis, and ultimately lead to the development of congestive heart failure. [5,6,11]Although the sources of these actuated fibroblasts remain under violent investigation and debate, the refinement of molecular markers and the development of new techniques for lineage tracing are helping to enhance our understanding of their origins. [5,6]Curcumin, a natural polyphenol and yellow pigment obtained from the spice turmeric, was found to have stronganti-oxidant andanti-inflammatory properties. Increasing evidence has shown that curcumin can be used to prevent and treat myocardial fibrosis, when the myocardium suffers pathologicalpro-fibrotic changes in vivo and in vitro. In this review, we've gathered several experimental studies in order to represent diverse impacts of this turmeric derivative on pathogenic factors of cardiac fibrosis.

[7,12] Curcumin significantly decreased interstitial and perivascular myocardial collagen deposition and cardiac weight index with reducing protein expression of collagen type I/ III in hearts($P<0.05$). [7,12]In addition, curcumin directly inhibited angiotensin (Ang) II- induced fibroblast proliferation and collagen type I/ III expression in cardiac fibroblasts($P<0.05$). Curcumin also inhibited fibrosis by inhibiting myofibroblast differentiation, decreased TGF-β1, MMP- 9 and TIMP- 1 expression($P<0.05$) but had no effects on Smad3 in Ang II incubated cardiac fibroblasts.

[10,12,13]Curcumin significantly ameliorated the inflammation process posterior to myocardial infarction, reflected by decreased expression of CD68 and CD3 cells, accompanied by dramatically improved cardiac function compared with the placebo group. [12] In addition, cardiac fibrosis is inhibited by curcumin administration. Interestingly, no significant reduction in fibrotic gene expression was observed when isolated cardiac fibroblasts were directly treated with curcumin in vitro; however, pro-fibrotic protein expression was

significantly attenuated in CF, which wasco-cultured with LPS- stimulated macrophages under curcumin treatment compared with the placebo group.

[12,13]Mechanistically, we discovered that curcumin significantly downregulatedpro-inflammatory cytokines in macrophages, which in turn inhibited IL18 expression inco-cultured cardiac fibroblasts using bulk RNA sequencing, and the TGF- β1- p- SMAD2/ 3 signaling network was also discovered as the eventual target downstream of IL18 in curcumin-mediatedanti-fibrosis signaling. administration of curcumin significantly ameliorated inflammation in the acute phase, as reflected by the promotion of macrophage apoptosis, accompanied by decreasedpro-inflammatory cytokine secretion, including IL- 6, IL1b, and TNF- α. The alteration of macrophage status is subsequently linked with resident cardiac fibrosis, resulting in decreased expression of IL- 18 in fibroblasts and hampered phosphorylation of SMAD2/ 3 in cardiac fibroblasts, reduced excessive collagen synthesis, and preserved long- term cardiac functionpost-MI. [12,13,14]The mechanisms of curcumin in treating different pathologic conditions, including ischemia, hypoxia/reoxygenation, pressure or volume overload, and hyperglycemia or high-fat-induced cardiac fibrosis.

[15,16]Novel analogs such as C66, B2BrBC, Y20, and J17 have been designed to maximize the therapeutic potentials of curcumin. These findings suggest that curcumin has implicit as a potent therapeutic target in treating adverse remodeling in ischemic heart disease. protein- coded fibrotic markers involving oxidative stress,pro-inflammatory cytokines, chemokine families, NLRP3 inflammasomes, and growth factors are being explored for therapeutic potentials in MF- induced heart failure.[15] Recently, based on the importance of novel factors in fibrogenesis, the potential roles of other protein- coding biomarkers, such as bone morphogenetic protein- 7 have attracted extensive attention. Although these protein- coding biomarkers have been investigated in the context of MF treatment, it's worth noting that protein- coding genes represent only 2 of the whole genomes. [16]Up to three quarters of human genome is transcribed intonon-coding RNA, which can be categorized into smallnon-codingmicro-RNAs (miRNAs) and longnon-coding RNAs. Targeting these ncRNAs may exert therapeutic effects on MF. In this review, we have gathered several experimental studies in order to represent diverse impacts of this turmeric derivative on pathogenic factors of cardiac fibrosis.

Rsults

Curcumin is a natural Indian medicine which currently has been declared to have therapeutic properties such as anti-oxidant and immunomodulatory activities, which prevents oxidative stress, inflammation, and mechanical stress involved in cardiac fibrosis. In this review, we have gathered several experimental studies in order to represent diverse impacts of this turmeric derivative on pathogenic factors of cardiac fibrosis as mentioned above.

Conclusion

Curcumin will open a new way in the field of cardiovascular treatment.

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Compulsion of Food Additives and it's Effect on Human health

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Introduction

In order to maintain the taste and quality of food products, food additives have acquired a social acceptance in the processing and preservation. These additives have now got legal and scientific sanctions to without changing the nutritional value of the food, they keep the quality, stability and appearance of food, in good order. The perishable foods which otherwise go waste are generally saved due to these additives within check the microbial development and quick oxidation, thus reducing the food wastage. Additives are substances which are added deliberately order to improve the quality or the preserve the food. It is also a substance or mixture of substances other than a basic food stuff which is present in a food as a result of any aspect of production, Processing, storage or packaging. Actually, naturally occurring substances are not necessarily non-toxic, For example: the causation of lathyrism, a serious neurological disease due to ingestion of khesari dal. (Lathyrus sativus) is well known and it's incidence in endemic form occurs in many parts of India such as Bihar, Uttar Pradesh, Madhya Pradesh abroad as well. It is a crippling disease accompanied by Paralysis of the leg muscles.

The Nutrition Research Laboratory at Hyderabad has done a painstaking and splendid piece of work to remove toxic factors from khesari dal and has evolved ways and means to remove toxic factors from this seed. Lathyrogens bring about alterations in collagen, elastin and mucopolysaccharides of connective tissue.

The existence of harmful fish toxins has been known for centuries. Teraodontoxin, the active principle of puffer fish, and saxitoxin from muscles are two of the best-known fish toxins. Vegetable cooking fat may contain 5 to 20 ppb of carcinogenic polycyclic aromatic hydrocarbons including benzopyrene.

A number of vegetables, especially the Brassica family and soyabean, contain goitrogens. In recent years many goitrogenous compounds in foodstuffs, for example crambe seed meal, have been detoxified by treatment with gaseous ammonia. It is well known that tapioca contains cyanogenic glycosides and steam treatment is necessary to remove these compounds. Peanut meal contains aflatoxin, which is one of most the carcinogenic compounds known today. Recently the Central Food Technical Research Institute, Mysore, has evolved methods of detoxification of aflatoxin containing meal by treatment with hydrogen peroxide under alkaline conditions. Unfortunately, this treatment may also affect the nutritive value of the meal to some extent.

Let us now consider the toxicity of food additives. Table 1 shows the list of commonly used food additives. They are additives added to foods with specific technological on nutritional objectives. In addition, food also contains other adventitious chemicals, as those from pesticides, from packaging materials and bacterial and chemical contaminants of various types. Some of these are listed in Table 2

Table 1: commonly used Food additives

Common Salt (NaCl)
•Sugar
•Spices
- Fruit extracts •Monosodium glutamate
- Maltol
- Saccharin

•Citric acid
•Cyclamates
- Phosphoric acid
- Fumaric acid •Malic acid
- Tartaric acid
- Adipic acid •Lactic acid •Succinic acid

•Vitamins (A, D, E, B1, B_2, B6, Niacin) •Minerals (iron, copper, calcium)
•Amino acids (lysine, threonine, methionine, tryptophan)
- Emulsifiers
- Stabilizers and thickeners (gum arabic, guar gum, CMC, gelatin, carrageenan, agar-agar, carobbean gum, etc.

•Firming agents (Ca salts)
- Anit-caking agents (Ca silicate, Ca3(PO4)2 Sod. Al. Silicate

- Moisture-retaining agents (Sorbitol, glycerol, propylene, glycol)
- Preservatives (Propionates, sorbic acid, Sod. Diacetate, benzoic acid, ethyl formate, ethylene and propionates oxides)

•Anti-oxidants (BHA, BHT, NDGA, tocopherol, ascorbic acid, erythorbic acid)

- Sequesntrants (Citric acid, sod. hexametaphosphate, EDTA)

Colours (saffron, cochineal, caramel, turmeric, Red No. 2. Yellow No. 5, Yellow No. 6).

- Flavouring agents (delta-decalactone)
- Leaching or contact additives

•Solvents (ethyl alcohol, glycerol, ethyl acetate, glycerol mono di and tri acetates, isopropyl alcohol, propylene glycol. diethylethar)

Table 2: Food Contaminants

- Aflatoxin (mainly from groundnut meal)
- Argemone oil (in mustard oil)
- Gossypol (in cottonseed meal)
- Solvent residues
- Pesticide, residues, Agricultural chemicals
- Defoaming agents and detergents
- Polymers, Plasticizers and Packaging contaminants
- Natural chemicals

By and large the additives do not increase the nutritional value of the food, rather they only improve the appearance and sale value and also the texture and flavour. However, commonly used food additives are usually excreted from the body,

The food additives may be broadly classified in two groups:

A. The incidental or indirect additives. B. The intentional or direct additives.

However, a third group, i.e., naturally occurring additives, may result from processing conditions, metabolic reactions and unanticipated chemical combinations. These include safrole and related compounds and contaminants as aflatoxins.

(A) Incidental or Indirect Additives:-

A food may contain minute traces of a chemical as a result of contact with a substance used in its production, processing or packing. Since its presence serves no useful purpose in the final food product, such a chemical may be considered to be an additive, e.g., food may pick up material from a wrapper or a container, either by dissolving it out or by abrasion from the container into the food. Detergents used for cleaning dishes or food equipments could be such an incidental source.

Of greatest concern are the pesticides which are used in crop production in order to avoid destruction by insects, nematodes, viruses, fungi or other plants peril. Residues of some of these pesticides sometimes may be present on fruits and vegetables even after careful washing. It is self-evident that these chemicals are toxic to some forms of life or they would not have their protective properties for the crop. Just what hazard they cause to man is a fundamental question. Since the minimum lethal dose of these chemicals for various species of animals is well known, the products must be used according to specified concentrations. More difficult to determine is the effect of built-up in the body when foods that contain such residues are eaten day after day for long periods of time-even a lifetime. Toxicological studies on animals throughout their life cycles, including the effects on reproduction and the next generation, are continuously being conducted to minimize such hazards.

(B) Intentional or Direct Additives:-

Chemicals are intentionally added to foods by processor in order to enhance the quality of afood such as texture, colour, flavour, nutritive value, or keeping properties to gain better consumer acceptance.

The intentional additives frequently used can be classified depending on their nature, composition and quality of preservation required:

1. Antioxidants
2. Emulsifiers
3. Enzymes
4. Flavoring agents
5. Colour and preservatives
6. Artificial sweetening agents

(a) Saccharin (b) Urea derivatives.

1. Antioxidants:-

The warm climate, temperature and humidity are such variable factors that they boost microbial infections to contaminate the food which, in due course, gets oxidized. The oxidation of food material makes the food unfit for human consumption. The dairy products such as cheese, butter, oils and fats etc. start stinking if exposed to air for a longer period. To check or reduce the oxidation of these food products, chemical antioxidants are used, of course, with following restrictions

(A) Such chemicals must be incorporated by the foodstuff and also must not tend any flavour of their own or the colour even during long storage.

(B) It must be fairly cheap and also active even at low concentrations. Only a little quantity of the antioxidants should be mixed with a diluting agent facilitating its proper and uniform mixing without affecting the food texture.

Not more than often, the antioxidants are also mixed with those agents which enhance their action and such substances are known as “synergists” though they have no or very little activity and only catalyse the action of antioxidant e.g, citric acid.

The natural antioxidants which are used in checking the oxidation of animal fats are oils of soyabean, rice, palm, peanut, olive, cotton seed. Coffee etc. Apart from this, certain spices and other natural products-e.g, oxalic, succinic, citric, malic, tartaric and amino acids like cystein, glycine and leucine or amides and proteins-are also used as antioxidants in food preservation

The commonly used antioxidants by food. To industries-butylated hydroxy anisole (BHA), in butylated hydroxy toluene (BHT) and propyl gallate been found to be the cheapest and best. The aforesaid antioxidants are frequently used to fl preserve chicken fat, meats, fishes, baked and fried. Food etc.

2. Emulsifiers:-

To accelerate the process of emulsification certain emulsifying agents are used to boost the desired emulsification. The most commonly used agents are glycerides of fatty acids and their esters, stearic acid, lecithin, and brominated vegetable oils. These agents, however, dilute the consistency of the products and thus, Agar, Gelatin, Pectin, Calcium, Sodium acetate and various starches are used to thicken food products as they possess the affinity with water.

3. Enzymes:-

Most harmless and without any toxic affect the enzymes have been found to play an important role in food preservation and are supposed to be the best commercially available agents. These are amylases, proteases, lipases, pectinases etc.

It is known to all of us that amylases hydrolyse starch and this reaction has been adopted by those engaged in the syrup production from various types of starches. Likewise, various fruit juices are also treated with peptide enzymes for clearing and there by reducing the viscosity, it also helps storage (wine and soft drinks).

The protein hydrolyzing enzymes have also been successfully picked up in dairy products e.g., cheese manufacturing and also in the preparation of bread and wines etc. Those who consume meat use raw papaya for meat tenderisation while cooking on large scale to make the meat soft and easily digestible. The animals are given intravenous injection of proteolytic enzyme before slaughter so that the enzymes may reach uniformly in each and every part of the body making the flesh soft and tender.

Since these additives are cheap and almost harmless, they are thought to be good ones.

4. Flavouring of food products:-

The specifically desired flavour and taste is the first and foremost prerequisite of the food products to attract large number of consumers. In order to improve the taste and increase or decrease the natural flavour of food the agents which are used are known as seasoners, enhancers, potentiators and flavour integers. Since long, scents and aromatic oils are being extracted from the leaves, flowers, fruits, barks and seeds of various aromatic plants. The most commonly used odorous plants are orange, lemon, grapes, rose, kewara, khas, vanilla etc. Even in the common houses pickles are made - a good number of raw spices are added to it to increase the taste and aroma. Apart from vegetative plant materials a good number of comparatively stronger synthetic flavour boosters have also been synthesized. However, such chemicals are used in very small quantity (not more than 300 ppm) yet they have been found quite effective and also easier in mixing. They are usually some acids along with sugar or certain salts and chemicals viz., Di-sodium 5'-inosinate and Di-sodium 5'quanylate (intensifiers). However, quite safe and most commonly used natural vegetable aromatic agents are coriander, ginger, garlic, onion, clove, cardamom, saffron. tejpatta etc. These

are freely used in our kitchens and houses without any toxic risk. Some of the fruits like mangoes, melon, banana etc. make other substances aromatic..

5. Colour and Preservatives:-

Colours have been found to be one of the important attractants in the finished food products Therefore, colours and preservatives are freely added in a variety of edibles e.g., icecream, sweets, candy, biscuits, soft and alcoholic drinks etc. Besides dairy, confectioneries and beverages, even the pulse like arhar (*Cajanus cajan*), masoor etc. are also coloured for an attractive food and consequently increased. sale (Phool Chap Dal).

Though only a limited number of colours are approved for colouring the food yet people utilize most randomly even the prohibited ones. The colours which are permitted are either of plant origin or synthetic. Some of the permitted colours are Erythrosine, Ponceau 4 R (red), Sunset yellow, In Carmine, Fast green, Amarnth etc. The permitted colours are either added to some of the edibleses aerated drinks, sauce, jams, syrups etc, or in go number of medicines. However, since the coloan (synthetic) are not quite safe, their doses should be carefully watched and monitored because they cause toxicity in animals. The excess use of some of these colours may cause diarrhea, lowering of RBC count (Ponceau), cholesterol disbalance, growth retardation etc. A higher concentration of amaranth has been reported to be carcinogenic, reduces fertility, induces abortion and fetal deformities. The ill-effect of most of the colours is because they do not decompose during digestion. Therefore, colours must be used with caution and care should be taken to restrict to the permitted ratio.

But it is most alarming that most of the sweet meat shops use certain nonpermitted dyes e.g. mentil yellow, auromine, orange II, rhodamine B, congo red, malachite green and methylene violet etc. which are highly toxic and cause irreparable harm to some of the most vital body organs e.g., kidney, spleen, liver, heart, eyes.

6. Artificial sweetener:-

The most commonly used natural sweetening agent is sugar ($C_{12}H_{22}O_{11}$) and is manufactured from sugarcane, palm, sugar beet, pineapple juice etc. In addition to sucrose, other natural sweeteners are glucose, fructose, maltose, lactose etc. But since these sugars are costlier, the industrialists use certain synthetic chemical sweeteners which are, on one hand, cheaper, and, on the other hand, 100 times sweeter than sugar. These chemical sweeteners are the compounds of amides, imides, nitrites, oximes, ureas and nitroaniline groups. Though they are excreted through urine they exert extra pressure on kidneys.

The most commonly used sweetening agents are:

1. Saccharin: This is a byproduct of coal-tar distillation and was developed during World War II (1939-45) by Germans and is chemically called o-sulphobenzimide. Itis about 700 times more sweet then natural sucrose. Since this chemical has obtained universal acceptance it is widely used in beverages and cold drinks, toothpastes and several such products. Like other food additives, saccharin has low acute toxicity in experimental animals. However, it may produce bladder tumors in rats.

2. Urea derive: Its commercial name is Dulcine and the chemical name is p-phenetyl. It is 300 times sweeter than sugar.

Besides, there are many more such chemicals in commercial use but they are not as safe as saccharin. These compounds have been reported to be toxic and cause damage to kidney, produce liver tumour and several more side-effects. Typical uses of various well-known intentional (direct) food additives by functionality is shown in Table 3.

Table 3:Well known Important Direct (intentional) food additives by Functionality

Si.No.	Agent's name	Description	Examples
1.	Anticaking agents and free flow agents	Substances added to finely powered or crystalline food products to prevent caking , lumping, or agglomeration	Glucitol, sodium,ferrocyanide, silicon dioxide
2.	Antimicrobial agents	Substances used to preserve food by preventing growth of microorganisms and subsequent spoilage, including fungistats,mold and rope inhibitors	Nisin, methyl-ethyl-propyl, or butyl ester of p-hydroxybenozoic acid: sodium benzoate; sorbic acid and its salts
3.	Antioxidants	Substances used to preserve food by retarding deterioration, rancidity or discolouration due to oxidation	Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate

4.	Colours and colouring adjuncts	Substances used to impart, preserve, or enhance the colour or shading of a food including colour stabilizers, colour fixatives, colour-retention agents	Tartrazine, β-carotene, annatto, turmeric
5.	Curing and pickling agents	Substances imparting a unique flavour and/or colour to a food	Calcium chloride, glucitol
6.	Dough strengtheners	Substances used to modify starch and gluten, producing a more stable dough, including the applicable effects	Calcium bromate, baker's yeast extract, calcium carbonate
7.	Drying agents	Substances with moisture-absorbing ability used to maintain an environment of low moisture	Calcium stearate, cobalt tallate
8.	Emulsifers and emulsifier salts	Substances that modify surface tension in the component phase of an emulsion to establish a uniform dispersion or emulsion	Phosphate esters of mono-and diglycerides, acctylated monoglycerides, calcium stearate
9.	Flavour inhancers	Substances added to supplement, enhance, or modify the original taste and/or aroma of a food without imparting a characteristic taste or aroma of their own	Monosodium glutamate, inositol
10 .	Flavour agents and adjuvants	Substances added to impart a taste of aroma in food	Cinnamon p-cresol, thymol zingerone

11.	Leavening agents	Substances used to produce or stimulate production of carbon dioxide in baked goods to impart a light texture, including yeast, yeast foods, and calcium salts	Carbon dioxide and Adipic acid
12.	Lubricants and release agents	Substances added to food contact surfaces to prevent ingredients and finished products from sticking to them	Mineral oil, acetylated monoglycerides
13.	Nonnutritive sweetener	Substances having less then 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity	Aspartame, saccharin
14.	Nutrient suppliment	Substances that are necessary for the body's nutritional and metabolic processes	Calcium carbonate
15.	Oxidizing and reducing agent	Substances that chemically oxidize or reduce another food ingredient, producing a more stable product	Calcium peroxide and hydrogen peroxide
16.	pH control agents	Substances added to change or maintain active acidity or basicity including buffers, acids, alkalis and neutralizing agents	Acetic acid, propionic acid, calcium acetate. Calcium carbonate, carbon dioxide
17.	Processing aids	Substances used as manufacturing aids to enhance the appeal or utility of a food or food component	Carbon dioxide, ammonium carbonate, ammonium sulphate, potassium bromide

18.	Surface finishing agents	Substances used to increase palatability, and inhibit discolouration of foods	Ammonium hydroxide
19.	Texturizers	Substances that affect the appearance or feel to food	Calcium acetate

Terms related to Adverse Reactions to Food:-

Various terms have been used for the adverse reactions of food. Their definitions are mentioned in Table 4 and description of very important terms generally used viz. food allergy, idiosyncrasy, pharmacological food reactions and metabolic food reactions are described in proceeding section.

Table 4: Adverse Reactions of food: Definition of Term

Si.No.	Term	Definition
1.	Adverse reaction (sensitivity) to a food.	Common terms that may be applied to a clinical abnormal response attributed to an ingested food or food additives.
2.	Food hypersensitivity (allergy)	An immunologic reaction resulting from the ingestion of a food or food additives. This reaction occurs only in some patients, may occur after only a small amount of the substance is ingested and is unrelated to any physiological effect of the food or food additives.
3.	Food anaphylaxis	A classic allergic hypersensitivity reaction to food or food additives.
4.	Food intolerance	A general term applied to describe an abnormal physiological response to an ingested food or food additives; this reaction may be an immunologic, idiosyncratic, metabolic. Pharmacological, or toxic response.
5.	Food poisoning	A term used to imply an adverse effect caused by the direct action of a

		food or food additives on the host recipient without the involvement of immune mechanisms.
6.	Food idiosyncrasy	A quantitatively abnormal response to a food substance or additives; this reaction differs from its physiological or pharmacological effect and resemble hypersensitivity but does not involve immune mechanisms. Food idiosyncratic reactions include those which occur in specific groups of individuals who may be genetically predisposed.
7.	Pharmacological food reaction	An adverse reaction to a food or food additives as a result of a naturally derived or added chemical that produces a drug-like or pharmacological effect in the host.
8.	Metabolic food reaction	Toxic effects of a food when consumed in excess or improperly prepared.

Table 5: symptoms of 1gE Mediated Food Allergies.

Cutaneous	Urticaria (hives), eczema, dermatitis. pruritus, rash
Gastrointestinal	Nausea, vomiting, diarrhea, abdominal cramps
Respiratory	Asthma, wheezing, rhinitis, bronchospasm
Others	Anaphylactic shock, hypotension, palatal itching, swelling including tongue and larynx.

Table 6: Known Allergenic Food Proteins

Food	Allergenic Protein
Cow's milk	α-Lactalbumin β-Lactoglobulin Casein
Egg white	Ovomucoid Ovalbumin
Egg yolks	Livetin Arah II
Peanuts	Peanut! β-Conglycinin
Soyabeans	Glycinin Gly mlA Gly mlB Kuniz trypsin inhibitor Gad cl
Codfish	Antigen II
Shrimp	Albumin fraction
Green peas	Glutelin fraction
Rice	Globulin fraction Glycoprotein fraction
Cotton seed	Several glycoproteins
Tomato	Albumin
Wheat	Gluten Gliadin Globulin
Okraα	Fraction 1

Table 7: Idiosyncratic Reaction to Foods

Food	Reaction
Fava beans	Haemolysis, sometimes accompanied by jaundice and hemoglobinuria; fatigue, nause dyspnea, fever and chills, abdominal pain
Chocolate	Migraine headache
Beets	Beetanuria: passage of red

	urine (often mistaken for hematuria)
Asparagus	Odorous, sulphurous-smelling urine.
Red wine	Sneezing, flush, headache, diarrhea, skin itch, shortness of breath
Choline-and carnitine containing foods	Fish odour syndrome; foul odour of body secretions
Lactose intolerance Fructose-containing foods	Abdominal pain, bloating, diante Abdominal pain, vomiting, diarrhea hypoglycemia

Table 8: Pharmacological Reaction to Food

Food	Reaction	Mechanism
Cheese Red wine	Severe headache Hypertension	Tyramine from endogenous or ingested tyrosine.
Coffee, Tea	Headache Hypertension	Methyloxantine (caffeine) acting as a noradrenergic stimulant
Chocolate	Headache, Hypertension	Methylxanthine acting as a noradrenergic stimulant

Other Contaminants of Food :

(Metals, Pesticides and antibiotics)

Various metals, pesticides, antibiotics etc. accidently or intentionally may contaminate the food stuffs as summarized in Table 9

Table 9: Deleterious Effects of some known Contaminants of Food (Metals, Pesticides, and Antibiotics

Si No.	Contaminants	Foods	Deleterious Effects
1.	Arsenic	Edible fruits sprayed by lead arsenate	Chills, Cramps, Paralysis
2.	Barium	Foods contaminated by rat poison	Muscular twitching and convulsions
3.	Cadmium	Fruit juices, soft drinks	Liver and kidney damage, excessive salivation prostate cancer
4.	Cobalt	Water, beer	Cardiac failure
5.	Copper	Acid foods in contact with tarnished copper ware	Vomiting, diarrhea, abdominal pain
6.	Lead	Some processed foods	Brain damage, Paralysis
7.	Mercury	Mercury fungicide treated seedgrains	Brain damage, Paralysis
8.	Tin	Canned foods	Colic, Vomiting
9.	Zinc	Foods stored in galvanised ironware	Dizziness, Vomiting
10.	Pesticides viz., DDT and BHC	All types of food	Damage to liver, kidney, brain, and nerves leading to death
11.	Antibiotics	Meat from animals fed antibiotics	Drug resistance, hardening of arteries, heart disease

Testing of Food additives:-

Unlike drugs, food additives are not used for their specific biological additives but are used for their specific technological effects and to ensure the maintenance of the natural quality of food. With food additives the hazard, if any, is more likely long term rather than acute.

The problem of testing food toxicity is much more complicated than that of testing drug toxicity. The composition of the food additives, the conditions and extent of usage and their biological properties-all factors have to be taken into account in evaluating the possible hazard or ensuring the safety in use of food additives.

Apart from chemical and physical specifications the main headings under which information should be sought are:

1. Acute toxicity data by oral and by injection in rats, mice and one additional species.
2. Short term studies (feeding for 90 days). In rat or mice and one additional species usually for a period of 10% of the life span. In this case a 1,000-fold margin of safety on non-effective levels in animals has been suggested in humans.
3. Long term (usually 2 years) tests which include studies on toxicity, with histopathological data, fertility and carcinogenicity. In this case a 100 fold margin of safety has been suggested in humans over non-effective levels in animals.
4. Metabolic effects on enzyme levels etc. Estimation of serum enzyme concentrations undoubtedly gives an excellent indication of tissue abnormalities and the acute effects of many hepatotoxic compounds. Elevation in urine enzyme levels can give some indications of kidney damage. These tests require sophisticated technologies and, apart from animal-house facilities, a good deal of inter-disciplinary collaboration between biochemists, physiologists, histochemists, electron microscopists, pharmacologists, and oncologists, in addition to analytical chemists, is necessary.

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Lead Toxicity on Human Health

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Introduction

The environment (water, soil and dust) contains different types of heavy metals and these cannot be degraded. Different types of anthropic activities such as mining, smelting and different kinds of industrial wastes are the main sources of heavy metals. Lead is a bluish-grey colour heavy metal with low melting point. It can be molded easily in to any shape and forms alloys with other metals. Lead toxicity is of prime concern in childhood because of environmental source in developing countries. Such poisoning occurs from different kinds of human related activities such as painting of home, smoking related activities, leaded petrol, contaminated food and drinking water, smelting and specially from the industries,which have been carrying out manufacturing processes. It is also found in human milk. Excessive exposure of the human body to lead results in disturbance of body function, which can be neurological, cardiovascular, hematologic and reproductive. Blood containing high level of lead causes inadequate functioning of the central nervous system (CNS) and consequently leads to encephalopathy and edema that mainly affects the cerebellum. In pregnant women, high amount of lead in the body can cause miscarriage. Prolonged lead exposure was found to decrease potency of males.

Five micro-organisms per deciliter is endorsed as a reference to identify blood lead levels (BLLs) in children by the centers for disease control and prevention (CDC) in the united states.

An effort has been made to decrease the amount of lead in the occupational environment for the reduction of lead poisoning.Particularly in children, even a small concentration of lead has been reported to induce dyscognitive behaviour.The CDC recommends testing for the presence of lead in the blood in all children at the age of 12 months and if possible once more at the age of 24 months. Novel chelating agent is used for treatment if the BLL is 45 mu g/dL or more.

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Possible sources of lead exposure

1-**Lead in paint:** Until 1978, in every house ,lead was used as common paint, later on consumer safety commission restricted lead in household paint. Before 1978, several buildings that were constructed had been painted with lead - based paints and approximately 74% of lead remains. Houses, which were built before 1950, pose a high chance of lead poisoning due to the use of lead based paint. Exposure of children to lead paints through direct and indirect way is common because of deferred maintenance and living in old house.Ingestible lead dust may be hazardous. Old toys, furniture and playground that are equipped with the lead-based paint have high lead content.

2-Lead in water: Some old well pumps may have the possibility of being contaminated by lead. During the last two decades, measures have been taken to reduce the lead exposure through the use of tap wateras per the safe drinking water act and a consequent Environmental Protection Agency Regulation (The Lead and Copper Rule) under the environment of the 1986 and 1996 amendments. The formulation prepared for the consumption of infants with lead contaminated water has a higher risk due to over intake of water as compared to their body size. If lead is suspected in plumbing, hot water taps should not be used and cold water taps should be flushed every morning for several minutes before use.

3-Lead in soil: The lead dust from the exterior of a house,which is painted with lead based paint, can also combine with soil. It may cause problem to the workers during remodeling of the old house. The mixture of lead contaminated soil can be stirred up by wind and may blow inside the house and surroundings. Metals melting and battery manufacturing are other important sources of soil contamination by lead. This easily flows in to the environment and thus easily mixes up with soil from the nearby homes. For children, lead contaminated soil has high risk of elevated BLL (EBLL), however the lead content of interior dust is of less importance. Children have a high BLL from exposure to play area soil and then from other locations.

4-Lead in the dust: Among children, common exposure source is the lead paint dust. Lead as dust is better and easily absorbed. Interior of the house can be contaminated with lead or lead dust due to damage to painted walls and airborne exposure can occur due to fall out of lead. When painted surfaces such as windows or doors rub against each other, fine lead dust is formed thereby contaminating the air. It can accumulate together on the surface of carpets, toys and other objects, speciallyon the floors, which later affects the children since they have a high chance of putting their contaminated hands in to mouth.

A study in young children shows that amount of lead in house paint has a greater association with the BLLs than the quantity of lead dust in the house. Statistical analysis of 12 new studies has shown connection between lead in the dust to among children (age group: 6-36 months) and also a linear involvement between lead dust stacking and threat of possessing an elevated BLL.

5-Lead in Tableware: Lead may be present in pewter, brass, handmade, imported or poorly glazed ceramic dishes and pottery. An acidic substances present in these pieces could act together with the glaze and increase the release of lead. Thus, the storage of acidic food such as tomato sauce, wine, orange, tomato and other fruit juices and vinegar in glazed containers is more hazardous.

6-Lead in folk medicines and cosmetics: Some folk medicines (Greta, azarcon and pay-loo-ah) may contain lead. These are frequently brought from Southeast Asia, India, Middle East, Dominican Republic or Mexico. These folk medications may contain high amount (approximately 90%) of lead.Various cosmetic products such as surma, kohl (alkohl), kajal and tozali also contain a high amount of lead. Application of Kohls results in successive ingestion of particles through hand to eye to mouth. Lead content of folk medicines and cosmetics is a vital predictor of the adult and childrens risk for EBLL.

7-Lead in metal costume jewelry: Lead poisoning in children has been most serious for the last decade. Children are encountered by lead toxicity from the metal costume jewelry containing lead.Charms are being manufactured without testing for lead. A high amount of lead(99%) is contained in charm and is considered dangerous as it raises BLLs up to three times the BLL seen in children.

8-Lead in toys: Toys and other useful goods for children are found with a high range of lead and pose lead poisoning threats. Maximum parents would not ever believe that their child’s sports accessory set could have a high amount of lead. The center for Environmental Health (CEH) discovered those lead toxic toys over the past few decades. CEH (Since 2007) has carried out trial on thousands of toys for lead and has exposed abundant hazardous products. All sources of lead exposure are summarized in table-1.

Table-1: Summary of sources of lead exposure

Sources of lead poisoning	Reason for poisoning
Lead in paint	Old building built before 1978 used lead based paint.
Lead in water	Old well pumps still in operation contaminating lead.
Lead in soil	Mixed lead-based paint with soil.
Lead in dust	Interior house dust contaminated with lead.
Lead in tableware	Old or poorly glazed ceramic dishes, pewter, brass and pottery may contain lead.
Lead in folk medicines and cosmetics	Some folk medicines Greta, azarcon and pay-loo-ah contained lead, some cosmetics such as surma, Kohl (alkohl), kajal, tiro and to zali also contained lead.
Lead in metal costume jewelry	Metal costume jewelry (fashion jewelry) containing lead.
Lead in toys	Toys and other useful goods for children found high range of lead.

Sign and Symptoms

At first, lead poisoning can be difficult or hard to detect because even people with high amount of lead in their blood might seem to be fully healthy.Usually, sign and symptoms are not noticeable until unsafe concentration has accumulated in the body. According to the age of patient, symptoms may vary. Given in Table-2.

Table-2: Common symptoms in different age group

Age group	Sign and symptoms of lead poisoning
Newborn (ages 0-4 weeks), Infant child (ages 1 week-1year) and Toddler (age 1-3 years)	Premature birth, lower birth weight and slowed growth.
Preschooler child (ages 4-6 years), School aged (ages 6-11 years) and adolescent (ages 12-19 years), Adults (ages 20-39 years) and young adult (ages 40-64 years).	Learning difficulties, irritability, weight loss, tiredness or sluggishness, abdominal pain, vomiting, hearing loss and seizures, high blood pressure, joint and muscle pain, trouble with memory storage, headache and sperms count decreases.

Effect of Lead toxicity

1-Neurologic toxicity: Neurotoxicity means exposure of the entire body to lead toxic agents and the imbalance in the neurophysiological function. There are two main symptoms caused by such exposures, they are psychiatric disturbances and neurocognitive symptoms.It predominantly influences the CNS, generally the developing brain and affects almost every organ symptoms.Therefore, children suffer more from neurotoxic effects than adults and are a greater risk of lead toxicity (Table-3).

Table-3: Cognitive effects of lead neurologic toxicity

Cognitive parameter	Effect of lead toxicity
Intelligence	IQ decreases
Memory	Decrease learning ability, memory scores capacity and other cognitive declines
Executive functioning	Decreases executive functioning abilities
Processing speed	Deficit processing speed
Language	Impede verbal concept formation, poor grammatical reasoning and imperfect command.
Visuospatial arts	Poorer copies of geometric figures and recalled visual patterns more weakly.
Motor skills	Vasomotor coordination affected.

2-Cardiovascular toxicity: Lead can cause hypertension and affect blood vessels. Lead blocked blood vessels can lead to immediate heart attack and death. Increasing BLLs significantly correlate with an increase in cardiovascular morbidity and mortality.Chronic and acute both type of lead poisoning can cause cardiac dysfunction and vascular damage. Recently, researchers have found an intriguing correlation between low blood leads concentration and cardiovascular toxicity and some studies suggest that low levels of lead may be associated with high blood pressure.

2-Nephrotoxicity: When lead affect the kidneys, medical experts call it lead related nephrotoxicity. Due to lead exposure, nephrotoxicity occurs because lead is eliminated through the kidney. In the renal tubules, lead is absorbed by

proximal tubular cells and it binds to specific lead binding proteins.These lead binding proteins produce intracellular inclusions of proximal tubular cells.

3-Hematologic toxicity: Due to lead toxicity,anaemia is the classic clinical manifestation in erythrocytes. Young and iron-deficient children have more likelihood of developing lead induced clinical anaemia. A high amount of lead exposure in the human body causes haemolytic anaemia. More than 99 % of lead is distributed in red blood cells rather than plasma.Lead causes anaemia by blocking the activity of ferrochelatase, aminolevulinic acid synthetase and ALAD. Lead also inhibits enzymes related to haemesynthesis. Haeme is an oxygen carrying moiety in haemoglobin which consists of a porphyrin ring that holds iron ion in the center.

4-Reproductive toxicity: Lead toxicity affects both the male and female reproductive systems. During the pregnancy, it crosses the placenta resulting in prematurity, intrauterine deaths and low birth weight. Different in vivo study proves that constant exposure to lead may cause inhibition of menstruation, ovulation and follicular growth, delayed vaginal opening and decrease in frequency of implanted ova. Usually, BLLs of >mug/dl are more damaging to the human reproductive organs, sometimes even at levels of <10 mu g/dl lead can be dangerous.

5-Bone toxicity: A significant reduction in the bone calcium content upon lead intoxication has been observed. This decrease in calcium content may be because of the increased bone resorption. Lead is one of the risk factors for the development of osteoporosis by altering bone mineral metabolism. Osteopenia, osteoporosis and osteomalacia with increased bone fragility in humans and experimental animals were observed because of lead exposure. Long term exposure to lead damages different body tissues.

6-Toxicity in human cells: Over exposure of lead in the human body influences the activation of mitogen by raising the secretion of pro-inflammatory cytokines interleukin-6(IL-6) and tumor necrosis factor-alpha and chemokines IL-8. Major cellular functions such as expression of cellular metabolic enzymes, metallothionein expression and protein kinase activity were affected by lead toxicity.

Prevention and treatment

In the United States, childhood lead toxicity prevention has taken a major concern in the public health for the reduction of blood lead concentrations in children. An excellent approach to lessen the lead toxicity is to suggest people, mostly uneducated people about CDC guidelines and by

creating awareness about lead poisoning. Every parent should frequently wash their children's hands and prevent children from placing their hands in mouth habitually. It is suggested that every family should use cold water because hot water contains high amounts of lead.Vitamin C has antioxidant properties, which is capable of removing free radicals and alleviating oxidative stress. Therefore, the physician should suggest that taking Vitamin C containing food in the diet regularly because they minimize lead toxicity easily. A chelation therapy can be recommended. Lead chelating agent has much more attraction toward lead than calcium and is excreted in urine.

