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Exploring Boundless Frontiers: Interdisciplinary Perspective in Research – Volume II

Exploring Boundless Frontiers Interdisciplinary Perspective in Research Volume II

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Mrs. Deepa K

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First Edition

Editors

Dr. P. Madhiyazhagan
Dr. Praseeja Cheruparambath
Mrs. Deepa K

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Foreword

Prof. Praveena Vijayan
Principal
SN college, Alathur
Palakkad, Kerala

It’s my pleasure to congratulate the authors for their initiative. It is more important than ever to use interdisciplinary approaches to comprehend and solve global issues as we negotiate the complexity of a world that is becoming more interconnected. May this publication give academics, researchers, and practitioners from various disciplines a place to interact, exchange ideas, and develop creative answers to today's most important problems. Also wishing good luck to all those who have contributed their academic thoughts to this book.

Preface

Welcome to the exploration of boundless frontiers through an interdisciplinary lens. In today's rapidly evolving world, the pursuit of knowledge knows no bounds, transcending traditional disciplinary boundaries. This book embarks on a journey to navigate these frontiers, offering a holistic view of research through interdisciplinary perspectives.

As we delve into the realms of various disciplines, we uncover connections, synergies, and novel insights that emerge at the intersection of diverse fields. By embracing this interdisciplinary approach, we transcend the limitations of singular viewpoints, fostering innovation and pushing the boundaries of human understanding.

Through the collaboration of scholars from diverse backgrounds, this book embodies the spirit of interdisciplinary research, bridging gaps and fostering dialogue across disciplines. From the sciences to the humanities, from technology to the arts, each chapter offers a unique vantage point, enriching our collective understanding of the world.

As we embark on this intellectual journey together, let us embrace curiosity, open-mindedness, and a willingness to challenge conventional wisdom. By exploring boundless frontiers through an interdisciplinary perspective, we embark on a quest for deeper insights, greater innovation, and a more interconnected world.

Join us as we embark on this exhilarating voyage of discovery, where the possibilities are limitless, and the frontiers are boundless.

Editors

Dr. P. Madhiyazhagan
Dr. Praseeja Cheruparambath
Mrs. Deepa K

About Editors

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

Dr. Praseeja Cheruparambath is currently working as an Assistant Professor in the Department of Zoology Sree Narayana College, Alathur, Affiliated to University of Calicut Kerala. She has 6 years of combined teaching and research experience, She is now recognised as research Guide under the university of Calicut with 3 Ph.D. research scholars with CSIR JRF fellows. She has established herself as an expert in the field of Microplastics, Conservation Biology, Dietary Supplimentation, entomology-particularly focusing on green synthesised Nano-based mosquito and agricultural pest control. She has an impressive publication record, having authored more than 18 research articles and 5 book chapters in highly reputed journals. Her contributions have garnered high impact factor, with high citations, h-index, and i-index . In addition to her research accomplishments, she has actively participated in the academic community. She served as a reviewer and editorial board member for various publications. She is

also a lifetime member of various scientific organizations. Furthermore, she has shared her expertise as a resource person in seminars and short-term courses. Her commitment to both research and education is highly appreciatable.

Ms. Deepa K is currently serving as an Assistant Professor in the Department of Botany at Sree Narayana College Erattakulam, Alathur, Kerala with 8 years of combined teaching and research experience, she has established herself as an expert in the field of Mushroom Research, Seed biology, Medicinal plant studies, particularly focusing on extension activities at the local level for the better agricultural productivity and biodiversity conversion. She has an impressive publication record, having authored research articles and book chapters in highly reputable journals. In addition to her research accomplishments, she has actively participated in the academic community. She is also a lifetime member of various scientific organizations. Furthermore, she has shared his expertise as a resource person in seminars and webinars. Her commitment to both research and education underscores her significant contributions to the field of Interdisciplinary biology.

Exploring Boundless Frontiers:
Interdisciplinary Perspective in Research
Volum II

Contents

AIRBORNE MYCOFLORA FROM THE OUTDOOR ENVIRONMENT OF SREE NARAYANA COLLEGE, ALATHUR

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Abstract

The present study aims to give an overview of varieties of fungi present in the air in outdoor environments viz. College ground, Ladies' washroom, Front of the college, and National Highway 544 of Sree Narayana College, Alathur, Palakkad, Kerala.The objective of this work is to identify the airborne fungi prevailing in the air which can cause numerous health issues. The identified fungus was *Rhizopus spp., Penicillium spp., Aspergillus niger, Curvularia spp, and Colletotrichum spp.* Among these *Rhizopus spp., Aspergillus niger, Colletotrichum spp., and Curvularia spp* were observed from the National Highway 544. It shows their capacity to survive even in the heavily polluted areas. And also, *Rhizopus spp and Aspergillus niger* were identified from the front of the college. *Penicillium spp* and *Aspergillus niger* were identified from the college ground. *Rhizopus spp*., *Aspergillus niger* were identified from Ladies' washroom. In this work, we made a comparison between the airborne fungus in different locations of outdoor areas.

Keywords: outdoor environments, airborne fungus, *Rhizopus, Penicillium, Aspergillus*

Introduction

Airborne pathogens in the environment and their risk factors

Exposure to airborne pathogens is a major risk to human health. Many microorganisms like bacteria, viruses, and fungi can be greatly dispersed through the air and inhaled and ingested by humans unknowingly. They are the major cause

of many respiratory diseases and can even cause allergic infections. Fungus disperses numerous spores in the air and causes health risks for humans. Airborne fungi are responsible for the majority of fungal infections in humans and animals. Outdoor air markedly influences the prevalence of fungal spore levels in indoor air and it is the major source of fungal infections. Air pollution from dampness and molds, chemicals, and other biological agents is one of the most public health problems with increasing importance due to the adverse health effects on humans, animals, and plants. The importance of airborne fungal contaminants has been dramatically increased given the health hazards caused by the spores themselves or by microbial metabolites. In addition to the risk of fungal infections, the allergic and toxic properties as well as the inflammatory effects may be considered as possible health impacts of fungal bioaerosols.Fungi can cause airborne diseases that need to consider in health plans due to its risk and threats of many communities worldwide. Airborne microorganisms scattered everywhere in both outdoor and indoor environments. (Ahmed et al., 2019)

An airborne disease is any disease that is caused by pathogens that can be transmitted through air. The relevant pathogen may be viruses, bacteria or fungi. They may be spread through breathing, talking, coughing, sneezing, raising of dust, spraying of liquids, and toilet flushing. Airborne fungi is the most important part of human pathogens which cause several human health problems such as allergic, contagious, infectious, and respiratory diseases (Gots et al., 2003). Human airborne diseases do not include conditions caused by air pollution such as volatile organic compounds, gases, and any airborne particles though their study and prevention may help inform the science of airborne disease transmission.

Airborne particles are major causes of respiratory ailments in humans causing allergies, asthma, and pathogenic infection of the respiratory tract. Airborne fungal spores are also important agents of plant disease and the means of dissemination of many common saprotrophic (saprophytic) fungi. Airborne fungi may be causative agents of adverse health effects and *Cladosporium* was the most dominant fungal genus followed by *Penicillium, Aspergillus, and Alternaria*(Fatih Kalyoncu & Sanver Ekmekci.2008).H.R.Pohekar & S.A.Kalkar.(2014) worked on Airborne culturable fungi in hospital environment of Nagpur. The study shows that

some of the dominant fungal forms encountered were *Cladosporium, Aspergillus, Penicillium, Alternaria, Curvularia* etc airborne disease can be caused by exposure to a source an infected patient or animal, by being transferred from the infected person or animal's mouth, nose, cut or needle puncture.

The sampling of air outdoors and indoors in a lecture hall in Cracow for culturable fungi revealed seasonal variations both outdoors and indoors. It confirms that the occurrence of *Cladosporium* was a major factor in the seasonal variation in the number of fungal propagules in indoor as well as outdoor air (E.Medrela-Kuder., 2003). Fungi are widely distributed over the world and are affected by various environmental factors such as temperature, moisture, wind, and geographical location.It has been reported that airborne fungi are the most common microorganisms that have adverse effects on human health causing asthma, rhinitis, and dermatitis besides they are considered a source of plant and animal pathogens. Zhiguo Fang et al (2005) worked on culturable airborne in an outdoor environment in Beijing, China., and concluded Airborne fungi adversely affect human health through allergy, infection, and toxicity moreover, they have a great influence on Urban air quality in Beijing. They identified *Penicillium* as the most abundant species, and *Cladosporium* were the most dominant fungal group.

The concentration of airborne microorganisms is correlated with the presence of dust from soil and human activities.As a result of air pollution, increasing carbon dioxide concentration in the atmosphere may induce the sporulation of common soil fungi.In addition, the concentration of fungal spores in the air is linked to the geographical region and seasonal variations; also meteorological parameters such as wind, humidity, temperature, rainfall, altitude, and vegetation affectthe numbers and type of airborne fungi.Airborne fungi like *Penicillium spp, Alternaria spp, and Aspergillus niger* respectively are one of the major sources of fungal disease (Nasrin Rostami et al. 2017).

The determination of airborne fungal concentration in an area is important because some of them may be aeroallergens. Our college campus is located at Erattakulam on Thrissur-Palakkad National Highway in Kavassery village, Palakkad. This location has having Latitude of 10.634185 and a Longitude of

76.507645. The college campus encloses 25 acres of land which has a wide variety of plant and tree species and is very rich in physiological and climatic conditions.

For this work, we selected four areas as outdoor locations i.e., College ground, Ladies washroom, Front of the college, and National Highway 544. These places were selected become of their pollution-causing tendency. The fungus was inoculated in PDA media. The project is carried out at a temperature of 26^0C. This project enables the comparison of species in the outdoorlocations of the college.

A systematic review of outdoor airborne fungal spore seasonality across Europe and the implications for health. Outdoor airborne fungal spores are associated with allergies. The most commonly reported genera were the *Alternaria* and *Cladosporium* (Samuel Anees–Hil et al 2022). The present review lights up on the airborne microflora in an outdoor environment. We identified *Rhizopus spp., Penicillium spp., Aspergillus niger, Curvularia spp.,* and *Colletotrichum ssp.* from the college outdoor environment and National Highway 544. There is no doubt that fungi produce allergens and cause some fungal diseases.

Study Area

Samples are collected in triplicates of culture plates from four different areas surrounding to the Sree Narayana College Alathur. They are collected outdoors during the period 20/08/2022 to 30/12/2022, the samples are collected in the Petri dish having PDA Media. The Petri dishwas then placed in an incubator at 25^0C for seven days.

TABLE 1-Areas of outdoor collections of fungal species

Sl. NO	AREAS	TRIPLICATES
1	College ground	G_1,G_2,G_3
2	Ladies washroom	L_1,L_2,L_3
3	Front of the college	F_1,F_2,F_3
4	National highway 544	C_1,C_2,C_3

METHODOLOGY

A. Preparation of Potato Dextrose Agar (PDA) Media.
B. Preparation of agar plate.
C. Sterilization of laminar air flow chamber and glasswares.
D. Detection of fungal growth in PDA Media.
E. Microscopic study of fungus in the culture of PDA Media.
F. Preparation of Potato Dextrose Agar (PDA) Media

Type: General purpose medium

Composition:

- Potato-250g/l
- Agar-15g/l
- Dextrose-20g/l
- Distilled water-1000ml
- Peel the potatoes, cut them into small pieces and boil 250ml water.
- Mash the boiled potatoes to form a thick paste.
- Stain through the cheese cloth.
- Add dextrose and agar and water to make upto a litre.
- A pinch of Amoxicillin (an antibacterial medicine)was added to the medium to prevent the bacterial growth to make this medium of devoid of contamination.
- Then, Prepared standard PDA was poured in to 500ml conical flask and sterilize (121^0C, 15 psi for 15 minutes) in pressure cooker.

A. Preparation of Agar Plate

25ml of sterilized PDA medium was poured in pre-sterilized borosil glass petridish. The petridish were allowed to cool at room temperature $25\pm^0$C. Triplicates are taken and placed in four different areas for 10-15minutes. Petridishes were incubated at room temperature for seven days. On eighth day, the fungal growth was examined under microscope for the preliminary determination of air-borne mycoflora.

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B. Sterilization of Laminar Air Flow Chamber and Glasswares

The Laminar air flow chamber is a device which is used for sterilization. This reduces the possibility of contamination while working with culture of fungus in a laminar air flow chamber. The working area was cleaned by using sanitizer containing 70% alcohol and cotton. After this UV radiation is provide for 15 minutes for sterilizing the environment.

Glasswares such as petridish, conical flask and inoculation needle were sterilized using hot air oven. The petridish were clean and dried with a soft non-abrasive dry cloth, then the petridish were wrapped using clean papers and they were kept in the oven 160^0C for 2 hours to be sterilized.

C. Detection of Fungal Growth in PDA Media

Among the petridish mycelial growth are seen. After seven days, different colour colonies are identified. The sticky surface carrying fungal spores and hyphae is carefully placed over the slide. And drops of Lactophenol is added. Then it is mounted with glycerine. After this it is observed under microscope.

D. Microscopic Study of Fungus in the Cultureof PDA Media

The detected fungus from the fungal colony is taken and observed under microscope. Then the type of fungus is identified.

Result

Most of the identified fungus were harmful to organism because they cause varieties of allergies.

1. *Rhizopus* sppis identified from National Highway 544, Ladies' washroom and from the front of the college.

Rhizopus spp.

Kingdom	:	Fungi
Division	:	Zygomycota
Class	:	Mucuromycotina
Order	:	Mucorales

Family : Mucoraceae

Genus : *Rhizopus spp*.

Morphological characteristic features

Colony texture deeply cottony, white becoming grey-brownish on surface. Growth rate is very rapid. The species grow as filamentous branching hyphae, rhizoids or stolon present. Rounded sporangia present inside the sporangiophore. The species grow as filamentous branching hyphae. In sexual reproduction, zygospores produced as colonies.

Symptoms

Sinusitis and pneumonia are the most common types of infection with disseminationprevalentin patients with underlying disease. Inhalation of spores, in addition traumatic implantation, can cause disease.

Penicillium spp**.** is identified from college ground.

Kingdom : Fungi

Division : Ascomycota

Class : Eurotiomycetes

Order : Eurotiales

Family : Trichocomaceae

Genus : *Penicillium spp.*

Morphological characteristic features

- They are initially white and become blue, green, grey-green ,olive grey, yellow or pinkish with time.
- Phialides may be produced singly in groups or from branched mature giving a brush like appearance.
- It resembles like a paint brush.
- Multicellular fungi are composed of filaments called hyphae.
- Hyphae may contain internal crosswalls called septa.
- The septa divide the hyphae into separate cells.

Symptoms

- General discomfort.
- Swollen lymph nodes.
- Difficulty in breathing, weight loss, fever.
- *Aspergillus niger* van Tieghem
- This fungus is identified from National Highway 544, ladies washroom, college ground and front of the college

Kingdom	:	Fungi
Division	:	Ascomycota
Class	:	Eurotiomycetes
Order	:	Eurotiales
Genus	:	*Aspergillus*
Species	:	*niger*

Morphological characteristic features

- *Aspergillus niger* the group which bear black spores.
- Surface colony is black.
- There is singleconidiophores that produces perpendicular to the long axis of the cell.
- Hyphae are septate, hyaline.
- Conidiophores are hyaline and smooth walled.
- Conidia are globose, brown to black coloured.

Symptoms

- Fever and chills.
- Chest pain or joint pain, headache.
- *Curvularia spp* is identified from National Highway 544

Kingdom	:	Fungi
Division	:	Ascomycota
Class	:	Dothideomycetes

Order : Pleosporales

Family : Pleosporaceae

Genus : *Curvularia spp.*

Morphological characteristic features

- Mostly pale brown, medium reddish brown to dark brown.
- Colonies are fast growing, suede- like to downy, brown to blackish brown with black reverse.
- Conidiophore erect, straight to flexuous septate, often geniculate (producing conidia in sympodial succession) sometimes nodulose.
- Conidia are ellipsoidal, often curved lunate rounded at the ends or sometimes tapering slightly towards the base.
- The species usually contain 3-5 septa.
- Conidial wall is smooth to verrucose.
- Hilum protruberant in some species.

Symptoms

- It causes nondermatophytic onychomycosis, pharohyphomycosis, mycetoma and infection of wounds, sinuses and eyes.
- It can cause central nervous system infections, postsurgical endocarditis, dialysis related peritonitis and a typical skin lesions.
- This *Colletotrichum spp*fungus is identified from National Highway 544.

Kingdom : Fungi

Division : Ascomycota

Class : Sordariomycetes

Order : Glomerellales

Family : Glomerellaceae

Genus : *Colletotrichum spp*

Morphological characteristic features

- Mostly white-greyish colony colour, texture of colony is velvety.
- It is characterised by oblong, sometimes slightly constricted, micro-guttulate conidia and simple obovoid to ellipsoidal appressoria.
- Conidia were fusiform in shape with both ends pointed or slightly acute.
- Colletotrichum is traditionally recognised as an asexual genus of fungi with a number of species linked to sexual morphs.
- They were septate, thick walled, acicular.
- Sporulation is one of the most important reproductive strategies in colletotrichum fungi and is responsible for disease epidemics.

Symptoms

Anthracnose of banana is caused by the colletotrichum species and is one of the most serious disease of ripe banana. It include black and sunken lesions with spore masses or acervuli in the lesion. It also cause keratitis and subcutaneous fungal infection in humans.

Table 2- Colony colours and morphological characterization of different fungal species

Sl. No	Name of the fungus	Identified area	Colony colour shape	Vegetative characters	Asexual characters	Sexual characters
				Septate/aseptate hyphae	Shape of Sporangia	Shape of conidia
1	*Rhizopus spp*	Ladies washroom, Front of the college, NH	Yellow Filamentous	Aseptate	Spherical structure	_
2	*Penicilium spp.*	College ground	Grey green Brush like	Septate	Club shaped	Brush like

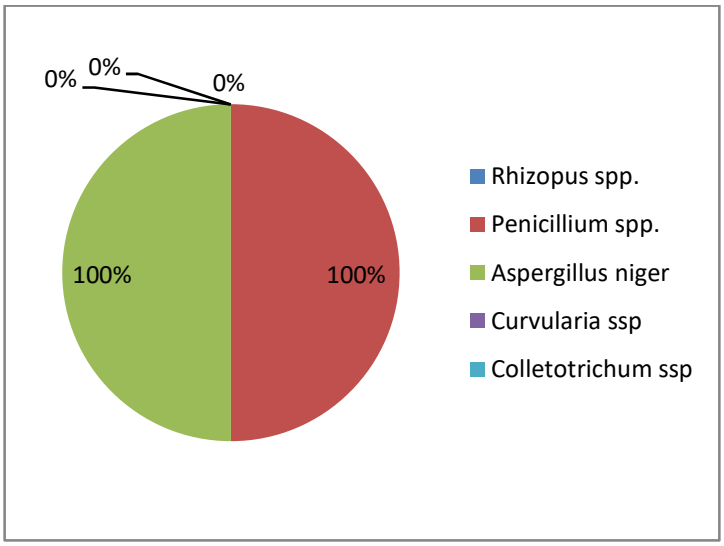
3	*Aspergillus niger*	College ground, Ladies washroom, Front of the college, NH	Black globular	Septate	Globular structure	Globose
4	*Curvularia ssp.*	NH	Pale brown, brown-dark brown, Velvety	Septate	Curved	Curved
5	*Colletotrichum spp.*	NH	White greyish Velvety	Septate	Spherical structure	Fusiform

Table 3- Analysis of dominantly observed fungal colony on different areas on the basis of their frequency

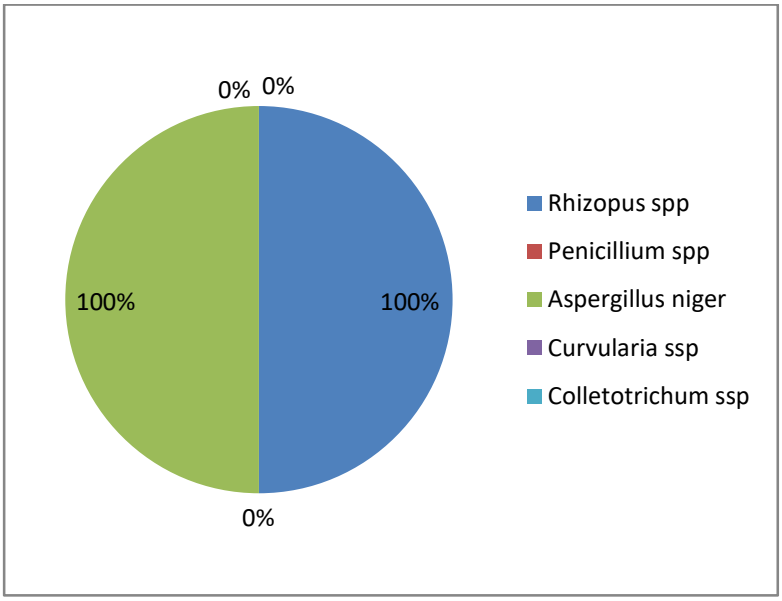
Sl. No	**Fungal colony**	**Study area**			
		College ground	**Ladies washroom**	**Front of the college**	**National Highway 74**
1	*Rhizopus spp.*	- (0%)	++++ (100%)	+++ (50%)	++++ (100%)
2	*Penicillium spp.*	++++ (100%)	- (0%)	- (0%)	- (0%)
3	*Aspergillus niger*	++++ (100%)	++++ (100%)	++++ (100%)	++++ (100%)
4	*Curvularia ssp.*	- (0%)	- (0%)	- (0%)	++++ (100%)
5	*Colletotrichum spp.*	- (0%)	- (0%)	- (0%)	++++ (100%)

Note: ++++ Maximum +++ Moderate Nil

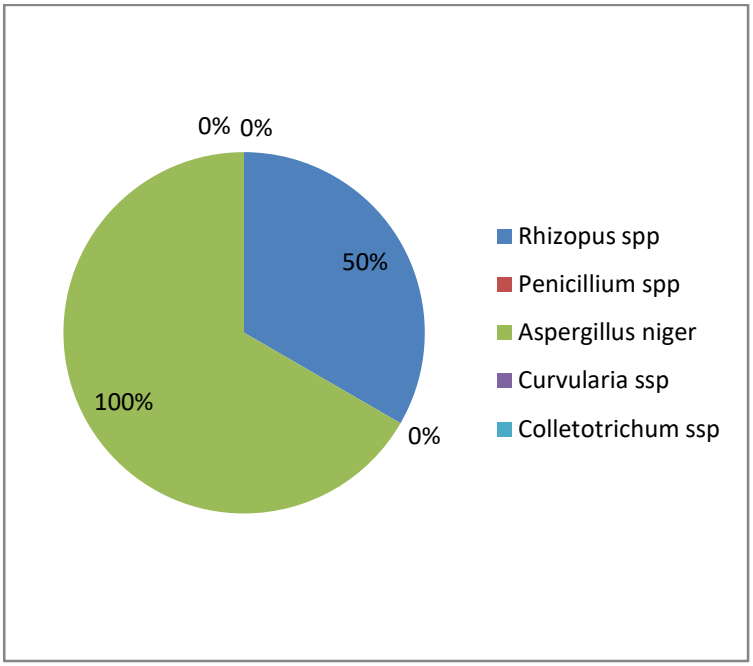
Graph 1- Fungal frequency of College ground (G)



Graph 2- Fungal frequency of Ladies Washroom (L)



Graph 3- Fungal frequency of Front of the college (F)



Graph 4- Fungal frequency of National Highway 544 (C)

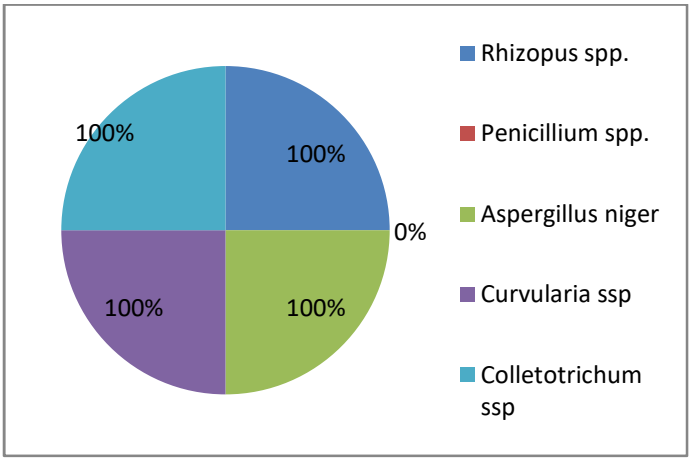


PLATE -1 Petridishes showing fungal growth from College ground (G)

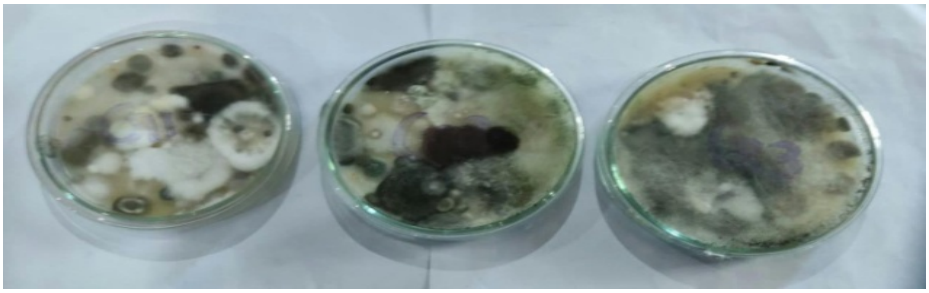


PLATE -2 : Petridishes showing fungal growth from Ladies washroom (L)

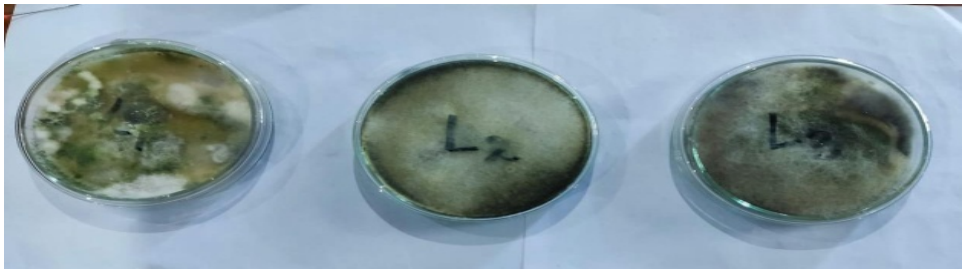


PLATE -3 : Petridishes showing fungal growth from Front of the college (F)

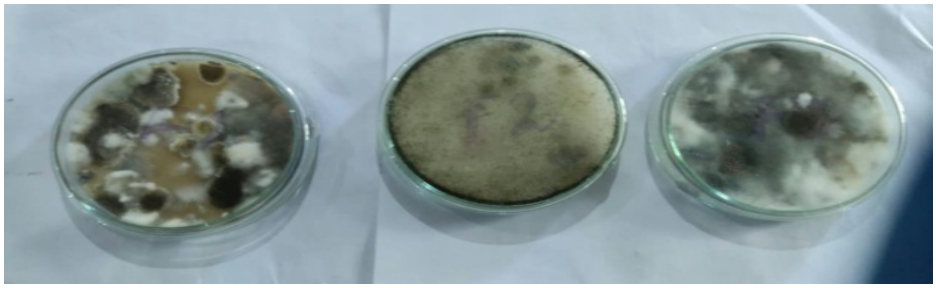


PLATE -4 : Petridishes showing fungal growth from National Highway 544 (C)

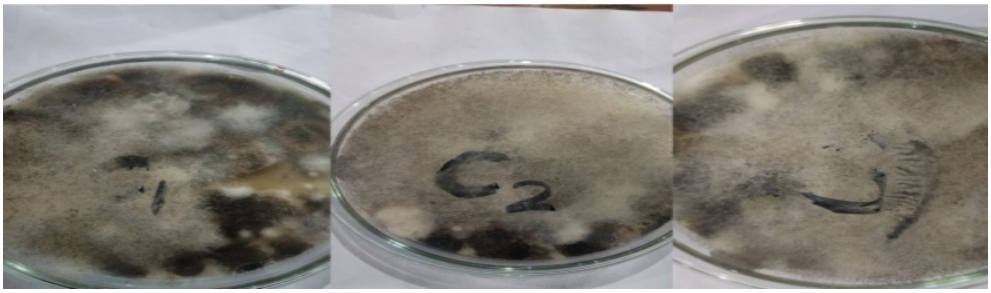


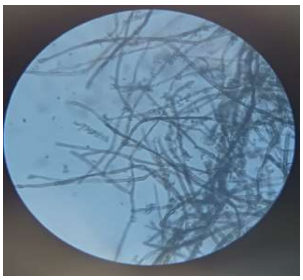
PLATE -5 : Microscopic images of identified fungus

Rhizopus spp

Penicillium spp

Aspergillus niger

Curvularia spp



Colleotrichum spp

Conclusion

Air-borne mycoflora is one of the most common types of fungus. There are many types of fungus can be seen in surroundings. Some are useful but most of the airborne fungi are responsible for the majority of fungal infection in humans and animals. It includes asthma, allergies etc. The genera *Penicillium* and *Aspergillus*, *Curvularia, Colletotrichum* comprise the major part of fungal community in the atmosphere. It has been demonstrated that abundance of airborne fungal spores in

outdoor air vary from place to place and it influence by mainly by climatic conditions and human activities. However, Aspergillus, Penicillium and Alternaria, called allergenic fungi, can colonize in indoor environments, thereby increasing fungi exposure levels. Human exposure to fungal aeroallergens is due to increased asthma severity (Gabriel et al., 2016). Aeromycological studies are important to understand and the diversity of fungi by air their distribution to derive strategies for to control fungal disease. This research is the first study in Sree Narayana College, Alathur, Kerala. In this work, we identified five fungus species such as *Rhizopus spp*, *Penicillium spp*, *Aspergillus niger, Curvularia ssp., Colletotrichum ssp* in the sampling air. The evaluation of air sampling results is currently based on comparison between outdoor environment of college premises and National Highway 544. Through this project, we identified that *Aspergillus spp* were dominant than other fungal species in outdoor environment of college premises and National Highway 544.

Our results indicate that outdoor air is a potential threat to public health because of harbouring a wide array of pathogenic and allergenic airborne fungal spores which can serve as the main source of contamination of indoor environment such as homes, offices and hospitals.

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PROTECTION OF MILD STEEL AGAINST CORROSION IN 1M HCL BY N_2O_4 TYPE SCHIFF BASE: AN ELECTROCHEMICAL APPROACH

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Abstract

The effect of a dicompartmental Schiff base derived from 3-ethoxysalicyladehyde and 2-aminobenzylamine on corrosion of mild steel in acid medium is investigated *via* electrochemical polarization and electrochemical impedance spectroscopic techniques. The compound exhibited promising inhibition against acid corrosion of mild steel *via* formation of a protective layer on the mild steel surface. The polarization measurements explicit the mixed type inhibition of the compound involving the suppression of anodic metal dissolution as well as the retardation of cathodic hydrogen evolution. However a predominant control over the anodic reaction has been observed for the Schiff base. The adsorption of the inhibitor molecules on the mild steel surface obeys Langmuir isotherms and the free energy change of adsorption give information regarding the chemisorption of the molecules on the metal surface through coordinate bond formation between the iron atom of the mild steel surface and the hetero atoms (N and O) of the inhibitors.

Keywords: Dicompartmental Schiff base; Langmuir adsorption isotherm; EIS studies; Corrosion inhibition.

1. Introduction

The significance of corrosion inhibitors applicable in HCl medium is widely increasing owing to their prevalent use in reducing the corrosion rates of metallic materials in the industrial area of acid pickling, acid cleaning, acid

rescaling and oil well cleaning [1]. Organic compounds having N, O, S and π bonds are found to be efficient corrosion inhibitors due to their capability to get adsorbed on the metal surface. The mechanism involved in the adsorption of organic compounds on the mild steel surface can be expressed in a general way as

$$Org_{(sol)} + nH_2O_{(ads)} \rightarrow Org_{(ads)} + nH_2O_{(sol)}.$$

It involves the exchange of water molecule already adsorbed on the metal surface with the organic molecules [2]. The adsorption process is affected by so many factors of the inhibitor molecules such as functional groups present in the molecule, steric factors, aromaticity, electron density at the donor atoms and π orbital character of donating electrons [3,4], the electronic structure of the molecules [5,6] *etc.*.

Among the vast number of organic compounds, Schiff bases deserve special mention owing to their promising corrosion inhibition activity in acid solutions [7]. There have been reports on the greater inhibition efficiency of Schiff bases than their corresponding aldehydes and amines due to the presence of C=N groups in the molecule which enable them to get adsorbed on the metal surface spontaneously therefore can act as efficient inhibitors [8-10].

The Schiff base for the corrosion inhibition studies was derived from 3-ethoxysalicylaldehyde and 2-aminobenzylamine. The main highlight of the prepared Schiff base is that it possesses two compartments for metal coordination. Presence of ethoxy groups in the benzene ring boosts the adsorption ability of the inhibitor molecules. As the synthesis and molecular structure of the compounds of concern is already reported [11, 12], the present study is limited to the electrochemical approach of the anti-corrosion properties only.

2. Experimental

2.1. Materials

The Schiff base was synthesized according to the previously published procedures. The Schiff base is prepared *via* condensation of 3-ethoxysalicyladehyde with 2-aminobenzylamine in 2:1 ratio in ethanol medium. The crystal structure is shown in Fig. 1.

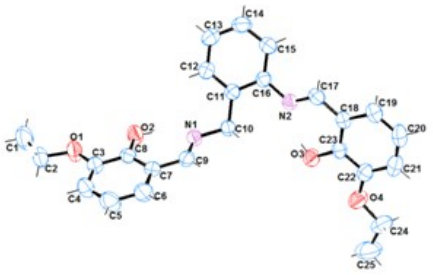

Fig. 1. Crystal structure of H_2L^1

The mild steel specimens for corrosion inhibition studies were first polished with emery paper of 400-1200 grade and washed thoroughly with acetone followed by double distilled water. The specimens were dried before immersion in acid medium. The acid medium, 1M HCl was prepared by diluting analytical grade 37% HCl with double distilled water. Different concentrations of inhibitors were made for the polarization techniques and the concentration range employed was 50-400 ppm in 1M HCl.

2.2. Electrochemical studies

Electrochemical experiments such as polarization and electrochemical impedance spectroscopy (EIS) were carried out with CH-Instruments. It involves a three electrode assembly. A saturated calomel electrode (SCE) and platinum wire was used as the reference and counter electrodes respectively. A thin sheet of mild steel as working electrode was employed in such a way that the exposed area of the specimen in the electrolyte was only 2 cm^2.The test solutions were prepared by dissolving the compounds in 5 mL DMSO followed by the addition of 1M HCl. Before measurement, the working electrode was immersed in the test solution for 30 minutes to attain a steady state indicated by a steady open circuit potential (OCP). The polarizationcurves were recorded by automatically changing the potential range of $\pm$200mV from OCP with a sweep rate of 10 mV per second. Corrosion current density i_{corr} was obtained by the extrapolation of the cathodic and anodic lines of the Tafel plots to the corrosion potential, E_{corr} value. EIS measurements were performed in the frequency range of 10^5 to 1 Hz with an ac amplitude of 5 mV at the OCP.

2.3. Morphological studies

The surface preparation for morphological studies were performed by immersing the cleaned mild steel specimens in 1 M HCl solution with and without inhibitors for six hours. After immersion, the specimens were taken out and cleaned well with acetone and double distilled water and dried carefully. The surface of each specimen was analysedby taking the SEM images with JEOL Model JSM - 6390LV.

3. Results and discussion

3.1. Electrochemical polarization studies

The potentiodynamic polarization behaviour of mild steel in 1M HCl in the absence and presence of various concentrations of inhibitors is shown in Fig. 2. The parameters such as corrosion potential (E_{corr}), cathodic Tafel slope (β_c), anodic Tafel slope (β_a) and corrosion current density (i_{corr}) obtained from these Tafel plots are tabulated in table 1. From the Tafel plots it is clear that as concentration of inhibitors increases, corrosion current decreases to a minimum value and then increases on increasing the inhibitor concentration. The inhibition efficiency was calculated using the equation

$$I.E._1\,(\%) = \frac{i_{corr} - i_{corr(inh)}}{i_{corr}} \times 100$$

where, i_{corr} and $i_{corr(inh)}$ are the corrosion current densities in the absence and presence of inhibitors respectively. In the case of uncomplexed ligand, maximum corrosion inhibition was observed for 300 ppm inhibitor concentration with an efficiency of 90.07% above which inhibition efficiency started to fall from the maximum value. Hence 300 ppm indicates the optimum concentration of H_2L^1 for better corrosion control of mild steel in 1 M HCl solution.

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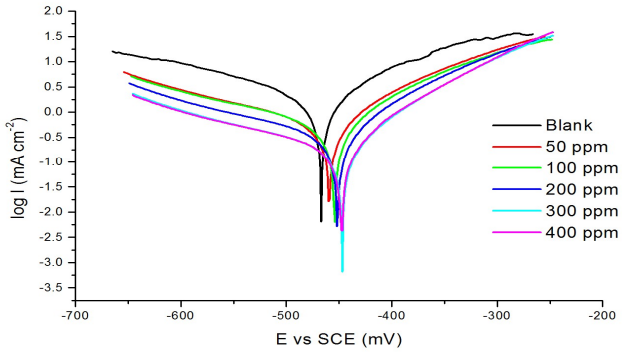

Fig. 2. Polarization curves for mild steel in 1 M HCl in the presence of H_2L^1at different concentrations.

Apart from the decrease in corrosion current on the addition of inhibitors, a shift in corrosion potential towards lower potential has also been observed. Corrosion inhibitors can be classified into anodic, cathodic and mixed type based on the effect of inhibitor on the anodic metal dissolution, cathodic hydrogen evolution or both respectively. Based on the changes occurring on the corrosion potential upon the addition of increasing concentration of inhibitors, corrosion inhibitors can be identified as cathodic, anodic and mixed type [13]. If the difference in the corrosion potential of the solution with and without inhibitor is larger than 85 mV, then the inhibitor falls into the category of either anodic or cathodic type. When it is less than 85 mV, the inhibitor qualifies as mixed type [14]. From Table 1, it can be seen that the shift in the E_{corr} values of all the inhibitors is less than 85 mV suggestive of mixed type inhibitors. So the addition of inhibitors suppresses the metal dissolution as well as makes slow the hydrogen evolution reaction. The Tafel plots of the inhibitors show a slight displacement of corrosion potential towards positive direction. This result indicates that the inhibitors have predominant control over the metal dissolution reaction (anodic) even though they act as mixed type inhibitors. This is done by forming a protective layer of inhibitors *via* adsorption over the mild steel surface and thereby blocking the anodic sites from acid attack [15]. The adsorption is enabled due to the presence of hetero atoms such as O and N as well as the aromatic π electrons in the inhibitor molecules. The parallel displacement of cathodic branch of Tafel plots upon the addition of inhibitors suggests that there is no change happened in the

mechanism of hydrogen evolution reaction on the cathodic sites of the mild steel surface which occurs mainly through a charge transfer mechanism [16]. Thus without modifying the mechanism of corrosion, the surface area exposed for acid attack is reduced by the inhibitor molecules and thereby suppress the metal dissolution as well as hydrogen evolution.

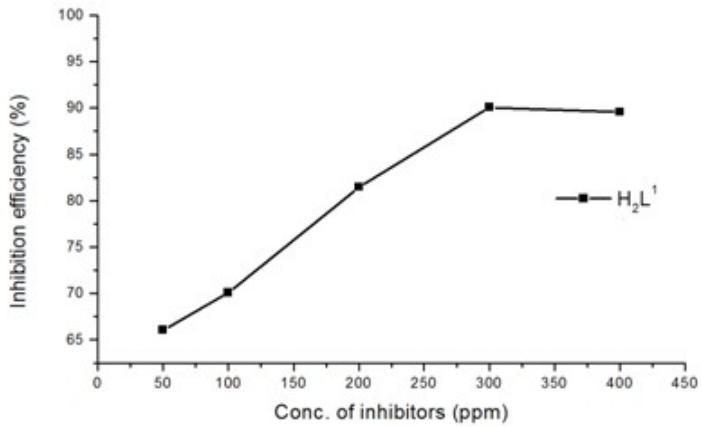

Fig. 3. Variation of inhibition efficiency with concentration of inhibitor

Table 1. Parameters obtained from polarization measurements

Inhibitors	Conc. (ppm)	$-E_{corr}$ (mV)	i_{corr} (mA cm^{-2})	R_p (Ω cm^2)	β_a(mV)	$-\beta_c$(mV)	R_{corr} (mmy^{-1})	$I.E._1$(%)
Blank		465	2.507	14	130.5483	185.7700	1.215×10^3	
H_2L^1	50	454	0.8515	36	109.5410	205.6343	4.128×10^2	66.04
	100	447	0.7500	42	108.6956	220.8481	3.636×10^2	70.08
	200	449	0.4645	62	95.8497	213.4472	2.251×10^2	81.47
	300	446	0.2489	104	84.3668	205.7613	1.206×10^2	90.07
	400	446	0.2619	102	86.2218	208.9864	1.270×10^2	89.55

3.2. EIS measurements

Fig. 4. presents the Nyquist plots of mild steel in 1 M HCl with and without various concentrations of inhibitor. It is clear that these plots yield slightly depressed semi-circular shape with one time constant. The deviation of the capacitive loop from a perfect semi-circle shape could be attributed to the inhomogeneityof the mild steel surface [17]. The diameter of these depressed semi-circles is commonly considered as the charge transfer resistance of the electrochemical system. The charge transfer resistance can be evaluated as the difference in real impedance at lower and higher frequencies and it must be corresponding to the resistance between the metal and outer Helmholtz plane. But the overall resistance corresponds to the metal/solution interface include charge transfer resistance (R_{ct}), diffuse layer resistance (R_{d}), accumulation resistance (R_{a}), film resistance (R_{f}), etc., hence all of them must be taken into account [18]. Therefore in the present study the diameter of the Nyquist plot is considered as the polarization resistance (R_p) instead of just charge transfer resistance *(R_{ct})*. The inhibition efficiency can be calculated from the EIS measurements as follows

$$I.E._2\,(\%) = \frac{R_{P(inh)} - R_P}{R_{P(inh)}} \times 100$$

Where $R_{P(inh)}$ and R_P polarization resistance in the presence and absence of inhibitor [19].

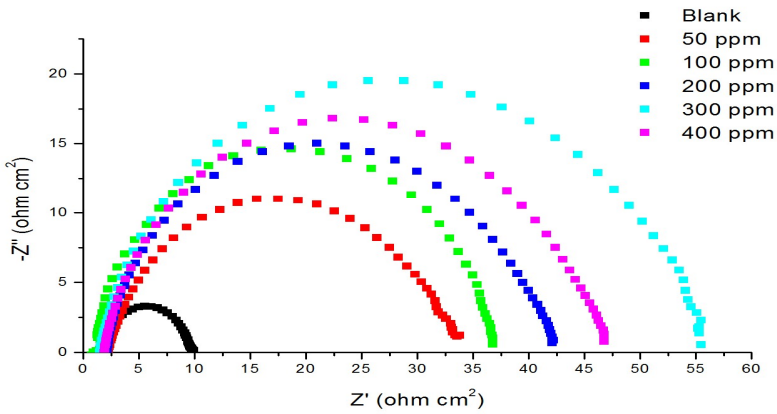

Fig. 4. Nyquist plots for mild steel in 1 M HCl in the presence of H_2L^1 at different concentrations.

Impedance results are depicted in Table 2. The Nyquist plots are in good agreement with the results obtained from the Tafel plots. As the concentration of inhibitor increases, the polarization resistance increases. When the concentration reaches its optimum value, inhibition efficiency attains its maximum. Above the optimum concentration, polarization resistance started to decrease suggestive of decreased inhibition efficiency. This observation can be justified as when the concentration of inhibitor increases, it covers the metal surface by forming a monomolecular layer *via* adsorption process and acts as a barrier for the charge and mass transfer. But the addition of inhibitors greater than the optimum concentration, leads to the perturbation of already formed monolayer results in the lessening of inhibition efficiency.

Table 2. Inhibition efficiency obtained from Nyquist plots.

Inhibitors	Conc.(ppm)	R_{ct}(Ω cm^2)	I.E. (%)
Blank		8.08	
H_2L^1	50	31.55	74.3898
	100	35.08	76.9669
	200	39.91	79.7545
	300	65.9	87.7390
	400	59.9	86.5108

Fig. 5 represents the Bode plots and Fig. 6 show the phase angle plots for the mild steel in 1 M HCl in the absence and presence of increasing concentration of inhibitor. Both the Bode plots and phase angle plots exhibit only one time constant. The high frequency region of the Bode/phase angle plots explicit the behaviour of the inhomogeneous surface layer while the low frequency region describes the kinetic response for the charge transfer reaction [20]. In the Bode plots, higher impedance in the low frequency region in the presence of additives indicates better protection of mild steel against corrosion. It is related to the adsorption of the inhibitor molecules on the mild steel surface. In the phase angle

plots, only one phase peak at the middle range of the plot is observed suggestive of one time constant for the inhibitors and it is related to the electrical double layer formation at the metal/solution interface [21]. As the concentration of inhibitor increases to the optimum concentration, the phase angle shifts to more negative value indicates that inhibition effect of the additives increases with concentration due to the adsorption of molecules on the metal surface [22].

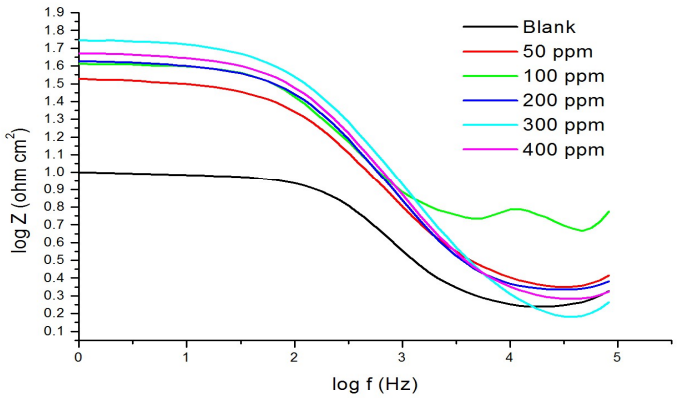

Fig. 5. Bode plots for mild steel in 1 M HCl in the presence of H_2L^1 at different concentrations.

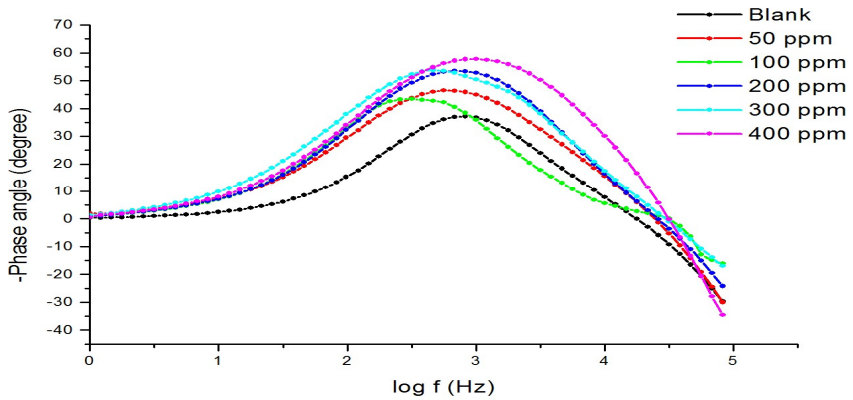

Fig. 6. Phase angle plots for mild steel in 1 M HCl in the presence of H_2L^1 at different concentrations

3.3. Adsorption isotherms

The inhibition efficiency of an inhibitor depends on many factors such as the type and number of active sites at metal surface, the charge density, the molecularsize of the inhibitor, and the interaction between the metal surface and inhibitor molecules [23]. Adsorption isotherms can shed light on the type of interaction involved in between metal surface and inhibitor molecules. The plot of surface coverage against concentration gives adsorption isotherm. The surface coverage can be evaluated from the polarization measurements as

$$\theta = \frac{i_{corr} - i_{corr(inh)}}{i_{corr}}$$

where, i_{corr} and $i_{corr(inh)}$are the corrosion current densities in the absence and presence of inhibitors respectively [21]. The Langmuir adsorption isotherm correlates θ with inhibitor concentration C_{inh} as follows

$$\frac{C_{inh}}{\theta} = \frac{1}{K_{ads} + C_{inh}}$$

where K_{ads} is the adsorption equilibrium constant. Fig.7 shows the Langmuir adsorption isotherm for the inhibitor at 25 °C. The linear behaviour of adsorption isotherm indicates that the adsorption of the inhibitor molecules on the metal surface obeys Langmuir adsorption isotherm. The reciprocal of the intercept denotes the adsorption equilibrium constant,K_{ads}.The free energy of adsorption $\Delta G_{ads}$of the inhibitors can be obtained from the following equation [24].

$$K_{ads} = \frac{1}{55.5} \exp\left(\frac{-\Delta G_{ads}}{RT}\right)$$

$$\therefore \Delta G_{ads} = -RT \ln(55.5\, K_{ads})$$

The adsorption parameters are presented in Table 3. It is observed that the free energy of adsorption is negative indicating the spontaneous adsorption of inhibitor molecules on the metal surface. The ΔG_{ads} value lower than -20 kJ/mol indicates physisorption whereas around -40 kJ/mol or greater value indicates chemisorption. Physisorption involves only an electrostatic interaction between the charged

molecules of the inhibitor and the charged metal surface while chemisorption involves charge sharing or charge transfer between the inhibitor molecules and the mild steel surface leading to coordinate bond formation [25,26]. From Table 3, it can be seen that adsorption of the free ligand H_2L^1 obeys a mixed type mechanism that involves both physisorption and chemisorption [27,28]. Physisorption occurs *via* attraction between the positively charged mild steel surface and the π bonds in the benzene rings whereas chemisorption happens *via* coordinate bond formation between the *d*-orbital of iron atoms of the mild steel surface and the lone pairs of N and O atoms of the inhibitor molecules.

