Zoologie Innovante

Series 1

Editors
Dr Dixy V A | Dr. Sheeba P | Sr Freny Jacob

PG Department of Zoology
Vimala College
(Autonomous)
Thrissur - 680 009

Vimala College Publications
Thrissur, Kerala, India 680 009

ISBN : 978-81-7255-135-3

Zoologie Innovante - Series 1

PG Department of Zoology

VIMALA COLLEGE (AUTONOMOUS)
THRISSUR - 9

ABOUT THE INSTITUTION

Vimala College (Autonomous), Thrissur, a first grade women's College under the CMC Management established in 1967, is affiliated to the University of Calicut. The Institution is under the Management of the Nirmala Province, Thrissur, of the Congregation of Mother of Carmel (CMC). True to the ideals and heritage handed over to the Congregation by her founder, Blessed Chavara Kuriakose Elias, the Institution aims at the "pursuit of intellectual and professional excellence and encourages a holistic approach to education that not only ensures academic excellence but also equips young women to face the challenges in life by fostering values, imbibing emotional maturity, creating civic responsibility and building global competencies in a dynamic environment. Ever since its inception, the Institution has been in the forefront of higher education in the State. The Institution offers 18 undergraduate and 18 postgraduate courses and is a centre for research in English, Commerce, Physics, Economics, Social Work and Malayalam. Accredited at the national level with a Five Star status in 2001 by the NAAC, the institution has undergone two subsequent cycles of re-accreditation in 2008 and 2014 and presently holds the top grade A with a CGPA of 3.50 on a 4 point scale. The University Grants Commission (UGC) conferred autonomy in 2016 and identified her as a College with Potential for Excellence in 2016. Vimala College has a full fledged DST - FIST funded laboratory and presently 6 science departments of college is supported under DBT-STAR college Scheme.The atmosphere of the Institution is charged with the noblest ideals of humanity and the spirit of secularism, justice and equality of opportunity enshrined in the Constitution.

ABOUT THE DEPARTMENT OF ZOOLOGY

The Department of Zoology became operational in 1967 and aims to provide quality education, to promote training in practical and conceptual skills in various sub-disciplines of biology and also inculcates the spirit of resource conservation and love for nature. The Department also never fails to motivate the students for self employment in various applied branches of Zoology and to orient the students in developing research skills. Both under graduate and post graduate courses are being offered by the Department, which is facilitated through excellent infrastructure and qualified faculty members. The Department of Zoology has one UG and two PG Programmes in Zoology. Self-financing M. Sc Zoology with specialization "Fishery Science" started in the academic year 2013-2014 and regular M. Sc Zoology with specialization in 'Structure, physiology, development and classification of animals' was introduced in the academic year 2020-2021. With the aim of educating students about the fish culture techniques, the department has initiated a start-up programme - "Aquaphilia" in June 2018. The department has tie up with various research organizations/institutions and has functional MoUs with them. The department is funded by DBT-STAR college scheme in 2020.

ZOOLOGIE INNOVANTE SERIES 1

PG Department of Zoology
Vimala College (Autonomous), Thrissur – 680009

Published by	:	Vimala College Publications
Month and Year of Publication	:	April 2021
ISBN	:	978-81-7255-135-3
Printed at	:	The Union Press, Ph : 0487 2973213
Distributors	:	Sooryagatha (Publishers), Kochi - 682035
Copy Right	:	PG Department of Zoology Vimala College (Autonomous) Thrissur - 9

Principal's Message

It is a great privilege and joy to publish this book **Zoologie Innovante Series 1-** a compilation of Research articles of our Post Graduate students. No doubt that this endeavor will bring out an array of scientific expressions with distinct individual signatures. I do appreciate the Head of the Department of Zoology and all faculty members for their effort. May God Bless you.

Dr. Sr. Beena Jose
Principal.
Vimala College (Autonomous)
Thrissur - 680009

Message

A deep sense of gratitude and joy surge through my heart as I greet you through the columns of this book Zoologie Innovante Series 1. I extend my heartfelt gratitude to Dr. Sr. Beena Jose, Principal for her continuous inspiration and support. I would like to express my sincere thanks to Dr. Sr. Beena T L and Dr. Minimol K, Vice Principals and the management for their support in publishing this Series. I also extend my thanks to all faculty members and students for their effort.

Dr. Honey Sebastian
HoD, Department of Zoology
Vimala College (Autonomous)
Thrissur - 680009

Preface

The main aim of research is to identify problems or to find answers to uncertainties and the purpose of writing and publishing is to disseminate knowledge and findings to other researchers in the respective fields. Innovative ideas and techniques should be imparted to the students so that they are motivated to give their best to the institution as well as themselves. Series -1 of this Book **'Zoologie Innovante'** is the outcome of an idea to publish the research works of PG students, with the implementation of the knowledge they gained from their research work during their PG programme. I would like to record an appreciation to my fellow workers in making this publication an enormous success. Continuing success of this book series means that planning can now proceed with confidence for the upcoming book series to be published in the next academic year.

Dr Dixy B A
Assistant Professor on Contract
Department of Zoology
Vimala College (Autonomous)
Thrissur - 680 009

CONTENTS

EFFECT OF CULTAR UPON SOIL FAUNA

BETSY THOMAS[1], KAYEEN VADAKKAN[2], INDU M S[3]

[1, 3] Department of Zoology, Vimala College, Thrissur

[2] Department of Biotechnology, St. Mary's College,Thrissur.

Abstract

Pesticides are the important agrochemicals used for prevention of crops from pests. The application of pesticide starts from the pre sowing stage. Repeated applications of pesticides contaminate the soil. The indiscriminate use of pesticides disturbs the soil environment by affecting flora and fauna including micro flora of soil, and also the microorganisms present in the soil. Here we study about effect of pesticide cultar upon soil fauna; this study aim to analyse the effect of cultar up on soil micro fauna (bacterial culture and counting technique) and *Pheritima posthuma* (earth worm) after the administration. The result showed that the increased concentration of cultar reduced the bacterial concentration and also observed that histological changes in earthworm.

Key words: Serial dilution, pouring plate, incubation, counting of colonies

Introduction

Soil is a dynamic living system and consists of a variety of micro and macrofauna and flora. They have a primary catabolic role in degradation of plant and animal residues in the environment which contributes to the cycling of nutrients. Insecticide residues usually occur in the top 15 cm layer of soil. It is also the region of greatest activity of soil fauna and flora, and provides a platform for interaction of insecticide residues with them. Microbial breakdown of pesticides is considered one of the most important activities in soil. Pesticides are the important agrochemicals used for prevention of crops from pests. Their use has been largely increased in last few decades. Laboratory and Field experiment was concerning the impacts of both organic and metals toxicity in a continuous process in order to detect their interaction behaviour in the environment which usually results due to several environmental factors leading to the reverse of their intended use. However, a grave environmental problem have aroused because of the indiscriminate usage of these chemicals in agricultural fields. Insecticides are known to be toxic to many non-target organisms and also cause serious sub increases and decreases in reproductive potential and growth rate (Vincyard and Scoot Stewart, 2017).