Conclusion

Since the 18th century humankind has known and experienced lead poisoning, lifestyle, socioecology and age are mainly associated with higher concentration of lead. The main complication of lead toxicity is impairment of the nervous system and blood disorders that may lead to encephalopathy and edema in the cerebellum. The brain and kidney are the main parts affected by lead toxicity in both adults and children.Even at low concentration, it can induce cognitive behavioural disturbances.Lead toxicity increases oxidative stress, neurological abnormalities, affects the Sodium ion concentration, other severe health complications and even death.Childrens are more prone to lead toxicity due to certain habits such as putting hands that might be contaminated in their mouth. Antioxidants, specially vitamin C are used for the treatment and improvement of oxidative stress-induced toxicity of lead until now. Chelation treatment is employed for blood lead toxicity if the level is 45 mu g/dl or greater. Lead toxicity and associated morbidity and mortality can be reduced by regulating ongoing medical diagnosis, creating health awareness and timely medical treatment.

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Determination of lethality in a freshwater Indian major carp *Labeo rohita*, exposed to Pyrethrum and atrazine

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Introduction

Pyrethrum (a plant based pesticide), and atrazine (herbicide) are frequently used in crop field to control different type of insects and various unwanted weeds respectively. They reach to the water bodies from agriculture fields through surface runoff and contaminate water bodies. Mostly organic farmers use pyrethrum as an insecticide for fruit and vegetable crops.

Pyrethrum belongs to the Asteraceae family and used for pyrethrum production. Atrazine is a triazine class herbicide. These chemicals control harmful insects and unwanted weeds but they are often toxic to non-target organisms (NTOs) like fishes (*Labeo rohita*).

The static renewal acute toxicity test was carried out to find out the 24h, 48h, 72h, and 96h, LC_{50} value of pyrethrum and atrazine, on the freshwater major carp *Labeo rohita*. The fish were exposed to different concentration of pyrethrum and atrazine for toxicity bioassay. All data were subjected to Finney's Probit analysis and processed with SPSS-21. The LC_{50} values of pyrethrum and atrazine for 24h, 48h, 72h, 96h were 0.75µg/l, 0.70 µg/l, 0.62 µg/l, 0.55 µg/l, and 34mg/l, 32.5mg/l, 31.2mg/l, 30.5mg/l respectively. On exposure to pesticide during bioassay fishes exhibited altered bahaviour. At higher concentration level of pyrethrum and atrazine, the fish showed changes such as higher mucus production, jumping, vertical position, sinking to the bottom, jerky movements, higher opercular rate due to higher respiration rate, and change in body colour.

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Materials and Methods

- Adult major carp were collected from local ponds.
- From collection point they were carefully transported to the laboratory and treated with 0.05% KMnO4 solution for 2 to 3 min to disinfect the fish.
- Then fish was kept in 200 litre plastic tank for 15 day to acclimatize under laboratory condition.
- Food materials given to them include rice bran, wheat flour, and oil cake. For the toxicity test feeding was stopped before 24h.
- Parameters such as DO, temperature, pH, and hardness of the water were recorded daily for the 24h, 48h, 72h, and 96h exposed period following the standard methods (APHA, 1998).
- The size of fishes about 15cm were selected for bioassay.
- Stock solution was prepared by dissolving pyrethrum and atrazine in absolute alcohol.
- A control set with equal number of fish was run at the same time.

The range finding tests prescribed by APHA (1998) was used to determine the concentration range of pyrethrum and atrazine.

100% mortality was found within 24h at the concentration of 1.10 µg/l for pyrethrum and 48mg/l for atrazine and no death was found at concentration below 0.25µg/l for pyrethrum and 20mg/l for atrazine for 96h.

After determining the test range of pyrethrum(0.25µg/l, 0.40µg/l, 0.55µg/l, 0.70µg/l, 0.85µg/l, 1.0µg/l and 1.15 µg/l) and test range of atrazine(20mg/l, 25mg/l, 30mg/l, 35mg/l, 40mg/l, 45mg/l, and 50mg/l) 8 acclimatized fish in four replicates, for each concentration were released in 10 litre water trough for 24h, 48h, 72h, and 96h acute toxicity test.

Fish were not given any food material during the experiment (Ward and Parrish 1982, Reish and Oshida, 1987).

During experiment fish behaviour were observed and recorded, besides mortality. LC50values were calculated according to Finney (1971), and SPSS-21.

Results

The exposure of fish to different concentration of pyrethrum and atrazine shows altered behavioural responses, like high mucus secretion, jumping, jerky movement, higher opercular rate, change in body colour, loss of balance, vertical position, sinking to the bottom and finally death.

The mortality of fish increases with the increase in the concentration of the pyrethrum and atrazine.

In present study the LC50 values for pyrethrum and atrazine is given in table-1.

Duration	24h con.	48h con.	72h con.	96h con.
Pyrethrum LC50 value	0.75 µg/l	0.70 µg/l	0.62 µg/l	0.55µg/l
Atrazine LC50 value	34 mg/l	32.5 mg/l	31.2 mg/l	30.5 mg/l

Table-1. LC50 value of pyrethrum and atrazine.

Conclusion

In the present study it is concluded that the *Labeo rohita* is very sensitive to these toxicants as evident from the behavioural responses. It was also found that the toxicity of pyrethrum is more toxic than atrazine.

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Status of net primary productivity in Indian grassland ecosystems

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Introduction

The global carbon cycle relies heavily on net primary productivity and an important variable in terrestrial ecosystems (Jian Ni 2003). Net primary productivity refers to the gross photosynthetic production minus respiratory losses per unit land area in vegetation. (Scurlock et al., 2002). Understanding carbon cycling and its feedback to climate change requires an understanding of the dynamics of NPP and carbon stocks. To better know the carbon cycle on the ground, the biosphere-atmosphere relationship, and ecosystem function responses to climate change and CO_2 fertilization, a better understanding of NPP is needed (Melillo et al. 1993; Cramer et al. 1999; Scurlock et al. 1999). Grasslands, in general, are one of the world's largest ecosystems, covering about 52 million km^2 or 40 percent of the ground surface except for Greenland and Antarctica (World Resources Institute, 2000, based on IGBP data). Woody savannah and savannah makes up approximate 13 percent of the world's land surface (without Greenland and Antarctica); Open and closed shrubs account for 12 percent, non-woody grassland for 8 percent, and tundra for 5 percent. Grasslands are found on all continents and in a variety of geological and climatic conditions, covering about 40 percent of the earth's crust devoid of Antarctica and Greenland (Suttie et al., 2005; White et al., 2000).

Grassland productivity is linked to grassland biomass, or the amount of organic matter produced by autotrophic organisms in a given unit of area over a given period of time (Fen Zhao et al., 2014). The climate and inherent soil properties limit the distribution and productivity of grasslands (Samuel Eze et al., 2018). Semi-arid grasslands account for 28 percent of grasslands worldwide, while arid grasslands account for 19 percent, humid areas for 23 percent, and cold areas for 20 percent (White et al., 2000). Grassland habitats provide over 20 percent of global net primary production (Grace et al. 2006; Hall & Scurlock 1991) and have the ability to have a substantial impact on CO_2

reserves around the world (San Jose et al. 1998). Grasslands have 3-5 times more below-ground biomass than above-ground biomass; despite the fact that below-ground biomass is 3-5 times greater (Titlyanova et al. 1999), in terms of grassland biomass and carbon stocks, the majority of studies have overlooked the underground portion (Peri & Lasagno 2010; Scurlock et al. 2002). According to Long et al. (1989), roots and rhizomes make up 50 percent of the biomass in tropical grasslands, and this proportion increases to 70 percent during the dry season. As a result, the root-to-shoot ratio (R: S) in tropical and subtropical grasslands is usually greater than 4 (Mokany et al. 2006), while woodland habitats have a root to shoot ratio of less than 1 (Cairns et al. 1997; Castro & Kauffman 1998).

Dynamic Land Ecosystem Model (DLEM) simulation over the country revealed major inter-annual fluctuations in terrestrial NPP between 1901 and 2010, ranging from 1.2-1.7 Pg C $year^{-1}$. Between 1901 and 2010, the terrestrial NPP with a mean of 460–530 g C m^{-2}, ranging from negative to 1380 g C m^{-2}. From the drier northwestern to the wetter northern-eastern zones, a positive gradient existed. in terrestrial NPP (Prasad et al. 2007). Forests (179–969 g C m^{-2}) had the highest NPP, followed by grasslands (324–927 g C m^{-2}), croplands (342–431 g C m^{-2}), and shrublands (407 g C m^{-2}). Forests contributed the most terrestrial NPP during the study period (0.63– 0.67 Pg C $year^{-1}$; 41–47 percent), followed by croplands (0.33–0.59 Pg C $year^{-1}$; 25–38 percent). However, for the period of the research time, grasslands (11–16 percent) and shrublands (8.3–9.1 percent) contributed a slightly lower amount of terrestrial NPP.

Around 34 percent of the world's carbon (C) is contained in grasslands, with a large portion the soil stores 89 percent of the carbon, sequestered in grassland (Ajtay et al., 1979; White et al., 2000), which is critical for ecosystem services and, in particular, climate control (Buckingham et al., 2013). In grassland habitats, this emphasizes the value of below-ground biomass.

In the last 24 years, Alpine grassland NPP in Northern Tibet has been very low, with a relatively wide yearly variation; however, the majority of the grassland region in Northern Tibet (88.61 percent) has not shown a substantial annual NPP improvement (Qingzhu Gao et al., 2009). The biomass generated per unit land area per year, or NPP, is utilized as a measure of potential C accumulation in an ecosystem. It calculates the pace at which radiant energy is deposited by means of organic matter in plants from an ecological standpoint (E.P. Odum et al., 1971). As a result of the global cycling of carbon, water, and nutrients, NPP serves as a connection between the biosphere and the climate

system (Roy et al. 2001; Gao et al. 2009). Carbon can be stored in savannas in large quantities (Buis et al. 2009), and most of it is kept underground (Liu et al. 2010). Climate, soil, vegetation type, and human activities all have an effect on NPP (S. D. Prince et al., 2001).

NPP is commonly considered as an important component in numerous ecological monitoring practices because it offers a thorough assessment of ecosystem status and resources, such as productivity potential, biodiversity and wildlife, and ecological footprint (R. Nemani et al. 2002; R. Crabtree et al. 2009). Because of the spatial heterogeneity of environmental conditions, NPP is not a clearly detectable ecosystem trait, and it is difficult to quantify reliably over large areas (S. J. Goetz et al. 1999; I. McCallum et al. 2009). A variety of NPP models have been designed for various ecosystems. These models can be divided into two categories: regression-based and process-based. The Miami model, for example, is an empirically derived relationship between climate values and NPP (Lieth, H. 2009).

This study focuses on grassland NPP trends and covers all forms of grasslands in India. Furthermore, the term "NPP" encompasses both above- and belowground efficiency of production.

Types of grassland in India

Grasslands are graminoids (grasses and grass-like plants)-dominated vegetation communities. Poaceae, Cypearaceae, Fabaceae, and Asteraceae are the major grassland families. The Caryophyllaceae, Gentianaceae, and Rosaceae families are all abundant in temperate grasslands. Tropical savannas, temperate grassland, and steppes are the three types of grasslands found around the world. The Savannas are vast expanses of tropical grasslands with scattered trees, the temperate grasslands, on the other hand, are long stretches of tall grasslands in temperate climates, and arid and semi-arid temperate high-altitude grasslands in wide stretches make up the Steppes. R. O. Whyte (1957) identified eight different types of grasslands based on their floral characteristics in India, which was the first attempt at classifying grasslands. Grass Covers of India (Dabadghao and Shankaranarayan, 1973), a landmark in the history of grassland ecological research, was later published based on a detailed survey. Dhadabgao and Sankarnarayan (1973) classified India's grasslands into the five broad cover types listed below. –

Table 1 India's major grassland types and their distribution

S.No.	Grassland Type	Elevation range (m.a.s.l)	Region
1.	*Sehima - Dichanthium*	300-1200 m	Deccan plateau, Chota Nagpur plateau and Aravallis
2.	*Dichanthium - Cenchrus - Lasiurus*	150-300m	Gujarat's northern regions, Rajasthan's Aravalli ranges, Uttar Pradesh's south-western region, Delhi, and Punjab's northern regions
3.	Phragmites - *Saccharum - Imperata*	300-500 m	The Gangetic Plains, the Brahamputra Valley, and the Punjab Plains
4.	*Themeda - Arundinella*	350-2000 m	Manipur's foothills and lower hills, Assam's northern parts, West Bengal's northern parts, Uttarakhand, Himachal Pradesh, and Jammu and Kashmir
5.	Temperate - Alpine	2000 m	Himalayan States and the temperate high-altitude areas of Nagaland, Manipur and Western Ghats

Source: Dhadabgao and Sankarnarayan (1973)

Methods used to measure net primary productivity

There are six commonly used algorithms for estimating biomass from grassland vegetation biomass measurements: peak live biomass, peak standing crop (live and dead matter that is still standing), maximum minus minimum live biomass, sum of positive increments in live biomass, sum of positive increases in live and dead plus litter biomass, as well as sum of changes in live and dead biomass with decomposition adjustment (Long et al. 1992; Scurlock et al. 2002). However, grassland NPP evaluates these six methods in a variety of ways. Some methods can only be used in specific biomes (Scurlock et al. 2002). For example, only temperate steppe grasslands can use the peak live biomass and maximum minus minimum live biomass methods (Milner and Hughes 1968; Long et al. 1989; Scurlock et al. 2002). Only the peak biomass way is used in this grassland. The current year's standing dead matter, litter fall, and dead roots are factored into the NPP calculation (the peak standing crop method). The advancement of remote sensing technology has encouraged studies on vegetation productivity and biomass (Li et al., 2013). Because of the ease of calculation and high accuracy of the method, remote sensing data has been broadly used to assess grassland biomass.

Tropical grassland's net primary production, Karnal (Haryana)

The grassland near here forced their beginning to abandon farming. As a result, they are seral in nature and are kept that way by grazing and annual burning. This grassland has been around for about 20 years, and it received no biotic intervention during the study other than insect and small mammal herbivory.

In India, research on primary productivity in grassland ecosystems began in 1967 (Singh 1967, 1968, Singh and Misra 1969), and Misra (1970, 1972) summarized the effort made until 1970. Singh and Yadava (1974) conducted research at the Kurukshetra University campus in the Karnal district of Haryana on the biomass and NPP of tropical grassland for the years 1970-1971. The aboveground plant biomass (live vegetation) varied significantly between months, according to Singh and Yadava (1974). From a low of 437 gm^{-2} in May 1970 to a high of 437 gm^{-2} in September 1970, it increased steadily (1,974 gm^{-2}). It's possible that in May 1971, there was a rise in the value of plant biomass was caused by unseasonably wet weather; otherwise, due to hot and drier conditions; the amount of biomass would have decreased. The amount of rainfall in different months, total density of vegetation, temperature, humidity, and solar radiation were all linked to the above-ground biomass. With increasing depth, the amount of belowground biomass

decreased. The 0-10 cm layer had the highest concentration, accounting for 54.5 percent (September) to 85 percent (November). Within the year, the belowground plant material in the 0-10 cm level ranged from 330 gm^{-2} - 996 gm^{-2}, 66 gm^{-2} - 231 gm^{-2} in the 10-20 cm layer, and 17 gm^{-2} - 155 gm^{-2} in the 20-30 cm layer. The rainy season produced the most aboveground net primary production (1,706 gm^{-2}) and the winter season produced the most belowground net primary production (785 gm^{-2}). The total net primary production for the year was estimated to be 3,538 gm^{-2}.

Table 2 Seasonal differences in net aboveground, belowground, and total primary production, as well as rates of production.

Season	Net production (gm^{-2})	Rate of production (gm^{-2})	Rainfall (mm)	BNP (gm^{-2})	Rate of production (gm^{-2})	TNP (gm^{-2})	Rate of production (gm^{-2})
Rainy (June - September)	1,706	13.9	651.1	137	1.1	1,844	15.1
Winter (October – February)	156	1.0	16.7	785	5.2	941	6.2
Summer (March – May)	544	5.9	45.3	209	2.2	754	8.1
Annual	2,407	6.0	713.1	1,131	3.1	3,538	9.7

Source: J. S. Singh and P. S. Yadava (1974)

Temperate grassland net primary production in Kumaun, India's Central Himalaya

In the Central Himalayan range, the Kumaun Grassland produced 361 gm^{-2} above-ground net primary production and 193 gm^{-2} total net belowground productions. Aboveground production was highest (84 percent) in the rainy season and lowest (less than 1 percent) in the winter season, according to the seasonal pattern. The winter season produced the most roots (52%) and the summer season produced the least (11 percent). This grassland's total net production was 554 gm^{-2}, with below-ground production accounting for about 35 percent of that.

The net accumulation peak growth rate is a measure of a plant's net photosynthetic efficiency under specific environmental conditions (Ram et al. 1989); It also denotes when most favorable augment setting occur at different locations (Sims and Singh 1978). In September, at the end of the monsoon season, peak growth rates in Indian tropical grasslands could reach 14-15 g $m^{-2}day^{-1}$ (Singh & Yadava 1974; Gupta & Singh 1982). The current grassland's peak growth rate of live shoot biomass (2.4 $gm^{-2}day^{-1}$) falls within Sims and Singh's (1978a) range of (1.2–6.5 $gm^{-2}day^{-1}$) for diverse temperate grasslands in Western North America. Biomass addition in belowground portion grew in a bimodal pattern, with a second peak in November. Singh and Krishnamurthy (1981) and Ram (1989) both reported bimodal belowground biomass trends in different grassland ecosystems of India.

In present grassland, the turnover of belowground biomass is quite high, with up to 62 percent of the new biomass is added to the ground every year. The above-ground production ranges from 51 - 679 gm^{-2} in the grasslands (Sims & Singh et al 1983). The current study's estimated above ground production (361 gm^{-2}) falls within this range and is lower than the value (1184 gm^{-2}) reported by Sah and Ram (1989) for a temperate grassland in the Central Himalaya.

Above ground production was higher than belowground production in this analysis, and it was higher than the values recorded by Kumar and Joshi (1972) and Sah and Ram (1989) for alpine and semi-arid grasslands, respectively, but lower than the values reported for tropical grasslands (Singh & Yadava 1974).

Table 5 Seasonal terms include aboveground net production (ANP), belowground net production (BNP), and total net production (TNP).

Season	Aboveground net production (ANP) gm^{-2}	belowground net production (BNP) gm^{-2}	Total net production (TNP) gm^{-2}
Rainy(June - September)	301.2	71.2	372.4
Winter(October – February)	1.0	100.6	101.6
Summer (March – May)	58.9	21.6	80.5
Annual	361.1	193.4	554.5

Source: Mukesh Joshi (1995)

Primary production in Western Garhwal Himalayan alpine grassland

Manoj Dhaulakhandi et al. (2000) conducted an alpine zone analysis between Bhojbasa and Gaumukh on the right bank of the Bhagirathi in Uttarkashi, Garhwal Himalaya, and found that aboveground net production varied from 39.7-164 gm^{-2} per month.The aim of this research was to look at the species composition, biomass, productivity, and system transfer functions of this alpine grassland at both the upper and lower altitudes. Aboveground biomass increased from May to August at both sites (upper and lower), then decreased later. In both the upper and lower sites, the monthly rainfall values were positively related to the live shoot biomass.

On steep slopes under which the climate was excessively cold in addition to harsh for tree growth, alpine and sub-alpine grasslands emerged (Yadava & Singh 1977). The diversity, productivity, and system transfer functions of tropical alpine grasslands have not been thoroughly investigated. Unplanned grazing in these grasslands has resulted in deforestation and placed biodiversity and ecosystems at risk. Several researchers in the Indian Himalaya have attempted to investigate the alpine grassland's phytosociological characteristics and productivity (Joshi et al. 1988; Ram et al. 1989).

On the upper and lower sites, the biomass in belowground part ranged from 1503-2005 g m^{-2} and 1805-2237 g m^{-2}, respectively. On the study place, aboveground net production varied from 39.7-164 g m^{-2} per month. On both the upper and lower point, monthly highest net belowground production was reported in September and June, respectively. The vegetation in alpine pastures resurfaces shortly after the snow melts in late April and early May. Alpine plants have a six-month life cycle, and most of them are perennated by seeds, suckers, rhizomes and rootstocks. Biomass output from live shoots reported from April to August demonstrated that the climatic surroundings for plant development were favorable. Snowmelt, which makes water available to plants, can explain why alpine sites produce more than Indian plains.

As the temperature drops before the upcoming rising season, addition in the belowground portion occurs. The pace of addition of biomass generated by living shoots peaked in August (around 5 g m^{-2} day^{-1}), indicates optimum growing conditions and is in the peak net category growth rates (1.7-7.9 g m^{-2} day^{-1}) for other Himalayan alpine populations (Joshi et al. 1988; Ram et al. 1989; Rikhari & Negi 1994). The fact that ANP and peak biomass differed so little suggests that most organisms assimilated peak biomass at the same time. The BNP levels in the current sites are higher than those recorded by Ram et al. for alpine grassland in the Himalaya (1989). Table 6 shows that the ANP and

TNP values for the current sites are similar to those for other Himalayan alpine grasslands.

Table 6 Upper and lower alpine sites' monthly fluctuations in aboveground and belowground biomass, standing dead, aboveground net primary productivity (ANP), belowground net primary productivity (BNP), and total net primary productivity (TNP).

Months	Site	Aboveground live biomass ($g\ m^{-2}$)	Standing dead ($g\ m^{-2}$)	Litter ($g\ m^{-2}$)	Belowground biomass ($g\ m^{-2}$)
May	Upper Lower	24.8 ± 8.3 12.3 ± 6.1	10.8 ± 3.4 11.2 ± 4.1	16.2 ± 4.9 12.8 ± 3.6	1503 ± 80 1805 ± 118
June	Upper Lower	78.2 ± 14.1 128.7 ±28.7	18.2 ± 7.1 16.2 ± 2.7	18.5 ± 6.1 27.7 ± 14.1	1584 ± 109 2108 ± 213
July	Upper Lower	121.4 ±26.7 168.4 ±26.6	36.2 ± 11.2 30.7 ± 7.3	32.8 ± 11.5 38.4 ± 12.9	1618 ± 118 2237 ± 368
August	Upper Lower	285.8 ±44.3 318.6 ±38.3	48.6 ± 10.3 38.8 ± 11.7	38.2 ± 8.2 42.5 ± 22.4	1566 ± 261 1903 ± 221
September	Upper Lower	177.1 ±24.2 284.3 ±35.1	42.8 ± 6.9 8.2 ± 19.3	52.5 ± 12.6 39.2 ± 16.7	2005 ± 311 2217 ± 196
October	Upper Lower	57.8 ± 13.5 108.7 ±31.9	63.2 ± 12.5 54.6 ± 18.8	61.5 ± 11.4 57.1 ± 23.2	1608 ± 128 1808 ± 133
Total	**Upper Lower**	**261 (ANP) 306 (ANP)**		**554 (BNP) 646 (BNP)**	**815 (TNP) 952 (TNP)**

Source: Manoj Dhaulakhandi et al. (2000)

Net Primary Production in an Imperata Grassland in Assam's Barak Valley

Barak Valley is in the southern part of Assam named after the river Barak, the Barak Valley (also known as the Cachar plain) is located between the North Cachar and Mizo hills to the north and south, respectively. The grass-land covered an area of 0.40 ha which was equally demarcated into two study plots: (i) burnt plot where burning was applied to imitate a traditional management system of annual burning and (ii) unburnt plot maintained for comparative study. The seasonal variations in biomass were studied during April 2003 to March 2005. Singh et al. (1975) defined the trough-peak study, which was used to measure aboveground net primary output (ANP). According to Singh and Yadava's process, belowground net primary output (BNP) was calculated by adding positive increments from successive sampling dates (1974). The sum of the production values for rhizome and root represented the estimate of BNP (Pandey 1988).

Aboveground net primary production (ANP) during 2003-04 was slightly higher in unburnt plot than burnt plot that contradicts to 2004-05. There were inter-annual variations in net primary productivity during the two years of study. Contribution of important species ($1 g m^{-2}) to ANP is presented in Table 7. During 2003-04, 11 and 12 species contributed $1 g m^{-2} to ANP in burnt and un-burnt plots respectively. The same in 2004-05, were 10 species in burnt plot and 15 species in un-burnt plot. *Imperata cylindrica*, the sprouting grass was the most important species in the built up of ANP as its share amounted to 69% in burnt plot and 73% in unburnt plot, during 2003-04. These values in 2004-05, were around 60% in two plots. Involvement of other species was not as noteworthy except for *Borreria pusilla* (co-dominant species). This species rapidly colonizes the fallow land other than sprouting species and form mosaic of patches soon after burning. Of the total ANP, *Borreria pusilla* shared about 21.9% to 23.7% in burnt plot and 15.8 to 15.1% in un-burnt plot during the two years of study. It was observed that burning had stimulated the germination and growth of *Borreria pusilla* and consequently ANP maximizes in burnt plot.

Annual net accumulation in rhizome (RH) showed higher values in burnt plot during the study period. Reduction in rhizome production from 2003-04 to 2004-05 corresponded to the decline in shoot production (Table: 7). Annual net root production (R) followed the trend of rhizome component during 2003-04 and contradicted in 2004-05. Inter-annual variation indicated

that burning favored root growth and distribution in 2003-04 which was not conspicuous in 2004-05 (Table: 7).

Table: 7 Aboveground net primary production (ANP), Rhizome (RH), Root (R) and belowground net production (BNP) in the *Imperata* grassland. All values are g m^{-2} $year^{-1}$

Study plots	Year of study	ANP	RH	R	BNP
Burnt plot	2003-04	877.96	1027.54	299.11	1326.65
Unburnt plot	2003-04	936.08	738.23	243.11	981.34
Burnt plot	2004-05	653.26	716.45	257.34	973.79
Unburnt plot	2004-05	527.12	476.56	287.56	764.47

Source: Ashim Das Astapati and Ashesh Kumar Das (2010)

Table: 8 Peak biomass (g m^{-2}) of important species to ANP in the *Imperata* grassland

Species	BURNT PLOT		UNBURNT PLOT	
	2003-04	2004-05	2003-04	2004-05
Achyranthes aspera L.	1.00	-	-	1.48
Borreria pusilla (Wall.) D.C.	206.91	154.80	153.88	86.40
Chromolaena odorata (L.) R. King & H. Robins	-	1.24	11.80	2.28
Cyperus halpan L.	1. 30	6. 72	3.72	14.68
Desmodium caudatum (Thunb.) D.C.	8.80	-	-	-
Desmodium triflorum (L.) D.C.	-	-	-	1.00
Digitaria ciliaris (Retz.) Koel	21.84	11.12	21.36	25.80
Digitaria longiflora (Retz.) Koel	4.20	5.76	8.00	28.80
Dioscorea alata L.	-	-	-	1.44
Imperata cylindrica (L.) Beauv.	652.81	397.38	709.89	357.68
Ipomoea pestigridis L.	2.72	4.20	1.56	1.94
Leucas plukenetii (Roth.) Spreng	-	-	1.00	-
Lindernia crustacea (L.) F. Muell	-	1.08	-	4.40

Lygodium japonicum (Thunb.) Sw.	3.12	-	-	-
Melastoma malabathricum L.	-	-	-	1.56
Mimosa pudica L.	-	-	3.44	-
Setaria glauca (L.) Beauv.	39.12	59.76	34.40	27.04
Setaria pallidefusca (Schum.) Stapf & Hubb.	-	-	-	1.16
Triumfetta rhomboidea Jacq.	2.64	11.60	3.16	16.14
Urena lobata L.	-	-	19.20	-

Source: Ashim Das Astapati and Ashesh Kumar Das (2010)

Cherrapunjee plateau's net primary production in humid subtropical grassland

The study took place on the Cherrapunjee plateau, which is located about 50 kilometers south of Shillong, Meghalaya's North-eastern India's capital. Via focus group discussions and interviews with experienced elderly people (over 60 years old) from the region, proprietors of mine, and employed people, the age of recovery, as well as the grassland's land use past was decided. The UG (undisturbed grassland) is the study's sites are representative of pre-disturbance vegetation and soil conditions, without any previous coal-dumping records. The MG location (grassland impacted by mining activities) is now being used as coal storage dumps. The RG15 (Grassland recovery by a 15-year-old) and RG50 (Grassland with a 50-year-old recovery) sites were grasslands that had previously been used for coal disposal and had been abandoned for 15 and 50 years, respectively, and have been recovering naturally since then.

The biomass of live and dead shoots differed significantly between places (p 0.05). UG (undisturbed grassland) has the largest biomass and the lowest possible biomass in MG. UG had slightly more live and dead shoot mass than the other strands, indicating that total aboveground biomass (AGB) was significantly higher in UG. AGB has raised overall dramatically with the dawn of the age of regeneration in the recovering grasslands (Tukey's HSD test, p 0.05). The fine root biomass ranged from 944.6-1499 g m^2, UG had a marginally higher value than MG, RG15, and RG50 (ANOVA, p 0.001) (Table 9). Fine root biomass decreased as the age of recovery increased among recovering grasslands. The RG50 had the highest value, while the MG and RG15 did not vary significantly from the UG in terms of value (Table: 9). Complete root biomass (roots, both fine and coarse) followed a similar pattern

to fine root biomass. From the fifteenth to the fifty-first year of rehabilitation, total biomass, identified as the amount of biomass levels both above and below ground have greatly increased (Table: 9).

Aboveground annual net production varied between 40.1 and 425.3 g m^2 per year, by means of UG having the maximum and MG was having the lowest possible values (Table: 9). The age of recovery was associated with a rise in ANPP. RG15 had the highest annual fine root productivity and MG had the lowest, while RG50 had the highest coarse root productivity and RG15 had the lowest (Table: 9). UG had the highest total annual NPP (aboveground + belowground) and MG had the lowest. The sum annual NPP increased as the regeneration age of the grasslands increased (Table: 9). The maximum pace of shoot yield was found in UG, while the highest pace of root turnover was found in RG15. MG had the fastest shoot turnover time, while UG had the fastest root turnover time (Table: 9). In grasslands impacted by mining, the aboveground total biomass and annual production were compressed to 16 and 9 percent, correspondingly, of the uninterrupted grasslands. Belowground biomass and production in its entirety of the undisturbed grasslands were reduced to just about 70 percent and 85 percent, respectively, suggesting a more extreme negative effect on the aboveground compartment of the vegetation than on the belowground. Overall, total biomass (aboveground + belowground) and net annual production in the grasslands impacted by mining decreased by 36 percent and 30 percent, respectively (MG). One of the factors that contribute to the decrease in biomass and production in grasslands impacted by mining was the vegetation removal earlier to the extraction of coal and disposal, which decreased density of plants (Huang et al. 2015). Biondini et al. (1998) also discovered that 7 years of intensive grazing (removal of about 90% of ANPP) resulted in a reduction in aboveground, belowground, and litter biomass, although poorer grazing pressure (removal of 50% of ANPP) had no impact.

Table: 9 Biomass, annual net primary productivity, turnover rate, and turnover time of various recovery ages in grasslands.

Compartments	UG	MG	RG 15	RG 50
Biomass (g m^{-2})				
Live shoot	176.66 ± 15.2a	23.44 ± 2.0d	73.63 ± 3.4c	122.50 ± 8.2b
Dead shoot	28.44 ± 5.7a	10.11 ± 1.3b	39.86 ± 7.9a	24.40 ± 6.0a
Fine roots	1499.04 ± 112.9b	1002.17 ± 68.8b	981.50 ± 95.0a	944.58 ± 61.3a
Coarse roots	210.96 ± 37.1b	191.04 ± 30.1b	227.67 ± 30.3b	359.46 ± 49.8a
Total	1915.11	1226.76	1322.66	1450.94
Net annual primary productivity (g m^{-2} $year^{-1}$)				
Live shoot	425.30	40.11	80.21	312.51
Fine roots	1213.00	1002.17	1496.17	1262.00
Coarse roots	434.17	402.13	362.29	544.29
Total	2072.47	1444.41	1938.67	2118.80
Turnover rate ($year^{-1}$) and turnover time (years)				
Turnover rate				
Shoot	2.45	0.30	2.06	2.08
Root	0.38	0.52	0.70	0.68
Turnover time				
Shoot	0.41	3.33	0.49	0.48
Root	2.60	1.93	1.43	1.47

Source: Wishfully Mylliemngap & S. K. Barik (2019)

In south-eastern Montana coal mine spoils, Wyatt et al. (1980) discovered less root biomass than in undisturbed soils. Wang et al. (2006) found that root biomass was poorer in highly ruined sites compared to uninterrupted and renewed sites in a study in the alpine Kobersia steppe meadow in Qinghai province of China. Gao (2008) found that in the grasslands of Inner Mongolia, there is a lot of grazing decreased total root biomass and BNPP, which could be explained by smaller carbon absorbing organs and increased root carbohydrate re-translocation to shoot meristems.

Throughout the undamaged and improving grasslands, the proportion of biomass assigned to aboveground and belowground compartments differed. The contribution of aboveground biomass and productivity to total biomass and production was 11% and 21%, respectively in undisturbed grasslands, although the proportion aid in MG (3%), RG15 (9%), and RG50 (9%), respectively, were comparatively low. To put it another way, the percentage contribution of

belowground biomass in grasslands that have been impacted by mining and are recovering was higher than in grasslands that haven't been disrupted. The belowground compartment contributed a larger percentage of total biomass in all grasslands. The optimum biomass distribution to various sections of plants, according to several researchers (Müller et al. 2000; Poorter and Nagel 2000; Craine et al. 2001), is determined by the structure/function that is most constrained in their environment. The mined area's nutrient-depleted conditions may explain the increased biomass supply to the subsurface part in this report. Plants put more biomass into belowground organs as a result, allowing for more effective nutrient absorption from the soil. Furthermore, the mining and recovering grasslands had a greater proportion of aboveground and underground compartments than the undisturbed grasslands, implying a elevated nutrient burden in the mining and improving grasslands.

Primary production along altitudinal and rainfall gradients in humid grasslands

This study was carried out in humid grasslands, distributed over Shillong plateau in the state of Meghalaya in northeast India at three sites namely, Burnihat (BU), Upper Shillong (US) and Cherrapunji (CH). These grassland ecosystems are perturbation dependent systems which have evolved from natural evergreen and deciduous forests due to an increase in the pace of the age-old practice of slash and burn agriculture, and are maintained by fire and grazing (Uma Shankar et al., 1991).

The rate of phytomass accumulation varied significantly between seasons, vegetation compartments and sites. Early in the rainy season, the pace of aboveground addition was highest, and during the winter, it was lowest. BU, however, had greater rate during winter compared to late rainy season. Annual accumulation rate for shoot was two-fold greater at US as compared to CH. Belowground accumulation rate increased from low to high altitude. Root accumulation rate was more at BU than US, but rhizome accumulation rate showed a reverse trend. Total accumulation rate did not differ between BU and CH =, but it was slightly higher at US.

At BU, total net primary output was 1710 g m^{-2} $year^{-1}$, 1759 g m^{-2} $year^{-1}$ at CH, and 2018 g m^{-2} $year^{-1}$ at Upper shilling. When statistical restraints were applied, it declined by 11, 4and 4%, respectively. For further discussion, estimates without statistical restraints are considered real. A major part of total production (44-66%) occurred during early rainy season. Belowground production constituted a major part of the total production, 75% at BU, 83% at CH and 75% at US. Belowground production was significantly lower at BU

(1277 g m^{-2}) than US (1513 g m^{-2}) and CH (1466 g m^{-2}). Rhizome contributed 23% at BU and 46% at US to the belowground production.

Table 10 Average (± SE, n = 130) standing crop of phytomass (g m^{-2}) in different vegetation compartments of three humid grassland in India. (* difference with next site significant at p < 0.05, # includes rhizome also).

Compartment	Burnihat	Upper Shilong	Cherrapunji
Aboveground phytomass	434 ± 15	467 ± 22	499 ± 21*
Live shoot	263 ± 25*	183 ± 34	185 ± 26*
Dead shoot	67 ± 10*	155 ± 14	163 ± 25*
Litter	103 ± 17	129 ± 15	151 ± 11*
Belowground phytomass	1026 ± 114*	1509 ± 116*	1274 ± 132
Live root	714 ± 73*	897 ± 100*	1216 ± 129*#
Live rhizome	158 ± 24*	297 ± 62	- - - -
Dead root	93 ± 31*	231 ± 71*	59 ± 13#
Dead rhizome	61 ± 17	84 ± 22	- - - -
Live belowground	872 ± 87*	1194 ± 116	1216 ± 129*
Dead belowground	154 ± 41*	315 ± 79*	59 ± 13*
Total phytomass	1460*	1976*	1773*

Source: P.S. Pathak and J. C. Dagar (2016)

Table 11 Seasonal phytomass accumulation rates (g m^{-2} d^{-1}) in different vegetation compartments of three humid grasslands in India. (# includes rhizome also)

Season	Days	Shoot	Root	Rhizome	Total
Burnihat					
Early rainy	103	2.18	3.04	2.10	7.32
Late rainy	79	0.70	4.03	0	4.73
Winter	135	0.95	0	0.30	1.24
Spring	65	0.37	5.49	0.51	6.37
Annual	383	1.13	2.59	0.76	4.48
Upper Shillong					
Early rainy	89	2.60	6.38	2.89	11.87
Late rainy	95	0.92	2.08	4.01	7.02
Winter	127	0.35	0.36	0.22	0.93
Spring	65	2.18	0.15	0.38	2.72

Annual	376	1.34	2.19	1.84	5.37
Cherrapunji					
Early rainy	91	1.60	10.69[#]	- -	12.30
Late rainy	86	0.57	3.41[#]	- -	3.97
Winter	131	0	0.55[#]	- -	0.55
Spring	71	1.39	1.80[#]	- -	3.20
Annual	379	0.77	3.87[#]	- -	4.64

Source: P.S. Pathak and J. C. Dagar (2016)

Conclusion

Grasslands are significant on a global scale because they occupy about one-fifth of the world's ground surface and account for 80 percent of all agriculturally productive land. Grassland ecosystem's spatial distribution, population composition, and net primary production affect regional climate by modulating evapo-transpiration flux. Precise data on NPP, carbon sequestration, and stocks are scarce, particularly in India's grasslands, where different grassland communities arise as a result of climatic and geographical factors that are highly variable.