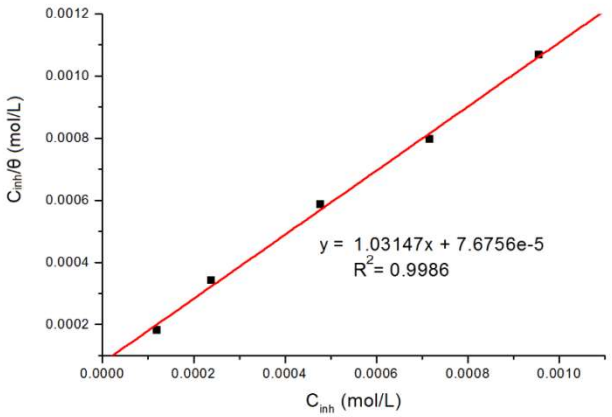

Fig.7. Adsorption isotherm of H_2L^1 obtained from polarization studies.

Table 3. Adsorption parameters of the compounds derived from isotherms.

Inhibitors	C (x 10^{-4} M)	C/θ (x 10^{-4} M)	K_{ads} (x 10^3 M^{-1})	$-\Delta G_{ads}$ (kJ/mol)
H_2L^1	1.1948 2.3896 4.7791 7.1687 9.5583	1.8092 3.4098 5.8661 7.9590 10.6737	13.0283	33.4256

3.4. Morphological studies

Comparison of the surface morphology of mild steel specimen in the corrosive medium with and without inhibitors yields valuable information regarding the inhibition capability of the synthesized inhibitors. In this study, surface was analysed by Scanning Electron Microscopy. The SEM images obtained for the mild steel specimen after 5 hours of exposure to corrosive medium with and without inhibitors are shown in Fig. 8. It is clear from the images that, the surface exposed to 1 M HCl without inhibitor (Fig. 8a) has been damaged to a large extent due to the metal dissolution in the acid medium. Fig. 8a shows large number of cracks and pits. However Fig. 8b shows less number of cracks and pits indicating less damage due to acid attack. The SEM images further illustrates that in the presence of inhibitors, mild steel surface was less attacked by HCl because of the protection enabled by the inhibitor molecules over the mild steel surface which acts as a barrier for acid attack. The molecule with better surface coverage capability forms better coating on the metal surface and thus provides maximum inhibition against corrosion.

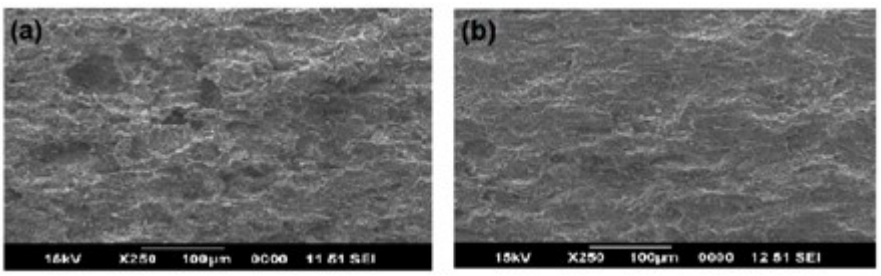

Fig. 8. SEM images of mild steel in the presence and absence of inhibitor. (a) 1 M HCl without inhibitor (b) in presence of H_2L^1

4. Conclusion

The Schiff base has exhibited promising inhibition against acid corrosion of mild steel in 1 M HCl medium. The polarization measuremets reveals that the compound is mixed type inhibitor with predominant effect on the anodic sites. They have shown good adsorbability over the mild steel surface *via* mixed effect of physisorption and chemisorption. The inhibition effect of the compound has ensued *via* formation of a protective layer over the surface which acts as a barrier between

the mild steel surface and acid medium. The present study illustrates the fact that application of dicompartmental type compounds increase the adsorption of the inhibitor molecules on the metal surface through coordinate bond formation between the hetero atoms of the compartment and the iron atoms of the mild steel surface and provide effective prevention against acid attack.

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STUDY OF AVIAN FAUNAL DIVERSITY IN COLLEGE CAMPUS (NSS COLLEGE OTTAPALAM) PALAKKAD, KERALA

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Abstract

This study investigated bird diversity in NSS College Campus, Ottapalam, India. A total of 36 bird species belonging to 25 families were recorded. Despite undergoing significant changes over the years, the campus remains a valuable habitat for birds. Understanding bird diversity is crucial for effective conservation efforts. This study provides baseline data on bird species present in different campus locations. Future research focusing on bird behavior and feeding ecology will enhance our understanding of these species and inform conservation strategies.

Key words: Bird diversity, college campus, ecology,conservation .

Introduction:

Birds, a highly diverse group of animals, play a crucial role in Earth's biodiversity. With over 10,000 species worldwide, they exhibit remarkable variation in form, function, and behavior. Adapting to diverse habitats, birds serve as scavengers, pollinators, seed dispersers, and predators, contributing significantly to ecosystem balance. India, renowned for its biodiversity, hosts approximately 13% of global bird species.

Birds are sensitive to environmental changes, making them valuable indicators of ecosystem health. Their presence and abundance can reveal pollution levels, habitat quality, and the overall well-being of ecosystems. As essential components of food chains and webs, birds contribute to maintaining ecological balance. Their role in pollination and seed dispersal further highlights their importance to biodiversity conservation

Based on the literature review done as a part of the study, it was noted that Meise(1934) listed 600 new species names of birds and its description.Newton (1995) made his contribution of some recent research on birds toecological understanding.Sushant Kumar (2001) did a survey of avifauna of Vazhachal, Athirapally reserve forest.Bijukumar in 2006 observed total of 140 species of bird in 49 families. Singha Roy (2012)did a study on the global diversity of bird size decreasing incessantly primarily due to anthropogenic disturbance and climate change.Sekercioglu et al (2012) stated that there was no surprise that the IUCN Red list of endangered birds has already recognized 1226 bird species globally and India with 88 threatened bird species ranked at 7th position.Ramachandra (2013) noted that a total of 151 species representing 15 orders and 45 families were recorded in newly formed habitats of Chandoli National park.Mhatre *et al* (2013) observed 131 birds which included residents,winter visitors and also summer visitors few rare in avifauna of Nigade in Raigad. Aggarwal *et al* (2015) studied the bird diversity at Indian Institute of forest management with a total 106 species of which 27 species were recorded as winter visitors.Pentewan 2018 identified a total of 56 birds near Sikara Dam.Gajanan Wagh *et al* (2020) recorded 221 species reported in the MlDC area of Amravati. Venkitachalam and Varsha in a study recorded a total of 35 bird species in Government college campus Chittur,Palakkad.Satish Harde *et al* (2020) recorded 97 species of birds belonging to 48 families.Gadekar *et al*.(2021) reported 114 species and 51 families detected from 7 habitats in 2 seasons in avifauna in the Konkan west of Western ghats biodiversity hotspot was reported by Dhananjai (2021)did a study on Avian diversity in Himalayas- bird species and diversity distribution in Indian Himalayan region.Bibha Kumari (2021) did a study on biomonitoring of bird species diversity on a college campus to assess the diversity of the ecosystem.

Major threats to the loss of biodiversity are the increased rate of population growth. Habitat change through land clearing and urbanization, Expansion of industrial-scale agriculture, human encroachment on habitats, the drying out of wetlands, deforestation, illegal or unattainable hunting and electrocution by power lines all due to increased rate of population growth. In case of migratory birds, Climate change adversely affects by reducing the availability of food at stopovers. Hunting and mobile signal towers are also adversely impacting migratory

birds.We should consider the consequences of our actions on birds. Thinking about the importance of birds for us culturally, aesthetically, economically and environmentally can help us feel that we have many genuine reasons to protect them. By protecting birds we are looking after the environment as a whole. (Kohli 2021)Thus the scope of the study was to do an assessment of the bird diversity in the campus, as not much documentation has been done, regarding the group in the present study area.

The major objectives of the study were:

1. To investigate the diversity of Avian fauna in NSS College campus,Ottapalam.
2. To assess the diversity of avian species based on their feeding guild.
3. To prepare the checklist of birds of NSS College campus,Ottapalam for future reference

Materials and Methods

The present study was conducted in NSS College Ottapalam (10.7659679 N,76.4060486E). Ottapalam is a town in Ottapalam taluk, Palakkad district, Kerala, India. Palakkad district, which is situated in the Kerala state. Palakkad is the largest district in Kerala. Palakkad is bordered on the northwest by the Malappuram District, on the southwest by the Thrissur District, on the northeast by The Nilgiris District, and on the east by Coimbatore district of Tamil Nadu.The district is nicknamed "the granary of Kerala".Palakkad is the gateway to Kerala due to the presence of the Palakkad Gap, in the Western Ghats.The climate is pleasant for most parts of the year, the exception is the summer months. There is sufficient rainfall and it receives more rainfall than the extreme southern districts of Kerala. The district is blessed with many small and medium rivers, which are tributaries of the Bharathapuzha River.The main type of vegetation in College campus is evergreen and shrub comprising of members predominantly belonging to the families: Apocynaceae, Anacardiaceae Poaceae, Fabaceae, Cyperaceae, Asteraceae, Euphorbiaceae, Verbenaceae, Solanaceae, Rubiaceae, Convolvulaceae, Caesalpiniaceae Amaranthaceae plants.

The analysis was done from September to January 2023.Observations were done thrice a week during the study period from 9AM to 9:30AM and 3.30 to 4.00pm. Identifying birds is a challenging process.Birds are active and energetic animals.Quick eye spotting is required in order to get possible details in a short span of time. The following techniques were used during bird watching. Birds were recognised by sighting. Continuous observation were made regarding their movement,songs,feeding ,habit and size.Simultaneously specific calls and songs were also identified. The sampling was done by direct search and observation method with random transect method of observation of birds from campus areas in the selected sites in college campus

Map showing the college campus with the line transects.

In this method target species or group of species were observed visually. Most species were photographed using mobile cameras(Apple 13 pro). Collected and photographed species were identified up to the genus level by bird identification applications in mobile like i-naturalist,e-bird, and Merlin. The birds were observed by using binoculars (20×50) and photographs were also taken for further confirmation by using a field guide Birds of Kerala (Neelakandan, 2019).The checklist of the birds was prepared based on the Aasheesh Pittie (2004).

Results and Discussion

A total of 36 species belonging to 25 families and 12 orders were recorded from the college campus .The highest number of bird species were recorded from FamilyColumbidae and Pycnonotidae which were represented by 3 species.The families Corvidae, Sturnidae, Nectariniidae, Accipitridae, Dicruridae, Megalaimidae, Cuculidae and Oriolidae were represented by 2 species. The family Leiothrichidae, Apodidae, Campephagidae, Muscicapidae, Picidae, Alcedinidae ,Phalacrocoracidae,Phasianidae,Meropidae,Passeridae,

Psittacidae,Tytonidae,Megalaimidae Monarchidae and Bucerotidae represented by Single species.The college campus was categorised into three sites for convenience of sampling ,site A near the women`s hostel; site B centre of the campus and Site C near the canteen. Bird diversity was observed in three sites, and site A and C showed more diversity. The roosting behaviour of birds were also noted.Behavior of birds to settle or rest at a place at specific times of day and night is called roosting.Here due to restriction in college time, the evening roosting behaviour have not be recorded in the sampling.Some birds prefer solitary roosting while others roost communally.The tree species in relation to bird roosting behaviour were observed.

Composition of bird type based on feeding guild

In the recent study, Most of the birds (28%)were Omnivore.Insectivore represented by 22%.Frugivores represented by 17%.Carnivores and granivores represented by 14%. Nectarivorous birds (5%) were the least abundant species based on classification by feeding guild.

Figure 1: Number of bird species in each Order observed from NSS College

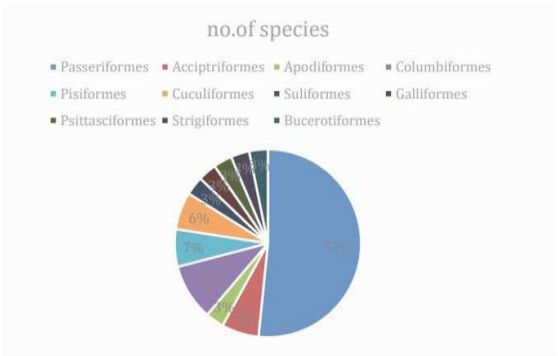

Figure 3: Percentage composition of bird type based on feeding guild

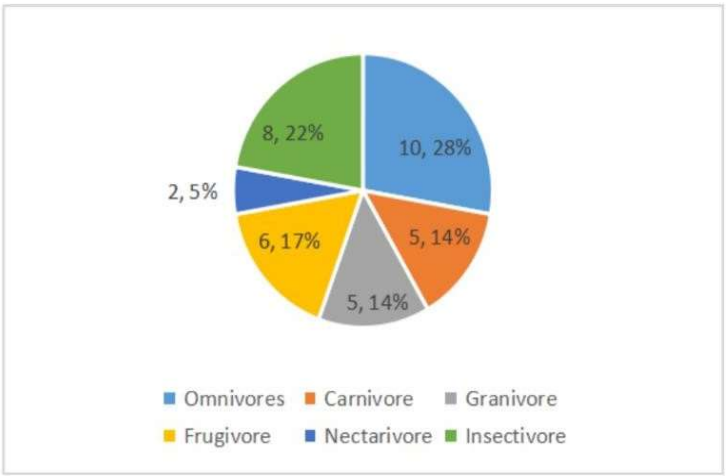

Figure 4: Abundance of birds observed in the college campus

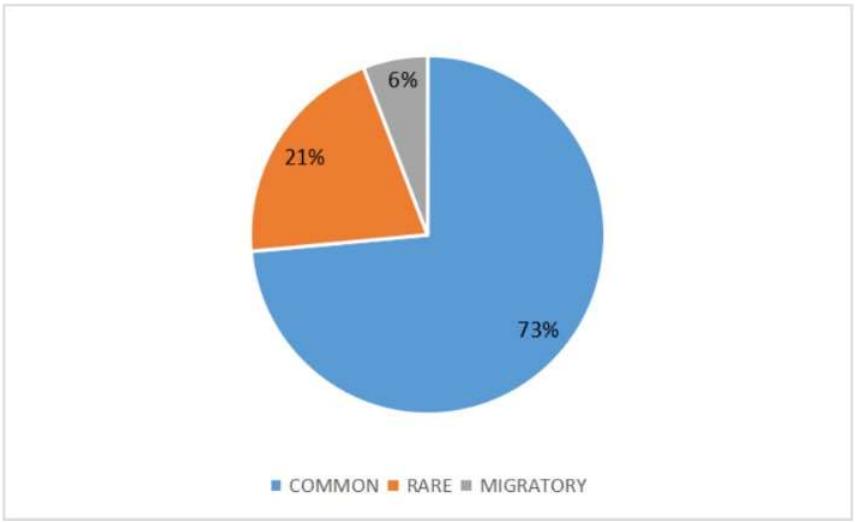

Table 1: Checklist of birds observed in NSS College Campus, Ottapalam

Family	**Order**	**Common name**	**Scientific Name**	**IUCN status**	**Feeding guild**	**Relative Abundance**
Corvidae	Passeriformes	Common crow	*Corvus splendens*	Least concern	Omnivores	C
	Passeriformes	Indian rufous treepie	*Dendrocitta vagabunda*	Least concern	Frugivorous	C
Leiothrichidae	Passeriformes	Jungle babbler	*Turdoides striata*	Least concern	Granivorous	C
Sturnidae	Passeriformes	Common myna	*Acridother es tristis*	Least concern	Granivorous	C
Nectariniidae	Passeriformes	Purple rumped sunbird	*Nectarinia zeylonica*	Least concern	Nectarivorous	C
	Passeriformes	Purple sunbird	*Nectarinia asiatica*	Least concern	Nectarivorous	C
Accipitridae	Accipitriformes	Black kite	*Milvus migrans*	Least concern	Carnivorous	R
	Accipitriformes	Brahminy kite	*Haliastur indus*	Least concern	Carnivorous	C
Dicruridae	Passeriformes	Black drongo	*Dicrurus macrocerc us*	Least concern	Insectivorous	C
	Passeriformes	Racket tailed drongo	*Dicrurus paradiseus*	Least concern	Insectivorous	C
Pycnonotidae	Passeriformes	Red whiskered bulbul	*Pycnonotu s jocosus*	Least concern	Frugivorous	C
	Passeriformes	Red vented bulbul	*Pycnonotu s cafer*	Least concern	Frugivorous	C
Dicaeidae	Passeriformes	Pale billed flowerpecker	*Dicaeum erythrorhy nchos*	Least concern	Frugivorous	R
Apodidae	Apodiformes	Asian palm swift	*Cypsiurus balasiensis*	Least concern	Frugivorous	C
Columbidae	Columbiformes	Spotted dove	*Streptopelia chinensis*	Least concern	Granivorous	C
	Columbiformes	Yellow footed green Pigeon	*Treron phoenicopt era*	Least concern	Frugivorous	R

	Columbiformes	Rock pigeon	*Columba livia*	Least concern	Granivorous	C
Megalaimidae	Piciformes	White cheeked barbet	*Megalaima viridis*	Least concern	Omnivorous	C
Oriolidae	Passeriformes	Black hooded oriole	*Oriolus xanthornus*	Least concern	Omnivorous	
	Passeriformes	Eurasian golden oriole	*Oriolus oriolus*	Least concern	Omnivorous	C
Muscicapidae	Passeriformes	Oriental magpie Robin	*Copsychus saularis*	Least concern	Insectivorous	R
Campephagidae	Passeriformes	small minivet	*Pericrocot us cinnamomeus*	Least concern	Insectivorous	C
Picidae	Piciformes	Black rumped flame back (golden backed woodpecker)	*Dinopium benghalense*	Least concern	Omnivorous	R
Alcedinidae	Coraciiformes	white breasted kingfisher	*Halcyon smyrnensis*	Least concern	Carnivorous	C
Phalacrocoracidae	Suliformes	little cormorant	*Phalacroc orax*	Least concern	Carnivorous	C
Meropidae	Coraciiformes	Small Bee-eater	*Merops orientalis.*	Least concern	Insectivorous	R
Passeridae	Passeriformes	House sparrow	*Passer domesticus*	Least concern	Granivorous	C
Psittaculidae	Psittaciformes	Rose ringed Parakeet	*Psittacula krameri*	Least concern	Frugivorous	C
Tytonidae	Strigiformes	Barn Owl	*Tyto alba*	Least concern	Carnivorous	R
Bucerotidae	Bucerotiformes	Indian Grey Hornbill	*Ocyceros birostris*	Least concern	Frugivorous	R
Monarchidae	Passeriformes	Asian paradise flycatcher	*Terpsipho ne paradisi*	Least concern	Insectivorous	R
Phasianidae	Galliformes	Indian peafowl	*Pavo cristatus*	Least concern	Omnivorous	C
	Galliformes	Grey Jungle	*Gallus sonneratii*	Least	Insectivorous	C

		fowl		concern		
Cuculidae	Cuculiformes	Common koel	*Eudynamy s scolopaceu s*	Least concern	Omnivorous	C
	Cuculiformes	Hawk cuckoo	*Hierococc yx varius*	Least concern	Insectivorous	C
	Cuculiformes	Greater Coucal/Crow Pheasant	*Centropus sinensis*	Least concern	Omnivorous	C

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THE VERSATILITY OF CLICK CHEMISTRY: FROM BENCH TO BIOMEDICAL APPLICATIONS

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Introduction

"The Nature generates substances by joining small molecularunits". Inspired by this natural truth, in 2001 K. Barry Sharplessintroduced Click chemistry as a chemical philosophy and described itas chemistry tailored to generate substances quickly and reliably byjoining small units together.[1] Click chemistry is not a specific reaction,it is a concept that mimics nature. A click reaction must be modular,wide in scope, give very high yields, generate only inoffensivebyproducts that nonchromatographic methods can remove, andbe stereo-specific (but not necessarily enantio-selective). The requiredprocess characteristics include simple reaction conditions (ideally, theprocess should be insensitive to oxygen and water), readily availablestarting materials and reagents, the use of no solvent or a solvent that isbenign (such as water) or easily removed, and simple product isolation.[2]Purification, if required, must be by non-chromatographic methods,such as crystallization or distillation, and the product must be stableunder physiological conditions.

Click chemistry can be classified as cycloaddition reaction,nucleophilic substitution reaction, carbonyl chemistry reaction,addition reaction, thiol-ene click reaction etc. Of these one of the bestclick reactiontodate is copper catalysed azide alkyne cycloadditionand which is the modified Huisgen 1, 3-dipolar cycloaddition of analkyne and an azide.[3]The active Cu (I) catalyst can be generated fromCu (I) or Cu (II) salt using sodium ascorbate as reducing agent. Theaddition of slight excess of sodium ascorbate prevents the formation ofoxidative homo-coupling products. This Cu (I) catalysed cycloadditionresults a regioselective 1, 4-disubstituted 1,2,3-triazole derivative asproduct which is a prominent privileged scaffold in drug discovery.[4]

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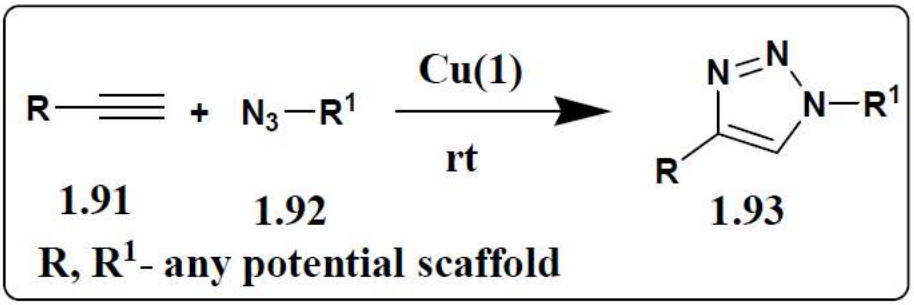


Scheme1. General synthetic scheme for Cu (1) catalyzed Azide-Alkyne [3+2]cycloaddition reaction.

This reaction applies to a wide variety of substrates with various functional groups. The catalytic process is insensitive towards the presence of air and pH changes in a solvent mixture of water and *t*-BuOH. Thisstrictly regioselective stepwise process selectively produces 1,4-disubstituted 1,2,3-triazole only and accelerates the reaction by a factor of up to compared to Huisgen's thermal procedure.[5]In addition to this, since large number of monosubstituted alkynes and organic azides are commercially available andmany others can easily be synthesized with a wide range of functional groups, it is easy to make large library of 1,2,3-triazoles derivatives for screening purpose.

Applications of Click Chemistry (CUAAC)

The popularity of the CuAAC is largely a result of the unique properties of both azides and the resulting triazoles.[6]The combination of the robustness of the triazole bond, the resemblance to an amide bond, and the potential biological properties it could endow make the triazole linkage not merely a benign, easily synthesized linker, but an integral part of the success of click chemistry. In addition to this, the simplicity, reliability and the bioorthogonality of the starting materials has made the CuAAC reaction an asset to a hugely varied range of scientific applications. The wide scope of CuAAC is firmly demonstratedby the use in different areas of life and material sciences such asdrug discovery,[7]bioconjugation,[8] polymer and materialsscience,[9]including supramolecularchemistry.[10] DNA labeling[11]and oligonucleotide synthesis,[12] assembly of glycoclusters[13] and glycodendrimers,[14] preparation ofstationary phases for HPLC column,[15] development of microcontact printing,[16] conjugation of molecular cargos to the

headgroup of phospholipids,[17] and constructionof bolaamphiphilicstructures[18]are a further examples of the use of CuAAC.It would be impossible to give a complete overview of the numerous applications of the CuAAC.For our purposes, the applications of 'click' chemistry have been summarized with illustrative examples in various categories; applications in materials science, for radiolabelling, for bioconjugation, and in drug discovery.

a) Material Science

The value of click chemistry for materials synthesis possibly becomes most apparent in thearea of material chemistry. Several recent reviews have described the use of CuAAC for the synthesis of macromolecular structures like dendritic, branched, linear andcyclic co-polymers.[19] Triazole-based dendrons can be divergently synthesized via CuAAC reaction. These dendrons were then anchored to avariety of polyacetylene cores to generate dendrimers. Since then, the CuAAC reaction hasbeen widely employed to synthesize or modify various dendrimers.[20]A click chemistry-based dendrimer is shown in Figure 1.

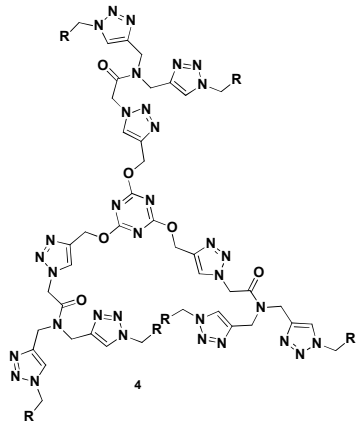


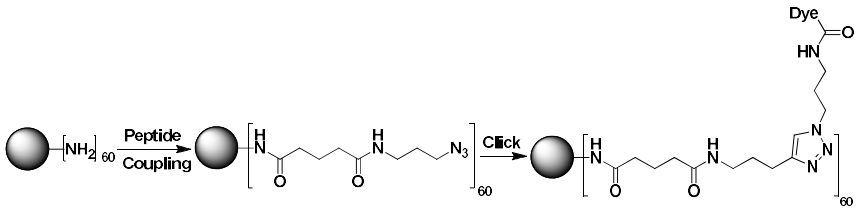
Figure 1.'Click' chemistry based dendrimers

b) Bioconjugation

Bioconjugation is the process by which synthetic molecules are attached to biological targets or by which biomolecules are linked together. It involves attachment of synthetic labels tobiomolecular building blocks, such as fusing two or more proteins or linking acarbohydrate with a peptide, and covers a wide range of science between molecular biologyand chemistry. The possibility of applying click chemistry inbioconjugation was first demonstrated by Meldal *et al* for the

preparation of peptidotriazoles*via* solid state synthesis. Their goal was to develop new and more efficient synthetic methodsto prepare various [1,2,3]-triazole pharmacophores for potential biological targets. This initialreport made possible the introduction of various novel functional and reporter groups intobiomolecules such as peptides and proteins,[21] for DNA labeling and modification,[22] andfor cell surface labeling.[23]

Finn and co-workers successfully labeled Cowpea mosaic virus particles (CPMV) with fluorescein with >95% yield.[24] Similarly, Tirrell and Link were able to modify *Eschericia coli* with an azide-bearing outer membrane protein C(OmpC). Schultz *et al* developed a method to genetically-encode proteins of *Saccharomyces cerevisiae* with azide- or acetylene-based synthetic amino acids.[25]The genetic modification was done by reacting an alkyne or an azide bearing protein with the counterpart unnatural amino acid. Click chemistry continues to attract attention for labeling of proteins and live organisms.



Scheme 2. Labelling of virus capsids by CuAAC

c) Radiochemistry

The CuAAC is an ideal ligation reaction forradiolabeling sensitive biomolecules.Alkyne or azide derivatives of radioisotopecontainingcompounds could be used forlabeling biomolecules such as folic acid,peptides, proteins, and glycopeptides.For example, an ^{11}C isotope label was introduced via converting [^{11}C]-CH_3I into[^{11}C]-CH_3N_3 by nucleophilic substitutionand subsequently reacting the azide withan alkyne-modified peptide. ^{18}F labelingfor PET imaging was achieved by clickingazidomethyl-4-[^{18}F]-fluorobenzene to amodified peptide.[26]CuAAC ligations have a significant impact on the synthesis and development of radiopharmaceuticals and it has vast application in the preparation of imaging agents for SPECT and PET, including small molecules, peptides, and proteins labeled with radionuclides such as ^{18}F, ^{64}Cu, and ^{111}In.[27] Various

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researchers have shown that CuAAC is a great approach for the construction of radiotracers also.

d) Drug Discovery

In history and even now a days, lead discovery and optimization had aided by combinatorial methods and high throughput screening to generate library of test compounds for screening. However, due to unreliability and new discoveries revealed click chemistry as a modular for the synthesis of drug-like molecules that can accelerate the drug discovery process by utilizing a few practical and reliable reactions. It is a new type of chemistry that able to synthesize complex molecule efficiently.It makes use of few chemical reactions for the synthesis and designing of new building blocks.Drug discovery based on Natures secondary metabolites is very slow and complex synthesis and thereby, click chemistry provides faster lead discovery and optimization.

Thiscommendably straightforward chemistry, which can be conducted in aqueous media, has been widely applied as a powerful tool for theselective modifications of enzymes[28] viruses[29]and cells.[30]Among the best-known examples of triazole-containing structures is, a β-lactamase inhibitor which is marketed in combination with the broad-spectrum antibiotic piperacillin. Indeed, when first described, tazobactam and related triazole-containing compounds (**5**, Figure 3) turned out to be potent β-lactamase inhibitors with higher potency than clavulanic acid and sulbactam, and the triazole ring appears to play a pivotal role for its potency.[31] In the antibiotics field, triazoles have been also used to improve pharmacokinetic properties of the desired drug. For example, cephalosporins endowed with good oral availability were obtained linking the triazoles moiety to the cephalosporin core (**6**, Figure 3).[32]Indeed; it is not just antibiotics which benefit from the triazole ring.

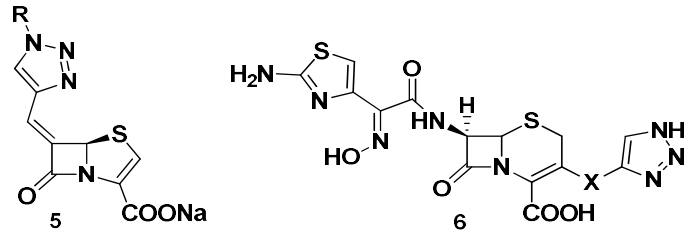

Figure 3. Example of β-lactamase inhibitors incorporating the 1,2,3-triazole moiety

Click chemistry, due to its highly modular and efficientreaction nature, has been identified as one of the most practicalmethods toward fragment-based enzyme inhibitor development. Using fragment pro-inhibitor library screening and click chemistry reaction, researchers developed a large class library of efficient enzyme inhibitors of various enzymes such as Protein Tyrosine Phosphatase Inhibitors, Protein Kinase Inhibitors, Transferase Inhibitors, Glycogen Phosphorylase Inhibitors, Serine Hydrolase Inhibitors, Metalloproteinase Inhibitors, Aspartic Protease Inhibitors, Oxidoreductase Inhibitorsand Glycosidase Inhibitors etc.[33]

e) Click Chemistry in Cancer treatment

Chemotherapy is considered as the most effective method among many other methods prevalent to treat cancer. Several nucleoside drugs have been developed ascancer treatment agents: cladribine, clofarabine, capecitabine, cytarabine, fludarabine, gemcitabine, decitabine, and floxuridine.[34]The development of new therapeuticapproach to breast cancer remains one of the mostchallenging areas in cancer research. Inhibitors of cyclin-dependent kinases (CDKs) are an emerging class of drugs for the treatment of breast cancers. Experimental evidence suggests that CDK inhibitors inhibit the cyclin D–dependent kinase activity and thus prevent tumor growth and/or at least partially revert the transformed phenotype. Several compounds arecurrently in clinical trials including flavopiridol (**7**), R-roscovitine(CYC202) (**8**), BMS-387032 (**9**), andUCN-01 (7-hydroxystaurosporine) (**10**).[35] CDK inhibitors are currently under evaluation in clinical trials as single agents and as sensitizers in combination with radiation therapy and chemotherapies.[36]

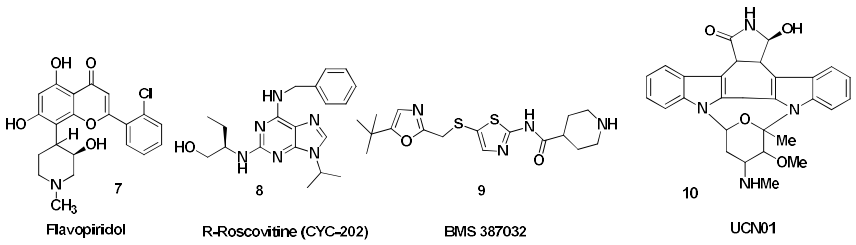

Figure 6. CDK inhibitors under clinical trials

In 2022, Carolyn R. Bertozzi, alongside Morten Meldal and K. Barry Sharpless, was awarded the Nobel Prize in Chemistry for her pioneering work in Click Chemistry, particularly for introducing bioorthogonal reactions. Bertozzi's groundbreaking synthesis enabled chemical reactions to occur in living systems without disrupting native biological processes. This innovation has transformed biomedical research, facilitating advancements in drug delivery, live-cell imaging, and diagnostics. By enabling selective and efficient molecular assembly in vivo, her work has broadened the scope of Click Chemistry, solidifying its relevance in therapeutic developments, including cancer treatment and targeted imaging, making it an indispensable tool in modern science.

Conclusions

In conclusion, this review summarizes the growing application of ''click'' chemistry in diverse areas such as bioconjugation, drug discovery, materials science, and radiochemistry. Click chemistry has found increasing applications in all aspects of drug discovery in medicinal chemistry, such as for generating lead compounds through combinatorial methods. Bioconjugation via click chemistry is rigorously employed in proteomics and nucleic research. In radiochemistry, selective radiolabeling of biomolecules in cells and living organisms for imaging and therapy has been realized by this technology. Click chemistry has proven itself to be superior in satisfying many criteria, thus, one can expect it will consequently become a more routine strategy shortly for a wide range of applications since it links various types of chemistry with biology and can tailor various useful syntheses in future.

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BUTTERFLY (LEPIDOPTERA) DIVERSITY IN NSS COLLEGE OTTAPALAM, CAMPUS, KERALA, INDIA

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Abstract

Butterflies, one of the most extensively researched insect groups, are essential indicators of environmental health due to their sensitivity to habitat changes and importance in pollination. This study examines the richness and quantity of butterfly species on the NSS College Ottapalam campus, which spans 41 acres of diverse vegetation. From September 2022 to March 2023, Pollard Walk sampling was used along four transects. A total of 32 butterfly species were identified, divided into five families: Nymphalidae, Pieridae, Papilionidae, Lycaenidae, and Hesperiidae. The Nymphalidae family has the most species diversity, whereas Pieridae had the most individuals. Butterfly abundance peaked following the monsoon season, particularly around January. The findings highlight the necessity of preserving native flora on institutional campuses to support butterfly populations, which contributes to both conservation efforts and ecological research. Additional research is recommended to broaden the species inventory and investigate the mutualistic links between butterflies and plants on campus.

Introduction

Butterflies are often recognized as one of the most well-studied groups of insects [Robbins and Opler 1997]. Butterfly species number over 28,000 worldwide, with tropical regions accounting for over 80% of the total. The Indian subcontinent, with its diverse topography, climate, and vegetation, is home to around 1,504 species of butterflies [Tiple, 2011]. The insect class encompasses numerous orders, with Lepidoptera being one of the largest [Tiple et al., 2007]. The Lepidoptera order contains butterflies and moths. There are currently 17,4250 Lepidopteron species recorded, of which 17,950 are butterflies and the rest are moths [Tiple et al. 2007]. Butterflies are the most taxonomically researched insect group, and they are excellent markers of environmental change. Because of their

diversity, vast distribution, specificity to vegetation type, rapid reaction to perturbation, taxonomic tractability, statistically significant abundance, and convenience of sampling, they are thought to be ideal creatures for monitoring environmental change. Butterflies are known for their beauty and grace. They are highly studied because of their diurnal behaviour, beautiful shape, vibrant colours, and graceful flying. They pollinate and serve as indicators of environmental change. They have excellent aesthetic and commercial values. Butterflies are sensitive to habitat degradation and climate changes, and they serve as good indicators of environmental change [Tiple, 2012]. They are an important food chain component. Butterflies provide ecosystem services by pollinating and serving as vital food chain components. As potential pollinators of their nectar plants as well as indicators of the health and quality of their host plants and the ecosystem as a whole, butterfly fauna exploration is critical in finding and maintaining threatened habitats. Recently, we have been fast losing flora in the name of development. There has also been an alarming increase in industrial and automobile pollution in major Indian cities. With the loss of vegetation and an increase in pollution, butterflies, birds, and all of our animals are rapidly disappearing. As a result, the ecology becomes completely imbalanced, and many species are extinct. Despite their rapid growth, Indian cities still have diverse serene habitats such as traffic island gardens in the middle of busy roads, parks or urban forest areas with mixed deciduous and non-deciduous trees, and scrubland that serve as ideal habitats for various types of insects, particularly butterflies. We chose this as our project topic since butterflies have a significant impact on the ecosystem. They are good markers of a healthy ecology (Evans, 1932). The primary goal of this study is to analyse the richness of different butterfly species on the N.S.S college Ottapalam campus. And to raise public knowledge about the need of preserving native plant species and the value of butterflies as indicators of ecosystem health.

Materials and Methods

Study Area

The NSS College Ottapalam campus (10° 45' 56" N and 76° 24' 22" E) is located on the grounds of Ottapalam Municipality and features a well-wooded campus surrounded by a mosaic of concrete buildings (IMAGE 1). The college campus covers 41 acres of lush green flora, including huge trees, bushy shrubs, and

long grasses that provide habitat for butterflies. The abundance of nectar, food plants suited for egg laying, open sunlit space, and less use of pesticides have all contributed to the area's butterfly species variety. The research area has a hot summer from March to early June, a humid south-west monsoon season from mid-June to August, a north-east monsoon (September to November), and a post-monsoon season from December to February.

Image 1. Satellite overview map of study locality

Study Design

Butterflies were sampled following a Pollard Walk [Kunte, 2000] across fixed transects (4X100m) in several habitats over two seasons, September 2022-February 2023. We placed four transects at various sites to cover the maximum amount of foliage; each transect was 100 m long. The findings reported here are based on random surveys conducted from September 2022 to March 2023. The entire college campus was surveyed from 9 a.m. to 4 p.m. using a Bushnell binocular (8x40). Butterflies were photographed from various perspectives as much as possible in order to collect enough pictures for accurate species identification. The photographs were captured with mobile cameras. Butterflies were first recognized in the wild using field guides, then photographed. The butterfly species of N.S.S College Ottapalam was observed for six months, from September to February. Since our goal was to record the seasonal diversity and number of butterflies, we divided our observations into two categories: the North East

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monsoon season (September-November) and the post-monsoon season (December-February). We examined the butterflies for half an hour in the morning (9-9.30am), afternoon (1-1.30pm), and evening (3.30-4.30pm).

Result and Discussion

A total of 32 species of butterflies (Appendix table 1) belonging to five families were identified from the college campus. The highest number of butterflies was recorded from the family Nymphalidae (9 species), followed by Pieridae (8 species), Papilionidae (6 species), Lycaenidae (6 species) and Hesperiidae (1 species).

Table 1. The sighted butterflies are listed with their occurrence status

Month	Total individuals	Nymphalidae	Pieridae	Papilionidae	Lycaenidae	Hesperiidae
September	16	6	6	2	1	1
October	17	2	11	1	0	1
November	13	3	10	0	0	0
December	9	2	7	0	0	0
January	48	13	19	9	5	1
February	4	1	11	1	0	0

Nymphalidae was the most dominant family comprising 9 species, and it constituted 28.125% of the total butterfly species in the campus. Nymphalidae was followed by Pieridae with 8 species, which constituted 25% of total butterfly species. Papilionidae and Lycaenidae were represented by 6 species which constituted 18.75% each of the total butterfly species. The family with least number of species was found to be Hesperiidae with 3 species constituted 9.375% of butterfly species. *Eurema blande* was the most dominant species sighted from Pieridae, followed by *Euploea core* was the most visited species from the family Nymphalidae. The total number of butterflies was recorded highest in the month of January (48 individuals) and lowest in the month of February (4 individuals).

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Table 2. Monthly abundance is shown

Family	No. of genera	Relative abundance	No. of species	Relative abundance
Papilionidae	4	14.81	6	18.75
Pieridae	6	22.22	8	25
Nymphalidae	8	29.62	9	28.125
Lycaenidae	6	22.22	6	18.75
Hesperiidae	3	11.11	3	9.375

Butterfly preferences for specific behaviors are linked to the availability of larval host plants and adult nectar sources. The enormous diversity of butterflies, particularly the Nymphalids and Pierids, suggests a diverse array of floral species. The flora on our campus is varied, with herbs and shrubs dominating the vegetation in the tropical environment. Trees are quite few in number. The study area is dominated by plant species belonging to families Annonaceae, Apocynaceae, Fabaceae, Malvaceae, Acanthaceae, Rubiaceae, etc., namely Ficus sp, Calotropis sp, Tridax sp, Polyalthia longifolia, Cassia fistula, Tabernaemontana sp, Alstonia scholaris, Ixora sp, Lantana camara, Cleome viscosa, Aegle sp, Citrus sp, Terminalia arjuna Butterfly variety varies by season. They are abundant for a few months and infrequent or absent the rest of the year [Kunte, 2000]. Wynter-Blyth (1957) recognized March-April and October as the peak seasons for butterfly abundance in India. The coming summer, high relative humidity, and increased rainfall all had a good effect on the quantity of various species. During the current study, butterfly populations peaked during the post-monsoon season (late August to October), which is consistent with the findings of [Tiple et al, 2007, Tiple, 2012, Tiple & Khurad, 2009]. Species abundance was lower during the monsoon. Aside from being one of the most visible biodiversity markers [Kunte, 2000], butterflies also serve as our native gardeners because they rely on indigenous plants to complete their life cycle. As a result, increased abundance of butterflies typically suggests a better habitat.

Out of the total 116 individuals recorded, 46 individuals were visited in monsoon. The October month holds the highest number of individuals visited, the

November the lowest (13 individuals). Family Pieridae is the most dominant with higher number of individuals (27 individuals). The post monsoon season (December – February) recorded a total of 70 individuals, which include 16 Nymphalidae individuals, 37 Pieridae individuals, 10 Papilionidae individuals, 5 Lycaenidae individuals and 1 Hesperiidae individual. January has the highest number of individuals visited (48) and December has the least number of individuals (13). Butterflies are important pollinators of both wild and cultivated plants [Tipple, 2006]. With the pressing needs of India's rising human population, natural forests are being cleared, giving way to urbanization, pollution, and overgrazing. Loss of excellent habitat is the greatest threat to all animals, including butterflies. In addition to these, human leisure activities, trampling, road runoff, trash deposition, and weeds are all common risks to butterfly populations. Although we cannot totally eliminate the negative consequences of urbanization and development, we can strive to mitigate them by planting endemic trees and plants that benefit the local species. This ensures that at least some common species do not become extinct.

Conclusion

The current study's findings highlight the relevance of institutional campuses as preferred habitat for butterflies. If the landscaping and garden maintenance are carefully managed, the diversity of butterflies on our college campus may expand, offering a fertile site for both butterfly conservation and research. This work will also contribute to our future efforts to understand the intricate nature of mutualistic interactions between butterflies and blooming plants, which is critical for the continuation of ecosystem services. This is the first attempt to explore the butterfly wealth of the NSS College campus. The current list of butterfly species is not complete and exhaustive, and further research will be conducted to update this checklist.

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Appendix Table 1. List of butterflies recorded from NSS College campus

Sl. No	Common Name	Scientific Name	Activity time	Month	Habitat	IUCN status	No. of individuals
Pieridae (8)							
1	Lemon Emigrant	*Catopsilia pomona*	M, A	Sep, Oct, Nov, Dec, Jan, Feb	O, G	LC	45
2	Three Spotted Grass yellow	*Eurema blande*	E	Sep	O, G	LC	1
3	Indian Cabbage White	*Pieris canidia*	M, E	Sep, Jan	O, G	LC	3
4	Painted Sawtooth	*Prioneria sita*	A, E	Sep	O	LC	1
5	Psyche	*Leptosia nina*	E	Oct	O, G	LC	3
6	Mottled Emigrant	*Catopsilia pyranthe*	M	Dec	O, G	LC	1
7	Spotless Grass Yellow	*Eurema laeta*	M	Dec	O, G	LC	3
8	Common Grass Yellow	*Eurema hecabe*	M	Jan	O, G	LC	1
Nymphalidae (9)							
9	Common Evening Brown	*Melantis leda*	M, E	Sep	O, G	LC	3
10	Blue Tiger	*Tirumala limniace*	M	Sep	O, G	LC	1
11	Common Crow	*Euploea core*	M, A, E	Sep, Nov, Dec, Jan, Feb	G	LC	7
12	Double Branded Crow	*Euploea sylvester sylvester*	M, E	Oct, Nov	G	LC	3
13	Common Castor	*Ariadne merione*	M	Dec	G	LC	1
14	Sullied Sailor	*Neptis soma*	M	Jan	G	LC	3

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15	Chocolate Pansy	*Junonia iphita*	M	Jan, Feb	G, PL	LC	4
16	Striated Five Ring	*Ypthima striata hampson*	M	Jan	O, G	LC	1
17	Striped Tiger	*Danaus genutia*	M	Jan	O, G	LC	2
Papilionidae (6)							
18	Blue Mormon	*Papilio polymnestor*	M, A	Sep, Oct, Jan	PL, G	LC	3
19	Common Mormon	*Papilio polytes linnaeus*	M, A	Jan	O, G	LC	3
20	Southern Birdwing	*Troides minos*	M	Sep	O	NE	1
21	Crimson Rose	*Pachliopta hector*	A, E	Jan	O, G	LC	3
22	Common Rose	*Pachliopta aristolochiae*	M	Jan	O, G	LC	3
23	Tailed Jay	*Graphium agamemnon*	A	Nov, Feb	O	LC	1
Hesperiidae (3)							
24	Water Snow Flat	*Tagiades litigiosa*	M	Sep	O	LC	1
25	Dusky Spotted Flat	*Celaenorrhinus fusca*	M	Oct	O	NE	1
26	Grass Demon	*Udaspes folus*	M	Jan	O, G	LC	1
Lycaenidae (6)							
27	Small Cupid	*Chilades parrhasius*	M	Sep	O, G	LC	1
28	Dark Grass Blue	*Zizeeria karsandra*	M	Jan	O, G	LC	2
29	Plains Cupid	*Luthrodes pandava*	M	Jan	O, G	LC	1
30	Ciliate Blue	*Anthena ciliata*	M	Jan	O	LC	1
31	Metallic Cerulea	*Jamides alecto*	M	Jan	O, G	LC	1
32	Red Pierrot	*Telicada nyseus*	M, A	Jan	O, G	LC	2

M: Morning **A**: Afternoon **E**: Evening **O**: Open area **G**: Garden **LC**: Least concerned **NE**: Not evaluated

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BIOSYNTHESIS OF SILVER NANOPARTICLES USING NEEM LEAF EXTRACT AND THEIR APPLICATION IN PHOTODEGRADATION OF METHYLENE BLUE

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Abstract

The synthesis of green nanoparticles offers significant advantages in environmental and medical applications by minimizing the use of hazardous chemicals [Ahmad *et al*, 2003]. Particularly, green synthesis utilizing biological materials such as plant extracts is considered safe and sustainable. In this study, silver nanoparticles (AgNPs) were synthesized through an eco-friendly and cost-effective method using neem leaf extract as both a reducing and capping agent. The synthesis involved the reaction of silver nitrate with neem extract, carried out at room temperature for 2 hours. The resulting AgNPs were characterized using UV-Visible spectroscopy and Powder X-ray Diffraction (XRD). Additionally, the photocatalytic performance of the AgNPs was assessed by evaluating the degradation of methylene blue (MB) dye under sunlight irradiation. The degradation of MB increased linearly with contact time, reaching its peak after 6 hours of exposure to sunlight. This enhanced dye removal is attributed to the increased availability of active sites on the nanoparticle surfaces, which significantly contributes to the higher degradation efficiency.

Key words: Photo degradation, biosynthesis, silver nanoparticles.

Introduction

Ensuring access to clean drinking water remains one of the most pressing global challenges, as water is essential for life and human well-being. The increasing demand coupled with declining water tables across various regions has intensified the need for innovative technological solutions to meet water needs. Currently, approximately one billion people lack access to safe drinking water, and waterborne diseases account for about 80% of infections in developing countries. In India alone, 10% of the population faces challenges in accessing clean water,

leading to health issues from waterborne diseases and cancer caused by industrial pollutants.

The textile and dye industries, which are significant water consumers, are major contributors to water pollution. These industries release various dyes that cause eutrophication, lower oxygen levels, and spread carcinogenic substances, contributing to severe water contamination. Traditional methods such as flocculation, electro-coagulation, activated carbon adsorption, and UV–Visible degradation have been employed to address these dye pollutants, but they often face limitations that hinder their broader application.

Recent advancements in nanotechnology have introduced silver nanoparticles as a promising solution for wastewater treatment. Due to their unique properties—such as high surface area, small size, and distinct morphology—nanoparticles exhibit remarkable catalytic abilities [Elumalai *et. al.,* 2010]. Biogenic synthesis methods for nanoparticles, particularly those utilizing plant extracts, are gaining attraction for their eco-friendly and cost-effective benefits [Gao *et. al.,* 2011]. Silver nanoparticles, known for their antibacterial properties, chemical stability, and catalytic efficiency, are particularly advantageous. They serve as effective photocatalysts under ambient conditions and visible light, offering a viable approach to degrade organic pollutants and dyes in wastewater.

Fig. 1.1. Neem Leaves

The green synthesis of nanoparticles using biological materials offers a sustainable and cost-effective alternative to conventional methods. Plant-based methods, such as using neem leaf extract, are increasingly recognized for their eco-friendly nature. Neem leaves, rich in natural reducing and stabilizing agents, provide an effective means for synthesizing silver nanoparticles without the need

for hazardous chemicals. This biogenic approach is advantageous as it not only reduces the environmental impact but also leverages the natural properties of plant extracts to stabilize nanoparticles during synthesis.

Various methods for synthesizing silver nanoparticles, including physical and chemical techniques, have been explored. While chemical reduction methods, such as using organic and inorganic reducing agents, are common, they often involve toxic chemicals and face challenges like nanoparticle stability and size control [Chakraborty *et. al.*, 2009]. In contrast, green synthesis methods using plant extracts, such as those from Camellia sinensis and neem leaves, offer a more environmentally friendly approach. These methods capitalize on the natural reducing agents present in the plant extracts, resulting in stable nanoparticles with potential applications in water purification and other fields.