This tremendous input of pesticides to the ecosystem ultimately will affect the soil fauna. In the present study, we have focused on the analysis of soil contamination

with paclobutrazole 23% sc (cultar) using laboratory techniques and its eco-toxicological effects on the earth worms (Song Y et al., 2015) micro fauna(bacterial colonies) population. There are no baseline data available about the soil contamination with pesticides and the soil fauna of the study area (Aebeed et al., 2018).

Materials and Method

Selection of sampling site and collection of soil for analysis and enumeration of soil microbes

Soil was collected from garden area to the frontage of St. Mary's College Thrissur. The soil samples were periodically collected based on the aim of investigation. The soil from surface was collected using a plastic scooper or corer into a plastic bag. Remove coarse materials (rocks and root) from the soil. Counting of soil bacteria can be achieved by direct microscopy as well as by cultural methods, the plate count technique.

Serial Dilution of soil sample and pouring plate technique for counting colonies

Shake up the soil suspension (dilution 10^{-1}) by hand, and transfer by means of a sterile pipette tip attached to a piston- stroke pipette 1 ml into a 9 ml water blank (dilution 10^{-2}). This process can be repeated until we reach a considerable dilution. Melt solidified nutrient agar. Select a range of 3 dilutions 10^{-1}, 10^{-4}, 10^{-7} into petridish. Subsequently, pour about 10 ml of sterile nutrient agar into plates. After solidification of agar, incubate the plates upside down in a pile in an incubator at 28^{0}C for 24 hours. After the incubation of 24 hours, inspect the plates at low magnification using a colony counter.

Preparation of stock solution for analyzing its effect on terrestrial environment

10ml cultar was accurately weighed. Make up this into 100 ml of distilled water. After administration, analysis of effect of the Azo dye by insitu analysis. The soil samples were taken into the laboratory after the administration of cultar and the culture plates are prepared. The stock solutions were administrated in the study area continuously for 3 days and the soil was collected and treated for culture.

Investigating the effect upon *Pheritima posthuma* (histological analysis)

Pheritima posthuma the study animal was procured from Kerala Agricultural University, Vellanikkara, Thrissur, Kerala. The solution was used perform mortality assay and to detect the time of paralysis. Varying concentration of test solutions were prepared ranging from (0, 1, 2.5, 5 and 10 mg/ml). At each sampling period, earthworms were removed from the soil and washed with distilled water.

Results and Discussion

Analysis and enumeration of soil microbes

Sl. Number	0 mg/ml	1 mg/ml	2.5 mg/ml	5 mg/ml	10 mg/ml
Bacterial Concentration (CFU)	28×10^{-6}	17×10^{-6}	10×10^{-6}	8×10^{-6}	5×10^{-6}

Table 1: Amount of soil microbes in different concentration of cultar

In situ analysis of effect of cultar upon soil micro-biome

A significant effect was seen upon soil microbes when treated with cultar. The increase in the concentration of cultar reduced the bacterial concentration. In higher concentration of pollutant, the presence of microbes was negligible which suggests that high concentrations of cultar contamination could probably inhibit the microbial growth in soil which indirectly effect the fertility of soil.

Effect of dye up on *Pheretima posthuma*

Concentration of cultar(mg/ml)	**Paralysis (minutes)**	**Death (minutes)**
1	131	145
2.5	69	99
5	46	67
10	29	45

Table 2: Concentration depended impact of cultar on Paralysis and death of *Pheretima posthuma*

Histopathological analysis of varying cultar concentration

Histopathological analysis suggested that there are notable aberrations in the tissue level organization of earthworms. Major change was the cell shrinkage and vaculation. Ectodermal spoilage was minimal at moderate concentration. However, the intensity of damage increased with concentration of the cultar.

BIBLIOGRAPHY

- Aebeed S. Aebeed, Abeer H. Amer. (2018). "Combined toxicity of cypermethrin, a pyerthroid insecticide and metal to earthworms Eisenia fetida". Med tox curr res. Vol.1 (1). Pp. 4-10.
- Song Y, Jianrong K, Xueying S, Wei Z, Lingling Li (2015). "Long term toxic effects of deltanethrin and fenvalerante in soil," Journal of Hazard matter. Vol. 30. Pp. 158 - 164. Pubmed website, doi; 10.1016/j.jhazmat.2015.02.057
- J Vineyard and Scott Stewart (2017). "Microbial Degradation of Neoricothnoid Innecticides in the soil and Potential implication on thrips control in cotton cory." The journal of cotton Science Vol. 21, Pp 128 - 133.

CHEMOMETRIC CHARACTERIZATION OF BIOACTIVE COMPOUNDS OF PLECTRANTHUS AMBOINICUS LEAF EXTRACT COMPLIMENTING MOLLUSCICIDAL ACTIVITY

JAMSHEELA V[1], KAYEEN VADAKKAN[2], MEERA K.M[3]

[1, 3]Department of Zoology, Vimala College, Thrissur.
[2] Department of Biotechnology, St. Mary's College,Thrissur.

Abstract

The present study was intended to delineate the molluscicidal property of aqueous extract of the plant *Plectranthus amboinicus* against the fresh water snail *Radix luteola.* Sample was extracted by simple decoction. Different concentrations of extract were added to petri dishes with the snail and their death rate was observed. Following this was optimization of the molluscicidal property of the extract at various temperature and pH. Histological analysis of subject exposed to leaf extract reveals that treatment with *Plectranthus amboinicus* extract altered the tissue level organization of the treated animal which includes formation of basal cell-basement membrane zone, vacuolation, muscular elongation, and formation of gaps and vacuoles also degradation of muscular cells and lose of integrity. The study proves that the aqueous extract of *Plectranthus amboinicus* is an effective molluscicide, it is eco friendly and causes no harm to the environment and is also easily available and easy to use.

Keywords: Molluscicide *Radix luteola, Plectranthus amboinicus,*

Introduction

Schistosomiasis is endemic in about 74 countries and more than 207 million people are infected worldwide, of which 85% of them live in Africa (WHO 2011).It is the second most prevalent disease in Africa after malaria. In Nigeria, 22 million people are infected, which include more than 16 million children (Otarigho Benson, 2012), In small children, *Fasciola hepatica* is one of the pathogenic worms developing resistance to most of the commercially available anthelmintic which became a severe problem nationwide.The vector snails are aquatic and act as an intermediate host for the development of the parasite to an infective free swimming larval stage, i.e. cercariae for schistosome or metacercariae for fasciola. There have been several synthetic chemical compounds and nanoparticles proven their Molluscicidal activities. However, it is also observed that these compounds are mostly toxic to the environment. In this juncture it is essential to develop suitable ethno botanical agents against this threat (Rajeswari, 2014). Biological control stands to be a better alternative to the chemical

controls aimed against snails as they do not produce any adverse effects in the non target organisms and are easily biodegradable.