In this chapter, we attempted to investigate and figure out systematic studies on net primary production and carbon stock in Indian grasslands from almost all grassland types. Destructive methods (algorithms for estimating biomass from grassland plant biomass measurements) and non-destructive techniques are used to estimate biomass and NPP. Estimation is also done with remote sensing data, but it needs field validation.

Tropical grassland had the highest overall net primary production, followed by subtropical, alpine, and temperate grassland.

The current chapter summarized various estimates of biomass and NPP, carbon pool, and sequestration from various grassland forms in India. The estimate can be used to create an information that can be used in favor of policymaking, especially in the areas of climate change mitigation and conservation.

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Recent advances in heavy metal tolerance through the transgenic plant development

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Abstact

Rapid urbanization has an impact on the environment that causes disturbance in the ecosystems and the normal biogeochemical cycles. The accumulation of heavy metals increases due to these ecological perturbations. Heavy metals are non-biodegradable, inorganic chemicals having an atomic mass above twenty. Plants require these metal ions in trace amounts for their growth and development, but in higher amounts impair normal metabolism and growth and may lead to death. The heavy metals cause the generation of reactive oxygen species (ROS) that cause damage to the cell membrane, enzymes, and DNA. The irreparable DNA damage may trigger apoptosis. Some wild plants are avoided or tolerate heavy metals because they either detoxify the toxic metals or accumulate them in their vacuoles where they may be harmless. To overcome the problem of heavy metal stress, transgenic plants are generated by transferring genes from their wild relatives or synthesizing artificially. The genes transferred in transgenic plants are codes for a protein, mainly enzymes that help in the detoxification of heavy metals and removal of ROS in oxidative stress.

Key Words: Heavy metals, DNA damage, ROS, stress, transgenic plants, etc.

1. Introduction

There are nine metals, Cr, As, Cu, Mn, Fe, Cd, Ni, Pb, Se, Hg, Zn, and Al categorize as heavy metals causing stress in plants when accumulates beyond optimal limit. These metals mostly create oxidative injury either directly or indirectly and generate ROS that cause further damage. Heavy metals can produce ROS directly via the Fenton and Haber–Weiss reactions (Manara, 2012). The reactive oxygen species generates free radicals likesuperoxide anion radical (O_2), hydroxyl radical (HO*) and hydrogen peroxide that cause oxidation of proteins and membrane lipids and as well as causing injury to DNA. Heavy metals also bind strongly to oxygen, nitrogen

and sulfur atoms thereby decreasing the solubility of these and inactivate biomolecules like enzymes (Schutzendubel and Polle, 2002).

1.1 Toxic effects of heavy metals

The heavy metals like chromium (Cr) enter into the plants through absorption by the roots. They accumulate in the root cell in higher concentrations and inhibits cell division and cell elongation and as a result the root growth is severely inhibited in *Helianthus annuus*, *Amaranthus viridis* restricted the absorption of water and nutrients (Shanker et al, 2005; Zou et a,l 2006; Fozia et al, 2008; Nematshahi et al, 2012; Emamverdian et al, 2015). Copper is essential redox-active metal ion involved in many physiological processes like electron transport chain in mitochondria and chloroplast. It is cofactor for many enzymes and play essential role in signalling (Yruela 2005). At higher concentration copper is toxic to the plants because it inhibits the growth by reducing chlorophyll contents and alteration of chloroplast structure. It impairs in various vital cellular process like photosynthesis and respiration by modifying the pigment and protein composition of membranes. (Prasad and Strzalka, 1999; Quartacci et al, 2000).

Copper ions are responsible for the destruction of oxygen-evolving complex polypeptide and the main target of Cu toxicity is the PSII reducing side, the Q_B binding site and the Pheo-Fe-Q_A (Yruela, 2005). Mn is required by the plants for their growth and many essential processes like photosynthesis, respiration and cofactor for many enzymatic reactions. Their toxicity symptoms include darkening of leaf veins, chlorosis, depressed root and shoot growth etc. (Reddy, 2006; Emamverdian et al, 2015). Cadmium ions are more readily absorbed by the plant roots (Wagner, 1993). From the roots Cd is translocated efficiently to the other parts of the plants. It accumulates in tissue and exerts its toxic effects there. Toxicity of Cd leads to growth inhibition and chlorosis of leaf. There is disturbance in plant water balance due to high Cd^{2+}doses. Some metabolic enzymes are sensitive to cadmium ions. Toxicity ofnickel hampered the activity of various hydrolytic enzymes like amylase that are involved in the germination of seeds. Growth of the plant in case of lateral root development plant height and leaf area are severely reduced. There is damage to membrane and disfunction of various enzyme occur due to the generation of ROS. Leaf chlorophyll contents also reduced in nickel toxicity (Emamverdian et al, 2015). The toxicity of zinc supersedes the availability of other ions like manganese and phosphorus. It also increases the production of reactive oxygen species. Aluminium is the most abundant metal in earth's crust, it is most phytotoxic in Al^{3+} form released when the soil become acidic

(Moffat, 1999). Al is inhibitory to the root elongation and growth because it inhibits the cell division. It interacts with the root cytoskeleton and induced alteration in microtubule and actin cytoskeleton structure (Barlow and Baluska, 2000; Sivaguru et al, 2000). Al toxicity hampers in the nutrient uptake efficiency of the root that affects arial parts also. Appearance of small necrotic spots on the border of young leaves and chlorosis of the young leaf, reduction in stomata and decrease photosynthetic activity are the common features of the Al toxicity (Emamverdian et al, 2015).

The main effect caused by the heavy metal toxicity includes generation of ROS, leaf chlorosis, growth retardation by impairing photosynthesis and respiration and ultimately death of the tissues that finely cause reduction in yield.

1.2 Plant defense to heavy metal poisoning

Plants have various defense mechanisms to combat heavy metal poisoning. These processes include the efflux of metals ions, detoxification, chelation and compartmentation in the vacuole. These operates at sensitive sites to protect the plants to build up toxic concentration of metal ions (Hall, 2002). There are some tolerant plants that hyperaccumulates metal ions are *Thlaspi* species and *Arabidopsis helleri* accumulates zinc and cadmium (Chiang et al. 2006). Plants can minimize the entry of the metal ion by precipitating or by complexing heavy metals with the root exudates (Hossain et al. 2012). In recent years large number of new metal transporters families has been identified that helps the plant to exit metal ions. These transporter families include ATP-binding cassette (ABC), P1B-type subfamily of p-type ATPases, natural resistance associated macrophage protein (NRAMP), multidrug resistance-associated proteins (MRP), Zn-regulated transporter and Fe-regulated transporter protein (ZIP family), ABC transporters of the mitochondria (ATM), cation diffusion facilitator (CDF), copper transporter (COPT),pleiotropic drug resistance (PDR), Ca-sensitive cross complementer 1 (CCC1), iron-regulated protein (IREG), yellow-stripe 1-like (YSL) subfamily of the oligopeptide transporter (OPT) superfamily, etc. These helps in the sequestration of metal ions in the vacuole by forming complexes with heavy metals like cadmium. In plants, these complexes are stored in the vacuoles of mesophyll cell and root cells (Cobbtte, 2000).

Another important mechanism of defense is the chelation of heavy metals. Phytochelatins (PCs) and metallothioneins MTs are cysteine-rich, heavy metal-binding proteins. The ultrastructure of PCs indicates that it is a family of peptides that contained repeated γ-Glu-Cys residue of the dipeptide

followed by a terminal Gly-the basic structure being (γ-Glu-Cys)n–Gly [(PC)n], where n is generally in the range two to five. Physiological, biochemical and genetic studies confirmed that GSH is the substrate for PC biosynthesis (Cobbett, 2000).

Metallothionins are the proteins which are categorized as metal binding proteins. The genes for the biosynthesis of MT proteins are found in almost all organisms. In plants, some metallothioningenes were isolated and characterized. The MT proteins contains various cystein rich motifs on the basis of which, these were classified into four types. There are Cys-Xaa-Cys motifs, Cys-Gly-Gly-Cys motifs, Cys-Gly-Asn-Cys-Asp-Cys motifs and Gln-Cys-Xaa-Lys-Lys-Gly motifs etc (Cobbett, 2000). MT proteins provides tolerance to copper and cadmium by their metal binding properties like PCs.

2. Genes involved in heavy metal tolerance

Due to the availability of new tools in genomics large numbers of genes related to heavy metals tolerance has been identified and characterized. New genes were discovered through *in silico* search from the data that become available in database.

2.1 Genes involved in amino acid biosynthesis

Some free amino acids accumulate in the plants under stress and act as an osmoprotectants and free radicle scavenger. These specific amino acids such as proline and histidine and polyamines like spermine, spermidine, putrescine play pivotal role in various environmental and heavy metal stress tolerance (Sharma and Dietz, 2006). Free proline act as an osmoprotectant, protein stabilizer, metal chelator, inhibitor of lipid peroxidation, hydroxyl radical scavenger and a singlet oxygen scavenger (Alia et al, 2001; Siripornadulsil et al, 2002).

The biosynthetic pathway of proline in plants can be divided into two, glutamate pathway and orinithine pathway. Mainly glutamate pathway is responsible for proline accumulation during osmotic stress. The pathway starts from glutamatic acid via intermediate Δ'-pyrroline-5-carboxylate (P5C) catalyzed by Δ'-pyrroline-5-carboxylate synthetase (P5CS) and Δ'-pyrroline-5-carboxylate reductase (P5CR). P5CS is encoded by two genes whereas P5CR is encoded by only one in most plant species. The second alternative pathway, proline can be synthesized from ornithine, which is transaminated to P5C by orinithine-δ-aminotransferase. Ornithine pathway for proline synthesis is used during seedling development and for stressinduced accumulation (Hayat et al 2012). The catabolism of proline occurs in mitochondria by the enzymes

named proline dehydrogenase or proline oxidase (PDH or POX) producing P5C from proline and P5C dehydrogenase (P5CDH) which converts P5C to glutamate. Two genes encode PDH, whereas a single gene encode P5CDH. PDH transcription is activated by rehydration but repressed during dehydration, thereby preventing proline degradation during stress (Hayat et al 2012).

Histidine synthesis is also increase during stress in some plants that accumulates metals. Nickel accumulators produced free histidine in their xylem sap. Transgenic plants were developed in *Arabidopsis thaliana* that showed tenfold increase in biomass production (Wycisk et al, 2004; Sharma, 2006). All the enzymes of histidine biosynthetic pathway are encoded by single genes. The pathway is composed of 10 enzymatic reactions and there are eight genes involved in it. In *Salmonella typhimurium* these genes are designated as *his*G, *his*D, *his*C, *his*B, *his*H, *his*A, *his*F and *his*I (Alifano et al, 2006; Stepansky and Leustek in 2006). *his*D and *his*B encoded bifunctional proteins. *hisA* is a homolog of *His6* in *Arabidopsis thaliana* (DeFraia and Leustek, 2004). The other amino acids involved in heavy metal tolerance are asparagine, cysteine, glutamine. Asparagine formed Zn-asparagine complex help to reduce Zn toxicity while other two amino acids help in nickel, copper and cadmium tolerance (Sharma, 2006).

The poly amines are ubiquitous in all living organisms, these are small, positively charged, organic molecules. Putrescine, spermidine, and spermine are the three common poly amines found most abundantly in plants. Polyamines play very important role in plant stresses they are the most of the time perfect modulators of stress but their mechanism is poorly understood. The polyamines biosynthetic enzyme genes have been expressed in various plants through the *Agrobacterium*-mediated genetic transformation. There are various genes like S-adenosylmethionine decarboxilase (SMDC) enzyme, ornithine decarboxilase (ODC), Ado Metdecarboxilase (ADC) are cloned and transferred to various plants under the control of CaMV 35S constitutive promoter or tissue specific promoter (Minocha et al, 2014).

Glutathione (GSH) is a powerful antioxidant and protecting the plants from heavy metal and various environmental stresses. plays an important role in various physiological functions that includes redox regulation, conjugation of metabolites, detoxification of xenobiotics and homeostasis and cellular signalling that trigger adaptive responses (Noctoret al, 2002; Grata õ et al, 2005) GSH is considered to be the precursor of metal binding PCs.

2.2 Genes involves in metal binding protein

The genes for the phytochelatins in plants and yeast has been identified and characterized. The PC synthase genes was isolated from *Arabidopsis* by *CAD i*.e. cadmium sensitive mutants and referred to as *AtPCS*1, similar genes in wheat are*TaPCS*1 and yeast *SpPCS*1. The phytochelatin biosynthesis genes are *Gsh1* (GCS/GSH biosynthesis), *CAD2/RML 1*(*GCS/GSH biosynthesis*), *Gsh*2 (GCS/GSH biosynthesis), *CAD*1(PC biosynthesis), *PCS*1(PC biosynthesis) (Cobbett, 2000). Another metal binding proteins is MTs The gene structure is divided into four types. All the MT genes in plants contains an intron present closely to the N-terminal cysteine-rich domain. There are four types of MT genes in which position of this introns differs. The single intron in Type 1 MT genes from monocots disrupts the codon after the last cysteine codon in the N-terminal cysteine-rich domain.This is the same position as the single intron in *Arabidopsis* Type 2 MT genes and the first of two introns in Type 2 MT genes in rice. All type 3 MT genes that have been characterized contain two introns, and the first lies in the same relative position after the end of the N- terminal cysteine-rich domain. Type 4 MTs lies in the codon preceding the last cysteine codon of the first domain. The seven members MT genes family in the *Arabidopsis* genome are organized as: The *MT1a* and *MT1c* genes lie within 4 kb as an inverted repeat on chromosome 1 *MT2a* and *MT3* are at distinct positions on chromosome 3; both *MT4a* and *MT4b* lie on chromosome 2 but are not closely linked; finally, *MT2b* is positioned on chromosome 5. One pseudo- gene, *MT1b*, has been identified in *Arabidopsis* and is also found on chromosome 5. In tomato and rice genome the MT genes are also distributed on different chromosomes. But in cotton there is a MT gene clustering has been found in cotton where three MT genes were identified within a 10-kb fragment of genomic DNA (Hudspeth et al 1996; Giritch et al. 1998; Cobbett and Goldberg 2002).

2.3-Genes for the transporter proteins

The plasma membrane and tonoplast transporter proteins families are mainly involved in metal uptake and homeostasis. These transporters belong to the heavy metal P_{1B}-ATPase, the NRAMP, the CDF (Williams et al. 2000), and the ZIP families (Guerinot, 2000).The ZIP family of transporter are involved in translocation of divalent cation like Fe and Zn. ZIP family of transporter have been identified in many plant species and in bacteria, fungi and animals. Another transporter family is NRAMP metal transporters have been shown to transport a wide range of metals, such as Mn^{2+}, Zn^{2+}, Cu^{2+},

Fe^{2+}, Cd^{2+}, Ni^{2+}, and Co^{2+}, across membranes.These transporters have been identified in bacteria, fungi, plants, and animals (Nevo and Nelson, 2006). In plants, NRAMP transporters are expressed in roots and shoots and are involved in transport of metal ions through the plasma membrane and the tonoplast (Krämer et al, 2007). NRAMPs in *A. thaliana* transport Fe and Cd, with *NRAMP*1 playing a specific role in Fe transport and homeostasis (Thomine et al, 2000). The AtNramp1 gene is induced under limiting Fe conditions. (Curie et al, 2000; Manara, 2012). The copper transporter family is the putative transporter responsible for the copper uptake. In *A. thaliana* COPT1 has been shown to transport copper, and it also has a role in growth and pollen development (Sancenón et al, 2004).The P-type ATPases also known as HMAs function not only as efflux pumps to remove metal ions from the cell, but also as internal transporters to load Cd and Zn metals into the xylem from the surrounding tissues.MATE is a family of membrane-localized efflux proteins involved in extrusion of multidrug and toxic compound from the cell. FRD3 is a MATE protein that participates in iron-citrate efflux, i.e., the loading of Fe^{2+} and citrate into the vascular tissue in the roots. Oligopeptide Transporters (OPT) is a superfamily of oligopeptide transporters including the YSL subfamily. The YSL family, specific for plants, takes its name from the maize Yellow stripe 1 protein (ZmYS1) that mediates Fe uptake by transporting Fe(III)-phytosiderophore complexes (Curie et al. 2001).ABC transporters can transport xenobiotics and heavy metals into the vacuole, and two subfamilies (MRP and PDR) are particularly active in the sequestration of chelated heavy metals. Plant cell vacuoles are, in fact, the major site for accumulation and storage of PC-Cd complexes.The AtHMA3 isa transporterprotein that belongs to the P_{1B}-adenosine triphosphate transporter family. It is involved in heavy metal transport that help in the intracellular sequestration of Cd (Gravot et al, 2004).

3. Transgenic plants expressing stress tolerance genes

Genes can be transferred to developed transgenic plants that are tolerant to various metal toxicants. Such typesof various gene were cloned and characterized from microorganism that have the mechanism of metal tolerance and detoxification. New genes can be identified in plants and characterized on the basis of the bioinformatic tool that hyper accumulates and sequester metal ions. These genes used to develop transgenic plants so that they acquired capacity to tolerate or accumulate metal ions and become an ideal plant for cleaning the environment to the metal toxicants. Generally, the genes can be over express in the transgenic plants using a constitutive promoters like actin,

ubiquitin, CaMV 35S, Maize alcohol dehydrogenase-1 etc. Most of the time the over expression of microbial genes in plants is an excellent way to engineer plants because there is no feedback inhibition occurs by the end product.

3.1 Transgenic plants tolerant to chromium

Transgenic plant was developed by transferring achromium (VI)reductase gene from heavy metal reducingbacteria *Pseudomonas aeruginosa* HP014 in tobacco by Jin et al in 2001. The transgenic tobacco plants were showed efficient chromium reduction, it also helps in the reduction of the toxic chromium (VI) in the soil. In yeast, (*Saccharomyces cerevisae*) chromium is taken up via sulfatetransporters, yeast transcriptional activator MSN1 expression in transgenic tobacco (*Nicotiana tabacum*) enhance the accumulation of chromium and sulfur (Kim et al, 2006)Transgenic mustered (*Brassica juncea*cv pusa jai kisan) is best example for tolerant to the chromium because it hyper accumulates the chromium from the soil (Unnisa et al, 2008).

3.2 Transgenic plants tolerant to cadmium

Metallothioneins (MTs) are metal-binding proteins that provides tolerance to heavy metal specially to cadmium and it promotes the accumulation of cadmium in vacuole. Cadmium tolerant transgenic *B. napus* and *N. tobaccum* plant was developed through the transfer of a chimeric gene human metallothionine II (MT-II) (Misra and Gedamu,1989).Transgenic tobacco lines can tolerate the 100M $CdCl_2$ as compared to the untransformed control plants. In another report metallothionine-I gene was fused with CaMV 35S promoter and nosterminator and transformed in tobacco plant using *Agrobacterium.* The transformed lines were tolerant to about 200UM cadmium as compared to control (Pan et al, 1994). Yeast Metallothionine genes (CUP1) is also transformed in *Brassica oleracea* var. botrytis (Hasegawa et al. 1997) and tobacco (Krystofova et al, 2012). *B. oleracea* was tolerant to about 400µM cadmium while tobacco plants were shown cadmium accumulation in roots and showed tolerance to 250µM cadmium. This gene (CUP1) is combined polyhistidine cluster(HisCUP) and was expressed in tobacco. The transgenic lines were assessed for the cadmium accumulation and tolerance by culturing different levels of cadmium. Transgenic tobacco (HisCUP) lines were accumulating cadmium and showed good growth untransformed control plants (Pavlíkováa et al 2004).

Reduced form of glutathione (GSH) is provided tolerance to heavy metals by protecting the cell from oxidative stress like lipid per oxidation etc. It is also a precursor of metal binding protein phytochelatins. γ-glutamylcysteine

synthetase gene is the rate limiting enzyme of glutathione synthesis because of the supply of cysteine. Overexpression of glutathione reductase II (*gsh*II) of *E. coli* in *B. juncea*showed threefold increase in Cd accumulation in shoots and more synthesis of GSH and phytochelatins (Arisi et al 1997). Similarly, another gene *gsh*Iencodingγ-glutamylcysteine synthetase was genetically transformed in Indian mustard (*Brassica juncea*). transgenic plants wereaccumulating more cadmium in shoots and shows cadmium tolerance than wild plants (Zhu et al 1999). The cytosolic cysteine synthase gene of rice (*Oryza sativa*) *RCS*1was transformed in tobacco. It was observed that this gene help in the production of sulphur compounds (various enzymes for the removal of ROS) for the metal detoxification and also help in the elimination of toxic metal from the plant system (Harada et al. 2001). *Phaseolus vulgaris* stress related genes (*PvSR*2) expressed in french bean upon the heavy metal exposure. The *PvSR*2 gene constitutively expressed in tobacco and the transgenic plants were showed higher tolerance to cadmium as compared to wild type. However, the accumulation of cadmium was lower in the roots of transgenic plants (Tuanyao et al. 2003). *Phaseolus vulgaris* stress related genes (*PvSR*2) expressed highly during heavy metal stress. It was expressed constitutively in tobacco and the seedlings of transgenic lines were showed higher tolerance to cadmium compared to wild type. Further, transgenic plant showed lower root Cd content at higher external Cd concentration than untransformed control (Chi et al. 2003).cDNA of Phytochelatin synthase (PCS) gene of *Arabidopsis thaliana* was expressed constitutively in Indian mustard (*B. juncea* L.) showed higher tolerance to Cd and Zn. However, the accumulation of Cd and Zn was not significantly increased (Gasic and Korban, 2006). *Arabidopsis thaliana*phytochelatin synthase (*AtPCS*1) gene expressing in Indian mustard under its native promoter and the transgenic lines were assessed for the Cd and As accumulation and tolerance. As compared to wild type plants the transgenic plants have higher tolerance to Cd and as and accumulates higher amounts of Cd but the accumulation ofAs was similar to untransformed plants (Gasic and Korban, 2007). *AtPCS from A. thaliana* and *CePCS*genefrom *Coenorhabditis elegance* was transferred in *N. tobaccum*L. *var.*Xanthi. The transgenic lines were showed moderately decrease cadmium-detoxification and increased cadmium sensitivity (Wojas et al, 2008). *Arabidopsis*phytochelatin gene (*AtPCS1*) over expressed in *Arabidopsis thaliana* wild-type plants (ecotype Columbia) and tobacco. Intransgenic lines of *Arabidopsis* at relatively low external Cd concentrations, the Cd tolerance of *AtPCS1* lines was lower than the untransformed control, while in tobacco over expressing *AtPCS1* was more tolerant to Cd than untransformed control.

At higher Cd concentration *Arabidopsis AtPCS1* seedlings were showed increase tolerance to Cd while tobacco seedlings are sensitive as compared to their respective untransformed controls (Brunetti et al 2011). Coontail (*Ceratophyllum demersum*) plants have shown highest accumulation (1293µg Cd/g at 10µm) of cadmium. A significant increase was also observed in non-protein thiols including PCs, cystein and glutathione. The activities of cystein synthase, glutathione-S-transferase, glutathione reductase and phytochelatin synthesisgene was increase at higher cadmium levels. This indicates that *C. demersum* has strong adaptation to heavy metal stress particularly with cadmium (Mishra et al 2009). Phytochelatin synthesis gene (PCS) was isolated and characterized from *C. demersum* and the expression of *CdPCS*1 gene in *E. coli*, *Arabidopsis* and tobacco enhanced PCs synthesis and metal accumulation (Shukla et al, 2013).

Transgenics were also developed through the transfer of the transporter proteins, that transport metal ions in the vacuole. In *Arabidopsis*, low affinity calcium antiporter AtCAX2 is related to calcium, and magnesium transport (Hirschi et al, 1996). Its expression, in tobacco results in increase Cd^{2+} and Mn^{2+} in isolated root tonoplast vesicles, indicating CAX2 has a broad substrate range transporter (Hirschi et al. 2000). Heavy metal transporter (Pb(II)/Cd(II)/Zn(II) pump) in *E. coli* encoded bygene, *Znt*A was genetically transformed in *Arabidopsis.* The transgenic protoplast accumulated less Cd and release faster preloaded Cd than wild-type protoplasts (Lee at al. 2003).

The yeast cadmium factor gene (*YCF1*) from *Saccharomyces cerevisiae*, is a type of ABC transporter and Other member of this group is human multi drug resistance-associated protein (MRP1). The gene encodes a protein of 1, 515-amino acid and provide resistance against heavy metals, like cadmium (Li et al. 1996). It is a vacuolar glutathione S-conjugate pump. Transgenic *Arabidopsis* plants were developed that over expressing *YCF*1 showed that plants have enhanced tolerance to Pb(II) and Cd(II) and accumulation of these metals was also higher (Song et al 2003). *3.3 Transgenic plants tolerant to copper*

The metallothionine-like genes were isolated form many plants that provide tolerance against heavy metals and promotes accumulation in vacuoles. One such gene is *Pisum sativum* metallothionine-like genes that analogous to plants methallothionin have role in metabolism and metal detoxification. The *PsMTA* gene was constitutively express in *E.coli* and *Arabidopsis thaliana* enhance copper accumulation andaccumulates several-fold Cu than transformed plants (Evans et al 1992). Yeast metallothionine gene was

transformed in tobacco and the transgenic plants were assessed for the Cu accumulation. The transforments accumulated seven times more copper in older leaves than the wild type (Thomas et al. 2003).

3.4 Transgenic plants tolerant to Aluminium

The major limitations for the production of agricultural crop are acid soil that contains toxic levels of aluminium. The best mechanism of Al tolerance in higher plants is the Aluminium-induced secretion of organic acid anions, mainly citrate, oxalate, and malate, from roots. citrate has the highest chelating activity among the three organic acid anions, for Al followed by oxalate and malate (Yang et al 2013). The bacterial (*Pseudomonas aeruginosa*) citrate synthase gene was expressed in *N. tobaccum.* The citrate synthase protein was expressed up to 100-fold higher than the previous lines (Delhaize et al 2001). Tesfaye et al (2001) over express using nodule-enhanced forms of malate dehydrogenase and phosphoenol-pyruvate carboxylase cDNAs under the control of CaMV 35S promoter in alfalfa. In the transgenic plants the activity of malate dehydrogenase increases with 7.1-fold increase of root exudation of citrate, oxalate, malate, succinate, and acetate than untransformed control whereas phosphoenol-pyruvate carboxylase trangenics were not showed increased root exudation.The over expression of mitochondrial citrate synthase gene in yeast and canola results in 2 to 3 fold increase in citrate synthase activity and enhanced levels of Al tolerance (Anoop et al 2003). The alfalfa plant was also engineered using *P. aeruginosa* citrate synthase gene under the control of Act2 constitutive promoter and tobacco RB7 root specific promoter. Two transformed lines showed more aluminium tolerance than untransformed control (Barone et al 2008). The Yuzu (Citrus junksSieb. ex Tanaka) plant secrete citrate upon exposure of Aluminium. The citrate synthase gene from Yuzu (*CjCS*) was cloned transformed into *N. benthamiana*showed increase expression of *CjCS* geneand Al^{3+} tolerance (Deng et al 2009). The efflux of the metal from the root is necessary and it is facilitated by members of MATE (multidrug and toxic compound exudation) family of transporter. Zhou et al (2014) was transformed barley (*Hordeum vulgare*) with two MATE genes to increase the Al^{3+} tolerance. These genes were *SbMATE* from shorgham and *FRD3* involved in iron nutrition in *Arabidopsis.* Transgenic lines expressing *Sb*MATE showed increased tolerance to Al^{3+} and aluminium activated citrate efflux from root apices. The *FRD*3 lines was also showed similar phenotype.

3.5 Transgenic plants tolerant to iron

The ferritin is iron storage protein that can stored iron molecule in the vacuole. The ferritin gene was cloned from soybean and first of all transferred in tobacco and rice plants under the control of CaMV 35S promoter and seed storage protein gluten promoter GluB-1. The accumulation of iron was 30% higher in the leaves of tobacco transformed lines than untransformed plants. The transgenic rice was also showed threefold increase iron contents compared to untransformed plants (Goto et al, 1998, 1999). Similar observation was made by Vasconcelos et al 2003 and they also observed the accumulation of zinc was improve in transgenic tissue. Soybean ferritin cDNA was transformed in rice and wheat and expressed constitutively. The transgenic lines were showed increase iron levels in vegetative tissues but not in seeds. The levels of ferritin mRNA and protein were lower in wheat and rice seeds (Drakakaki et al, 2000).

3.6 Transgenic plants tolerant to selenium

The toxicity of selenium is a serious problem, to overcome this new biotechnological approach has been used to generate transgenic plants. The transgenic plants are showed enhanced tolerance and selenium accumulation. Sulphur transporters and enzymes are used by the plants for the uptake and assimilationof Se (Pilon-Smits and LeDuc, 2009). The strategy used to generate transgenic plants is through modulation of sulphur assimilation enzymes and transporters. Selenate aassimilation occurs through the reduction step is mediated by ATP sulpfurtlase gene *Arabidopsis APS*1gene was transferred in Indian mustard (*Brassica juncea*). The ATP sulfurylase activity was 2 to 2.5-fold higher in the seedling and 1.5 to 2-fold higher in shoots but not in roots of mature plant as compared to untransformed plants (Pilon-Smith et al. 1999). This was may be because of their enhanced Se assimilation and tolerance indicating thatATP sulfurylase is involved in selenate reduction and the assimilation of selenate. Mouse (*Mus musculus*) Se-Cys lyase specifically catalyzes the decomposition of Se-Cys into elemental Se and alanine. The Se-Cys lyase gene was expressed in the cytosoland chloroplast of *Arabidopsis* and the transgenic plants were showed enhanced activity of the enzyme (2-fold in and 3-fold respectively). The *cytSL* transgenics display both higher Se tolerance and higher shoot Se concentrations. The same strategy may be used to create SL transgenics of other plant species (Pilon et al 2003). The enzyme Cystathionine-gamma-synthase (CGS) is catalyzing the synthesis of Se-cystathionine from Se-cysteine, the first step in the conversion of Se-cysteine to volatile dimethylselenide. Cystathionine-gamma-synthase from *Arabidopsis*

thaliana (L.) Heynh. was overexpressed in Indian mustard [*Brassica juncea* (L.) Czern&Coss], The transgenic CGS lines were showed 10-fold enhanced CGS levels were compared with wild-type Indian mustard with respect to Se volatilization, tolerance and accumulation. The CGS transgenics showed 2- to 3-fold higher Se volatilization rates than wild-type plants when supplied with selenate or selenite. Transgenic CGS plants contained 20-40% lower shoot Se levels and 50-70% lower root Se levels than the wild type when supplied with selenite. CGS seedlings were more tolerant to selenite than the wild type. Indian mustard is used for the phytoremediation purposes. *Arabidopsis* and mustard was transformed with the gene encoding selenocysteine methyltransferase (SMT) from the selenium (Se) hyperaccumulator *Astragalus bisulcatus.* The SMT transgenic seedlings tolerated Se, significantly better than the untransformed plants and producing 3- to 7-fold greater biomass and 3-fold longer root lengths. Also, SMT transgenic plants had significantly increased Se accumulation and volatilization. This indicates, that SMT gene increase the phytoremediation potential of the crop (LeDuc et al 2004). The Se tolerant transgenic was developed by Hoewyk et al (2005) by over expressing chloroplasticNifS-like protein in *Arabidopsis thaliana* (*AtCpNifS*) that can catalyze the conversion of cysteine into alanine and elemental sulfur (S^0) and of selenocysteine into alanine and elemental Se (Se^0). The transgenic plants showed significantly enhanced selenate tolerance (1.9-fold) and Se accumulation (2.2-fold). Moreover, the transformed lines were showed significantly reduced Se incorporation into protein, which may explain their higher Se tolerance. The sulfur accumulation was also enhanced by approximately 30% in CpNifS over expressors, both on media with and without selenate. The double transgenic of Indian mustard was also developed using SMT and APS genes that showed nine-fold increase in Se accumulation (LeDuc et al 2006).

3.7 Transgenic plants tolerant to lead

Nicotiana tabacum calmodulin-binding protein (NtCBP4) and its truncated version (NtCBP4-C) (from which its C-terminal, with the calmodulin-binding domain and part of the putative cyclic nucleotide-binding domain, was removed) was over express in tobacco and to *Arabidopsis thaliana* ecotype Wassilewskija. The transgenic that express truncated genes were showed improved tolerance to Pb^{2+} than the normal gene. In another case, the disruption of the *ArabidopsisCNGC*1 geneby T-DNA insertion mutagenesis, which encodes a homologous protein, also conferred Pb^{2+} tolerance (Sunker et al 2000). Another case was *Triticum aestivum*phytochelatin gene (*TaPCS*1) that was identified cloned and

characterised, its cDNA encodes 55kDa protein. The protein show homology with *Arabidopsis thaliana, Schizosaccharomyces bombe and coenorhabditis elegance* (Clemens et al, 1999). *N. galuca*was genetically modified to produce the *Ta*PCS protein and the transformed lines were showed increased tolerance to lead and cadmium. The accumulation of these metals was also higher in these transgenic lines (Gisbert et al. 2003).

3.8 Transgenic plants tolerant to multiple metal ions

Nicotiana tabacum calmodulin-binding protein (NtCBP) that can modulate plant tolerance to heavy metals. Tobacco transgenic plants overexpressing this protein was developed that showed higher level of NtCBP4. the transgenic lines exhibited tolerance to Ni2+ and hypersensitivity to Pb2+, which are associated with reduced Ni2+ accumulation and enhanced Pb2+ accumulation, respectively (Arazi et al. 1999).Bacterial ACC (1-aminocyclopropane-1-carboxlic acid) deaminase was constitutively, and root specifically expressed in transgenic plants of tomato (*Lycopersicon esculantum*) cv. Heinz 902. In both cases the transgenics have good impact of transgene on shoot and root development and the chlorophyll contents. The transgenic plants that root specific expression of ACC accumulates metals (Cd, Co, Cu, Mg, Ni, Pb and Zn) higher than the control (Grichko et al, 2000). The over expression of ATP sulphurylase (APS) in India mustard and at seedling stages APS transgenics were more tolerant to As(V), Cd, Cu, Hg and Zn. The mature plants contained 2.5-fold higher concentration of Cd, Cr, Cu, Mo, V and W in the shoots as compared to the untransformed plants (Wangeline et al 2004). AtMHX is an *Arabidopsis tonoplast* transporter that electrogenic exchanger of protons with Magnesium and Zinc ions. The mRNA is localized in close association with the xylem tracheary elements, suggests that AtMHX may control the partitioning of Mg^{2+} and Zn^{2+} between the various plant organs (Shaul et al 1999). It was expressed in tobacco and the transgenic plants were showed increased expression and activity of vacuolar proton ATPase (Berezin et al. 2008). Copper and Zinc transporter (*tcu*-1 and *tzn*1)from*Neurospora crassa*was cloned and transferredin tobacco (*Nicotiana tabacum* L. cv Havana). The transgenic plants were showed that higher accumulation of Cu in both shoots and roots compared to control plants at higher Cu. concentrations tested as compare to untransformed tobacco plants. Zn transporter transgenic plants also showed higher accumulation of Znin roots as compared to shoots and enhanced tolerance to Zn as compared to untransformed plants (Singh et al 2010). γ-glutamyl cysteine synthetase-glutathione synthetase (*GCS-GS*) gene with limited feedback inhibition from *Streptococcus thermopiles* was over expressed in sugar beet (*Beta vulgaris*) showed enhanced tolerance to Cd, Zn

and Cu ions compared to wild type. The accumulation of these metal ions was also higher in these lines and represents a good way of phytoremediation (Liu et al 2015).

4. Conclusion

Our agricultural crop plants are susceptible to various stress specially to metal toxicant that cause damage to the plants and reduce the yield. The problem of heavy metal pollution rising day by day because a wide variety of toxic chemicals are being released into the environment. We can manipulate certain genes, related to the detoxification of heavy metals, to developed transgenic plants. The transgenic plants are hyper accumulates of metal ions and are able to tolerate the higher concentration of these metal ions or may exude these ions out from the systems with the help of ion transporters. The transgenic plants capable of fighting against many toxic metal ions should be better than one type of metal ion. So those genes are preferably transferred that give tolerance to multiple metal ions as they can grow everywhere. The other strategy used to develop transgenic plants against multiple ions is through transferring genes for oxidative stress because almost all metal cause oxidative damage.

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Parameters Affecting Quality of Soil and Irrigation Water

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Introduction

Soil Quality Introduction

India is a country which have plenty of soil types, from the fertile alluvial to laterite along with marshy soil to desert land. To understand more about soil we should know whatsoil is made up of? Soil is a mixture of inorganic particles made up of silicates, along with organic matter containing humus, water and air trapped in those particles. The varying concentration of different minerals and nutrients completely changes the quality of the soil.

The higher concentration of slats of calcium and magnesium results in the alkalinity of soil and lower concentration may give rise to the acidity. The type of soil affects the flora and fauna of the ecosystem up to a great extent. So it becomes important to understand the quality of soil to know more about ecosystem there. Thus, the quality of soil can be classified in three main parameters viz. physical, chemical and biological properties of soil.

Physical Parameters of Soil

The physical properties of soil consist of two characteristic properties which are soil texture and its structure, these when combine with other properties determine the quality of soil in terms of its porosity and bulk density, which ultimately results in water retention in the soil.

1. Soil Texture: When we analyse soil of a particular area it may have coarse particles in large as compared to other area or it may be more gritty or smooth, having different soil textures.

Thus the soil texture corresponds to the relative-proportion of various size granules in an amount of soil. There may be various minerals, sands of different size, clay and slits etc. found the sample of naturally occurring soil.

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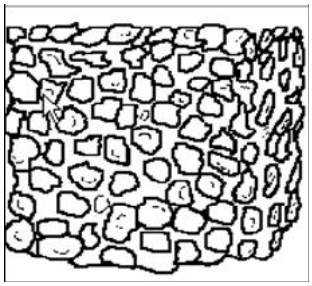
The ideal soil texture for wheat cultivation is clay loam because of its good structure and water holding capacity.