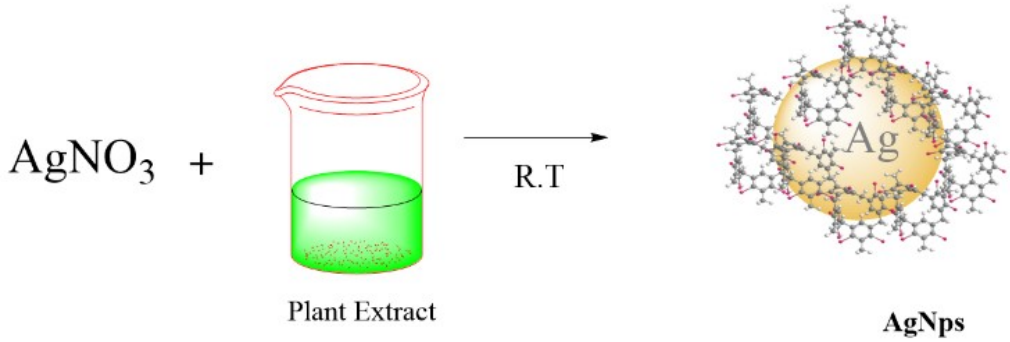

Fig.1.2. Green Synthesis of Silver nano particles

Since the early 1990s, silver nanoparticles (AgNPs) have garnered significant attention for their potential in environmental remediation, particularly for treating water contaminated with organic and inorganic pollutants due to their high surface reactivity. Traditional chemical and physical synthesis methods for AgNPs, while effective, often involve toxic, corrosive substances and expensive equipment, leading to concerns about safety and cost. In contrast, green synthesis methods using natural extracts have emerged as viable, eco-friendly alternatives [Huang H & Yang X, 2024]. Plant extracts, such as those from neem leaves, Datura metel, and Capsicum annuum, have been successfully employed to synthesize silver nanoparticles. These biogenic methods leverage natural reducing agents and stabilizers present in plant extracts, resulting in stable nanoparticles with promising

applications in water treatment and antimicrobial activities. For instance, neem leaf extracts and other plant-derived materials have demonstrated the ability to produce nanoparticles of various sizes and shapes, which show strong antibacterial properties against pathogens like E. coli and Staphylococcus aureus.

The use of plant-based extracts in synthesizing silver nanoparticles offers several advantages, including cost-effectiveness and environmental sustainability [Raveendran *et al,* 2003]. Various studies have highlighted the effectiveness of different plant extracts in producing nanoparticles with specific properties and sizes. For example, leaf extracts from Euphorbia hirta and Acalypha indica have been shown to produce nanoparticles with significant antimicrobial activity, while extracts from Cacumen platyclade and Cinnamon zeylanicum have yielded nanoparticles with narrow size distributions and high antibacterial efficacy. The biosynthesis of silver nanoparticles using these natural extracts not only provides a green alternative to traditional methods but also enhances the potential applications of nanoparticles in fields such as textile and medical industries [Hebeish*et. al*., 2011]. Advances in green synthesis techniques, including the use of bio-organic molecules and plant extracts, continue to offer innovative solutions for nanoparticle production, aligning with the growing demand for environmentally friendly and economically viable processes. In the present study, we aim to synthesize silver nanoparticles by using the leaf extract of neem. Phytochemicals composition of neem was found to be the major reducing and stabilization agent during the synthesis of Ag nanoparticles.

Singh *et al.* (2021) emphasized the use of neem leaf extract, which not only facilitated the synthesis of AgNPs but also imparted antimicrobial properties to the nanoparticles. Similarly, Sharma *et al.* (2022) explored the use of *Coriandrum sativum* (coriander) and *Murrayakoenigii* (curry leaf) extracts, revealing their potential in producing AgNPs with controlled size and shape, which are important for various applications including catalysis and medicine. These studies underscore the diverse phytochemicals present in plant extracts that play a key role in the reduction and stabilization of silver nanoparticles.

Moreover, recent literature highlights significant advancements in optimizing synthesis conditions and enhancing the properties of plant-derived AgNPs. For instance, Kumar *et al.* (2023) investigated the impact of varying

extract concentrations and reaction conditions on the size and morphology of AgNPs derived from *Tinospora cordifolia* (giloy). Their findings suggest that fine-tuning these parameters can lead to nanoparticles with enhanced functional properties and stability. Patel *et al.* (2024) demonstrated the scalability of plant-based AgNP synthesis, making it feasible for large-scale applications. This body of work collectively illustrates the growing interest in plant-mediated synthesis of AgNPs as a sustainable and cost-effective approach, with promising implications for environmental and biomedical applications.

Recent research has demonstrated the efficacy of plant-extract synthesized AgNPs in the photocatalytic degradation of Methylene Blue (MB) dye, offering a sustainable solution for dye pollution [Trieu *et al*., 2023] These biosynthesized AgNPs exhibit high photocatalytic efficiency due to their unique physicochemical properties, including a high surface-to-volume ratio and optimal light absorption capabilities. The degradation process is driven by the generation of electron-hole pairs under light, which accelerates the oxidation and reduction reactions necessary for breaking down Methylene Blue. For instance, recent studies have shown that AgNPs not only facilitate rapid dye decolorization but also maintain their catalytic activity over extended periods, underscoring their potential for effective environmental remediation. This approach leverages the green synthesis of nanoparticles, making it a promising and eco-friendly strategy for addressing water pollution caused by textile dyes.

3. Materials and methods

Fresh neem leaves (Azadirachta indica) were procured from a local herbal garden. Silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich. The chemical was of analytical grade, with a purity of 99.9%, and was supplied in a crystalline form. All solutions and dilutions were prepared fresh from the stock solution to ensure the accuracy and reliability of the experiments. Methylene Blue (MB) was obtained from Merck. Solutions were prepared in distilled water immediately prior to use, ensuring the dye's efficacy and minimizing any potential changes in concentration.

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3.1 Preparation of leaf extract

Prior to use, the neem leaves were thoroughly washed with distilled water to remove any dirt or contaminants. They were then air-dried in a shaded area at room temperature until completely dry. Once dried, the leaves were ground into a fine powder using a laboratory-grade grinder and stored in an airtight container to maintain their quality until use. Into 100 ml of double distilled water in a 250 ml beaker was added 10 g of leaves and boiled for 20 min at 80 °C. The extract was allowed to cool down at room temperature, filtered through Whatman filter Paper (no.1) and stored in a refrigerator at 4 °C.

3.2 Synthesis of Ag nanoparticles

For the biosynthesis of silver nanoparticles, 350 ml of leaf extract was added to 150 ml of 1 mM $AgNO_3$ and incubated in water bath at 80 °C for 20 min for the reduction of Ag^+ ions. Bio reduction was observed by colour change from yellowish to dark brown. The nanoparticle solution was then centrifuged at 10,000 rpm for 20 min and pellet obtained was washed two times with double distilled water and one time with absolute ethanol. The synthesis of AgNPs was confirmed by Powder XRD and UV–visible spectroscopy having a wavelength in the range of 200–800 nm.

3.3 Photocatalytic degradation

Photocatalytic degradation of Methylene Blue (MB) dye was evaluated by biosynthesized silver nanoparticles in the presence of sunlight. An accurately weighed 10 mg of the MB dye was dissolved in 1000 ml of double distilled water to prepare a stock solution. Approximately 20 mg of AgNPs were added to 50 ml of MB solution and continuously stirred at magnetic stirrer for 20 min to ensure the equal distribution of AgNPs in solution for catalytic degradation. A control of dye solution without silver nanoparticles was maintained. Subsequently, the tubes were placed in sunlight exposure for the photocatalytic degradation of dye. After irradiation, the catalyst was separated by centrifugation and the absorbance of MB was determined by the UV-Vis. spectrophotometer at the absorption wavelength of 665 nm. The photocatalytic efficiency (%) was calculated using the formula

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$$\%\ Degradation = \frac{A_0 - A_t}{A_0} \times 100$$

where A_0 and A_t are the absorbance of MB solution before and after the photocatalytic reaction.

4. Results and discussion

The silver nanoparticles were successfully synthesized from neem leaf extract by 1 mM $AgNO_3$. Before the addition of $AgNO_3$ the colour of the leaf extract was yellowish however, when 1 mM $AgNO_3$ solution was added to the aqueous leaf extract initially no colour change was observed in the reaction medium. However, after incubation in water bath at 80 °C for 20 min the colour of the solution changed to dark brown that indicated the formation of AgNPs.

4.1. UV–Vis spectroscopy

UV–visible spectroscopy was used to investigate the formation of nanoparticles as it provides information about the size, structure, stability, and aggregation of the nanoparticles. The confirmation of silver nanoparticles synthesis was monitored by UV–Vis spectrophotometer as it is the most convenient tool for measuring the reduction of metal ions based on optical properties called (SPR) surface plasmon resonance, SPR is produced by resonant oscillation of conduction electrons at the interface of nanoparticles stimulated by incident light. Therefore, metallic nanoparticles display characteristic optical absorption spectra in UV–visible region. UV–visible spectra showed the single and strong band absorption peak at 423 due to excitation of (SPR) thus indicating the nanoparticles were isotopic and uniform in size.

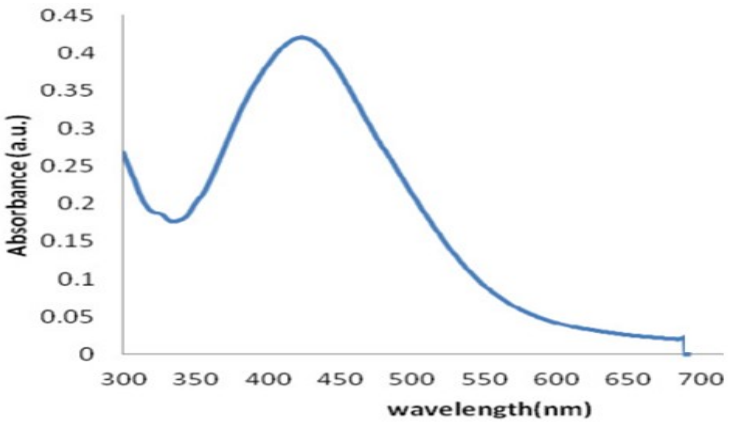

Fig. 4.1. UV–Vis absorption spectrum of AgNPS,

4.2 Powder XRD of Ag NPs

The Powder X-ray Diffraction (PXRD) pattern of AgNPs synthesized using neem leaves reveals distinct crystalline peaks characteristic of silver. Notable peaks observed at 2θ values of 38.1°, 44.3°, 64.6°, and 77.5° correspond to the (111), (200), (220), and (311) planes of the face-centered cubic (FCC) crystal structure of silver, respectively [Chandra *et. al*., 2005]. The sharpness and intensity of these peaks confirm the high crystallinity and well-defined structure of the nanoparticles. The peak at 2θ = 38.1° is the most prominent, indicating a high degree of crystallinity and suggesting that the majority of the nanoparticles are oriented along the (111) plane [Rafique *et.al*., 2019]. This PXRD pattern signifies the synthesis of silver nanoparticles with a stable FCC structure.

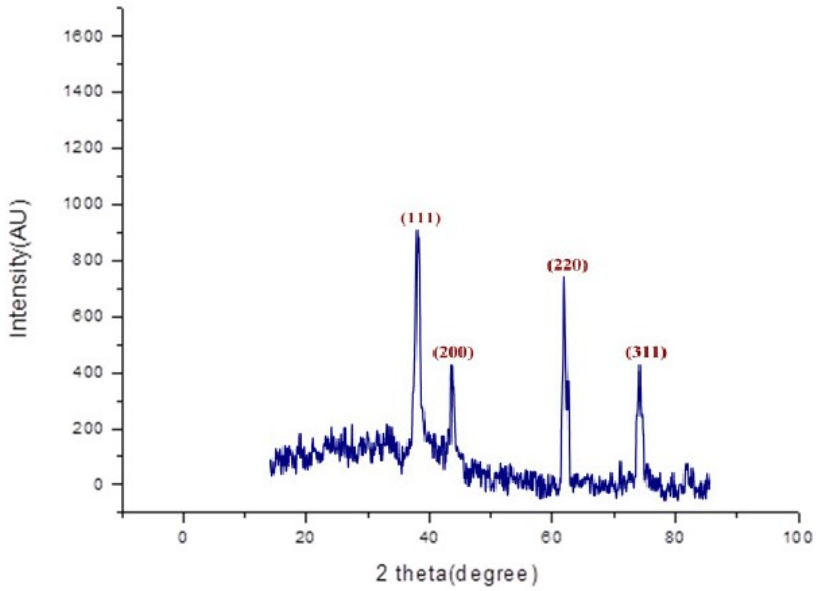

Fig. 4.2. Powder XRD Pttern of Ag NPs

4.3 Scanning Electron Microscopy (SEM)

SEM analysis of the silver nanoparticles synthesized using neem leaves reveals a diverse range of morphological features. The SEM images depict predominantly spherical particles with sizes ranging from approximately 80-120 nm, exhibiting a relatively uniform distribution. The particles are seen to aggregate in clusters, which is a common occurrence due to van der Waals forces and the interactions between particles during synthesis. The high-resolution images further

illustrate that the nanoparticles possess smooth surfaces, with few noticeable irregularities or defects, suggesting a controlled and consistent growth process [Swati Jaast & Anita Grewal, 2021]. This morphology indicates that neem leaves effectively stabilize the nanoparticles and promote the formation of well-defined, spherical silver nanoparticles with minimal agglomeration.

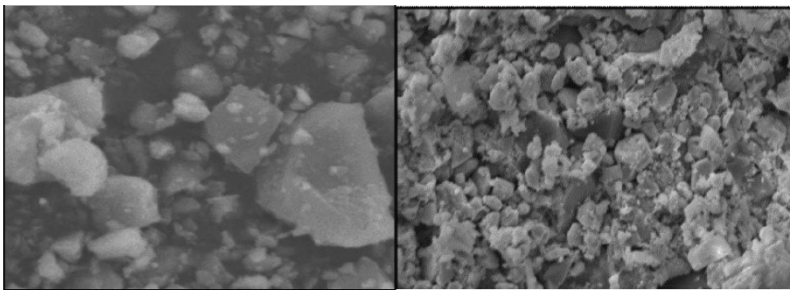

Fig.4.3. SEM Images of Ag NPs

4.4 Photocatalytic degradation of MB dye

The release of dye effluents from textile industry is a major source of water pollution. Dyes along with other organic compounds are released as waste products by the various industries, which leaves harmful effects on human, animals as well as plants. Methylene Blue is very stable, basic dye. AgNPs are successfully used as photocatalysts because of high surface to volume ratio, non-toxic, cost - effective and novel way of treatment of several dye pollutants [Marimuthu *et al*, 2020]. Photo catalytic degradation of dye was assessed by biosynthesized silver nanoparticles in the presence of sunlight. The colour of the suspension faded along with the increase of reaction time, which is indicative of dye degradation. The fact behind the dye degradation is that light illumination assisted the electron-hole pair generation that was responsible for the increase of reduction and oxidation process with the dye. UV–Vis spectrometry was employed to determine the degradation behaviour of MB dye and was manifested by decolorization of MB solution. The characteristic absorption peak of MB solution was found to be around 450 nm and the decrease in peak intensity was observed visually with increase of exposure time [Vanaja *et.al.,* 2014]. The contact time is an essential parameter in dye degradation during the catalytic process. It was observed that as the contact time increased, the degradation of dye increased linearly and reached maximum at 6 h of incubation in presence of sunlight and this increase in dye removal was because of higher

existence of active sites on nanoparticles surface that leads to greater dye removal [Edison &, Sethuraman, 2012].

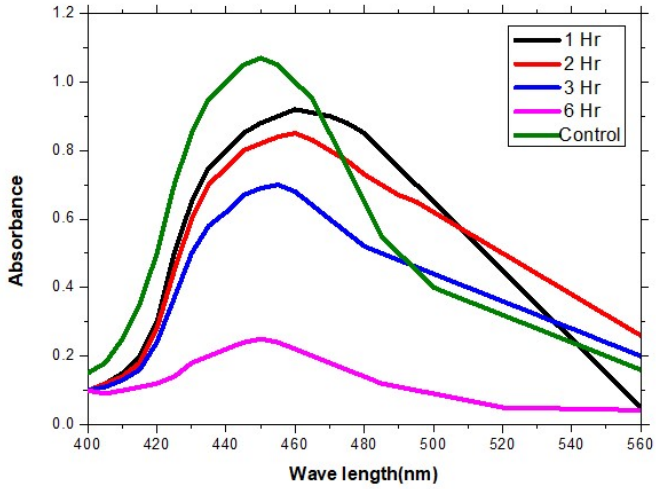

Fig.4.4. UV–vis absorption spectrum of MB dye treated with silver nanoparticles biosynthesized by neem leaf extract at different time intervals.

It was observed that AgNPs have the effective catalytic potential for the reduction of MB dye and suggested that photocatalytic activity is related to the surface area as the size of silver nanoparticles decreases the number of coordinated Ag atom increases that enhanced the absorption of reactant MB dye on the catalyst surface. Many factors such as light absorption, contact time, pH temperature etc. play significant effect on the photocatalytic degradation [Tahir *et al,* 2015]. Sphere structure and particle size also effect on the degradation activity of silver nanoparticles as the size increases, more active side, and surface area increases, which enhance the binding area. Previous studies have reported that compared to other irradiation techniques, solar light was found to be faster in decolorizing the dye in presence of metal catalyst. Ag nanoparticles are good, highly efficient and stable photocatalysts under ambient temperature with visible light illumination for degrading organic compounds and dyes.

Conclusion

The present study successfully demonstrates the green synthesis of AgNPs using neem leaf extract as both a reducing and capping agent. The resulting AgNPs were characterized by UV-Vis spectroscopy and Powder X-ray Diffraction (XRD),

confirming their high crystallinity and well-defined face-centered cubic (FCC) structure. The SEM analysis revealed predominantly spherical nanoparticles with a size range of 80-120 nm, exhibiting a controlled and consistent morphology with minimal agglomeration. These results underscore the effectiveness of neem leaf extract in producing stable and uniform silver nanoparticles, providing a sustainable alternative to conventional synthesis methods.

The photocatalytic performance of the biosynthesized AgNPs was evaluated through the degradation of Methylene Blue (MB) dye under sunlight. The study revealed that the AgNPs shows significant photocatalytic activity, with the degradation efficiency increasing linearly with contact time, peaking after 6 hours of sunlight exposure. This enhanced performance is attributed to the high surface area and active sites on the nanoparticles, which facilitate the generation of electron-hole pairs necessary for dye degradation. The results highlight the potential of plant-extract synthesized AgNPs as effective, eco-friendly photocatalysts for addressing water pollution, offering a viable solution for the treatment of dye contaminants in wastewater.

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SYNTHESIS AND CHARACTERIZATION OF COPPER(II) COMPLEXES OF A SCHIFF BASE DERIVED FROM 2-AMINO PHENOL AND BENZIL (BOAP).

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ABSTRACT

This study investigates the synthesis and detailed characterization of copper(II) coordination complexes formed with a Schiff base ligand derived from 2-amino phenol and benzil. The primary focus is on the formation and analysis of copper(II) chloride and copper(II) thiocyanate complexes, which were synthesized through well-established chemical procedures. Comprehensive characterization of these complexes was carried out using an array of analytical techniques to gain a thorough understanding of their properties. Elemental analysis was performed to determine the composition and stoichiometry of the complexes. Magnetic susceptibility measurements were used to assess the magnetic properties and confirm the oxidation state of copper. Molar conductance studies provided insights into the ionic nature of the complexes in solution. Thermal analysis was conducted to evaluate the thermal stability and decomposition characteristics. Infrared (IR) spectroscopy was employed to identify functional groups and confirm the coordination of the Schiff base to the metal center. Proton nuclear magnetic resonance (1H NMR) spectroscopy was used to investigate the environment of hydrogen atoms in the ligand and the metal-ligand interactions. X-ray diffraction (XRD) analysis helped in elucidating the crystal structures and confirming the coordination geometry around the copper ion. Electronic spectral analysis was performed to understand the electronic transitions and the bonding nature within the complexes. The combined data from these techniques provided a comprehensive overview of the structural, magnetic, and spectral properties of the copper(II) Schiff base complexes. This detailed characterization contributes to the broader understanding of their potential applications in bioinorganic chemistry and

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materials science, highlighting their utility in modeling biological copper systems and exploring their functional behaviors.

Keywords: Schiff Base Ligand, Physico-chemical studies, Elemental Analysis, Magnetic Susceptibility, Infrared (IR) Spectroscopy, X-ray Diffraction (XRD)

Introduction

In coordination chemistry, Schiff bases are regarded as some of the most flexible and well-explored ligands. Their notable biological activities include the formation of metal complexes, which significantly enhances their antibacterial and antifungal effects. The effectiveness of these activities is heavily influenced by the arrangement and structure of the chelating moieties.

Copper is an essential element present in all forms of life, and earlier studies have extensively explored its various biological roles. Cu(II) complexes are known for their Jahn-Teller distortion and can also exhibit notable magnetic properties. Recent research on copper(II) complexes aims to model biological copper-containing molecules. Schiff base copper(II) complexes are of particular interest. This study addresses the synthesis and characterization of Cu(II) complexes with chloride and thiocyanate ligands, using a Schiff base derived from 2-amino phenol and benzil. Characterization methods included elemental analysis, magnetic moment and molar conductance measurements, thermal analysis, IR spectroscopy, 1H NMR spectroscopy, XRD, and electronic spectral data.

Preparation of the Ligand

Schiff base derived from 2-amino phenol and Benzil (BOAP)

The ligand BOAP was synthesized by the condensation of 2-aminophenol and benzil. An ethanolic solution of 2-amino phenol (0.10M, 20mL) was added to an ethanolic solution (0.05M, 10 mL) of benzil. The mixture was shaken vigorously and heated over a water bath for 4-5h. The resulting brown solid was washed with methanol and dried.

The compound is expected to exist in the form given below

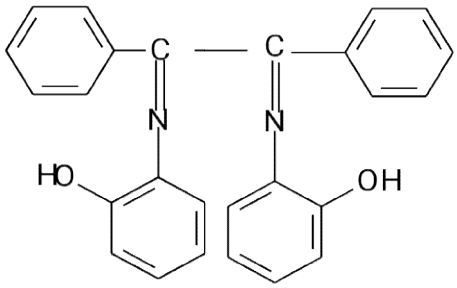

Fig (1)

BOAP (melting point >300^{o}C)

Synthesis of Metal Complexes

[Cu(BOAP)$_2$Cl$_2$] and [Cu(BOAP)$_2$(Cl)(NCS)]

The following general method was adopted for the preparation of the above complexes, A hot methanolic solution of copper(II) chloride (2 mmol) was added to a hot methanolic solution of ligand (2 mmol). The resulting solution was then refluxed on a water bath for ~5 h. The solution was then concentrated so that a solid complex separates out. It was filtered and washed with aqueous methanol followed by ether and finally dried over calcium chloride in a desiccator.

[Cu(BOAP)$_2$(NCS)(Cl)]

To the methanolic solution of copper(II) chloride (2.5 mmol), ~0.5 g of ammonium thiocyanate was added, stirred well and filtered. The filtrate was then refluxed with the hot methanolic solution of ligand (2.5 mmol) for ~3-4 h on a water bath. This solution was then allowed to concentrate in order to separate the solid complex. It was then filtered and washed with aqueous methanol followed by ether and then dried over calcium chloride in a desiccator.

General Properties of the Complexes

All the complexes are deeply coloured, dark brown and are non-hygroscopic solids. The complexes are soluble in ethanol and methanol, partially soluble in benzene and insoluble in ether.

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Analysis

Physicochemical Studies

Conductance measurements of the complexes in nitrobenzene, acetonitrile and methanol were done using an Elico direct reading conductivity meter. The magnetic susceptibility measurements were carried out at room temperature by Gouy's method. The IR spectra of the ligand and the complexes were recorded on a Schimadzu DR-43S spectrophotometer and FTIR impact 410 spectrometer using KBr pellets in the range 4000-400cm^{-1}. Electronic spectra of the ligand and the complex were recorded in methanol on a Systronics UV-Vis spectrophotometer model 117 in the range 250nm-900nm. ^{1}H NMR of the ligand was recorded in deuteriated methanol on a JOEL GSX 400 NBFT NMR spectrometer using TMS as reference material. The TG curves of the complex were recorded on a 2960 SDT V2.2B TG/DTA at a heating rate of 10^{o}C/min, in oxygen atmosphere with sample mass ~ 3 mg in the temperature range 0-1000^{o}C.

Results and Discussion

The empirical composition of the complexes have been confirmed on the basis of analytical data. The analytical data suggest that the complexes have the general formula,

$[ML_2X_2]$ [M = Cu(II), 'L' is a neutral bidentate ligand and X=[Cl^- and NCS^-].

CHNS Analysis

Carbon, hydrogen, nitrogen and sulphur contents of the complexes were determined by micro analytical method. The analytical data are presented in Table I (1).

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Table I (1)

ANALYTICAL DATA OF COPPER (II) COMPLEXES OF BOAP

Complex	Colour	% of Cu	% of Cl	% of S	% of C	% of H	% of N
		Obs. (Calc.)	Obs. (Calc.)	Obs. (Calc.)	Obs. (Calc.)	Obs. (Calc.)	Obs. (Calc.)
$[Cu(L)_2(Cl_2)]$	Brown	7.40 (7.46)	8.21 (8.26)		72.98 (72.78)	4.71 (4.75)	6.55 (6.49)
$[Cu(L)_2(NCS)(Cl)]$	Brown	6.75 (6.73)	3.76 (3.73)	3.40 (3.36)	67.58 (67.63)	4.28 (4.31)	7.43 (7.45)

Where , L – BOAP

Electrical Conductance

The molar conductance of 10^{-3} M solution of the complexes in nitrobenzene, acetonitrile and methanol were determined and are presented in table I (2). The molar conductance of a 1:1 electrolyte in nitrobenzene, acetonitrile and methanol for the corresponding concentrations are in the range 20-30, 120-160 and 80-115 $ohm^{-1}cm^2\ mol^{-1}$ respectively. A comparison of the observed conductance of all the complexes with the data available shows that all the complexes are non-electrolytes.

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Table I (2)

Molar Conductance Data of Copper (II) Complexes of Boap

Complex	Nitrobenzene	Acetonitrile	Methanol	Nature of solution
	Molar Cond*	Molar Cond*	Molar Cond*	
$[Cu(L)_2Cl_2]$	3.6	37.1	24.1	Non-electrolyte
$[Cu(L)_2(Cl)(NCS)]$	4.1	38.9	27.3	Non-electrolyte

Where , L - BOAP

*Molar conductivity, 10^{-3} M solvents at 298 K.

*(ohm^{-1} cm^2 mol^{-1})

Magnetic Behaviour

In the present investigation, all the Cu(II) complexes have magnetic moment values in between 1.69-1.88 B.M, which indicates that the complexes have a distorted octahedral configuration. TABLE I (3) shows the effective magnetic moment values of all the complexes determined by Gouy's method under the condition of sufficient magnetic dilution.

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Table I (3)

Magnetic Moment Values of Copper (II) Complexes of Boap

Complex	μ_{eff} in BM
$[Cu(L)_2Cl_2]$	1.81
$[Cu(L)_2(Cl)(NCS)]$	1.69

Where , L - BOAP

Infrared Spectra

The IR spectral bands of the ligand BOAP and its complexes along with probable assignments are given in Table I (4).

In the IR spectrum of free ligand BOAP, a strong and very broad band centered around 3060 cm^{-1}, which is characterization of strong hydrogen bonded O-H vibration in the Schiff base ligand.

The C=N stretching vibration exhibits a negative shift from ~1613cm^{-1} and 1591 cm^{-1} to ~1604 cm^{-1} and 1572 cm^{-1} in the complexes indicating the participation of azomethine nitrogen in bonding. The IR spectrum of the complexes also shows band at ~474 cm^{-1} which may be attributed to $\upsilon_{Cu-N.}$ Thus from the IR spectra it is clear that ligand is bonded to the metal ion in a bidentate fashion through the azomethine nitrogens.

Thiocyanate complex

The thiocyanate group is an ambidentate ligand. It can coordinate to the metal atom through nitrogen(I) or through sulphur(II). It even act as a bridging group(III).

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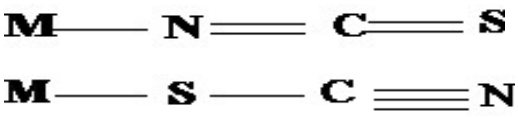
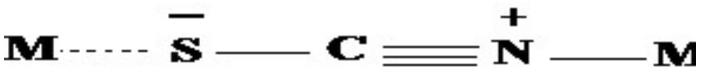


(I) & (II)



(III)

In the IR spectrum of copper(II) thiocyanate complex of BOAP, a strong band observed at 2069 cm^{-1} can be assigned to the thiocyanate group co-ordinated through nitrogen. Also a medium intensity band present at 774 cm^{-1} in the spectrum of the thiocyanate complex can be attributed to ν_{CS} vibration. The medium intensity band at 525 cm^{-1} in the spectrum of thiocyanate complex is assignable in the –NCS bending vibration. From these data, it is evident that in the present complex the thiocyanate ion is co-ordinated unidentately through the N atom. Co-ordination of the thiocyanate ion agrees with the non-electrolytic nature of the complex.

Table I(4)

Infrared Spectral Bands of Boap and its Copper (II) Complexes

L	$[Cu(L)_2(Cl_2)]$	$[Cu(L)_2(Cl)(NCS)]$	Assignments
	3385s	3373s	υ_{OH} free
3060b	-	-	υ_{OH} hydrogen bonded
-	-	2069s	$\upsilon_{(NCS)}$ of thiocyanate
-	-	-	$\upsilon_{4(NO3)}$ coordinated
1369m	1595m	1604m	υ_{C-N} stretching
1375m	1576m	1580m	υ_{C-N} of azomethine
1329m	-	-	
-	-	-	$\upsilon_{4(ClO4)}$coordinated
-	-	-	$\upsilon_{2(NO3)}$coordinated

-	-	-	$\upsilon_{1(SO4)}$coordinated
-	-	-	$\upsilon_{1(ClO4)}$coordinated
-	-	774m	$\upsilon_{(C-S)}$ of thiocyanate
-	-	525	υ_{NCS}
-	-	754m	$\upsilon_{(C-S)}$
-	-	-	$\upsilon_{2(ClO4)}$
-	423	431	υ_{Cu-O}
-	-	-	$\upsilon_{3(ClO4)}$ coordinated
-	-	-	$\upsilon_{5(ClO4)}$
-	457m	463m	$\upsilon_{(Cu-N)}$

s=strong, m=medium, b=broad Where , L - BOAP

^{1}H NMR

The ^{1}H NMR spectrum of the Schiff base ligand BOAP in deuteriated methanol shows a sharp and singlet peak at δ 10.02 ppm, due to the OH proton. The aromatic protons appear as mutiplets within the range 6.69-7.64.

Electronic Spectra

The copper(II) complexes of the ligand BOAP are dark brown in colour. Since Cu(II) is susceptible to Jahn Teller distortion, formation of a regular octahedral complex never occurs. Instead, weakly or strongly distorted octahedral complex with the latter approaching square planar configuration is obtained. Thus the symmetry will be lowered from O_h to D_{4h}. For the ligand field of D_{4h} symmetry, the energy level diagram predicts three transitions, $^2B_{1g}\rightarrow{}^2A_{1g}$, $^2B_{1g}\rightarrow{}^2B_{2g}$, $^2B_{1g}\rightarrow{}^2E_g$. Because of the overlap of three bands, only one broad band is generally observed in the visible region of many Cu(II) complexes.

The electronic spectral bands of the Cu(II) complexes of BOAP recorded in methanol are given in Table I(5).

TABLE I(5)

Electronic Spectral Bands of Copper (II) Complexes of Boap

Ligand/Complex	Spectral bands in Methanol		Assignment
	λ_{max}(nm)	ν cm^{-1}	
L	273	37027	$\pi \rightarrow \pi^*$ transition
	364	27322	$n \rightarrow \pi^*$ transition
$[Cu(L)_2(Cl)_2]$	265	37735	$\pi \rightarrow \pi^*$ transition
	410	24390	CT transition
	677	14771	$^1B_{1g} \rightarrow {}^2E_g$

Where, L – BOAP.

The electronic spectra of ligand show an intense band at ~273 nm which may be due to $\pi \rightarrow \pi^*$ transition. Similarly, a band at ~364 nm in the ligand spectrum may be assigned to $n \rightarrow \pi^*$ transition.

The electronic spectra of the complexes show absorption band at ~270 nm, 418 nm and 678 nm. The former band at 270 nm can be attributed to the $\pi \rightarrow \pi^*$ transition; where as absorption band at 418 nm may be due to charge transfer transition. The absorption band at 678 nm observed in the spectra of complexes is assigned to $^1B_{1g} \rightarrow {}^2E_g$ transition. The possibility of distorted octahedral geometry for all the complexes is confirmed by the occurrence of a weak band ~678 nm corresponding to the transition $^2B_{1g} \rightarrow {}^2E_g$. The electronic spectral data are in conformity with the magnetic moment values.

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X-RAY POWDER DIFFRACTION STUDIES

The X-ray diffraction patterns of the complex $[Cu(BOAP)_2(NCS)Cl]$ recorded using CuK_α radiation λ =1.54056 A^o is shown in the Fig 1(6).

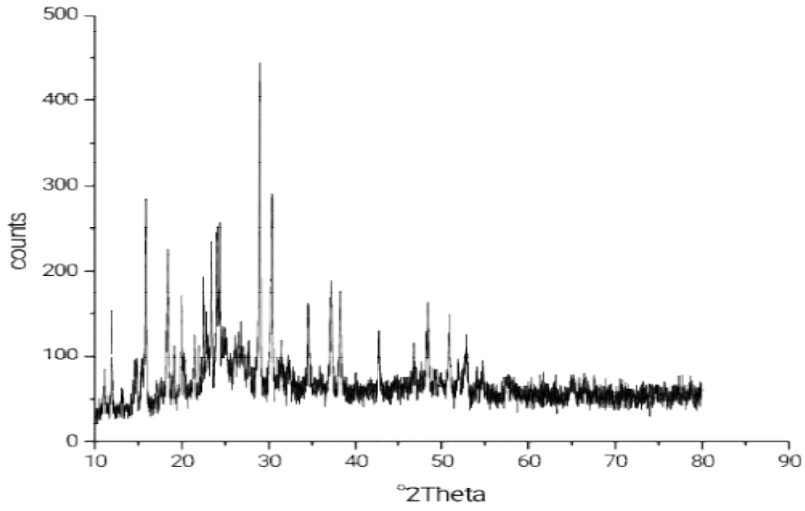

FIG 1(6)

X-ray Powder Diffraction of $[Cu(BOAP)_2(NCS)Cl]$

The diffraction pattern was indexed by the method developed by H. Lipson and the complex was found to be orthorhombic. The $\sin^2\theta$ values were calculated using the equation.

$$\sin^2\theta(hkl) = Ah^2+Bk^2+Cl^2$$

Where A, B, C are lattice constants. The unit cell a, b, c have been calculated from the relation

$$A = \lambda/4a^2,\ B = \lambda/4b^2,\ C = \lambda/4c^2.$$

The observed and calculated $\sin^2\theta$ values for the corresponding hkl values together with the relative intensities of the peaks are given in the Table 1(7). The calculated and observed $\sin^2\theta$ values are in good agreement.

The lattice constants for the complex are A=0.006, B=0.019, C=0.009. The unit cell diamensions calculated are a=9.94062 A^o, b= 5.5858A^o, c=8.1164 A^o.

TABLE 1 (7)

The Experimental and Theoretical $Sin^2\theta$ VALUES, hkl Values and Relative Intensities of $[Cu(BOAP)_2(NO_3)_2]$

2θ	hkl	$Sin^2\theta$(exp-erimental)	$Sin^2\theta$(Theo r-etical)	Relative Intensity	
				%	cps
11.13	001	0.009	0.009	17.67	76
18.39	110	0.025	0.025	50	215
21.47	111	0.034	0.034	28.13	121
24.05	210	0.043	0.043	58.37	251
19.91	201	0.036	0.033	35.81	117
34.57	021	0.088	0.085	35.58	153
26.49	211	0.052	0.052	28.37	122
35.87	121	0.094	0.091	19.06	82
28.95	112	0.062	0.061	100	430
15.73	300	0.018	0.018	32.32	139

Thermal Studies

In the present investigation, thermal studies were carried out to supplement the IR and percentage composition data of the complexes. The TG curve of $[Cu(BOAP)_2(Cl)_2]$ were studied in oxygen atmosphere [Fig. I(2)] and the thermal data is given in Table I (8)

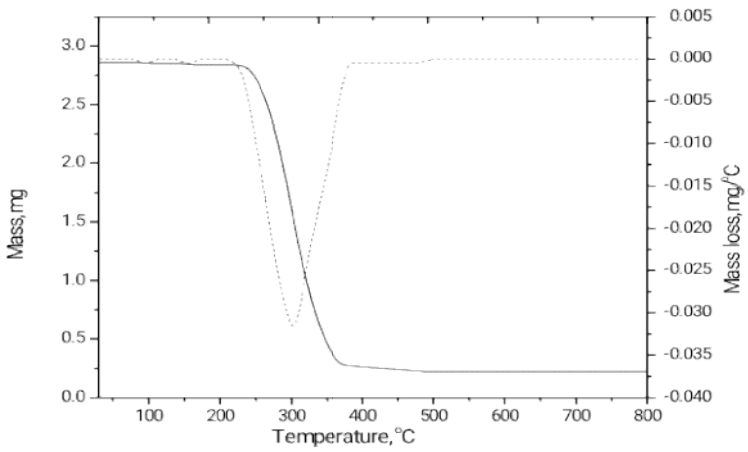

Fig I (8)

TG and DTG curves of $[Cu(BOAP)_2(Cl)_2]$

TABLE I (8)

Thermal Decomposition Data of $[Cu(BOAP)_2(Cl)_2]$

Stage of decomposition	T_i(K)	T_f(K)	T_s(K)	Mass loss	
				TG	Theoretical
1	230	370	303	90.21%	90.73%

The complex $[Cu(BOAP)_2(Cl)_2]$ shows a plateau upto 230^{o}C indicating that the complex is stable upto this temperature and no coordinated water molecule is present. The TG curves shows one weight loss curve indicating that the complex has undergone single stage decomposition. The decomposition starts at 240^o C and completed at 370^0 C. At this stage the oxidative decomposition of the complex to CuO takes place. The percentage mass loss obtained from TG during this process is 90.21 % which is also in good agreement with the theoretical value 90.73 %. The

mass loss observed agrees with value calculated for the conversion of the complex to its metal oxide.

Evaluation of Kinetic Parameters from TG/DTG

There are three different approaches for the evaluation of kinetic parameters namely differential methods, integral methods and approximation methods. Of this integral method used in the present study is reviewed here. The computational details and the kinetic parameters of the single stage thermal decomposition of the complex $[Cu(BOAP)_2(Cl)_2]$ are given in the Table I(9) and I(10)

TABLE I (9)

Computational Details of the Single Stage Thermal Decomposition of $[Cu(BOAP)_2(Cl)_2]$

n = 2 r = 0.99873

T^0C	mg	TK	A	gα 2	CR2
270	2.43	543	0	0	-
280	2.19	553	0.13115	0.14879	-14.53592
290	1.92	563	0.27869	0.37335	-13.6518
300	1.61	573	0.44809	0.76	-12.97488
310	1.27	583	0.63388	0.76101	-12.3029
320	1.01	593	0.77596	1.54261	-11.71031
330	0.78	603	0.90164	2.8866	-10.89548
340	0.6	613	1	6.74195	-

TABLE I (10)

Kinetic Parameters for First Stage Thermal Decomposition of $[Cu(BOAP)_2(Cl)_2]$

Stage	Peak Temp°C	Activation Energy(E) kJ/mol	Pre-exponential Term (A)/S	Entropy of Activation (ΔS) J/K/mol
1	303	195.71572	$6.175x10^3$	-172.4739

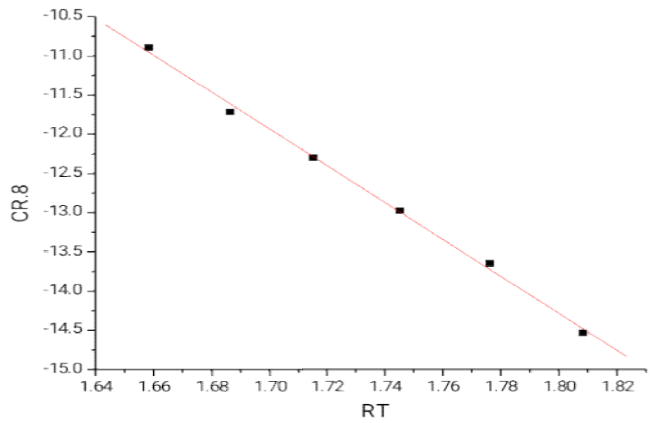

Fig II

Coats-Redfern plot of stage I decomposition of $[Cu(BOAP)_2(Cl)_2]$

Geometry of the Complexes

In the present work, complexes of copper with Schiff base prepared from 2-amino phenol and benzil were synthesized. These complexes have the composition $[Cu(BOAP)_2(Cl_2)]$ and $[Cu(BOAP)_2(Cl)(NCS)]$. The instrumental methods of analysis and other experimental studies revealed that the anions are in the coordination sphere of the metal ion. The complexes show normal magnetic behavior expected for the presence of one unpaired electron of a d^9 configuration. This indicates the absence of metal-metal interaction in complexes. The molar conductance values of the complexes in nitrobenzene, acetonitrile and methanol

lies in the range 3.6 to 4.4, 37.1 to 43.2 and 24.1-31.7 respectively, which reveals that all the complexes are non-electrolytes in nature which suggest the coordinating nature of anions present in all the complexes. The magnetic moments of all the complexes are in the range 1.69-1.88 B.M which indicates that the complexes have a distorted octahedral geometry. The IR spectra shows that a broad medium absorption band observed at ~ 3060 cm^{-1} due to hydrogen bonded OH group in the free ligand, is found to be absent in the spectra of all the complexes. The C=N stretching vibration exhibits a negative shift from ~1613 cm^{-1} and 1591 cm^{-1} to ~1604 cm^{-1} and 1572 cm^{-1} in the complexes indicating the participation of azomethine nitrogens in bonding. Thus from the IR spectra it is clear that, in all the complexes the ligand is bonded to the metal ion in a bidentate fashion through two azomethine nitrogens. The anions are linked in a unidentate fashion in all the complexes. The IR spectral data is in favour of a hexa coordinated and distorted octahedral geometry.

The electronic spectra also suggest the possibility of distorted octahedral geometry for the complexes by the occurrence of a weak band ~678 nm corresponding to $^1B_{1g} \rightarrow {}^2E_g$. On the basis of all these physico-chemical studies a distorted octahedral geometry is proposed for all prepared complexes of copper(II) with BOAP.

Suggested structures of Cu(II) complexes with BOAP

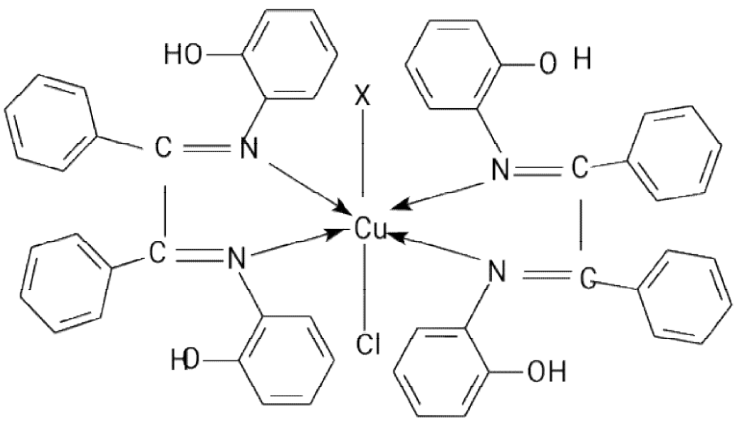

Fig III

[X=Cl, NCS]

Conclusion

Cu(II) complexes having the composition $[Cu(BOAP)_2(Cl_2)]$ and $[Cu(BOAP)_2(Cl)(NCS)]$ were prepared and characterized by elemental analysis, magnetic moment determination, conductance measurements, thermal analysis, IR, and electronic spectral data. The complexes are found to be stable in air and the conductance measurements reveals that all the complexes are non-electrolyte. The complexes show normal magnetic behavior expected for the presence of one unpaired electron of a d^9 configuration. This indicates the absence of metal-metal interaction in complexes. The magnetic moments of all the complexes are in the range from 1.69 to 1.88 B.M which indicates that the complexes have a distorted octahedral geometry. The IR spectral data suggests that two azomethine nitrogens are participating in coordination. The anions chloride, thiocyanate, nitrate and perchlorate are monodentately coordinated.

The electronic spectra also suggest the possibility of distorted octahedral geometry for all the complexes by the occurrence of a weak band ~ 678 nm corresponding to $^2B_{1g} \rightarrow {}^2Eg$. Thermogravimetric studies show the absence of any water molecule in the complex. The complex shows a plateau upto 220^oC indicating that the complex is stable upto this point of temperature. The first stage corresponds to the decomposition of the anionic part of the complex and during second stage of decomposition, the ligand part was completely removed and CuO is formed as final residue. The percentage mass loss obtained from TG during both the stages agreed well with the theoretical value.

On the basis of all the above mentioned physico-chemical studies revealed the formation of stable copper(II) complexes with BOAP and all these complexes $[Cu(BOAP)_2(Cl)_2]$, and $[Cu(BOAP)_2(Cl)(NCS)]$ have a distorted octahedral geometry. All the complexes are non electrolytes with paramagnetic behavior.

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STUDY ON THE EFFICIENCY OF MILLIPEDES IN ORGANIC COMPOSTING IN COMPARISON WITH EARTHWORMS

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ABSTRACT

The present research work was carried out with the aim of analysing the efficiency of millipede and earthworm, and exploration of millicomposting and vermicomposting process with the help of millipede, *Trigoniulus corralinus* and the earthworm *Megascolexmauriti* by using litter waste. Both millicompost and vermicompost are ecofriendly and economically viable to farmers to replace the chemical fertilizers.

Methods: Millicompost and vermicompost were produced with litter waste of Sree Narayana College, Alathur. Macronutrients such as Carbon, Nitrogen, Phosphorus, Potassium and Calcium were estimated at the 15^{th} and the 30^{th} days of experiment and compared with the control soil.

Results: Millicompost showed rich in macronutrients Nitrogen, Phosphorus, Potassium and Calcium (Nitrogen 77.33, Phosphorus 49.86, Potassium 69.08 and Calcium 68.34) at 30^{th} day followed by vermicompost (Nitrogen 64.20, Phosphorus 30.56, Potassium 66.01 and Calcium 67.63).

Conclusion: The chemical analysis of harvested millicompost showed an outstanding composition of macronutrients when compared to vermicompost.

Key words: *Trigoniulus corralinus*, *Megascolexmauriti,* Millicompost,

Introduction

In modern era of advanced development, a prominent issue currently facing is the increasing amount of solid waste materials in developing and developed countries. This crisis of generation of waste materials depends mainly on increased urban population, and, also on living standards, growth and human resources. The common solid wastes produced by the towns and cities are mainly the organic waste, which includes kitchen waste, vegetable market waste, sewage sludge,

animal excreta, weeds, coir waste, leaf litter, paper and pulp, agricultural residues, feed and fodder waste, and, aquatic biomass (Gopalakrishnan, N., 2005).

Compost derived from different organic wastes could act as effective organic manure to the crop plants and eco-friendly alternative source to minimize the usage of synthetic fertilizers. Composting is a biological process of controlled aerobic decomposition that transforms organic waste in to nutrient rich and stable organic products (Bernal *et al.*, 2009) which can be used as agricultural fertilizers (Tejada *et al.,* 2008).

Earthworms are most studied and used soil organisms in composting (vermicomposting) along with microbial Earthworm excrete products that constitute nutrient-rich organic fertilizers, which are rich in humus, stabilized organic matter, macro and micro nutrients, beneficial soil microorganisms and growth hormones (Bernal *et al.*, 2009, Adhikary S 2012, Bhat *et al*., 2018).

Similarly, millipedes are known to be macro detritivores, terrestrial arthropods feeding on decaying vegetable matter and mineral soil and are represented by more than 80,000 species. Millipedes are essentially important soil animals and in some ecosystem, they are more important than earthworms as agents of soil and nutrient turn-over (Martens *et al.,* 2002).

The earthworms belong to the Oligochaeta class and millipedes to the Diplopoda class. Diplopods are widely distributed in tropical, subtropical, and temperate regions and play an important role in improving soil fertility, as they are able to mobilize nutrients trapped in litter and enrich the soil with N, C, Ca, Mg, P, and K in microcosm conditions (Antunes *et al*., 2019). This nutrient enrichment results from a high litter consumption capacity that is associated with a high microbial activity present in the feces of diplopods. When the litter passes through the digestive tract, it is crushed; this process increases its specific surface, it moistens it, and it enriches it with microorganisms (Correia; Aquino, 2005). Diplopods can metabolize up to 0.3% to 0. 7% of the ingested material and the microbial activity continues taking place in their fecal pellets, which increases the bioconversion of plant residues (Ambarish, Sridhar, 2013).

Millicomposting is a new, little known, and environmentally friendly biotechnology, which facilitates the biotransformation of plant residues into stable

organic matter; this process is aided by the activity of diplopods, commonly known as millipedes. The final product of millicomposting is millipede humus, which has been called millicompost (Antunes *et al*., 2018).

Both millicomposting and vermicomposting are eco-friendly and economically viable to farmers to replace the chemical fertilizers.

In this scenario, aim of work is to evaluate the efficiency of millipedes and earthworms in composting the waste. Since there is only limited knowledge on millicomposting, this study is an attempt to give more consideration on millipede composting

Significance

Millicomposting, similar to vermicomposting, is an organic waste decomposition process using millipedes. This method can be highly effective for managing organic waste and producing nutrient-rich compost. Millipedes help break down complex organic matter into simpler forms, enhancing soil fertility and structure. The resultant compost improves soil aeration, water retention, and provides essential nutrients for plant growth. Millicomposting also contributes to sustainable agriculture by recycling organic waste, reducing landfill use, and lowering greenhouse gas emissions associated with conventional waste disposal methods.

Millicomposting can serve as a viable alternative to chemical fertilizers, offering numerous benefits for sustainable agriculture. The compost produced through millicomposting is rich in essential nutrients, such as nitrogen, phosphorus, and potassium, which are crucial for plant growth. Unlike chemical fertilizers, which can lead to soil degradation and pollution over time, millicompost improves soil structure, increases water retention, and enhances microbial activity, promoting long-term soil health.

Materials and Methods

Collection of compost materials

The raw materials for the present study, dried leaves of jamun tree were collected from college campus. They were shredded in to pieces and dried for long hours.