The new approach to the search of plant molluscicides in this study is the use of aqueous extract of *Plectranthus amboinicus.* It is a large succulent aromatic perennial herb, much branched with distinctive smelling leaves (Kirtikar and Basu 2005). *Radix luteola* were selected for the anti molluscoidal activity in *Plectranthus amboinicus.* It is a species of fresh water snail, an aquatic gastropod mollusc in the family Lymnaeidae. *R. luteola* is a widespread species in south Asia and south East Asia.

Materials and Method

Preparation of the plant extract

The fresh leaves of *Plectranthus amboinicus* was collected from in and around Thrissur during April 2019.Plants were identified and authenticated by botanist Dr. Sr. Meena K Cheruvathur, Assistant Professor, Department of Botany, St. Mary's College, Thrissur, Kerala . The plant material were dried in shade at ambient temperature, and made it in to powder by electrical blender. The crude aqueous extract of *Plectranthus amboinicus* was prepared using decoction method. 20 g of dried powder was extracted with 100 mL of deionized water at 100 °C for 30 min in a water bath.

Collection and maintenance of study animal *Radix luteola*

Fresh water snail *Radix luteola* were collected locally from fresh water ponds in and around Thrissur, Kerala. The snails were acclimatized to laboratory condition for 72 h and used as experimental animal. Ten experimental animals were kept in glass aquaria containing 3L of dechlorinated tap water at room temperature. The pH of the water was 7.2-7.4 and bicarbonate alkalinity, dissolved oxygen, free carbon dioxide were 102.0-105.0, 6.8-7.4, 5.4-6.1 and mg/l respectively.

Histological analysis of subjects treated with *Plectranthus amboinicus* leaf extract

The histological analyses were performed blindly in order to avoid bias. The study animals were relaxed in 3% magnesium chloride and the shell was partly incised to facilitate the penetration of the fixative. The animals were fixed in Bouin's solution for 14 days to decalcify the shell, and transferred to 80% ethanol for 2 days. Processing of paraffin embedding was performed as four to five animals were embedded per paraffin cassette, and continuous serial sections of 2–3 μm with distance of 10 μm each were cut using a rotating microtome. For routine histology, sections were stained with haematoxylin and eosin for 1 and 10 min, respectively.

Analysing the effect of variables in Molluscicidal activity of *Plectranthus amboinicus.*

Various factors such as temperature, pH, and concentration were checked for its effect in maximizing Molluscicidal activity of *Plectranthus amboinicus.*The solution

was prepared by adding varying concentration (0- 40 mg /ml) of plant extract in distilled water. Snail mortality was identified by the contraction of the body within the shell; no response to a needle probe was taken as evidence of death. Similarly, the effect of pH was understood by varying the pH from 5 to 9 in a constant concentration (20mg/ ml) of *Plectranthus amboinicus* leaf extract.pHwas was maintained by three different buffers; acetate (3.6-5.6), phosphate (5.8-7.4), bicarbonate (9.2-10.6). The effect of temperature was understood by incubating reaction mixture in various temperatures (20 - 60/ °).

Results and Discussion

Molluscicidal activity of *Plectranthus amboinicus* against *Radix luteola*.

The molluscicidal effect of active plant extracts was evident from the anatomical and physiological changes observed in the treated group .High dosage of active plant extract caused the cephalopodal mass of each snail to become severely swollen, turgid and failing to mechanical stimulus with blunt needle. Mucous secretion was observed over most of the foot. 100% mortality is observed in all treated concentrations however the time taken for activity varied depending upon the concentration of antagonist (figure 1).Death was observed after 46 minutes of administration when the concentration was 40mg/ml however the time taken to kill subject and concentration of extract was inversely proportional to each other. In higher concentrations the time of death was less where in the presence of low concentration the time taken to cause death was more. As per the observations obtained it took about 103 minutes to cause death in animals when treated with 5 mg/ ml concentration.

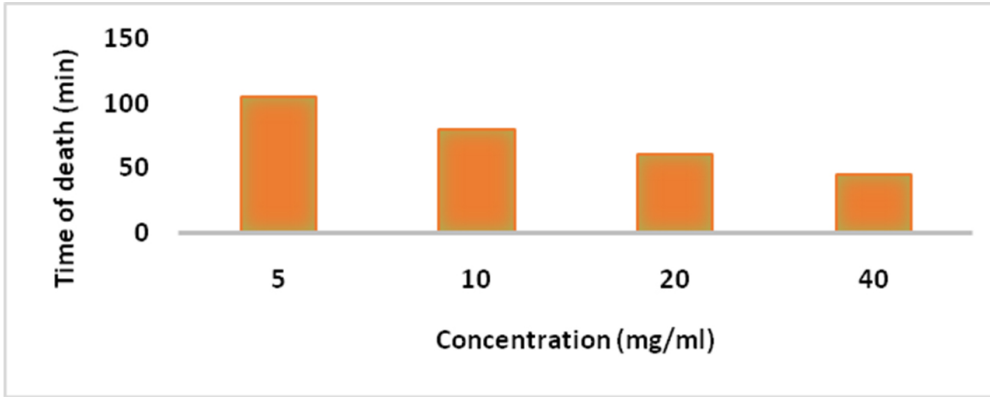


Figure 1: Molluscicidal activity of *Plectranthus amboinicus* against *Radix luteola*

Histological analysis of study subjects exposed to *Plectranthus amboinicus* extract.

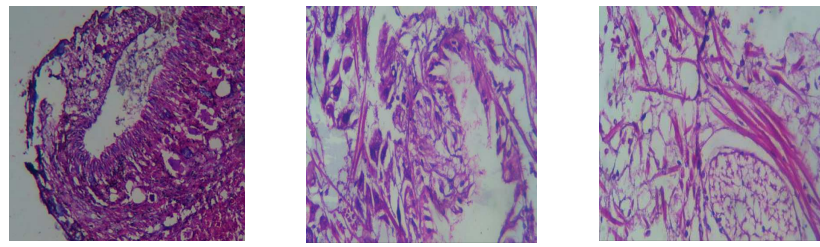


Figure 2 : Histological analysis of effect of *Plectranthus amboinicus* extract on *Radix luteola*. A: photomicrograph of normal animal that shows undisturbed epithelium with tightly arranged connective cells; B: treated subjects suffering from formation of basal cell-basement membrane zone and vacuolation; C: Muscular elongation and formation of gaps and vacuoles also degradation of muscular cells and lose of integrity.