• To figure out the particle size of the soil, we can use graded sieves to sieve the particles having diameter more than 0.05mm. Apart from it we can use sedimentation method for the finerparticles in which a soil sample is dispersed in the aqueous solution.

• Measuring the radius of particle by measuring the terminal velocity (with which particles settle down) of a sample having known viscosity is termed as sedimentation technique. Thecoarse fragments which range from 2 to 75mm in diameter are known as pebbles.

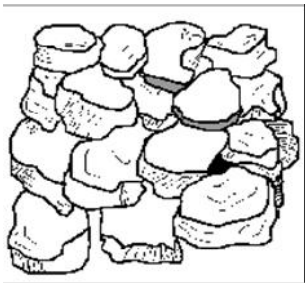
2. Soil Structure: It can be described as the arrangement in which the soil granules clump or bind together to form aggregates which have pores responsible for its porosity. The porosity in the soil influences the biological activity, aeration along with other major properties like water retention in the soil which ultimately affects the soil quality. There are mainly six categories of soil structure which have different levels of porosity depending upon the spatial arrangement of soil granules.

1. Granular is a typical arrangement in which the basic units are approximately spherical or polyhedral which are joined by the irregular or rough faces. The micro capillaries are responsible for the water movement in this arrangement.



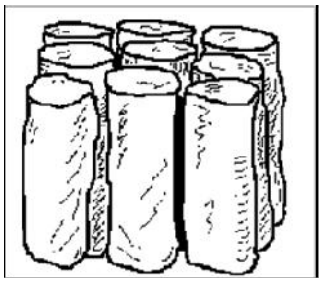
Granular

2. Blocky arrangements have units like polyhedral or block like structures which are bounded by flat or smooth surfaces in major. The flat surface of these should be porous for proper movement of water.



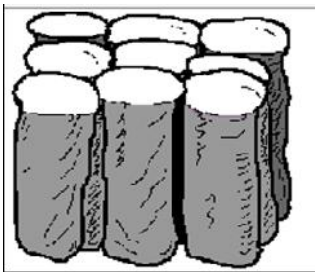
Blocky

3. Prismatic have vertical long units and are adjoined in the form of long vertical moulds or casts.



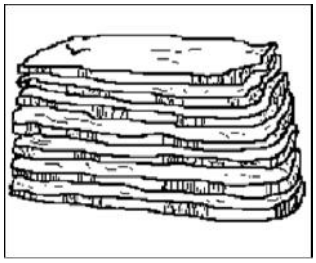
Prismatic

4. Columnar arrangement is very similar to the prismatic arrangement the only difference is the round top of basic column like units. This arrangement offers highly porous soil. For good aeration soil should not be compact.



Columnar

5. Platy arrangement has flat plate like units which are generally arranged horizontally. The movement of water is more on the surface of plates if the porosity is low.



Platy

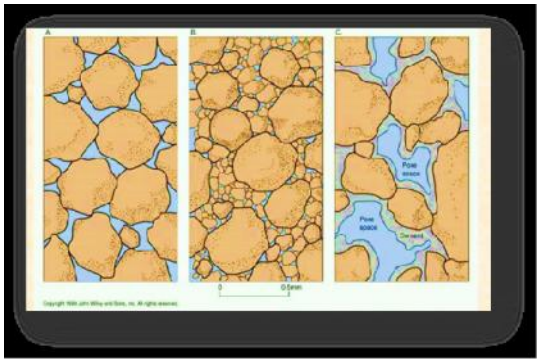
6. Single Grained It is a type of soil in which the soil particles are not bound together and the single grained particles are spherical or polyhedral in shape. This type of soil cannot retain water in it.

Single grained

Factors Affecting Soil Quality

1. Soil Compaction: The compaction of soil takes place when the soil particles are pressed together to form the low porous soil which has less water and air retention capacity for e.g. compaction of soil caused by the continuous heavy traffic passage over roads for long periods of time.

2. Porosity: It is the gap in the packing arrangement of soil particles which can be expressed as the percentage of the space occupied by the pores in the soil structure to the total space occupied by the soil sample. Generally, these pores contain water and air molecules trapped in them. These pores also contribute in the drainage of water. The higher percentage of clay in the soil results in the higher water retention capacity and higher level of slit and pebbles in the soil results in the lower water retention capacity because the water under gravity pull may easily pass through the bigger pores of pebbles as compared to the smaller pores of clay.



3. Water Movement and Availability: water retention in the soil has a characteristic role in the plant growth. The porosity is the very important factor to measure the water retention in the soil. In general, higher the porosity higher will be the water movement if the intake of water is more, the main three forces gravity, adhesive forces (between soil and water), cohesion forces (between water molecules) also plays a characteristic role in water movement. Apart from theses percolation (downward flow of water due to gravity), interflow (lateral seepage of water from more to less pervious soil) also affect the water availability in soil. Generally, the rate of flow of water in the soil of various textures is in the following sequence.

Sand > Loam > Clay

Chemical Parameters of Soil

There are several chemical constituents whose presence in varying concentration can immensely affect the soil quality3. The deviation from the given standard concentrations may lead to adverse effect on the plant growth. There are several factors which can be described as

1. pH Value: pH value is the measure of free Hydrogen-ion concentration in the soil. The pH variation from neutral soil having pH 7 can be due to the alkalinity or acidity of the soil.

Soils can be distinguished in three categories based on their pH value:

• 6.5 - 7.5 - neutral soil

• More than 7.5 - alkaline soil

• Lower than 6.5 - acidic, the soil having pH lower than 5.5 is considered as strongly - acidic soil.

Naturally the soil pH depends upon the original rock and clay chemical composition because the water soluble chemicals present in the soil composition tend to change the pH of soil by forming ionic species.

The highly acidic soil may be due to the presence or deficiency of following ions like Magnesium toxicity, Aluminium toxicity, Calcium deficiency, Magnesium deficiency.

The high levels of alkalinity often observed due to the higher concentration of sodium ions in high concentration or it may be due to the deficiency of copper and manganese. The highly alkaline soil may have a pH of about 9. Whereas pH of neutral soil is about 7.0. The pH of soil can be measured by the pH meters or the analytical methods like titration. The ideal pH of soil for wheat cultivation should be in 6.3-7.0

2. Soluble Salts: There are several salts which are in ionic form in the soil. Soluble salts which are generally found in soils calcium, magnesium, sodium, chloride, sulphate, and bicarbonate along with some salts in smaller quantities like Potassium, ammonium, nitrate, and carbonate.

Basic Soil Salinity: The method for this is to check the electrical conductivity of the soil to water mixture in 1:2. The electrical conductivity method includes ions of magnesium, calcium, potassium and sodium etc.

Electrical Conductivity (mmhos/cm)	Soil Rating	Interpretation from Conductivity Values
0 to 0.15	Very Low	Plant may be starving of nutrients
0.15 to 0.50	Low	Soil is deficient in organic matter and needs organic compost
0.51 to 1.25	Medium	Good for plantation
1.26 to 1.75	High	flora can be planted but soil is not good for seedling and cutting
1.76 to 2.00	Very High	Plants of this soil are often chlorotic/stunted
Over 2.00	Excessively high	Dwarfed plants with rooted cuttings destroyed by soil pH

The value of soil salinity by electrical conductivity measures expressed is supposed to be the ideal value if the method is followed by 1:2 dilution of soil to water. The values changes when the super saturation method is used.

Nutrient Holding Capacity

The soil may have variation in its nutrient holding capacity place to place. The major decisive factors for the nutrient holding capacity of soil are soil texture, pH, drainage and aeration, moisture and temperature. These factors affect the soil nutrients as follows.

Moisture

Appropriate Moisture content is very important for the organic decomposition.The organic components upon decomposition gives nitrogen, oxygen and phosphorous like compounds which increases the fertility of soil. Appropriate moisture content also increases uptake of nutrients by roots thereby increasing the mutual interaction.

Temperature

The ideal temperature for soil to hold nutrients is about 18-24° C. At this temperature the decomposition of microorganismsand insects living in soil yields the most nutrient, atlower temperatures the decomposition takes more time.

pH

The slight change in pH can affect the nutrient holding capacity of soil immensely. The lower or higher pH from the ideal standards may result in the inhibition of bacterial growth thereby decrease the organic decomposition. The ideal range of soil pH is from 5.5 to 7.5. For wheat production soil should have a pH ranging from 6.3 to 7.

Texture

The soil texture effects the water and nutrient holding capacity of the soil. The higher percentage of clay in the soil results in the more water retention in the soil as compared to the soil having more percentage of sand or pebbles in it. For wheat production soil should have a good clay loamy texture. The major part of the soil for wheat production should be about 72.2%.

Structure

Soil structure affects the water movement in the soil ultimatelywhich affects the quality of soil. The dissolved nutrients in the waterif moves freely in soil then the nutrient supply to it’s surroundings will also be good. The soil structure also affects the aeration the soil which affects the growth of aerobically growing microorganisms.

Biological Parameters of Soil

Soil is habitant of a large number of microorganisms, insects and animals, these organisms upon decomposition produce nutrients for soil. Generally, these provide nitrogen, sulphur, oxygen and phosphorous to the soil along with in compound form they serve as the food for other microorganisms. The biological parameters are categorised in the following bullets as follows-

1. **Macro-fauna**
2. **Micro-fauna**
3. **Micro-organism**
4. **Biological Activity**
5. **Organic Matter**

Now we will be discussing the effects of these factors-

1. Macro-fauna

These are those small creatures which are more than 1cm long but generally smaller as compared to earthworms.12 Macro fauna includes centipedes, millipedes, slugs, snails, fly larvae, beetles, beetle larvae, and spider etc. These insects are responsible for the aeration of the soil, upon decomposition either they act as food for micro-organisms or they provide nutrients to the soil. These macrofauna upon also play role in the oxygen and nitrogen fixation of the soil while alive and upon decomposition provide nutrients like phosphate and complexes of nitrogen which are further degraded into smaller molecules by microorganisms which are absorbed by plants for their growth. In all macro fauna are very important part of the ecosystem which completes the whole cycle and increases the quality of soil.

Living entities having size more than certain limits, this category is known as Mega fauna.

2. Micro-fauna

These are very small, generally microscopic animals, inhabiting in the soil. These may be Single-celled protozoans, small nematodes, small un-segmented worms, and tardigrade. These microorganisms feed upon the decaying organic matter or the smaller microorganisms.

These microfauna are further divide into three categories which arebacteria, fungi and viruses. Bacteria are the microorganisms which are single celled and doesn't have any separate nucleus in them. They are present in billions in number in just one gram of soil. These play their role in decomposition of organic matter and weathering of rocks and minerals. These microfauna increase the soil quality in many aspects.

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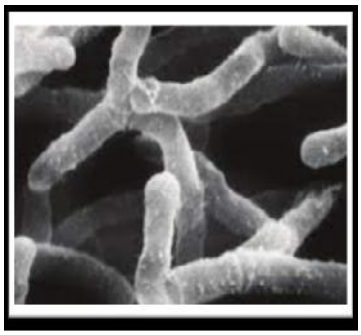
These are the smallest of the soil fauna having size less than 0.1 mm, and so are not visible with naked eyes, we need a microscope to see them. These micro creatures are responsible for the aeration of soil up to a great extent.

3. Micro-organisms

These micro-organisms are responsible for major part of nutrient release in the soil from organic matter. When these microorganisms decompose the organic matter present in the soil, they utilise the carbon and nutrients in the obtained from decomposition for nurturing themselves. Indirectly these microorganisms release a number of nutrients in the soil which can be utilised by other microorganisms for their growth or by the plants. Bacteria and fungi differently recycle the nutrients in the soil. Generally, fungi act more efficiently in storing and consuming the nutrients than bacteria[1,19] One reason for being more efficient is the cell wall of fungi which are made up of melanin and chitin which are polymers not easy to decompose, these microorganisms sore more calcium like micronutrients which are consumed by plants

further[1,19]These also contributes in making macro aggregates which allow and water to pass through them thereby increasing drainage which ultimately increase the quality of soil.



Actinomyces isrealii Photo credit to Graham Colm

4. Biological Activity:

Soil Bacteria are extremely diverse in their biological activitieswhich improves the soil quality by nitrogen fixation and carbon dioxide fixation and these activities can be categorised in the four major classes as follows-

• **Photo-heterotrophs** are those bacteria which obtain energy from the photosynthesis by one electron transfer process. These cannot get enough carbon from CO_2 gas present in the soil fortheir growth.

• **Photo-autotrophs:** These obtain energy from photons and carbon from carbon-dioxide. Bacteria like cyanobacteria also contribute in the nitrogen fixation.

• **Chemoautotrophs** use inorganic compounds to get energy by reducing them. These micro-organisms get energy from the compounds like hydrogen, ammonia, nitrite, hydrogen sulphide derived from decomposition of other microorganisms.

• **Chemo-heterotrophs** are those bacteria which feed upon some special organic molecules for their nurture. Some of these bacteria require common carbon containing compounds suchas glucose and succinate, whereas other hetero autotrophs decompose complex organic compounds such as proteins and carbohydrates to convert them into simpler molecules to usethem further.

5. Organic Matter

The presence of organic matter increases the quality of soil because upon decomposition they supply nutrients to the soil, these organic compounds

may be humus and small plant residues long with small living organisms. These were some of the physical, chemical and biological parameters which affect the quality of soil. These microorganisms can be retained in the soil by mixing the organic waste in the soil like tea leaves and molasses. The micro nutrients in the soil also increase the growth of these microorganisms which make the soil fertile.

Water Quality Introduction: water is as necessary for plants as it is for humans. There are several standards for the irrigation water which are pH, Total salt concentration, Sodium adsorption ratio, Bicarbonate ion concentration and total Boron content. These factors can be described as follows-

1. Total Salt Concentration: Total salt concentration is a measure of the free ions which can be detected by measuring the electrical conductivity. If the EC of irrigation water is more than 10mmhos/cm then the water can be categorised ad saline and this water is not suitable for irrigation on saline soil.

• If it's value is less than 1.5mmhos/cm then the water can be treated as normal water and is suitable for all types of soil.

• If this value lye in between 1.5-3mmhos/cm then the water is of low salinity and can be used for irrigation on light and medium texture soil.

• If the value of electrical conductivity lye in between 3-5mmhos/ cm then the medium salinity water is suitable for irrigation on the medium texture soil for semi tolerant crops.

• If the value lye in between 5-10mmhos/cm then the water is categorised as saline water and can be used for irrigation on low saline soil for tolerant crops.

2. Sodium Adsorption Ratio (SAR): It is measured as the sodium ion concentration relative to the calcium and magnesium ion concentration. The SAR value is obtained by the following formula:

$$SAR = Na2^{+}/ (Ca2+/2+Mg2+/2)1/2$$

Where the symbols have following meaning

SAR = Sodium Adsorption Ratio

Na2+,Ca2+,Mg2+= Na2+ion concentration, Ca2+ion concentration,

Mg2+ion concentration in me/I

Where, me/I = milli-equivalent/litre.

The following four categories have values for SAR as follows-

If SAR value is below 10 then the water has low Sodium Adsorption Ratio. If the value of SAR is between 10-18 then the water has medium SAR ratio and if in between 18-26 then high SAR value. If the value exceeds 26 then the value of SAR ratio is very high.

3. Bicarbonate Ion Concentration: This index of irrigation water is used to describe the level of alkalinity in the soil. This index is used to check the suitability of irrigation water for particular type of soil like clay soil.

It can be determined as following-

$$RSC = (Co_3^{2-} + HCO3^{-}) - (Ca^{2+} + Mg^{2+})$$

Residual sodium carbonate included ion concentration of Carbonate ions, bicarbonate ions, calcium ion concentration and magnesium ion concentration.

Where, me/I = milli-equivalent/litre

If the value of Bicarbonate ion concentration is less than 1.5me/I then it can be categorised as having low bicarbonate ion concentration, whereas if value lye in 1.5-3.0 or 3.0-6.0 me/I these are categorisedas medium and high bicarbonate ion concentration. Further if the value exceeds from 6.0me/I the irrigation water is supposed to have very high concentration of bicarbonate ions. For wheat cultivation the bicarbonate ion concentration should be less than 8me/litre at 40me/litre salt concentration.

4. Boron Content: Boron serves as a nutrient in for the soil but up-to some permissible limit; above those limits the higher concentration of boron may be toxic to the soil. For irrigation water there are certain limits which can be categorised in low, medium and high levels as follows-

Category Boron concentration in ppm

i Low Below 1.0

ii Medium 1.0-2.0

iii High 2.0-4.0

iv Very High Above 4.0

These were some of the standard parameters according to the Indian standards for irrigation water. Generally, we can use water ranging from 5.5 to 6.5 for irrigation purposes, this value may change from place to place

according to the nature of soil. For wheat cultivation the boron content in irrigation water should be atleast 0.02ppm/ litre and should be less than 0.3ppm/litre.

Conclusion

In irrigation we use a number of water bodies to supply water needs to plants which may be contaminated by the trace elements, pathogens, Nano particles, trace elements etc. These elements excess may not be good for the production of crop but under limits may not affect the growth and production of plants. The higher boron concentration more than a prescribed limit in India under BIS guidelines can also result in to the boron contamination, whereas the higher concentration of salt causes the water highly saline which is not good for irrigation purposes. Similarly, soil has its own set of different parameters which includes some physical parameters, chemical parameters and biological parameters. The soil quality highly affects the crop production in a particular and area. The lower level of moisture in the soil or the less water supply retards the production and growth of plants similarly less organic decomposition may reduce humus in the soil. The phenomenon such as nitrogen fixation by the microorganisms is very important quality parameter for the soil because reduced levels of nitrogen may result in the less production of the land.

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Industrial toxicants effluent and their effect

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Introduction

Human activities like agriculture and industry, which result in higher demand for water and lead to a rise in the generation of industrial wastes, are caused by an increase in population size. Water, air, and soil contamination are all results of these wastes. It includes chemicals that are both biodegradable and inert.

Waste discharged by industries, municipalities, agriculture and households among other sources, is polluting the water supply more and more. The sort of industry is what mostly determines how much and what kind of potable water is reduced. The quantity and quality of drinkable water may be improved by treating wastewater before dumping it into a river. Industrial effluent that is not treated and is released directly into bodies of water contaminates the groundwater, has a negative impact on animal health, and seriously harms the environment.

Pollutants emitted from industrial facilities, oil spills, heavy metals, animal and chemical waste, sewage leaks, eroded sediments, deforestation, littering, fertilizers, herbicides, pesticides, etc. are the main causes of water pollution. Water contamination is receiving increasing attention today as a result of growing industrialization and negative health effects.

Pollutants of industrial discharge

Copper, iron, chromium (Cr), cadmium (Cd), nickel (Ni), arsenic (As), mercury (Hg), phenol, and phenolic compounds are only a few of the contaminants found in industrial effluent. The paint and dye, textile, paper, pharmaceutical, and fine chemical industries all generate heavy metal pollution. Both directly and indirectly, these heavy metals have toxic, harmful effects on people and aquatic flora and animals.

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In general, wastewateris of two types:

1] Sewage water: Discharge from places like houses, schools, hospitals, hotels, restaurants, public toilets etc.

2] Non–sewage wastewater

Produce from commercial activities such as that generated by industries and after rainfall or flood events.

Polluted water not only harms aquatic organisms but also harms the entire environment directly or indirectly connected to all life forms.

Types of wastewater

Domestic agricultural industrial storm runoff

Toilet
Washing
Bathing } **GRAY WATER**
Kitchen

Urine
Faeces } **BLACK WATER**

Toxic effects of industrial discharges

The amount of contaminants in the environment has dramatically risen during the previous several decades as a result of increasing industrialization. Environmental and health risks were brought on by toxic industrial water. Emergency personnel and the impacted populations are harmed by these chemicals. Serious health problems might be caused by some toxins. such as cancer, birth defects, genetic mutations, physical deformities, alterations in behaviour, and physiological malfunctions. In some cases, these dangerous substances may also irritate the skin or eyes, making it difficult to breathe, or they may cause nausea, headaches, or other unpleasant side effects.

Toxins in Industrial water kill organisms in a lake or river, destroying animals and plants, and hormonal imbalance in aquatic animals.

The consumption of harmful heavy metals by aquatic organisms can lead to adverse health issues in animals and ultimately in people via the food chain, which causes an imbalance in the environment. They may be teratogenic and carcinogenic, and they may also harm the neurological system, and organs, or induce oxidative stress and organ damage. The main chemical pollutants are phenolic compounds, which are harmful because they prevent normal microbial activity that affects the biological decomposition of wastes. Phenolic chemicals can also lead to respiratory failure, reflex loss, sweating, low body temperature, and cyanosis. Mutagenicity and genotoxicity are caused by the effluent of the paper and pulp industry, which includes resins, tannin, and chlorinated organic compounds.

Lignin and its derivatives are typical effluents from the paper and pulp industry. During the biological treatment of wastes, they are transformed into toxic compounds that can disrupt fish reproduction by binding to the oestrogen receptors in fish and disrupting the hormonal balance in aquatic organisms.

Colloidal substances made of chromium complex and greasy waste scum hinder sunlight from penetrating water, causing a reduction in the ability of the water to dissolve oxygen, which is also carcinogenic.

(a) Effect of industrial discharge and its toxins on freshwater

Diverse water bodies, including rivers, lakes, and groundwater, are contaminated by individual discharges. Water quality and quantity are crucial since humans use it extensively for drinking, washing, bathing, and irrigation. These industrial discharges pose a threat to aquatic life as well as people who are part of the food chain and may decrease agricultural productivity and plant development.

(b) Effect of industrial discharge and its toxins on the seawater

Industrial discharges and their toxins flow through rivers and finally reach the coastal and ocean some are settled with sediments. It contaminates the aquatic environment and enters in human food chain accumulating in fishes, mollusks (octopus, cockle, shellfish) crustaceans (shrimp, lobster and crab), sea cucumber, seaweed etc.

(c) Effect of industrial discharges and their toxins on the land

The land is polluted by untreated industrial discharges that are disposed of onto the soil surface. It also contaminates groundwater. This may affect human activity, the growth of the plant as well as human health.

(d) Effect of industrial discharges and their toxins on human health

Nowadays human health is a major concern of WHO. According to WHO, around 80% of diseases are water born. Several water-borne pathogens proliferate in industrial discharges and produce toxins. Heavy metal and organic matter also will bring adverse health impacts to humans.

The health hazards to humans describe the following:

Metals

Chromium

Chromium is an essential nutrient for animals and plants because it has effective metabolism but hexavalent chromium is the most toxic form of chromium and also carcinogenic. It can irritate the nose, throat lungs. Prolonged exposure can damage the mucous membrane of the nasal passage, resulting in ulcers, lung cancer, and kidney and liver damage.

Aluminium

A high level of Aluminium affects children's brain function and memory loss with kidney disease. Some studies say Al is linked to Alzheimer's disease.

Iron

Iron is an essential element for the biochemical and enzymatic process. It helps in the transport of oxygen to the cells. Its high concentration may increase the free radicals which are responsible for degenerative disease and ageing.

Cadmium

Cadmium is hazardous to both human health and the aquatic environment. Cadmium is carcinogenic, mutagenic, teratogenic, and embryotoxic. It may also cause hyperglycemia reduced immune potency and anaemia. Acute exposure to cadmium can result in flu-like symptoms (chill, fever and muscle pain) and can damage the lungs. It also damages kidneys and liver and deformation of bone structure.

Lead

Continuous exposure to a high level of lead may cause anaemia, weakness, and kidney liver, bone and brain damage. Lead can cross the placental barrier which can damage the developing body's nervous system.

Zinc

Zinc is an essential element to plants and animals. High doses of zinc cause acute intoxication, muscular pain, and intestinal haemorrhage. It also interferes with the uptake of copper.

Nickel

It is an essential element to both flora and fauna but continuous exposure to this metal may cause an allergic reaction, chronic bronchitis and reduced lung function. Its high exposure can lead to cancer in organs of cardiovascular, breathing system and kidney diseases. Drank water containing a high amount of nickel had a stomach ache and adverse effects on kidneys and blood.

Mercury

The nervous system, immune system. Inorganic and mercury through biological Mercury can damage the brain heart, kidney, lungs liver, the nervous process can convert into MeHg. MeHg can cross the placental barriers and cause fetal brain damage.

Organic or inorganic matters

Potassium

Exposure to potassium fumes can cause sores of the nasal passage and contact with solid potassium can cause nervous and digestive disorders,kidney, heart disease, diabetes, hypertension, and coronary artery disease.In infants, it causes immature kidney function and renal reserves.

Fluoride

A high concentration of fluorides can lead to skeletal and dental fluorosis such as deformation of ligaments, bending of the spinal cord, and bone may become hardened and less elastic so increasing the risk of fractures.

Nitrate

Consuming too much nitrate cause methemoglobinemia (also known as a blue baby syndrome) in infants and cause cancer. In the blood, nitrate converts haemoglobin to methemoglobin. This methemoglobin does not carry oxygen to the body cells.

Sulphate

A high concentration of sulphate may reduce lung function, asthmatic symptoms, alimentary canal problems and death in people who have chronic heart or lung disease.

Corrective Action

There are some methods to reduce the effect of industrial discharges and their toxins.

These methods are:

(a) Bioremediation
(b) Biosorption
(c) Phytoremediation
(d) Green monitoring and green application of green chemistry

Bioremediation

Bioremediation is the use of bacteria, fungi, yeast etc. In remediating the contaminant while phytoremediation uses plants.

Example:Bioreactors, Biofertilizers, Bioventing, Composting, Land Forming, Bioaugmentation, Biostimulation.

Biosorption

Biosorption is the sorption of metals on biological material. Its a way to remove toxic and recover useful metals from waste.

Example: Brown Algae and Yeast

Phytoremediation

Phytoremediation refers to the use of plants in surface and submerged aquatic plants for industrial discharges. It updates the metals from contaminated areas -a bio clean-up technology.

Green Chemistry and Green Monitoring

Green chemistry and green monitoring are alternative options to prevent the toxicity of industrial discharge.

Conclusion

The rising pollutant emissions from expanding industrial sites are presently having a harmful effect on the ecology. One of the industrialization's biggest adverse consequences is water pollution. Hazardous chemicals created

by industrial sectors put the health of people, animals, and aquatic life in danger. These pollutants also have a significant impact on the portability and hygiene of drinking water.

Despite the fact that health is a top priority, industrial development is essential for a thriving economy. The methodologies utilised to carry out various industrial programmes and interventions are always being improved upon and evaluated. Understanding how pollutants interact with biotic and abiotic systems and how they impact the environment and human health is crucial before creating successful remedial methods. In a word, it's critical to take remedial action to implement industry pollution cleaning. Even with the corrective steps mentioned in the prior sections, there are still challenges.

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Survey of acute symptoms among rural pesticide spray workers following work with pesticides

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Abstract

The present study is performed to evaluate the occurrence of illnesses by pesticide exposure among farmer workers of district Bathinda. One hundred and seventy (170) professional farm workers engaged in spraying various pesticides participated in the study. A questionnaire to know their age, weight, symptoms for illness, personal protective equipment used, years of exposure to pesticides and name of pesticides used. The majority of farm workers complaint about occurrence of common symptoms likes nausea (60.00%), headache (47.06%), backache (24.12%), and respiratory dysfunction (19.41%). Numbness of limbs (17.06%), joint pain (15.29%), jaundice (14.11%), abdominal dysfunction (14.11%), burning sensation in urine (11.76%) and chest pain (11.17%) were also very prominent. There is need for more health education programs and pesticide toxicity programs that made to stress farm workers the need for precautions.

Keywords: Pesticide spray workers; pesticides and symptoms.

Introduction

Pesticides have significant economic, environmental and public health impacts. Their application helps to improve human nutrition through greater availability, longer storage life and lower costs of food[1]. Usage of pesticides in the ecosystem leads to development of various types of morphological, physiological, biochemical and behavioral changes in individuals[2]. The degree of toxicity depends upon the nature of the pesticides, their environmental concentration and factors such as temperature, humidity, pH, oxygen concentration etc[3].

Farmers are using pesticides in large quantity to save their crops from pest and to get higher yield. Thus, farmers may be inadvertently exposed to pesticides through skin contact and inhalation for prolonged period during the growing season[4]. Exposure to pesticides both occupationally and

environmentally causes a range of human health problems. It is estimated that nearly 10,000 deaths annually to use of chemical pesticides worldwide, with about three-fourths of these occurring in developing countries[5]. Besides effecting immune and endocrine system of humans, pesticides also cause a variety of dermatological, gastrointestinal, genitourinary, respiratory, musculoskeletal, and cardiological problems[6].

At present, India is the largest producer of pesticides in Asia and ranks twelfth in the world for the use of pesticides with an annual production of 90,000 tons[7]. Farmers in India use pesticides regularly on large scale to save their crops from pest attack. Exposure to pesticides results in acute and chronic health problems. Several studies have shown that farmers exposed to pesticides for prolonged period are more likely to develop leukemia, brain and prostrate cancers than the general population[8].

The occurrence of symptoms soon after work with pesticides does not necessarily imply poisoning. Such illness could be a chance coincidence or perhaps trigged by psychological mechanisms. However, if acute toxicity from pesticides were common among users, even if only mild, there would be a need to review the current approach to risk assessment and the capacity of operators to follow instructions for use[9].

To explore the frequency and nature of acute symptoms following the use of pesticides, we analyzed data from Bathinda district of Punjab.

Materials and Methods

This was a cross-sectional study to investigate the prevalence of pesticides exposure and its risk factors. The study was done in three villages of district Bathinda (viz. Mahinangal, Jajjal and Balloh), which fall under major cotton producing area of Punjab. Other major crops produced here are rice, wheat and sugarcane.

A total of 170 farm workers participated in this study. All participants were male farmers in age group of 18-60 years. A questionnaire was designed to know the health impacts of pesticide use and practices and self reported poisoning symptoms. The farmers were interviewed during their hours for their convenience. The farmers were informed about the purpose of study and the data was collected with their consent.

Results

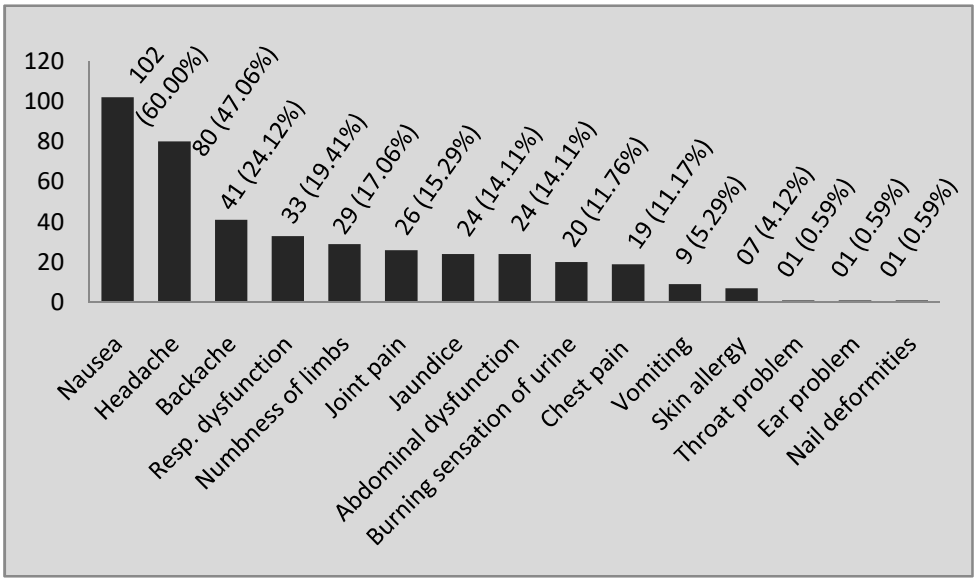
This study included 170 occupational pesticide sprayers. Table-I shows demographic data of the spray workers which includes age, weight, education and years of exposure to pesticides. Most of the sprayers were young and were in age group of 26-35 years (37.05%) but few sprayers were older (≤56 year, 2.36%). Likewise 61.76% sprayers were found in 51-65 kg. Out of 170 individuals 47.65% workers were illiterate and 9.41% got education up to 12^{th} standard. These workers were exposed to pesticides from 1 to many years. Most sprayers started working since childhood. So (54.11%) sprayers were exposed to pesticides for more than 11years. Only 18.78% of workers have less than 5 years of exposure to pesticides.

Table-1 Shows the demographic data of Farm Workers.

Parameters	Exposed population N=170	% age
Age		
≤25 years	43	25.29
26- 35 years	63	37.05
36-45 years	46	27.06
46-55 years	14	8.24
≥56 years	04	2.36
Weight		
≥50 Kg	07	4.12
51-65 kg	105	61.76
66-80 kg	44	25.88
≤81 kg	14	8.24
Education		
Uneducated	81	47.65
$1\text{-}8^{th}$	35	20.59
$9\text{-}11^{th}$	38	22.35
$\geq 12^{th}$	16	9.41
Years of exposure		
≤5 years	31	18.24
6-10 years	47	27.65
≥11 years	92	54.11

Figure-1 shows the list of main symptoms as reported by farmers. Similarly the weight of most of sprayers (61.76%) was between 51-65kg. The most prevalent symptoms were nausea (60.00%), headache (47.06%), backache (24.12%), and respiratory dysfunction (19.41%). Numbness of limbs (17.06%), joint pain (15.29%), jaundice (14.11%), abdominal dysfunction (14.11%), burning sensation in urine (11.76%) and chest pain (11.17%) were also found to be very common among these sprayers.

Fig-1 Showing various symptoms shown by sprayers.



Discussion

The present study is to estimate the extent of acute pesticide poisoning among agricultural workers who use pesticides.

These workers are daily exposed, used little protection due to cultural and economic reasons, and underestimate the toxicity of pesticides[10]. Although farmers claim knowledge of health risks from pesticides, they do not generally use personal protective measures, the predominant reasons given being that the protective equipment is out of their financial reach and uncomfortable to use under the prevailing hot and humid climatic conditions[11]. Despite these, people treat pesticides as something useful and ordinary in that they are using it in their everyday normal dealing, with less safety precautions including storage and disposal, concurring to early findings[12].

During spraying, the farmers sprayed in the direction where he was going and therefore he walked in a cloud of spray. This worsened if the farmer sprayed against the wind. There was a strong tendency for some individuals to report multiple symptoms and symptoms related to multiple types of pesticide. However, no distinctive clusters of symptoms were apparent in relation to specific types of pesticide[13]. Most farmers reported that they had felt some ill effects of pesticide application such as headache, dizziness, and vomiting that normally occur during or after spraying[14].

Several flu like symptoms such as headache, aching limbs and fever or chills that have previously been suggested often to occur after dipping sheep, a phenomenon known as dipper flu[15]. The prevalence of headache, blurred vision and nausea were respectively 2.7, 7.5 and 3.3 times more in US OP applicators than non-exposed. The prevalence of these symptoms was 3.6, 2 and 15 times higher in Indian OP formulators[16].

In the Community Canvass Survey in the Prairie Ecosystem Study (PECOS) study, whose aim, in part, was to understand the short-term effects of environmental pesticides on lung function, the most commonly reported health diseases for children agedv18 years were a history of bronchitis (19.8%), asthma (10.2%) or skin allergies (9.7%)[17]. Several incidents occurred where people, including children, living near to fumigant-treated surfaces experienced respiratory symptoms[18]. Exposure to organophosphates may be related to respiratory diseases in children, through deregulation of the autonomic nervous system by their anticholinesterase activity[19,18]. Conversely, pyrethroid derivatives are allergens, causing asthma-like attacks and anaphylactic reactions[18].

Some farmers had complaints of headache, dizziness, nausea, vomiting and respiratory problems after spray [21,20]. General symptoms (weakness, fever, lethargy) were the predominant abnormal manifestations among those examined (63.8%). HEENT symptoms (blurring of vision, deafness, and headache) were also predominant among the farmers. Involvement of the skin was also noted, with 21% of farmers having integumentary abnormalities. Specifically, headache was the most frequently reported symptom (48%) closely followed by easy fatigability (46.1%) and cough (40.2%). Blurring of vision and palpitations were also common (36.3% and 33.3% respectively). Similar symptoms were found by Strong, Thompson, Coronado, Griffith, Vigoren, Islas, in 2004 [22] among farmers exposed to organophosphates.

There is need to avoid spraying under strong sunlight, avoid spraying against wind, and avoid smoking during spraying and wear personal protective equipment like cap, face mask, full sleeve shirts and shoes while spraying.

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Climatic change and Its Effect on Sericulture Industry

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Introduction

Sericulture is science which deals with the rearing of silkworm for the production of silk.

Silkworm is the most important domesticated insects, which produces silk thread in the form of cocoon by consuming mulberry leaves during larval period. The growth and development of silkworm is greatly influenced by environmental condition. The ideal temperature for healthy growth of silkworm varies according to developmental stage and the breed.

Increase in the temperature up to 27-28 ºC increases the growth of silkworm. However, more then 30ºC imbalances the metabolic activities and the silkworm, thus become unhealthy.

Sericulture and silk industry has long history of thousands of year as a conventional trade of India. Climate change has varied implication and its effects have touched the silk industry in India. Of the different varieties of silk produced in the country like Muga silk is produced mostly in the Assam. The state produces 94% of the total Muga silk production in the country.

The pride and glory of Assam has, however come under severe threat due to changing climatic condition .India is the second largest producer of silk in all over world, about 35,468 MT in year 2018-2019 has presented a promising future in coming year.

The silk production is reduced in country during 2020-2021 due to caused by the covid -19 pandemic. The total raw silk production of the country during 2020-2021 was 33,739 MT.

The silkworm are poikilothermic animal whose body temperature is not stable, approximately similar to the environment. Therefore, the change in atmospheric temperature influence behaviour, development, growth and reproduction.

The environmental factor like temperature and humidity determines for a successful rearing of the silkworm and production of the silk outcome of this industry. Since, the silkworm are sensitive to the changing atmospheric temperature and humidity level a slight fluctuation in the surrounding environmental condition, leads to the complete loss of the crop.

Because a wide fluctuation in the surrounding environmental condition, some insects show wide range of adoptation to those fluctuation at a tolerable limit but silkworm are unable to survive extreme natural fluctuation rising temperature and day to day changing weather links to global warming which becomes the threat to the sericulture industry not only India but for the other countries which are associated with sericulture.