Pre digestion of Compost Materials

The dried leaves waste was mixed with equal amount of fresh cow dung and kept in plastic troughs separately and allowed for pre digestion by sprinkling water. After 15 days, the predigested materials were subjected to composting with millipedes, and, earthworms, separately.

Collection of experiment animal

Earthworm, known as 'Nature's ploughman', especially, the South Indian species Megascolex (plate 1.1) was collected from Sree Narayana College, Alathur campus, and, introduced in pot for vermicomposting. The 'thousand legger' red millipedes, Trigoniulus corralinus (plate 1.2) was collected from Kavil Village, Kozhikode district.

Plate -1.1: *Megascolex mauriti*

Plate 1.2: *Trigoniulus corralinus*

Preparation of compost bed

Closed systems were used in the experiments in order to control the main parameters that can affect the organic matter decomposition, such as aeration and humidity, and to simulate small-and medium-scale composting**.** Three garden pots (Figure 2.1) were taken in which two of them used as composting system and the other one as control. Composting systems 1 and 2 contains millipedes and earthworms respectively. Third pot chosen as control with no addition of detritivores animals (invertebrates).

Two composting bins were filled with soil and predigested leaves in layers alternatively. The raw materials for the present study, dried leaves of jamun tree were collected from college campus. The leaf waste was mixed with equal amount of fresh cow dung and added to the pot alternatively with soil followed by sprinkling water for keeping moisture. The pot mixture is subjected to composting by addition of millipedes and earthworms, in each of the 2 system, other than the control.

Plate 2.1

Pots were covered with wet muslin cloth in order to avoid the invasion of foreign materials and escape of earthworms and millipedes. Water was sprinkled at regular intervals to maintain moisture content in the mixture for easy decomposition. The biocomposting process was allowed to occur for 30 days.

Biochemical estimation : Spraying of water was stopped before two days of the harvest. Samples of millicompost, vermicompost and control were collected for analysis of physico- chemical characters at 0^{th} day ,15^{th} day and 30^{th} day.

To quantify the organic Carbon in the compost, Walkley and Black's rapid titration method was followed (Jackson, 1973). Total Nitrogen was determined using Micro Kjeldhal method (Umbreit *et al.*, 1974). Phosphorus was estimated by Vogel's technique (Vogel 1963). Available Potassium and Calcium were detected using Flame photometer (Elico; Model CL 378).

Designing a pot experiment to assess the quality of compost

30 days processed compost were prepared. To assess the efficiency of different composts on plant growth, a pot experiment was conducted.

Three pots were planted with spinach plants each and analysed it's progression of growth. One pot was taken as control and other two filled with 30 days older millicompost and vermicompost respectively. Measurements of growth of each plant were taken daily. After 30th day, growth parameters, such as, height of plant, and, number of leaves were measured.

Result

The appearance of compost in the form of fecal pellets were more clearly observed in millicompost bin when compared to vermicompost bin. The size of the pellets is larger in millicompost than vermicompost.

The compost harvested from each bin was undergone physicochemical analysis. The amount of micronutrients is presented in the table. The increasing percentage of nutrients is in millicompost followed by vermicompost, which is, compared with control, and, is depicted in the Table 1.1.

At the end of 15th day (Table 1) and 30th day (Table 2) period, significant difference was found in all biochemical components of millicompost compared to vermicompost and control.

Higher Nitrogen content was observed in millicompost (77.3%) than vermicompost (64.20%) and control (44.92). Similarly, higher Phosphorus content was found in millicompost (49.86%) compared to vermicompost (30.56%) and control (26.70%). The Potassium content was maximum in millicompost (69.08%). A similar trend was observed in Calcium content. High amount of Calcium was found in millicompost (68.34%) followed by vermicompost (67.63%). There was remarkable decrease in organic carbon content in millicompost (0.66%) after 30

days. It was 0.70 % in vermicompost since carbon content in first day was 0.78%. At the end of 30th day pH is found to be decreased. It was 6.56 and 6.90 in compost prepared by millipedes and earworms, respectively.

The effect of different composts processed by two types of detritivores on plant growth in comparison with control is shown in the Table 1.1. The result reveals that the spinach grown in the compost produced by millipedes attained maximum height compared to vermicompost plant.

15th Day analysis			
PARAMETERS	**Cc**	**Vc**	**Mc**
pH	5.21±*0.10*	7.0±*0.01*	6.98±*0.05*
Carbon (%)	54±*0.01*	*52.2* ± *0.02*	51.4 ± 0.04
Nitrogen (%)	44.67±1.26	59.86±5.05	65.22±13.89
Phosphorus (%)	25.68±0.36	28.90±1.90	45.34 ±0.02
Potassium (%)	51.19±2.44	56.21±43.43	58.15±26.48
Calcium (%)	60.48±1.22	64.23± 1.24	65.50 ±1.27
Magnesium (%)	19.75±2.07	57.02± 0.05	58.09± 0.05

Table – 1 **Nutrients composition (%)of 15 days old control soil, vermicompost and millicompost Value represented as mean±SD**

Cc - control compost **Vc –vermicompost** **Mc –millicompost**

30th Day analysis			
PARAMETERS	**Cc**	**Vc**	**Mc**
pH	5.2±*0.09*	6.90 ±0.02	6.56 ±0.03
Carbon (%)	*52.2*±0.12	49.3± 0.1	46.4± 0.55
Nitrogen (%)	44.92±1.03	64.20 ±0.08	77.33± 1.47
Phosphorus (%)	26.70± 0.51	30.56 ±0.13	49.86± 0.09

Potassium (%)	48.10±3.42	66.01±20.15	69.08±*12.03*
Calcium (%)	60.50±1.21	67.63±1.62	68.34± 0.09
Magnesium (%)	21.01± 0.01	62.38±1.43	70.0± 1.2

Table -2 **Nutrients composition(%) of 30 days old control soil, vermicompost and millicompost Value represented as Mean±SD**

The growth of Amaranthus plant in composts processed by millipedes and earthworms in 30th day is shown below:

a) Millicompost plant b) Control plant c) Vermicompost plant

Figure -1 Effect of organic waste processed by earthworms and millipedes on *Amaranthus dubius* compared with control

The height of millicompost plant is more than both vermicompost and control plant. Similarly, the number of leaves is seen greater compared to vermiplant and control.

PARAMETERS	Control	Vermicompost	Millicompost
Height of plant (cm)	8.96±3.81	12.28 ±3.56	15.62±7.34
Number of leaves	9.3±2.51	12.12 ±3.46	28.5± 4.38

Table -3 Effect of leaf waste residues composed with earthworms and millipedes on growth parameters of *Amaranthus dubius* after 30 days - Each value represents mean and standard deviation.

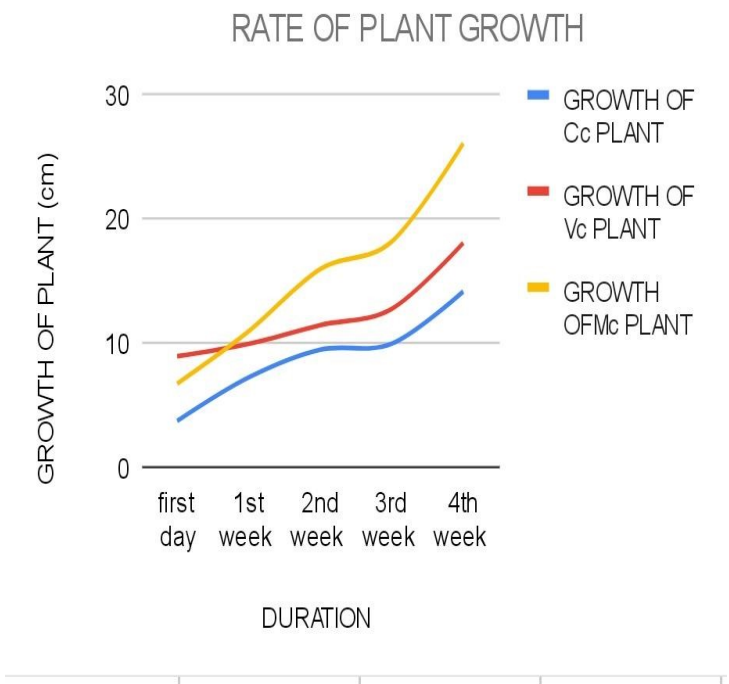

Fig 1.1

Growth Rate of Plants in Different Composts

The initial heights of the plants were as follows: the control plant was 3.8 cm, the vermiplant was 9 cm, and the milliplant was 6.8 cm. By the end of the first week, the control plant grew to 7.3 cm, the vermiplant to 10 cm, and the milliplant to 11 cm. In the second week, the control plant reached 9.53 cm, the vermiplant 11.5 cm, and the milliplant 16 cm. By the third week, the control plant had grown to 10 cm, the vermiplant to 12.8 cm, and the milliplant to 18.2 cm. Finally, by the

end of the fourth week, the control plant measured 14.2 cm, the vermiplant 18.1 cm. and the milliplant is 26.1cm.From these data it is seen that growth of plant in vermicompost is greater than control. Similarly, milliplant has higher growth compared to vermiplant. (Fig1.1).

Discussion

The soil macrofauna are known to play a significant role in disintegration and decomposition of organic materials added to the soil. Saprophagous invertebrates change the micro environmental conditions of litters through separation and vigorous mixing of litter in the soil. Vermicomposting is a simple biotechnological process of composting, in which certain species of earthworms are used to enhance the process of waste conversion and produce a better end-product. Similarly, millicomposting is a novel technique, in which millipedes employed for composting organic waste. Earthworms consume various organic wastes and reduce the volume by 40-60%. Generally earthworm eats organic wastes equivalent to its body weight and produces cast about 50% of the waste it consumes in a day, but mature millipedes eat organic waste about five times their weight.

Millicompost showed rich in macronutrients N, P, K, Ca and Mg (Nitrogen 77.33, Phosphorus 49.86, Potassium 69.08, Calcium 68.34 and Magnesium 70.0) at 30^{th} day followed by vermicompost (Nitrogen 64.20, Phosphorus 30.56, Potassium 66.01, Calcium 67.63 and Magnesium 62.38) than in control was (Nitrogen 44.92, Phosphorus 26.70, Potassium 48.10, and, Calcium 60.50). The results of the present investigation revealed that Nitrogen, Phosphorus, Potassium and Calcium were higher in millicompost followed by vermicompost than conventional compost. Hence, the following factors have been found to be different in millipede faces versus the original litter. (i) More available carbohydrates and amino acids: (ii) higher nitrogen levels, (iii) more moisture due to compaction of the pellets or otherwise; (iv) a pH of 6.5 and (v) the change in physical structure of the pellet. compared to the parent litter. The presence of millipedes positively changes the concentration of many nutrients like phosphorus, calcium and magnesium. Higher concentration of elements was found in compost derived from millipedes than that from earthworms. However, the concentration of nitrogen and phosphorus increased due to digestion and fecal pellet formation in millipede.

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The present study manifested that among the composting species chosen for the present study, millipede highly efficient in composting the waste into useful organic manure than earthworm. Ambarish and Sridhar reported increase in the concentration of N, P, K and Ca in compost produced with the help of millipede and earthworm. Prabhas *et al.,* showed that millicompost is superior and has a positive effect on plant growth over vermicompost and ordinary compost. Nicholson *et al.,* observed that ash and phosphorus were found to be high in fecal pellets of millipedes. The rise of pH and the availability of ammonia make millipede feces very hospitable microflora and other organisms. Similarly McBrayer reported that the fecal pellets of millipedes also increased pH, moisture, and, bacterial count, decreased fungal count, and, carbon, than undigested leaf litter.

Macro-micronutrients were significantly higher in millicompost compared to vermicomposts and microbial composts. There are many advantages in making use of millipedes for preparing compost. The most important advantage is the ability of the millipedes to feed upon raw organic materials without having to wait for them to decay microbially. The high gut microbial load of millipedes helps turn the raw organic waste into a highly nutrient-rich compost. Confining millipedes to a chamber is very cumbersome as they have high locomotory powers compared to earthworms. If taken care of this major handicap, it will be possible to produce millicompost on a commercial scale.

Many nutrients increase in quantity and diversity on faeces after they are dropped which is also evidence of the increase in microbial activity in faeces. Our results indicate that the nitrogen, phosphorus, potassium and calcium were higher and Carbon lesser in millicompost than vermicompost. This is mostly caused by the increase in nitrogen compounds with relative changes taking place in the amount of carbon compounds. The increasing trend of N, P, K and Ca and decreasing trend of C have been found by many researchers.

Earthworms have organic material, equal to their own body weight per day. One kilogram of worms can consume one kilogram of residues every day. The castings are rich in nitrate, available forms of phosphorus, potassium, calcium and magnesium. The passage of soil through earthworms promotes bacterial and actinomycetes growth. Misra *et al.,* (2003) pointed that the major drawback of the

vermicomposting process is that the temperature is not high enough for an acceptable pathogen kill (unlike the thermophilic composting methods), as it must be maintained at less than 35°C during the vermicomposting processes.

All the fertilizers tested in study supported the growth of plant selected. All the fertilizers contributed to the attainment of height by the plants that received the fertilizer treatment. After 4 weeks of growth, maximum growth was recorded in plant fertilized with millicompost.

Foliar proliferation is a measurement in plants to indicate the state of nutritional status. Healthy, well-nursed plants produce large number of leaves compared to less nutritional ones. Anilkumar *et al.,* (2011) studied the effect of different composts processed by *A. magna* and *E. fetida* on the growth parameters of *Capsicum annuum* (Carl Linnaeus); higher yield (10.6 ± 0.95), was recorded for millicompost compared to other composts (4.94±0.39). Similarly, we also found that, millicompost plant produced large number of leaves (28.5± 4.38) compared to vermiplant (12.12 ±3.46), and, control (9.3±2.51).

Maynard (1993) also reported increases in yield of compost-amended plants compared with those growing in soil alone. Millicompost produced by millipedes from selected waste materials resulted in better yield when applied to plants. Chemical fertilizers should be avoided and focus on natural organic fertilizers like millicompost as well other traditional eco-friendly farming methods should be promoted. Thus millicompost is an alternative method to minimize the waste quantity and produce mineral rich organic fertilizer.

Millipede composts derived from various organic substances showed significant enhancement in the physiochemical and biochemical constituents. Millipedes, among other soil fauna, are important components of ecosystems because of their role in nutrient cycling. Suresh *et al.,* (2014) stated the millicompost is not only ecologically safer than ordinary compost but also it is better associated with other eco-friendly soil fauna. The results obtained from our present investigation revealed that millicompost prepared from organic wastes processed by the millipedes, were highly effective than the compost produced by conventional methods, in terms of nutritional quality and also their effect on the growth of the vegetable plant Spinach.

Conclusion

Composting is an alternative and effective method for the burning issue of enhancing sedimentation of waste and their impact on human health and environment. It is the process of decomposing organic matter, such as kitchen scraps and yard waste, into a nutrient-rich soil amendment called compost. This natural process involves microorganisms, bacteria, fungi, and invertebrates breaking down organic materials into simpler compounds.

Both millipedes and earthworms are important in composting. Millicompost comprises of high level of nutrients (N, P, K) when compared to the compost processed by earthworms. It improves the water retention, soil texture and fertility than vermicompost. Since it is an asset in field of agriculture. It is significant to aware farmers the importance of millicomposting in waste recycling. The produced millicompost had a thick black colour, oval shape, mull-like soil odour and was homogeneous. The resulting millicompost had significant level of plant nutrients than vermicompost, indicating the success of getting an environment friendly nutrient rich manure for the agricultural crops. It is suggested that, future research should explore the potential for producing millicompost using different types of waste products and compost, benefitting both the ecosystem and humanity.

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SYNTHESIS, CHARACTERIZATION, AND APPLICATIONS OF FE_3O_4 NANOPARTICLES IN FERROFLUIDS: ADVANCES AND FUTURE PROSPECTS

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Abstract

This chapter explores the synthesis, characterization, and applications of Fe_3O_4 nanoparticles (NPs) within ferrofluids. Fe_3O_4 NPs were synthesized using the chemical co-precipitation method, yielding nanoparticles with a characteristic spinel structure, as confirmed by X-ray diffraction and UV-visible spectroscopy. Scanning electron microscopy revealed uniform spherical morphology and consistent size distribution. The ferrofluids, formulated with sunflower oil and oleic acid shows exceptional stability over a 15-day period, with no observable aggregation.

Key Words : Ferrofluids, Iron oxidenanoparticles, Powder XRD.

1. Introduction

Ferrofluids are colloidal liquids containing ferromagnetic nanoparticles (NPs) that exhibit unique properties when exposed to a magnetic field [1]. These fluids, composed of magnetic NPs suspended in a carrier liquid with surfactants to prevent agglomeration, have diverse applications due to their ability to alter physical characteristics under magnetic influence. The main types of magnetic fluids are ferrofluids and magnetorheological (MR) fluids, differentiated primarily by particle size [2]. Ferrofluids utilize nanoparticles in the nanometre range, leading to higher suspension stability compared to MR fluids with micron-sized particles. This stability is crucial for various applications such as dynamic loudspeakers, heat dissipation, and magnetic resonance imaging (MRI), where ferrofluids' distinct behaviour in magnetic fields manifests as self-organized structures like the Rosensweig instability.

The synthesis of ferrofluids involves producing iron oxide NPs, typically magnetite (Fe_3O_4) or maghemite (Fe_2O_3), through methods such as coprecipitation, thermal decomposition, and mechanical milling [3-6]. Coprecipitation is favoured for its simplicity and scalability, involving the reaction of iron sources in a water bath with a base to form magnetite. Thermal decomposition, using iron carbonyls or organometallics, offers precise control over NP size but is more complex. Mechanical milling provides an economical method for large-scale production but may result in less controlled NP size distribution and aggregation. Each synthesis method impacts the quality of ferrofluids, affecting their magnetic properties and stability.

Surfactants play a critical role in ferrofluid stability by coating NPs to prevent agglomeration and facilitate dispersion in the carrier liquid [7]. The choice of surfactant depends on the carrier liquid and the intended application. For instance, oleic acid is commonly used with nonpolar liquids like kerosene, while surfactants with hydrophilic tails, such as acrylic acid or chitosan, are chosen for polar media. The surfactants create a protective layer around the NPs, balancing attractive and repulsive forces to maintain stability. Additionally, the carrier liquid influences ferrofluid properties like viscosity and stability; common choices include water and kerosene, with water being preferred for biomedical applications due to its nontoxic nature[8-10].

Ferrofluids have a broad range of applications across various fields. In biomedical research, they enhance imaging techniques like MRI by serving as contrast agents that improve image quality [11-12]. Ferrofluids are also utilized in drug delivery, where their ability to cross biological barriers and target specific areas enhances the efficacy of treatments. In cancer therapy, magnetic fluid hyperthermia uses ferrofluids to generate localized heat in tumours, aiding in treatment. Other applications include antimicrobial and antifungal activities, water treatment for contaminant removal, and improving heat transfer in various engineering fields [13-15]. The versatility and effectiveness of ferrofluids continue to drive research and development in these diverse areas.

Recent studies highlight the versatility of iron oxide nanoparticles (IONPs) in various applications. Yusefi *et al.* (2021) demonstrated that IONPs coated with Punica granatum peel extract effectively manage temperature during hyperthermia

treatments [29], while Mazario et al. (2017) and Fotukian *et al.* (2020) found that IONPs and $CuFe_2O_4$, respectively, show promising results for magnetic hyperthermia[12,15]. Additionally, altering synthesis conditions, such as doping with cobalt, can enhance hyperthermia properties. IONPs are also prominent in cancer therapy, with drug-loaded IONPs coated with materials like hydroxyapatite, PEG, and citric acid showing strong potential. Notable drugs attached to IONPs include doxorubicin, paclitaxel, and methotrexate.

Beyond medical uses, IONPs offer significant advantages for environmental applications. Hatamie *et al.* (2016) showed that surfactant-free IONPs could reduce water turbidity and remove pollutants, including color and harmful ions, while also reducing chemical oxygen demand [7]. Additionally, IONPs effectively remove heavy metals, pharmaceuticals, and dyes from water and soil. Recent advancements include Fe_3O_4/bentonite composites for dye adsorption, Fe_3O_4/Au NPs for removing disulfine blue, and Fe_3O_4/CuO NPs for arsenic removal. These developments underscore the diverse utility of IONPs in both environmental and therapeutic contexts [16].

The present study is highly relevant in the context of ongoing advancements in nanotechnology and its applications across various fields. The synthesis and characterization of Fe_3O_4 nanoparticles and their incorporation into ferrofluids address current demands for high-performance materials with advanced properties for use in both biomedical and environmental applications. The study's scope encompasses the development of stable ferrofluids, essential for applications such as drug delivery systems, magnetic hyperthermia, and contaminant removal, where stability and efficacy are critical. The primary objective is to enhance the understanding of ferrofluid behaviour and stability, thereby paving the way for optimized and innovative uses of these materials.

2. Materials & Methods

2.1 Materials

Ammonia (NH_3), iron (III) chloride hexahydrate (FeCl3.6H2O), Iron (II)sulphateheptahydrate (FeSO4.7H2O) and oleic acid used were of analytical grade and procured from Merck, India. All the aqueous solutions were prepared by

using distilled water. Sun flower oil used to make ferrofluid was procured from local market.

2.2. Methods

2.2.1. Synthesis of Fe_3O_4 nanoparticles

Fe_3O_4 nanoparticles were prepared by the chemical co-precipitation methodas reported earlier [17]. AR grade FeSO4 and $FeCl_3$ were used as starting materials. For synthesis, 3ml of 2M FeSO4 and 12 ml of 1M$FeCl_3$ were mixed and homogenized at room temperature. The pH of the solution was adjusted to 11 by adding an ammonia solution (25%) dropwise. The mixture was then stirred for about one hour. The black precipitate formed was then centrifuged and washed several times with double distilled water to remove the salt residues and other impurities. The samples were annealed at 300 ˚C for one hour.

2.2.2. Synthesis of iron oxide ferrofluids using sunflower oil

Briefly, the synthesized magnetic nanoparticles (Fe_2O_3) were initially coated with oleic acid by mixing 5% (w/v) Fe_2O_3 nanoparticles with 10% (v/v) oleic acid and the resultant viscous solution was stirred vigorously for 1 hour at 40 °C. After stirring, the viscous solution was transferred into a beaker and diluted to 100 ml by adding sunflower oil to obtain colloidal solution. This colloidal solution was further stirred for 1 h at 70 °C to obtain stable nanofluid. After stirring, the prepared nanofluid was kept in a bottle and the stability of the ferrofluid was observed with respect to time.

2.3. Characterization Studies

Electronic spectrum of the synthesized nanoparticle emulsion in water was recorded on a Systronics UV–Visible spectrometer in the range 200-700 nm.The phase purity of nanoparticles was investigated using X-ray diffraction technique (XRD). Powder XRD was recorded on a PANalyticalX'Pert Pro Powder X'Celerator Diffractometer with Cu Kα (λ= 0.15406 Å) radiation. The morphology of the nanoparticles was studied bySEM analysis using Carl Zeiss EVO 18 research instrument.

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3. Result & Discussion

3.1. Powder XRD

The structural analysis of samples was done by a powder X-ray diffraction technique using CuKα radiation. Fig. 1 shows the powder X-ray diffraction patterns for as synthesized sample. The d values and intensities of the observed diffraction peaks match with the single crystalline spinel form of Fe_3O_4 nanoparticles. The X-ray diffraction pattern shows broad peaks indicating the ultrafine nature and small crystallite size of the particles[18]. The lattice parameters were calculated for these samples. The 'd' values and intensities of the observed diffraction peaks match with the single crystalline spinel form of Fe_3O_4 nanoparticles (JCPDS Card No. 019-0629). The discernible peaks can be indexed to (220), (311), (400), (511) and (440)[19,20]. X-ray diffraction pattern shows broad peaks indicating ultrafine nature and small crystallite size of the particles. The lattice parameter is 8.4090 Å. The observed lattice parameter is slightly higher than the standard value indicating that samples are under strain.

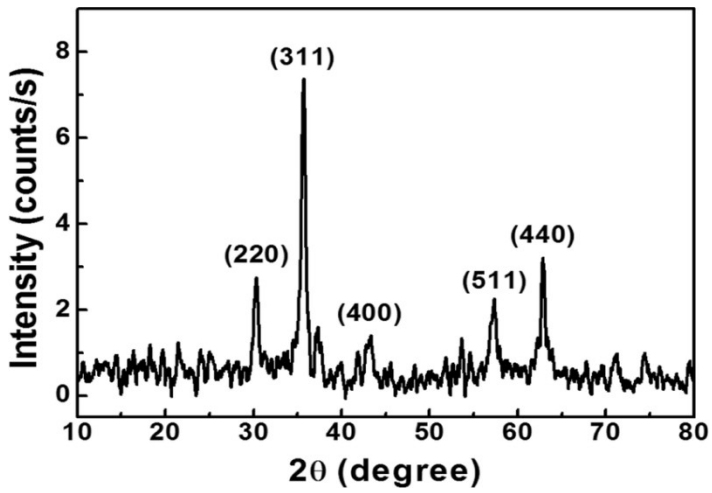

Fig 1: PXRD Pattern of Fe3O4 Nano Particles

The strain induced in the material and the crystallite size were determined by Williamson-Hall method;

$$\beta \cos\theta = 4\varepsilon \sin\theta + \lambda/D$$

where D is the crystallite size, λ is the wavelength of the X-ray, β is full width at half maximum (FWHM) measured in radians, ε is the induced strain in system, and

θ is the Bragg angle. The induced strain in the crystallites was 0.00155. The crystallite size of as-synthesized and annealed samples was then determined by using the Scherer relation. For the synthesized sample, the average crystallite size is 11.5 nm [21-22].

3.2 UV- Visible Spectrum

UV-Vis absorption spectrum of Fe_3O_4 nanostructure is shown in Fig. 2, represents the absorption band below 300 nm. From previously reported studies, it is ascertained that the peak below 300 nm is due to the Fe x O y (Fe_2O_3 and Fe_3O_4) NPs [23-25].

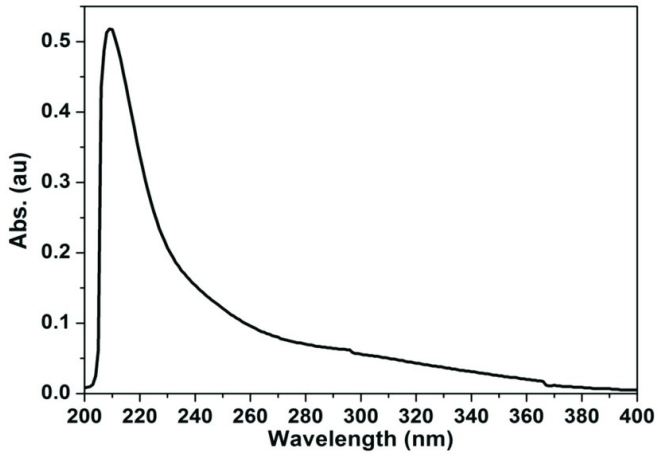

Fig 2: UV-Visible spectrum of Fe_3O_4 Nano Particles

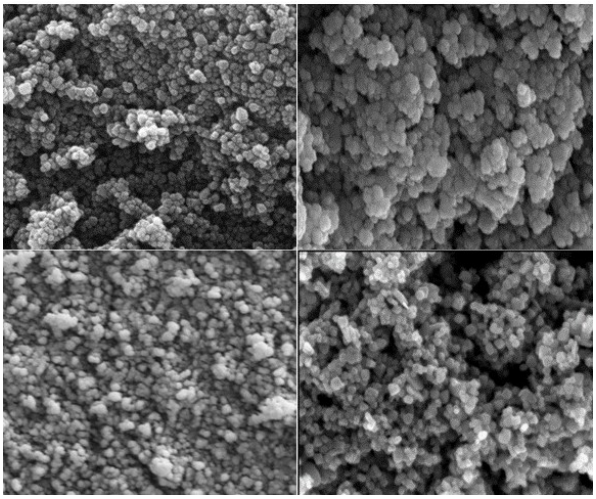

Fig 3: SEM images of Fe_3O_4 Nano Particles

3.3 SEM Analysis

The SEM images of the synthesized nanoparticles given in Fig. 3 reveal that they exhibit a relatively uniform spherical morphology. The particles appear well-defined, with a consistent size distribution that aligns with the intended synthesis parameters. The spherical shape of the nanoparticles is indicative of the successful formation of magnetite, as irregular shapes or agglomeration would suggest incomplete synthesis or a need for optimization in the process [26]. The smooth surfaces of the particles, as shown in the images, further support the high purity and well-controlled synthesis of the Fe_3O_4 nanoparticles [28-29].

3.3. Stability of ferrofluids

Since stability is the most important characteristics of ferrofluid, the detailed stability analysis of prepared ferrofluids was conducted by observing the aggregation behaviour of ferrofluids with respect to time [27]. In this work, the stability analysis of 1.5 vol% nanoparticle based ferrofluids was conducted by storing the ferrofluids in a beaker in an open ambience for 15 days. Digital images of the ferrofluids were taken in regular intervals of time to observe the aggregation behaviour of nanoparticles in fluid. The digital images of ferrofluids with respect to time were shown in Fig.4. No sign of aggregation has been noticed in the beaker even after 15 days. From the figure, it is clear that flocs are not formed and the ferrofluid is stable.

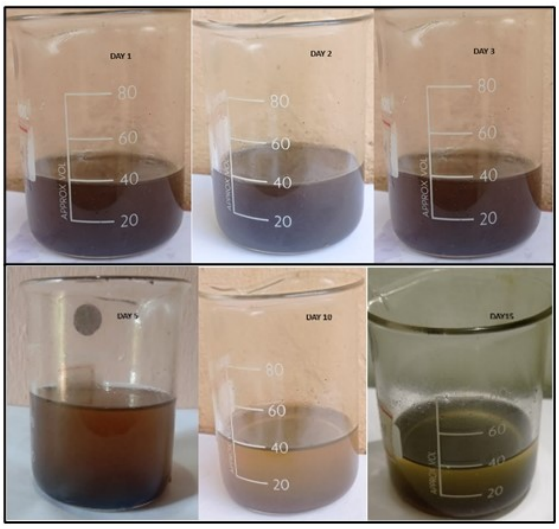

Fig. 4: Stability of Ferrofluid in Sunflower oil medium.

4. Conclusion

The synthesis and characterization of Fe3O4 nanoparticles and their subsequent formulation into ferrofluids demonstrate promising results for both stability and application potential. The Fe3O4 nanoparticles were successfully synthesized using the chemical co-precipitation method, exhibiting characteristic X-ray diffraction patterns and UV-visible absorption features indicative of high purity and fine particle size. The stability of the ferrofluids, prepared with sunflower oil and oleic acid, was rigorously tested over a 15-day period, showing no aggregation or instability, which highlights their suitability for practical applications.

The findings underscore the effectiveness of the synthesized ferrofluids for various applications, particularly in biomedical and environmental contexts. The stable ferrofluids retain their properties over time, which is crucial for their use in drug delivery systems, magnetic hyperthermia, and contaminant removal. This study paves the way for further exploration of ferrofluids in advanced applications, demonstrating their versatility and reliability as functional nanofluids. Future work could focus on optimizing the synthesis methods and exploring additional applications to fully harness the potential of these materials.

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FROM SCIENCE TO STORY TELLING: REIMAGINING MEDICINE THROUGH THE HUMANITIES

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Medical humanities is an interdisciplinary field that explores the intersection of medicine and the humanities, encompassing various disciplines such as literature, philosophy, ethics, history, anthropology, sociology, art, and cultural studies. It seeks to provide a broader perspective on medical practice, education, and research by examining the human experience of illness, suffering, healing, and caregiving through the lens of the humanities. The field of medical humanities aims to enhance healthcare professionals' understanding of patients as individuals with unique stories, beliefs, and cultural backgrounds. It also emphasizes the importance of effective communication, empathy, and ethical decision-making in medical practice. By incorporating the humanities into medical education and training, healthcare providers can develop a more holistic approach to patient care and a deeper appreciation for the social and cultural contexts of health and illness.

Medical Humanities: An Introduction defines "Medical Humanities as an inter- and multidisciplinary field that explores contexts, experiences, and critical and conceptual issues in medicine and health care, while supporting professional identity formation."(Carlin et al, ix) Evans in *Reflections on the Humanities in Medical Education* (2002) defines as "an integrated, interdisciplinary, philosophical approach to recording and interpreting human experiences of illness, disability, and medical intervention…"and as an interdisciplinary field concerned with understanding the human condition of health and humanities in order to create knowledgeable and sensitive health care providers, patients and family care givers. (Klugman 422) It draws on the "creative and intellectual strengths of diverse disciplines including literature, art, creative writing, drama, film, music, philosophy, ethical decision making, anthropology and history in pursuit of medical educational goals."(Heady et al 492)

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The field of medical humanities aims to foster a deeper understanding of the human dimensions of health, illness, and healthcare practices. It recognizes that medicine is not solely a scientific endeavour but is also influenced by social, cultural, ethical, and historical factors. By incorporating humanities perspectives into medical education, research, and practice, medical humanities seek to enhance healthcare providers' ability to engage with patients, empathize with their experiences, and provide compassionate and patient-centred care.(Carlin et al 8)

Medical humanities explore a wide range of topics, including the patient-doctor relationship, the experience of illness and suffering, the social and cultural dimensions of health and healing, the history and ethics of medical practice, medical narratives in literature and film, the portrayal of medicine in the arts, and the role of technology in healthcare, among others. By integrating the humanities into medical education, medical humanities programs encourage healthcare professionals to reflect critically on their own beliefs, values, and assumptions, fostering self-awareness, empathy, and ethical decision-making skills. This interdisciplinary approach also allows for a broader understanding of health and illness, encouraging a more holistic and humanistic approach to patient care. Medical humanities contribute to the humanization of medicine, providing insights and perspectives that enrich medical education, research, and practice, and promote a deeper appreciation of the complex and multifaceted nature of healthcare. (Carlin et al 9)

The core elements of physicianship are anchored in the arts and humanities including forming deeper connections with patients, maintaining joy and meaning in medicine, and developing empathy and empathy and resilience. Integration of humanities / arts into medical education, it is suggested, can support learners developing essential qualities such as professionalism, self awareness, communication skills and reflective practice.(Wald et al 492)

Medical humanities aim to enhance the understanding of medical practices and patient experiences through the study of literature, philosophy, history, ethics, and other humanities disciplines. Medical ethics, medical history, narrative medicine, literature and medicine, medical anthropology, philosophy of medicine, medical sociology, medical education, visual and performing arts in medicine, health humanities, cultural competence, end-of-life care and palliative medicine,

and patient advocacy are just a few of the many sub-disciplines and specialised areas that make up the field of medical humanities.

Medical Ethics explores the complex ethical challenges within medicine, healthcare, and biomedical research, delving into critical topics such as informed consent, end-of-life care, organ transplantation, and the rights of patients and healthcare providers.

Medical History investigates the historical development of medicine, medical practices, and healthcare systems. It explores how medical knowledge and technologies have evolved over time and how past medical practices impact present-day healthcare.

Narrative Medicine emphasizes the importance of storytelling and patient narratives in understanding illness and healing. It encourages healthcare professionals to engage with patients' stories to provide more compassionate and patient-centered care.

Literature and Medicine explores literary works (e.g., novels, poetry, drama) that depict medical themes, patient experiences, and the human condition in the context of illness and healthcare. This sub-discipline helps healthcare providers develop empathy and insight into patients' emotional struggles.

Medical Anthropology studies the cultural, social, and behavioral aspects of health, illness, and healing practices in different societies. It examines how cultural beliefs and practices influence medical decision-making and health outcomes.

Philosophy of Medicineanalyzes the fundamental concepts and philosophical questions related to medicine, health, and disease. It explores topics such as the nature of health and illness, the role of medicine in society, and the concepts of disease and disability.

Medical Sociology investigates the social factors that influence health and healthcare. It examines issues like health disparities, access to healthcare, patient-doctor relationships, and the impact of societal norms on health behaviours.

Medical Education focuses on the pedagogy and training of healthcare professionals. It seeks to improve medical education through the integration of humanistic approaches, promoting empathy and compassion in medical practice.

Visual and Performing Arts in Medicine explores the use of visual arts, music, theater, and other performing arts to enhance healing, patient well-being, and medical education.

Health Humanities is an umbrella term that encompasses a range of interdisciplinary approaches to understanding health and healthcare through the humanities. It integrates diverse perspectives to deepen insights into health and illness, enhance cultural competence in healthcare settings, and address complexities in 'End-of-life care and Palliative medicine.' Additionally, it emphasizes the critical importance of Patientadvocacy in supporting patients' rights and needs.

Medical humanities programs can be found in various academic institutions, and they often involve collaborations between healthcare professionals, humanities scholars, and artists. The integration of medical humanities into medical education and clinical practice fosters a more compassionate and patient-centered healthcare system. Additionally, it encourages a critical examination of the ethical and societal implications of medical advancements and healthcare policies.

Pathography is an essential aspect of medical humanities as it contributes to the understanding of the human experience of illness. Through pathographies, healthcare professionals, students, and researchers can gain insights into patients' perspectives, which are not always fully captured in medical records or clinical data. Pathographies help to humanize the medical experience, fostering empathy and a deeper appreciation for the lived experiences of those facing illness.

Dunglison introduced the term pathography in his *Medical Lexicon* in 1853,as a description of disease. (648) In medical humanities, the classic text on pathography is Ann Hunsaker Hawkin's *Reconstructing Illness*. She describes the book as "a study of the myths and attitudes and assumptions that inform the way we deal with illness." Hawkins takes pathographies to be autobiographies and biographies of illness- in other words, narratives of illness: path (illness) + graphy(narrative). (Carlin 2)

The term "pathography" was used by medical sociologist Arthur Frank in his book *The Wounded Storyteller: Body, Illness, and Ethics*(1995). In this

influential work, Frank explores the narratives of illness and the role of storytelling in coping with and making sense of the experience of illness. He introduced the concept of "pathography" to describe personal accounts of illness written by the individuals who have experienced it, as well as those written by their close family members or caregivers. Frank classified illness narrative as chaos narratives, quest narratives and restitution narratives. The restitution narrative, in which the doctor is presented as a hero. The chaos narrative, in which one terrible thing after another happens. Quest narratives are more hopefuland are written by patients who survive the illness and reconstruct a new future. (Dosani 275)

Four different groups of pathographies emerge when one analyzes them according to authorial intent. The first might be called "didactic pathographies." These narratives are motivated by the explicit wish to help others. A second category consists of "angry pathographies." Authors of these are motivated by a strong need, based on personal experience, to point out deficiencies in various aspects of patient care. A third type of narrative, the "alternatives pathography," is also critical of our medical system, but without angry denunciations or doctor-bashing. Like their angry counterparts, these pathographies stem from dissatisfaction with medicine. A fourth and very recent group, which might be called "ecopathography," links a personal experience of illness with larger environmental, political, or cultural problems.(Hawkins 128)

Frank's groundbreaking work shed light on the importance of patient narratives and their impact on the experience of illness, medical practice, and the doctor-patient relationship. Since then, the term "pathography" has become widely used in medical humanities and related fields to refer to autobiographical writings and personal stories about illness and health challenges. These narratives play a significant role in fostering empathy, understanding, and communication between healthcare professionals and patients, as well as in raising awareness about the complexities of the human experience of illness.

Medical humanities scholars often analyze and study pathographies to explore themes such as patient empowerment, coping mechanisms, the impact of illness on identity, the doctor-patient relationship, and the ethical considerations related to storytelling and patient privacy. By incorporating pathographies into medical education and research, the field of medical humanities can further its

mission of promoting patient-centered care and enhancing healthcare providers' ability to engage with and understand their patients on a more profound level. Additionally, pathographies can contribute to the broader cultural understanding of illness and inspire discussions about healthcare practices, policy, and patient advocacy.

Pathographies come in various forms, and individuals may choose different ways to share their experiences with illness. Memoirs are autobiographical accounts of a person's life, often focusing on specific aspects, such as their experience with a particular illness or health condition. In a medical context, these memoirs delve into the emotional and physical journey of the author through their illness, providing a deeply personal perspective. Diaries and journals are written records that chronicle an individual's thoughts, feelings, and experiences over a period of time. In the context of pathography, people may keep diaries or journals to document their daily struggles, symptoms, treatments, and emotional responses to their illness.

With the advent of the internet and social media, many people share their pathographies through blogs. These online platforms allow individuals to reach a wider audience and connect with others who may be going through similar experiences. Blogging can serve as a form of self-expression and a means of providing support to others.Books can range from deeply personal narratives to more informative and advocacy-oriented pieces, addressing issues related to healthcare systems, patient rights, and medical ethics.Some pathographies take the form of poetry, short stories, or other creative writing formats. These artistic expressions allow individuals to convey their emotions and experiences in a unique and evocative way.

While not strictly written pathographies, documentaries, web series and films can also be considered as visual pathographies. In these mediums, individuals may share their journeys through illness or explore the experiences of others facing health challenges.Online forums and support groups provide spaces for individuals to share their pathographies with others who have similar health conditions. These platforms encourage dialogue, understanding, and mutual support among those facing similar challenges. Some individuals share their pathographies through shorter posts on social media platforms like Facebook, Twitter, or Instagram. These

concise accounts can be powerful in raising awareness and connecting with others. Regardless of the form they take, pathographies play a crucial role in the medical humanities by providing insights into the human experience of illness, promoting empathy, and fostering a deeper understanding of the challenges and triumphs faced by individuals living with health conditions.

Pathography and illness narrative are related concepts that both involve personal accounts of individuals' experiences with illness. While they share similarities, they have distinct characteristics and purposes. As mentioned earlier, a pathography is a personal account of illness written by the individual who has experienced it or by someone close to them, such as a family member or caregiver. It is an autobiographical narrative that provides a firsthand perspective of the challenges, emotions, and coping mechanisms associated with living with a particular health condition.

An illness narrative is a broader term that encompasses all forms of storytelling related to the experience of illness, including both pathographies and narratives told by others about a person's illness. Illness narratives can be composed by patients, family members, friends, healthcare providers, or even authors and filmmakers who create fictional accounts of illness experiences. They aim to communicate the complexities of the illness experience, highlight the emotional and psychological impact of illness, and often serve as a means of seeking understanding, healing, and connection.

While pathography specifically refers to the patient's own account of their illness, illness narratives can include multiple perspectives and may incorporate different voices and viewpoints. For example, a family member might write an illness narrative from their perspective, offering insights into the challenges faced by the patient and the family as a whole.Both pathography and illness narrative play significant roles in medical humanities and healthcare contexts by humanizing medicine, fostering empathy, and promoting patient-centered care. They allow individuals to share their stories, provide insights into the lived experience of illness, and contribute to a more comprehensive understanding of health, suffering, and healing. Additionally, they can raise awareness about specific health conditions, challenge stigmas associated with illness, and inspire advocacy efforts to improve healthcare systems and patient support.

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In “Past, Present and Imaginary: Pathography in All Its Form” (2021) Jutel and Russell have the opinion that, we live in the age of diagnosis. The culture of storying and explaining lives by using diagnostic language shows the degree to which diagnosis is used in an explanatory capacity in high income countries of early twenty first century…Pathographies are a form of medicalisation because they simultaneously expand the signifiers of illness and disorders, and extend the diagnostic language we have to talk about them, reducing to individuals body and brains what may have been politically or contextually driven.(13)

Narrative medicine is an interdisciplinary approach to healthcare that emphasizes the importance of storytelling and narratives in medicine. It integrates the principles and methods of literary studies and the humanities with clinical practice, medical education, and healthcare research. It emphasizes the importance of storytelling and narrative skills in medicine, recognizing that narratives play a central role in the way we understand, communicate, and make meaning of our experiences, including illness and healthcare encounters. It was developed by physician and literary scholar Dr. Rita Charon in the late 1990s. Narrative medicine seeks to integrate the principles of the humanities, particularly narrative and storytelling, into medical practice, education, and research.

The core premise of narrative medicine is that effective healthcare relies not only on clinical expertise and technical skills but also on the ability of healthcare providers to listen to and understand their patients' stories. By engaging with patients' narratives, healthcare professionals can gain deeper insights into the individual's experience of illness, their values, beliefs, and emotions related to their health, and the impact of illness on their lives.

Close Reading and Interpretation : Narrative medicine encourages healthcare providers to carefully listen to and "read" patients' narratives with attention to both the explicit and implicit meanings. By paying attention to the details and nuances of the patient's story, healthcare professionals can better understand the patient's unique perspective and experiences.

Empathy and Perspective-Taking : Narrative medicine emphasizes the cultivation of empathy and the ability to see the world from the patient's point of

view. This understanding helps healthcare providers connect more effectively with their patients and deliver patient-centered care.

Reflective Practice : Practitioners of narrative medicine are encouraged to reflect on their own beliefs, biases, and emotions in response to patients' narratives. This self-awareness can help healthcare providers provide more compassionate and non-judgmental care.

Narrative Ethics: The field of narrative medicine also explores ethical considerations related to storytelling in medicine, such as issues of confidentiality, consent, and the use of patient narratives in research and education.

Teaching and Learning : Narrative medicine is integrated into medical education, enabling students to develop skills in narrative competence. This includes learning how to elicit patients' narratives, interpret them effectively, and use storytelling as a tool for diagnosis and treatment planning.

By embracing narrative medicine, healthcare providers can foster better communication with their patients, enhance the doctor-patient relationship, and improve patient outcomes. It also allows for a more holistic understanding of health and illness, recognizing that illness experiences are not just medical conditions but deeply intertwined with the patient's personal, cultural, and social contexts. Additionally, narrative medicine contributes to the humanization of medicine and supports the emotional and psychological well-being of both patients and healthcare providers.

Narrative medicine plays a significant role in understanding and addressing mental disorders. By incorporating narrative approaches into mental healthcare, practitioners can gain deeper insights into patients' experiences, foster empathy, and enhance therapeutic relationships. Here's how narrative medicine can be applied in the context of mental disorders:

Patient-Centered Care : Narrative medicine places the patient's story at the center of healthcare. In the case of mental disorders, it encourages clinicians to actively listen to patients' narratives of their struggles, symptoms, and emotions. This patient-centered approach helps in developing a more comprehensive understanding of the individual's unique experience of their mental health challenges.

Therapeutic Storytelling : Encouraging patients to share their experiences through storytelling can be therapeutic. It allows patients to give voice to their feelings and thoughts, leading to increased self-awareness and a sense of empowerment. Storytelling can also help patients create meaning out of their mental health journey, which can be crucial for recovery and resilience.

Narrative Assessment: Instead of solely relying on standardized assessments, narrative medicine allows clinicians to use patients' own stories to assess their mental health. Understanding patients' narratives can provide context and additional information that may not be captured in traditional assessments, leading to more accurate diagnoses and treatment plans.

Breaking Stigma: Narrative medicine can challenge the stigma surrounding mental disorders by sharing authentic stories of individuals living with mental health conditions. By listening to these narratives, healthcare providers and the broader society can develop more empathetic and compassionate attitudes towards those experiencing mental health challenges.

Understanding Lived Experience: Mental disorders can be complex and diverse, and each person's experience is unique. By embracing the narrative approach, mental healthcare providers can gain a more nuanced understanding of the lived experiences of individuals with mental health conditions. This understanding can inform personalized treatment plans and support strategies.

Therapeutic Writing: Writing therapy, a form of narrative medicine, can be beneficial for individuals with mental disorders. It allows patients to express their emotions, process trauma, and explore their thoughts in a safe and structured manner.

Support Groups: Narrative medicine can be integrated into support groups for individuals with mental health conditions. Sharing stories in a group setting can foster a sense of belonging, reduce feelings of isolation, and provide validation and support.

Provider-Patient Communication: Narrative medicine can improve communication between mental healthcare providers and their patients. By actively engaging with patients' narratives, providers can develop deeper connections, which can enhance treatment adherence and outcomes.

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It provides a holistic and humanistic approach to mental healthcare, recognizing the importance of patients' stories in understanding and addressing mental disorders. By incorporating narrative approaches into mental health practice, clinicians can provide more compassionate, individualized, and effective care to their patients.

Bradley Lewis in "Narrative and Psychiatry"(2011) discusses about the importance of narrative in psychiatry. Psychiatry is perhaps the most "narrative" of all medical specialties, but here as elsewhere clinical skills are in danger of being lost as evidence-based medicine becomes the dominant paradigm in medical culture. **Narrative psychiatry** provides an opportunity to put science in perspective and to keep empathetic and meaningful connections with patients at the centre of psychiatric education, research and practice.

Narrative psychiatrists have picked up these insights from narrative medicine and narrative psychotherapy and started applying them to psychiatry. Like narrative physicians, narrative psychiatrists practice in a person-centered style that puts first and foremost an empathic connection with patients and clinical stories. And, like narrative psychotherapists, narrative psychiatrists recognize that the stories people tell about themselves not only describe their lives, but also shape their lives. These culturally located 'self' stories scaffold narrative identity and provide a compass for living. They tell us where we have been and where we are now, and they provide us with a trajectory into the future. If the stories are not working, then new stories may be needed… Beyond medications, the larger implication of narrative psychiatry is that there are many ways to tell the story of psychic difficulties and differences. There is not just one right way and many other wrong ones. All the models of mental illness involve a process of story telling and story retelling. Each provides an alternative understanding of our past and our present and each suggests different routes forward. These different routes include different meanings, different practices, different rituals, different communities, different locations, different side-effects, different costs, etc.(491)

The intersection of narrative medicine and film highlights the role of storytelling in exploring the complexities of illness, healthcare, and the human condition. Medical documentaries, through their authentic depictions of real-life medical scenarios, patient experiences, and healthcare systems, bridge the gap between clinical detachment and the subjective realities of patients. These visual

narratives illuminate ethical challenges, emotional struggles, and societal implications, making them valuable tools in medical education for fostering reflection, empathy, and critical thinking among healthcare professionals. Similarly, fictional films and adaptations of real-life experiences delve into the emotional, psychological, and social dimensions of illness, offering nuanced portrayals of patient struggles while addressing themes such as healthcare delivery, patient advocacy, and the broader impacts of disease on individuals and families. Both genres provoke critical discussions on the moral and ethical dimensions of caregiving and inspire a patient-centered approach to medicine. By humanizing healthcare and advocating for systemic change, films and documentaries align with the principles of narrative medicine, emphasizing the importance of understanding patients' stories to enhance empathy, improve medical education, and ensure compassionate care.