Several changes were observed when *Radix luteola* is treated with *Plectranthus amboinicus* extract. There were notable formations of basal cell-basement membrane zone area which lead to vacuoles. Muscular cells were found elongated and separated with unusual gaps; there were depletion of connective tissue due to muscular degradation. From the observations it was evident that treatment of *Plectranthus amboinicus* extract altered the tissue level organization of study animal.

Analysis and optimization of variables for maximizing activity

The maximum molluscicidal activity was observed in the highest concentration of plant extract (40 mg/ml) where death occurred in 46th minute. Maximum activity was complimented in neutral pH however activity tends to decline in the decrease and increase of pH.Optimum temperature was found to be 40°C where in the activity found reducing with the increase of temperature.

Bibliography

- Benson, O. (2012). Efficacy of Chromolaena odorata leaf extracts on Biomphalaria pfeifferi eggs in control of Schistosomiasis. Zoology and Ecology, *22*(3-4), 236-239.
- Kirtikar, K. R., & Basu, B. D. (2005). Indian Medicinal Plants. Dehradun, India. International Book Distributors, *1*, 478-479.
- Rajeswari, V., 2014. Anthelmintic activity of plants: A review. Res. J. Phytochem. 8, 57–63.
- World Health Organization (2011). Schistosomiasis: Number of people treated worldwide in 2009. Weekly Epidemiological Record 86: 73–80.

ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESIS OF IRON NANO PARTICLE USING *PIPER BETLE* LEAVES EXTRACT

NIVA ANTONY[1], KAYEEN VADAKKAN[2], INDU M S[3]

[1, 3] Department of Zoology, Vimala College, Thrissur.
[2] Department of Biotechnology, St. Mary's College, Thrissur.

Abstract

In the present study, an effort is made to synthesize iron nanoparticles using leaves extract of medicinal *piper betle* as reducing agent. The characterization of green synthesized iron nanoparticles was characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), also antibacterial activity was studied against human pathogenic gram-negative and gram-positive bacterial strains.

Key words

Iron nanoparticles, UV-Vis spectroscopy, SEM, TEM, XRD analysis, antibacterial activity.

Introduction

Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and their potential use for human benefits. Although chemical and physical methods may successfully produce pure, well-defined nanoparticles, these are quite expensive and potentially dangerous to the environment. The nanoparticles are of great interest due to their extremely small size and large surface to volume ratio and they exhibited utterly novel characteristcs compared to the large particles of bulk material (Latha and Gouri, 2014). Use of biological organisms such as microorganisms, plant extractor plant biomass could be an alternative to chemical and physical method for the production of nano particle (Dixon M B *et al*., 2012).

Materials with dimensions 1-100 nm offers the wide range of bioremediation application because nanoparticles provide increase surface area to mass ratio. It can be divided into three categories: treatment and remediation, sensing and detection, and pollution prevention. Iron nanoparticles have recently gained great research interest in environmental applications since it offers high surface reactivity due to high surface area. Removal of organic pollutants, inorganic pollutants and pathogenic bacteria are among the most important applications. Moreover, iron nanoparticles have been applied for the catalytic degradation of chlorinated hydrocarbons such as trichloroethene, tetrachloroethene, and carbon tetrachloride from aqueous solutions (Enshirah *et al*., 2018).

The synthesis of iron oxide nanoparticles using plant materials offer several benefits of eco friendliness and compatibility for various applications as they do not use toxic chemicals for the synthesis protocol.(Amutha and Sridhar., 2018). Bacterial resistance to various antibiotics is a serious clinical dilemma, so different antimicrobial activities were performed using plants as a source. The development in the field of green chemistry has delivered different nanomaterials as substitute antibacterial agents. In this study, an effort is made to synthesize iron nanoparticles using leaves extract of medicinal *piper betle* as reducing agent.

Materials and Method

Collection of plant materials:

Leaves of *piper betle* Willd were collected from Thalore, Thrissur district of Kerala and the taxonomic identification was done by taxonomist Dr Sr Meena K Cheruvathur, Assistant Professor, Department of Botany, St.Marys College, Thrissur.

Preparation of plant extract:

The washed leaf were dried using hot air oven at 60^{o}C and grinded into fine powder. 2.5g of fine powder was weighed and boiled with 100mL of de-ionized water at 80^{o} for 15 minutes using water bath. The plant extract was filtered and collected separately.

Synthesis of iron nanoparticles from plant extract:

Take 10ml of water extracts of *piper betle* in a beaker. Add 90ml of aqueous solution of 1M ferric chloride solution into it. If iron nanoparticle is present, there will be a change in colour from yellowish brown to brownish or greenish black after certain time period of incubation at room temperature.

Characterization of iron nanoparticles:

The synthesis of nanoparticles was confirmed and monitored with the help of the following analytical methods, which help to investigate distinctive properties of iron nanoparticles that were synthesized biologically.

UV-Visible spectra analysis:

UV-Visible spectroscopy is a widely used technique to investigate the optical properties of the particles. This analysis specifies the time point of maximum production of FeNPs by taking absorption spectra between 200 to 400nm wavelength range.

SEM Analysis:

Scanning Electron Microscope (SEM) is a type of electron microscope that images the sample by scanning it with high energy beam of electrons. The morphological features such as size of the synthesized iron nanoparticles from Saraca bark was studied by SEM (JOEL - MODEL 6930) at an accelerating voltage of 20KV.

TEM Analysis:

Transmission Electron Microscope (TEM) technique was used to visualize the morphology of the FeNPs. This method helps to observe the particle size of a material in nano-dimensions and study the crystal structure meticulously. In a high resolution transmission electron microscope, a thin sample or specimen is irradiated with a sharp high electron beam (usually in the range of 100-200KV).

XRD Analysis:

X-Ray diffraction is an analytical technique that reveals the detailed information about the chemical composition, crystallographic structure and physical properties of particles. The crystalline structure of iron nanoparticles was determined by XRD

Antibacterial Activity of Iron nanoparticles.

A human pathogenic gram-negative (*Pseudomonas aeruginosa*) and gram-positive (Staphylococcus aureus) bacterial strains were used for antimicrobial study of iron nanoparticles by well-diffusion method.

Results and Discussion

Synthesis of FeNPs:

The green synthesis of iron nanoparticles was carried out by adding 1Mm $FeCl_3$ solution into the extract of *Piper betle* results a colour change from yellowish-brown to greenish-black .