The effect of climatic change on silkworm are of great significance because they use to contribute a significant amount to the GDP of the country.

Present Status of Climatic Change

Climatic is defined as a long term statistied expression of short term weather. Compared to the other planet, our earth's climate is highly unstable as well as unpredictable.

A key indicator for climate change is expected global-mean surface temperature increase.

According to the current reports of the intergovernmental panel on climate change, the global average surface temperature over the 20^{th} century has increased by around 0.60ºC.

This rise in the temperature is mainly due to the concentration of green house gases like CO_2, CH_4 and NO_2. On the basis of increasing the green house gases, climatic model predict a 1.4 ºC to 5.8ºC average increase in global warming from 1990 to 2100, probably leading to a more repaid increase in temperature.

Impact of Climatic Change on Sericulture

The silkworm *Bombyx mori* is a poikilothermic, highly sensitive to environmental temperature due to artificial domestication and indoor rearing. The most suitable temperature for silkworm development is approximately 24-

28 ºC. It is predicted that, global warming affects the cultivation area of various crops including mulberry.

Mulberry (*Morus alba*) is a C_3 plant and it is inefficient in ultilizing the atmospheric CO_2 where as enzyme of C_4 plant located in the mesophyll cell are efficient in fixing CO_2. In C_3 plant CO_2 react with Ribulose biphosphate in the presence of enzyme ribulose biphosphate carboxylase, which is an inefficient enzyme with low substrate specificity. To overcome this inefficiency, stomata in C_3 plant remain open for longer period leading to increased evapotranspiration. Hence, C_3 plant grow better in cooler moist environments with elevated CO_2 concentration increased levels of CO_2 will effect plant yield through photosynthesis and stomatal conductance. Where as the growing evidence suggesting that C_3 crops, may respond positively to increased atmospheric CO_2 in the absence of other stressful condition, but the beneficial direct impact of elevated CO_2 can be offset by other effects of climate change, such as elevated temperature, higher troposheric Ozone concentration and altered patterns of precipitation. The direct and indirect effect of climate change includes-

1. Direct effects from changes in temperature, precipitation or CO_2 concentration and
2. Indirect effects through changes in soil moisture and the distribution and frequency of infestation by pests and diseases.

In recent years, many pests and diseases have been reported to be major limiting factors affecting production and productivity of mulberry leaves due to intensive cultivation practices and indiscriminate use of nitrogenous fertilizers and pesticides. There is also a change in the insect pest scenario in mulberry due to changes in climate and agroecosystem.

Effect of Climate Change on Cocoon Production and Silk

For the economical growth of the sericulture industry, quality and quantity of cocoon and silk play an important role. Proper shape, size and compactncss of a cocoon is neccssary for filament length as well as the quality of reeled thread. Different characters of silkworm as well as the cocoon are not only influence by the genes but are also by the temperature, humidity, air current etc. quality of the cocoon and silk is highly influenced by ambient temperature, seasons and other environmental condition. Change in temperature level affects the quality of the cocoon which result in variation of filament size and quality of reeled thread.

According to Srivastava et.al (1998), variations occur in cocoon weight, shell weight, filament length, silk yield, denier and sericin percentage due to change in environmental condition. The influence of temperature on cocoon and reeling characteristics of new bivoltine hybrids during spinning period (Gowda and Reddy 2007).

Conclusion

The creation of nature cannot be changed. The use of pesticides industrial growth is some of the factors contributed to change in climate. Man has to develop alternatives to cope up with this climate change has its impact on sericulture industry in various ways. To avoid crop loss during rearing in case of Muga silkworm indoor rearing can be conducted for early stage worms. Rearing can be done inside nylon nets to avoid heavy rainfall and pest infestation.

For mulberry silkworm rearing, temperature control system can be installed to avoid crop loss.

Now a day, shifting of rearing season in case of Muga is accepted by the farmers to mitigate the loss regarding the food plants, frequent care should be token by checking soil quality and pest infestation, providing adequate amount of manures and fertilizers pruning and pollarding, Care should also be taken during spinningof cocoons by maintaining required temperature and humidity throughout the period. As the exact effect of climate change on sericulture industry is not yet proven, so on the basis of available research findings future researchs need to be conducted in this areas for the benefit of this industry. There is dire need to combat this emerging problem with some sustainable measures to safeguard aur resources.

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Global warming and climate change: Causes, impacts and mitigation strategies

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Abstract

Many scientists, engineers, and environmentalists are gravely concerned about changes in the planet's overall climate. Electricity is continuously produced using fossil fuels. Global warming is caused by gases like carbon dioxide, methane, and nitrous oxide that are produced when fossil fuels are burned. The warming trend is also a result of deforestation. The environment of Earth is continuously suffering significant harm as a result of the threat of global warming. The majority of people are still ignorant of global warming and do not believe it will pose a serious threat in the near future. The paper provides an overview of global warming, explains its origins and dangers, and offers some options to address this pressing problem.

Keywords: Deforestation, global warming, fossil fuels, climate change, sea level rise.

1. Introduction

It's really troubling that the planet's temperature is constantly rising. The main factor causing this is global warming. Sunlight hitting the Earth causes global warming to start. About 30% of the sunlight is then reflected back into space by clouds, atmospheric particles, reflective ground surfaces, and ocean surfaces, with the remainder being absorbed by the sea, the air, and the land. The planet's surface and atmosphere subsequently warm as a result, making life possible. The solar energy released by thermal radiation and infrared rays radiate directly out to space and cools the earth. However, some of the emitted radiation is transmitted back to the Earth's surface after being re-absorbed by gases in the atmosphere such as carbon dioxide, water vapour, ozone, and methane. Because of their ability to trap heat, these gases are frequently referred to as greenhouse gases. It should be mentioned that this process of re-absorption is beneficial since without greenhouse gases, the Earth's average surface temperature would be quite low. The problem arose as

a result of humankind purposely increasing the quantity of greenhouse gases in the atmosphere during the previous 200 years at an alarming rate. Over the past 100 years, the planet's surface temperature has increased the most. The average surface temperature of the Earth increased by 0.6 to 0.9 degrees Celsius between 1906 and 2006. Landfills and agricultural decomposition of biomass and animal manure produce millions of pounds of methane gas. Different nitrogen-based fertilisers, such as urea and diammonium phosphate, as well as other soil management practises release nitrous oxide into the atmosphere. These greenhouse gases remain in the atmosphere for decades or even longer after being released. Since the industrial revolution of 1750, the levels of carbon dioxide and methane have risen by 35% and 148%, respectively, according to the Intergovernmental Panel on Climate Change (IPCC). As a result of scientific information about the rising concentration of greenhouse gases in the atmosphere and the changing climate of the Earth, environmental issues have grown to be mankind's top concern today. The amount and distribution of rainfall are changing, along with the global temperature. A significant global issue that transcends regional and national boundaries is global warming and climate change. We are effectively warming up as a result of the continued overproduction of greenhouse gases, which has led to an increase in the amount of heat being trapped in the earth's atmosphere. This is referred to as global warming.

2. Evidences of Climate change

According to the IPCC, climate change is any change in the climate over time, whether it is brought on by natural variability or human action. The United Nations Framework Convention on Climate Change (UNFCCC) uses the term "climate change" differently than this, referring to a change in the climate that can be directly or indirectly linked to human activity that modifies the composition of the earth's atmosphere in addition to natural climate variability seen over comparable time periods. The fundamental cause of climate change is the rise in greenhouse gas concentration in the atmosphere as a result of several anthropogenic and natural activities. Since 1750, human activity has significantly boosted the levels of carbon dioxide, methane, and nitrous oxide in the atmosphere, and they now significantly exceed pre-industrial levels as estimated from ice cores dating back thousands of years. The use of fossil fuels and changes in land use are the main causes of the global increases in carbon dioxide concentration, whereas agriculture is the main cause of the increases in methane and nitrous oxide. Increases in average global temperature (global warming), changes in cloud cover and precipitation,

especially over land, melting of ice caps and glaciers, reduced snow cover, and increases in ocean temperatures and ocean acidity—caused by seawater absorbing heat and carbon dioxide from the atmosphere—are the main features of climate change.

3. Global warming as an emerging threat to national security

All aspects of national security, including military security, economic security, resource security, border security, demographic security, disaster security, energy security, information security, food security, health security, ethnic security, environmental security, and cyber security, can be impacted by global warming. Economic conditions would deteriorate due to global warming, which could eventually provide favourable conditions for the growth of terrorism and insurgency. In addition to human activity, natural factors also contribute to the planet's temperature rising.

4. Climate change evidences from physical system

i. Rise in atmospheric CO_2, CH_4, N_2O, and atmospheric temperature

The most significant manmade greenhouse gas is carbon dioxide. In 2005, the amount of carbon dioxide in the atmosphere was 379 ppm, up from a pre-industrial value of roughly 280 ppm. According to ice core data, the atmospheric carbon dioxide content in 2005 is significantly higher than the natural range for the previous 650,000 years (180 to 300 ppm). Despite year-to-year variability, the yearly growth rate of carbon dioxide concentration was higher over the past ten years (1995-2005 average: 1.9 ppm per year) than it had been since the start of continuous direct atmospheric measurements (1960-2005 average: 1.4 ppm per year).

ii. Increase in sea water temperature

The average temperature of the world's oceans has risen to depths of at least 3000 m since 1961, according to observations,the ocean has been absorbing more than 80% of the heat that has been supplied to the climate system. Sea level rise is a result of seawater expansion brought on by such warming. Over the past century, an increase in surface air temperatures of 0.4° C has been noted at the national level. Along the west coast, in the interior peninsula, central India, and north-eastern India, a warming trend has been noted. However, north-western India and some regions of south India have shown cooling trend.

iii. Melting of mountain glaciers and snow

In both hemispheres, the average amount of mountain glaciers and snow cover has decreased. The melting of ice caps and glaciers has raised sea levels on a large scale (ice caps do not include contributions from the Greenland and Antarctic Ice Sheets). Since the publication of the Third Assessment Report, new data have emerged that strongly suggest that the melting of Greenland's and Antarctica's ice sheets contributed to the rise in sea level between 1993 and 2003. Some Greenland and Antarctic outlet glaciers, which remove ice from the ice sheets' interiors, have seen an increase in flow rate. Thinning, shrinking or disappearance of ice shelves, or disappearance of floating glacier tongues have frequently been followed by an increase in the mass loss of the ice sheet.

iv. Evidences of floods, droughts and earthquakes

Natural disasters including floods, droughts, earthquakes, super cyclones, and others have become more frequent and intense than in the past, which has resulted in the destruction of property and the loss of life. Since the 1970s, larger areas have experienced droughts that are more severe and last longer, especially in the tropics and subtropics. The severity of the drought has changed as a result of increased drying brought on by higher temperatures and less precipitation. Droughts have also been connected to variations in wind patterns, sea surface temperatures, and snow cover and snowpack. Floods have become more frequent in most geographical areas due to heavy precipitation events, which is consistent with global warming and measured increases in atmospheric water vapour.

5. Climate change evidences from biological system

Evidence of climate change in biological systems includes changes in fish, plankton, and algae populations as well as in plants, sand trees, insects, and other animals. These research' observations reveal a very strong connection to climatic variations. Populations move into locations where the environment is more hospitable and vanish from areas where the climate is unsuitable. Frequently, this entails moving the ranges poleward. Blooming happens sooner. But it also means that there are discrepancies between the breeding of migratory birds and the availability of specific caterpillars or insects. The migratory birds still arrive at the usual time and do not find the typical food for their young because the caterpillars or insects respond to the increased temperatures by emerging sooner. Agriculture has already experienced changes such as early planting, which lengthened the growing season, as well as crop

failures brought on by altered rainfall patterns. The relationship between the altered temperature and variations in insect invasion patterns and forest fire behaviour is evident in forest management.

i. Advance onset of flowering in trees

Trees are typically a very strong bio-indicator of climate change because perennial trees only flower after they have reached the necessary thermal unit/thermal period or degree-days for that type of crop. Mango trees are excellent examples since they bloom in accordance with the local thermal season. In south India, the mango tree typically blooms in October to November, in eastern and central India, it blooms in December to January, and in north India, it blooms in the middle of February to March. However, there has been some evidence of mango tree flowering in December in north India in 2004, which was probably caused by the higher regime that month. As a result, the blossoming behaviour of fruits like the mango, cherry, apple, and others could be utilised as a very good bio-indicator for climate change.

ii. Cropping pattern change: There have been some signs of spatial changes in cropping pattern, especially in Himachal Pradesh's mountainous mountain regions. Some apple-growing regions have moved to higher altitudes as their traditional belts of climate are warming and may no longer be appropriate for their growth. In addition, Kashmir and Bihar both have prosperous winter wheat and maize farming industries. Apples require a particular thermal regime for optimal growth, similar to how the yield of apples in the Kashmir region may decrease as a result of recent warmer winters.A rise in temperature that may be favourable for agricultural cultivation causes the development of arable land poleward in areas where low temperatures are a barrier to crop cultivation.

6. Greenhouse Effect

The surface of the Earth has comparatively mild, stable temperatures compared to other planets in the Earth's solar system, which are either scorching hot or freezing cold. The thin layer of gases that covers and shields the globe, known as the atmosphereallow the Earth to experience these temperatures. However, 97 percent of climate scientists and experts concur that during the past 200 years, humans have dramatically altered the Earth's atmosphere, contributing to global warming. The greenhouse effect must first be understood in order to comprehend global warming. In contrast to the human-enhanced greenhouse effect, which causes global warming, the natural greenhouse effect typically traps some amount of heat to prevent our planet from freezing. This results from the burning of fossil fuels, which raises the

atmospheric concentrations of greenhouse gases (carbon dioxide, methane, and nitrogen oxides).Because a greenhouse functions similarly, the exchange of incoming and outgoing radiation that heats the Earth is frequently referred to as the "greenhouse effect." In a greenhouse, incoming UV light is easily transmitted through the glass walls and is absorbed by the plants and hard surfaces. However, weaker infrared light finds it difficult to travel through the glass walls and is held within, warming the greenhouse as a result. This result enables tropical plants to thrive in a greenhouse even in the winter.It is anticipated that the greenhouse effect, rising greenhouse gas concentrations, and the ensuing global warming would have philosophical repercussions. Global warming will result in severe climate change, a rise in sea levels, extreme weather occurrences, and other brutal natural, environmental, and societal effects if it is left unchecked and nothing effective is done to stop this evil.

7. Greenhouse gases

The Earth warms as a result of various greenhouse gases. Carbon dioxide is the most significant gas. Power stations that burn coal and natural gas are the source of CO2. When cars consume gasoline, they also release CO2. Each year, the Earth's atmosphere receives about 23 billion tonnes of greenhouse gases, including carbon dioxide. Compared to 1750, the amount of CO2 in the air has increased by around 48%.In the last 20 years, mankind has released about 75 percent of the CO2 into the atmosphere as a result of burning fossil fuels like coal or oil. The remainder is primarily the result of modifications to how land is used, such as tree removal.Deforestation, or the removal of trees in order to acquire land, also contributes significantly to the atmospheric concentration of carbon dioxide. When calcium carbonate is heated it produces lime and carbon dioxide, cement production also adds carbon dioxide to the atmosphere. Methane, sometimes known as natural gas, is the second harmful gas. It is created as a result of agricultural practises such the digestion of cattle, the cultivation of paddy rice, and the usage of manure.

Methane is the second-most significant greenhouse gas, sometimes known as natural gas, is the second harmful gas. It is created as a result of agricultural practises such the digestion of cattle, the cultivation of paddy rice, and the usage of manure. Fertilizers are the principal source of nitrous oxides. Additionally, refrigeration and numerous industrial activities are the main causes of fluorinated gases like chlorofluorocarbons (CFCs). These gases are contributing in global warming. They are continuously raising the temperature of the planet.

8. Causes of Global Warming

There are many things that have an impact on the earth's climate. These include the sun's energy output (which has a warming effect), volcanic eruptions (which have a cooling effect), the amount of GHGs in the atmosphere (which has a warming effect), and aerosols (cooling effect). Additionally, exhaust emissions from various forms of transportation create a diverse mixture of contaminants that are either aerosols straight out of the gate or are converted into aerosols by atmospheric chemical reactions.

Following are the major causes of global warming

Manmade cause

i.Deforestation

The primary source of oxygen is plants. They maintain environmental balance by absorbing carbon dioxide and exhaling oxygen. For a variety of domestic and commercial uses, forests are being destroyed. This has caused an imbalance in the environment, which has resulted in global warming.

ii. Use of Vehicles

Even over extremely short distances, using a car produces a variety of gaseous pollutants. When fossil fuels are burned in vehicles, a lot of carbon dioxide and other poisons are released into the atmosphere, raising the temperature.

iii.Chlorofluoro carbon

Humans have been introducing CFCs into the environment through the excessive use of air conditioners and freezers, which has an impact on the ozone layer in the atmosphere. The ozone layer shields the surface of the earth from the sun's harmful ultraviolet rays. By causing the ozone layer to thin and make space for ultraviolet light, CFCs have raised the earth's temperature.

iv. Development of Industry

The earth's temperature has been rising quickly since the beginning of industrialization. The earth is getting hotter because to the manufacturers' damaging emissions.The global temperature rose by 0.9 degrees Celsius between 1880 and 2012, according to a 2013 report from the Intergovernmental Panel on Climate Change. The difference from the pre-industrial mean temperature is 1.1 degrees Celsius.

v. Agriculture

Several farming practises result in the production of methane and carbon dioxide. These raise the earth's temperature by increasing the amount of greenhouse gases in the atmosphere.

vi. Overpopulation

More individuals breathing equals more people in the population. As a result, the atmospheric concentration of carbon dioxide, the main gas responsible for global warming, rises.

vii. Increase on the use of fossil fuel

The proportion of fossil fuels burned is rising with the advent of the industrial period. GHG emissions have increased due to a growing reliance on energy produced by burning fossil fuels like coal, oil, and gas for transportation, residential usage, and industrial use.

Natural causes

i. Volcanoes

Volcanoes are among the main natural causes of global warming. When a volcano erupts, ash and smoke are sent into the sky, which has an impact on the climate. Massive amounts of carbon dioxide are released during volcanic eruptions, and these gases eventually function as GHGs and cause global warming (www.bionomicfuel.com).

ii. Clouds of water

A particular type of greenhouse gas is water vapour. More water is evaporating from water bodies and remaining in the atmosphere due to the planet's rising temperature, contributes to global warming.

iii. Forest Fires

Large amounts of smoke containing carbon are released by forest fires or forest blazes. Global warming is caused by these gases, which are released into the atmosphere and raise the earth's temperature.

iv. Rotation of earth

The amount of sunlight that reaches higher latitudes, especially the polar areas, is impacted by the Earth's rotation, which alters the orientation of the Earth to the Sun (Stricherz, 2014). Because of how the globe rotates, some regions may receive more or less solar energy, which can change the

temperature (www.bionomicfuel.com).Carbon dioxide (CO2), followed by methane, has been the main cause of the rise in global warming since the Industrial Revolution (i.e., 1750). Greenhouse gases are the primary culprits behind global warming. They consist of gases such as carbon dioxide, methane, nitrous oxides, and occasionally compounds including chlorine and bromine. The radiative balance in the atmosphere is altered by the accumulation of these gases. Because greenhouse gases absorb part of the Earth's emitted radiation and reflect some of it back towards the surface, they have the overall effect of warming the Earth's surface and the lower atmosphere. Joe Farman of the British Antarctic Survey published an essay in 1985 demonstrating the drop in ozone levels over Antarctica at the beginning of the 1980s. The response was startling: extensive international research projects were launched to demonstrate that CFCs, which are employed as aerosol propellants in industrial cleaning solutions and refrigeration equipment, are to blame for the issue. The loss of ozone is the second main factor contributing to global warming. The main cause of this is the existence of source gases containing chlorine. These gases split apart in the presence of ultraviolet light, producing chlorine atoms that then catalyse the destruction of ozone.

Industrial emissions do not always contribute to global warming. By preventing solar energy from reaching the earth's surface, aerosols produced by industrial pollutants have helped to counterbalance around 26% of greenhouse warming. However, a lot is still unknown about how much of an impact aerosol have on climate. Compared to aerosols, GHGs (especially CO2) have a longer residence duration in the atmosphere.

The greenhouse effect happens when infrared thermal radiation from solar energy that strikes the earth's surface is reflected back into the atmosphere. The wave frequency of this radiation is lower than that of solar energy. Low frequency heat radiation is absorbed by GHG molecules, causing these molecules to vibrate. The energy from these greenhouse molecules is then released as infrared photons, many of which hit the earth's surface again. Nitrogen and oxygen are non-GHGs and do not absorb heat.

Watts per square metre (W/m2) are used to measure the greenhouse impact in terms of radiative forcing (RF). Since the Industrial Revolution, the net effect of rising GHG and aerosol concentrations in the atmosphere is projected to have raised the overall RF by around 2.3 W/m2 (1.1 W/m2 - 3.3 W/m2; 90% confidence interval).

9. Global warming: The Effects

One of the most challenging issues facing climate researchers is predicting the effects of global warming. This is because a variety of different elements affect the natural processes that result in rain, snowfall, hailstorms, and sea level rise. Furthermore, it is extremely difficult to forecast the volume of greenhouse gas emissions in the coming years because this is largely influenced by technical improvements and political actions. Many detrimental repercussions of global warming are produced, some of which are described here. First, excess water vapour in the atmosphere that is present falls again as rain, causing floods in many parts of the world. The evaporation process from both land and sea rises as the climate warms. In locations where higher evaporation is not offset by increased precipitation, this results in drought. This may cause crop failure and hunger in some parts of the world, particularly in regions with already high temperatures. The additional amount of water vapour in the atmosphere will precipitate again as further rain, resulting in a flood. Drought and a lack of water supply may affect towns and villages that depend on snow-covered mountains melting. The most lethal result of global warming is a rise in sea levels because glaciers and ice sheets are melting quickly due to the higher temperatures. This would cause the water levels in the oceans, rivers, and lakes to rise, which might trigger devastating floods. The regions that will be impacted by a drop in melting water are home to nearly 1/6 of the world's population, according to the Intergovernmental Panel on Climate Change (IPCC). More heat waves, more intense rainfall, as well as an increase in the severity of hailstorms and thunderstorms, are all predicted to result from the environment being warmer. The most lethal result of global warming is a rise in sea levels because glaciers and ice sheets are melting quickly due to the higher temperatures. This would cause the water levels in the oceans, rivers, and lakes to rise, which might trigger devastating floods.

i. Floods and drought

On more than half of the earth's surface, floods are predicted to happen more regularly. Mid-latitudes are predicted to get less snowfall over the winter, which will lead to less severe snowmelt floods in the spring.

ii. Rising sea level

Sea level rise isa natural consequence of the warming of our planet. When water heats up, it expands.When ice is exposed to heat,it melts.And when ice on land melts sea level rises. The tides are up to three feet higher than they were fifty years ago in some areas, such the U.S. Eastern Coast. Rising sea

levels will have increasingly detrimental effects close to the coasts, including floods, shoreline erosion, and submergence of low-lying areas, putting people, infrastructure, animals, and vegetation in danger. Rising ocean levels, floods, and more violent storm urges pose a short-term threat to the destruction of low-lying regions (like Bangladesh) and entire islands (like the Maldives and Kiribati).

iii. Changes in Ecosystem

Since millions of years ago, climate change has happened naturally at a slower rate, allowing ecosystems to adjust. However, many claim that the Anthropocene has begun in the 20th century. The pace of species loss has increased up to 100 times the"Normal" tempo (i.e., without anthropogenic impact). There is a serious issue in biodiversity todayand perhaps a sixth "mass extinction" is about to begin.

iv. Effect on living beings

The health of living things is significantly impacted by global warming. Stress from too much heat can raise blood pressure and create cardiac problems. A loss in human body resistance to viruses and illnesses can be brought on by crop failures and famines, which are a direct result of planet warming. As people move from hotter places to those with relatively cooler temperatures, global warming may also spread numerous diseases to other areas. Warmer surface waters and oceans may cause serious cholera outbreaks and dangerous infections in specific species of seafood.[7]Due to warmer and longer summers, researchers have already noted an increase in mosquito-borne illnesses like dengue fever and malaria. Another harmful illness, Lyme disease, is mostly spread by the bites of specific tick species.

• Extreme weather occurrences have immediate effects, such as elevated mortality and disability risks.

• Environmental and ecosystem changes have an indirect impact on human health, such as greater rates of death and disability during periods of excessive heat or a higher prevalence of waterborne infections brought on by higher temperatures. The prevalence of illnesses will increase as a result of climate change, particularly in areas with brittle healthcare systems and limited capacity for adaptation. Poor areas are predicted to be the most at risk for health problems due to climate change, especially poor children.

v. Spread of Diseases

Heat and humidity patterns alter as a result of global warming. This has caused the movement of disease-carrying mosquitoes.

vi. High Mortality Rates

The average death toll typically rises due to an increase in floods, tsunamis, and other natural calamities. Additionally, such occurrences may lead to the spread of diseases that may endanger human life.

vii. Natural Habitat Loss

Numerous plants and animals lose their habitats as a result of a worldwide climate change. The creatures in this situation are forced to leave their native habitat, and many of them even go extinct. This is another significant effect of climate change on biodiversity.Furthermore, it is well known that increased temperatures produce dehydration, which is a key contributor to kidney stones. Due to warmer and longer summers, researchers have already noted an increase in mosquito-borne illnesses like dengue fever and malaria.Animals are impacted by global warming as well. To live, they must relocate to cooler locations. This phenomenon has been seen in a number of locations. There have been reports of fish moving northward in the North Sea as well. The effects on species are becoming so noticeable that changes in their behaviour can be interpreted as evidence of global warming. They are the unspoken observers of the rapid changes affecting the planet.According to scientists and researchers, global warming is slowly harming the ecosystems of many species. For example, the orangutan, the only ape found in Asia, is in dire straits. A number of factors, including climate change, are threatening its last surviving strongholds in the Indonesian rainforests, placing the animal in danger of going extinct within the next several decades. Bushfires are occurring more frequently in these extensively logged forests resulting drought which last longer and occur more frequently due to global warming.Another consequence is that the biosphere started losing different types and species of wildlife and vegetation. Animals that become acclimated to historically low temperatures compared to the realities of today cannot survive.A few of them mutate. Along with this result, ongoing dry conditions cause the extinction of other animal and plant species. Human suffering also occursdue to health issues and increasingly frequent harvest failures.In conclusion, the world is constantly evolving. Due to the material advantages, human beings have made every effort to worsen the environment through scientific and technological advancement. The primary cause of global warming is human activity.

10. Mitigation strategies

All human actions that lessen greenhouse gas sources and incorporate GHG sinks are referred to as mitigation. Some of the strategies are as below:

i. Lowering greenhouse gas emissions by **t**he Energy Conservation Act of 2001 gives the government the authority to establish requirements for energy audits, energy conservation building rules, and the enforcement of standards and norms for energy consumers. According to the law, big commercial consumers are required to submit energy audits, which include action plans to minimise consumption as well as technical reports and cost-benefit analyses that verify, monitor, and analyse energy use.

ii. Energy efficiency and conservation programmes being implemented by the Bureau of Energy Efficiency (BEE) and the Central Public Works Department (CPWD) include energy efficiency performance contracting projects in nine government buildings with an estimated annual savings of approximately 30 GWh with a simple payback of less than two years.

iii. Green rating of buildings is covered by several schemes including the Indian chapter of the Leadership in Energy and Environmental Design (LEED) and the indigenous systems, namely green rating for integrated habitat assessment (GRIHA), The Ministry of Environment & Forests (MoEF) technical manual for environmental appraisal of buildings, and the Energy Conservation Building Code (ECBC). The Ministry of New and Renewable Resources (MN&RE) has also taken up development of an indigenous rating system for green buildings.

iv. The Ministry of New & Renewable Sources (MNRE), along with a specialised financial institution called the Indian Renewable Energy Development Agency Ltd (IREDA), and other institutions, ensures the promotion of renewable energy by implementing a comprehensive programme covering the entire range of new and renewable energies. The 11th Five-Year Plan seeks to add 14,500 MW to installed renewable power capacity (excluding large hydropower), or 20% of the total growth in installed utility-based capacity (78,577 MW).

v. The National Mission on Bio-diesel seeks to build biodiesel (jatropha) plantations in 26 states during the first (demonstration) phase. The second phase will result in the production of enough biodiesel to permit a 20 percent blend in vehicle diesel in 2011–12.

vi. The National Urban Transport Policy prioritises non-motorized modes and significant public transportation infrastructure above private vehicles. Steps in its implementation include the expansion of the Delhi MRTS and other mass transport projects like the Metro Bus project in Bangalore. The strategy also stimulates R&D for the commercialization of cleaner technology and supports cleaner fuels like CNG.

vii. Use of beneficiated coal unless the plants are based on clean-coal technologies, use of beneficiated coal is required for coal-based thermal power plants located more than 1000 kilometres from pit-heads, in urban, ecologically sensitive, or highly polluted areas.

11.Steps to reduce global warming

Kyoto protocol

Some individuals consume less fossil fuel. Less greenhouse gas emissions are a goal for nations. The Kyoto Protocol was signed in 1997. The goal was to bring greenhouse gas concentrations in the atmosphere down to levels below those of 1990. In 1997 the Kyoto Treaty, which has now been signed by more than 160 countries, is, to date, the most comprehensive global effort to decrease CO_2 emissions. Though the agreement was signed by the U.S. and then President Clinton consented to decrease greenhouse emissions in the U.S. by 40 percent, it has been dismissed by the Bush administration and has yet to be ratified by the U.S. Additionally, people can employ non-fossil fuel energy sources including solar panels, nuclear power, wind power, and electricity. Alternately, they can use a technique known as carbon capture and storage (CCS) to prevent the carbon dioxide from entering the environment (CCS).

Alternative fuels

The most abundant of the greenhouse gases is carbon dioxide (CO2). The emissions from motor vehicles that are fuelled by fossil fuels account for the majority (70 percent) of man-made CO2 release. The use of alternative fuels could be the answer to reducing vehicle emissions.The conversion of beets or corn into ethanol is touted by scientists today as an economically and environmentally sound solution to global warming concerns. (Boles, 2005).Vegetable oil can be used in place of diesel fuel and ethanol can be used as a petrol additive, both of which represent promising potential substitutes for crude oil. A form of alcohol that can be produced using crops like corn, wheat, or sugar beets is ethanol.Nuclear power is another option because it produces fewer pollutants, but it should be used carefully because it can cause serious accidents. If

this option is to become realistic, the biggest challenge is to get past nuclear power's security, spread, waste management, and high prices [9].

i. Solar Energy

Solar energy is already widely used and is expected to grow in popularity as an energy source in the future. Today, hundreds of thousands of people throughout the world receive their electricity from solar energy.

ii. Saving energy

Saving energy requires a number of critical components, including education and awareness. Utilizing energy wisely and only when absolutely necessary can be quite beneficial. Some examples include turning off our electrical equipment when not in use and using appliances like CFL lights.

iii. Driving green

Most GHG emissions to the environment come from automobiles. The best method for combating global warming is to use fewer cars, clean energy-powered cars and public transportation systems.

iv. Organic farming

By increasing soil organic matter content, reducing soil erosion, and enhancing soil physical structure, sustainable and organic agriculture works to combat climate change. Organic farming also minimises global warming while preserving crop yields since it does not utilise chemical fertilisers that produce significant amounts of NO2 and CH4 into the atmosphere.

v. Practices that reduce, reuse, and recycle

Today's materialism culture drives people to buy and discard. Energy and resources are utilised in the production, packaging, delivery, and retail of each thing we buy and use, as well as in its final disposal. Adopting a Reduce, Reuse, recycle lifestyle can significantly lessen the effects of climate change.

vi. Reforestation

The most effective and environmentally friendly way to remove CO2 from our atmosphere is actually free. The earth's capacity to absorb carbon dioxide from the atmosphere was significantly lowered by deforestation. In order to combat the effects of climate change, reforestation and reducing the consumption of wood products are essential. Reducing Emissions from Deforestation and Degradation (REDD), a new worldwide carbon trade mechanism, has been proposed to reduce greenhouse gas emissions (GHGs).

vii. Education

Education is the most effective tool for getting results. Individuals, groups, organisations, and the entire country need to become informed and aware of this issue.

12. Climate change program

The Government of India launched National Action Plan on Climate Change (NAPCC) on 30thJune, 2008 outlining eight National Missions on climate change. These include:

1. National Solar Mission
2. National Mission for Enhanced Energy Efficiency
3. National Mission on Sustainable Habitat
4. National Water Mission
5. National Mission for Sustaining the Himalayan Eco-system
6. National Mission for a Green India
7. National Mission for Sustainable Agriculture
8. National Mission on Strategic Knowledge for Climate Change

13. Recommendations

The use of fossil fuels must be decreased in order to minimise GHG emissions, whichcan be accomplished through reducing global warming. Investigating alternative energy sources can help us become less dependent on other countries. This will boost our economy, which will subsequently help to support our national security. With a decrease in GHG, our spending on national health will go down, and the development of environmentally friendly companies will lead to the creation of jobs that will stop the brain and muscle drain.The government must prioritise REDD(Reducing emissions from Deforestation and Degradation) more and support the construction of more biogas facilities, which will safeguard the environment and aid with carbon trading.

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Migration in Fishes and its Factors

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Introduction

In fishes, the life history needs of a population can not met by a single habitat.This is due to variability in the habitat condition that is temperature or to the changing needs of the population itself that is foraging habitat vs spawning habitat. In such cases, the fitness of individuals benefits from movement to an alternate habitat.So many fishes have evolved a life history that include coordinated movement from one habitat to another. This directed movement of part or all of a population between discrete habitat is called migration.Though all the fish do not migrate.Approximately 2.5% of a fish species undertake migration.

Cohen defined migration as it is periodic passing of animals from one place to another.Usually it is travelling from feeding grounds to breeding grounds.According to Cohen (1970) about 8000 fresh water species, 12000 marine and 120 diadromous species show regular migration.

Heape (1931) defined migration as a class of movement which impels migrants to back to the region from where they migrated.

Migration may take place in vertical direction as from the deeper to the surface water or may be in horizontal direction either upstream or downstream.

Types of Fish Migration

Typically fish migrations are grouped in to three categories. It is based arbitrarily on their relationship to the sea water or freshwater boundary.

1: Oceanodromous migration: It is a category of migration is which all migratory activity occur in sea water. It is performed by tunas, white sharks (*Carcharodon carcharias*) and plaice (*Pleuronectes platessa*) which entirely migrate with in sea water.

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2: Potamodromous migration: It is a category migration in which all migratory activity in freshwater. It is performed by Salvelinusnamaycushwhich migrate solely in lakes and by Lampetra which migrate solely in rivers.

3:Diadromous migration: It is a category of migration in which all migratory activities crosses the sea water or freshwater boundary.

It is further subcategorised in to three types-

i- Anadromous migration: When feeding and growth of fish occurs in saltwater and fully grown adults move towards freshwater to spawn, it is known as anadromous migration. So an anadromous fish, born in freshwater, spends most of its life in the sea and returns to freshwater to spawn Ex.- Salmon, Shad, Stripedbass and Sturgeon.

ii- Catadromous migration: When feeding and growth of fish occurs in freshwater and fully grown adults move towards saltwater to spawn, it is known as catadromous migration.So a catadromous fish born in saltwater, spends most of its life in freshwater and return to saltwater to spawn Ex.-eels.

iii- Amphidromous migration: It is an uncommon subcategory of diadromous migration in which there is a brief excursion from freshwater to seawater during the juvenile stage, but the majority of feeding and growth and spawning occurs in freshwater. It is most common in fishes inhabiting islands in the tropics and subtropics. Ex.- Gobies and Mullets.

Causes of migration

According to Heape, primary causes of migration are as follows-

1-Gametic migration: Gametic or spawning migration is undertaken by a fish to ensure better survival and proper development of their eggs and larvae.Just prior migration,migrants either stop feeding completely or their food intake is drasticallyreduced.The energy requirement for movement is provided by accumulated nutrients,chiefly in the form of fat deposits of the body.Catadromous and anadromous migrations are examples of gameticmigration.

2-Alimental migration: Alimental or feeding migration is undertaken mainly due to shortage of suitable food supply in the wintering or spawning grounds. After spawning, the fishes are exhausted and food requirement of their body probably provides the stimulus for feeding migration. The juvenile migrates in search of food resources and besides enjoying better food facilities, the individuals have a better chance of survival due to faster growth rate which helps them to escape predation.Better fed individuals mature earlier and be able

to produce more eggs at the time of spawning which is necessary for better survival of the species.Cod, Herring, Tuna, Grass Carp, Chinese roach and Mackerel etc. shows feeding migration.

3-Climatic migration: Climatic or wintering migrations are stimulated by inactive physical conditions and low BMR either after feeding or before spawning. It depends on both that is condition of fish and environmental changes. Ex.-Sturgeons, Roaches and Perches.

Advantages of Fish Migration

The feeding and spawning grounds may not have enough food to maintain both mature and juveniles of a species. So migration is advantageous in order to have separate spawning and feeding grounds. Migration also provides the proper environmental condition for spawning .

Migratory Behaviour of some Fishes

Migration of Salmon: Salmon lives in northern Atlantic and Pacific oceans and are anadromous fish which spend a part of life in the sea, feed and grow in this habitat and ascend the river for spawning purpose. So this type of migration is also known as upward migration.There are nine species of Salmon belonging two genera, Salmo and Oncorhynchus.Salmo spawn in the cold, clear water of lakes or upper streams. Eggs are laid in gravel beds.The young of the Atlantic Salmon remains in freshwater for 2-3 years and sometimes for six years. Pacific Salmon usually remains in freshwater for one year. Adults Salmon usually remain in the sea for two or three winters but sometimes only for one winter. The adult return to freshwater for spawning after changes occur in colour and other external features.Some Atlantic Salmon die soon after spawning,thus they spawn once in their life time but their youngonesinherently know their way back to the sea.

The migration in Atlantic Salmon (Salmo) is described in following steps-

1-The Atlantic Salmon spawn during November and December. As the breeding period approaches, they ascend the river after travelling several thousand kilometers in the sea.