Medical humanities hold transformative potential for enriching healthcare by bridging the gap between medicine and the humanities through an interdisciplinary lens. By integrating fields such as literature, philosophy, art, sociology, history, and cultural studies, medical humanities provide a comprehensive framework to examine the human dimensions of health, illness, and caregiving. This interdisciplinary approach encourages healthcare professionals to move beyond a purely scientific understanding of medicine, fostering empathy, critical reflection, and ethical decision-making. Sub-disciplines like narrative medicine, medical ethics, and medical anthropology demonstrate the potential of storytelling, cultural analysis, and ethical reasoning in shaping patient-centered care and improving health outcomes. Pathographies and the use of visual and performing arts further emphasize the importance of capturing lived experiences, enhancing communication, and building resilience among both patients and providers.

The interdisciplinary nature of medical humanities makes it uniquely positioned to address complex healthcare challenges by drawing on diverse intellectual traditions to inform practice, education, and policy. It promotes a holistic understanding of the social, cultural, and psychological contexts of health and illness, paving the way for a more inclusive and compassionate healthcare system. Additionally, healthcare workers benefit directly from engaging with

medical humanities, as it fosters self-awareness, resilience, and emotional intelligence, reducing burnout and enhancing their ability to navigate the complexities of patient care. By humanizing medical practice and nurturing the professional identity of caregivers, medical humanities contribute to a healthcare system that values both scientific rigor and profound human connections, engaging with broader ethical and societal implications in innovative and impactful ways.

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MICROPLASTICS (MPS) AND NANOPLASTICS (NPS) AS EMERGING CONTAMINANTS IN THE ENVIRONMENT

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Abstract

The present study examines the distribution and contamination produced by microplastics and nanoplastics, which are extensively dispersed throughout the environment. Microplastics seriously threaten human survival and marine life in both terrestrial and marine ecosystems. Environmental media contain nanoplastic samples, and their toxicity has an undifferentiated effect on living things. Local governments ought to enact stringent laws, reduce the use of single-use plastics, encourage recycling, outlaw the main microplastics found in cosmetics, and enhance waste management procedures.

Keywords: Microplastics, pollution, nanoplastics, environmental media, waste management.

Introduction

Sources of Microplastics and Nano Plastics

The Greek word Plastikos meaning capable of being shaped or molded was discovered due to itsvaried properties (Hammer et al., 2012). The term "microplastics" refers to small plastic particles discharged into marine habitats that measure less than 5 mm in size (Duis and Coors, 2016). Different marine biota, such as corals, plankton, marine invertebrates, fish, and whales, can ingest these microplastics, which ultimately go up the food chain (Sharma and Chatterjee, 2017). In addition to directly endangering marine life, these plastic polymers indirectly impact the environment by absorbing other marine contaminants. Microplastics can easily absorb hydrophobic contaminants from the aquatic system because of their huge surface-to-volume ratio. Due to its detrimental effects, notably on the health and biota of the ocean, microplastic contamination will be a serious problem shortly.

Due to the cost-effectiveness and availability, its production and use have dramatically increased, leading to anaccumulation of non-recycled syntheticplastic polymers in terrestrial and aquatic ecosystems.Wastewater treatment plant effluent, industrial manufacturing, synthetic textiles, andthe shredding of larger anthropogenic litter into smaller pieces are allsources of plastic litter to aquatic ecosystems.The World's ever-increasing use of plastics and especially the lack of proper disposal strategies and infrastructures have created large areas of floating waste in the oceans, the so-called plastic soup. An astonishing 10% of the 8.3 billion tons of plastic produced globally to date has accumulated in marine and freshwater systems as plastic debris, making plastic a fast-growing environmental concern.

In addition, if the current rate of plastic waste generation continues then by 2050 oceans will have more plastics than fish (Geyer *et al.,* 2017). As the global manufacturing and application of plastic has increased, microplastics have been identified as emerging hazardous contaminants persistent in marine habitats. The primary sources of microplastic contamination in the aquatic ecosystem include residential runoff, which contains microbeads and microplastic pieces (used in cosmetics and other consumer goods), as well as the shattering of huge plastic garbage (Andrady, 2011).

The main sources of synthetic organic polymers used to make plastics are oil and gas, The two categories of polymers are thermosets and thermoplastics, The thermoplastic group includes materials like polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyesters, while thermosets include polyurethane (PU) and epoxy resins. Among the various polymer kinds, PE and PP have shorter life cycles and hence are overrepresented in the garbage (Geyer et al., 2017).

Microplastic contamination in the marine ecosystem is also a result of coastal human activities, such as fishing, tourism, and marine businesses. Once introduced to the aquatic environment, microplastics are subject to variousphysico-chemical processes, including weathering, biofouling and the leaching or assimilation of secondary pollutants. The floating debris in water bodies andplastic waste on land gradually fragment into smaller particles thateventually become microplastics (MPs) and even nanoplastics(NPs). For many years, researchers thought that the oceanacted as the primary global plastic sink. However,

researchers recentlyconcluded that terrestrial ecosystems andsoil in particular are also MP sinks.Microplastics, with sizes lower than 5 mm, are fragmented in the environment into thousands of nanoplastics, with sizes lower than 1 micron. Nanoplastics have ahighersurface area to volume ratio they also exhibit significantly higher reactivity and adsorption capacity. Being more abundant than microplastics, nanoplastics can reach in distant locations, they also can form biofilms with microbes, organic matter and pollutants. Plastic particles present in the environment may reach individualsthrough inhalation of airborne particles, via food products, and via dermal exposure. MPs and NPs can also be consumedindirectly via personal care products such as toothpaste, body wash, face scrub, lip balm,or other cosmetics.

Formation of Microplastics

A combination of several environmental factors such as sunlight and temperature, and the properties of the polymers such as size and density are some of the factors that influence the disintegration of macroplastic debris. The photodegradation of the plastics is caused by the exposure of larger plastic debris to UV radiation from the sun that oxidizes the polymer matrix in turn leading to the cleavage of chemical bonds. plastics are found in many different types of media including surface water, soils, and sediments all over the world (Ostle et al., 2019). Small-sized plastic formation is more on beaches by the influence of high UV radiation, wave actions and turbulence results in brittle and cracks on the plastic surface followed by yellowing. Once these fragments submerge into surface waters, or deeper environments, the cooler temperatures, and reduced exposure to UV light render their breakdown that continues until the fragments become smaller over time and become microplastics and even nano fractions in size (Cole et al., 2011).Plastics that reach land or aquatic bodies gets deteriorate and fragment into varied sizes by the action of some external environmental factors and for which standardized categorisation has been proposed: mega-plastics (> 1 m), macroplastics (2.5 cm – 1 m), mesoplastics (5 mm - 2.5cm), microplastics (1 μm - 5 mm), and nano plastics (< 0.1 μm) (Cordova, et al., 2018).

Oceans are considered as the ultimate sinks of microplastics because the plastic debris that generally originates on land accounts for 80% of the plastics found in marine litter (Andrady, 2011). Marine industries, recreational and

commercial fishing, maritime transportation, and coastal tourism are all sources of plastic pollution thatcan contaminate the ocean and endanger its biota. A variety of plastics that have been thrown on the beaches and coastal resorts by tourism activities will later be carried out by the in-shore and ocean currents leading to the accumulation of marine debris.

Another source of microplastic contamination is the release of microplastics from car tires, road markings, marine coatings, city dust, shoes, toothbrushes, plastic pellets, straws, fishing nets, etc. Discarded fishing gear is a significant and frequent source of plastic trash in the maritime environment. Unintentional plastic leaks during shipping and direct discharge from processing plants are two more major sources of plastic waste on land and at sea. However, because it is challenging to measure the amount, density, and distribution of small plastic debris in the marine environment, the research focuses primarily on this type of waste.

Distribution of microplastics in the marine environment

Plastics are capable of lasting for tens to hundreds of years, during which time they will change and break up into smaller pieces (Andrady, 2011). According to Teuten et al. (2009), about 60% of all plastic and its derivatives are less dense than saltwater. Microplastics accumulate in many ecosystems as a result of the direct or indirect transportation of plastics in aquatic systems via winds, contaminated effluent emissions, soil leaching, and other comparable processes. Biofouling activity can cause microplastics to sink to the bottom of a sea, while de-fouling activity can bring them back to the surface. The majority of the microplastics found in the maritime environment of Asia were classified as secondary microplastics. China ranks among the top three Asian nations. The Central Pollution Control Board study from 2021 states that India generates roughly 160000 metric tonnes of trash per day (TPD). 95.4% of the garbage is appropriately collected, 50% is handled, 18% is dumped in a landfill, and roughly 32% is left untreated or unaccounted for.

Primary microplastics are a significant source of plastic in the oceans

Primary microplastics from human activity are discharged into the world's oceans, while the rest are likely trapped in sewage sludge or soil. Their long-term fate and impact are uncertain and contingent on local conditions and customs. The

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contribution of primary sources may be substantial when comparing these releases of primary microplastics to recognized sources of secondary microplastics, such as plastic debris and abandoned fishing nets. The degradation of synthetic tyres and textiles accounts for two-thirds of the emissions; city dust and personal hygiene products also play a significant role.

Occurrence of micro- and nano-plastics in food

Nowadays, the extensive use of plastics and theirwidespread occurrence in the environment has resulted in thepresence of MPs in the food chain and exposure of consumers.While studies have reported the presence of MPs in drinking water,seafood, sea salt, sugar, honey and beer, there is generally littleinformation on the occurrence of MPs in food.MP contamination is reported in a variety of fish and seafood products, namely seabass, whiting, flatfish, lizardfish, grouper, mullet, barracuda, tuna, mackerel, haddock, plaice, sardines, anchovies, sprats, as well as mussels, clams, oysters, shrimps, prawns, squids, crabs, scampi, urchins, scallops, cockles, and periwinkles.

Another potential risk of microplastics and nanoplastics is their colonization by microorganisms such as the formation of bioflms (Wang et al. 2021). Biofilms typically contain bacteria, fungi, and algae. bacteria attach to plastic surfaces, extracellular polymeric compounds are released, and bacteria proliferate within biofilms. Proteins, nucleic acids, carbohydrates, and lipids are examples of extracellular polymeric materials that shield microorganisms in biofilms from physical abrasion, water shearing, and photodegradation. Microbial development in biofilms may be influenced by plastic properties like surface functional groups and roughness.

Fig 1: Micro- and nano-plastics in food

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Detection and identification of micro- and nano-plastics in food

Emerging environmental contaminants are micro and nanoplastics, which vary in size, shape, density, and polymer type. The complex nature of these not only provides issues for chemical identification and quantification but also requires the employment of modern analytical methods

Fourier-transform infrared spectroscopy (FTIR)

The most used analytical technique for assessing MP contamination in food and beverages. Investigating the chemical makeup of unknown plastic pieces has been more popular recently in microplastic (MP) pollution studies. It is also used to evaluate the degree of ageing of MPs collected from the environment. The degree of ageing can be assessed using different indexes known to be related to changes in MPs: Carbonyl Index (CI), Hydroxyl Index (HI), and Carbon-Oxygen Index (COI). Recent developments in μ-FTIR (micro-FTIR) imaging have made it possible to automatically identify MPs that are concentrated on the filter membrane without the need for pre-sorting. Focal plane array-based μ-FTIR imaging, which objectively measures the entire filter surfaces, significantly reduced analysis time and improved result accuracy. As of right now, FTIR remains the most popular method for routine MP analysis. Because Reflectance-FTIR is a non-contact method, it is time-efficient, automatable, and non-destructive. Reflectance-FTIR, as opposed to transmission-FTIR, does not require infrared-transparent substrates and can analyze thicker or more opaque samples that would normally absorb all incident infrared light. Reflectance-FTIR spectroscopic imaging can therefore screen microplastics over a wide region of filter paper when utilized in an imaging configuration (such as a multi-pixel array detector), removing the need for an initial visual inspection of the particles. However, because identification was based on a small number of absorption bands, these methods were confined to screening for a small number of plastic kinds.

Raman spectroscopy

Our civilization now cannot overlook the environmental issue of microplastic contamination. The detection of microplastics has made extensive use of Raman spectroscopy technology because of its non-contact, non-destructive chemical specificity**.** Single-point detection is used in traditional point confocal

Raman micro-spectroscopy technology, which results in lengthy measurement durations to scan the usual sample's wide areas of interest. The line-scan An advancement in rapid imaging technology is Raman spectroscopy. Its excitation is a line that is focused on the diffraction limit only in one of the two transversal directions. This allows all points on the line to be interrogated simultaneously, increasing speed by the number of resolved points along the line, usually by two orders of magnitude. The most produced and often found nanoplastic in the natural environment is trace polystyrene (PS), which may be easily identified using surface-enhanced Raman spectroscopy (SERS). SERS mapping may be used to acquire a series of Raman spectra containing chemical information when PS nano plastics were encircled by SERS-active silver nanoparticles (AgNPs). A rapid and thorough examination of the nanoplastics was made possible by this map, which displayed the possible PS distribution of the particles on a silicon wafer. The ability of SERS to examine nanoplastics by detecting hitherto undetected plastic particles as small as ~50 nm spiking in actual water. However, some obstacles can make it challenging to analyze microplastics and nanoplastics using Raman spectroscopy, especially when using Raman imaging. Because spontaneous Raman scattering is often faint, the strength of the signal data that is gathered is limited. Raman analysis sample preparation can be challenging. It is difficult to separate target particles from environmental components, which frequently results in insufficient interference removal (e.g., some organic waste and particles). Furthermore, coexisting components in plastics, such as dyes or pigments, biofilms that form on the plastic surface, and derived surface groups from weathering or aging, can make it difficult to understand the Raman data by significantly altering the Raman spectra or obfuscating polymer signals. Raman's analysis can be made more successful in some ways. For instance, it has been shown that optimizing the optical structure of a Raman spectrometer can greatly improve the signal-to-noise ratio. Another crucial element is the interpretation of data. From a statistical perspective, the massive matrix dataset produced by Raman imaging by its very nature offers a higher signal-to-noise ratio than the data from a single Raman spectrum.

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Optical microscopy

Optical microscopy made it possible to quantify particle size ranges and fibre lengths, as well as morphological classification of the types of particles or fibres present in the sample. Either optical microscopy or the naked eye can be used, and tweezers are used as a pre-screening technique to reduce the number of particles that need to be examined and, consequently, the likelihood of error. In actuality, optical microscopy is a ubiquitous instrument in almost all labs and is essential to the single-particle study of MP particles. The most widely used methods in the context of MPs are addressed here: stereoscopic and fluorescence microscopy. However, optical microscopes are still not very accurate at detecting microplastics. Under standard light microscopy, the identification error rate for microplastics is still more than 20%, and when the microplastics are transparent, the error rate rises to over 70%. Furthermore, microplastics with a particle size of less than 100 μm are challenging to identify and analyze. To lower the mistake rate of microplastic identification, optical and electron microscopy are frequently used in tandem. The PE particles are generally distributed relatively uniformly, according to the optical microscope mapping, although there may be some particle clustering. Particle detection enables accurate identification of particles as small as 50 μm, and interference with the filter's background signal is noted, presumably as a result of a high concentration of particles smaller than 20 μm. The type and composition of the particles cannot be identified microscopically, so contaminations cannot be excluded.

Scanning electron microscopy (SEM)

The ability of scanning electron microscopy (SEM) to image particle surfaces at high resolution makes it a useful tool for microplastic analysis. SEM can be used to detect microplastics in marine settings and to investigate how they weather over time. Through the imaging of surface features and element signatures, SEM can assist in the identification of microplastics. For instance, because of their distinct elemental signatures, SEM can assist in identifying chlorinated plastics such as PVC. Moreover, it can offer information regarding the chemical makeup of the materials because it can be fitted with EDS (Energy Dispersive X-ray-Spectroscopy) detectors. Different (elastic and inelastic) scattering processes are created when the primary electrons enter the solid object, and various detection

systems gather the resulting signals to create an image. The back-scattered electrons that pass through the specimen produce an increasing intensity that provides information about the topography and contrast of the material based on the atomic number (Z), while the secondary electrons, in particular, provide a detailed image that aids in understanding the morphology of objects. More back-scattered electrons are produced by samples containing elements with a higher atomic number (Z) than by those with a lower Z. This phenomenon can be utilized to identify variations in sample composition. The FE-SEM is a low-voltage alternative to the SEM that enables the acquisition of high-quality, high-magnification pictures of MP samples without the need for additional sample preparation. Because it may be used with a STEM holder to study samples on a grid, the SEM is a flexible tool that also enables the characterization of NPs. Small particles can be seen without the high voltage and energy required for traditional TEM. Due to their distinct elemental signatures, which include chlorine, chlorinated plastics like polyvinyl chloride (PVC), and mineral species that optical microscopy mistakenly identifies as plastics could be easily recognized using SEM/EDS. Pretreatment is a challenging procedure. The cost is high and the work efficiency is low. The amount of microplastics cannot be accurately estimated. Their colour cannot effectively identify microplastics. This technique is mostly employed to identify particular microplastics.

Pyrolysis Gas Chromatography – Mass Spectroscopy (Py- GC-MS)

Pyrolysis Gas Chromatography – Mass Spectroscopy can perform microplastic analysis in a system. Thermoanalytical approaches offer mass-quantitative data and the identification of microplastic contamination, in contrast to spectroscopic methods. Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) is a potent technique in which the material is separated and subjected to GC-MS analysis after being thermally broken down in an inert atmosphere. Samples, such as solids, organic compounds, or viscous liquids, can be added to the pyrolyzer and thermally evaporated into a gas using a tiny furnace. Depending on the size of the particles, sample preparation usually entails either density separation or filtration to isolate the particles. If the sample is made up of cryo-milled sediments, direct analysis is feasible.

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Soil ecosystems like agricultural fields are one of the major sinks of microplastics, and the presence of microplastics like high-density polyethene (HDPE) will reduce the germination rates of seeds, the shoot length of grass, and the biomass (Boots et al. 2019). It can carry microplastics, especially polyethene microbeads into some depths and is capable of leading microplastics into the groundwater.

Fig 2: Plastics in the agricultural fields and Ocean

Microplastic Pollution in the Indian Environment

In recent years, plastic pollution has drawn increased attention and grown to be a significant problem. They will affect our bodies differently depending on their size, shape, and type of plastic. Because of the chemicals used during production to give them unique features including strength, stiffness, flexibility, and reactivity to environmental factors, micro- and nanoparticles (NP) may pose a chemical risk. Additives may migrate when food comes into contact with plastics, but environmental pollution can also result in plastic particles being deposited on the food. The unchecked manmade plastic in this area may be the cause of the elevated concentration of microplastics in the coastal waters.

India produces about 26 million metrictonnes of plastic garbage yearly (Law et al. 2020), making the country asignificant contributor to global plastic waste generation. The country'sper capita consumption is about 11 kg of plastics (Centre for Science andEnvironment, 2019), making it a big consumer of the material whencompared to global average (28kg). Considering the negative impacts of microplastics, it is recommended that every coastal village adopt suitable solid

waste management practices. As a result, less non-biodegradable waste will enter coastal and marine environments. To sum up, stringent laws and policies need to be implemented immediately to lessen the quantity of microplastic pollution in coastal waters and its biota; if they are not, there is a risk that these contaminants may continue to increase unchecked over the coming years. Among the primary solid contaminants that threaten the water, sediment, and marine ecosystem biota globally are plastics. The fragmentation of larger plastics, either as a result of external environmental factors or from products that use those smaller polymers for industrial applications, produces tiny microplastic particles (less than 5 mm). Since microplastic contamination threatens ecosystems and can harm the entire food chain, it is a global environmental concern in India.

Conclusion

In 2015, India consumed about 13.4 million metric tonnes of plastics; by 2020, that amount had risen to 22 million metric tonnes. India's average annual per capita usage of plastic is 11 kilograms, while the global average is approximately 28 kg. (Baztan et al 2014) To avoid the most extensive use of plastics, three current solutions should be implemented: reduce, reuse, and recycle. To reduce plastic pollution or usage, solutions for proper plastic disposal must be developed. Despite research, recycling, and new technologies, alternative packaging materials should be employed to reduce reliance on plastic products. Programs should be put in place to prevent plastic littering and to increase public awareness of the risks that microplastics pose to human health and marine ecosystems. According to published research, the marine and terrestrial environments in India are becoming more and more contaminated with microplastics these days. One of the biggest obstacles to reducing plastic pollution in the future is India's lack of scientific solid waste treatment. India's land and water resources, as well as the ecosystem worldwide, will be seriously threatened if we do not address it scientifically and suitably.

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PRELIMINARY STUDY OF FORENSICALLY IMPORTANT INSECT FAUNA PRESENT IN FORMALIN TREATED AND UNTREATED FISH BLACKSPOT BARB, *DAWKINSIA FILAMENTOSA*

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Abstract

Preliminary investigation on the incidence, abundance and succession of some forensically important insects associated with formalin treated and untreated fish (Dawkinsiafilamentosa). And their role in decomposition of carrion were carried out at AnjumoorthyMangalam, a small village near Alathur block of Palakkad district in Kerala. A total ten insect species under eight insect families belonging four orders were collected and identified during this study. Eight families through Calliphoridae, Sacrophagidae, Muscidae, Piophilidae, Drosophilidae under the order of Diptera; Histeridae under the order of Coleoptera; Formicidae under the order of Hymenoptera; Alydidae under the order of Hemiptera; were found in formalin untreated fish. Formicidae only one family under the order of Hymenoptera; were found in formalin treated fish. The most abundance insects were Formicidiae and followed by Calliphoridae and Muscidae. Formicidae in fish laced with formalin occur when there is decrease in chemical effect and pungent smell. Formicidae is the family have capability of antibiotic resistance. Formaldehyde is highly toxic to all animals regardless of the methods of intake. This study on fish system followed by researchers and might contribute to the medico- legal purposes as well as could impart knowledge to develop a biomonitoring model and similar studies can light path in forensic entomology; also more detail study can provide for food and nutrition department for good health and safety to our society.

Keywords : Fish carcass without formalin, Formalin treated fish, Insect succession, Relative abundance of insects in each carcass

Introduction

Forensic entomology is an area of entomology, which applies insects and other arthropods that sequentially colonizes a corpse as decomposition progresses and on the rates at which the various stages of their offspring develop.Carrion decomposition and succession studies are important in forensic entomology, supports a diverse organisms, majority of them are insects. Apart from ecological interest, an analysis of the arthropod fauna on a carcass can be used to determine time since death in legal investigation (Anderson and Van laerhoven, 1996). In addition if an insect can be found exclusively in a rural or urban habitat, analysis of the carrion associated fauna may help to determine whether the remains have been moved from an urban to a rural environment or vice versa.

Insects are usually the first organisms to arrive on a body after death, and they colonise in a predictable sequence. The sequence of decomposition stages of the body from fresh to skeletal, is attractive to a different group of sacrosaprophagus arthropods, preliminary insects (Anderson and Van laerhoven, 1996). The occurence of the insects forms a succession of colonising species which are eliminated as carrion decay progresses. Succession is the idea that as each organisms or group of organisms feeds on the body, the corpse changes thereby making it more attractive to another group of organisms (Goff and Flynn, 1991). The insect found in a decomposing vertebrates corpses or carrion can be used to estimate the time of death ie, time interval between death and corpses discovery, also called postmortem index (PMI), movement of the corpse, manner and cause of death and association of suspects at the death and scene (Sukontason*et.al,* 2007).

The nature and time of the insects succession depends on several factors including the size of the, carrion seasonal and climatic conditions, and the surrounding non– biological environment, such as soil type. The organisms involved in the succession vary according to whether they are upon or within the carrion (Gullan and Cranston, 2010). This stems from the fact that carrion of different animals have different fat and muscle composition. Furthermore, each succession will comprise of different species in different geographic areas, even in places with similar weather factors. This is because few species are very widespread in distribution and each biogeographics are will have its own specialist carrion fauna. Blowflies are the most common dipterans seen in abundance around

carcasses, which serves as oviposition sites and larval food sources. Adult flies are attracted to the carrion until it is nearly dry, but other species visit the carcasses only during specific stages of decomposition; thus a succession of species can be observed (Goddard and Lago, 1985).

While a wide variety of insect species are attracted to decomposing remains and play an active roll in the decay process, two groups, the flies (Diptera) and beetle (Coleoptera) are of major importance in most circumstances. Diptera (flies) whose larvae are capable of living in semiliquid medium are the first insects to be attracted to and colonize decomposing remains. Fly larvae (maggots) are responsible for the dramatic consumption of the cadaver's organ and tissues. According to (Gullan and Cranston, 2010) during decomposition, the first waves of insects involves certain blowflies (Dipteran: calliphoridae) and house flies (Diptera: Muscidae) that arrive within hours or few days at the most. The second wave is of flesh flies (Diptera: Sacrophagidae) with additional house flies and blowflies that follows shortly thereafter, as the carrion develops an odour. All these flies either lays eggs or ovipositor on the carrion. At this blowfly activity ceases as their larvae leaves the carrion and pupate in the ground. When the fact of the carrion turns rancid, a third wave of species enter this modified substrate, notably more Dipteran, such as Phoridae, Drosophilidae and Formicidae in the liquid parts. As the carrion become butyrie a fourth wave of Diptera ,Piophilidae and related flies also cal the body. A fifth wave occurs as the ammonic smelling dries out, adult and larvae of Dermestidiae and Cleridae (Coleoptera) become abundant, feeding on the keratin . In the final stages of dry decay some Hymenopteran and Hemiptera (bugs) feeds on the remnant skin.

The rather predictable sequence of colonization and extinction of carrion insects allows forensic entomologist to estimate the age of corpses, which can have medico–legal application in homicide investigation. Difference in decomposition of carrion in relation to biogeography and ecology of necrophagous insects communicates have been the subjects of several field studies.

Fish and other seafoods are highly perishable and can only be kept fresh in ice for eight to fourteen days. In order to keep the freshness of fish and seafoods, fishermans and fish vendors tend to use formalin as preservative agents. Formalin is usually used for the preservative of tissues. It makes the fishes stiff and keeps

them fresh for longer period of time. Inadequate freezing facilities and ice factories and time consuming transport force the fish traders to resort to such malpractise. Available reports suggests that formalin is sometimes added or sprayed to the fishes by the fish traders while transporting to domestic marketing chain to prevent spoilage and increase shelf life (Yeasmin*et.al*, 2010). Owing to the increasing demands for fishes, the world market farmers continue to use formalin and other chemicals to maximized harvest efficiency of the fish, although this may be detrimental to human health (Andem*et.al*, 2015).

Formaldehyde is colourless, strong smelling, naturally occurring organic compound with formula CH_2O (HCHO) (Butlerov, 1859). Formalin is a 37–40 percent aqueous solution of dissolved formaldehyde CH_2O. It was used in fish culture by (Leger, 1909). Formalin was brought into common usage by fish (1940) to control ectoparasiticdisesase. Formaldehyde is metabolized naturally in our bodies by normal metabolisim and can also be found in the air, natural food, some skin care products as well as preservatives in processed food, especially in dried and frozen food. If the amount of formaldehyde is small, it doesnot harm health. However, it can cause minor to serious problems such as pain, vomiting, coma and possible death when large doses of formaldehyde taken. Formaldehyde has an acceptable daily intake (ADT) of 0.2 mg/kg body weight set by united state environmental protection agency (Nash, 1953).

Formaldehyde is a major by product of the manufacturing industry, a common environment hazards, and a product of the cellular metabolism of many methylated compound. Formaldehyde is listed as a probable human carcinogen (Yeasmin, 2010) studies relating to formaldehyde have focused almost exclusively on its toxicology in animals and humans. The carcinogenic properties and determined effects of formaldehyde exposure on growth and reproductive development have been described and summarized extensively (Golden *et .al*, 2006; Tang *et.al*, 2009; Zhang *et.al*, 2009; Szende and Tyihak, 2010; Duong *et.al*, 2011; Tulpule and Dringen, 2013). Formaldehyde also highly toxic to microbes and it has widespread application as a disinfectant for sterilization.

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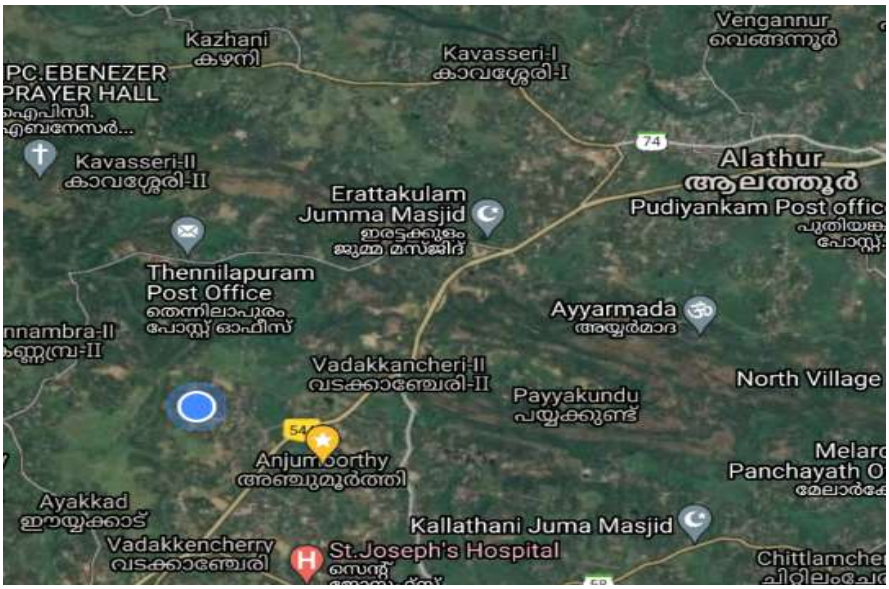
Materials and Method

Study site: The study was carried out at AnjumoorthyMangalam, a small village near Alathur block of Palakkad district in Kerala. The study site was in a natural habitat. The area was surrounded by trees and there was paddy field beside the study area. The site was selected with limited public access to minimize potential human interference, allowing natural insect association with fish carcass.



Fishcarrion: In order to understand the insect associated with fish carcass, the fish sample were slightly dissected and were hanged in plastic box covered with aerated bowl [Fig 1] to prevent the attacks from birds, and other vertebrates.Freshwater river fish Dawkinsiafilamentosapunitus species raised in MangalamRiver was selected for the study. The species vernacular name is “paral” and other common name are filament barb or black spot barb. About 100g of fish carcass was taken for the study. The fish carcass treated with formalin and untreated with formalin was placed separately to investigate the arrival of forensic insects. The carcass samples were placed in the courtyard for insect arrival.

Fig 1; Carcass placement

SAMPLING

The incidence, succession, abundance and decomposition were studied by recording the timing of arrival and duration of stay of carrion insects and these data were compared to the insect activities in carcasses. During each trial, insect were collected using sweeping net and also hand-picked using forceps. Using these methods insects were sampled from fish carcasses every day until the complete decomposition of fish. All insects were collected and subsequently preserved in the 70% alcohol. Sample were sorted and identified to family level.

Daily observation were carried out three times a day (early morning, afternoon, evening) for a period of 27 days (multiple trials were done).

Tempature and Humiditysince arthropod activities and the decomposition of the supplied items are influenced by temperature and humidity. Temperature and humidity data were recorded every day during trials from 7:00 AM to 6:00PM.

Result and Discussion

Incidence and succession of insects and decomposition of fish carrion:- The fresh stage of decomposition of fish carrion started from the first day of placement of dead fish . The average temperature and humidity during each trail (December) 24˚C and 75.2˚F of humidity 75%; (January) 27.6˚C and 81.7˚F of humidity 76%; (February) 26˚C and 78.8˚F of humidity 57%; (March) 29˚C and 84.2˚F of humidity 51%respectively. The fresh stage of decomposition was characterized by the arrival of necrophagous blowflies and flesh flies.

A total of 9 species of the families namely Calliphoridae, Sacrophagidae, Muscidae, Piophilidae, Drosophilidae, Histeridae, Formicidae, Alydidae were collected from fish untreated with formalin (Table 1) and a species of the family Formcidiae were collected from fish treated with formalin (Table 2). Among the families Formicidae, Muscidae and Calliphoridae were most abundant insects found on carcasses. Their incidence, succession and the decomposition rate present in formalin treated and untreated fish carcasses were recorded.

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Fish carcass without Formalin

1st week: The arrival of insects from the first day of placement of carcass at the study site. Calliphoridae, were the first to arrive among the insect (Plate 1). They appeared within ten minutes of placements. The arrival of other insects occurred within half an hour of placement of the carrion. The adult Calliphorids has colonized and oviposited on all fish carcasses. On the first day the dominant species was Calliphorids in all the trails. The incidence of other insects includes flesh fly, house fly (Plate 2 and 3) in fish carrion. The adult Calliphoridae were observed all over the plastic jar and in rope and also in surrounding area. Egg and early and late instars larvae of Calliphorids extensively covered the head area of the carcass. Eggs and larvae were especially concentrated in and around the mouth and abdomen.

Adult Muscadomestica, Sacrophagasps. andLuciliasps., were observed and collected from the carcasses throughout the first week in all trails. Drosophilidae under the order Diptera were also observed throughout the decay process in all the trails. At this time, the carcasses showed expanded head and abdominal area and the yellowish colour on day 3 and bloated on day 4. Decay started on day 6 and Piophilidae were observed flying and crawling on top and underneath the carcasses. Black ants and yellow crazy ants under the family Formicidae (Plate 4 and 5) were dominant species in fish carrion throughout the decay process. They were observed to act as predators and feed on larvae and pupae of Calliphorids, Sacrophagids, Muscids.

Some carcasses were extremely distended in the area of abdomen and the was subsequently filled with eggs and larvae. Adult Calliphorids, eggs and first and second instar larvae were observed in the mouths, and in the abdomen area of all the fish.

2ndweek : The peak maggots period occurred during 6 days in all trails. Majority of Calliphorids larvae were found within the second week of fish placement. In 2nd week advanced decay occur and much of the scale of the fish carcass was dried on the head and decreased the number of insect activity.The pupae of Calliphorids were observed on day 8 and the completion of development for these flies took 14 to 22 days. Many other insects, such as Piophilids,

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Formicidae, Muscids, Drosophilidae, Histerids were actively around in the pasty areas of the carcasses.

3rd week: Calliphorid larvae were still observed on day 15 and 16 but very less number. By day 15, maggots activity in the carrion had decreased dramatically. Pupae of Calliphorids and Muscids were observed and collected by hand-picking throughout the 3rd week in all the trails. Histerids (Plate 6) were first observed in fish carcasses on day 13 and remained prevalent until decay process completed.

4thweek: Period was the final stage of decomposition. Fish carcasses were found completely decomposed within the beginning of the 4th week. Species composition and diversity were found very less in this period. Alydidaesps. Activity was observed during this period.

Formalin treated Fish

Formalin is applied as a bath treatment for 20 minutes. Observation made on sensory characterization through general appearance, colour of gills, eyes, fins etc. Colour of gills are characteristic red, skin are bright, blood spot on gill cover was none, stiff in roger mortis, bellies are firm, eye are clear and clarity, eye shapes are normal, having a pungent smell. Fins and tails become shriveled and ready to brittle. Fish get dried up fastly. Fishes might not have any foul smell.

The arrival of insects during 1st week is absent. On the 2nd week during 8th to 12th day there observed the insect under the family Formicidae (Plate 7). Adult Formicidae extensively found at the wound areas of carcass. The head area of the carrion was colonized by the small ants. It was fewer in number than the Formicidae found in formalin untreated fish. Here, there is no any decomposing stage, directly to the dry stage. No other insect succession were found. Peak number of Formicidae observed during 9th day. By day 14, activity of Formicids decreased substantially and there was less number ofFormicids during 4th week of carcass placement.

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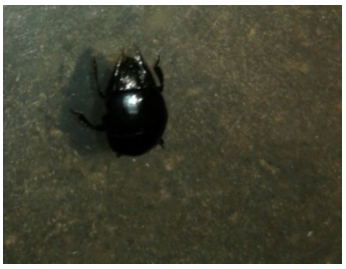
Insect Succession in Formalin Untreated

1.Calliphoridae

2. Sacrophagidae

3. Muscidiae

4. Formicidae



5. Formicidae

6. Histeridae

Insect Succession in Formalin Treated

7. Formicidae

Table 1. Abundance of insects in formalin untreated fish carrionon week basis

Order	Family	GENERAL NAME	Week 1	Week 2	Week 3	Week 4
Diptera	Calliphoridae	Blow fly	235	170	30	0
Diptera	Sacrophagidae	Fresh fly	30	12	0	0
Diptera	Muscidae	House fly	250	210	50	15
Diptera	Drosophilidae	Drosophila	0	220	0	0
Diptera	Piophilidae	Cheese skipper fly	25	30	0	0
Hymenoptera	Formicidae	Ant	350	300	60	30
Coleoptera	Histeridae	Hister beetle	0	3	15	7
Hemiptera	Alydidae	Tree bug	0	0	0	2

Table 2. Abundance of insects in formalin treated fish carrion on week basis

Order	Family	General Name	Week 1	Week 2	Week3	Week 4
Hymenoptera	Formicidae	Small ant	0	45	30	5

Fig 3; Chronology of insect succession in formalin untreated fish

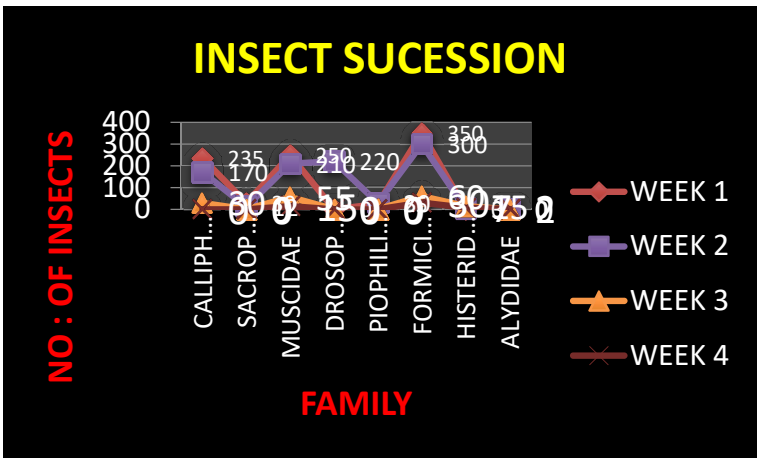


Fig 4; Chronology of insect attraction in formalin treated fish

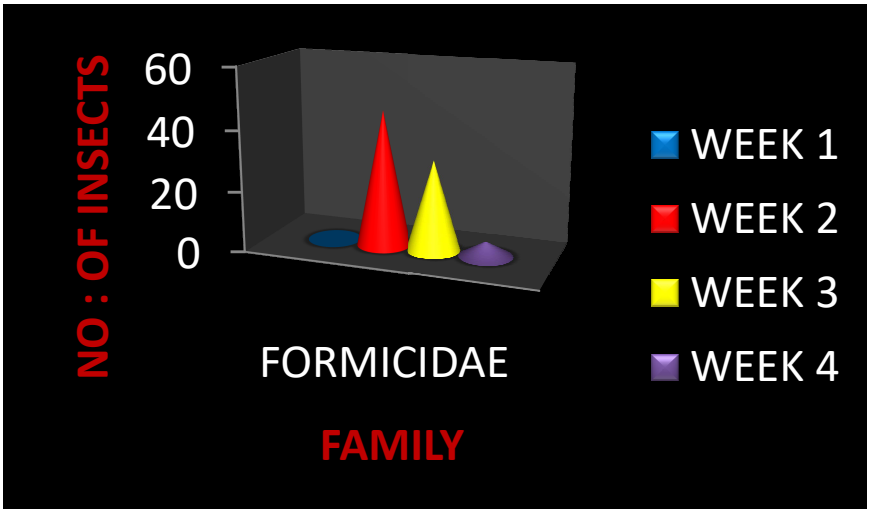


Table 3. Checklist of insect identified from Formalin untreated fish carcass

FAMILY	GENERAL NAME	GENUS / SPECIES	Decomposition status of carcass	Arrival day	Stay in days	Decomposition status of carcass
Calliphoridae	Blow fly	Luciliasps	Fresh	1st	18-21	Advance decay
Sacrophagidae	Fresh fly	Sacrophagasps	Fresh	1st	11-15	Advance decay
Formicidae	Ant	Anoplolepsissps	Fresh	1st	10-12	Post decay
Muscidae	House fly	Muscadomestica	Fresh	1st-2nd	12-15	Advance decay
Drosophilidae	Drosophila	Drosophila sps	Fresh	1st	11-12	Advance decay
Formicidae	Ant	-	Bloat	6th-8th	20-24	Post decay
Piophilidae	Cheese skipper fly	-	Bloat	7th	7-9	Decay
Histeridae	Hister beetle	-	Decay	13th	10-15	Post decay
Alydidae	Tree bug	-	Decay	17th	2-3	Decomposed

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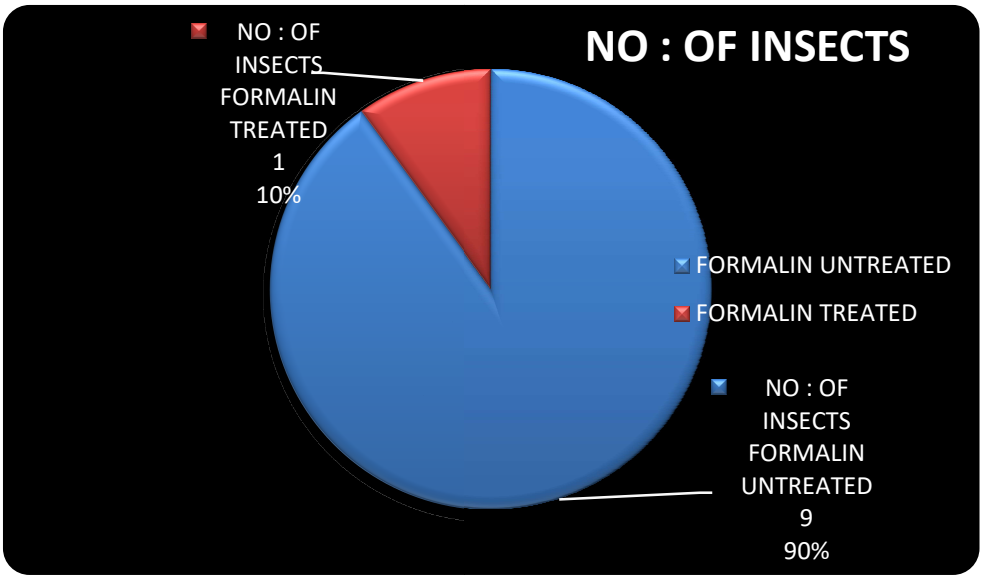
Table 4. Checklist of insect identified from Formalin treated fish carcass

FAMILY	GENERAL NAME	GENUS /SPECIES	Decomposition status of carcass	Arrival day	Stay in days	Decomposition status of carcass
Formicidae	Small ant	Solenopsissps	Dry remaining	8th	8th - 14h	Dry

Relative Abundance of Insects in Each Carcass

Comparing the above reports the insects arrival were different in each carcass. Insects succession in fish without formalin has a relevance in forensic entomology, that belongs to following orders Diptera, Hymenoptera, Coleoptera, Hemiptera under families; Calliphoridae, Sacrophagidae, Muscidae, Drosophilidae, Piophilidae, Formicidae, Histeridae, Alydidae (Fig. 3) and there is only a species present in formalin treated fish is Solenopsis. sps under family Formicidae (Fig. 4). The incidence of insects in both carcass shows the difference (Fig 5) that realize the fact is toxicity in fish treated with formalin. The formalin treated fish carcass shows the incidence of insect only during the 2nd week that due to the decrease in the concentration of formalin when time passes and this because only a fraction of the applied formalin was absorbed by fish protein.



Discussion

Throughout the period of study the carrion observed to follow the normal pattern of decomposition. These were divided into four stages as fresh, bloated, decay and dry (Reed, 1958; Tantawi*et.al*, 1996).The carrion stayed fresh for about a day or two and several insects, mainly Calliphoridae, were attracted to all of them. Usually, fresh stage begins at the moment of death and continues until bloating is first evident. During this stage the process of autolysis, the breakdown of complex protein and carbohydrates molecule to simpler chemical compound (Gill-king, 1997). The bloated stage, which lasted for about a week commenced with the onset of noticeable swelling on the carrion and ended when they deflated. The decay stage began with the detection of gases especially carbon dioxide, ammonia and hydrogen sulphide. The carrion deflated and cracks were observed on fish carrion in one or more places by feeding dipterous larvae. The stage ended when most of the remnants were relatively dry (Reed, 1958). The dry stage was the final stage of decomposition.

The forensically important insects are found on both carcasses belong to the following orders, Hymenoptera respectively. The Calliphoridae, Sacrophagidiae, Muscidae, Drosophilidae, Piophilidae, Formicidae, Histeridae, Alydidae are the eight different families seen on fish carrion without formalin and it may give some idea about insect fauna of that geographical region. The composition of species and succession of insects on carcass varies with respect to geographical region and season (Bornemissza 1957 and Reed 1957).

The number of flies are higher at the initial stage, are gradually decreasing as the decomposition progresses and completely disappear at the final stage of decomposition. It may be due to the strength of necrophagic wave to oviposit is lowering with decomposition. The dry stage was the final stage consisted of only dry skin, cartilage and bone. Odour was typically of dried fish skin. Only 10% of weight remains Wolf (2001).

The present study showed that there is a delay of one week is seen in the arrival of insect on the fish carrion treated with formalin. However, the formalin treated fish sample demonstrated longer shelf life compared to that of the non-adulterated fish samples. The antibacterial/antifungal nature of the formaldehyde

solution and the existing residual formalin treated fish may cause the longer shelf life. It is clear that insect arrival occur when the formalin concentration decrease when time passes and this is because only a fraction of the applied formalin was absorbed by fish protein.

Formalin penetrates cells quickly but its reaction with protein starts slowly (Rooban, 2012). This may be the reason of gradual decrease of formalin content in fish sample. Moreover, formaldehyde acts as an antibacterial/antifungal agent; therefore, treating fish sample with formaldehyde solutions may denature the microorganisms on the fish skin or in fish fillet, and delay the spoilage of formalin treated fish sample (Mezbah*et.al*, 2014).

Only insect species arrived on the formalin treated fish was Solenopsissps. under family Formicidae. Present study pinpointed a promising new source of antibiotic resistance ants. Antibiotic resistance is a growing problem constant pressure from parasites and pathogen have cause ants to evolve several lines of defense, prevention, tolerance and cure by the behavioural adjustment and or physiological response (Sadd&Schmid-Hempel, 2009). Among the social insects, ants and bees have been measured, making it difficult to assess whether true medication takes place (Castella*et.al,* 2008; Simone-Finstrom&Spivak, 2012). Ants can potentially, come in contact with ROS (Reactive Oxygen Species) in multiple ways during foraging. Firstly, ants often scavage for cadavers, which have been shown to contain an increased amount of ROS in their bodies (Packowski&Schutz, 2011). Secondly, ants forage on nectar, which also contain high level of ROS (Carter *et.al*, 2007).

Ant have an extraordinary amount of sensilla on their antennae with which they can smell the medicine. The ants did this, using a substance called Reactive Oxygen Species (ROS). These molecule contain oxygen capable of suppressing infection by killing off the pathogen. While some animals can produce this themselves, the ants gets it from the food they eat. 4% Hydrogen peroxide (H_2O_2), which is a ROS.

Conclusion

The present study is a preliminininary approach to find out Forensically important insect fauna of a particular area, along with a simple comparative study

on fish carcass treated with or without formalin. Many insect fauna were collected belongs to four order; Diptera, Coleoptera, Hymenoptera, Hemiptera. The families noted where Calliphoridae, Sacrophagidae, Muscididae, Drosophilidae, Piophilidae, Formicidae, Histeridae, Alydidae. Of these families Calliphoridae, Sacrophagidae, Muscidae have a great role in Forensic Entomology and Histeridae ,Alydidae in a smaller extend. The simple comparative approach also provide a considerable result. The fish carrion with formalin is attracted by less number of families than the formalin untreated fish. Only family belong to formicidae were observed.

When fishes treated formalin, it penetrate cells quickly but its reaction with protein starts slowly. This may be the reason of gradual decrease of formalin content fish carrion. This results the arrival of Formicidiae on the carcass. Moreover formalin acts as an antibacterial and antifungal agents. This may denature the microorganisms on the fish and delay the spoilage of formalin treated fish carcass.Solenopsissps under family Formicidae were observed during the gradual decrease in concentration of formalin in fish carcass.

The preserving agent often used by distributors is very same formalin even though its harmful effects are well known, owing to its ability to maintain the shelf life of products and arrest decay, as “fresh looking” fish always has better market value. Continuous ingestion of formalin might have catastrophic consequences for public health. The Kerala Government launched ‘Operation Sagar Rani’ with the objective of ensuring safety and hygiene at fish handling and distributing centres and seizing the fish with formalin content.

This study can alert the Governments to seize the formalin laced fishes and prevent customers from consuming toxin-laced fish product, for ensuring safety and public health.

From this study, we can says that patients suffering from trauma, intensive care and burns units are under particular risk, since ants may be in direct contact as disease vectors and considering as resistant bacteria strain. This revealed that the ants supplemented with H_2O_2 (ROS) they can seek out potentially toxic compound to “medicate”. So many scientist proven and identify the bacteria associated with these ants and evaluate their resistance levels to antibiotics.

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A PRELIMINARY INVESTIGATION OF THE INSECT POLINATORS VISITING INVASIVE PLANTS IN SELECTED SITES OF THRISSUR DISTRICT, KERALA, INDIA

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Abstract

Insect pollinators play a crucial role in the agriculture and in global biodiversity. Presence of invasive plants may have positive, negative or neutral effects on the pollinator visitation rates and seed output of native species. These effects mainly depend on the abundance of the flowers and reward to the visiting pollinators. Competing with native species for pollinators is critical way in which invasives hinder the plant-pollinator mutualism and thus destroys the ecosystem services by these pollinators. In the present study four invasive plants (Lantana camara L., Bidens sulphureus, Sphagneticola trilobata and Tridax procumbens)were selectedto study their pollinator interaction.It was found that L. camara attracted more pollinators towards it, even in low flowering state.Thus, it shows that the presence of L. camara affected negatively on the native plant pollinator interaction.Few flowers on B. sulphureus positively affects the native plant pollinator interaction of the area under study otherwise affects negatively. The presence of high number of flowers in S. trilobita negatively affected the native plant pollinator interaction. The presence of T. procumbens had a neutral effect on the native plant pollinator interaction as there was no reduction in the insect foragers to the nearby non invasives.