Characterization of FeNPs:

The biologically synthesized iron nanoparticles were analysed using UV-Vis spectroscopy, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Selected area electron diffraction (SAED) and X-ray diffraction analysis. The characterization of FeNPs by these techniques obtained the following results:-

UV-Visible Spectral analysis:

The SPR phenomenon arises when nanoparticles are irradiated with visible light, because of the collective oscillations of the conduction electrons. In our results, a sharp peak specific for the synthesized iron nanoparticles was obtained at 200 –250 nm by UV Visible spectroscope.

XRD Analysis

The crystalline nature of biologically synthesized FeNPs has been investigated by X-ray diffraction (XRD). Typical XRD pattern monitored, which indicates the formation of iron nanoparticle. Table.1 shows the experimentally obtained and standard diffraction angle.

Experimental diffraction angle (2θ in degree)	Standard diffraction angle (2θ in degree)
34.6	35.2

Table 1 : Experimental and standard diffraction angles of FeNPs

SEM analysis:

SEM technique was employed to characterize the size, shape and morphology of iron nanoparticles. SEM images were obtained for the iron nanoparticles. The SEM micrograph revealed that the synthesized iron nanoparticles were aggregated as irregular spherical shapes and ranges from 100-200 nm with inter particle distance. The larger size of particle is due to capping agent binding the FeNPs.

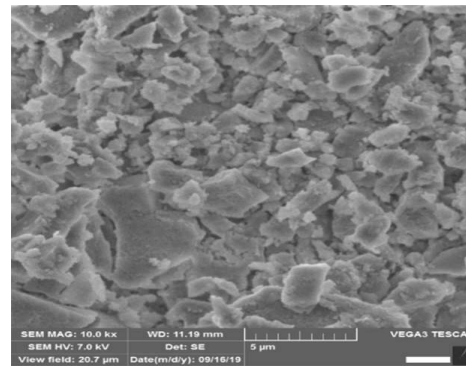


Figure1: SEM Micrograph of FeNPs synthesized from the leaf extracts

TEM and SAED study of FeNPs:

Transmission electron microscopy (TEM) provided further insight into the morphology and size of iron nanoparticles as shown in the fig.2. The irregular shrinkled nature of the FeNPs from aqueous extracts was visualized by TEM analysis with average diameter ranging from 50-80 nm

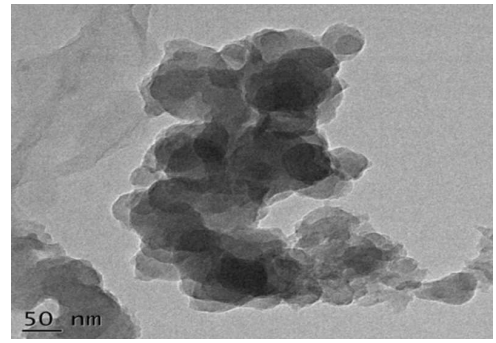


Figure2: TEM analysis of FeNPs

Antibacterial Activity of Iron nanoparticles.

Iron nanoparticles were synthesized using *Piper betle* leaf extract. It showed high degree of antibacterial activity against gram positive and grams negative strains. *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains were found to be inhibited by the administration of FeNPs.

	ZONE OF INHIBITION (ZOI)	
Bacterial strains	IRON NANOPARTICLE	Amoxicillin
Staphylococcus aureus	18.33333333	21
Pseudomonas aeruginosa	17	20

Table 2: showing average diameter of zone of inhibition of iron nanoparticle and amoxicillin.

Bibliography

- Amutha, S., & Sridhar, S. (2018). Green synthesis of magnetic iron oxide nanoparticle using leaves of Glycosmis mauritiana and their antibacterial activity against human pathogens. Journal of Innovations in Pharmaceutical and Biological Sciences, Vol. 5, 22-26.
- Latha, N., and Gauri, M (2014). Biosynthesis and characterisation of Fe_3O_4 nanoparticles using Caricaya Papaya leaves extracts. Synthesis, Vol. 3 Pp. 1551 - 1556.

ISOLATION AND CHARACTERISTICS OF PHOSPHORUS SOLUBILIZING BACTERIAL STRAIN AND ITS EFFECT UPON GROWTH REGULATION IN *CUCUMIS SATIVUS*

REMYA KRISHNA K.K[1], KAYEEN VADAKKAN[2], MEERA K.M[3]

[1,3]Department of Zoology, Vimala College, Thrissur.
[2] Department of Biotechnology, St. Mary's College,Thrissur.

Abstract

Current soil management strategies are mainly dependent on inorganic chemical based fertilizers which caused serious threat to human health and environment. The exploitation of beneficial microbes as a biofertilizer has become paramount importance in agriculture sector for their potential role in food safety and sustainable crop production. Hence we introduced a new strain of phosphorus bacteria as a biofertilizer. The strain of phosphorus bacteria was isolated and incubated then screening was done. Here we introduced two different pots; a control and test of *Cucumis sativus*. The inoculums prepared from the phosphorus bacterial broth culture was introduced in the test. The comparison between the control and test was done. The result indicated that test showed maximum phosphorus solubilizing capacity promoting its growth

Keywords; Serial dilution, pouring plate, 16S rRNA screening, phosphorus broth culture

Introduction

The excess uses of chemical fertilizers in agriculture are costly with adverse effects on physico-chemical properties of soils. Therefore, in the recent years several organic fertilizers have been introduced that act as natural stimulators for plant growth and development (Khan *et al*., 2009). The knowledge of such natural stimulator or microbial inoculums has long history started with culture of small scale compost production and passes from generation to generation of farmers (Abdul Halim, 2009). A specific group of this kind of fertilizers includes products based on plant growth-promoting microorganisms named biofertilizer or 'microbial inoculants' that are preparation containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulytic microorganisms. These are used for application of seed, soil or composting areas with the objective to enhance the numbers of such microorganisms and accelerate certain microbial process to augment the extent of the availability of nutrients in a form which can assimilated by plant (Khosro and Yousef, 2012). Such biofertilizers are important components of integrated nutrients management in soil, while play key role in productivity and sustainability of soil. With every passing days, these biofertilizer replacing chemical fertilizers due to cost

effectively, ecofriendly and renewable source of plant nutrients. The viable microbial preparations possessing P-solubilizing activity are generally termed as microphos. The phosphate-solubilizing microbes showing greater solubilization (both qualitatively and quantitatively) of insoluble P under in vitro conditions are selected for field trials prior to production in bulk for ultimate transmission as a biofertiliser. In this work, we aim to carry out isolation of bacteria that may have the potential to act as a potential bio fertilizer which may play a vital role in sustainable development of agriculture. Here we are taking nitrophosphorus solubilizing bacteria as a potential biofertilizer.