2-On entering freshwater habitat, they stop feeding, bright silvery colour changes to a dull reddish brown shade and skin becomes spongy and thick. The male Salmon become spotted with red, orange and large black spots, called as Red fish. The female Salmon becomes darker and called as black fish.

3-When these fishes select a suitable spawning ground,they segregate in to pairs. After spawning, the adults usually die but some Atlantic species do survive and begin return journey to sea. Generally males do not survive for second breeding but females are able to reach the sea. They start feeding and soon recover their normal conditions and silvery colouration.

4-In freshwater the Salmolives on the reserve food accumulated in the body in the form of fat.

5- In freshwater, after hatching the first freshwater phase is Alevin stage which stay in breeding ground and use remaining nutrient in their yolk sacs. During this developmental stage,their young gills develop and they become active hunters. Next developmental stage is fry stage and now fish grow and leave the breeding ground in search of food. They move to areas with higher prey concentrations.The final freshwater stage is parr and now they prepare themselves to migrate to the Atlantic ocean and after reaching the home grounds,they become the adult.

It has been reported that young Salmon hatched in freshwater could not return to the sea until their salt secreting cells have developed. Huntsman and Hoar reported that changes in body behaviour may expose the Atlantic Salmon to different environmental condition to which they react in specific way. Probably it results travelling up freshwater streams by adults and downstreams by juveniles.

Migration of eel

The eel is a long,thin bony fish that live and feed in the freshwater and descend to marine water for spawning so called as catadromous fish. This type of migration is also called as downstream migration. The European eel, *Anguilla anguilla* and *American eel, Anguilla rostrata* represent the best example of catadromous migration.

The Danish Prof.J.Schmidt solved the mystery of eel migration. The eel have two distinct phases in their life history and looks morphologically different from each other.

Yellow coloured eels represent the feeding phase.Their length vary from few inches to five or six feet and are found in both salt and freshwater. As the breeding period approaches, they stop feeding and shows following changes-

i-The eye become large.

ii-The lips become thinner and snout becomes sharper.

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iii-The alimentary canal shrinks.

iv-The gonads become fully matured.

v-The colour becomes metallic silvery along the sides.

vi-Pectoral fin become more points.

These metallic silvery coloured eel represent breeding phase and migrate down the rivers to reach sea. The metallic silvery eel travels a distance about 4500-6000 km in the Atlantic ocean to reach in their breeding ground, south of Bermuda. Eel spawn at the depth of about 500 metres with the temperature ranging between 10-12 .The adult die immediately after spawning.

The egg hatches in to transparent leaf like pelagic larva, called protocephaline. These larva retain yolk sac and ascend to a depth of about 200 metres.After reabsorption of its yolk content,protocephalinelarva changes to leptocephalus. The leptocephali are confined to a depth of about 100 metres.Theyhave leaf like, glassy transparent body, long and pointed teeth and large silvery eyes. These are about 5 cm in length. Theleptocephali starts homeward journey. During their journey,they grow rapidly during the first few months.They reach middle of Atlantic by their second summer and finally touches the coast of Europe by the third summer. Now they attain a length of 8-9 cm and become 2 years old. Duringautumn of third year, leptocephali undergo metamorphosis during which they stop feeding. The sharp needle like teeth are lost and body becomes cylindrical with a decrease in length.Now they are called as Glass eel or elvers.Elver may remain in coastal water for 1-2years till they are strong enough to ascend the rivers. 3 year old elever starts ascending the river in large number and reach their suitable resting places.Now they start feeding and growing and on attaining maturity, they change in to silver eel before starting the spawning migration to the sea.

Maintenance of Catadromous and Anadromous fish in changed environment

When Salmon reaches the freshwater habitat during its upstream migration, it faces problem of salt depletion and overhydrationas compared with tendency towards dehydratIon and salt excess in the ocean. It can cope up the problem of over hydration by producing dilute urine and depletion by absorbing salts through chloride cells of gills.In this way Salmon can withstand with freshwater habitat.

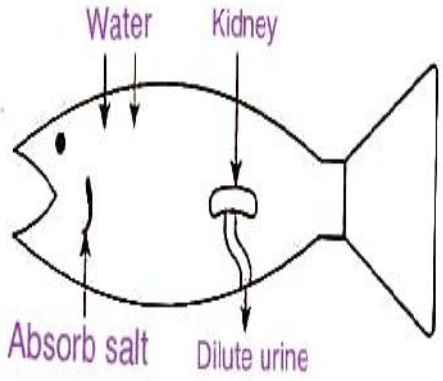

Fig.1: Diagrammatic representation of maintenance of Salmon in freshwater habitat

When Anguilla reaches the ocean habitat during its downstream migration, it faces problem dehydration and salt excess as compared with the tendency towards overhydrationand salt depletion in freshwater.It can cope up the problem of dehydration by drinking water and by producing concentrated urine and salt excess by secreting salt through chloride cells of gills.In this way eel can withstand with saltwater habitat.

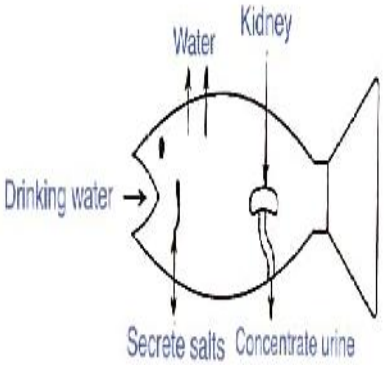

Fig.-2:Diagrammatic representation of maintenance of Anguilla in sea water habitat

Factors influencing fish migration

Migration is influenced by various factors which may be physical, chemical or biological.Physical factors include temperature,light intensity and photoperiod,currents and tides etc.Temperature is an important abiotic factors responsible for fish migration.In summers higher temperature of sea water provides stimulus to Salmon for migration and it moves upstream for spawning.

The intensity and light duration also influences migration of various fish species. Sturgeon migrate during night where as herrings migrate during full moon.Light condition also influences the speed and direction of migrants.

The chicas factors include the salinity, pH, dissolved oxygen, smell and taste of water. Salinity of water is an important factor. Salmo, Anguilla and Hilsa can with stand with wide range of salinity.These are therefore migrates from freshwater to sea water and vice versa. The CO_2 gradient in water is an important determining factors for migration. In Salmo accelerated metabolism at spawning time will need more oxygen so they ascend the stream to fulfil the demand of more oxygen. The biological factors such as sexual maturity, blood pressure, effect of endocrine secretion and physiological changes in the body system and behaviour influences the migration.

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Effect of different sound frequencies on growth and antibiotic susceptibility on microorganism

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Abstract

All the different life forms are known to be influenced by various physical, chemical and biological environmental factors. Generally the organisms in the ecosystem respond to the external environmental factors to which they are exposed to by varying degrees. Sound is one such physical factor which has a wide distribution in all ecosystems. Sound of different frequencies has been shown to affect the growth of microorganisms and to observe the interaction of sound and organisms, its physical signals for intracellular communication and study how cells sense and respond to different sound frequencies. In our research we have used *Escherichia coli* as our test organism.The organism was exposed to sound frequency upto 1000Hz i.e. The audible range. Further effect of antibiotic susceptibility was also checked using streptomycin with different sound frequencies. The growth was studied in the form of colony forming units and antibiotic susceptibility changes were studied in context to zone of inhibition. We could observe that *Escherichia coli* had shown changes in growth and antibiotic susceptibility. The maximum number of colonies were obtained at 600Hz. Optimum range for inhibition of *E.coli* by Streptomycin is between 400 to 600 Hz. The reduction in inhibition at higher frequencies (above 1000Hz). Thus microorganisms responded to sound. Thus, sound/music affect on immune system, endocrine, psychological responses etc. So base on sound we can either increase or decrease microorganisms to treat patients suffering from various diseases.

Key words: Audible sound, Sound frequency, *Escherichia coli*, Growth, Antibiotic susceptibility

Introduction

Microorganisms are ubiquitous. They live in environments where life is even impossible to imagine existence. They can reproduce in environments where no other organism can survive. They are found to survive in the extreme harsh environments like hot springs, deep underground veins of water, in volcanic rock beneath the ocean floor. Bacteria such as *Bacillus infernus* are able to live over 2.4 km below earth surface without oxygen and at temperatures above 60^{o}C (Willey et al, 2014). They are also found in extreme environments like Great Salt Lake and Dead Sea with high sodium chloride concentrations (Oren, 2008).These microorganisms are referred to as extremophiles and can exist in such environments due to their extraordinary adaptations. They are even found to exist on human body as normal flora (Willey et al, 2014) including skin, nose and throat, gastrointestinal tract, urinogenital tract etc. However these microorganisms are considered as "Normal" and they exist in moderate conditions.

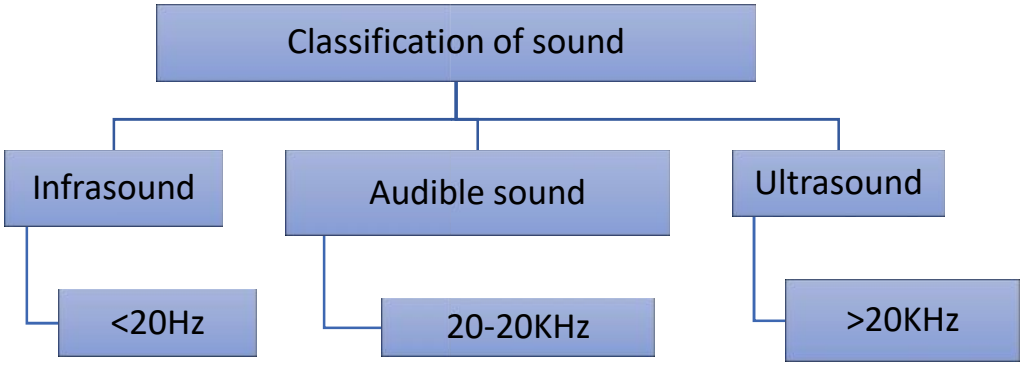
All the habitats pose certain changes to which microorganisms respond constantly in order to sustain. These changes may be a stimuli that can either promote their growth and reproduction or can kill them, however it also depends on the organism as an environment that is harmful to one organism may be beneficial to another. For example, providing high sugar concentrations may be detrimental to bacterial growth but promotive to moulds. The major factor affecting microbial growth is its nutritional components that mainly include carbon source, energy source and additional materials like macronutrients, micronutrients and electron donors. Other than nutritional components certain environmental factors are also known toinfluence microbial growth. These can be broadly classified into physical and chemical factors. Physical factors include temperature, pH, moisture, light, Pressure, Radiations (UV, Gamma and X-rays), Metal ions, Pulsed Electric Field (PEF), Oxygen Concentration and SOUND. Chemical factors include growth influenced by major nutritional components like Carbon, Nitrogen, Phosphorus, Sulphur, Hydrogen etc (Kaiser, 2022).

What is sound?

Sound is a repeated pressure wave travelling through matter (Banerjee et al, 2018). It is a vibration that propagates as an acoustic wave through transmission medium such as gas, liquid and solid. It is a physicalstimuli to which almost all life forms are exposed to varying degree (Kothari and Sarvaiya, 2017). As it requires a medium to be transported and moves via particle interactions, this disturbance can be characterized as a mechanical

wave (Gu et al, 2010). In the biological world, organisms that are exposed to sound have been shown to respond to it. Perception of received sound waves occurs in human brain. These waves are however limited to the audible frequency (Garuba et al, 2021).

Depending upon the frequencies, sound waves are broadly classified into Infrasound (10^{-4} to 20 Hz), Audible sound (20-20kHz) and Ultrasound (>20kHz) (Gu et al, 2016).



The above figure shows the classification of sound frequencies. Frequency is the speed of vibration and determines the pitch of sound. It is denoted in Hz (Hertz). The infrasound waves are in audible range to elephants and others like rhinos, hippos and whales while audible frequency is heard by humans. The ultrasound frequencies are heard only by a few animals like bats that use it to see trees and insects at night.

Effect of music on various forms (Mortazavian, 2012; Exbrayat and Brun, 2019):

Music is an organised sequence of sound. It effect on Organisms, cells, plants, animals. On human being music effect on emotions, stress, immune system etc. (Exbrayat and Brun, 2019). Sound in the form of music has been studied to affect organisms ranging from the primitive unicellular forms to the most developed plants and animals including humans.

Effect on Unicellular organisms: All unicellular forms from prokaryotic to eukaryotic cells are sensitive to music showing changes in their growth and activity. It influences the production of metabolites and has also been reported to impair the membrane permeability.

Effect on cell culture: cells like RBCs has shown an increased deformability and reduced aggregation due to music. Exposure of low sound frequencies to human osteoarthritic chondrocytes grown invitro improved cell proliferation and did not generate Reactive Oxygen Species (ROS). At cellular levels, sound vibrations can increase solubility of polyamines and sugars and regulate transcription of certain genes modifying the activity of various proteins.

Effect on plants: Sound waves have shown to influence the germination rates and increment in growth and development increasing their yield. It can also enhance its immunity against pathogens and tolerance to drought.

Effect on animals: The effect on animals has been studied on varied aspects. They are as:

1. It has been proved to induce relaxation thus reducing stress and anxiety levels.
2. It effects on embryonic development has been studied in chicks and shown to decrease the fluctuating symmetry i.e. a small random deviation from a perfect bilateral symmetry resulting due to genetic or environmental stress during development. In humans it has shown to facilitate neurogenesis and repair of neurons during embryonic development.
3. Patients suffering from stroke, Alzheimer's disease or Parkinson's disease had shown to have an increased percentage and activity of Natural Killer cells of the immune system irrespective of their pathological state. Thus it improves the immune responses.

Understanding the influences of environmental factors on microbial growth helps to gain an insight on the physiology of microorganisms and also paves a way to control their growth. In this chapter we have focus on the effect of Sound on the Growth and Antibiotic susceptibility of microorganisms using *Escherichia coli* as the model organism.

1. Effect of various sound frequencies on growth of *Escherichia coli:*

Test organism:

We have used *Escherichia coli* as the test organism as it has simple nutrient requirements and a lower replication time due to a smaller genome size. It is also a common bacteria present in our small intestine that aids in digestion.To study its effect we had used sound frequencies of audible sound ranging upto 1000Hz.

Media:

Nutrient agar was used as the growth medium to provide essential nutrients and support the growth of organism under laboratory conditions.

Procedure (Banerjee et al, 2018):

1. Awell-developed colony from 24 hours young culture of *E. coli* was taken and its suspension was prepared.
2. Five specific sound frequencies at an interval of 200Hz were selected.The respective frequencies were: 200Hz, 400Hz, 600Hz, 800Hz and 1000Hz. Also a control plate (no frequency) was used.
3. Thus for each round of experiment, 6 nutrient agar plates (control + 5 frequencies) were taken and were streaked with young culture suspension (0.1ml).
4. The experiment was performed in triplets.
5. The frequencies were delivered through mobile phones which were connected to earphones.
6. The earphones were adhered to petri plates such that the inoculated organisms suffer the effect of necessary frequencies (Fig.1).
7. These plates were incubated at 37^{o}C for 24 hours. Also utmost care was taken to prevent any interference of external sound.
8. The final number of colonies for each frequency was taken as an average of the number of colonies on the three plates for each frequency.

Fig.1: Experimental set up-plates with selected frequency and control plate (Without frequency)

Table.1: No. of cololonies of *E.coli* at different frequencies

Different frequencies (Hz)	No. of colonies
Control	120
200	122
400	131
600	229
800	199
1000	106

Results and discussions

As shown in the Table.1, there was a consecutive increase in the number of colonies upto 600Hz while there was a significant decrease in this number at frequencies above it i.e. 1000Hz. The maximum number of colonies were obtained at 600Hz. Our result supports Kothari et al, 2018, where it was shown that microorganisms can respond to various sound stimuli and this response is a function of level of sound frequencies.Banerjee et al, 2018 also studied effect of sound on *E.coli* with different frequencies and he found maximum colonis at 432 Hz (46 Cfu) and sound effect on antibiotic susceptibility with 20mm zone size with same frequncey i.e. 432Hz. So, 432 Hz is good frequency for beneficial effect. Also 432 Hz is a frequency of classical music. In our study we have also found maximum number of colonies up to 600Hz.

The increase in the number of colonies compared to control may be due to one of the reasons like: increment in nutritional uptake due to changes in fluidity of membrane wall and membrane lipid increasing the permeability and genetic material synthesis in S-phase (Gu et al, 2016), sound exposure could significantly promote synthesis of the intracellular protein and RNA in the early treatment stages (Gu et al, 2016). The decrease in the colony number may be due to the production of Reactive Oxygen Species (ROS) due to higher frequency (Banerjee et al, 2018). It can also affect lipid peroxidation and lead to oxidation of amino acids in proteins.

After studying how various sound frequencies affect the growth of microorganisms, effect of antibiotic susceptibility on microorganism *(E.coli)* with different sound frequencies were studied.

2.) Effect of antibiotic susceptibility on *E. coli* at different sound frequencies:

Antibiotics have been widely used since decades by humans for curing diseases of humans as well as plants (Martinez, 2017). Antibiotics at all times may not be degradable and thus pass from the human or animal body into different habitats like river water or soils and these are polluted by antibiotics that act as an ecological factor which could potentially affect microbial communities in terms of structure and productivity of microbiota colonizing such habitats (Martinez, 2017). It also affects phylogenetic structure and ecological functions including nitrogen transformation, methanogenesis and sulphate reduction (Ding and He, 2010).

Antibiotics are compounds produced by microorganisms or similar chemically synthesized substancewhich in very low concentrations can inhibit the growth of other microorganisms (Patel R and Patel K, 2006). Most pathogenic bacteria are highly sensitive and susceptible to a new antibiotic. However due to a continuous prolonged exposure of these antibiotics, many microorganisms are becoming resistant to it which is defined by the term "AMR- Antimicrobial Resistance or Drug Resistance." The ineffectiveness of medicines due to resistance of microorganisms has increased the difficulty to treat infections and enhanced the risk of disease spread leading to severe illness and even death (World health organization, 2021).

Sound in the form of different frequencies has shown to aid in this problem by enhancing the uptake of these drugs due to membrane permeability changes.This was seen by us in our research.

Test organism: *Escherichia coli* is used as the test organism.

Media: Nutrient agar were used a growth medium to promote microbial growth under laboratory conditions.

Antibiotic: Streptomycin (0.75% W/V) was used to study antibiotic susceptibility.

Procedure:

1. A young culture of *E. coli* was used to prepare suspension.
2. To check antibiotic susceptibility 3 different sound frequencies were selected, i.e. 200Hz, 600Hz and 1000Hz.

3. The experiment was performed in triplets. Also a control plate was used. Thus for each experiment, 4 plates were required (3 frequencies + control).
4. The prepared culture suspension (0.1ml) was spread on the respective plates.
5. Antibiotic was delivered by Kirby-Bauer's disc diffusion method (Banerjee et al, 2018) where discs (6mm in diameter) were soaked in the prepared streptomycin solution and placed on middle of the plates.
6. Allow the plates to stand at refrigeration temperature for 30 minutes to permit uniform diffusion of antibiotic before allowing growth of the organism.
7. The respective frequencies (200Hz, 600Hz and 1000Hz) were delivered to the respective plates through mobile phones connected to the earphones. The earphones were adhered to petri plates such that the inoculated organisms suffer the effect of necessary frequencies as shown in fig. 2.
8. These plates were incubated at 37^{o}C for 24 hours. Also utmost care was taken to prevent any interference of external sound.

Fig. 2: Experimental set up showing petri plates applied with specific frequencies

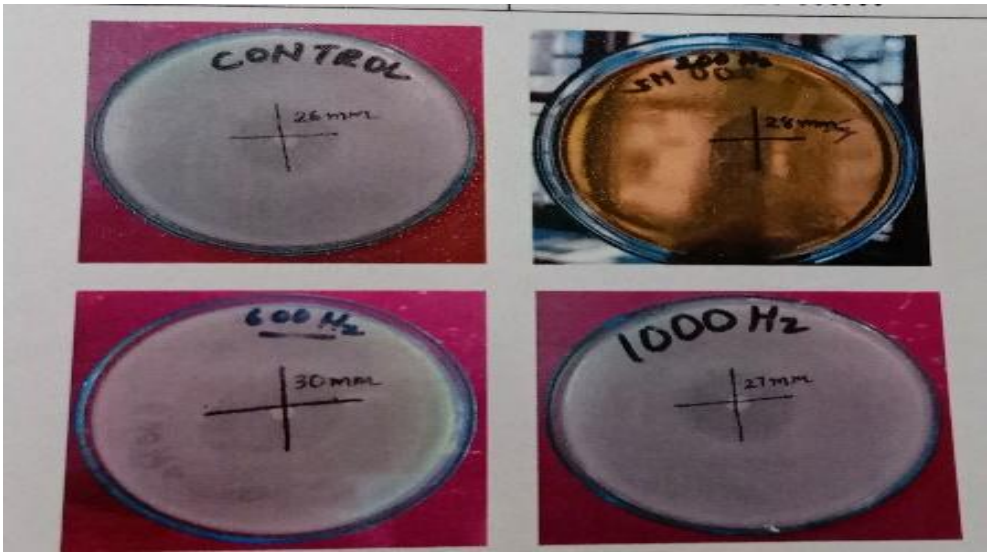


Fig. 3: Zone of inhibition at different sound frequencies against streptomycin.

Table.2: Zone of inhibition at different frequencies

Different frequencies	Zone of inhibition
Control	26mm
200Hz	28mm
600Hz	30mm
1000Hz	27mm

Results and discussions:

The result was observed in the form of zone of inhibition which was measured by zone-scale. (Table.2, Fig.3). The control plate has a zone of inhibition 26mm, while, zone of inhibition with 400 Hz sound frequency has 28mm, 600Hz has 30mm. Whereas the plate with 1000Hz shows a zone of inhibition 27mm. Thus among the selected frequencies, the lowest zone was observed in the plate exposed with 1000Hz frequency and greater zone of inhibition was found at 600 Hz.

Banerjee et al, (2018) had observed a greater zone of inhibition at 432Hz while a lower zone at 4000Hz.Thus it can be said that the test organism has a decreased antibiotic susceptibility at higher frequencies.

From the above data and the research by Banerjee et al (2018), we can conclude that the optimum range for inhibition of *E.coli* by Streptomycin is between 400 to 600 Hz. The reduction in inhibition at higher frequencies (above 1000Hz) may be due to tentative development of drug resistance to normal dosage or loss of susceptibility (Banerjee et al, 2018).

Thus, both the experiments i.e growth and antibiotic susceptibility at different sound frequencies have shown that organisms have a decline in growth as well as antibiotic susceptibility at higher frequency. So, exposure to higher frequencies is detrimental as it impairs not only with the growth of useful organisms but also leads to the loss of antibiotic susceptibility thus enhancing pathogen growth.

Our result also support kothari et al, 2018 work, where they studied mono-frequency sound for gram negative microorganisms *(Chromobacterium)*and bacteria respond to sonic stimuli. And it influence growth, metabolism, antibiotic sensitivity etc. (Sarvaiya and Kothari, 2015).

The frequency range of classical music is of about 425 Hz (Kothari and Sarvaiya, 2017). In our study we also found maximum number of colonies up to 600Hz. This can be used to treat patients with decreased metabolism to increase number of beneficial organisms, while higher frequency can be provided to patients affected by pathogens to decrease number of hazardous organisms.

Audible sound increase *E. coli* CFC under normal condition and enhance inhibitory effect of osmotic stress (Gu et al, 2010). Activity of NK cells were also increases in patients with sound (Hasegawa et al, 2001). Research suggest that type of sound has beneficial effect but not all kind of sound do the same, e.g. Noise or unpleasant sound causes inhibitory effect on cells (Clark, 1991).

Thus, sound/music affect on immune system, endocrine, psychological responses etc. So base on sound we can either increase or decrease microorganisms to treat patients. Much of work needs to be done in this area as it has been studied very little. Very less effort have been made to study the effect of sound on various living forms (Exbrayat and Brun, 2019). Thus this area could prove to be demanding and a vocation for future researchers.

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Biofertilizers for better Agrosystem

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Introduction

The production of mineral fertilizers is an expensive process, since it requires high energy consumption, and cannot be produced by small farmers. Recently the use of bio-fertilizers in agriculture has received considerable attention because of the environmental problems associated with alternative disposal methods and the harmful effect on human health. Bio-fertilizers are frequently recommended firstly for improving biological, physical and chemical properties of soil and secondarily to get high and clean agricultural yield products free from undesirable high doses of heavy metals and other pollutants. For the production of good quality biofertilizer, an efficient nitrogen fixing strain is required. Beneficial strains of microorganisms are selected and are used as inoculum. While producing Biofertilizers, the standard laid down by Bureau of Indian Standard (BIS) must be taken into consideration. A commercial protocol of Azotobacter biofertilizer is as follows:

Material and Method:

a. Growth of Azotobacter: Usually Azotobacter is grown on a solid medium free of nitrogen. After some times (6 months) old growth of Azotobacter is transferred to a fresh solid medium to renew the growth. This procedure is repeated periodically so that the culture can be maintained in good condition.

b. Production:

i. Mother culture: A pure growth of any organism on a small scale is called as a mother culture. Mother culture is always prepared in a conical flask of 500- or 1000-ml.capacity and then this mother culture is used for further production.

For this purpose, one-liter conical flasks are taken to which 500 ml of broth of nitrogen free medium is added and these flasks are then plugged with non-absorbent cotton, sterilized in an auto clave for 15-20 minutes at 75 lbs. pressure for 15 minutes. Flasks are then inoculated with mother culture with the help of inoculating needle aseptically. The flasks are transferred to shaker and shaking is done for 72-90 hours so as to get optimum growth of bacteria in broth. Bacteria are multiplied by binary method i.e., cell division. After about 90 days, the number of per milliliters comes to about 100 crores. Total growth of bacteria in this broth means starter culture or mother culture, which should carefully be done, since further purity of biofertilizer or quality of biofertilizer depends upon how mother culture is prepared.

ii. Production on a large scale: Azotobacter is multiplied on a large scale by two ways viz. Fermenter and Shaker. The fermenter is most automatic and accurate method of multiplication of any micro-organism. In this method, the medium is taken in a fermenter and then sterilized. After this pH of the medium is adjusted and 1% mother culture is added. In order to get an optimum growth of the Azotobacter required temperature and oxygen supply is adjusted so that concentrated broth is made. This concentrated broth of the culture is then mixed with a carrier previously sterilized and bio-fertilizers is prepared. Depending upon the demand and supply suitable fermenter is selected.

In the 2nd method i.e., shake method, a suitable medium is prepared transferred to conical flask of suitable capacity. These flasks are then sterilized in an autoclave at 15 lbs. pressure for 15 minutes. Each flask is inoculated with 10 ml mother culture and they are transferred to shaker for multiplication where they are kept for 72-90 hours. This broth is mixed with a suitable carrier previously sterilized. Thus, biofertilizer is prepared, filled in plastic bags and stored in cool place.

Selection of carrier:

A carrier is nothing but a substance which has high organic matter, higher water holding capacity and supports the growth of organism. In order to transport the biofertilizer and becomes easy to use the suitable carrier is selected. Generally, Lignite cool, compost and peat soil are suitable carriers for Azotobacter. Out of these carriers lignite is most suitable for this organism, since it is cheaper, keeps organism living for longer period and does not lower the quality of bio-fertilizers.

The lignite comes in clouds and hence it is ground in fine powder by grinding machine. Its finesses should be 250-300 mesh. The pH of the carrier is adjusted to neutral by adding $CaCO_3$. The lignite naturally has a variety of micro-organism and hence it is sterilized in autoclave at 30 lbs. Pressure for 30 minutes. After this the broth is mixed with lignite 1:2 proportion by following method.

Galvanized trays are sterilized and used. To these trays, previously sterilized lignite is transferred and broth is then added (lignite 2: broth 1) and mixed properly. Trays are then kept one above the other for 10-12 hours for allowing the organism to multiply in the carrier. This mixture is then filled in plastic bags of 250 g or 500 g capacity. Plastic bags are properly. Trays are then kept one above the other for 10-12 hours for allowing the organism to multiply in the carrier. This mixture is then filled in plastic bags of 250 g or 500 g capacity. Plastic bags are properly sealed. All the required information such as name of biofertilizer, method of use expiry date, etc. is printed on plastic bags. In this way biofertilizer is ready to sell or use. If biofertilizer is used immediately then bags are stored in cool place otherwise they should be stored in cold storage in order to keep biofertilizer in good quality.

As per ISI standards, one gram of biofertilizer immediately after it is prepared should have one crore cells of bacteria and 15 days before expiry date one gram of biofertilizer should have 10 lakh bacteria. If biofertilizer is stored at 15-20 ^{0}C then it will remain effective for 6 months. However, at 0 to 4 ^{0}C (cold storage) the bacteria will remain active for 2 years. The storage periods are decided after testing the biofertilizer for that particular storage conditions, such as temperature and humidity.

Use of Biofertilizer:

A plant needs nitrogen for its growth and Azotobacter fixes atmospheric nitrogen non-symbiotically. Therefore, all plants, trees, vegetables, get benefited. However, especially cereals, vegetables, fruits, trees, sugarcane, cotton; grapes, banana, etc. are known to get addition nitrogen requirements from Azotobacter. Azotobacter also increases germination of seeds. Seeds having fewer germinating percent if inoculated can increase germination by 20-30%.

How to apply bio-fertilizer?

a. Seed inoculation:

On the basis of efficiency of Azotobacter, other micro-organisms present in the soil, benefits obtained from biofertilizer and expenditure it has been fixed to use Azotobacter - bio-fertilizer at the rate of 250 g biofertilizer for 10-15 kg. If one knows this proportion then take a definite quantity of seed to be inoculated. The required quantity of fresh biofertilizer is secured and slurry is made by adding adequate, quantity of water. This slurry is uniformly applied to seed, seed is then dried in shed and sown. Some stickers are used in order to adhere biofertilizer to seeds. Viz. Jaggery or gum arebia.

b. Seedling inoculation:

This method of inoculation is used where seedlings are used to grow the crop. In this method, seedlings required for one acre are inoculated using 4-5 packets (2-2.5 kg). For this, in a bucket adequate quantity of water is taken and biofertilizer from these packets is added to bucket and mixed properly. Roots or seedlings are then dipped in this mixture so as to enable roots to get inoculum. These seedlings are then transplanted e.g., Tomato, Rice, Onion, Cole, Crops, flowers.

c. Self-inoculation:

In this method 50 liters of water is taken in a drum and 4-5 kg of Azotobacter biofertilizer is added and mixed properly. Sets are required for one acre of land are dipped in this mixture. Potato tubers are dipped in the mixture of biofertilizer and planting is done.

d. Soil application:

This method is mostly used for fruit crops, sugarcane, and trees. At the time of planting fruit tree 20 g of biofertilizer mixed with compost is to be added per sapling, when trees became matured the same quantity of biofertilizer is applied.

In sugarcane, after two to three months of planting i.e., before earthing up, 5-6 kg of biofertilizer per acre is applied by mixing with compost or soil. Although, Azotobacter fixes nitrogen non-symbiotically, it also fixes atmospheric nitrogen in the rhizosphere region i.e., soil around the seedlings or

trees. Biofertilizer applied to seed or seedlings bacteria remain around seeds or seedlings and use organic carbon for their metabolism. When seeds are germinated or seedlings set in soil they leave or exude root exudates which become food of these bacteria. They grow on these substances which include sugars, organic acids, amino acids and fix atmospheric nitrogen most efficiently. Nitrogen so fixed by these bacteria becomes available to plants after death and degradation of bacterial cells.

Conclusion

1. Azotobacter contributes moderate benefits.
2. Azotobacter is heaviest breathing organism and requires a large amount of organic carbon for its growth.
3. It is poor competitor for nutrients in soil and hence its growth promoting substances, fungistatic substances.
4. It can benefit crops by Nitrogen fixation, growth promoting substances, fungi static substances.
5. Azotobacter is less effective in soils with poor organic matter content.
6. It improves seed germination and plant growth
7. It thrives even in alkaline soils.
8. Azotobacter is tolerant to high salts.

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Prevalence of HPV in oral and Oropharyngeal cancer

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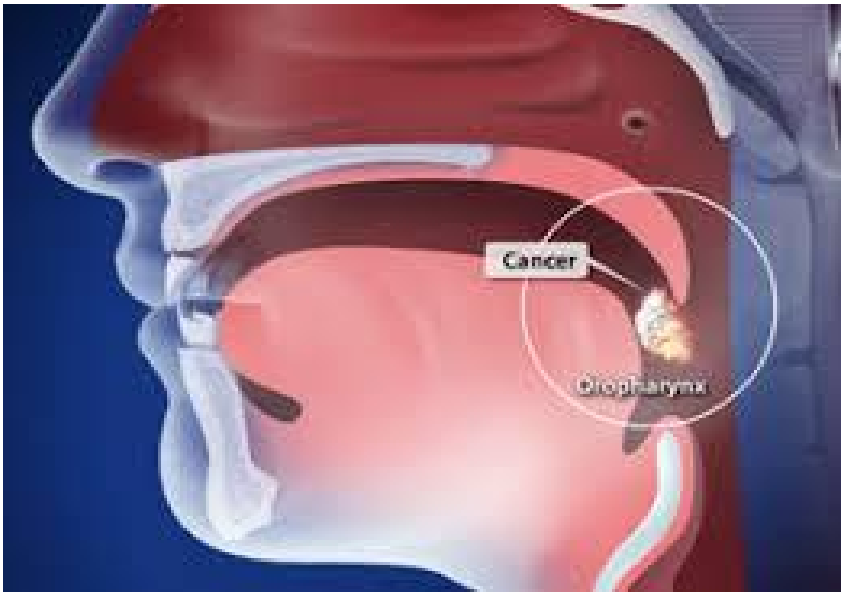
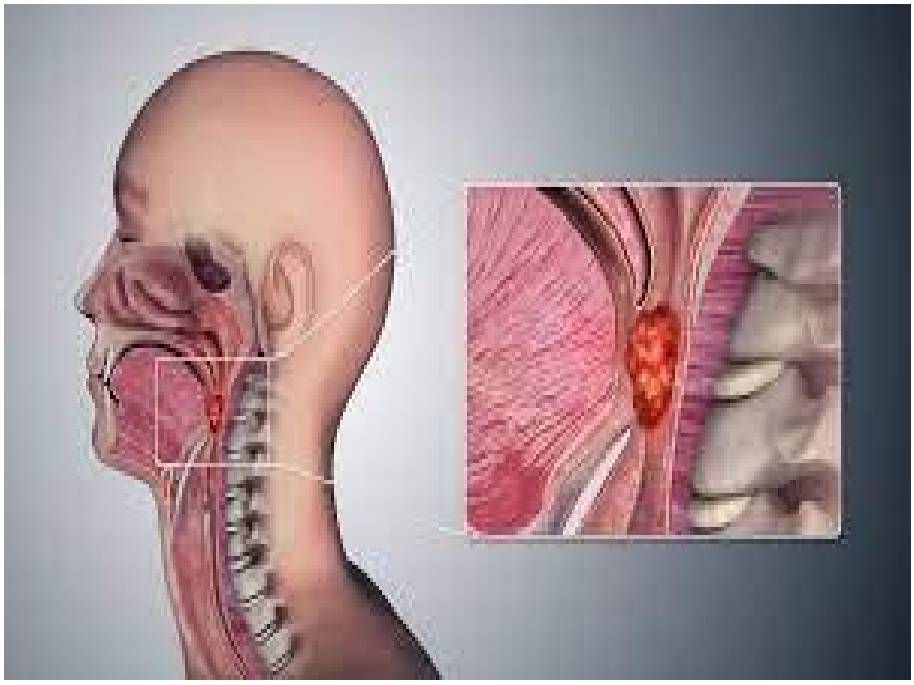
Introduction

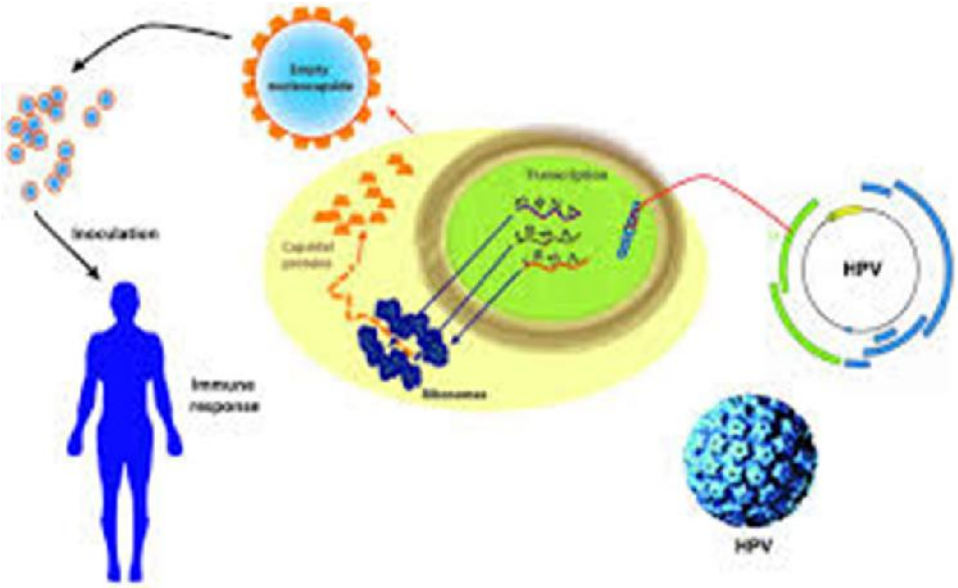
Dr. Maura Gillison was the head of the study which tested 253 patients diagnosed with head and neck cancer. In 25% of these cases, the tissue taken from tumors was HPV positive. HPV-16 was present in 90% of the positive SPB tissues. This information helped to confirm that there is a strong link between HPV and oral cancer. We have studied 30 patients with oral and oropharyngeal cancers. The tissue from the region was sent for HPV-16 and HPV-18 detection and it was found that 18 out of 30 patients were HPV positive regardless of tobacco and alcohol habits. This small study indicates a strong association between HPV infection and oropharyngeal cancer in the Indian population and needs the detailed study.