Key words: Plant pollinator interaction, invasive plants, positive, negative, neutral effect

Introduction

Plant-pollinator interactions are valued mutualisms in agricultural food production and provide essential ecosystem functions that support global biodiversity (Ollerton, 2017). It is estimated that 87.5 percent of flowering plants rely on animal pollinators for reproduction (Klein *et al*., 2007). Pollinators are a

diverse group of animal species that transfer pollen from flowering plants (Ollerton, 2017). Insects are the most diverse and abundant pollinators, with more than 140,000 species estimated (Wardhaugh, 2015), Coleoptera around 77,300, and Hymenoptera around 70,000. (Wardhaugh, 2015). Diptera and Thysanoptera are the least diverse groups of insect pollinators (Eliyahu *et al*., 2015; Orford *et al*., 2015). In fact, Thysanoptera are commonly regarded as pests, and their role in pollination has been largely overlooked (Eliyahu *et al*., 2015). Hoverflies from two families, the Syrphinae (about 1800 species) and the Eristalinae (about 3800 species), are documented as pollinators among the Diptera (Rotheray and Gilbert, 2011).

Insect pollinators have an impact on the stability, diversity, and function of natural and agricultural plant communities (Tepedino, 1979; Biesmeijer *et al*., 2006; Garibaldi *et al*., 2011). Reproductive success in natural plant communities has been found to be positively correlated with pollinator functional diversity (Albrecht *et al*., 2012; Fründ *et al*., 2013). Plants that are visited by a functionally diverse pollinator community produce high quality and quantity of seeds (Gómez *et al*., 2007; Celep *et al*., 2020). Several researchers have proposed that functionally diverse pollinators may improve gene flow and genetic diversity (Cusser *et al*., 2016; Kumar *et al*., 2020). Thus diverse pollinators provide not only pollination services but also are important to environmental safety, human health, culture and aesthetics.

Common Insect Pollinators

Insect pollinators play a significant role in the agricultural sector especially in Indian scenario where more than 85 percent of the plants require pollinators for their propagation. It includes bees, wasps, ants, butterflies, moths, beetles, flies Among the pollinator groups, bees 2 have been considered a priority group. A wide diversity of native bees pollinates wildflowers, cultivated flowers, orchards and vegetables. The bees which come under order: Hymenoptera are included in seven different families namely Apidae, Megachilidae, Andrenidae, Colletidae, Halicitidae, Melitidae and Stenotritidae.Second to bees are the members of Order: Lepidoptera, the butterflies and moths. Common Lepidopteran pollinators include *Euploea core, Tirumalalimniace, Papilio clytia, Catopsilia pomon, Delias eucharis, Danaus plexippus, Pieris rapae, Macroglossum spp*. Beetles, coming

under Order: Coleoptera also serve as pollinators. *Haptoncurina motschulskyi* is one of the common beetle pollinators in India.

Invasive Alien Plants and Their Effect on Native Plant

Invasive plants are intentionally or unintentionally introduced (Sakai et al., 2001; Marbuah et al., 2014) for agricultural purposes or for their aesthetic beauty (Pimentel et al., 2005). Pollinators may be a critical resource for both native and invasive plants (Levin and Anderson, 1970; Bastolla et al., 2009). Many invasive species rely on pollinators to establish themselves in new areas (Memmott and Waser, 2002; Bartomeus et al., 2008), resulting in changes in pollination patterns such as the decline of certain pollinator species and the disappearance of certain plant pollinator interactions (Aizenet al., 2008). Presence of invasive plants may have positive, negative or neutral effects on the pollinator visitation rates and seed 3 output of native species (Brown et al. 2002; Albrecht et al. 2016). Competing with native species for pollinators is an important factor which hinders the plant-pollinator mutualism and thus destroys the ecosystem services by these pollinators.

Materials and Methods

In the present study, we explored the diversity of potential pollinators on four selected invasive plants, *Lantana camara L., Bidens sulphureus, Sphagneticola trilobata* and *Tridax procumbens*. The plants were selected on the basis of abundance in the particular locality and the availability of native plants near its premises. The investigation was carried out during the period from October 2021 to February 2022; five different localities in Thrissur district, Kerala, India were selected for the study. (Sites: Thaikkad, Blangad, Kottapadi, Kunnathur and Pulinchode). *L. camara* – Thalikkad;*S. trilobita* – Kottapadi; *B. sulphureus*- Blangad; *T. procumbens*- Thalikkad. Methodology described by Munoz et al. (2005) was followed for observing the visitation patterns, involving 15 minutes observations in which the number of visitors to a known number offlowers was carefully recorded. The observation period (9:30 AM and 5:00 PM) of a day was divided into three phases: Early Phase (9:30 AM – 12:00 PM), Middle Phase (12:01 PM – 2:30 PM) and Late Phase (2:31 PM – 5:00 PM). Insects that landed on the inflorescence and moved over the stigma or anthers were counted as floral

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visitors and observations were made only on these insects. Identity of these insect visitors and the frequency of the visitors were duly recorded.

Results and Discussion

1. *Lantana Camera*

Lantana camera also known as wild sage is mostly native to subtropical and tropical America, but a few taxa are indigenous to tropical Asia and Africa. Itis a noxious weed, hasbeen expanding and now established in many regions of the world over wide geographical rangeinneotropics (Day *et al*.,2003;GISIN, 2011) including India.

Fig 1 : Insect visitors on *Lantana camera* a.*Amegillazonata* (Hymenoptera: Apidae); b.*Tirumalalimnaceae* (Lepidoptera: Nymphalidae); c.*Papiliopolytes* (Lepidoptera:Papilionidae); d.*Catopsiliapomona* (Lepidoptera:Pieridae)

A total of 4 different species of insect visitors were recorded visiting *Lantana camara*, from the two orders (Table1, Fig. 3) viz., Lepidoptera and Hymenoptera. Even though diverse Lepidopteran members visited the plant, it was encountered by more number of Lepidopterans compared to Hymenopterans (Fig 1).Of the insect visitors *Amegilla zonata* L. belonging to Family Apidae in Order Hymenoptera was the most dominant visitor to *L. camara.* It was followed by *Papilio polytes,* a member of Family Papilionidae of Lepidoptera; *Catopsilia Pomona* (Lepidoptera: Pieridae) and *Tirumala limnaceae* (Lepidoptera: Nymphalidae) (Fig 2). Lepidopterans spent longer duration feeding on nectar of the florets than *Amegilla zonata*

Table 1.Frequency of insect visitation on *Lantana camara*

Sl.No	Insect visitors	Order	Family	Number of insect visitors			
				Early	Middle	Late	Total
1	*Tirumalalimnaceae*	Lepidoptera	Nymphalidae	12	15	11	34
2	*Papilio polytes L.*	Lepidoptera	Papilionidae	16	13	14	43
3	*Catopsilia Pomona*	Lepidoptera	Pieridae	13	15	13	41
4	*Amegilla zonata L.*	Hymenoptera	Apidae	18	20	17	55

From the study it was found that *L. camara* attracted more pollinators towards the site of study, but those pollinators were concentrated more on *L. camara*. Thus it shows that the presence of *L. camara* affected negatively on the native plant pollinator interaction.

2. *Sphagneticola trilobata*

It is native to tropical America and is an herbaceous perennial plant with leaves that areirregularly toothed or serrate with paired lateral lobes. It is reported that mainly butterflies, moths and bees (both apids and non apids) are attracted to this plant. (Fig 2).

Fig 2: Insect visitors on *S. trilobite* a.*Chiladesparrhasius* (Lycaenidae);b.*Pelopidasmathias*(Lepidoptera:Hesperiidae);c. *Hylephilaphyleus* (Lepidoptera:Hesperiidae); d.*Amblyscirtesvialis* (Lepidoptera:Hesperiidae)e. *Apis mellifera* (Hymenopera: Apidae); f. *Lassioglossum spp.* (Hymenoptera:Halicitidae) ; g.*Apisdorsata*; h.*Lassioglosum spp* (Hymenoptera:Halicitdae); i.*Ceratinahieroglyphica* (Hy menopera:Apidae)

A total of 9 different species of insect visitors were recorded visiting *Sphagneticola trilobite*, which belong to two orders (Table 2, Fig. 2) viz., Lepidoptera and Hymenoptera. Even though diverse Lepidopteran members visited the plant, it was encountered by more number of Hymenopterans compared to Lepidopterans (Table 2). Of the insect visitors *Apis dorsata* and *A. mellifera* belonging to Family Apidae in Order Hymenoptera was the most dominant visitor to *S. trilobite.* It was followed by *Lassioglossum spp,* a member of Family Halicitidae of Hymenoptera; *Ceratina hieroglyphica* (Hymenopera: Apidae) *Amblyscirtes vialis* (Lepidoptera: Hesperiidae); *Chilades parrhasius* (Lepidoptera: Lycaenidae) *Hylephila phyleus* (Lepidoptera: Hesperiidae) and *Pelopidas mathias* (Lepidoptera: Hesperiidae)

Table 2: Frequency of insect visitation on *Sphagneticola trilobite*

Sl. No	Insect visitors	Order	Family	Number of insect visitors ± S.E			
				Early	Middle	Late	Total
1	*Hylephila phyleus*	Lepidoptera	Hesperiidae	12	11	11	34
2	*Amblyscirtes vialis*	Lepidoptera	Hesperiidae	13	12	11	36
3	*Pelopidas mathias*	Lepidoptera	Hesperiidae	10	11	11	32
4	*Chilades parrhasius*	Lepidoptera	Lycaenidae	12	11	11	34
5	*Apis mellifera*	Hymenoptera	Apidae	15	23	26	64
6	*Apis dorsata*	Hymenoptera	Apidae	14	23	29	66
7	*Lassioglossum*	Hymenoptera	Halicitidae	13	19	20	52
8	*Lassioglossum*	Hymenoptera	Halicitidae	12	14	22	48
9	*Ceratina hieroglyphica*	Hymenopera	Apidae	11	18	15	44

In the case of S. trilobite, its flowers attracted more insect pollinators, especially bees towards the study site both at low and high flowering time. In high flowering time majority of the insects were found to forage over this invasive plant, but during low flowering condition this plant attracted the pollinators and a comparatively more amount of pollinators were dispersed to the nearby vegetation. Thus it is concluded that the presence of low number of flowers in S. trilobita will positively affect the native plant pollinator interaction

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3.B. sulphureus

B. sulphureus is native to Mexico, and northwestern South America (USDA-ARS, 2016).It is recorded as an environmental weed. Despite being recorded as an invasive species in a number of countries around the world, there is very limited information available about its environmental impact especially on pollinator visits of native plants.

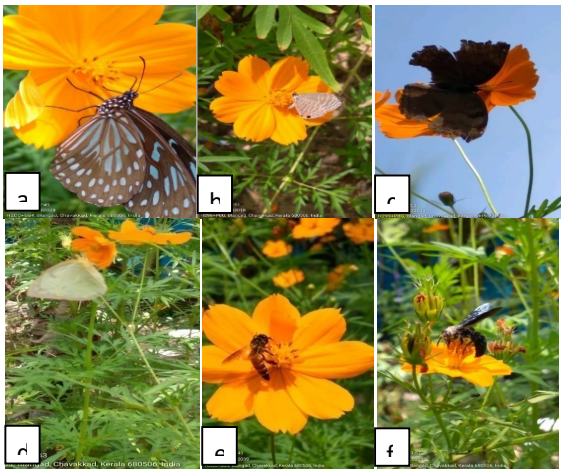

Fig 3: Insect visitors on *B. sulphureus*a. *Tirumala limniace* (Lepidoptera: Nympalidae)*; b. Chilades parrhasius* (Lepidoptera: Lycaenidae)*; c.Junoniaiphita; d.Catopsilliapomonae* (Lepidoptera:Pieridae) .e.*Apisdorsata* (Hymenoptera:Apidae)*; f.Xylocopa spp* (Hymenoptera:Apidae)

A total of 6 different species of insect visitors were recorded visiting *Bidens sulphureus*, which belong to two orders (Table 3, Fig. 3) viz., Lepidoptera and Hymenoptera. Even though diverse Lepidopteran members visited the plant, it was encountered by a greater number of Hymenopterans compared to Lepidopterans (Table3).The flower was encountered mostly by *Apis dorsata* (Hymenoptera: Apidae) followed by *Xylocopa spp.* (Hymenoptera: Apidae), *Junonia iphita* (Lepidoptera: Nymphalidae), *Chilades parrhasius* (Lepidoptera: Lycaenidae), *Tirumala limniace* (Lepidoptera:Nympalidae) and *Catopsillia pomonae* (Lepidoptera: Pieridae) (Table 3).

From the study site it was noticed that the presence of more flowers of B. sulphureus attracted more pollinators towards the site but majority of them foraging

over its flowers only. But when the number of flowers was less it attracted the pollinators which were found to forage over the native plants too (data not shown). So, less flowers on *B. sulphureus* posively affect the native plant pollinator interaction of the area under study.

Table 3: Frequency of insect visitation on *Bidens sulphureus*

Sl.NO	Insect visitors	Order	Family	Number of insect visitors ± S.E			
				Early	Middle	Late	Total
1	*Tirumala limniace*	Lepidoptera	Nymphalidae	12	11	11	34
2	*Junonia iphita*	Lepidoptera	Nymphalidae	13	12	11	36
3	*Catopsillia pomonae*	Lepidoptera	Pieridae	10	11	11	32
4	*Chilades parrhasius*	Lepidoptera	Lycaenidae	11	13	11	35
5	*Apis dorsata*	Hymenoptera	Apidae	16	19	17	52
6	*Xylocopa spp*	Hymenoptera	Apidae	13	19	16	48

4.*Tridax procumbens*

T. procumbens is an invasive plant, occurring mainly in tropical and subtropical environments, and occupying pastures, meadows, fields, side of highways and degraded areas.Balasubramaniam (1989) reported that honey bees, flies, ants and thrips acted as facultative pollinators of *T. procumbens*. But the impact of this invasive plant on native plant pollinator interaction is not studied.

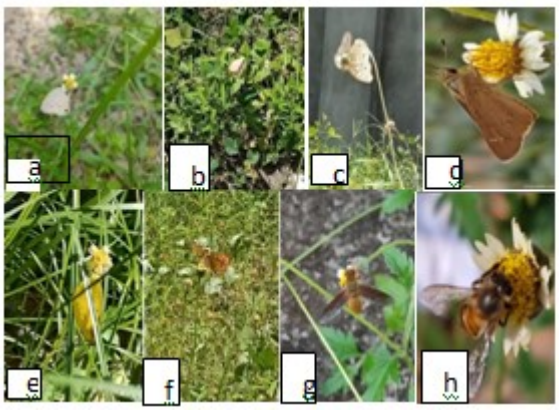

Fig 4: Insect visitors on *T. procumbens* a.*Chiladesparrhasius*; b.*Junoniaspp.*; c.*Junoniaatlites*; d. *Pelopidasmathiase. Euremahecabe*;f. *Junoniaalmana*; g.Unidentified sp. (Diptera:Bombylidae);h.*Apisdorsata*

A total of 8 different species of insect visitors were recorded visiting *Tridax procumbens*, whichbelong to two orders (Table 4, Fig. 4) viz., Lepidoptera and Hymenoptera. The flower was encountered mostly by *Apisdorsata* (Hymenoptera:Apidae) followed by anunidentified Hymenopteran species belonging to family Syrpidae., *Eurema hecabe* (Lepidoptera: Pieridae), *Pelopidas mathias* (Lepidoptera:Hesperiidae) *Junonia almana*, *Junonia atlites*, *Junonia sp* .(Lepidoptera:Nymphalidae), *Chiladesparrhasius* (Lepidoptera:Lycaenidae) (Table 4)

Table5.Frequencyofinsectvisitationon*Tridaxprocumbens*

Sl.NO	Insectvisitors	Order	Family	Number ofinsectvisitors±S.E			
				Early	Middle	Late	Total
1	*Chilades Parrhasius*	Lepidoptera	Lycaenidae	12	16	13	31
2	*Junoniaspp*	Lepidoptera	Nymphalidae	11	12	12	35
3	*Junoniaatlites*	Lepidoptera	Nymphalidae	11	14	11	36
4	*Pelopidas Mathias*	Lepidoptera	Hesperiidae	13	16	12	41
5	*Euremahecabe*	Lepidoptera	Pieridae	13	18	11	42
6	*Junoniaalmanac*	Lepidoptera	Nymphalidae	12	15	12	39
7	*Bombylid* fly	Diptera	Bombylidae	12	15	25	52
8	*Apisdorsata*	Hymenoptera	Apidae	18	20	17	55

Flowers of *T. procumbens* had a neutral effect on the native plant pollinator interaction as there was no reduction in the insect foragers to the nearby non invasives (data not shown), as it did not attract more pollinators towards it during its high blooming time.

Out of the five plants selected for the study *Sphagneticolatrilobite* and *Tridaxprocumbens* were encountered with diverse insect pollinators. It is followed by *Bidenssulphureus* and *Lantana camera.* This difference in the pollinator encounterismainly due to flower abundance, shape, colour, symmetry and corolla size (Gibson et al.,2012; Junker et al., 2013). Among the several factors, floral colour acts as an important cue forflower selection by the pollinators. (Wolfe and Sowell, 2006; Lazaro et al., 2008)31 Individualpollinators show remarkable plasticity and are known to switch plants in response to changes inpollen ornectar levels(Heinrich,1979).

A sum total of 20 different insect pollinators were identified to pollinate the selected five invasive plants.The pollinators fall under two orders viz., Lepidoptera and Hymenoptera.

Conclusion

Presence of invasive plants may have positive, negative or neutralef fect sonthe pollinator visitation rates and seed output of native species. These effects mainly depend on theabundance of the flowers and reward to the visiting pollinators. Competing with native speciesfor pollinators is critical way in which invasives hinder the plant-pollinator mutualism and thusdestroystheecosystem servicesbythesepollinators.

From the study it was found that *L. camara* attracted more pollinators towards the site ofstudy, but those pollinators were concentrated more on*L. camara*. Thus, it shows that the presence of *L.camara* affectednegativelyonthenativeplantpollinator interaction.Inthecaseof*S. trilobite*, its flowers attracted more insect pollinators, especially bees towards the study site both at low and high flowering time. In high flowering time majority of the insects were found to forage over this invasive plant, but during low flowering condition this plant attracted the pollinators and a comparatively a greater number of pollinators were dispersed to the near by vegetation. Thus, it is concluded that the presence of low number of flowers in *S. trilobita* will positively affect the native plant pollinator interaction. Flowers of *B. sulphureus* attracted more pollinators towards the site but majority of them foraging over its flowers only. But when thenumber off lowers was lessitattracted the

pollinators which were found to forage over the native plants too (data not shown). Few flowers on *B. sulphureus* posively affects the native plant pollinator interaction of the area under study otherwise affects negatively. Flowers of *T. procumbens* had a neutraleffect on the native plant pollinator interaction as there was no reduction in the insect foragers tothe nearby non invasives (data not shown), as it did not attract more pollinators towards it duringitshighbloomingtime.

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ESTIMATION OF FEEDING RATE OF DIFFERENT INSTARS OF MANGO LEAF WEBBER (*ORTHAGA EXVINACEA* HAMP.) AND THE FEEDING DETERRENCE OF DIFFERENT LEAF EXTRACTS ON ITS SIXTH INSTAR LARVA

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Abstract

The mango leaf webber(*Orthaga exvinacea* Hamp) has in the recent past become a serious pest across India.The current study aims to estimate the feeding rate of its different instars.The objectives also include the estimation of feeding deterrence of neem, adhatoda and guava leaf extracts on sixth instar larva of *Orthaga exvinacea*.The feeding rate in gram per hour (g/h) of each larval instars(1-7) of the pest were calculated.Effect of each leaf extracts mixed to their normal feed showed a slow and steady fall in the feeding rate compared to their normal feeding rate. Among the three extracts comprising of neem, adhatoda and guava it was evidently observed that neem leaf extracts caused a significant decrease in their rate of feeding.

Key words: Mango leaf webber, *Orthaga exvinacea,* feeding deterrence.

Introduction

Orthaga exvinacea, defoliator pest of mango trees is a lepidopteran insect responsible for low productivity (Ranjini *et al.,* 2016).The mango leaf webber has in the recent past become a serious pest across India. Two leaf webber species, viz. *Orthaga euadrusalis* Walker and *O. exvinacea* Hampson, have been recorded from north and south India, respectively (Butani, 1979). It results in crop yield decrease and defoliation during the caterpillar stage. Extensive infestation of this parasite negatively impacts both new flush growth and flowering (Kavitha *et al.,* 2005). The caterpillar feeds on the green material in the leaves of the mango tree, which results in defoliation. It thrives by weaving webs inside the leaves. Severe infection gives the trees a burned appearance (Verghese, 1998).

The development of these pest species from a cluster of eggs to fully grown moths through different larval stages isa notable characteristic of the leaf webber (Ranjini *et al*, 2016). After hatching, the first and second instars, or early instars, scrape the leaf chlorophyll content. The larvae are highly active in their movement within the web, where they will have tunnels made of silken webs to escape, hide, and pupate inside the webbings itself in a silken cocoon-like case covered with its excreta outside. Later instars begin forming the webs by webbing three to four leaves together. Thus, this intense infection causes the entire flower initiation process to fail, which ultimately affects the yield (Anon, 2017).

Significantly higher production losses due to the mango leaf Webber result in severe economic losses. It wasn't previously regarded as a serious pest of mango in India, but it subsequently wreaked devastation in the Gujarati middle regions (Sisodiya*et al.,* 2003).The preservation of production and economics is contingent upon the discovery, analysis, and appropriate management of these factors. The magnitude of the pest's harm is typically economic, necessitating quick management response (Kannan & Rao, 2006).

Several studies have been reported regarding the use of plant extracts to control insect pests. A study to control the pest *Orthaga exvinacea*, a major defoliator of mango trees, using botanicals to assess its effect on protein and amino acid concentration of fat body of the pest was carried out by Ranjini *et al*., 2016. The present study aim to test the effect of neem, adhatoda and guava leaf extracts on the feeding rate of sixth instar larva of*Orthaga exvinacea.*

Meterials and Methods

Collection and rearing of pest

At the outset the larvae and pupae of *O. exvinacea* along with its webbings were, manually picked from afflicted mango trees from Tirurangadi Locality. The rearing troughs were provided with fresh and tender mango leaves for feeding. The cultures were kept in rooms with ideal temperatures (30 + 2° C).

After the pupahatched, the cages were reoriented by moving the adult moths in to separate cages, they were left for mating and were fed with 50% honey. After few days eggs were laid on the leaves of twigs as well as on the covering clothes of

the containers. These severed as the primary culture. Different containers were set up and eggs were relocated into it which contained 10 eggs per troughs.

By estimating feeding rate of different larval instar, the most feeding larval instar was determined. This larval instar was further used for feeding deterrence experiments using botanical extracts.

Estimation of feeding rate

The feeding rate of the larvaewere estimated by an exploratory set up. Different instars were kept in different plastic troughs with 10 larvae per trough. Each trough wasprovided with fresh and young mango leaves for feeding (100 grams of mango leaves in each trough, which implies 10 grams with respect to each larva in the trough).Their feeding behaviour and nature of damage they cause to leaves with respect to each instar was observed. Feeding rate of the larva was calculated from the obtained cumulative weight of ingested leaves (in grams) to time (in hours) taken for feeding. Weight of leaves ingested were determined from the weight of fresh leaves and weight of leaves remaining before and after feeding for period of one day. Feeding rate of the larva in gram per hour were obtained.

$$Feeding\ rate = \frac{Weight\ of\ leaves\ ingested\ by\ larvae\ (in\ gram)}{10 * Time\ (in\ hours)}$$

Preparation of leaf extracts of neem, adhatoda and guava

For the aqueous extraction, 20.0 g of various powdered samples are added individually to a 250 ml conical flask along with aqueous (100ml) as the solvent. The primarymixtures were then left to stand at room temperature for two days, being periodically agitated with an electric agitator. Each conical flask was plugged with non-absorbent cotton and tightly covered with an aluminium foil. The solvent will separate the soluble components of the extract during this whisking period (avoid the direct contact of light with samples).After 2 days,the samples werefiltered by using Whatman No 1 filter paper. From the final product, a 50% stock solution was prepared by diluting 50 ml into 100 ml. A different concentration of 1%, 2%, 3%, 4% and 5% of each plant extracts were obtained from the 50% stock solution. All three of the primary powered samples were made into solutions using this preliminary technique and was employed in subsequent feeding deterrence experimental research.

Assessment of feeding deterrence

The assessment of inhibition in feeding occurred to the voraciously feeding larvae due to ingestion of the plant extracts prepared. Freshly collected mango leaves were dipped in the different concentrationspreparedfrom stock solution of three types of leaves, for a span of 2 days. Dipped leaves weredried in room temperature and provided as feed for the larvae for a period of 24 hour. The feeding rate was estimated after one day which on comparison withthe normal feeding ratesgave a better idea of using plant extracts inpest management of *O.exvinacea.*

Spectral analysis of leaf powders and formulations (KBr Disc-FTIR analysis)

Potassium Bromide - Fourier-transform infrared (KBr-FTIR) was used to reveal the complex absorption spectral details related to organic constituents present in the leaf extracts. Here in IR spectroscopy the samples were mixed with KBr. A very less quantity of each target sample powder to be analysed is ground with KBr powder and pressed into a disc or pellet. It is then followed by spectra reading. The spectra reading of corresponding samples were collected over 4000 to 400cm^{-1}range. The end spectra were analysed by comparison with similar data's available.

RESULTS

Estimation of feeding rate

The feeding rate in gram per hour (g/h) of each larval instars of the pest were calculated and analysed with repeated trials during all four life cycles observed. Each trough containing the particular stages of larval instar were provided with mango leaves(weighing 10 grams) for feeding. It was found that young larval instarsobserved to feed in a slow manner such that the rate of feeding seems to be low in the initial phase. Larval instars including third and fourth stages were voraciously feeding such that the rate of feeding seemed to approach a maximum rate. The sixth stage larvawasfound to the most feeding instar among all the larval stages.

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Table 1: Showing the feeding rate of ea ch larval instar of Mango leaf webber

Larval stage of moth	**Feeding rate (gram/hour) Mean± SD**
1st instar	0.18±0.02
2nd instar	0.20±0.03
3rd instar	0.25±0.02
4th instar	0.26±0.04
5th instar	0.30±0.03
6th instar	0.37±0.03
7th instar	0.28±0.01

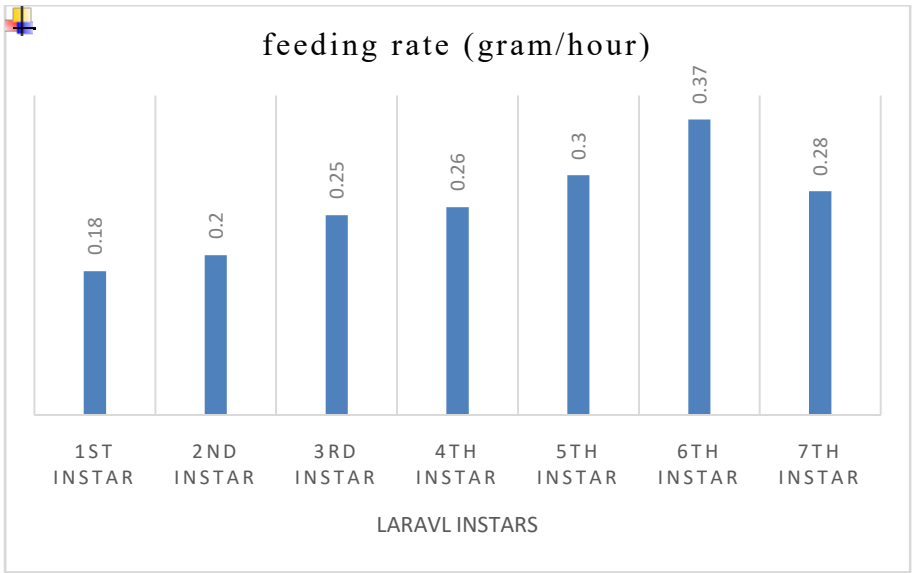

Fig.1: Representation of normal feeding rate (g/h) of each larval instar

Estimation of feeding deterrence of Neem (*Azadirachta indica*) leaf extract on sixth instar larva of *Orthaga exvinacea* Hamp.

Neem leaf extracts showed a considerable effect in the feeding of the sixth instar larva than the other two leaf extracts. Each concentration of neem showed different levels of drop in feeding, but with high concentration such as 4% and 5% the larva showed maximum decline in feeding rate(Table 2).Observation of gorging behaviour showed that the larva started to feed the neemextract treated leaves only in the initial phase but as time progressed larva ceased feeding and went into a quiescent state of non-feeding. Hence further feeding of larva were interrupted such that even added on leaves also remained unconsumed for more than day. From the examination of feeding rate with respect to present observation and comparison with normal rate of feeding it was evident that neem is a strong inhibitor of feeding.

Table 2: Showing effect of different concentrations of Neem extracts on feeding
rate of sixth instar larva

Concentration of neem extract	Feeding rate (gram/hour) Mean± SD
Control	0.37±0.03
1%	0.30±0.05
2%	0.29±0.03
3%	0.18±0.12
4%	0.07±0.04
5%	0.04±0.04

Estimation of feeding deterrence of Adhatoda (*Justaciaadhatoda*) leaf extract on sixth instar larva of *Orthaga exvinacea* Hamp.

Adhatoda leaf extract showed feeding deterrent effects differ from neem extract, but it shows significant fall in consumption rate in case of 4% and 5% concentrations like that of neem. Feeding rate with respect to different concentrations of plant extracts were used for these comparisons (Table 3). The feeding behaviour during having the leaves with low concentration seemed to be normal but after a concentration of 3% feeding rate started to drop. Newly added

fresh leaves were not fed by the larva for a period of 24 hours of treatment but later they began to chew slowly. It was revealed that the crude leaf extract of adhatoda could kill B. brassicae nymphs and adults and the extract had a potential application as insecticides because it contains flavonoids, volatile oil, tannins, saponins, glycosides, alkaloids, and resins (Haifa and Salim, 2016). The antifeedant and repellence effect of adhatoda was studied by Kumar*et al.* (2017) on a thrip *Acrotelsa collaris* and was found that adhatoda aqueous extract demonstrated substantial repellence against *the pest*, although no mortality was noted throughout the trial. Hence it is evident that *Adhatodavasica* had the strongest repulsive action against insect pests.

Table 3: Showing effect of different concentrations of Adhatoda extracts on feeding rate of sixth instar larva

Concentration of Adhatoda extract	Feeding rate (gram/hour) Mean± SD
Control	0.37±0.03
1%	0.35±0.15
2%	0.34±1.03
3%	0.28±0.12
4%	0.13±0.05
5%	0.08±0.12

Estimation of feeding deterrence of Guava (*Psidium guajava*) leaf extract on sixth instar larva of *Orthaga exvinacea* Hamp.

Effect of guava leaf extract in feeding deterrents with respect to feeding rate showed that guava has less impact on overall feeding of larva when compared to neem and adhatoda. Analysing the variations in feeding rate with respect to different concentration provided these inferences (Table 4). Feeding during treatment with initial concentrations did not show significant change from normal feeding. However, consumption of mango leaves was intermittently stopped to a

limited extend at 4% and 5% concentrations of leaf extract. Reintroduced fresh leaves were fed by the larva after 12 hours of subsequent treatment. Guava leaf extracts were observed to contain suggests the presence of an aromatic ring in tannins which give it a significant feeding inhibitor effect (Britto*et al.,* 2015).

Table 4: Showing effect of different concentrations of Guava extracts on feeding rate of sixth instar larvae

Concentration of Guava extract	Feeding rate (gram/hour) Mean± SD
Control	0.37±0.03
1%	0.37±0.15
2%	0.35±0.03
3%	0.32±0.02
4%	0.24±0.05
5%	0.18±0.04

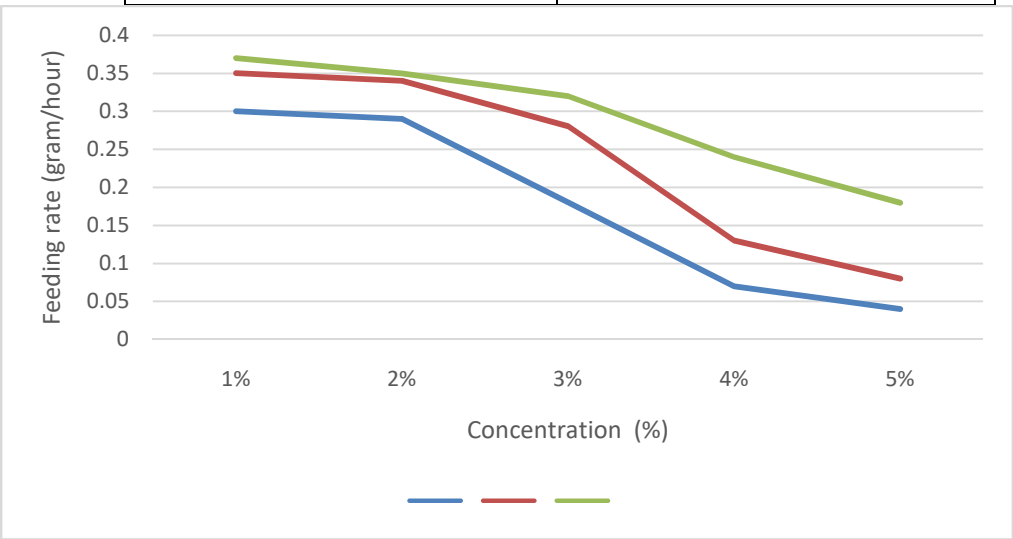

Fig. 2: Effect of all three leaf extract at various concentrations on feeding rate (gram/hour) of sixth larval instar

KBr Disc-FTIR spectral analysis of leaf powders of neem, adhatoda and guava

The Potassium bromide Disc Fourier transmission infrared spectrum (KBr-Disc FTIR) analysis of leaf powders of Neem, Adhatoda and Guava revealed the vibrational spectrum of functional groups in the extracts.FTIR Spectral analysis of powderform of leaves showed many absorption peaks regarding the presence of various functionalgroups contained in the finely grinded powder. The common functional groups identified in FTIR Spectrum were O-H/N-H stretch, C-H stretch, S-H stretch, C=O stretch, N-O stretch – asymmetric, N-H bend,C-H bend, NO2 stretch - symmetric, C-O/Si-O stretchand C-O/P=O stretch.

KBr Disc-FTIR Spectral analysis of Neem leaf

Neem powder spectrum showed the highest or strongest absorption peak between the wavelength of 2800 to 3000 cm^{-1}. Lowest absorption peak was observed from 500 to 1100cm^{-1}. Highest peak of absorption at a wavenumber of 2921 cm^{-1} indicates the presence of C-H functional group. Uptake at wavenumber of 3459 cm^{-1} indicates the presence of O-H(alcohol) functional group while 1648 cm^{-1} indicates C=O (ketone or aldehyde or ester) functional group. The absorption peak at a wavenumber of 1033 and 1159 cm^{-1} represents C-O group presence, while the absorption at a wavenumber of 1400 cm-1 with low intensity indicates N-O groups presence.The present observations regarding the presence of unique functional groups in neem powder acquired by spectral analysis using FTIR showed similar results in the work conducted by Nair *et al.,* (2013) on neem extracts. In the study conducted by Mohan, 2001 the extremely potent absorption band measured at2848 cm^{-1} and 3400 cm^{-1} indicated presence of C-H and O-H functional group that could be caused by amides and amines with bound N-H/C-H/O-H stretching. In a similar way, the band including the region 1621–1635 cm^{-1} is where the chelated C=O stretching vibrations lie (Konwar *et al.,* 2011). In astudy it was noted that traces of C-H, O-H and C-Ocorresponded to the presence of saponin, alkaloid and flavonoid contents in neem powder (Seriana *et al*., 2021)

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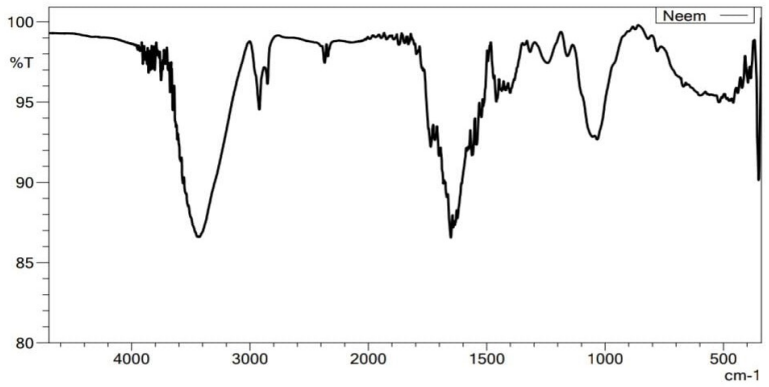
Fig 3: Representing the FTIR spectrum of Neem



KBr Disc-FTIR Spectral analysis of Adhatoda leaf

Spectrum of Adhatoda powderacquired by FTIR showed absorption peaks similar to that of neem. The broad peak at 3400 cm^{-1} range corresponds to hydroxyl groups(O-H) in the sample and peaks between2800 to 3000 cm^{-1} showed the presence of C-H functional group. Absorption 1651 cm^{-1} indicates C=O (ketone) or C=Nfunctional groups. The absorption peak at a wavelength between 1239 and 1156 cm^{-1} represents C-O group presence. The results concerning the present observation are similar with study conducted by Murali*et al.,*(2016)which concluded that O-H functional group in the sample of Adhatoda leaves extracts are responsible for the main peak at 3500 to 3100 cm-1, whereas the imine (C=N) functional group in the extract is responsible for the other peak at 1635 cm^{-1}.

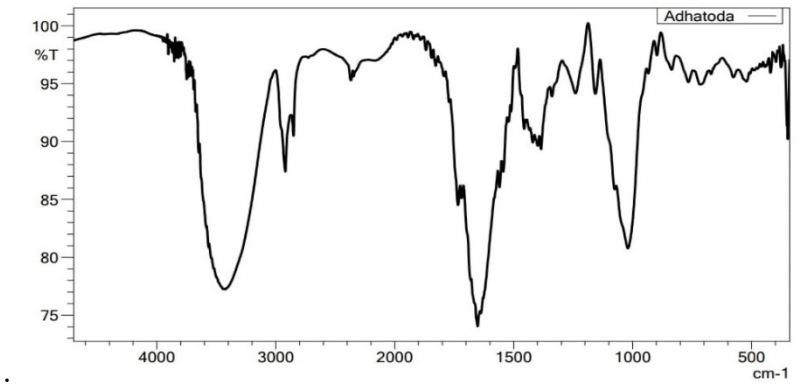


Fig 4: Representing the FTIR spectrum of Adhatoda

KBr Disc-FTIR Spectral analysis of Guava leaf

FTIR spectral analysis of Guava leaves showedthat its wavenumber of absorption peaks wascomparable to both Neem and Adhatoda spectrum. absorption peaks at 3425 cm^{-1} represented OH functional group while 2922 cm^{-1}showed CH-O groups. Wavelength of 1622 $cm^{-1}$and 1443 cm^{-1} indicated the presence of C=OandN-O groups.The FTIR showed in the study matches with the work conducted by Falcão and Araújo, (2013) in whichGuava leaf extract's infrared spectra displayed a large, strong absorption band in the 3000–3700 cm−1 region, which is indicated the stretching vibrations of the hydroxyl groups (O–H). This suggests that phenolic and alcoholic groups with a range of hydrogen bonding are present. A distinct peak corresponding to –C–H stretching, seen at 2855 cm−1 and 2924 cm−1, suggests the presence of an aromatic ring in tannins (Forato*et al.,* 2015). It was found that Guava leaf extract is rich in phenolic–OH and has a high tannin content (Somchaidee, 2018). The lack of absorbance in the 2220–2260 cm–1 range indicate the absence of cyanide content, which demonstrates that the sample used in these extracts were free of any harmful materials (Ragavendran *et al*., 2011)

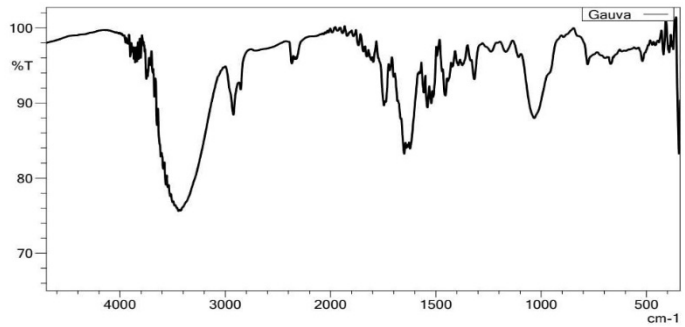


Fig 5: Representing the FTIR spectrum of Guava

Statistical analysis using ANOVA (analysis of variance)

Feeding deterrence effect of leaf extracts of neem, adhatoda and guava based on feeding rates showed by sixth instar larvae wasstatistically analysed using ANOVA (Analysis of variance). One-way ANOVA was used to find the variance among the three leaf extracts (neem, adhatoda and guava). This analysis aimed to investigate whether there are significant differences in the feeding deterrence effect

caused by the three extracts to the larvae. The one-way ANOVA allowed to compare the means of the feeding rate in the larvae by feeding on different concentrations of three different extracts and to determine whether these differences were statistically significant. By performing this analysis, it gained insights into the potential variations in the feeding behaviour of pest and assess whether the plant extracts respond in same or different rates to the treatment, providing valuable information for effective management strategies against the pest.

The overall F-statistic tests whether the means of the groups were equal or not. The p valuewas extremely small when compared to valuelevelsignificance (α) where α was 0.05. The small p value indicated that there'sstrong evidence to reject the null hypothesis of equal means among the groups. In other words, it showed that the alternate hypothesis which can be accepted that shows significant differences among the variance groups.

When the table values of ANOVA were analysed,certain inferences weremade.

Degrees of Freedom:degree of freedom for a factorof 5 groups(concentration) were obtained as 3, corresponding to the 4(n) groups (df = n-1). This showed the degree of freedom between groups. While the degree of freedom within groups is obtained as 16, corresponding to the total number of observations (total observation –number of groups that is, 20-4 = 16).

For "sum of squares" to be obtained, the total variability in the data was partitioned into the variability between groups and the variability within groups.Between groups, the "sum of Squares" represented the variation between the group means. And that was observed to be 0.190735 with degrees of freedom (df) of 3, resulting in a "Mean square" of 0.063578. This variation indicated the differences between the mean of feeding rates of different extracts.

Within Groups, the "Sum of squares" represented the variation within each group, that was the variabilityof individual observations around their group means. It is 0.14592 with a "degree of freedom" of 16, resulting in a "Mean square" of 0.00912.

F critic and F value were the main parameters other than p value that tested if the results showed significant variances. If F statistic obtained is greater than the F critical value, then it is meant that the variability between groups is higher than within groups and hence the null hypothesis can be rejected; this means that there's a significant difference between the group variances. Here the F value is 6.971308 and F critic is 3.238872 which means F critic < F statistic and hence the null hypothesis can be rejected.

The p-value (0.003259)associated with the f statistic and a very small value is obtained Pr< (F), which is much lesser than the common significance level (e.g., 0.05). This indicates that the differences between the group means were statistically significant.

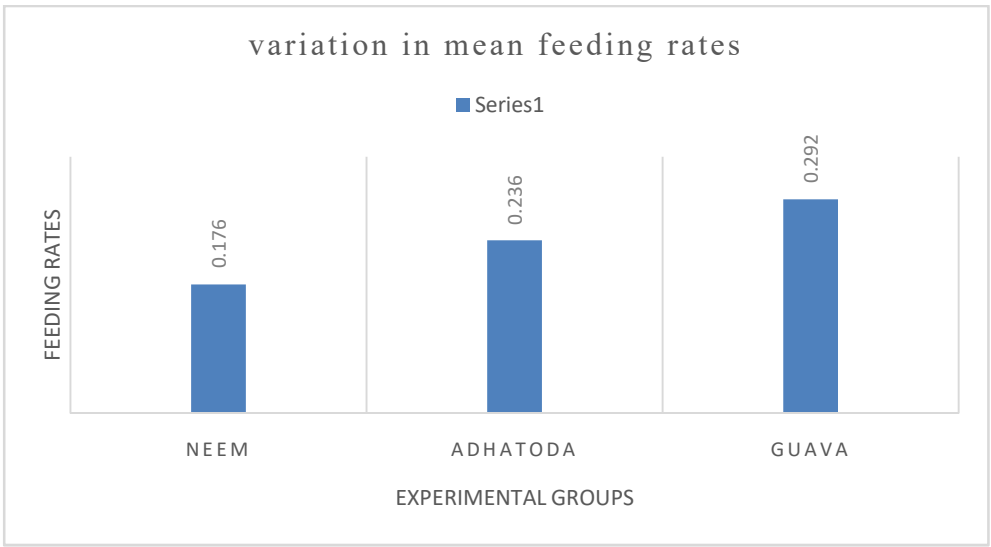


Fig. 6:ANOVA graph showing variation in mean feeding rate parameter of each experimental groups.

From the statistical data it is evident that the p value (0.003259) is very small when compared to common level of significance (0.05)and hence null hypothesis can be rejected. This implies that there are astatistically significant differences in the mean levels of the measured parameters. Hence it can be concluded that all three extractsshow different effects on the feeding inhibition of larvae. From the ANOVAtable data and graph 4.7 it can be understood that neem has high drop in the feeding rates of the larvae than both adhatoda and guava and hence, extracts Neem shows higher feeding deterrent in the sixth instar larvae of

Orthaga exvinacea while adhatoda is close to neem in its deterrent effect and guava shows roughly a slight variation from neem and adhatoda.

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DATA SCIENCE AND MACHINE LEARNING

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Abstract: This paper defines what data is, data science and machine learning. We introduce python; the facto language for data science and some of its vast libraries. We also cover the mathematics behind data science & machine learning and also some algorithms.

Key words: Statistics, data science, machine learning, python libraries, Matrix operations, EDA, linear regression, diamentionality reduction.

1. INTRODUCTION

Data science is the study of data to extract meaningful insights for business. It is a multidisciplinary approach that combines principles and practices from the field of mathematics, statistics, artificial intelligence and computer engineering to analyse large amounts of data. Data scientists use three main types of maths - linear algebra, calculus and statistics. Data sets usually takethe form of matrices. Data scientists store and manipulate data inside them and they use linear algebra during the process. Linear algebra is the core component of data processing. Calculus, the mathematics study of continuous change. At its basic level, calculus allows you to use variables and models to arrive at a solution. Overall data science we mainly tend to focus on thepractical application of maths to solve real-world problems.

2. PRELIMINARIES

Python

Python is a versatile and popular programming language known for its simplicity and readability.Python has become the de facto language for data science and machine learning due to its extensive libraries. In Data Science we use the libraries like NumPy, Pandas, and Matplotlib are essential for data manipulation, analysis, and visualization. Libraries such as Scikit-learn, Tensor Flow, and PyTorch provide tools for machine learning model development and deployment. Here are the basics of Python programming:

```
In [4]: 1 print("Hello, world!")
Hello, world!
```

Figure 2.1: print a variable

```
In [2]:  1 a=3
         2 a
         3 #print(a)

Out[2]: 3
```

Figure 2.2: Variable name

```
In [3]:  1 #to give input through userlevel
         2 name=input("enter the name\n")

enter the name
Riya
```

Figure 2.3: To give input

```
In [8]:  1 marks1=int(input("enter mark1\n"))
         2 marks2=int(input("enter mark2\n"))
         3 print(marks1+marks2)

enter the mark1
66
enter the mark2
66
132
```

Figure 2.4: To give different input

To check the datatypes

```
In [4]:
1 a=6
2 print(type(a))
```

```
<class 'int'>
```

```
In [5]:
1 x=True
2 print(type(x))
```

```
<class 'bool'>
```

```
In [4]:
1 ab=6.5
2 print(type(ab))
```

```
<class 'float'>
```

```
In [6]:
1 s="hello world"
2 print(type(s))
```

```
<class 'str'>
```

Figure 2.5: Datatypes

```
In [25]:
1 #Get the character at position 1 (remember that the first character has the position 0):
2 a = "Hello, World!"
3 print(a[5])
```

,

Figure 2.6: Position

```
In [14]:
1 a = "Hello, World!"
2 print(len(a))
```

```
13
```

Figure 2.7: Length

```
In [3]:
num1=float(input("enter the number\t"))
num2=float(input("enter the number\t"))
```

```
enter the number        4.5
enter the number        5
```

```
In [4]:
print("addition", num1+num2)
print("subtraction", num1-num2)
print("multiplication", num1*num2)
print("division", num1/num2)
print("floor division", num1//num2)
print("remainder", num1%num2)
print("exponent", num1**num2)
```

```
addition 9.5
subtraction -0.5
multiplication 22.5
division 0.9
floor division 0.0
remainder 4.5
exponent 1845.28125
```

Figure 2.8: Arithmetic Operatios

```
In [6]:
fixed_amount=100000
withdrawn=int(input("enter the withdrawing amount \t"))
balance=fixed_amount-withdrawn
print("your balance is",balance)
```

```
enter the withdrawing amount    20000
your balance is 80000
```

Figure 2.9: ATM Receipt

3. INTRODUCTION TO DATA-SCIENCE AND MACHINE-LEARNING

3.1 Statistics

Statistics is the process of Gathering data, Describing and visualizing data,Making conclusions and it is a Science of analysis and interpretation. Two types of Statistics:

1. Inferential Statistics:Inferential statistics is the practice of using sampled data to draw conclusions or make predictions about a larger sample data sample or population.

2. Descriptive Statistics: It describes or summarizes the data and helps to describe and organize known data using charts, bar graphs, etc : It consists of three basic categories of measures:

- Measures of central tendency describe the center of the data set (mean, median, mode).
- Measures of variability describe the dispersion of the data set (variance, standard deviation).
- Measures of frequency distribution describe the occurrence of data within the data set (count).

3.2 Data Science

Data Science is a process of extracting, manipulating, visualizing, maintaining data as well as generating predictions. Data science is a deep study of the massive amount of data, which involves extracting meaningful insights from raw, Structured and unstructured data that is processed using the scientific method, different technologies, and algorithms. Data science is all about

- Asking the correct questions and analyzing the raw data.
- Modelling the data using various complex and efficient algorithms.
- Visualizing the data to get a better perspective.
- Understanding the data to make better decisions and finding the finalresult.