Materials and method

Collection of soil

A sufficient amount of soil sample is taken from the sampling site. The soil from surface was collected using a plastic scooper or corer in to a plastic bag. Remove coarse materials (rocks and root) from the soil. The plastic must have kept open in order to avoid compaction during transport to the laboratory (Navarro-Noya et al., 2012)

Isolation of phosphorus solubilizing bacteria

One gram of rhizosphere soil was mixed thoroughly in 100 ml sterile water and was processed following serial dilution agar plate technique. About 1 gm of soil was taken from the plastic bag and then the soil was diluted to 100 ml of water in a conical flask which served as stock solution. Remaining 9 test tubes were filled with 9 ml of water. Transferring of 1 ml of water from the stock solution to 9 ml of sterilized distilled water with the help of pipettes yielded 10^{-1} dilutions and the series continued up to 10^{-9} dilutions. Sterility is the hallmark of any bacteriological isolation so the entire process was carried in the laminar airflow. Suitable dilutions (10^{-1}, 10^{-4} and 10^{-7}) of rhizosphere wereplated on pikovaskyas medium. Pikovaskyas medium is a selective medium for the isolation of Phosphorus solubilizers. The plates were incubated at room temperature 37°C for 48 hours and the colonies exhibiting clear zones were selected, purified by four-way streak plate method.

Screening of bacterial isolates

Strains by studying the external morphological structures were further confirmed by their ability to be grown on Pikovaskyas agar media which is the most important test for the phosphate solubilizing bacteria. The bacterial colonies were picked from the pure culture slants by the help of the inoculating needle and were streaked in the Pikovaskyas agar media plates and were incubated at 37° C for 48 hrs. In the next day the bacterial colonies showed a clear halozone formation which confirmed them to be Phosphorus solubilising bacterial species. For further studies these colonies were again grown in nutrient agar media.

In-situ field trial to analyze the effect of Phosphorus Solubilizing Bacteria in the growth of *Cucumis sativus.*

Inoculum was made by bacterial broth culture that has attained its log phase of growth. Two study groups were maintained and denoted as control and test group. The soil was placed in pots (10cm x 10 cm), each contained 1.5 kg soil and each treatment was represented by three replicates using a fully randomized design. Plant seedlings were planted in the pot carefully and the test group was inoculated with bacterial cultural broth on day 3, 7, and 10. The study was carried out in controlled environment for 30 days. After study span the plants were analyzed for the effect of PSB in it.

Results and Discussion

Isolation and selection of phosphorus solubilizing bacteria (PSB)

Bacterial strains were isolated from soil as mixed culture (Figure 1). The bacterial colonies were picked from the pure culture slants by the help of the inoculating needle and were streaked in the Pikovskayas agar media plates and were incubated at 37° C overnight. In the next day the bacterial colonies showed a clear halozone formation which confirmed them to be Phosphorous solubilizing bacterial species (Figure 2).

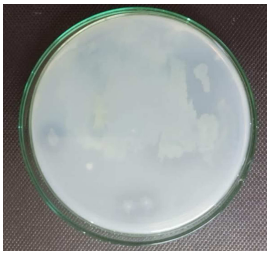


Figure 1: Bacterial mixed culture isolated from soil

Figure 2: Positive bacterial isolate showing halo zone in PKV agar media plates.

Field analysis of bacterial inoculum in plant growth

The growth parameters of *Cucumis sativus* in each test was taken and compared

with the growth of the control. The growth parameter determination was done using the standard protocols. Parameters such as height and number of leaves was determined to analyze the effect of PSB upon growth of *Cucumis sativus*

	Height of plant (cm)	**Number of leaf (n)**
Treated plant	51	20
Control	43	13

Table 1: Effect of Bacterial inoculum in growth parameters

Bacterial inoculum had a great impact on the growth of plant. It was exhibited that growth parameters such as height of the plant and number of leaves was highly influenced by bacterial inoculums.

Bibliography

- Abdul Halim NB, 2009. Effects of using enhanced biofertilizer containing N-fixer bacteria on patchouli growth. Thesis. Faculty of Chemical and Natural Resources Engineering University Malaysia Pahang. pp.145.
- Navarro-Noya, Y. E., Hernández-Mendoza, E., Morales-Jiménez, J., Jan-Roblero, J., Martínez-Romero, E., & Hernández-Rodríguez, C. (2012). Isolation and characterization of nitrogen fixing heterotrophic bacteria from the rhizosphere of pioneer plants growing on mine tailings. *Applied Soil Ecology*, *62*, 52-60.
- Khan W, Rayirath, UP, Subramanian S, 2009. Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.,* 28: 386-399.
- Khosro M, Yousef S, 2012. Bacterial biofertilizers for sustainable crop production: A review. *ARPN J. Agric. Biol. Sci.,* **7**: 307-316.

NUTRACEUTICAL EFFECT OF *Averrhoa bilimbi* IN ANTAGONIZING CONTAGIOUS HELMINTHIASIS

SREELAKSHMI T.M[1], KAYEEN VADAKKAN[2], DIXY B A[3]

[1, 3] Department of Zoology, Vimala College, Thrissur
[2] Department of Biotechnology, St. Mary's College,Thrissur.

Abstract

The present experimental work was done for the search of anthelminthic activity of *Averrhoa bilimbi*. Sample was extracted by simple decoction. Different concentration of extract was added to petri dishes with the earthworms and their death rate was observed. Following this was the anthelminthic property of the extract at various temperature and pH. Histological analysis of subject exposed to leaf extract reveals that treatment with *Averrhoa bilimbi* extract altered the radial thickness of body wall. It also showed the neighboring cells in circular and longitudinal muscles to be discontinuous, separated by narrow to large gap junctions. The prominent damage seen was the degradation of connective tissues. The study proves that the aqueous extract of *Averrhoa bilimbi* has an effective anthelminthic activity, it is ecofriendly and causes no harm to the environment and is also easily available and easy to use.

Key words: Anthelminthic activity, *Averrhoa bilimbi* , *Pheretima posthuma*

Introduction

Helminthiasis is among the most important animal diseases infecting heavy production losses. The diseas is prevalent in third world countries (Das et al., 2011) due to poor management practices. The present experimental work was done for the search of anthelminthic activity of plant. Leaf extract of plant of oxalidaceae family were conducted in this study. *Averrhoa bilimbi* is commonly known as bilimbi or irumpanpuli or vilimbipuli in India. The fruits of bilimbi are commonly used in some medicines and food items. In Phillipines, the leaves serve as paste on itches , swelling , rheumatism , mumps or skin eruptions. They are used for bites of venomous creatures. A leaf intrusion is used for after birth tonic, while flower infusion is used for thrush, cold and cough. In some villages in the Thiruvananthapuram district of Kerala, the fruit of bilimbi was used in folk medicine to control obesity. The leaf extract of bilimbi contain alkaloids, tannins, saponins, flavonoids, phenols etc. The major nutrients include vitamin C, iron, vitamin B2, vitamin B3 and phosphorus. Anthelminthic activity on *Averrhoa bilimbi* is performed for the first time and the objective of the study was to determine anthelminthic activity of leaf extract of bilimbi.