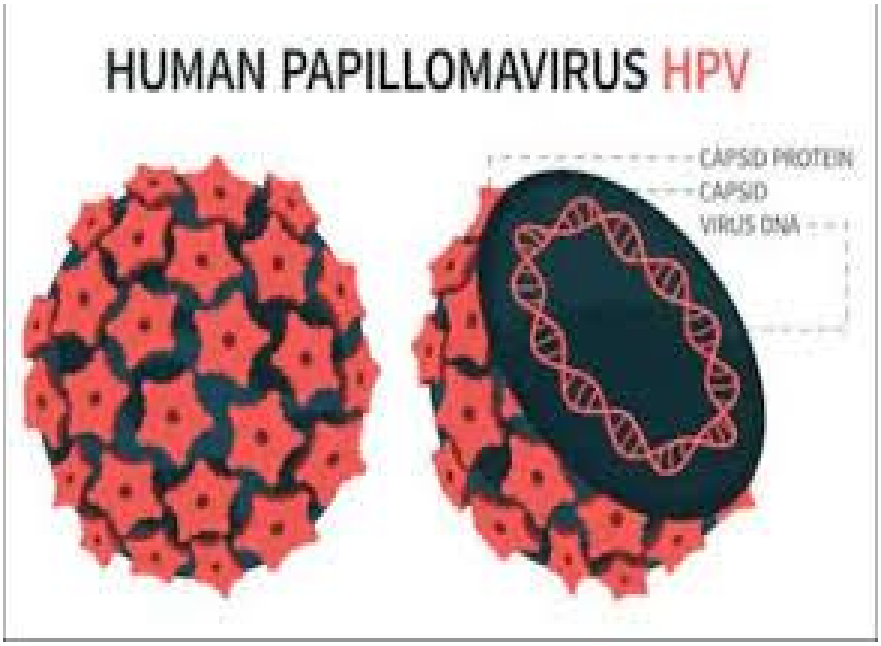
Human Papilloma viruses (HPV's) are a group of more than 100 related virus. They are responsible for benign (non cancerous) tumors. Some types of HPV are associated with oncogenic risk. HPV may also play a role in Cancer of the anus, vulva, vagina, penis as well as oropharyngeal cancer. Incidences of oropharyngeal cancer in India are the highest in the world (20 to 30 lakhs). In 80 to 90% of cases oropharyngeal cancers are associated with either tobacco or tobacco and alcohol consumption both. In 10 to 20% of cases, tobacco and alcohol are not the culprits. Sites of HPV-associated cancers of the oropharynx and oral cavity are tonsils most common site (42 to 43.6%) based on tongue 38.4%. Recently HPV infection has been found an important positive factor in oral and oropharyngeal cancer.

It is well established that HPV-16 and HPV-18 are causative factors in cervical cancer, now these viruses have also been linked to oral and oropharyngeal cancer.


Cancer
Oropharynx




Inoculation
Immune response
HPV
HPV


HUMAN PAPILLOMAVIRUS HPV
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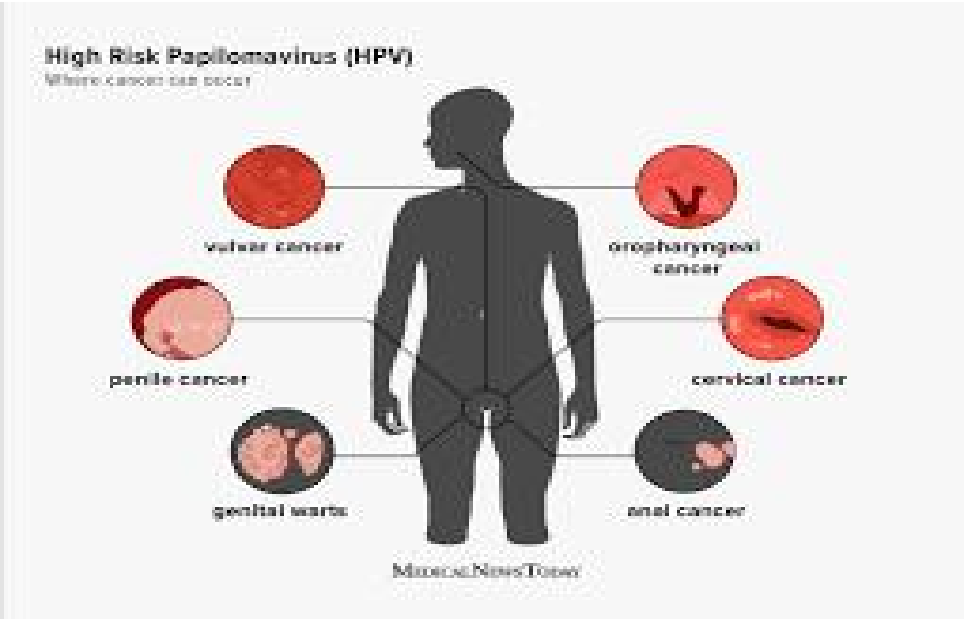
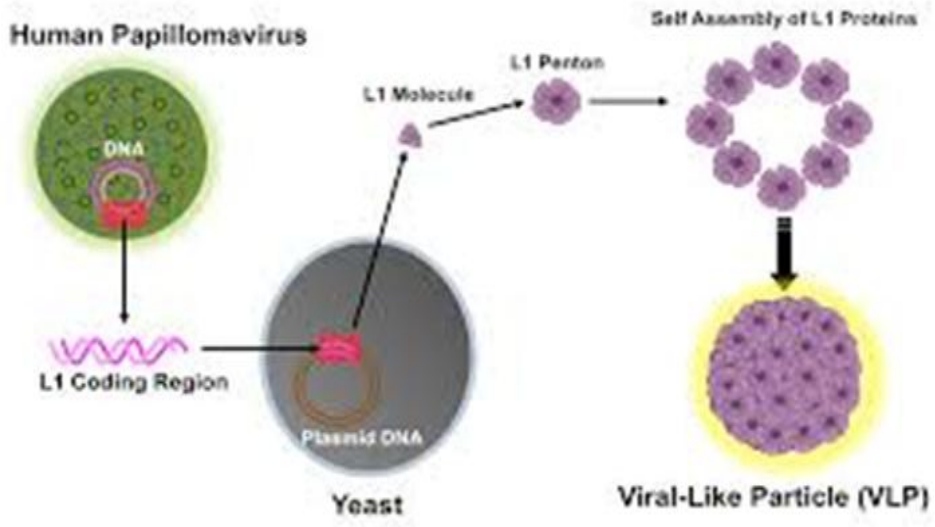
VIRUS DNA





Materials and Methods

Specimens were collected from 30 case patients before therapy and oral mucosal specimens were collected with the use of saline oral rinse and 5 to 10 strokes of cytology brush on the posterior oropharyngeal wall. Serum samples were collected and Stored at- 80° C.

For case patients formalin fixed, paraffin-embedded tumor specimens were obtained for detection of HPV.

All patients completed an audio, computer-associated self-administrated interview that obtained information about demographic characteristics, oral hygiene, family history of cancer, lifetime history of tobacco and alcohol use.

Laboratory studies

For HPV-16 and HPV-18 in formalin-fixed and paraffin-embedded tumors from all case subjects. The HPV-16 and HPV-18 status of a tumor was defined as specific stain of tumor cell nuclei for HPV-16 by dacogen point amplification.

Serological analysis

Serum antibodies to the HPV-16 protein were detected with the use of **ELISA (an enzyme linked immuno sorbent assay) based.**

Table-1:

SEX	
Male	25
Female	5
Age	
<40yrs	11
40-50	16
>50yrs	3
Oral hygiene	
Good	0
Poor	30
Toothbrush	
Yes	5
No	25
Tobacco or Tobacco+Alcohol	25
No Tobacco	5

Table-2:

Result		Alcohol+Tobacco	No Tobacco
HPV virus +	18	14	4
HPV absent	12	11	1

Result:

30 Consecutive male patients were enrolled with newly diagnosed oropharyngeal cancer in 2009.In the Univariate analysis, patients were similar with regard to age and sex.

A history of heavy tobacco (20 years or more) history of heavy alcohol use (15 drinks/weaker for 20 years or more) associated with Oropharyngeal cancer caused by HPV-16 and HPV-18 was reported in 60% of cases.This small study is alarming for Indians as this epidemiologic study support for the association between HPV-16 and HPV-18 in males as 60% cause for oropharyngeal cancer.

Conclusion

Incidence of oropharyngeal cancer in India is highest in the world(20 to 30/100,000 in population).In 80 to 90% of cases these cancers are associated with either tobacco or tobacco and alcohol consumption in 10 to 20% of cases tobacco and alcohol are not the culprits. Recently HPV infection has been found an important causative factor in oropharyngeal cancers.

It is well established that HPV-16 and HPV-18 are positive factors in cervical cancer. Now this virus are also being linked to oro and oropharyngeal cancer. A study done by Dr. No-Hee-Park showed that the mouth, at the cellular level, is structurally very similar to vagina and cervix. Both organs have the same type of epithelial cells that are the target of HPV-16 and HPV-18.

The majority of oral cancer and cancers of epithelial cells, primarily squamous cells carcinomas are not Unlike the cancers that affect the cervix. This first step in understanding the similarities of these tissues and the diseases that affect them led to research that was able to link the oral cancer to HPV Dr. Park's study also showed that smoking and drinking alcohol help to promote HPV invasion. Alcoholic beverages contain ethanol alcohol ethanol is known to inhibit the production of the P 53 protein. The carcinogens in tobacco have been shown to damage cell DNA, the precursor event to malignancy.

Combined tobacco and alcohol with HPV and epithelial cells in the mouth, may have the formula for the development of oral cancer. A recent study conducted by Johns Hopkins Oncology centre furthered the premise HPV is linked with certain types of oral cancer.

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Sustainable Green Environmental Technology

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Abstract

The hydrosphere, atmosphere, lithospheric quality, and biosphere all suffer from environmental contamination. Contrary to organic molecules, heavy metals are industrial pollutants that cannot be broken down, therefore they build up in the water, soil, bottom sediments, and living things. "Phytoremediation," the practice of using plants to eliminate heavy metals, has positive effects on the environment and the economy. Using plants to partially or significantly remove certain chemicals from polluted water and soil is known as phytoremediation. It makes use of several plant biological processes as well as plant physical traits for site rehabilitation.Over the course of an organism's lifespan, bioaccumulation occurs, resulting in a larger concentration in older people.In a food chain, biomagnification occurs when chemicals move from lower trophic levels to higher trophic levels, increasing the concentration of apex predators.

Keywords: Phytoremediation, Sustainable Green Environmental Technology, Environment

Introduction

A significant environmental risk is soil contamination with toxic metals. Chemical decontamination techniques for heavy metals (HMs) such as heat treatment, electro remediation, soil replacement, precipitation, and chemical leaching are often highly expensive and not suitable for agricultural fields.Both bioaccumulation and biomagnification are distinct processes that frequently take place simultaneously. Biomagnification is the process by which toxins are transmitted from one trophic level to the next within a food web (and hence rise in concentration), as opposed to bioaccumulation, which is the

process by which poisons enter the food web by building up in individual organisms.

To clean up contaminated surroundings, several methods are employed. One of these is phytoremediation, which relies on the utilisation of hyper-accumulator plant species that can withstand significant concentrations of hazardous HMs in the air, water, or soil. In such a method, harmful metals are removed, degraded, or detoxified using green plants. For soil decontamination, five different phytoremediation procedures have often been used: phytostabilization, phytodegradation, rhizofiltration, phytoextraction, and phytovolatilization.

Traditional phytoremediation method presents some limitations regarding their applications at a large scale, so the application of genetic engineering approaches such as transgenic transformation, nanoparticles addition and phytoremediation assisted with phytohormones, plant growth-promoting bacteria and AMF inoculation has been applied to ameliorate the efficacy of plants as candidates for HMs decontamination.

The health of people, animals, plants, and the ecosystem at large is significantly harmed by excessive metal concentrations in soil and water. Human activities, particularly those connected to mining, industrial emissions, disposal or leaking of industrial wastes, application of sewage sludge to agricultural soils, and use of manure, fertiliser, and pesticides, have frequently resulted in the contamination of soil with dangerous metals. Metal pollution of soil and water is an environmental issue that needs a practical and economical solution due to its potential toxicity and high persistence.

Bioaccumulation and Biomagnification

When considering bioaccumulation and biomagnification, synthetic (man-made) substances known as Persistent Organic Pollutants, or POPs, are the main cause for worry. These substances are difficult to degrade in the environment and can accumulate in the fatty tissues of living things. You may be familiar with POPs such as PCBs and DDT, a pesticide that was widely employed after World War II (flame retardants).

Although the production of these chemicals was banned during the 1970s and 1980s, they can still be found in the oceans as well as in the tissues of many marine animals because of their ability to

(1.) persist in the environment for long periods of time,

(2.) move within the water, and

(3.) dissolve into the fatty tissues of living organisms.

For all of these reasons, POPs like DDT and PCBs are especially good at bioaccumulating and biomagnifying.

At the base of a food chain, basic producers like phytoplankton are often where bioaccumulation takes place. These tiny photosynthetic organisms immediately take up POPs from the saltwater and gradually build them up in their bodies. Because they are absorbed from the water at a pace that is quicker than their ability to be digested, the poisons accumulate in their tissues.

When slightly bigger creatures known as zooplankton eat the contaminated phytoplankton and then absorb POPs into their own tissues at a greater concentration, the process is known as biomagnification. A zooplankton's body will contain more contaminants the more polluted phytoplankton it consumes. In other words, the POPs can be transferred from producer to consumer. The process of biomagnification can go on across the entire food chain or web. Some of the ocean's top predators run the risk of developing potentially lethal levels of POPs in their bodies due to the increasing concentration of POPs at each trophic level.

The orca is one significant apex predator that is adversely affected by the bioaccumulation and biomagnification of POPs. The blubber of Arctic orcas has been shown to have unusually high amounts of PCBs, making them "the most poisonous mammal in the Arctic." Additionally, Japanese researchers discovered that mother orcas are feeding these toxins to their young through their high-fat milk. Studies are being done to determine whether and how POPs are affecting orcas in other ways, as PCBs are known to interfere with reproduction.

Governments are gradually realising how important it is to mitigate these pollutants' harmful effects. In the United States, the manufacture of DDT was outlawed in 1972, and new chemicals are being prohibited every year. The manufacture of PCBs and other dangerous compounds is now prohibited globally thanks to the Stockholm Convention on Persistent Organic Pollutants, which went into force in 2004. The ambient levels of several of these poisons have already started to substantially decline as a result of these prohibitions, which have shown to be largely effective.

Phytoremediation

Although a variety of methods have been developed to remove metals from polluted soils, many sites still contain contaminants because doing so would be too expensive economically and environmentally. Therefore, there is a significant potential risk that heavy metal contamination poses to both the environment and human health. Groundwater, surface water, and soil pollution can be removed, transferred, stabilised, and destroyed using a series of procedures called phytoremediation .

One of the key elements of green technology is phytoremediation technology, which is straightforward, affordable, sustainable, compatible, and eco-friendly. These heavy metals can be naturally degraded by plants through a variety of mechanisms, including bioaccumulation, translocation, and pollutant storage/degradation. Since phytoremediation is carried out in situ, is powered by solar energy, and requires little maintenance after it has been established, it is approximately ten times less expensive than conventional engineering techniques .

The words "phytoremediation" and "remedium," which both refer to the restoration of balance or remediation, are derived from Greek and Latin, respectively. Using plants that can capture, break down, or remove metals, pesticides, solvents, explosives, crude oil and its byproducts, as well as other pollutants from the medium they are present in, phytoremediation reduces pollutant concentrations in polluted soils and water.

Phytoremediation is the use of green plants to eliminate contaminants from the environment or make them harmless. Three procedures that can assist lower the metal content of, say, stormwater are included in phytoremediation: phytoextraction, phytostabilization, and phytofiltration.

1. Phytoextraction (Phytoaccumulation):

The process through which soil-borne metal pollutants are ingested by plant roots and transferred to their tissues above the soil surface is known as phytoextraction. Many different plants can be employed since they all have varying capacities for absorbing and resisting high concentrations of contaminants. This is especially crucial for sites that have been contaminated with several metal contaminants. Due to their tolerance for relatively high levels of pollution, hyperaccumulator plant species—species that absorb more contaminants than the majority of other species—are planted in numerous locations.

Phytoextraction is the utilization of plants to translocate and collect pollutants in their aboveground biomass by absorbing them from soil or water. The most significant phytoremediation method used today to remove heavy metals and metalloids from contaminated soil is phytoextraction.

Phytoextraction is a long-term method for removing heavy metals from contaminated soil, in contrast to phytostabilization, in which plants only momentarily retain heavy metals that nonetheless stay belowground.It is, therefore, more suited for commercial use. There are a few steps involved in the phytoextraction of heavy metals:

(i) mobilisation of heavy metals in rhizosphere,
(ii) uptake of heavy metals by plant roots,
(iii) translocation of heavy metal ions from roots to aerial parts of plants, and
(iv) sequestration and compartmentation of heavy metal ions in plant tissues The plants are collected and carefully disposed of once they have grown and absorbed the metal contaminants.

To bring the level of contamination down to acceptable limits, this process is done numerous times. The metals can occasionally be recycled by a procedure called phytomining, but this is often only done with valuable metals. Zinc, copper, and nickel have all been successfully removed using plants, but lead and chromium-absorbing plants are the subjects of the exciting new study.

2. Phytostabilisation:

Utilizing certain plants to immobilise pollutants in soil and water is known as phytostabilization. Roots absorb and collect contaminants, which are either adsorbed into the roots or precipitated in the rhizosphere. This lessens or even stops pollutant mobility, reducing migration into the groundwater or air, as well as contaminant bioavailability, inhibiting transmission through the food chain.

In locations that have been denuded by heavy metal pollution, this approach can also be utilised to re-establish a plant community. The possibility of wind erosion (and consequently the spread of the pollutant) is decreased after a community of tolerant plants has been developed, and the leaching of the toxins from the soil is also minimised. Heavy metal precipitation or a decrease in metal valence in the rhizosphere, absorption and sequestration within root tissues, or adsorption onto root cell walls can all result in phytostabilization.

3. Phytofiltration (Rhizofiltration)

The concept behind phytofiltration is similar to that of phytoextraction, except the focus is on cleaning up dirty groundwater as opposed to polluted soils. Either the pollutants are absorbed by the plant roots or they are adsorbed onto the root surface. Instead of being planted immediately in the intended location, plants used for phytofiltration are first acclimated to the pollutant. In place of dirt, plants are hydroponically cultivated in clear water until they have acquired a substantial root system. To acclimate the plant, a clean water source is substituted once a substantial root system has developed. After acclimating, the plants are placed in the polluted region, where the roots absorb the contaminated water and its toxins.

The roots are collected and carefully disposed of as soon as they get moist. As seen in Chernobyl, where sunflowers were grown in radioactively polluted pools, repeated treatments of the site can reduce pollution to acceptable levels (Baker and Brooks, 1989).

According to Mesjasz-Przybyowicz et al. (2004), phytofiltration is the process of removing contaminants from polluted surface waters or wastewaters using plant roots (rhizofiltration), shoots (caulofiltration), or seedlings (blastofiltration). Heavy metals are either absorbed by the roots or adsorbed onto the root surface during rhizofiltration. Root exudates have the ability to alter the pH of the rhizosphere, which causes heavy metals to precipitate on plant roots, further reducing the flow of heavy metals to groundwater.

Limitations of phytoremediation

According to Erakhrumen and Andrew (2007); there are some merits and drawbacks of the phytoremediation process which are as follows:

The cost of phytoremediation is cheaper than that of conventional methods both in situ and ex-situ, and it is adaptable to a wide variety of organic and inorganic pollutants including many metals with few alternatives. It is simple to keep an eye on the plants. Is cost-effective for huge quantities of water with low pollutant concentrations; it does not require expensive equipment or highly skilled workers.The potential for important metals to be recovered and reused (by businesses that specialise in "phytomining").

Because it utilises naturally occurring organisms and maintains the environment's natural condition, it is the least destructive technique.In large-scale applications, the potential energy stored may be used to create thermal energy; plant absorption of polluted groundwater can stop off-site migration.

Limitations:

Phytoremediation is only possible at the depth and surface area that the roots occupy. Because of their slow development and low biomass, polluted sites generally require lengthy cleanup processes that might last for several years. Plant biomass that has been harvested using phytoextraction may be categorised as hazardous waste; therefore, proper disposal is required. It is not feasible to entirely stop the leaching of pollutants into the groundwater with plant-based remediation methods (without the complete removal of the contaminated ground which in itself does not resolve the problem of contamination).Climatic circumstances are a limiting element; they may limit the pace of growth of plants that can be used.

Numerous types of pollutants, such as metals, pesticides, explosives, and oil, can be cleaned up by plants. Additionally, the plants aid in preventing pollutants from locations from being transported to nearby regions by wind, rain, and groundwater. In the lake ecosystem, phytoplankton and macrophytes that live in the littoral zone play a significant role in the biogeochemical cycling of elements by acting as either sources or sinks for contaminants that enter the lake. However, the development stage, surface-to-volume ratio, physiological state, seasonal fluctuation of growth, and water chemistry are the key determinants of the plant's absorptive properties. It is sometimes challenging to compare the metal content of macrophytes since various living forms have demonstrated a predisposition to collect certain metals.

So, it is impossible to generalise metal accumulation data from one species to another or even within the same species under various environmental circumstances. Macrophytes and phytoplankton have recently been employed as biomonitoring instruments and for the improvement of water bodies polluted with toxic metals due to their tolerance and sensitivity to toxic metals. The amount of metal in plants is often largest during the cold season and rapidly decreases during the period of maximum development.

Macrophytes are regarded as an essential part of the aquatic environment because they serve as an effective heavy metal accumulator in addition to providing food for aquatic animals. They are permanent biological filters that are crucial to the upkeep of the aquatic ecology. Aquatic macrophytes are aquatic phanerogams, which are aquatic phanerogams, which exist in a completely different habitat but are taxonomically closely related to terrestrial plants. They provide fascinating study subjects for testing and simulating ecological hypotheses on evolution, plant succession, as well as nutrition and metal cycles because of their propensity to store metals.

Understanding the roles played by macrophytes in the aquatic ecosystem is crucial.

Conclusion

Environmental pollution affects the hydrosphere, atmosphere, lithospheric quality, and biosphere. Heavy metals, which cannot be broken down like organic molecules, are industrial pollutants that accumulate in the water, soil, bottom sediments, and living beings. Utilizing plants to remove heavy metals is called "phytoremediation," and it benefits the environment and the economy. Phytoremediation is the use of plants to partially or significantly remove certain pollutants from contaminated water and soil. For site rehabilitation, it takes advantage of a variety of biological functions unique to plants as well as their physical characteristics. Bioaccumulation takes place throughout an organism's lifespan, leading to a higher concentration in the elderly.Because of the possible effects on human and animal health, the pollution of soil, water, plants, and air by heavy metals is a major issue. To safeguard priceless natural resources and biological life, more affordable and efficient technologies are required. In comparison to other physicochemical procedures, phytoremediation offers a number of benefits and has been shown to be a potential method for replanting heavy metal-polluted water and soil. An efficient mitigating strategy to revegetate heavily metal-polluted water and soil is phytoremediation, an environmentally beneficial process.

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Nutritional Significance of *Basella alba* Linn an underutilized medicinal green leafy vegetable

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Abstract

Around the world, particularly in India and China, different plant components are utilized for various healing activities for humans and animals as well as for the treatment of ailments. It can be used as an aperient, a rubefacient, and to treat catarrhal infections. Basella saponins, kaempherol, betalin, and other substances are some of the compounds that are mainly found in plants. For various pharmaceutical applications, a variety of extracts including aqueous, chloroform, ethanol, and petroleum have been employed. Since it has anticancer, antiviral, antioxidant, anti-inflammatory, anti-cholesterol, anti-ulcer, anti-microbial, anti-hypoglycemic, wound healing, and androgenic properties, it has the potential to treat a variety of ailments.

Keywords: *B. alba*, Morphology, Medicinal properties

Introduction

Nutraceuticals are a combination of pharmaceutical and nutritional substances that function as medications and are gaining popularity as a complement to traditional therapies. Because they include a variety of plant secondary chemicals, including anthocyanins, tannins, carotenoids, flavonoids, phenols, and antioxidants, many of these "folk-medicines" provide health

benefits beyond their nutritional value. Although phytochemicals, also known as antinutritional factors, are typically not necessary for the body to operate normally, they have significant therapeutic purposes. Biochemical studies have shown a strong, positive relationship between frequent consumption of natural food products and lower rates of degenerative diseases, which has made eating fruits and vegetables more popular.

Basella alba L. (*B. alba*) is a perennial plant of the Basellaceae and is also known as Malabar spinach, Indian spinach, Ceylon spinach, and vine spinach. It is widely farmed around the world. The stems and leaves are used both as food and medicine (Zhao *et al.,* 2014). It is a medicinal and succulent plant; the stem and leaves contain a sizable amount of mucilage, which has a variety of possible uses in the culinary, cosmetics, and pharmaceutical industries. Additionally, *B. alba* has several useful qualities, such as the extract of *B. alba* which has antioxidant, anti-inflammatory, and anti-microbial properties. It has also been used to treat hypercholesterolemia and generate a substance that inhibits Sitophilus zeamais (Baskaran *et al.,* 2015). Due to its strong capacity to suspend substances and high viscosity, its mucilage can be utilized as a thickening agent and tablet binder (Kumar *et al.,* 2013).

Leafy vegetables are made from tender shoots with succulent stems and thick, semi-succulent, and mucilaginous leaves. It has exceptionally heat-tolerant, frost-tender, and short-day plant varieties. It is an herbaceous perennial with quick growth and twining or creeping growth patterns. *B. alba* is essential for food, nutritional, and economic security and has major economic, nutritional, and medical significance (Reddy *et al.,* 2014). The fruit extract of *B. alba* can be used as a natural dye for fabrics since it contains pigments (Mitra and Das, 2015).

Origin

B. alba is widely available in India, China, Malaysia, the Philippines, Brazil, South America, tropical Africaand Bangladesh. This leafy vegetable is now widely farmed because of its nutritional properties and is grown almost everywhere as a medicinal herb (Deshmukh *et al.,* 2014). It is a soft-stemmed vine that grows quickly and can get as long as 10 metres (33 feet). Its heart-shaped leaves have a moderate flavor and a mucilaginous texture. *B. alba* requires direct sunlight to develop, while low temperatures slow down its growth. As a result of its untapped potential as a vegetable or as a component in the creation of functional foods, *B. alba* might be regarded as an under appreciated green leafy vegetable. The plant produces a significant amount of flesh along with a tiny number of extremely few fruits, which are typically not

consumed. AlthoughBasella offers a significant number of vitamins and minerals, it is not regarded as a field crop.

Figure 1 *Basella alba*

Morphology

A perennial vine with fibrous roots is called Malabar spinach. The leaves of Malabar spinach are arranged spirally, and the stem is meaty, succulent, slender, silky, and bright. The stem measures 8 to 10 metres. The length of the leaf is greater than its breadth, the leaf is oblong and has a short stalk. Depending on the cultivar, the *B. alba* has white, red, or pink flowers. The seed's exterior is brilliant and rough, and it is colored black and brown. The seed is surrounded by thick testa. Under ideal circumstances, seeds can survive for four years (Mahr, 2014). Malabar spinach has the potential to be employed as one of the plant species for phytoremediation of Metalloids because of its quick development, large thick leaves, high biomass production capacity, and ease of adaptability to a variety of soil and climatic conditions. However, there is currently little knowledge about the Malabar spinach’s ability to withstand metalloid stress and undertake phytoremediation.

Cultivation

The family Basellaceae includes the plant usually referred to as "poi," Indian spinach (*Basella alba* L.). It is a large-scale grown fleshy annual or biennial in Bangladesh and India. Bangladeshi farmers grow this crop under

their personal preferences because standard production methods are either absent or unavailable there. They consequently do not receive an adequate income and return on investment. Given the aforementioned facts, the current study was conducted to establish the ideal plant spacing for optimum growth and increased output (Islam *et al.,* 2014).

The moist tropics are home to the *B. alba* plant, which can be found there at heights of up to 1,500 metres. It can handle temperatures between 10 and 35°C but thrives in environments with yearly daytime temperatures between 23 and 27°C. It tolerates the rain between 700 and 4,200 mm but prefers a mean annual rainfall between 2,000 and 2,500 mm (Ciju, 2021).

The tribal people, small farmers, and marginal farmers in South India are cultivating the landraces of Malabar spinach in their peri-urban, rural, and tribal vegetable agricultural systems (Reddy *et al.,* 2014). Its output can be increased by expanding cultivation into new viable locations or by enhancing cultivar yield.

The plant species *Basella alba* Linn., also known as Ceylon Spinach or Phak Plung in Thai, belongs to the Basellaceae family and is one of the most widely farmed cool-season vegetables. Favourable weather between 55 and 75 degrees Fahrenheit, which corresponds to late winter, early spring, late summer, early fall, and early winter (Manosroi *et al.,* 2020). A plantcan produce 1.5–2 kg of fresh leaves.Climate, socioeconomic conditions, regional production techniques, soil type, geographic characteristics, crop types, human activities, and other factors influence Ceylon spinach growth (Acikgoz and Adiloglu, 2018).

Medicinal properties

Numerous chemical constituents in *B. alba* contribute to its several medicinal qualities. It is utilized as a culinary product in addition to being used medicinally. The plant can be used as medicine since it contains biologically active substances such as proteins, carbohydrates, enzymes, lipids, and oils. Along with other substances, *B. alba* also has carotenoids, saponin, quinines, and simple phenolic glycosides (Adhikari *et al.,* 2012).Since ancient times, medicinal plants have been utilized to heal physical diseases and play a significant role in traditional medical practices. Recently the research focused on the use of naturally occurring bioactive chemicals with therapeutic value which are affordable and available. As a result, there has been an increase in the usage of herbal treatments throughout the world, with many of them now being used in mainstream medical procedures.

Antioxidants activity

The leaves of *B. alba* contain bioactive compounds such as saponin, tannin, cardiac glycosides, terpenoids, and steroids; the stem of the plant contains flavonoids, saponins, glycerine, terpenoids, and steroids. Alkaloids, flavonoids, phytosterol, saponin, cardiac glycosides, fixed oil, glycerine, phlorotannin, and terpenoids are all present in various extracts of the *B. alba* fruit (Revathi and Sudha, 2018).These plant extracts can serve as effective electron donors and prevent the formation of free radical chain reactions, due to the antioxidant capacity. Therefore, the pharmaceutical and nutraceutical industry may rely onthe Basella specieswhich hasa highly significant source of natural antioxidants.The methanol extraction of the phenoliccompound from *B. alba* leaves proved thatit can be a good source of unprocessed natural antioxidants (Jayswal *et al.*, 2021).

B. alba helps in the prevention of damage to heart muscles, arteries, and even nerves from free radicals because it contains powerful antioxidants. It has less amount fat and calories (100 g of raw leaves provides 19 kcal) and has anadequate amount of antioxidants, vitamins, and minerals. The ß-carotene, lutein, and zeaxanthin are one of the essential carotenoidpigment anti-oxidants that are abundant in fresh leaves. These substances work as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS), which have a beneficial effect on the aging process and several disease processes(Grubben, 2004).

Antidiabetic activity

Kumari *et al* (2021)found the effects of methanol extracted *Basella alba* leaf (MEBA) against diabetic nephropathy in rats, resulted in significant improvements in blood sugar, ALP, ALT, total protein, serum lipid profile, including total cholesterol, triglyceride, and HDL levels, as well as renal functions like urine volume, urinary protein excretion, serum creatinine, blood urea. It can cure the aberrant thickening of the glomerular basement membrane in the kidney. According to the study by Bamidele *et al* (2020) *B. alba* is excellent in reducing stress due to its hypoglycaemic impact, antioxidant capacity, and role in reversing immune system chronic activation, As stress is an inescapable event, the consumption of *B. alba* as part of an individual's daily diet should be highly advocated across varied groups.Mohanty *et al.*, 2021 also proved that the *B. alba* leaf extract has a variable anti-diabetic effect in alloxan-induced diabetic rats. *B. alba* leaf consumption as a leafy vegetable ought to be promoted for the treatment of diabetes mellitus.

Hepatoprotective activity

Basella alba leaves, traditionally are used in the treatment of anaemia and have hepatoprotective potential.Hemolytic, expectorant, anti-inflammatory, and immune-stimulating activities arevital properties of the phytochemicals called saponins present in *B. alba* leaf extract. The considerable variations in the plasma concentration of AST, ALT, TBA, and LDH that result after paracetamol overdose may be caused by these bioactivities, which may reduce the harmful effect of liver inflammation (Das *et al.,* 2015).

Antiulcer activity

The ethanolic extract of *B. alba* (EEBA) has dose-dependent ulcer preventive properties. The ulcer index significantly decreased when the EEBA was given at doses of 200 and 400 mg/kg. There was no discernible difference between the 400 mg/kg dose of EEBA and the animals treated with ranitidine. Also, it has antiulcer potential, anti-inflammatory activity, and hypoglycemic activity and a natural antioxidant can also provide greater protection against age-related alterations (Mohanty *et al.,* 2021). The effects of aqueous and ethanol extracts of *B. alba* leaves on antiulcer activity in the rats subjected to pylorus ligation and ethanol-induced ulcer models were found that both the extracts increased the mucosal defense and decreased the gastric acidity very significantly which indicates the *B. alba* has gastroprotective activity as well as statistically significant antiulcerogenic activity due to improvement in gastric cytoprotection and inhibition of acid secretion (Kumar *et al*., 2012). *In vitro* and *in vivo* studies on flavonoids show that *B. alba*has gastroprotective properties against peptic ulcers. A study in rat models of aspirin-induced and pyloric ligation ulcers resulted that *B. alba* has anti-ulcer properties due to the occurrence of bioflavonoid in it. It shows that the ethanolic extract of *Basella alba* (EEBA) has dose-dependent ulcer preventive properties. The ulcer index significantly decreased when the EEBA was given at doses of 200 and 400 mg/kg. There was no discernible difference between the 400 mg/kg dose of EEBA and the animals treated with ranitidine (Bamidele *et al.,* 2014)

Anti-microbial activity

Aqueous extract of *B. alba* leaves was found to have potential antibacterial activity against Gram-positive bacteria like *Staphylococcus aureus* and *Bacillus* species and Gram-negative bacteria like *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, but the *E.coli* was found to be resistant to the extract. However, the *B. alba* leaves extract was more active

against Gram-positive than Gram-negative bacteria. The presence of phytochemicals such as phospholipids, fatty acids, sterols, tannin, terpene, steroid, saponin, and anthroquinone is responsible for the antibacterial activity of the crude extract (Adesina *et al.,* 2017). Besides, the results of aqueous methanol extracts of *B. alba* had no impact on *Clostridium sporogenes* or *Streptococcus faecalis*at 30 mg/mL and 35 mg/mL (Akinniyi *et al.,* 2019).

Nutritional properties

Malabar spinach has been used to cure a wide range of conditions from the beginning of time, including androgenic, anticancer, antiviral, antioxidant, anti-inflammatory, anticholesterol, anti-ulcer, antibacterial, and hypoglycemic ailments (Shade *et al.,* 2017). Additionally, this herb is utilized as a laxative, to cure several skin issues, diarrhea, and dysentery. The Basella plant also possesses anti-proliferative qualitydue to the presence of betacyanin, carotenoids, bioflavonoids, β-sitosterol, and lupeol (Moutusi *et al.,* 2019).

It is a green leafy vegetable that may be consumed and is frequently added to stews and soups as a thickening agent. The nutrients such as iron, calcium, soluble fibre, vitamins A and C are all abundant in *B. alba.* The mucilage from this plant has also been utilized in traditional Thai remedies for the treatment of irritations, bruising, ringworm, and delivery. Its leaves and stem are used as a mild laxative, antipyretic, and diuretic (Manosroi *et al.,* 2020).Fresh *B. alba*leaves have high quantities of ash (0.70±0.31), fat (0.49±0.01), fibre (7.52±0.57), carotene (0.02±0.00) and vitamin C (95.01±2.50), protein (1.35±0.03), and carbohydrate (3.48), according to Abdulrasheed *et al*(2021).

It is an edible leafy vegetable and has long been used as a thickening agent in starters. However, there was a reduction in nutrients due to thermal treatment such as blanching (90 – 92 °C for 2 min), boiling (100°C water for 25 min) and shade drying at 27°C. The process like blanching and boiling has an adverse effect on the β-carotene and vitamin C content of *B. alba*. The reduction in vitamin C content could be attributed to the fact that it is water-soluble and also heat-labile. Therefore, the processing method affected the nutritional composition of *B. alba*leaves. Though, shade drying is the best processing method for retaining the nutrients. In addition, it reduces microbial attack, has a longer shelf life, and thus is a preservation method for these leaves (Putriani *et al.,* 2022).

Iron is abundant in the leaves of Malabar spinach (*B. alba*), the amount of iron present in 100 g of fresh leaves is around 1.20 mg or 15% of the daily requirement. The human body needs iron, a vital trace element, to produce sufficient red blood cells (RBCs). Additionally, during cellular metabolism, this element functions as a co-factor for the oxidation-reduction enzyme (cytochrome oxidase) and it has significant concentrations of various B complex vitamins, including riboflavin, folate, and vitamin B6 (pyridoxine). Around, 140 g or 35% of the folates is present in 100g of fresh leaves which is akey element for the growth and formation of DNA. Neural tube abnormalities in the newborn baby could occur from a folate shortage in the early stages of pregnancy. Therefore, it is recommended that expectant and pregnant women consume a lot of fresh greens in their diet to prevent neural tube problems in children (Opabode and Adebooye, 2005).

Table 1 Nutritional composition of *Basella alba* L.