3.3 Machine Learning

Machine learning is backbone of data science. It is all about to provide training to a machine so that it can act as a human brain. In data science, we use various machine learning algorithms to solve the problems. Machine learning can be classified into three types:

1. **Supervised Learning**

   It is classified into Classification and Regression. Supervised learning involves training a machine learning model on a labeled dataset, where the correct outputs are already known. The goal of supervised learning is to learn a function that can accurately predict the correct output for new, unseen inputs.

2. **Unsupervised Learning**

   It is classified into Clustering and Association. Unsupervised learning involves training a machine learning model on an unlabeled dataset, where the correct outputs

are not provided. The goal of unsupervised learning is to discover patterns or relationships in the data without the need for human intervention or labelling

3. **Reinforcement Learning**

   Reinforcement learning involves training a machine learning model to make decisions in an environment by receiving feedback in the form of rewards or punishments based on its actions. The goal of reinforcement learning is to learn a policy that maximizes the cumulative reward over time.

Regression

1. **Linear Regression**

   Linear regression is one of the easiest and most popular Machine Learning algorithms. It is a statistical method that is used for predictive analysis.

   Linear regression makes predictions for continuous/real or numeric variables such as sales, salary, age, product price, etc. It shows a linear relationship between a dependent (y) and one or more independent (x) variables, hence called as linear regression.

2. **Logistic Regression**

   It analyze the relationship between a set of independent variables and the dependent binary variables. It is a powerful tool for decision-making. For example email spam or not.

4. PYTHON LIBRARIES

4.1 Python libraries

Python libraries are collections of pre-written code modules that provide additional functionality to Python programmers: It contains bundles of code that can be used repeatedly in different programs. It makes Python Programming simpler and convenient for the programmer. As we don't need to write the same code again and again for different programs. Python is a popular choice for data analysis because of its extensive library of tools for: Manipulating data, Visualizing data, and Training machine learning models. Some of the python libraries that are widely used for data analysis are: TensorFlow, Numpy, Pandas, Matplotlib, Scikit-learn etc.

4.2 NumPy

(Numerical Python) is a powerful Python library for numerical computing. The library supports working with arrays, matrices, and various mathematical functions. NumPy is used for scientific computing, engineering, data science, and machine learning projects. Pandas is a Python library used for working with data sets. It has functions for analyzing, cleaning, exploring, and manipulating data. The name "Pandas" has a reference to both "Panel Data", and "Python Data Analysis".

4.3 Matplotlib

Matplotlib is a popular data visualization library in Python. It's often used for creating static, interactive, and animated visualizations in Python. Matplotlib allows you to generate plots, histograms, bar charts, scatter plots, etc., with just a few lines of code.

4.4 Scikit-learn

Scikit-learn is an open-source Python library that helps implement machine learning models. It's considered the gold standard for machine learning in Python.

5. EXPLORATORY DATA ANALYSIS

Machine LearningWork Flow

- Step 1 :- Problem and Data Identification.
- Step 2 :- EDA,Feature Engineering and Feature Selection.
- Step 3 :- Model selection and training.
- Step 4 :- Hosting of the solution.

5.1 EDA

Exploratory Data Analysis (EDA) is a critical phase in any data analysis project. It involves examining and visualizing data to understand its key characteristics, uncover patterns, detect anomalies, and formulate hypotheses.

Steps

1. Understand the Data:
2. Data Cleaning:
3. Explore the Structure of the Data:
4. Summary Statistics:
5. Univariate Analysis:

6. Bivariate Analysis:
7. Multivariate Analysis:
8. Feature Engineering (if applicable):
9. Visualization:
10. Iterate and Refine:
11. Document Findings:
12. Communicate Results:

General outline for Feature Engineering

- **Step 1:- EDA**
    1. Preview data - head(),tail(),describe(),info()
    2. Check total number of entries and column types.
    3. Check any null values
    4. Check duplicate entries
    5. Check outlier values
    6. Plot distribution of numeric data (univariate and pairwise joint distribution) different graphs.
    7. Plot count distribution of categorical data - histogram/box-plot.

- **Step 2:- Handling Missing Values**
    1. Mean/ Median/Mode replacement.
    2. Random Sample Imputation.
    3. Capturing NAN values with a new feature.
    4. End of Distribution imputation.
    5. Arbitrary imputation.
    6. Frequent categories imputation.

- **Step 3:- Handling Outliers**
    1. Approach 1 : Outlier Detection with Standard Deviation
    2. Approach 2 : Outlier Detection with Percentiles

- **Step 4: Handling Imbalance Datasets**
    1. SMOTE - Synthetic Minority Over-sampling Technique.

- **Step 5:- Categorical Encoding**
    1. **Nominal Encoding**
        a. OneHot Encoding
        b. OneHot Encoding with many categories
        c. Mean Encoding
    2. **Ordinal Encoding**
        a. Label Encoding
        b. Target guided Ordinal Encoding
- **Step 6:- Normalization and Standardization**
    1. Normalization And Standardization
    2. Scaling to Minimum And Maximum values
    3. Scaling To Median And Quantiles

General outline for Feature Selection

Approach 1: Correlation

Approach 2: Forward Elimination

Approach 3: Backward Elimination

Approach 4: Random-forest Importance

Approach 5: Decision Tree Feature election

Handling Outliers

1. Outlier Detection with Standard Deviation

In [ ]:
```
#Dropping the outlier rows with standard deviation
factor = 3
upper_lim = data['column'].mean () + data['column'].std () * factor
lower_lim = data['column'].mean () - data['column'].std () * factor

data = data[(data['column'] < upper_lim) & (data['column'] > lower_lim)]
```

Figure 5.1: Outlier Detection with Standard Deviation

2. **Outlier Detection with Percentiles**

In [ ]:
```
#Dropping the outlier rows with Percentiles
upper_lim = data['column'].quantile(.95)
lower_lim = data['column'].quantile(.05)

data = data[(data['column'] < upper_lim) & (data['column'] > lower_lim)]
```

Figure 5.2: Outlier Detection with Percentiles

6. MACHINE LEARNING ALGORITHMS

Data science careers require mathematical study because machine learning algorithms, performing analysis and discovering insights from data require maths.Algorithms are the set of finite rules or instructions to be followed in calculation or other problem solving operations. Machine learning uses the concept of calculus to formulate the functions that are used to train algorithms.

Machine Learning is trained with data sets with multiple feature variables. Hence getting familiar with multivariable calculus is important for building a suitable model.
Mathematical modelling is fundamental to translate a business problem into data science problems. It provides a framework to represent relationships, make predictions and optimize solutions. Also various maths are involved in estimating the parameter of a model and to evaluate the models performance.Matrices are instruments in solving system of linear equations which arise in various data science scenarios such as in regression analysis and also employed in feature extraction techniques such as Principal Component Analysis (PCA) and Singular Value Decomposition (SVD).

6.1 Linear Regression

Linear regression is a fundamental algorithm in machine learning used for predictive modeling. It models the relationship between a dependent variable (target) and one or more independent variables (features) by fitting a linear equation to the observed data

1. Simple Linear Regression

Simple linear regression involves one feature and one target variable.

The relationship is modeled as a straight line:

$y = mx + c$

- y : Predicted value (dependent variable).
- x : Feature (independent variable).
- m : Slope.
- c : Intercept.

2. Multiple Linear Regression

Multiple linear regression involves multiple features:

$y = a0 + a1x1 + a2x2 + ... + anxn$

- y : Predicted Value.
- $x1, x2, ..., xn$: Features.
- $a1, a2, ..., an$: Coeffients.
- $a0$: Intercept.

Applications

- Predictive Analytics: Forecasting sales, stock prices, and other trends.
- Risk Assessment: Predicting default rates, insurance claims, etc.
- Marketing: Understanding the impact of marketing efforts on sales.

Example 1. *Simple linear regression in Home price prediction with area as independent variable and price as dependent variable.*

In [1]:
```
import numpy as np
import pandas as pd
import matplotlib.pyplot as plt
%matplotlib inline
from sklearn.linear_model import LinearRegression
```

Figure 6.2: Importing libraries

In [2]:
```
df = pd.DataFrame({
    'area': [2000, 2500, 3500, 4500],
    'price': [425000, 532000, 714500, 937550],
})
df
```

Out[2]:

	area	price
0	2000	425000
1	2500	532000
2	3500	714500
3	4500	937550

Figure 6.3: Data frame

We can see the relationship between price and area from the figure 5.4 . It is clear that the relation is linea

```
In [3]:
1 plt.xlabel('area')
2 plt.ylabel('price')
3 plt.scatter(df.area,df.price,color="red",marker="*")
4 plt.show()
```

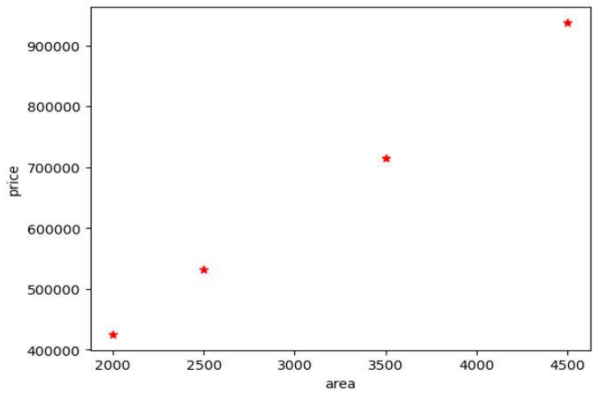

Figure 6.4: Scatter plot

We set a new dataframe with independent values.

```
In [17]:
1 new_df=df.drop('price',axis=1)
2 new_df
```

Out[17]:

	area
0	2000
1	2500
2	3500
3	4500

Figure 6.5: new d

```
In [6]:
1 price=df.price
```

Figure 6.6: price

Then we create the Linear regression model as follows:

```
In [8]:
1 lr=LinearRegression()
2 lr.fit(new_df,price)

Out[8]: LinearRegression()
```

Figure 6.7: Linear regression model

```
In [9]:  1 lr.predict([[3300]])

C:\Users\sride\anaconda3\Lib\site-packages\
ression was fitted with feature names
  warnings.warn(

Out[9]: array([687686.94915254])
```

Figure 6.8: Prediction

```
In [13]:  1 lr.coef_

Out[13]: array([202.42542373])

In [14]:  1 lr.intercept_

Out[14]: 19683.050847457722
```

Figure 6.9: Coefficient and Intercept

Confusion Matrix

A confusion matrix is a useful tool in machine learning and statistics for summarizing the performance of a classification algorithm. It is a specific table layout that allows visualization of the performance of an algorithm, typically in the context of binary or multiclass classification.

In a binary classification problem, the confusion matrix consists of four parts:

- True Positives (TP): The number of correct positive predictions.
- True Negatives (TN): The number of correct negative predictions.
- False Positives (FP): The number of incorrect positive predictions (Type Ierror).
- False Negatives (FN): The number of incorrect negative predictions (Type II error).

The layout of a Confusion matrix is given below:

-	Predicted Positive	Predicted Negative
Actual Positive	True Positive (TP)	False Negative (FN)
Actual Negative	False Positive (FP)	True Negative (TN)

Now let's see an example of a confusion matrix:

Let's say you have a dataset where you want to classify emails as "spam" or "not spam." After running your classification model, you might get the following results:

- 50 emails are correctly classified as spam (TP).
- 10 emails are incorrectly classified as spam (FP).
- 5 emails are incorrectly classified as not spam (FN).
- 100 emails are correctly classified as not spam (TN).

The confusion matrix would look like this:

-	Predicted Positive	Predicted Negative
Actual Spam	50	5
Actual Not Spam	10	100

```
In [1]:
from sklearn.metrics import confusion_matrix, ConfusionMatrixDisplay
import matplotlib.pyplot as plt

# Example data
y_true = [0, 1, 0, 1, 1, 1, 0, 0, 1, 1]  # True labels
y_pred = [0, 1, 0, 0, 0, 1, 1, 0, 1, 1]  # Predicted labels

# Compute the confusion matrix
cm = confusion_matrix(y_true, y_pred)

# Display the confusion matrix
disp = ConfusionMatrixDisplay(confusion_matrix=cm, display_labels=[0, 1])
disp.plot()
plt.show()
```

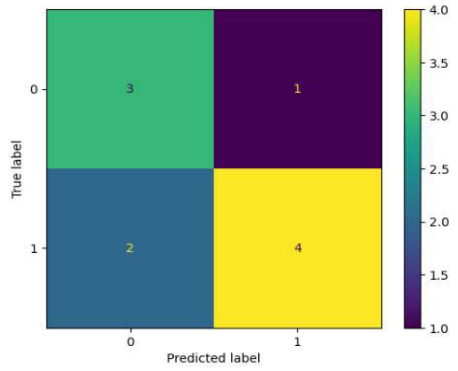

Figure 6.10: Visual example of Confusion matrix using python

Regularization

It is a technique to prevent the model from over fitting by adding extra information to it. Regularization works by adding a penalty or complexity term to the complex model. Let's consider the simple linear regression equation:

$y = a0 + a1x1 + a2x2 + ... + anxn$

In the above equation, y represents the value to be predicted, $x1, x2, ..., xn$ are the features of y, $a1, a2, ..., an$ are the weights or magnitude attached to the features, respectively. Here represents the bias of the model, and $a0$ represents the intercept. Linear regression models try to optimize the intercept to minimize the cost function. The equation for the cost function for the linear model is given below:

$$\sum_{i=1}^{m}(Y - Y')^2 = \sum_{i=1}^{M}(yi - \sum_{j=0}^{m} aj, xij)^2$$

Now, we will add a loss function and optimize parameter to make the model that can predict the accurate value of y.

There are mainly two types of regularization techniques, which are given below:

1. Ridge Regression

   Ridge regression is a regularization technique, which is used to reduce the complexity of the model. It is also called as L2 regularization. In this technique, the cost function is altered by adding the penalty term to it. The amount of bias added to the model is called Ridge Regression penalty. We can calculate it by multiplying with the lambda to the squared weight of each individual feature. The equation for the cost function in ridge regression will be:

$$\sum_{i=1}^{m}(Y - Y')^2 = \sum_{i=1}^{M}(yi - \sum_{j=0}^{m} aj, xij)^2 + \lambda \sum_{j=0}^{n} aj^2$$

   In the above equation, the penalty term regularizes the coefficients of the model, and hence ridge regression reduces the amplitudes of the coefficients that decrease the complexity of the model. As we can see from the above equation, if the values of λ

tend to zero, the equation becomes the cost function of the linear regression model. Hence, for the minimum value of λ, the model will resemble the linear regression model

2. Lasso Regression

   Lasso regression is another regularization technique to reduce the complexity of the model. It stands for Least Absolute and Selection Operator. It is similar to the Ridge Regression except that the penalty term contains= only the absolute weights instead of a square of weights. It is also called as L1 regularization. The equation for the cost function in lasso regression will be:

$$\sum_{i=1}^{m}(Y - Y')^2 = \sum_{i=1}^{M}(yi - \sum_{j=0}^{m} aj, xij)^2 + \lambda \sum_{j=0}^{n} aj^2$$

6.2 Dimentionality Reduction

Dimentionality reduction is a process of transforming high-dimensional data into a lower-dimensional space that still preserves the essence of the original data.Techniques for dimensionality reduction including principal component analysis (PCA), singular value decomposition (SVD) and and linear discriminant analysis (LDA).

Principal Component Analysis (PCA)

Principal-component analysis, or PCA, is a technique for taking a dataset consisting of a set of tuples representing points in a high-dimensional space and finding the directions along which the tuples line up best. The idea is to treat the set of tuples as a matrix *M* and find the eigenvectors for MM^T or M^TM.

The matrix of these eigenvectors can be thought of as a rigid rotation in a high dimensional space. When you apply this transformation to the original data, the axis corresponding to the principal eigenvector is the one along which the points are most “spread out,” More precisely, this axis is the one along which the variance of the data is maximized. Put another way, the points can best be viewed as lying along this axis, with small deviations from this axis. Likewise, the axis corresponding to the second eigenvector (the eigenvector

corresponding to the second-largest eigenvalue) is the axis along which the variance of distances from the first axis is greatest, and so on.

We can view PCA as a data-mining technique. The high-dimensional data can be replaced by its projection onto the most important axes. These axes are the ones corresponding to the largest eigenvalues. Thus, the original data is approximated by data that has many fewer dimensions and that summarizes well the original data. If M is a matrix whose rows each represent a point in a Euclidean space with any number of dimensions, we can compute M^TM and compute its eigenpairs. Let E be the matrix whose columns are the eigenvectors, ordered as largest eigenvalue first. Define the matrix L to have the eigenvalues of M^TM along the diagonal, largest first, and 0's in all other entries. Then, since $M^TMe = \lambda e = e\lambda$ for each eigenvector e and its corresponding eigenvalue λ, it follows that $M^TME = EL$.

We observed that ME is the points of M transformed into a new coordinate space. In this space, the first axis (the one corresponding to the largest eigenvalue) is the most significant; formally, the variance of points along that axis is the greatest. The second axis, corresponding to the second eigenpair, is next most significant in the same sense, and the pattern continues for each of the eigenpairs. If we want to transform M to a space with fewer dimensions, then the choice that preserves the most significance is the one that uses the eigenvectors associated with the largest eigenvalues and ignores the other eigenvalues. That is, let E_k be the first k columns of E. Then ME_k is a k-dimensional representation of M.

Example 2. *Since PCA is a technique for taking a data set consisting of a set of tuples representing points in high-dimensional space, here the data is two-dimensional. ie, the number of dimensions is too small, this is for the easy learning of PCA.*

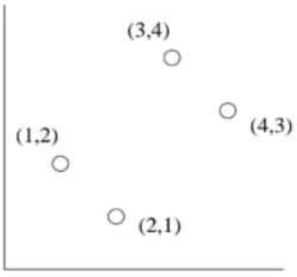

Figure 6.11: Four points in a 2-dimensional space

Representing the points by a matrix M.

$$M = \begin{bmatrix} 1 & 2 \\ 2 & 1 \\ 3 & 4 \\ 4 & 3 \end{bmatrix}$$

Compute $M^T M$.

$$M^T M = \begin{bmatrix} 30 & 28 \\ 28 & 30 \end{bmatrix}$$

(To find the eigenvalues of a square matrix A: Find its characteristic equation using $|A - \lambda I| = 0$*, where I is the identity matrix of same order A. Solve it for* λ *and the solutions would give the eigenvalues.)*

We may find the eigenvalues of the matrix above by solving the equation.

$$(30 - \lambda)(30 - \lambda) - 28.28 = 0$$

The solution is $\lambda = 58$ *and* $\lambda = 2$.

Then we must solve $\begin{bmatrix} 30 & 28 \\ 28 & 30 \end{bmatrix} \begin{bmatrix} x \\ y \end{bmatrix} = 58 \begin{bmatrix} x \\ y \end{bmatrix}$

When we multiply out the matrix and vector we get two equations; $30x + 28y = 58x$

$28x + 30y = 58y$

From both equations we get same thing $x = y$.

But the vector we get is not a unit vector. So we multiply the vector with $1\sqrt{2}$ *to get unit vector.*

Thus the unit vector corresponding to the principal eigenvalue 58 is $\begin{bmatrix} 1\sqrt{2} \\ 1\sqrt{2} \end{bmatrix}$

For the second eigenvalue 2, we perform the same process $\begin{bmatrix} 30 & 28 \\ 28 & 30 \end{bmatrix} \begin{bmatrix} x \\ y \end{bmatrix} = 2 \begin{bmatrix} x \\ y \end{bmatrix}$

Solving and we get 2 equations

$30x + 28y = 2x$ $28x + 30y = 2y$

Both the equations tell us the same thing $x = -y$.

Thus the unit eigenvector corresponding to the eigenvalue 2 i is $\begin{bmatrix} 1\sqrt{2} \\ 1\sqrt{2} \end{bmatrix}$

Normally we write eigenvectors with their first component positive, we choose the opposite here because it makes the transformation of coordinates easier.

Now let's construct the matrix E of eigen vectors for the $M^T M$ *equations*

$$E = \begin{bmatrix} 1\sqrt{2} & -1\sqrt{2} \\ 1\sqrt{2} & 1\sqrt{2} \end{bmatrix}$$

Any matrix of orthonormal vector represents a rotation or reflection of the axes of a Euclidean space. The matrix above can be viewed as a rotation 45 degrees counter clockwise. Now computing ME.

$$ME = \begin{bmatrix} 1 & 2 \\ 2 & 1 \\ 3 & 4 \\ 4 & 3 \end{bmatrix} \quad \begin{bmatrix} 1\sqrt{2} & -1\sqrt{2} \\ 1\sqrt{2} & 1\sqrt{2} \end{bmatrix} \quad = \quad \begin{bmatrix} 3/\sqrt{2} & 1/\sqrt{2} \\ 3/\sqrt{2} & -1/\sqrt{2} \\ 7/\sqrt{2} & 1/\sqrt{2} \\ 7/\sqrt{2} & -1/\sqrt{2} \end{bmatrix}$$

*This data has only two dimensions, so the only dimensionality reduction we can do is to use k = 1; i.e., project the data onto a one dimensional space. That is, we compute ME*1 *by*

$$ME_1 = \begin{bmatrix} 1 & 2 \\ 2 & 1 \\ 3 & 4 \\ 4 & 3 \end{bmatrix} \quad \begin{bmatrix} 1\sqrt{2} \\ 1\sqrt{2} \end{bmatrix} = \quad \begin{bmatrix} 3\sqrt{2} \\ 3\sqrt{2} \\ 7\sqrt{2} \\ 7\sqrt{2} \end{bmatrix}$$

The effect of this transformation is to replace the points of M by their pro- jections onto the x-axis.

The first two point's project to the same point, as do the third and fourth points, this representation makes the best possible one-dimensional distinctions among the points.

7. REFERENCE

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DRONE-BASED INNOVATIONS IN MOSQUITO CONTROL: PIONEERING SUSTAINABLE SOLUTIONS FOR VECTOR MANAGEMENT

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ABSTRACT

Mosquito-borne diseases such as malaria, dengue, and Zika virus continue to pose significant public health challenges worldwide, especially in regions with limited resources for vector control. Traditional methods of mosquito control, including chemical spraying and habitat elimination, are often ineffective, costly, and environmentally damaging. This study explores the potential of drone-based innovations as a sustainable, efficient, and precise alternative for mosquito management. Drones equipped with advanced sensors, GPS technology, and targeted spraying mechanisms can identify mosquito breeding sites, apply larvicides, release biological control agents, and monitor populations in real-time. The integration of Artificial Intelligence (AI) and the Internet of Things (IoT) further enhances the accuracy and adaptability of these interventions. Through a review of case studies and pilot projects, the study highlights the successful application of drones in reducing mosquito populations and disease transmission in various global settings. Despite challenges such as regulatory concerns and technological limitations, drone-based mosquito control represents a transformative approach that offers scalable, cost-effective, and environmentally friendly solutions to vector management. This study concludes by emphasizing the importance of continued research, international collaboration, and policy development to optimize and expand the use of drone technology in global mosquito control programs.

INTRODUCTION

Mosquitoes are among the deadliest vectors of human and animal diseases, responsible for transmitting pathogens that cause illnesses like malaria, dengue fever, chikungunya, Zika virus, and yellow fever. According to the World Health Organization (WHO), vector-borne diseases account for over 17% of all infectious diseases globally, with millions of cases reported annually. This alarming statistic underscores the critical need for effective mosquito control strategies.

CHALLENGES IN TRADITIONAL MOSQUITO CONTROL

Conventional mosquito control methods, such as fogging, insecticide spraying, and breeding site elimination, have been widely employed for decades. While these techniques have been moderately effective, they face significant limitations:

- **Inaccessibility of breeding sites**: Remote, swampy, or urban areas with high vegetation density often hinder ground-based operations.
- **Environmental impact**: Overuse of chemical insecticides leads to pollution and non-target species harm.
- **Insecticide resistance**: Increasing mosquito resistance to traditional chemical control agents poses a major obstacle to their efficacy.
- **Cost and manpower**: Traditional operations demand significant human and financial resources, limiting scalability in resource-constrained regions.

EMERGING TECHNOLOGIES FOR VECTOR CONTROL

The integration of advanced technologies like Geographic Information Systems (GIS), remote sensing, and biological control agents into mosquito control programs has shown promising results. However, these approaches often require complementary delivery systems that can operate with precision and efficiency

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across diverse environments. This is where drone technology offers a transformative solution.

The Promise of Drone Technology

Drones, or Unmanned Aerial Vehicles (UAVs), have revolutionized several industries, including agriculture, disaster management, and logistics. Their application in mosquito control is a relatively new but rapidly evolving field. Drones provide an innovative platform for both surveillance and control by enabling:

- **High-resolution monitoring**: Drones equipped with cameras and sensors can identify breeding hotspots, even in hard-to-reach areas.
- **Precision spraying**: Automated larvicide and adulticide delivery minimizes chemical wastage and environmental damage.
- **Speed and scalability**: Large areas can be covered in significantly less time compared to ground-based methods.

OBJECTIVES OF DRONE-BASED MOSQUITO CONTROL

Drone-based mosquito control aims to enhance the efficacy and sustainability of vector management programs. This approach aligns with global health priorities, including:

1. **Reducing disease transmission**: Effective targeting of mosquito populations lowers the incidence of vector-borne diseases.
2. **Minimizing environmental impact**: Drones support precision application, reducing the overuse of insecticides.
3. **Supporting data-driven decisions**: Drones integrated with GIS and AI provide actionable insights for vector control strategies.

SCOPE OF THE CHAPTER

This chapter explores the technological and operational aspects of drone-based mosquito control, highlighting their advantages over traditional methods. It delves into real-world applications, emerging innovations, and the challenges of scaling up this technology in public health programs. By presenting case studies and discussing future directions, this chapter aims to provide a comprehensive understanding of drones' role in modern mosquito management.

With drones offering a powerful blend of efficiency, adaptability, and environmental sensitivity, they represent a significant leap forward in the fight

against mosquito-borne diseases. This technology has the potential to transform vector control from a reactive process into a proactive, precision-oriented strategy for safeguarding public health and ecosystems.

DRONE TECHNOLOGY IN VECTOR CONTROL

The application of drones, or Unmanned Aerial Vehicles (UAVs), in vector control represents a cutting-edge technological advancement. These versatile tools provide innovative solutions to long-standing challenges in mosquito management, such as inaccessibility to breeding sites, inefficiencies in traditional methods, and the need for environmentally friendly control measures. This section explores the components, types, and benefits of drones used in vector control.

Unmanned Aerial Vehicles (UAVs)

KEY COMPONENTS OF MOSQUITO CONTROL DRONES

Drones used in vector control are equipped with advanced systems tailored to perform specific tasks efficiently:

1. **Navigation Systems**:
    - **GPS Integration**: Ensures precise geolocation and mapping of breeding sites for targeted interventions.
    - **Autonomous Flight Programming**: Enables pre-set flight paths to cover specific areas with minimal human intervention.
2. **Payload Delivery Systems**:
    - **Spray Mechanisms**: Designed to release liquid larvicides or adulticides with precision, minimizing chemical wastage.

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- **Biological Agent Dispensers**: Specialized systems for deploying bio-control agents such as sterile mosquitoes or bacteria like *Bacillus thuringiensis israelensis* (*Bti*).

3. **Sensors and Imaging Equipment**:
    - **High-Resolution Cameras**: Capture aerial images to identify potential breeding sites in stagnant water or vegetation.
    - **Thermal Sensors**: Detect temperature variations that may indicate mosquito habitats.
    - **Multispectral Imaging**: Used to differentiate vegetation, water bodies, and other mosquito-prone areas.
4. **Data Transmission and Processing**:
    - **Real-Time Data Streaming**: Enables instant transmission of field data for analysis.
    - **AI Integration**: Uses machine learning algorithms to analyze data for optimized intervention strategies.

TYPES OF DRONES FOR MOSQUITO CONTROL

Different types of drones are employed depending on the terrain, control requirements, and operational scale:

1. **Fixed-Wing Drones**:
    - Best suited for covering large areas such as agricultural fields or wetlands.
    - Can stay airborne for extended periods, ideal for surveillance and mapping.

Image source: https://www.jouav.com/fixed-wing-drone

2. **Rotary-Wing Drones (Quadcopters)**:
    - Highly maneuverable, making them effective for small-scale, localized applications.
    - Suitable for spraying in confined areas, such as urban environments with dense vegetation.

Image source: https://www.environmental-expert.com/products/superfox-model-6-lidar-and-photogrammetric-drone-652853

3. **Hybrid Drones**:
    - Combine the benefits of fixed-wing and rotary-wing drones.
    - Ideal for operations requiring a balance of range and precision.

Image Source: https://advancedaircraftcompany.com/news/startup-aac-launches-hybrid-propulsion-drone

APPLICATIONS IN VECTOR CONTROL

1. **Surveillance and Monitoring**:
    - Drones equipped with imaging and GPS technology are used to map mosquito habitats and track changes in breeding sites.
    - GIS integration provides detailed maps to guide ground teams for targeted interventions.
2. **Targeted Chemical Application**:
    - Precision spraying systems apply larvicides and adulticides directly to breeding sites, reducing environmental contamination.
    - Drones are especially effective in hard-to-reach areas like marshlands, forested regions, and urban rooftops.
3. **Deployment of Biological Control Agents**:
    - Sterile Insect Technique (SIT): Drones release sterile male mosquitoes to reduce population levels through unsuccessful mating.
    - Biopesticides: Controlled release of natural larvicides such as *Bti* ensures eco-friendly mosquito control.
4. **Environmental Monitoring**:
    - Regular drone surveys help monitor environmental conditions such as water levels, vegetation growth, and temperature changes that favor mosquito proliferation.

ADVANTAGES OF DRONE TECHNOLOGY IN VECTOR CONTROL

1. **Precision and Efficiency**:
    - Accurate application of control agents minimizes wastage and ensures maximum impact.
2. **Accessibility**:
    - Drones can easily navigate challenging terrains like swamps, densely populated urban areas, or thick forests.
3. **Cost-Effectiveness**:
    - Over time, drones reduce the need for extensive manpower and repeated chemical applications, lowering operational costs.

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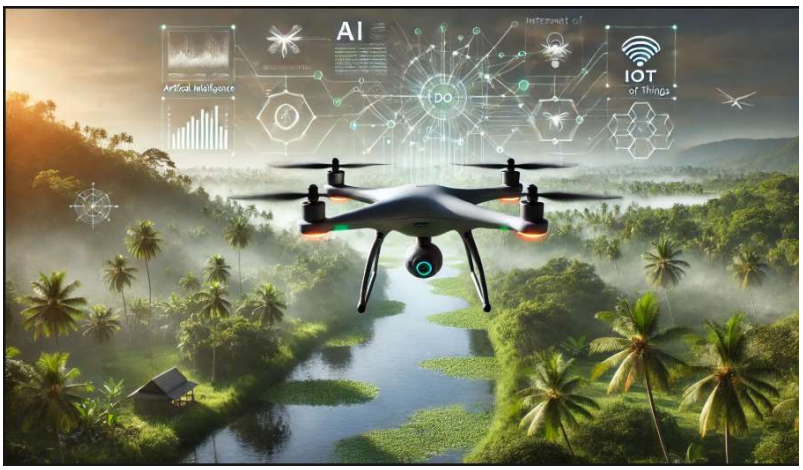
4. **Environmental Benefits**:
    - Targeted interventions reduce the overuse of chemicals, minimizing harm to non-target organisms and ecosystems.
5. **Scalability**:
    - Drones are adaptable for use in both small-scale and large-scale vector control programs.
6. **Real-Time Feedback**:
    - Immediate data collection and processing enable dynamic response strategies based on current field conditions.

APPLICATIONS OF DRONES IN MOSQUITO MANAGEMENT

1. **Surveillance and Mapping**:
   Using drone-mounted sensors, mosquito breeding hotspots such as stagnant water, paddy fields, and urban drains can be identified efficiently. GIS tools enable detailed mapping of these areas, supporting targeted interventions.
    - Example: Drone surveillance in Brazilian favelas helped identify mosquito habitats inaccessible by ground teams, reducing dengue cases significantly.
2. **Larvicide and Adulticide Spraying**:
   Drones can precisely apply eco-friendly larvicides like *Bacillus thuringiensis israelensis* (BTI) to water bodies, minimizing collateral damage to non-target species. Similarly, drones can deliver adulticides to areas experiencing outbreaks.
    - Case Study: In Tanzania, drones were used to spray larvicides over 30 acres of marshland, reducing mosquito populations by over 90%.
3. **Biological Agent Release**:
    - Drones are effective in releasing sterile male mosquitoes as part of the Sterile Insect Technique (SIT) to reduce reproduction rates.
    - They can also deploy other bio-control agents like fungi or predatory organisms that target mosquito larvae.
    - Example: The World Mosquito Program's use of drones to release Wolbachia-infected mosquitoes to curb dengue transmission.

INTEGRATION WITH EMERGING TECHNOLOGIES

1. **Artificial Intelligence (AI)**:
    - Machine learning algorithms process environmental data from drones, predicting mosquito population surges based on weather patterns, breeding site density, and historical data.
    - AI optimizes flight paths and spraying patterns for maximum efficiency.
2. **Internet of Things (IoT)**:
    - Drones can be integrated with IoT-enabled environmental sensors to monitor temperature, humidity, and water stagnation, creating a feedback loop for dynamic control.
    - Example: Smart mosquito traps sending real-time data to drones for targeted interventions.



CASE STUDIES AND SUCCESS STORIES

1. **Zanzibar Malaria Elimination Project**:
    - Drones mapped rice paddies, applied larvicides, and reduced malaria transmission rates by 50%.
2. **Singapore's Dengue Control Initiative**:
    - Rotary drones used to apply larvicides in urban construction sites, a major breeding ground for mosquitoes.
3. **India's Smart Vector Control**:

- Pilot programs in Kerala used drones to identify mosquito breeding hotspots in flood-affected areas, leading to a 40% drop in dengue cases.

CHALLENGES AND LIMITATIONS

1. **Regulatory and Ethical Concerns**:
    a. Many countries have strict regulations on drone use, particularly in urban areas or near airports.
    b. Ethical considerations include data privacy and community concerns about over-surveillance.
2. **Technical Constraints**:
    a. Limited battery life and payload capacity restrict drone operations.
    b. Adverse weather conditions, such as strong winds or rain, can hinder drone performance.
3. **Economic Barriers**:
    - High initial investment costs for drone equipment and training can be a challenge for resource-constrained regions.

ENVIRONMENTAL AND SOCIETAL IMPACTS

1. **Positive Impacts**:
    - Precision spraying reduces the overuse of chemicals, protecting non-target organisms and ecosystems.
    - Improved public health outcomes through better control of mosquito-borne diseases.
2. **Potential Risks**:
    - Over-reliance on drones may reduce community involvement in traditional vector control measures.
    - Mismanagement or malfunction of drones could lead to environmental harm or ineffective control.

FUTURE DIRECTIONS AND RECOMMENDATIONS

1. **Scaling Up**:
    - National and regional integration of drone technology into existing vector control programs.

2. **Innovative Research**:
    - Development of solar-powered drones for extended operations.
    - Exploring the use of biodegradable materials for drone components to minimize environmental impact.
3. **Policy Recommendations**:
    - Harmonizing international regulations for drone use in public health.
    - Creating frameworks for public-private partnerships to fund and implement drone technology.

CONCLUSION

Drone technology offers a promising, sustainable solution to mosquito control, providing precision, efficiency, and scalability that traditional methods lack. By enabling targeted interventions in hard-to-reach areas, drones reduce pesticide use, minimize environmental impact, and improve public health outcomes, particularly in regions burdened by mosquito-borne diseases. While challenges such as regulatory barriers and technical limitations remain, ongoing advancements in AI, IoT, and drone capabilities will further enhance their effectiveness. As global adoption of drone-based mosquito control grows, it has the potential to significantly reduce disease transmission, offering a smarter, more sustainable approach to vector management and contributing to healthier, disease-free communities.

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SEED GERMINATION STUDIES AND SEEDLING GROWTH PERFORMANCE OF SEVEN CONSUMER-ACCEPTED MICROGREENS

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Abstract

Studies on seven microgreens, including radish red, radish white, yellow mustard, pak choi, and arugula Plantlet growth performance, black mustard, and seed germination were all studied in lab conditions. The experiment was planned using a randomized block design, and data on the number of days needed for seed germination, seed characteristics, and seedling growth performance were recorded. According to the data, black mustard exhibited a lower percentage of germination (74.5%) than radish-white seeds, which offered the highest germination (96.75%). Black mustard had the longest roots and radish white had the longest shoots according to the seedling growth characteristics. Less than one gram is the biomass of pak choi, whereas radish white has the maximum biomass (i.e., 14.55). Seven to twenty-one days after germination, the precise section of the vulnerable plant's growing stem, the cotyledon leaves, and the likely true leaves are being harvested.

Keywords: Microgreens**,** Seed dimensions, seed propagation, germination percentage, growth parameters

INTRODUCTION

Microgreens, a hypothesized term used for the emerging food product that is developed from various commercial food crops, such as vegetables, grains, and herbs, consist of developed cotyledons along with partially expanded true leaves. These immature plants are harvested between 7–21 days. In recent years, microgreens are on demand from high-end restaurant chefs and nutritional researchers due to their potent flavours, appealing sensory qualities, functionality, and abundance of vitamins, minerals, and other bioactive compounds (1). Globally, in the last decade, especially during- and post-COVID-19 pandemic, the

growing interest of society in eating fresh, healthy, and functional foods, such as sprouted seeds and microgreens, has been on the rise Sprouts and microgreens are so advantageous that their growth period is much shorter, and their maintenance is considerably lower compared to matured green plants and their produce, such as vegetables and fruits (2). The commonly grown vegetables were used to cultivate mi-crogreens that belong to various families, such as Amaranthaceae (amaranth, beet, quinoa,spinach, buckwheat, chard), Amaryllidaceae (garlic, onion, leek), Apiaceae (parsley, carrot,fennel, celery, dill, carrot, chervil, cilantro, coriander), Asteraceae (lettuce, radicchio, chicory,endive, tarragon, common dandelion), Boraginaceae (phacelia), Brassicaceae (radish, water-cress, arugula, broccoli, cauliflower, cabbage, chicory, wild-rocket), Convolvulaceae (water convolvulus), Cucurbitaceae (melon, cucumber, squash), Malvaceae (jute mallow/Nalta jute), Poaceae (corn, lemongrass), Lamiaceae (chia), Leguminosae (chickpea, alfalfa, bean,green bean, fenugreek, fava bean, lentil, pea, clover), Onagraceae (evening primrose).

Although the cultivation of microgreens is simple, their production and use are limited by their short shelf life. Therefore, one of the main challenges of the microgreens industry is storage, i.e., maintaining quality after harvesting. Microgreens are difficult to store due to their high surface area to volume ratio and their high rate of respiration and transpiration as well as accelerated senescence When stored at room temperature, the shelf life of microgreens is three to five days.Seed germination depends on both internal and external conditions. The most important external factors include the right temperature, water, oxygen or air and sometimes light or darkness.

Nutrient content

Microgreens are often grown indoors in chambers with artificial lights and under controlled conditions, enabling their cultivation throughout the year and providing protection from potential pollution in nature. It has been shown that red and blue lights stimulate plant growth and affect plant quality by stimulating the accumulation of secondary metabolites such as ascorbate, flavonoids, and anthocyanins(3,4) Microgreens are largely associated with micro and macronutrients, such as Fe, Zn, K, Ca, N, P, S, Mn, Se, Mo, and other. Apart from these mineral components, microgreens are rich in biological phytochemicals,

which have an immense potential to enhance human health and also aid in improving diseases. The major bioactive compounds, such as ascorbic acid, phylloquinone, α-tocopherol, β-carotene, phenolic antioxidants, carotenoids, anthocyanins, glucosinolates, and sugar content, are reported to be present in the microgreens in larger contents. The secondary metabolites that are present in the microgreens help promote metabolic activity, preventing free radical oxidation, and reducing inflammation**.**It was also found that the total phenolic content in the microgreens ranged between 10.71–11.88 mg/g, especially in broccoli, which was 10 times higher than that of the respective mature counterparts and sprouts. These phenolic contents in the microgreens are responsible for the improvement of glucose homeostasis and various other metabolic reactions in the body(3).

SEED GERMINATION STUDIES OF SEVEN MICROGREENS

The study was carried out over 6 months from October 2022 to March 2023 on the campus of Sree Narayana College Alathur, experiments were conducted to study growth performance, dormancy status and to understand the storage behavior and germination of seven consumer-accepted microgreens.Seeds used for experimental conditions were obtained from retailers in Kochi, Kerala. Healthy, viable, and disease-free seeds are selected. The germination trials were carried out with four replications of 100 seeds from each category. The seeds have no dormancy and pre-treatment is not necessary. Seeds from each category were sown individually in cocopeat as a medium for seed germination. The germination trials were conducted in a completely randomized design. After commencing the germination, the details of emergence from the date of first sprouting (First count) up to the date of culmination of germination (Final count) were recorded.

The germination of seeds was observed daily and the total number of seeds germinated was counted up to 10 days from the days after sowing. The influence of seed dimensions and seed weight were evaluated in the saplings originating from the seed germination experiment. After seedling development time, plantlets were uprooted from the medium to determine the length and mass of the shoot system and root system.

The plant height was measured from ground level to the tip of the longest leaf at 10 days after sowing and at harvest from ten plants and their mean was worked out.

The length of root from ten randomly selected plants in a tray was recorded usinga scale from apex to the base of the root and the average is expressed in centimetres. The root and shoot ratio was also calculated for ten randomly selected plants from each tray by measuring the root and shoot length and the average expressed in centimetres.

Germination Parameters: Different types of germination parameters viz., percentage of germination, time taken for initial and completion of germination, and peak have been shown here following under mentioned methodologies.The seeds that germinated normally during the germination period were counted and the total number of seeds that germinated was divided by the total number of seeds sown and the result was expressed as a percentage.

The germination percentage was calculated at germination and at the various stages of growth,using the formula

$$\mathbf{GP\% = (g \times 100)/G}$$

Where,

GP = Germination percentage

g. = No. of germinated seeds

G = Total no. of seeds

Number of days taken to initiation of germination and number of days taken from sowing to completion of germination were recorded for each species. The seeds were considered germinated only when the sprouted plumule along with the cotyledons had protruded about 0.5 cm above the bed. The weight of the seeds was measured usingan electronic balance.

Since microgreens do not grow more than 1 to 1.5 inches long it becomes very easy to grow the greens in small plastic containers which are compact in size and can be placed in a very small area. Flat trays with good drainage are used for germination studies.For the growth of healthy and strong microgreens, Coir pith was used as the growing mediumbecause it is free of bacteria and fungal spores, and is easier to work with than soil.

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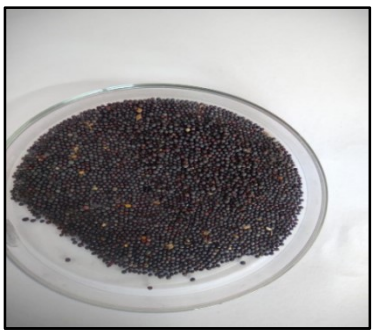
A moderate setup of indirect sunlight of 5 hours was provided to avoid longer stems and fewer leaves. Microgreens tend to grow best if the pH value of the water is a bit acidic ranging from 5.5 to 6.0. use a spray bottle for watering the seeds of microgreens. It is important to maintain the moisture in the seeds therefore cover the seeds with a damp kitchen towel or cotton swabs. Once the sprouts reach the height of the container, it will become difficult to spray water from the top as all the water will stick to the leaves and the roots will not get any. Thus to water the plants let the plastic containers sit on a water basin a let the soil absorb water from the basin this way the roots get an ample amount of water. This process needs to be repeated at least twice a day or as required by the plant.

Experimental design and statistical analysis

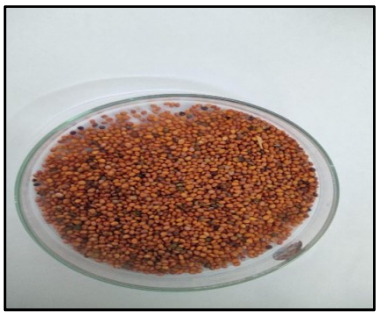
Germination tests were performed in four replicates (100 seeds per batch), the measurements of height and biomass were obtained in 10 seedlings per replicate using a complete randomized design.

1.Growing tray 2Growing medium 3. Organicseeds 4. Light 5. Water 6. Weighing balance



(a) Pak choi seeds

seed germinated

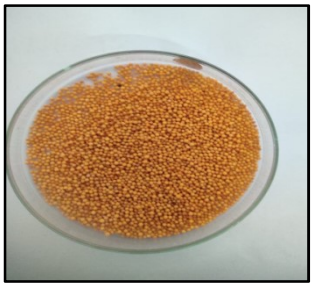


(b) Arugula seeds

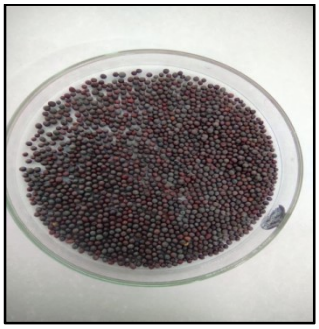
seed germinated

(c) Radish white seed germinated

(d) Radish red seed germinated



(e) Yellow mustard seed germinated



(f) Black mustard seed germinated

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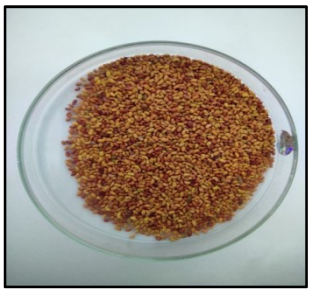

(g) Alfalfa seed germinated

1.*Pak choi*

Chinese cabbage, (*Brassicarapa* var.*chinensis*) are important vegetable grown in Asia. It accounts for 30-40% of the vegetable production area in China and also Taiwan. The plant is being used mostly for its leaves and leaf stalks. These plants are an excellent salad material when they are young and little (15 cm and shorter leaves) and it is being used as food ingredients when the leaves are grown ripe (when they reach 30 cm plant height).It is also used for soups and meals, as well as decorating purposes.Pak choi leaves have a sweet taste and crisp and juicy texture which contains 2.18 g of carbohydrates, 1.5 g proteins and 1.0 g dietary fiber in 100g of fresh matter.

Health Benefits: Pak choi microgreens are rich in vitamins A, K, C, iron, calcium and magnesium. They can also provide a daily recommended 3.7% potassium. Apart from these vitamins, pak choi microgreens contain folic acid, calcium, magnesium, manganese, thiamine, zinc and niacin.Pak Choi micro greens are known for their ability to build and maintain bone strength and structure. Their iron and zinc content plays an essential role in collagen growth, and vitamin K maintains the calcium balance in bones.These microgreens also keep blood pressure and heart health in check as they have zero cholesterol. The choline in pak choi microgreens helps improve sleep, memory and muscle movement. As an essential antioxidant food, pak choi microgreens boost collagen production in the body, smooth out wrinkles and improve skin texture. These microgreens have a robust flavour and are slightly sweet. Along with it being a tasty and crunchy green, it is also full of fibres and antioxidants. Overall, it’s a vegetable that provides valuable nutrients and is well-suited to a healthful diet.

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2. Arugula

Also known as salad rocket or Italian cress, arugula (*Eruca sativa*) is of Mediterranean origin and belongs to the Brassica family of microgreens that include cabbage, kale, broccoli, and radish.

Health benefits: Every 100 grams of raw arugula leaves contain important macronutrients such as carbohydrates, fibre, and proteins; essential micronutrients such as vitamins A, C, K, B_2, and folate; and minerals like phosphorus, magnesium, and especially high amounts of potassium and calcium. Among Brassica microgreens, arugula is thought to have the highest amount of vitamin C and beta carotenes. It also contains indoles, which are phytochemicals with powerful cancer-fighting abilities. Arugula also packs in vitamin K, another requirement for good bone health. Arugula extracts were also found to possess cytoprotective properties – they fortify the gastric mucosa by enhancing prostaglandin activity. This in turn increases gastric mucus secretion and thus prevents ulcer formation. Arugula contains the carotenoids lutein and zeaxanthin, both essential for preventing age-related macular degeneration, cataracts, and other eye diseases. Studies reveal that lutein has antioxidant and anti-inflammatory properties. Additionally, lutein concentration in the eyes helps fend off harmful blue light emissions from mobile, computer and TV screens, thereby protecting your eyes from damage. Scientists strongly recommend incorporating this “eye vitamin” into our daily diets to maintain its concentration in the human lens and retina (6,7). Arugula can not only keep the body on track regularly, but it also shows potential as a remedy to fight certain illnesses. Here are some promising lines of research:

Arugula is being studied for its potential as an antidiabetic treatment. One study that tested the effect of arugula leaf extracts on insulin-responsive cells found that they have significant antidiabetic properties.Arugula’s anti-inflammatory compounds protect against heart disease. Additionally, in animal studies, arugula has been shown to prevent the formation of blood clots(8).

Radish

Radish (*Raphanus raphanistrum*) is an annual and biennial cool-season crop of ancient origin. Radish is a root crop particularly grown for its taproot that varies in

colour from white, pink, red, purple, and black and has an appetizing flavour. The taproot is edible as raw as it is palatable, juicy and crunchy with a pungent peppery pleasing taste. It is also well known to cure various ailments and used for centuries in Southeast Asian countries as traditional medicine. With the increasing interest of researchers in this root over the last few decades to confirm its health claims, natural molecules such as glucosinolates, isothiocyanates, phenols, flavonoids, anthocyanins and other bioactive substances are believed to be responsible for its medicinal activities. Leaves, seeds, peel and sprouts of radish are rich sources of many functional molecules that may have possible medicinal values(9).Radishes have different skin colours (red, purple, black, yellow, and white through pink), while their flesh is typically white. In addition, the edible root of radish varies in its flavour, size, and length throughout the world. Radish was found to have unique bioactive compounds that have been recently recognized to have potential health benefits for humans. The main bioactive compounds that have been quantified in radish are glucosinolates (e.g., glucoraphanin, glucoraphanin, 4-hydroxy glucobrassicin,glucoerucin, glucoraphanin, glucobrassicin, 4-methioxyglucobrassicin, and neo glucobrassicin) and isothiocyanates (e.g., sulforaphane, sulforaphane, and indole-3-carbinol) (10,11).

Selected Radish types :

3. White radish: Daikon also known as winter, white, oilseed and icicle radish is a variety of radish native to China and Japan.