Materials and Methods

Collection of plant and preparation of plant extract

The plant of interest *Averrhoa bilimbi was* collected from local areas of Thrissur, plants were identified and authenticated by botanist Dr. Sr.Meena K Cheruvathur, Assistant professor, Department of Botany, St. Mary's College, Thrissur, Kerala and kept in herbarium under voucher number SMC/BOT/MCAR/2019/45. The crude aqueous extract of *Averrhoa bilimbi* was prepared using the powdered plant material by mixing it with 200mL of distilled water in a flask and boiled for 30 minutes.

Collection of *Pheretima posthuma*

All the experiments were carried out in Indian adult earthworms (*Pheretima posthuma*) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. *Pheritima posthuma* the study animal was procured from Kerala Agricultural University, Vellanikkara, Thrissur, Kerala. The animals were maintained in moisture soil in the lab.

Experimental Design for screening of *Averrhoa bilimbi* for anthelmintic activity

Pheretima posthuma was placed in petri dish containing 25 ml of test solution of varying concentrations of *Averrhoa bilimbi aqueous* extract ranging from 0 to 40 mg/ml. Each Petri dish was placed with 3 worms and observed for paralysis or death.

Optimizing the effect of limiting factors in anthelminthic activity.

Various factors such as temperature, pH, and concentration were checked for its effect in maximizing anthelmintic activity of *Averrhoa bilimbi.* The solution was prepared by adding varying concentration (0- 40 mg /ml) of plant extract in distilled water. The effect of pH was understood by varying the pH from 5 to 9 in a constant concentration (10 mg/ml) of *Averrhoa bilimbi* leaf extract. The effect of temperature was understood by incubating reaction mixture in various temperature 20 - 60^0C.

Characterization of Bioactive Fraction of *Averrhoa bilimbi* leaf extract

The bioactive constitutes present in leaf *Averroha bilimbi* extracts of leaf extract was determined by Gas Chromatography (Agilent 6890 series) equipped with HP-5MS column mass spectrometer operated at initial column temperature of 30/ °C and heated up to 300/ °C at 10/ °C /5min.

Analyzing the phytochemical constituents of *Averrhoa bilimbi* leaf extract.

Standard protocols were practiced for the identification of phytochemical constituents of *Averrhoa bilimbi* leaf extract.

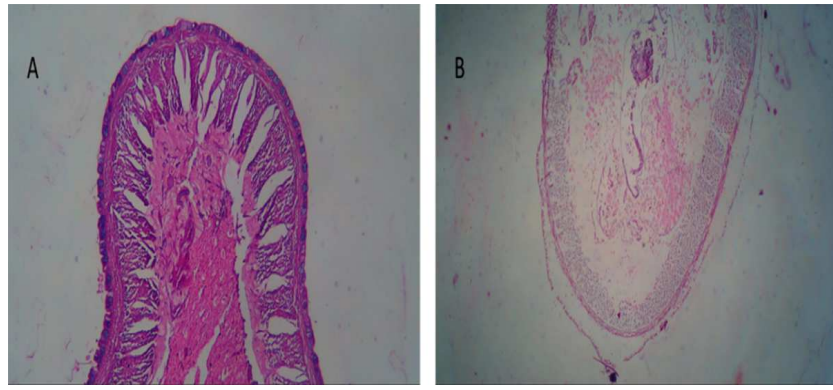
Free radical scavenging assay

Free radical scavenging activity of the plant extract was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH), by

a modified method. The free radical scavenging capacity of the ethanol extract of *Averrhoa bilimbi as* determined using DPPH assay.

Results and Discussion

The fresh petioles of *Averrhoa bilimbi* was collected from local areas of Thrissur, plants were identified and authenticated by a botanist Dr. Sr. Meena K Cheruvathur, Assistant professor, Department of Botany, St. Mary's College, Thrissur, Kerala and kept in herbarium under voucher number SMC/BOT/MCAR/2019/45. All the experiments were carried out in Indian adult earthworms (*Pheretima posthuma*) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. The effect of *Averrhoa bilimbi* aqueous extract in anthelminthic activity was analyzed by taking *Pheretima posthuma* as a model organism at varying concentrations that of 0 to 40 mg/ ml. It was observed that subjects exhibited diverse anatomical, morphological and behavioral changes in response to the treatment. There were notable anatomical changes observed in subjects such as multiple concussions, tissue inflammation, necrosis, posterior elongation and damage in mucoid layer, it was noted that tissue integrity was damaged to a greater degree in subjects treated with high concentrations (Das et al., 2011). There were several clinical observations done in the histological analysis of earthworm treated with *Averrhoa bilimbi* extract wherein the major change being the significant decrease in the radial thickness of body wall. It also showed the neighboring cells in circular and longitudinal muscles to be discontinuous, separated by narrow to large gap junctions. The prominent damage seen was the degradation of connective tissues.



Histological analysis of effect of *Averrhoa bilimbi* extract upon Pheretima posthuma. A: Photomicrograph showing histology of normal earthworm. B: Photomicrograph showing histology of earthworm treated with *Averrhoa bilimbi*

Phytochemical screening of leaf extract observed that there was presence of carbohydrate, phenol, flavonoids, tannins, terpinoids, steroids, sugar and reducing sugar were present in extract.

The maximum anthelminthic activity was observed in the highest concentration of plant extract). From the analysis it was observed that maximum activity was complimented in neutral pH however activity tends to decline in the decrease and increase of pH. Similarly, the optimum temperature was found to be 40°C wherein the activity found reducing with the increase of temperature. GC-MS analysis of is carried out to determine the possible chemical compounds in it. It was observed that there were two compounds present in the bioactive fraction however the major component was found to be pentanoic acid The effect of antioxidant on DPPH is believed to be due to their hydrogen-donating ability. The DPPH assay measures the antioxidant activity of water soluble phenolics. It was evident that the extracts showed proton-donating ability and this could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

BIBLIOGRAPHY

- Das, J. K., Choudhury, S., Adhikary, S., Das, B., Samanta, S., Mandal, S. C., & Dey, S. P. (2011). Anthelmintic activity of Clerodendrum viscosum. *Oriental Pharmacy and Experimental Medicine*, *11*(2), 119-122.
- Das, S., Akhter, R., Huque, S., Anwar, R., Das, P., Tanni, K. A., & Shahriar, M. (2017). In vitro anthelmintic activity of leaf extracts of four different types of Calamus Species. *Pharm. Pharmacol. Int. J*, *5*, 00118.