Nutrients	Indian Food Composition Table 2017	Kenya Food Composition Table 2018
Energy (Kcal) KJ	(19.59) 82	(28) 117
Water (g)	92.68	90.4
Protein (g)	1.57	3.40
Fat (g)	0.75	0.70
Carbohydrates (g)	2.01	0.20
Dietary Fiber(g)	2.21	3.70
Ash (g)	1.09	1.60
Ca (mg)	93.89	267
Fe (mg)	4.58	10.9
Mg (mg)	153	40
P (mg)	37.26	56
K (mg)	337	446
Na (mg)	18.74	8
Zn (mg)	0.39	0.50
ß-carotene (mcg)	5824	2213
VitC (mg)	63.35	79.8

Source:Longvah *et al.*, 2017 and Mwai *et al.*, 2018

Therapeutic properties

B. alba is a quickly growing, highly medicinal leafy vegetable plant that is grown all over the world (Adhikari *et al.*, 2012). It is traditionally used as an antidote, aperients, astringent, demulcent, diuretic, febrifuge, laxative and rubefacient.It has vital nutrientslike fibre, ash, calcium, vitamins, thiamine, riboflavin, and niacin (Kumar *et al.,* 2013; Deshmukh and Gaikwad, 2014). Inflammation, atherosclerosis, stroke, heart disease, diabetes mellitus, multiple sclerosis, Parkinson's disease, Alzheimer's disease, etc. are other conditions for which *B. alba* is effective (Prashant *et al.,* 2011). Additionally, the *B. alba* leaves showed the capacity to reverse male infertility (Manfo *et al.,* 2014).

According to Divya *et al* (2020), the *B. alba* extract (BAR) at doses of 100 mg/kg and 200 mg/kg demonstrated a statistically significant dose-dependent increase in urine volume and excretion of electrolytes; hence, BAE has a diuretic effect. The Rotarod test is used to examine how the medicine affects an animal's ability to coordinate its movements. This test can be used to assess the sedative effect of the medicine since sedation results in a loss of motor coordination. According to research on the sedative activity model, all BAE-treated groups experienced shorter falls than the control group. Consequently, the BAE also has sedative properties.

Betacyanin, carotenoids, bioflavonoids, β-sitosterol, and lupeol are found in Basella plant species, and they are said to have antioxidant, antiproliferative, antimicrobial, anti-inflammatory, and other properties. They are also said to have anticancer, antiviral, antioxidant, anti-inflammatory, anti-inflammatory, anti-inflammatory, anti-cholesterol, anti-ulcer, antimicrobial, and anti-hypoglycemia (Shade *et al.*, 2017).

Other benefits

Testosterone levels in males are desirably improved by the *B. alba*. Rats treated with both fresh and dried leaf extracts experienced a significant rise in the weight of seminal vesicles, blood testosterone level, in *vitro* testosterone synthesis, prostatic acid phosphatase activity, and concentration of spermatozoa in the lumen of the seminiferous tubules (Moundipa *et al* 2005).

Haneefa *et al* (2012) examined the ability of *Basella alba* to heal wounds in male albino rats. They inflicted burn wounds on the backs of rats and treated them for roughly 20 days with *B. alba* leaf extract in glycerin. The result demonstrated that *B. alba* had a maximum ability for wound healing with substantial wound closure in rats treated with aqueous leaf extracts.

The effect of aqueous leaf extract of *B. alba* on hematological and biochemical parameters was studied in Wistar strain albino rats, and found that *B. alba* dramatically increased platelet count, hemoglobin concentration, packed cell volume, red blood cell and white blood cell counts. However, the extract considerably decreased the activity of liver enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Therefore, the consumption of *B. alba* leaves may help to stay healthy and reduce the risk of anemia (Bamidele *et al.*, 2010).

The study revealed that *B. alba* methanolic extract has intriguing anticancer and antibacterial capabilities. The methanolic extract exhibits a significant zone of inhibition against the clinical pathogen of the human cancer cell (Sushila*et al.*, 2010).

Children and pregnant women can safely use a leaf decoction as a laxative. The mucilaginous leaf is administered externally to burns, scalds, and urticaria after being crushed. *B. alba* is used as a treatment for burns because of its cooling effect. To treat burns and scalds, the juice derived from its leaves is administered topically. Before applying the liquid extract to the burn, it is frequently combined with ghee butter.In every region of India, it is eaten in the form of dal, chutney, soup, curry, and several other dishes.Reactive oxygen specieswhich are abundant in *B. alba*, helps in delaying the aging process. The leafy vegetable is also a good source of natural antioxidants like beta carotene, that shield cells from damage caused by circulating free radicals.

Food products

The species of basella plants have not been commercialized, due to a lack of information on their therapeutic uses, minimal planting area and not being fully explored, but still have economic potential. Few products like noodles, candyand oil were developed by the researchers.

1. Noodles:

The *B. alba* egg noodles were discovered through a dehydration process that contains a considerable amount of ash and crude protein. Three formulations of fresh egg noodles were made using the resulting powder at substitution levels of 10%, 15%, and 20%, with 100% wheat flour acting as the control. These noodles are good to particularly children, pregnant women, nursing mothers, seniors and those whoconsume the product can assist meet the recommended dietary requirement of vitamin A. The *B. alba* leaves powder can also be added to other goods that require fortification because it has a relatively high total carotenoid content (Soriano *et al.*, 2020).

2. Candy:

Raw cane sugar and honeywere used for the preparation of *B. alba* candy. The sugar syrup was heated at 110°C and mixed with *B. alba* leaf extract, then stir till reaches hard crack consistency. Using silicon moulder the candies were developed by Pui *et al.,* (2020). *B. alba* candy remained safe for human consumption throughout 42 days of storage at ambient temperature since *B. alba* is also an excellent source of protein whereby it contains amino acids such as arginine, isoleucine, leucine, lysine, threonine and tryptophan. The leaves were reported to have bioflavonoids, carotenoids, organic acids, water-soluble polysaccharides and vitamin K.

3 B.alba seed oil:

Vitamin A and vitamin E can be found in *B. alba* seed oil. Additionally, this oil has excellent edible, cosmetic, and dietary qualities due to its considerably larger level of polyunsaturated fatty acids (PUFA), which are primarily made of linoleic acid. *B. alba* seeds may be seen as a new source of unconventional oils with all these possible applications in the food, cosmetic, and medicinal industries (Diemeleou *et al.,* 2014).

Conclusion

The discovery and development of new drugs are increasingly influenced by naturally occurring chemicals. In reality, most anticancer and anti-infectious drugs are derived from nature. Due to the medications that are ineffective and have adverse effects, treating it is quite difficult. To prevent and treat peptic ulcers, natural medicinal ingredients are grown. The leaves are a good source of vitamins A and C, calcium, potassium and iron, with many other vitamins and minerals. It has over 3 times more vitamin C than spinach, and over 1.5 times more vitamin A than kale. Shoots have many medicinal properties, and are febrifuge, diuretic and laxative, whereas roots are used to treat diarrhea. *B. alba* contains important components such as vitamins, proteins, carbs, lipids, minerals, fibre, carotenoidand organic acid.

Various ailments are reportedly treated in numerous nations using *B. alba* and its extracts or formulations, according to ethnopharmacological accounts. According to scientific sources, *B. alba* has a wide range of significant phytochemicals and pharmacological properties. Androgenic, anti-diabetic, anti-inflammatory, antimicrobial, antioxidant, antiulcer, anti-viral, CNS depressant, hepatoprotective, and wound healing activities stand out among the biological effects. Moreover, the presence of sitosterol and lupeol in the plant is said to have antioxidant, anti-inflammatory, and anticancer

properties.Inclusion of leafy vegetables in the diet will likely result in higher intakes of dietary fibre, which promotes regular bowel movements, balances the digestive system, and prevent obesity.

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Medicinal and Therapeutic use of *Justicia adhatoda* L.

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Abstract

Over 2000 years have passed since the usage of *Justicia adhatoda* L. in traditional medicine. It is found throughout India's tropical and subtropical regions. It is renowned for having advantageous qualities.It belongs to the family Acanthaceae and is widely known for its efficacy in curingrespiratory problems. Consequently, there has been an increase in interest in the study of medicinal plants and their long-standing use in various nations during the past few decades. Today, it is necessary to provide scientific evidence to support whether using a plant or its active ingredients is reasonable.Vasica is an expectorant and antispasmodic that has been used for millennia to successfully treat respiratory disorders like asthma and chronic bronchitis. It has a tremendous potential to be developed as a medicine by the pharmaceutical industry. This study reviews the pharmacological and general therapeutic applications of *Justicia adhatoda* components. The general therapeutic applications and pharmacological properties of numerous plant components have been reviewed in this work.

Keywords: *Justicia adhatoda*, *A. vasica*, medicinal plant, pharmacological uses, phytoconstituents

Introduction

For thousands of years, plants have been essential to preserving human health and improving the standard of living. Over ten percent of the approximately 258,650 species of higher plants known throughout the world are used to treat sick communities, and the use of plants as medicine dates back to the beginning of human civilization Many of the currently practiced medical

systems, including Ayurveda, Unani, Homeopathy, Naturopathy, Siddha, and other alternative medical systems, have been using plants as potent medications to treat a variety of hazardous conditions (Prasad *et al.,* 2011). According to estimates from the World Health Organization (WHO), the majority of these therapies and 80 percent of all people on earth rely on traditional medicine. Adhatodavasica plant has a vital medicinal role in our country (India) and other countries. This plant has been widely employed in the Ayurvedic medical system. Vasaka plants contain a wide variety of phytochemical constituents that have been isolated. These constituents have been shown to have several different health benefits, including antifungal, antiviral, hepatoprotective, antibacterial, anti-inflammatory and antiulcer, abortifacient, thrombolytic, radio modulation, cardiovascular protection, hypoglycaemic, antitubercular, antioxidant, antimutagenic, and reproductive action.

Figure: 1 *Justicia adhatoda* L.

Classification

Kingdom	- Plantae
Subkingdom	-Trachebionta
Division	- Angiospermae
Super division	- Spermatophyta
Class	- Dicotyledoneae
Subclass	- Asteridae

Order	-Tubiflorae
Family	-Acanthaceae
Genus	-Adhatoda
Species	-VasicaNees.

Indigenous names

English- Malabar Nut, Telugu- Addasaramu, Hindi- Arus, Kannada- Adusoge, Tamil- Atatotai, Gujarati- Aradusi, Malayalam- Aatalootakam, Bengali- Basak, Manipuri- Nongmangkha, Nepali- Asuro, Sanskrit- Atarusa, Oriya- Basango, Marathi- Adulasa, Konkani- Adulasha, Mizo- Kawl-dai, Assamese- Boga.

Plant Description

The plant can be found growing throughout the Indian peninsula up to an elevation of 1300 metres (m), as well as in Nepal, Pakistan, Myanmar, Sri Lanka, and Germany. It typically thrives in waste areas, frequently close to villages, and frequently as a weedy hedge plant. It is a small to medium-sized shrub with opposite ascending branches. The leaves are simple, opposite, and measuring 7–19 cm long by 4–7 cm wide. The blossoms have a distinctive odor and harsh flavor and are either white, pink, or purple. A transverse section of the leaves revealed diacytic stomata and two layers of palisade cells. There are trichomes on both the grandular and nongrandular surfaces of leaves. The flowering season in central India lasts from December to March, and the fruit-bearing season is from January to April(Claeson UP, 2000)

Temperament	- Warm and dry in the first degree
Taste	- Bitter taste
Colour	- Leaves are dark green above and pale yellow below, flowers are white
Odour	- Unpleasant smell
Main Actions	- Bronchodilator and Expectorant

Parts used in Unani

- Stem-to increases uterine contraction during childbirth, antispasmodic, mudir-e-haiz (emmenagogue) and abortifacient.

- Rheumatic fevers, urinary tract infections, dysentery, and diarrhea are treated with leaf-decoction of leaves. The mudir-e-haiz (emmenagogue) activity is also present.
- Zeeq-un nafas specifically recommends fresh leaf juice.
- Flower-used to treat whooping cough, bronchitis, asthma, and other upper respiratory tract illnesses like the flu and the common cold. Qulqand, a sugar and petal sweet, is supposed to be helpful for all respiratory conditions (Claeson UP *et al.*,2000)
- Its fruit and seeds are used to make oil that is used to relieve ear pain. Whooping cough and tuberculosis root usage.
- In fever, the entire herb-decoction is utilized as a blood purifier (habis-u-dam, or hemoptysis), particularly for haemoptysis and epistaxis.

Whole plant

Adhatoda vasica plant extracts are combined with ginger and tulsi to create a variety of formulations that can be used as expectorants and antispasmodics. The entire *Adhatoda vasica* pant was used in Sri Lanka to cure a variety of conditions, including menorrhagia, profuse phlegm, bleeding piles, sexual abnormalities, and impotence (Dymock *et al.*, 1890).

Figure: 2 *Justicia adhatoda* Whole Plant

Root

The roots of *Adhatoda vasica* were used to make a variety of remedies, including powder, decoction, and paste, which were used to cure a wide range of illnesses, including diabetes, cough, some liver disorders, TB, diphtheria, malarial fever, leucorrhoea, and eye conditions. The pubic and vaginal regions are treated with an extract made from macerating the root. The extract aids in the baby's expulsion from the vagina during childbirth. The macerated root extract can be used to treat gonorrhea (Roberts, 1931).

Figure: 3 *Justicia adhatoda* Root

Fruit

Adhatoda vasica fruit extract is active against like cold, fever, bronchitis, diarrhea, jaundice, antispasmodic, and dysentery (Kumar *et al.*, 2005). Flower The *Adhatoda vasica* plant contains constituents like triterpenes Flavonoids they are apigeninastragalinkaemferolvitexinquercetin. The plant blooms in January-march. In South-East Asia fresh Flowers are used to cure diseases like cough, bronchitis, phthisis, asthma, and are also used as antiseptic to improveblood circulation (Kirtikar and Basu, 1975).

Figure: 4 *Justicia adhatoda* Fruit

Leaves

The leaf of *Adhatoda vasica* is a dicot leaf that is covered by a single layer of epidermis on both sides of the leaves. They are amphistomatic and contain trichomes on both the sides of leaves. For clearing the respiratory passages before an active yogic practice, the tender shoots of the plant with very little ginger are chewed (Adnan *et al.*, 2010). In most parts of Southeast Asia, various extracts are prepared from the leaves and are used in many ways such as wound healing agents, expectorant, anti-leprotic, jaundice, headache, snakebites (bruised leaves in Sri Lanka and India) and in various skin diseases (Pushpangadan *et al.*, 1995). They are also used in the treatment of constipation, rheumatoid arthritis, gout and constipation. The leaves are burned and the smoke from the leaves is used in treating asthma.

Figure: 5 *Justicia adhatoda* Leaves

Important formulations

Important formulations containing Asgandof Unani, and Ayurveda formulations are as follows, Syrup Basakarista, Basadikwath, Sarbat Ejaz, Sarbat Tulsi, Sarbat Sadar, Sarbat Vasac Basaboleho (Hossain and Hoq, 2016).

Therapeutic Uses

Cough and bleeding - Internally take 1 tablespoon of leaf juice with honey.

Excessive menstruation - Juice of leaves 15ml with 15 gmjaggery twice daily internally

Bleeding piles - Decoction of the whole plant with sugar internally

Chemical Constituents

It contains phenols, Flavonoids, saponins, tannins,anthraquinones, amino acids, reducing sugars, triterpenes, vasicolinone, vasicol, anisotine, steroids, betaine, 7-hydroxyvasicine, quinazoline,alkaloids vasicine, 3-deoxyvasicine, steroids, carbohydrate and alkanes (Kumar *et al.,* 2005).

Table 1 Important phytoconstituents of *Adhatoda vasica*

Main alkaloids	Vasicine Vasicol Vasicinone Peganine
Minor alkaloids	Adhatonine Vascinol Vasicinolone
Flower content	Kaempferol Quercetin
New moiety in flower	2,4dihydroxychalcone and glucoside
Leaves	Vsicoline Adhatodine Vasicolinine Anisotine
Inflorescence	VasiconineVasicol

***Source: Prathiba and Giri (2018)**

Adhatoda vasica Used Plant Parts

This plant's bark, roots, blooms, and stems are all employed in traditional medicine.

Potential Interactions and Side Effects

In the recommended dosage and usage, *Adhatoda* is regarded as safe. This herb should be avoided unless specifically prescribed by a medical professional because the safety of its use in kids has not been studied. It is not advised to take this supplement when pregnant. When combining this herb with medications or dietary supplements that have expectorant or antispasmodic properties, caution should be taken.

Pharmacologicaland Medicinal use of *Justicia adhatoda*

It is most known for its ability to effectively cure respiratory ailments. Vasica leaves have an energizing effect on the respiratory system. Vasica has been successfully used for millennia to treat asthma, chronic bronchitis, and other respiratory problems because it possesses antispasmodic and expectorant properties. To treat ear infections and stop bleeding, herb powder is cooked with sesame oil. The pain from urinary tract infections and rheumatic pain are both treated with boiled leaves. Additionally, it is thought to have abortifacient qualities. In some regions of India, it is used to amplify uterine contractions and quicken labor.

It leaf juice can be used to cure diarrhoea and dysentery in addition to a number of other disorders like influenza, TB, bronchitis, and other conditions. Radio modulation, hypoglycaemic impact, cardiovascular protection, antitubercular effects, antiviral, hepatoprotective, and antioxidant action have all been observed.

The scientific evidence supports the promise of *Justicia adhatoda* crude extracts as a promising antiviral agent and treatment for the influenza virus. Treatment with synergistically active antiviral compounds that have various mechanisms of action may have multiple benefits over treatment with a single molecule, including higher potency, fewer side effects and toxicity, and superior clinical studies. The above results support the necessity for clinical trials to examine the therapeutic and preventative potential of *Justiciaadhatoda* extracts and to broaden this research to include other viruses.

Staphylococcus aureus and *Escherichia coli* were used to assess the antibacterial effect of the manufactured AgNPs, and it was determined that it may be a hazardous nanomedicine for respiratory tract diseases. It has also

been suggested that the *Justicia adhatoda* alkaloids have antiviral properties. One of the principal pharmacological targets for COVID-19 treatment is the main protease (Mpro), which is essential for the cleavage of the viral polyprotein. Vasicine may be a viable therapeutic target for COVID 19. It has been suggested that several phytochemicals, including polyphenols and alkaloids, are effective inhibitors of Mpro. Pemirolast and *Justicia adhatoda* anisotine and vasicoline are both excellent inhibitors. Consequently, *J. adhatoda* extract might be among the finest choices for creating novel natural treatments for COVID-19.

Bronchodilator and anti-asthmatic action

Traditional medicine has utilized *Adhatoda* to treat respiratory issues. The two main alkaloid components of *Adhatoda*, vasicine and vasicinone, have a long history of use as respiratory therapeutics. Bronchitis, other lung and bronchiole problems, as well as typical coughs and colds, can all be effectively treated using extracts of the leaves and roots of *Adhatoda*. Adhatoda leaf decoction has a calming effect on throat irritation and functions as an expectorant to clear mucus from the respiratory tract. *Adhatoda* extract was tested for its antitussive properties in anesthetized guinea pigs, rabbits, and unanesthetized guinea pigs, and the results indicated that the plant had a good antitussive action (Dorsch W, Wagner H., 1991).

The anti-asthmatic properties of *Adhatoda vasica* by extracting the powdered leaves with ethanol and observing the effects on guinea pigs that were given acetylcholine and histamine to induce bronchospasm, as well as by conducting in-vitro tests on isolated guinea pig ileum. The extract has demonstrated positive effects by inhibiting the bronchial structure in a dose-dependent manner (Dangi *et al.*, 2015).

Wound healing Process

Vertebral columns from buffalo calves were used, along with powdered alcoholic and chloroform extracts of *Adhatoda*. The calves treated with *Adhatoda vasica* displayed significantly better healing when compared to the control animals. Vasica enhanced the tissue used to treat wounds in terms of breaking strength, tensile strength, absorption, and extensibility. *Adhatoda*-treated mice also had significantly higher levels of elastin, collagen, hydroxyproline, hexosamine, and zinc. It was discovered that the herb's alcoholic extract worked best.

Anti-Ulcer properties

Adhatoda vasica was investigated for its ability to prevent ulcers brought on by ethanol, pylorus, and aspirin. Compared to controls, adhatoda leaf powder demonstrated significant anti-ulcer efficacy in experimental rats. The ethanol-induced ulceration model showed the highest level of activity. *Adhatoda vasica's* potential as an anti-ulcer medication is enormous. Additional studies revealed that *Adhatoda* syrup reduced the symptoms of dyspepsia.

Action against allergies

At a dosage of 5 mg, the extract containing the alkaloid vascinol and 20% vasicine reduced ovalbumin-induced allergy responses by roughly 37% (Paliwa, JK *et al.*, 2000)

Tubercular prevention

Bromhexine and ambroxol, two commonly used mucolytics, are produced by the chemical composition of *Adhatoda* alkaloids known as vasicine. Both of these substances limit the growth of Mycobacterium TB in a pH-dependent manner. Increased levels of lysozyme and rifampicin in bronchial secretions, lung tissue, and sputum are two examples of how *Adhatoda* indirectly affects tuberculosis, suggesting that it may be an essential supplementary treatment for the disease.

Uterotoxic and abortifacient activity

Adhatoda vasica is beneficial for causing abortion and for promoting uterine contractions to quicken labor since it has abortifacient and uterotonic effects. The presence or absence of specific estrogens appears to have an impact on this activity. Human myometrial strips collected from the uteri of both pregnant and non-pregnant women were treated with Adhatoda as part of a study on the activity of vasicine in generating uterine contractions. It was discovered that the plant may cause uterine contractions with equal efficiency to the medication oxytocin.

Insecticide usage

Adhatoda vasica has been used for centuries in India as an insecticide. Its leaves have been shown to control insect pests in oil seeds, in both laboratory and warehouse conditions. Vasicinol, an alkaloid found in *Adhatoda*, blocks the oviduct and has an antifertility effect on several insect species. *Adhatoda's* usefulness as an insect repellent has also been supported by research.

Bacterial resistance

Using the paper disc and dilution procedures, a leaf extract was examined for antibacterial activity. *Adhatoda's* alkaloids have a substantial antibacterial effect on *Pseudomonas aeruginosa*, according to in-vitro testing. Significant antibacterial activity was also shown against gram-negative *E. coli* and the Gram-positive bacteria strains *Streptococcus faecalis*, *Staphylococcus aureus*, and *Staph epidermidis* (Thirumagal and Pricilla, 2020).

Cardioprotective activity

In combination withvasicine and vasicinone significant reduction in cardial depressant effects was observed. No effect was shown by vasicinone (Dl-form) however, Lform was found to be weakly effective in stimulating cardiac muscles (Atal, 1980).

Hepatoprotective activity

In the evaluated research on traditional medicine, the Indian Council of Medical Research, New Delhi, identified liver disorders as one of the prevalent thrust areas. Various degrees of hepatoprotection against the CCl4-induced liver damage in rats have been documented for *Adhatoda vasica*. The goal of the current study was to determine whether *Adhatoda vasica* whole plant powder could protect the Wister rat model's liver from damage caused by the chemical CCl4. The hepatoprotective activity of the liver has been assessed using blood and tissue biochemical markers. GOT, GPT, Alkaline Phosphate, Glucose, Bilirubin, Triglycerides, GT, Cholesterol, DNA, RNA, and total protein are these biological markers. By using a standard procedure, the effect of *Adhatoda vasica* whole plant powder is compared with Silymarin and is found to have superior hepatoprotective effects (Shirish S Pingale, 2009)

Anti-inflammatory activity

Adhatoda vasica's primary alkaloid, vasicine, had anti-inflammatory properties. By using the modified hen's egg chorioallantoic membrane test, the anti-inflammatory activity of the methanol extract, the non-alkaloid fraction, the saponins, and the alkaloids was assessed. At a dose of 50 /pellet, which is similar to the dosage of hydrocortisone, the alkaloid fraction showed strong activity, whereas the MeOH extract and the other fractions displayed weaker activity (Chakraborty and Bratner, 2001).

Immunity Booster

The immune system is in charge of defending the body against foreign invaders like harmful bacteria and viruses as well as eradicating malignant cells when they develop inside the body. Greater infections, sluggish recovery from injuries and illnesses and increased vulnerability to immune system dysfunction symptoms and problems are all consequences of poor nutrition. Research indicates that immune function declines with aging and that adequate eating may be able to reduce or even reverse this decline. Studies also demonstrate that immune function declines often as we age. The immune system may be strengthened and colds and the flu may be avoided by eating a few certain foods (Mehak *et al.,* 2022).

Anti-diabetic activity

In mice with diabetes induced by alloxan, the effects of ethanolic extracts of *Justicia adhatoda* L. leaves and roots were investigated. Compared to the root extracts, oral administration of *Justicia adhatoda* L. leaves extract caused a significant decrease in blood glucose levels. Additionally, notable improvements were seen in the experimental animals' body weight, glycosylated hemoglobin, glucose tolerance, and serum lipid profiles, which supported the hypothesis that *Justiceia adhatoda* L. has a defense mechanism (Gulfraz *et al.,* 2011).

The EPG count and percentage worm recovery rate were both shown to be decreased by 79.57 percent and 16.6 percent, respectively, by leaf extract. The results were even discovered to be superior to praziquante therapy.

Anti-cancer activity

Justicia adhatoda L. methanol extract significantly inhibited cancer cells. The methanolic and ethanolic extracts of *Justicia adhatoda* L. were tested for their ability to cause cytotoxicity using the MTT assay. Ag-Au/CeO_2 nanoparticles were shown to have the strongest anticancer effects when compared to Ag/CeO_2, CeO_2, and Au/CeO_2 nanoparticles (Pandiyan *et al.*, (2011).

Anti-diarrheal activity

Castor oil was given to Swiss-albino mice of either sex to test the methanolic extract of *Justicia adhatoda* L. roots for anti-diarrheal properties. Compared to conventional loperamide, which showed 58.62 percent defecation inhibition, *Justicia adhatoda* L. showed 29.31 percent defecation inhibition (Hussain *et al.,* 2016).

Rheumatoid arthritis

Male Swiss albino mice were used to test the protective effects of a methanolic extract of *Justicia adhatoda* L. leaf on collagen-induced arthritis. It was discovered to recover chronic inflammation throughout the detrimental growth of rheumatoid arthritis and pro-inflammatory mediator release, which suggested that it could be used for upcoming drug innovations and advances in the fields of rheumatology. Post-oral administration of leaf extract dosages also reduced arthritic manifestation and footpad inflammation (Abikary *et al.*, 2015).

Anti-depressant activity

The effects of *Justicia adhatoda* L. leaf extract was investigated in rats with co-occurring depression and diabetes produced by alloxan. The extract treatment successfully reduced the elevated blood glucose, stopped the co-morbid depressed behavior, and demonstrated a potential protective effect (Gupta *et al.*, 2014).

Natural remedy for COVID-19

The continuation of the prolonged pandemic due to SARS-CoV-2 retrovirus is provoking researchers to devote themselves to pursuing research in the field of drug designing and vaccine making for COVID-19 disease.

The failure of market-available vaccines and the continued rise of COVID-19, particularly in the form of a novel variant, have heightened public and governmental concern. The COVID-19 pandemic's existence for a year and full or partial lockdowns everywhere else in the world has severely harmed the global economy, therefore extending the lockdown would not be a solution. The only option to get out of this terrifying scenario is to discover affordable and widely available COVID-19 medication. Drugs based on the *Justicia adhatoda* (*Justicia*-M/30/200 in homeopathy, VASAKA in Ayurveda) fully satisfy these requirements. *Adhatoda's* clinical trial report is presented here with great enthusiasm (Sobitri, 2021).

Conclusion

The medicinal plant’swide range of biological activities is well recorded, as well as the therapeutic benefits of *Adhatoda vasica* in treating various illnesses. More activity screening and structural and medicinal use of plants. The primary biochemicals in *Justicia adhatoda* L., such as those that are hepatoprotective, anti-fungal, anti-depressant, anti-bacterial, anti-diabetic,

anti-allergic, anti-diarrheal, cardioprotective activity, insecticide activity,etc., contribute to the substance's potent pharmacological profile.

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Current Herbal drugs trends: A Review

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Introduction

Herbal medications, often known as plant materials or herbalism, comprise the application of either the entire plant or a plant portion in the treatment of injuries or illnesses[1]. Herbal remedies are the practice of using medicinal plants to diagnose, treat, or prevent illness, as well as to promote overall health and well-being[2]. These are pharmaceuticals or other preparations that are derived from a plants or species and are employed for any of the aforementioned functions[3]. Herbal medicine is the first kind of treatment for disease that modern man is aware of. Numerous herbal remedies on the market make the claim that they can alleviate the signs and symptoms of a wide variety of illnesses, ranging from anxiety and insomnia to the common cold and the flu.

Herbal medications are defined by the WHO Organization (WHO) as full, labeled medical products that contain active substances, aerial or secretive portions of the plant, or other plant products or mixtures. The guidelines that have been established by the World Health Organization for determining the efficacy, quality, and safety of herbal medicines are very specific. According to

estimates provided by the WHO, approximately 80 percentages of people around the world currently use herbal medicines for serious medical treatment[4].

Traditionally, herbal medicines in some places may also include naturally occurring inorganic or organic active ingredients that are not derived from plants. This is a rare exception, but it does occur. Traditional medicine relies heavily on herbal drugs, which are also frequently used in ayurveda, homoeopathic, naturopathic, and other medical systems[5]. Since herbs come from natural sources, they are almost often thought of as being risk-free[6].

The cytotoxicity as well as adverse reactions of allopathic medicines has led to a surge in the use of herbal drugs, which in turn has led to a rapid development in the number of makers of herbal products. People have been using more and more herbal remedies without a prescription for the past several decades. This trend is expected to continue.

Humans have relied on nature for medicines, shelters, food, scents, clothing, flavors, fertilizers, and transportation for centuries. This is especially true in developing nations, where herbal therapy has a long history of use. Both developed and developing nations are recognizing these plants' medical and financial benefits. Traditional medicine has been based on plants for thousands of years. Plants still offer new medications[7].

Some plant benefits are faulty, and medical plant treatment is founded on tens of thousands of years of experimentation. Fine compounds, cosmetic, pharmaceutical and drugs. Vinblastine, which is used to treat leukemia in kids, Hodgkin lymphoma choriocarinoma, non-Hodgkin’s lymphomas, testicular and neck cancer, was isolated from *Catharanthus rosesus*. This plant is also used to treat Non-Hodgkin’s lymphomas. In Japan, the *Nothapodytes nimmoniana* tree, also known as *Mappia foetida*, is often utilized for the treatment of cervical cancer[8].

Medicinal herbs are vital to medication research. Medicinal plants have proven their function in treating cancer and viral disorders like Hepatitis, AIDS, etc. During 1950-1970, 100 plant-made medications were introduced to the US market, including vincristine, reseinnamine, vinblastin, deseridine, and reserpine. Fresh medications, including artmisinin, guggulsterone, ginkgolides, lectinam, teniposide, ectoposide, plaunotol, and nabilone, were introduced throughout the world between 1971 and 1990[9]. Irinotecan, toptecan, paclitaxel, and gomishin, among other drugs, are among the 2% of drugs introduced between 1991 and 1995. An innovative development in the management of

hypertension and blood pressure lowering was the 1953 isolation of serpentine from of the Indian plant rauwolfia serpent root.

Even in the modern era, plants remain not only vital in the field of medicine, but also the most promising potential source for future medicines. In despite the fact that we are already in possession of a number of cutting-edge medications, it is nonetheless of the utmost importance that we find and create brand new therapeutic agents[10].

Most major medications of the past fifty years have been extracted from plants. These chemicals have plant and animal drug-like characteristics. The WHO advocates adding herbal pharmaceuticals to national health care programmers since they are easily accessible, affordable, and safer than current synthetic drugs[11].

Current trend

Recent work in phytochemical and pharmacological research has produced effective remedies to certain diseases, which the synthetic drug industry has been unable to provide because to financial constraints. The scientific work that was done on *Artimisia annua*, *Cathranthus roseus*, *Taxus spp*., *Lantana camara*, and *Baccopa spp*., etc., is among the most important of these studies. In the past, such plants were thought to be either dangerous or of no use; however, recent research has revealed that they contain compounds with high therapeutic value and as a result, they are now regarded as herbal remedies of significant importance[12].

American Indians used medicinal plants like bonest, mayapple, and ginseng. These plants are highly valued for their beauty. Maya Indians in Mexico, Miskitos and Sumus in Honduras and Nicaragua, Pech, Lencas, and Xicaques in Honduras, Pipiles in El Salvador, *Talamancas* in Costa Rica, and Guaymis and Kunas in Panama use medicinal herbs[13].

Aspects of distribution

The distribution analysis of medicinal herbs reveals that these plants are dispersed across a wide variety of habitats and other components of the landscape. The tropical forests of the Eastern and Western Ghats, the Chota Nagpur plateau, the Aravalies, the Vindhyas, and the Himalayas are home to around 70–75% of India's medicinal plant species. Among the Himalayas, the Kashmir Himalayan region can be found tucked away only within Northwestern slopes of the Himalayas' recently proclaimed status as a global hotspot for diversity[13].The

therapeutic flora of Kashmir, on the other hand, has not received the attention it deserves, and it is possible that Kashmir alone has at least twice as many as this figure.

Status of Herbs

Herbal medicine is now maintaining its popularity in the majority of underdeveloped countries, and its use is quickly spreading throughout industrialized countries as well. It is believed that seventy percent of all medical practitioners in the countries of France and Germany regularly prescribe herbal medication to their patients[14]. Additionally, there has been a meteoric rise in the number of people searching for herbal treatments. In China, 30%-50% of all drugs are herbal. Herbal medications comprise 60% of first-line treatment in Ghana, Mali, Niger, and Zambia[15]. Over 50% of the population in Europe, Central America, and other affluent countries has taken herbal medicine at least once.70%-90% of Canadians and Germans have used herbal medication[16]. 158 million adult Americans take herbal medications and their use is growing.Adults tend to utilize both conventional and natural treatments equally. Since this demographic has a higher frequency of chronic diseases, sophisticated traditional medication regimens generally discourage long-term use due to adverse effects.Herbal remedies have no long-term adverse effects. With such features, herbal medicine is gaining worldwide popularity[17].

Regulatory requirements for Herbal drugs

In South Africa, herbal medicines are regulated like synthetic medications. It has a shorter time-to-market for pharmaceuticals with known safety, efficacy, and quality in humans and no obligation to even further refine extracts. The new approach encompasses Current Good Manufacturing, Good Laboratory Practices, and Good Agricultural Processes for plant-derived medicine manufacturing. To prepare for a Phase 1 or 2 medical trials with a herbal product, companies must provide data on the active constituent or marker chemical, documentation on the historic and present use of those elements, and some safety datainformation on the chemical, manufacturing, and control processes. Depending whether the medication has previously been used as a nutritional supplement, further chemical, manufacturing, and control information on preclinical safety as well as toxicological investigations are required for Phase 3 studies.

Value of Herbs

***Allium sativum* Linn-** Alliin, ajoene, diallylpolysulfides, vinyldithiins, and S-allylcysteine are some of the sulfur-containing compounds that can be extracted from fresh or crushed garlic. Additionally, garlic can secrete enzymes, tannins, flavones, as well as Maillard metabolic byproducts, which are not sulfur-containing compounds[18].

***Mentha spicta* Linn-** *Mentha spicta* has R-(-)-carvone, the component with the highest concentration in spearmint oil, is what gives the herb its characteristic aroma. A large amount of limonene, dihydrocarvone, and 1,8-cineol are also present in spearmint oil[19]. In contrast to oil of spearmint, oil of mint has very little menthol and menthone. It occasionally goes into shampoos and soaps. Is used as a flavoring for tooth paste & candy. Spearmint essential oil works well as a fumigant and pesticide against adult moths[20].

***Mimosa pudica* Linn-** Mimosine, a poisonous alkaloid, is anti-proliferative and apoptotic. *Mimosa pudica* seeds produce D-glucuronic and D-xylose mucilage[21].

***Withania somnifera* Linn-** Traditional Indian medicine has used the plant's long, brown tuberous roots for generations[22]. In Yemen, where it's called ubab, the dried leaves are mashed into a paste for burns and wounds. *Withania somnifera* leaves reduce edoema and joint pain[23].

***Curcuma longa* Linn-** In Ayurveda and Siddha practices, turmeric has been used both internally and externally as a potential treatment for a wide range of illnesses, including indigestion, throat infections, the common cold, and liver conditions. Turmeric has also been used to cleanse wounds and heal skin sores[24].

***Ficus racemosa*-** is an Ayurvedic herb. Fruits, bark, and root of *F. racemosa* are used in traditional medicine to cure diabetes. *F. racemosa* has anti-inflammatory, hepatoprotective, and hypoglycemic properties[25].

Future scope of medicinal herbs

It is anticipated that medicinal plants will have a prosperous future because there are approximately 500,000 plant species all over the world, the majority of which have not yet been researched for their potential medical applications, and the fact that these plants' undiscovered medical applications may prove to be critical in the treatment of both ongoing and upcoming research[26].

Medicinal herbs have played an important role in human civilization, including religions and rites. Many modern drugs, like aspirin, are made from medicinal plants. Garlic has therapeutic properties. Medicinal plant research helps humans and animals avoid natural poisons. Plant secondary metabolites cause therapeutic effects. Considering this, natural product chemistry research has gained popularity[27]. This interest can be due to therapeutic needs, the diversity of chemical structure and bioactivities of natural sources bioactive substances, the utility of innovative natural bioactive substances as biological probes, the advancement of innovative as well as responsive methods for detecting biological active items, enhanced methods to separate, detoxify, as well as structurally characterise such active components, and advance[28]. WHO has produced strategies, guidelines, & criteria for herbal remedies in recognition of traditional medicine's value. Agro-industrial technologies are used to grow, process, and make herbal medicines. Many contemporary pharmaceuticals are derived from medicinal plants[29].

Conclusion

Nearly every single known human culture has used herbal medicines for ages to cure dermatitis, injuries, skin problems, swelling, ageing, psychiatric disorder, cancer, asthmatic, diabetic, jaundice, scabies, venereal illnesses, snakebite, and gastric ulcers. This is because people believe herbal treatments are safe, affordable, and easily accessible. As herbal products become more popular, policymakers, health experts, and the public are concerned about their safety, standardization, efficacy, quality, accessibility, and commercialization. Herbal remedies are not as heavily controlled as conventional drugs in many nations, including the US. Globalization has made herbal remedies from all around the world more accessible. Efforts must be coordinated to assess the effectiveness and efficacy of herbal medications. Many researchers are studying herbal medications based on its Ayurvedic, Unani, TCM, and other uses. Recent advent of cutting-edge analytical tools and research methodologies has resulting in herbal medical use as per modern drug standard. Due to increased quality checks and regulatory measures, herbal remedies will be integrated into mainstream medical systems soon, as the "herbal age" approaches.

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