4. Red radish: The most popular radish variety in the US and resembles a ping-pong ball with a small tail.

Health benefits: In Unani, Greeko-Arab, and Indian folk medicine, radish is used as a household remedy for the treatment of many diseases such as jaundice, gallstone, liver diseases, rectal prolapse, indigestion, and other gastric pains(12,13). Boosts the immune system: Rich in vitamin C, radishes help to protect the body's overall immune system. It acts as an antioxidant by stabilizing volatile molecules that can damage cells. Vitamin C helps with collagen production which helps heal wounds and maintain strong blood vessel walls. Radishes contain plenty of vitamin A. This vitamin helps promote healthy skin and maintain vision. It also converts food into energy and helps to bolster the immune system.As radishes have zero fat,

and are low in carbs and calories, it can be a great addition to your weight loss diet. The chemical compounds glucosinolate and isothiocyanate are found in radishes. These help to maintain healthy blood sugar levels. In addition, an antioxidant called coenzyme Q10 helps to prevent the onset of diabetes. Compounds found in radishes help the kidneys cleanse the body of toxins, and protect the liver from damage. Isothiocyanates found in radishes prevent tumours from forming and cleanse the body of unhealthy cancer-causing compounds. Radishes are high in antioxidants that can help reduce inflammation throughout the body as well as protect against diseases such as arthritis.Radish microgreens are very high in vitamin E which is a powerful antioxidant that helps protect cells and lipids from damage. A diet rich in vitamin E is a great way to ward off Alzheimer's disease. Radishes are a great source of dietary fibre. This promotes healthy bowel movements which may help relieve common digestive problems such as constipation, bloating, gas and indigestion.

Mustard

Mustard is a natural antimicrobial preservative with unique emulsifying and binding properties and contributes pungency, heat, flavour and nutritive value to foods. Mustard is a versatile food ingredient available in many forms: whole seeds, flours, meals, and brands. Mustard is used in packaged meat products, condiments, sauces, baked goods and evenBeverages. Canada is the world's largest exporter of condiments Yellow and Brown mustards. Mustard is one of the oldest spices according to records dating back to 3000 BC. It was recognized for its therapeutic and condiment value, historically being used to treat scorpion bites and entomb kings and as a flavouring agent to disguise degraded food.

Globally, three types of mustard seeds are used as condiments: Yellow or White mustard, Brown and Oriental mustard.

Mustard greens are widely used as a salad, as an oilseed crop in India, for green manure, as a fodder crop or for industrial oil purposes.Mustard seeds are composed of protein,oil,carbohydrate and micronutrients (14).

5.Yellow mustard

Yellow mustard has become popular because of its unique, strong and spicy flavour and is used as a condiment in hotdogs, salads, sandwiches and burgers around the

world. Mustard is rich in protein, fibre, vitamin C and many of the B-complex vitamins. There are several health benefits of mustard for the body like relief from muscular pains, ringworm, and respiratory disorders and also helps in treating cancer and diabetes.

Health benefits: Mustard is known for its rubefacient properties which can help in relieving any kind of muscle spasm. Yellow mustard powder can help in maintaining strong bones, joints and muscles as it is a good source of potassium and calcium. It is a wonderful decongestant that helps in clearing the mucus in the air passage. Inhaling the steam of heated mustard seeds or gargling with mustard tea, helps expel mucus out of the throat and lungs.Mustard oil made from yellow mustard seeds helps to get quick relief from any kind of pain, including arthritis pain. Yellow mustard is rich in monounsaturated and polyunsaturated fats that help in balancing cholesterol levels. It reduces the LDL or 'bad' cholesterol level and increases the HDL or 'good' cholesterol level in the body, thereby reducing the risk of cardiovascular diseases. Yellow mustard is an effective home remedy to get rid of bad breath. Yellow mustard contains an abundance of phytochemicals called glucosinolates that have been scientifically proven to fight against various types of cancer like bladder, cervical, and coloncancer. Yellow mustard is an excellent source of magnesium, which plays an important role in metabolic processes and is in charge of the synthesis of protein. The phosphorous content of yellow mustard contributes to the metabolism of carbohydrates, protein, and fats in the body. Yellow mustard is loaded with beta-carotene, protein, iron and calcium which help in promoting hair growth and keep your hair healthy. Massaging your scalp regularly with yellow mustard oil helps promote stronger and faster hair growth by increasing blood circulation in the scalp(15).

6.Black mustard

Black mustard Microgreens are young seedlings of the black mustard plant. Mustard is a very quick-growing crop and can be ready to harvest in as little as 7 days. The maximum height can be up to 1.5 inches. The first leaves are light green, frilly textured leaves edged with green mustard Black microgreens have a wonderful sweet hot mustard flavour from its lacy lime green leaves, some say it tastes like a mild version of horseradish. Black Mustard Microgreen has a spicy, tangy taste, and can be used as a universal seasoning in various dishes. They are

great on sandwiches or on salads the greens have a fresh crunch.These greens are dense with Vitamins A, B6, C, K, E, calcium, fibre, potassium, and antioxidants.

Health benefits :

• improves immunity, normalizes metabolism, promotes energy storage

• helps to strengthen the walls of blood vessels, and prevents the development of cancer

• Microgreens have antiseptic and anti-inflammatory properties(16)

7.Alfalfa

Alfalfa is a very nutritious microgreen with many benefits. A natural detoxifying agent, Alfalfa has been used for centuries to support good health. The Arabic word Alfalfa means "father of all foods." It stands to reason, that Alfalfa is extremely nutritious. Traditional Chinese Medicine has used Alfalfa leaf to stimulate the appetite and relieve ulcers. The Alfalfa microgreen is mild in flavour. Alfalfa is rich in vitamins, minerals and other nutrients that play a vital role in the strength and growth of our bones and in the maintenance of a healthy body. It contains protein and vitamins A, B1, B6, C, E, and K. It also contains calcium, potassium, carotene, iron, and zinc.

Nutrients:People typically consume alfalfa as a herbal supplement or in the form of alfalfa sprouts they are typically a fair source of vitamin K and also contain many other nutrients, including vitamin C, copper, manganese, and folate (17).

- Reduces risk of breast cancer.Minimizes menopause symptoms and excessive menstruation.
- Prevents osteoporosis, Lower blood glucose levels and treats diabetes.
- Contains antioxidants and other anti-ageing agents.
- Alfalfa is one of the best cholesterol-lowering foods.
- Contributes to detoxifying the urinary tract and purifies blood and liver.
- Has a strong alkaline effect on the body.
- Eases general digestive problems.
- Contains high levels of enzymes for food digestion and assimilation.

RESULTS AND DISCUSSION

The absence of readily available, uniformly produced seeds was a constraint and we depended on seed stores meant for planting and food stores. Seeds meant for consumption did not give information on variety names, expiry dates, expected germination rates and chemical/ fungicide treatment, while all these data were available for the seeds meant for planting. As harvesting of microgreens commences at day 7 (Xiao *et al*., 2012) germination at day 7 was determined (Table 1).

The germination values (Table 1) indicate that germination rates were fastest in the selected microgreens. Among factors that are attributed to poor germination are unfavourable temperature, quality and quantity of light,lack of moisture and inherent factors (Baskin and Baskin, 2014). High germination may indicate high seed vigour. Seedling emergence occurred within 3 days in all species, with the time required to reach harvesting height ranging from ~6 to 10 days after sowing.Radish white exceeded the maximum height limit (11.15 cm) within 6 days and true leaves emerged on day 8. Pak choi and alfalfa have shown moderate rates of growth and did not exceed the maximum height within 14 days, however, true leaves emerged within 8-10 days.

GROWTH PERFORMANCE

Species	Pak choi	Arugula	Alfalfa	Yellow mustard	Radish red	Radish white	Black mustard
Mean root length	2.66	2.13	3.74	4.07	4.74	3.96	6.42
Mean shoot length	6.18	9.27	6.2	7.29	11.15	12.63	10.97
Biomass	0.51	3.36	3.7	3.07	11.35	14.55	2.22

Table 1. shows root length shoot length and total biomass of microgreens

The mean of shoot length and root length of selected microgreens are measured. Black mustard has the highest root length and radish white hasthe highest shoot length. Also, arugula has the lowest root length and alfalfa and pak choi have the

lowest shoot length The table of biomass shows the total biomass of each microgreen. Pak choi has the lowest biomass which is less than 1 gram but radish white has the highest biomass (ie.14.55).

Germination

Species	No .of seeds in one gram	Dry weight of 1000 seeds (g)	G%
Pakchoi	**658**	**2.0**	**73.5 ± 0.86**
Arugula	**299**	**3.6**	75.5 ± **2.08**
Alfalfa	**352**	**3.0**	81.75 ±**0.5**
Yellow Mustard	**477**	**2.6**	96 ±**2.16**
Radish Red	**352**	**3.0**	89.25 ±**0.96**
Radish white	**81**	**11.7**	96.75 ±**0.5**
Black mustard	**198**	**5.5**	74.5 ±**1.91**

Table 2. showing germination percentage of microgreens with standard deviation

Out of 7 microgreen seeds sown all of them germinated. There are no seeds germinated on the first and second days. On the third day, all of them germinated. Radish red was most highest in number and pak choi was the least. On the fourth day, some of the radish red were perished while No.of yellow mustard takes the lead.On the fifth sixth and seventh day the no seeds were newly germinated. the number remains the same only the growth of microgreens takes place

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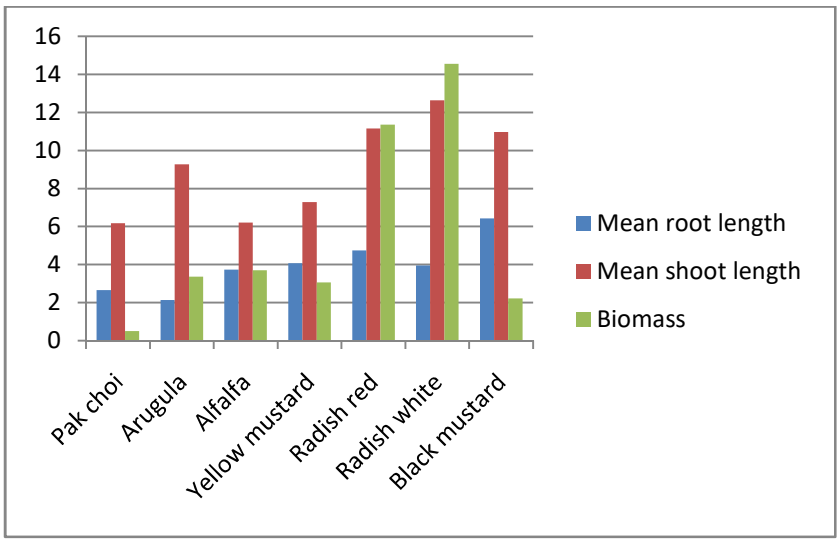
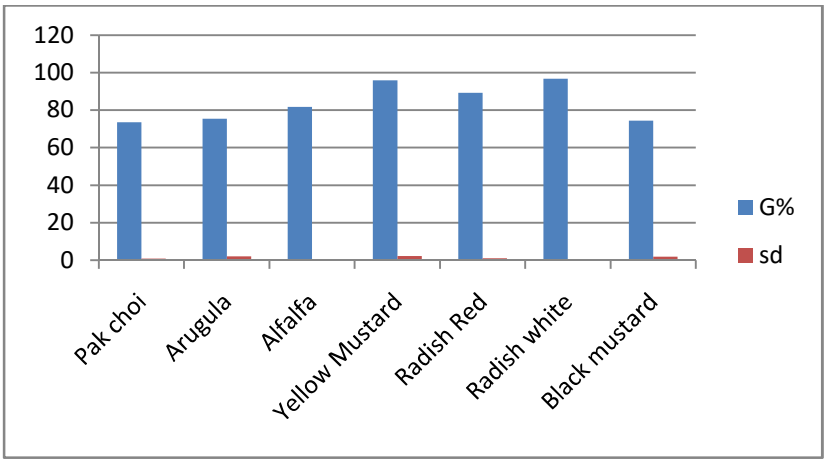


Graph 1. showing root length shoot length and total biomass of microgreens



Graph 2. Showing germination percentage of microgreens with a standard deviation

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IMPACT OF WATER QUALITY ON AQUATIC BIODIVERSITY: LINKING POLLUTION TO ECOSYSTEM HEALTH

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Introduction

The intricate relationship between water quality and aquatic biodiversity is a critical area of research, particularly as anthropogenic activities continue to exert pressure on freshwater and marine ecosystems. Water quality, defined by physical, chemical, and biological parameters, directly influences the health and diversity of aquatic organisms. The degradation of water quality due to pollution has been shown to have profound effects on aquatic biodiversity, leading to shifts in community composition, loss of species, and alterations in ecosystem functions (Hale et al., 2018; Sobhana et al., 2021; Wang & Yin, 2023). This chapter aims to elucidate the mechanisms linking water quality to aquatic biodiversity, focusing on the sources of pollution, its impacts on species diversity, and, potential mitigation strategies.

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Sources of Water Pollution

Water pollution arises from various anthropogenic sources, including agricultural runoff, industrial discharges, and, urban wastewater. Agricultural practices, particularly the use of fertilizers and pesticides, contribute significantly to nutrient loading in aquatic systems. Fertilizer runoff - containing nitrogen and phosphorus - is a primary driver of eutrophication (see *Effects on Species Diversity* for details) (Tian et al., 2017; Umi et al., 2020; Wang & Yin, 2023).

A review by Springmann et al. (2018) clarifies the challenges of balancing food production with environmental sustainability, noting the significant contribution of chemical fertilizers to nutrient runoff and subsequent water quality degradation.

Similarly, the effects of nutrient pollution on carbon dynamics in freshwater ecosystems are significant. Rosemond et al. (2015) reveal that excess nitrogen and phosphorus not only stimulate algal growth but also disrupt carbon pathways essential for riverine food webs, adversely affecting aquatic biodiversity.

And studies have demonstrated that eutrophication can result in functional homogenization of aquatic communities, where species with similar functional traits dominate, leading to reduced biodiversity (Dunck et al., 2022; Wang & Yin, 2023).

Furthermore, the introduction of plastic waste into aquatic environments poses additional threats, as it not only harms individual organisms but also disrupts essential ecosystem services (Freese et al., 2019; Hale et al., 2018; Vijayakumari et al., 2018).

Plastic pollution, particularly microplastics, represents another critical endangerment to aquatic ecosystems. Windsor et al. (2019) discuss how rivers act as conduits for plastic transport, highlighting the ecological impacts of microplastics on aquatic biodiversity. The review reinforces the need for comprehensive research to quantify the sources and effects of plastic pollution, especially in freshwater systems (Windsor et al., 2019). Bellasi et al. (2020) further elucidate the interactions between microplastics and sediments, stressing the importance of understanding their fate in freshwater environments to assess their scope on benthic organisms.

In addition to nutrients and plastics, heavy metals and other toxic substances from industrial activities further compromise water quality. Research indicates that heavy metal contamination can lead to decreased biodiversity and altered community structures in aquatic ecosystems (Staninska-Pięta et al., 2020; Venturieri et al., 2017). For example, the presence of heavy metals has been linked to reduced functional genetic potential in microbial communities, which are essential for nutrient cycling and ecosystem health (Staninska-Pięta et al., 2020).

Aziz et al. (2023) detail the sources and toxicological impacts of heavy metals, which can lead to substantial ecological risks. The urgent need for effective remediation strategies to protect biodiversity and ecosystem health is brought into focus, as heavy metal contamination can lead to bioaccumulation in aquatic organisms.

Moreover, the cumulative effects of multiple pollutants, including pesticides and heavy metals, can synergistically exacerbate the magnitudes on aquatic biodiversity, accentuating the need for comprehensive assessments of water quality (Ito et al., 2020).

Effects on Species Diversity

The outcomes of poor water quality on species diversity are multifaceted and can lead to significant ecological consequences. Eutrophication caused by nutrient enrichment (as discussed in *Sources of Water Pollution*) leads to harmful algal blooms which further deplete oxygen levels in water, creating dead zones

detrimental to aquatic life; ultimately reducing species diversity (Rosemond et al., 2015; Tian et al., 2017; Umi et al., 2020; Wang & Yin, 2023).

Nutrient pollution also alters the availability of essential organic carbon sources for aquatic organisms, as spotlighted by Rosemond et al. (2015). This disruption of food webs can have cascading effects on species diversity and ecosystem services.

The dominance of a few species, particularly cyanobacteria, can lead to a decline in overall biodiversity, as these blooms can produce toxins harmful to fish and other aquatic life (Bestion et al., 2020; Tian et al., 2017). In parallel, the loss of biodiversity, also, can have ripple effects on food webs and ecosystem stability, ultimately affecting human communities that rely on these ecosystems for resources (Hale et al., 2018; Sobhana et al., 2021; Wang & Yin, 2023).

The effects of microplastics on primary producers, notably cyanobacteria, further illustrate the broader ecological consequences of plastic pollution. Yokota et al. (2017) indicate that microplastics

can alter algal growth and photosynthesis, critical processes for maintaining aquatic biodiversity. This alteration may affect entire food webs, linking pollution directly to ecosystem health.

Climate change further complicates the relationship between water quality and biodiversity. The review by Dallas and Rivers-Moore (2014) amplifies the ecological disturbances of climate change on freshwater ecosystems, including changes in aquatic health and biodiversity. Changes in the abiotic factors such as temperature and precipitation patterns can alter water quality parameters, such as dissolved oxygen levels and nutrient concentrations, which significantly influences aquatic biological assemblings, thereby affecting aquatic organisms (Drouineau et al., 2018; Sobhana et al., 2021).

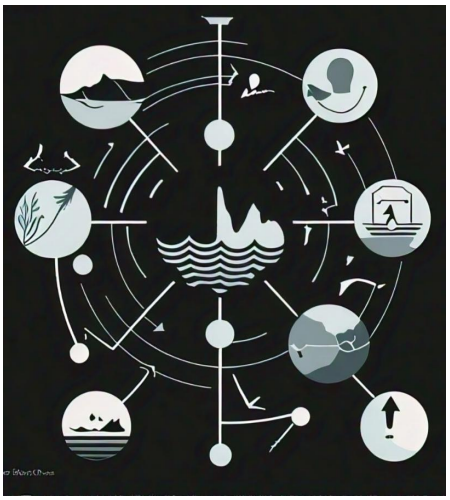
To exemplify, research has shown that increased temperatures can enhance the effects of eutrophication, leading to more severe algal blooms and further reductions in biodiversity (Bestion et al., 2020; García et al., 2018). The interconnectedness of water quality, climate change, and aquatic biodiversity, underscores the importance of understanding how these factors interact to influence aquatic ecosystems.

The research on the combined effects of multiple stressors, including pollution, is crucial for understanding the overall health of aquatic ecosystems. Schäfer et al. (2016) showcase the significant role of organic toxicants in contributing to ecological risks alongside habitat degradation and nutrient overload. This calls for integrated management approaches to address these challenges effectively.

Ecological Risk Assessment and Monitoring

To mitigate the impacts of water pollution on aquatic biodiversity, effective monitoring and risk assessment strategies are essential. The Smart Integrated Monitoring (SIMONI) approach point out the use of bioanalytical methods to assess water quality and identify chemical hazards threatening aquatic ecosystems (Sobhana et al., 2021; Van Der Oost et al., 2017; Vijayakumari et al., 2018). This proactive strategy allows for the early detection of pollution sources and the implementation of management practices aimed at preserving aquatic biodiversity. Besides, the development of biodiversity change indicators can help evaluate the effects of anthropogenic stressors on ecosystems, facilitating targeted conservation efforts (Branquinho et al., 2019; Sobhana et al., 2021).

Knowledge Gaps and Future Research Directions



Despite significant advancements in understanding the relationship between water quality and aquatic biodiversity, several knowledge gaps persist. A case in point is, while the effects of microplastics are increasingly recognized, the understanding of their long-term ecological consequences in freshwater systems is still rudimentary (Windsor et al., 2019). Future research should focus on quantifying the sources, fates, and effects of microplastics on various trophic levels in aquatic ecosystems.

Another case is, while the effects of specific pollutants have been well-documented, there is a lack of comprehensive studies examining the cumulative impacts of multiple pollutants on biodiversity. Understanding the interactive effects of diverse pollutants, such as pesticides and heavy metals, is critical for assessing their collective repercussions on aquatic biodiversity and informing mitigation strategies (Ito et al., 2020). Additionally, recognising the specific mechanisms through which climate change alters water quality and its subsequent effects on aquatic organisms is crucial for developing effective conservation strategies (Drouineau et al., 2018; Sobhana et al., 2021).

Moreover, the role of innovative remediation techniques, such as phytoremediation, in improving water quality and protecting aquatic biodiversity is an area that warrants further investigation. Obinna and Ebere (2019) emphasize the potential

benefits of using aquatic plants for removing pollutants, which could provide sustainable solutions for alleviating the fallouts of pollution on aquatic ecosystems.

Enhanced understanding of how to lessen the ramifications of industrial and agricultural pollution on water quality will be essential for preserving aquatic biodiversity in the future (García et al., 2018).

Additionally, there is a need for more comprehensive studies that explore the cumulative contributions of multiple stressors, including climate change, nutrient pollution, and heavy metals, on aquatic biodiversity. The study by Dallas and Rivers-Moore (2014) points to the importance of considering these interactions to develop effective management strategies.

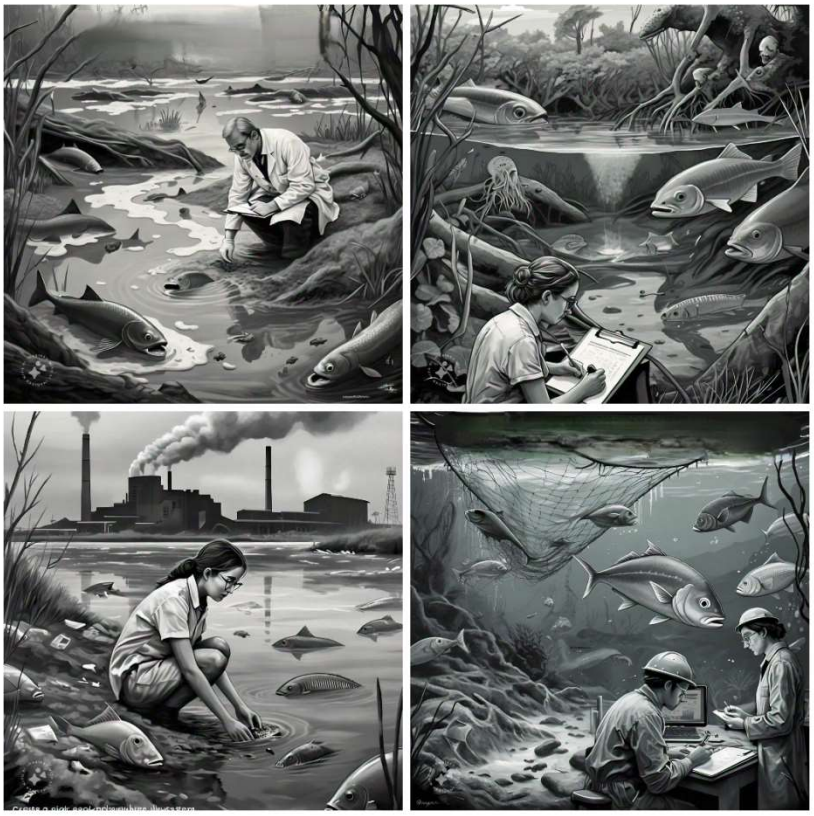
Furthermore, interdisciplinary approaches that integrate ecological, chemical, and social sciences can provide valuable insights into the complex interactions between water quality, biodiversity, and human well-being (Drouineau et al., 2018; Isbell et al., 2022).

Conclusion

The relationship between water quality and aquatic biodiversity is complex and influenced by various pollutants and environmental factors, with significant implications for ecosystem health and resilience.

This literature review accents the significant impacts of nutrient pollution, microplastics, and heavy metals on aquatic ecosystems and biodiversity.

Addressing the challenges posed by water pollution requires a holistic approach that integrates pollution management, climate change adaptation, and conservation strategies. As aquatic ecosystems continue to face various anthropogenic pressures, maintaining clean water will be essential for the health of aquatic life and the overall ecological balance. Prospective studies



and policy efforts must prioritize these interconnections to safeguard aquatic biodiversity for generations to come.

Future research, also, should aim to fill existing knowledge gaps regarding the long-term effects of these pollutants, and, as mentioned, should explore integrated management strategies to defend aquatic ecological balance in the face of ongoing environmental changes. Addressing these challenges is crucial for ensuring the health and sustainability of aquatic ecosystems worldwide.

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ANT DIVERSITY AS BIOINDICATORS OF HABITAT DISTURBANCE: A COMPARATIVE STUDY IN VATAKARA MUNICIPALITY, KERALA

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Abstract

Ants (Hymenoptera: Formicidae) form a crucial ecological group in terrestrial ecosystems, contributing significantly to ecosystem structure and functioning. Representing 15–25% of terrestrial animal biomass, ants are sensitive to habitat disturbances, making them valuable bio-indicators of ecological health. This study compares ant diversity in two transects: a disturbed industrial area and an undisturbed agricultural area in Vatakara Municipality, Kozhikode District, Kerala. Using hand-picking methods and expert identification, 14 species belonging to 4 subfamilies were identified. Diversity indices such as the Shannon-Weiner and Simpson indices were calculated to compare species richness and diversity. The results reveal a lower diversity of ants in the disturbed area, highlighting the adverse effects of habitat disturbances. This study emphasizes the importance of ants in ecosystem monitoring and the ecological consequences of anthropogenic activities.

Key words: Ant diversity, Bioindicators, Habitat disturbance, Species richness, Ecosystem health and Habitat fragmentation

Introduction

Ants (Hymenoptera: Formicidae) are among the most abundant and ecologically significant components of terrestrial ecosystems. They exhibit universal distribution, barring extreme regions such as Antarctica, Greenland, and Iceland, and account for 15–25% of terrestrial animal biomass, with their abundance being particularly pronounced in tropical regions (Hölldobler & Wilson,1990). Ants play keystone roles in ecological processes, including soil turnover, nutrient cycling,

predation, and mutualistic relationships with plants and other organisms (Andersen, 1997).

Morphologically, ants possess a tripartite body structure: the head, mesosoma, and metasoma. Their heads are equipped with compound eyes, ocelli for detecting light and movement, and tactile antennae. Mandibles serve various functions, including food processing, object manipulation, and defence. The mesosoma, the "power core" of the ant, enables locomotion, while the metasoma houses vital systems such as respiration and reproduction. Ants undergo complete metamorphosis, progressing through egg, larva, pupa, and adult stages (Wheeler, 1910) and (Hölldobler & Wilson, 2009).

Social organization is a hallmark of ants, with colonies exhibiting division of labor among castes, including queens, workers, and males . Workers, the sterile females, maintain colonies, forage, and defend against threats, while soldiers are specialized workers with enhanced mandibles for combat. Reproductive queens, supported by workers, lay millions of eggs annually. Ant colonies demonstrate complex communication and coordination, often mediated by chemical pheromones (Wilson, 1971) and (Bourke, 2011)

Ant diversity and community structure are highly sensitive to habitat changes, making them effective bio-indicators (Gotelli & Ellison, 2002). Habitat disturbances, whether anthropogenic (urbanization, industrialization) or natural (fire, flooding), significantly influence ant populations. Disturbed habitats tend to favour generalist species, while undisturbed areas support greater species richness. Given their ecological importance and sensitivity to environmental changes, ants provide critical insights into ecosystem health (Agosti & Alonso, 2000).

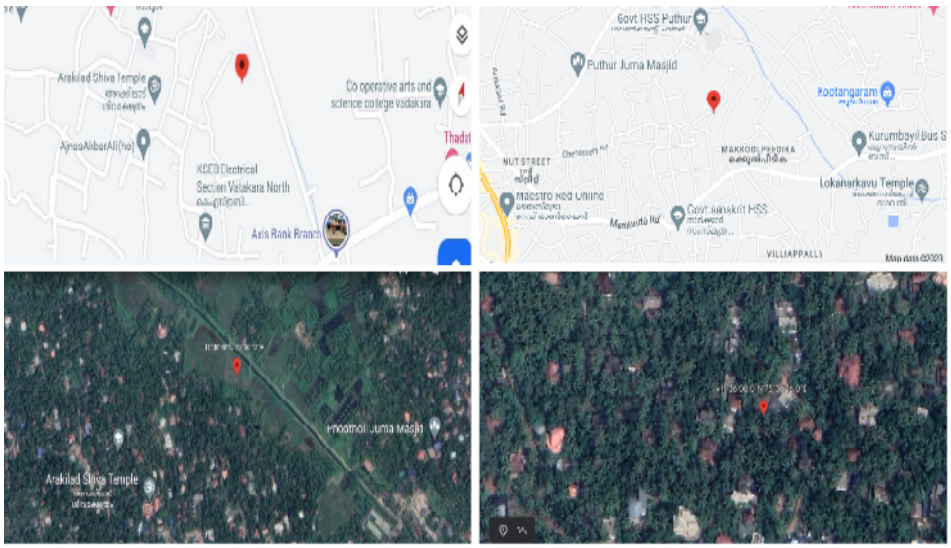
The present study investigates the diversity of ant assemblages in two contrasting habitats within Vatakara Municipality: a disturbed industrial area and an undisturbed agricultural area. By comparing diversity indices, the study aims to assess the ecological health of these areas and understand the impact of habitat disturbances on ant diversity.

Materials and Methods

Study area: The study was conducted in two distinct habitats within Vatakara Municipality, Kozhikode District, Kerala: an undisturbed agricultural area and a disturbed industrial area.

Site 1: Undisturbed Habitat (Kulangarath Thazhekuniyil): This area, located 100 meters from the KSEB 110 KV substation in Vatakara, is primarily used for coconut cultivation, with occasional vegetable cultivation. Historically, this land was extensively used for paddy cultivation. It experiences minimal human interference, qualifying it as an undisturbed habitat. **Geographic coordinates:** 11°36’46°N latitude, 75°36’12°E longitude.

Site 2: Disturbed Habitat (Puthan Purayil Oil Mill Premises): This site comprises 10 cents of land near the Puthan Purayil Oil Mill, a small-scale industrial facility established in 2021. It is located 750 meters from Govt. Sanskrit HSS Meppayil and Govt. HSS Puthur. The area is characterized by significant human interference and habitat fragmentation, making it a disturbed habitat. **Geographic coordinates:** 11°36′08″N latitude, 75°36′26″E longitude.



Site 1 Site 2

Ants were collected using the hand-picking method. Forceps, fine moistened brushes, or fingers (for non-stinging species) were used for collection. The collected ants were placed in plastic or glass tubes of appropriate capacity (1.5–3.0 ml for small ants and 5–8 ml for larger ants) containing 70% ethanol for preservation. For long-term storage, 90% ethanol was used. Each tube was labelled

with collection details, including time, date, and location, which were recorded in a field book. Plastic tubes were preferred for their durability and lightweight properties.

Identification

Collected ant specimens were identified to the species level with the assistance of an expert in ant taxonomy.

Diversity Analysis

Two diversity indices, Shannon-Weiner Index and Simpson Index, were used to analyze the species richness and diversity in the selected areas.

Shannon-Weiner Index (H')

This index accounts for species richness and evenness, providing a comprehensive measure of species diversity. It is calculated as follows:

$$\textbf{Shannon Index, } H' = -\sum pi \ln pi$$

Where:

pi = ni/N

ni = Number of individuals of a species.

N = Total number of individuals.

A higher value indicates greater species diversity.

Simpson Index (D)

This index measures species richness and accounts for the relative abundance of species. It is expressed as

Where:

$$\textbf{Simpson Index, } D = 1 - C$$

$C=\sum(pi)^2$

pi = ni/ N

The index ranges between 0 and 1, with 0 indicating no diversity and 1 indicating infinite diversity. Both indices were used to compare the diversity of ants between the disturbed and undisturbed areas, providing insights into the ecological impact of habitat disturbances.

Results

The study, conducted from February 2023 to April 2023, examined ant diversity in two selected areas within Vatakara Municipality, Kozhikode District, Kerala. A total of 14 species belonging to four subfamilies were collected. Seven species from three subfamilies were recorded from Site 1 (Oil Mill Area), while 12 species from three subfamilies were recorded from Site 2 (Agricultural Area).

Ant diversity in site 1: oil mill area

The Oil Mill Area, a disturbed habitat, documented seven ant species belonging to three subfamilies: Dolichoderinae, Formicinae, and Myrmicinae. Among these, one species belonged to Dolichoderinae, three species to Formicinae, and three species to Myrmicinae. Notable species included *Camponotus mitis* and *Camponotus parius* from the genus *Camponotus*.

Table 1. Identified Ant Species and Subfamilies from the site 1

SL.NO	Species Name	Sub-Family
1	*Tapinoma melanocephalum*	Dolichoderinae
2	*Camponotus parius*	Formicinae
3	*Camponotus mitis*	Formicinae
4	*Myrmicaria brunnea*	Myrmicinae
5	*Monomorium pharaonis*	Myrmicinae
6	*Pheidole sps*	Myrmicinae
7	*Anoplolepis gracilepis*	Formicinae

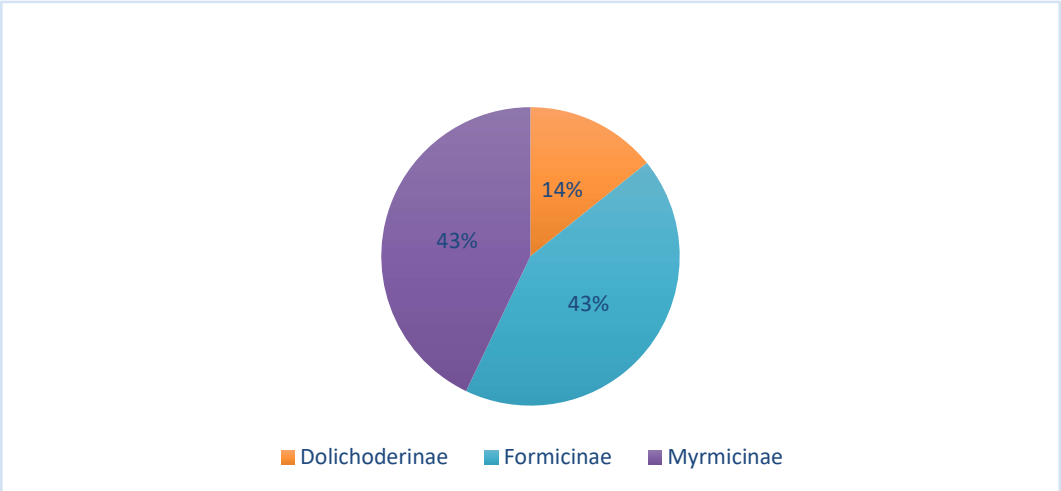


Figure 1. Relative abundance of ant sub families in site 1

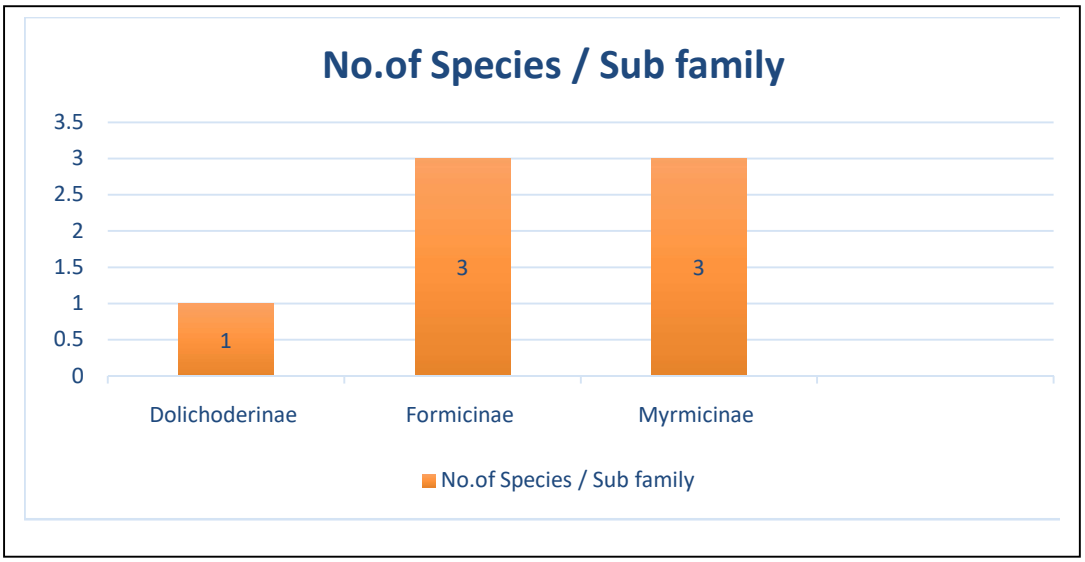


Figure 2. Relative abundance of ant sub families in site 1

Table 2. Biodiversity indices of ant species of site 1.

SL.No	Sub Family	No.of Species	Pi	lnPi	PilnPi	$(Pi)^2$
1	Dolichoderinae	1	0.1428	-1.9463	-0.2779	0.0203
2	Formicinae	3	0.4285	-0.8474	-0.3631	0.1836
3	Myrmicinae	3	0.4285	-0.8474	-0.3631	0.1836
Total		7			-1.0041	0.3875

Shannon- Weiner Index, H' (-Σ PilnPi) is **1.0041** and Simpson Index (1-C) is **0.6125**

Ant Diversity in Site 2: Agricultural Area

The Agricultural Area, an undisturbed habitat, recorded 12 ant species from three subfamilies: Formicinae, Myrmicinae, and Ponerinae. Each subfamily contributed four species. Notable species included *Camponotus mitis*, *Camponotus parius*, and *Camponotus sericius* from the genus *Camponotus*.

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Table 3. Identified Ant Species and Subfamilies in site 2

Sl.No	Name of Species	Sub Family
1	*Monomorium pharaonis*	Myrmicinae
2	*Odontomachus haematodes*	Ponerinae
3	*Diacamma scalpatrum*	Ponerinae
4	*Myrmicaria brunnea*	Myrmicinae
5	*Meranoplus bicolor*	Myrmicinae
6	*Pseudoponera sps*	Ponerinae
7	*Anoplolepis gracilepis*	Formicinae
8	*Camponotus mitis*	Formicinae
9	*Camponotus parius*	Formicinae
10	*Camponotus sericius*	Formicinae
11	*Crematogaster sps*	Myrmicinae
12	*Lobopelta sps*	Ponerinae

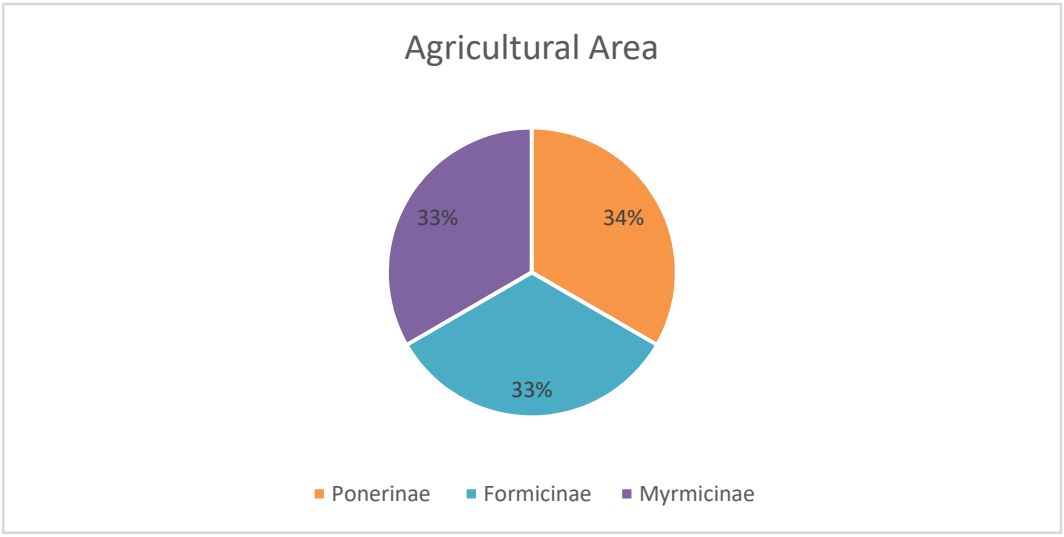

Figure 3. Relative abundance of ant sub families in site 2.

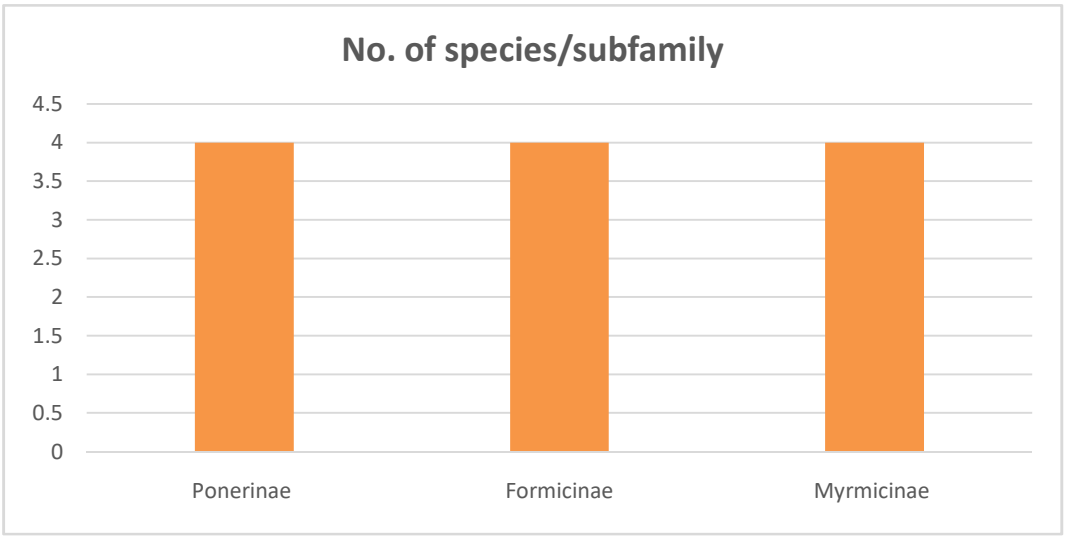

Figure 4. Relative abundance of ant sub families in site 2

Table 4. Biodiversity indices of ant species of Agricultural Area.

SL.No	Sub Family	No. Of Species	Pi	lnPi	PilnPi	$(Pi)^2$
1	Formicinae	4	0.3333	-1.0987	-0.3661	0.1110
2	Myrmicinae	4	0.3333	-1.0987	-0.3661	0.1110
3	Ponerinae	4	0.3333	-1.0987	-0.3661	0.1110
Total		12			-1.0983	0.3332

Shannon- Weiner Index, H' (-Σ PilnPi) is **1.0983** and Simpson Index, D (1-C) is **0.6667**

Table 5. Comparison of ant diversity indices of both the areas

	Shannon-Weiner Index	Simpsons Index
Agricultural Area	1.0983	0.6667
Oil Mill Area	1.0041	0.6125

Discussion

A total of 14 species belonging to 4 subfamilies were documented in the present study. The study revealed that the Agricultural Area, an undisturbed habitat, exhibited higher species diversity (Shannon-Weiner Index: 1.0983; Simpson Index: 0.6667) compared to the Oil Mill Area, a disturbed habitat (Shannon-Weiner Index: 1.0041; Simpson Index: 0.6125). The greater diversity in the Agricultural Area can be attributed to its minimal human interference, allowing for stable ecological conditions and habitat heterogeneity (Tilman, 1999). Conversely, the reduced diversity in the Oil Mill Area may be due to habitat fragmentation and frequent human disturbances, which often limit the abundance and richness of ant species (Fahrig, 2003). The study highlights the critical role of habitat stability in maintaining biodiversity, underscoring the importance of conserving undisturbed ecosystems to support species diversity.

The diversity and distribution of ant species provide insights into the ecological balance of an area. Ants, being one of the most ecologically important groups of insects, serve various functions, including decomposition, seed dispersal, and predator-prey dynamics (Hölldobler & Wilson, 1990). The differences in species composition, distribution, and habitat preferences reflect the varying ecological characteristics and anthropogenic influences on these habitats (Folgarait, 1998).

Subfamily: Ponerinae

Diacamma scalpatrum: This species demonstrates unique reproductive behaviors through gamergates and mutilation of nest mates (Peeters & Ito, 2001). Its presence in both tropical and subtropical zones highlights its adaptability to varied climatic conditions (Andersen, 1995). The distinctive striated head and powerful mandibles indicate an evolutionary advantage in predatory activities and colony defense (Hölldobler & Wilson, 1990).

Pseudoponera sp.: Characterized by its compact build and slit-shaped propodeal spiracle, *Pseudoponera* displays morphological similarities to related genera such as *Austroponera* (Schmidt & Shattuck, 2014). Its distribution in tropical regions signifies its adaptability to warm, humid climates, with nesting often observed in soil (Bolton, 1994).

Odontomachus haematodes: Known as the trap-jaw ant, this species' large head and strong mandibles serve an ecological function in prey capture and nest defense (Dejean & Corbara, 2003). Its preference for open habitats like coastal areas and lawns, while avoiding dense forests, suggests a reliance on human-altered landscapes (Blüthgen & Feldhaar, 2010).

Lobopelta spp.: The ability of *Lobopelta* ants to produce audible stridulatory sounds emphasizes their unique communication mechanisms, which might be advantageous for colony coordination and defense (Hölldobler, 1999). Their broad distribution in Ethiopian, Indo-Malayan, and Australian regions points to their ecological resilience (Bolton, 1994).

Subfamily: Myrmicinae

Crematogaster spp.: Commonly called acrobat ants, their morphological adaptations, such as a flexible gaster, facilitate venom application as a defensive strategy (Longino, 2003). This species' omnivorous nature and aggressive behavior make it a significant component of tropical and subtropical ecosystems (Wilson & Hölldobler, 2005).

Meranoplus bicolor: The striking coloration of this species and its unique posteriorly directed spines serve as distinguishing features (Andersen, 1995). Their presence in bare lands and grasslands highlights their adaptability to open habitats and their potential role in soil aeration and seed dispersal (Folgarait, 1998).

Myrmicaria brunnea: With a wide range of habitats, from fruit gardens to well-developed forests, this species' shiny, chestnut-brown body and varied nest-building activities reflect its ecological versatility (Wilson, 1987). Its ability to form large soil mounds signifies its contribution to soil turnover and ecosystem engineering (Folgarait, 1998).

Monomorium pharaonic: The pharaoh ant, being a global pest, exemplifies how ants can thrive in human-altered environments (Buczkowski & Bennett, 2008). Its nesting within buildings and symbiotic relationship with aphids indicate its opportunistic behavior and potential as a bioindicator of human impact (Peeters & Ito, 2001).

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Pheidole spp**.**: Known for their major and minor worker castes, *Pheidole* ants showcase a hyper-diverse genus (Wilson, 2003). Their robust mandibles and adaptive behaviors underline their ecological roles as harvesters, influencing seed dispersal and nutrient cycling in their habitats (Folgarait, 1998).

Subfamily: Dolichoderinae

Tapinoma melanocephalum: The ghost ant, with its pale and translucent abdomen, is a characteristic tramp species widely distributed in tropical regions (Wetterer, 2009). Its small size and cryptic nesting behavior make it an invasive species capable of spreading across urban and natural landscapes (Holway et al., 2002).

Subfamily: Formicinae

Anoplolepis gracilipes: Commonly referred to as the yellow crazy ant, its long legs and multi-queen colonies make it a dominant invasive species (Abbott, 2005). Its ecological impact, such as predation on native species like red land crabs, emphasizes the need for monitoring and controlling its spread (O'Dowd et al., 2003).

Camponotus mitis*:* This carpenter ant is ecologically significant due to its omnivorous feeding habits and ability to form large colonies (Hölldobler & Wilson, 1990). Its strong mandibles are crucial for nest construction and food transport, making it a key player in forest ecosystems (Andersen, 1995).

Camponotus parius: The black exploding ant is notable for its defensive strategies and dense silk pilosity (Schmidt & Shattuck, 2014). Its wide distribution across Asia, Australia, and Sri Lanka reflects its adaptability to diverse environments (Bolton, 1994).

Camponotus sericeus: The silky sugar ant, with its golden pubescence and robust build, is a generalist species with a wide distribution (Hölldobler & Wilson, 1990). Its role in seed dispersal and symbiotic relationships with plants highlights its ecological significance (Wilson & Hölldobler, 2005).

The agricultural area, with its higher ant diversity indices, signifies a less disturbed habitat compared to the oil mill area (Magurran, 2004). The dominance of generalist species like *Anoplolepis gracilipes* and *Camponotus spp.* across both sites suggests their adaptability to anthropogenic influences (Fahrig, 2003).

Conversely, the presence of species like *Lobopelta* and *Diacamma scalpatrum* in specific habitats indicates ecological niches and microhabitat preferences (Hölldobler & Wilson, 1990).

These results highlight the importance of ants as bioindicators of ecosystem health and underline the need for further studies on their ecological roles and conservation.

The high ant species diversity was observed in agricultural area and minimum numbers of species diversity were recorded in Industrial area (Gayathri and Roopavathy, 2019)

Conclusion

This comparative study on ant diversity in selected areas of Vatakara, Kozhikode, underscores the ecological importance of ants as bioindicators and highlights the adverse effects of habitat disturbances on biodiversity. The results reveal a clear difference in species richness and diversity between the undisturbed agricultural area and the disturbed industrial area. The agricultural area, with its higher Shannon-Weiner and Simpson diversity indices, exhibited a richer and more stable ant community, indicative of minimal human interference and more favourable ecological conditions.

In contrast, the lower diversity in the industrial area reflects the negative impact of habitat fragmentation and anthropogenic activities. Generalist species, such as *Anoplolepis gracilipes* and *Camponotus spp.*, dominated disturbed habitats due to their adaptability, while specialist species, such as *Lobopelta spp.* and *Diacamma scalpatrum*, were more prevalent in the undisturbed area, showcasing their preference for stable microhabitats.

This study emphasizes the critical role of conserving undisturbed ecosystems to maintain biodiversity and ecological balance. Furthermore, the findings advocate for the use of ants as a cost-effective and efficient means of monitoring environmental health and anthropogenic impacts. Future research should focus on long-term studies across multiple habitats to better understand the complex interactions between ants, their environment, and ecological processes.

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