STUDY ON NEUTRACEUTICAL ASPECT OF *Psophcarpus tetragonolobus* BY ANALYSIS PHYTOCHEMICAL AND ANTIHELMINTHIC SCREENING

SHERIN VARGHESE[1], SANDHYA P.V[2], AND SR. FRENY JACOB[3]

[1, 2, 3] Department of Zoology, Vimala College, Thrissur.

Abstract

Neutraceuticals are essentially prophylactic or preventive in contrast to drugs which are active chemical substance used for pre-protection or treat an illness. Hence food as a medicine is most important aspect in the present world, the core basis of neutraceutical study. The plant derived neutraceuticals was used by ancient Egyptians because of their ability to cure variety of health conditions and used as dietary food supplements (Sumanthi *et al*., 2019).This activity can be tested by using earthworm *Pheretima posthuma* due to its anatomical and physiological similarity to the intestinal round worm, parasites on human beings (Awad.,2004)in extract of *Psophocarpus tetragonolobus* taken at different concentration showed presence of primary and secondary metabolite such as carbohydrates, diterpenes, triterpenes, saponins and phenolic compounds. The degree of activity can be noted based on analyzing movements like curling, wriggling followed by paralysis in regard with time. The histopathological analysis of tested earthworm concretes the experimental observations.

Introduction

Neutraceutic is a term derived from nutrition and pharmaceutics, are naturally derived health promoting bioactive compounds that are found in foods, dietary supplements and herbal products and have disease preventing and medicinal properties (Apri Dey and Laxmipriya Nampoothiri., 2019). Which help to improve health, delay the aging process, and prevent chronic diseases and disorders related to oxidative stress including allergy, cardiovascular, cancer, diabetes, immune-inflammatory diseases as well as increase life expectancy and support the structure and function of the body. Neutraceuticals has been used as a alternative for pharamaceuticals on the basis of prevention and cure. The most common helminthiasis is those caused by infection with the intestinal helminthes, ascarisis, trichuriasis, and hook worms, followed by schistosomiasis and Lymphatic filariasis. Weakness due to malnutrition and anemia is the major complains of infection with worms (Jones and Berkley, 2014).mostly affecting school and preschool children characterising poor educational performance, stunted growth and diminishing physical fitness as well as impaired memory and cognition. A number of medicinal plants have been used to treat parasitic infections in man and animals (Akhtar *et al*., 2000).

The experiments were conducted in the Indian earthworm *Pheretima posthuma* due to its anatomical and physiological similarity to the intestinal round worm, parasites on human beings (Awad, 2004). The leaf of *Psophocarpus tetragonolobus*, plant material used is one of the versatile edible legumes of tropical origin, that is cultivated mainly at a subsistence scale in hot and humid countries across India, Southeast Asia, and the Western Pacific islands, with a presence in a number of African countries as well (Kant et al., 2018). The plant is a climber in the Fabaceae family and closely related to the pole beans.

Plants produce phytochemicals in order to protect themselves against environmental threats like predatory insects, pollution and diseases. They are largely metabolized by the enzymes that metabolize food and drugs. Generally phytochemicals have been classified to six major categories based on their chemical structures and their characteristics. These categories include carbohydrates, lipids, phenolics, terpenoids and alkaloids and nitrogen containing compounds.

Anthelminthic activity can be used to treat against contagious helminthiasis, because there is a considerable death and economic loss are caused by such parasitic infections and helminthes (Mehlhorn., 2014). Currently synthetic anthelmintics are the primary means of controlling parasitic infections. One promising area of investigation is the use of plant based antihelmintics.

Materials and Methods

Collection of plant material and preparation of plant extract

The mature green leaves of *Psophocarpus tetragonolobus* was collected from St.Mary's college hostel Thrissur and it was identified and authenticated by a botanist. The plant material were dried in shade at ambient temperature, and made it in to powder by electrical blender. The powdered material was stored in tightly closed glass bottles for further use. The crude aqueous extract of *Psophocarpus tetragonolobus* was prepared using the powdered plant material by mixing it with 250ml of distilled water in a flask and boiled for 30 minutes. It is reduced to 50 ml and cooling to 37 degree Celsius, residues were filtered using What man filter paper 1.

Collection of *Pheretima posthuma*

The adult *Pheretima postuma* (earthworm) was used to evaluate anthelminthic activity *in vitro*. Earthworms were collected from Kerala Agricultural University, Vellanikara. The average size of earthworm was 6-8 cms. The worm was washed with cold water and they kept in water for one hour.

Phytochemical screening

The Phytochemical analysis of plant material was done by conducting various tests using the plant extract, for primary and secondary metabolites present in the selected plant.

a) Test for Carbohydrate

a. 1 Molisher's reagent test: 1 ml of plant extract was taken in a clean test tube and add 2 drops of alcoholic alpha naphthol solution. Then add con.sulphuric acid through the sides of the test tube. Formation of violet ring at the junction.

a. 2 Benedict's test: To 1 ml of plant extract add 2-3 drops of Benedict's reagent. Heat gently formation of orange red precipitate indicate the presence of carbohydrate.

a. 3 Fehling's test: The plant extract was hydrolysed with dilute Hcl, dilute with alkali, heated with Fehling's A and Formation of red precipitate indicates the presence of reducing sugar.

b) Test for Alkaloids

b. 1 Mayer's test: Take 1ml of plant extract. Add 1% of Mayer's reagent. Formation of yellow precipitate indicates the presence of alkaloids**..**

b. 2 Wagner's test: Take 1ml of extract. Add 1ml of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

b. 3 Dragendroff's reagent test: Take 1 ml of plant extract add 1ml of Dragendroff's reagent. Reddish brown precipitate indicates the presence of alkaloids.

c) Test for Tannins

Take 1 ml of plant extract and add 1 ml of con. HCl. Formation of red precipitate indicates the presence of tannins. Take 1ml of plant extract, add 1ml of methanol. Boil the solution. Then add 1 ml of ferric chloride. Green color precipitate indicates the presence of tannins. Take 1ml of dil.NaoH to the equal volume of plant extract taken. Intense yellow colour obtained. To that add dil.Hcl. The disappearance of yellow colour indicates the presence of tannins.

d) Test for Saponins

1 ml of methanolic plant extract was dissolved in boiling water. Allow it to cool for the formation of vigorous froth. Height of froth measured to get the saponin content.

e) Test for Proteins

To 1ml of plant extract add Ninhydrin reagent. Formation of purple color indicates the presence of proteins. Proteins stained red on warming with Million's reagent.

f) Test for Diterpenes

The plant extract dissolved with water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

g) Test for Triterpenes

Salkowi's test: To 1ml of plant extract add 1ml of chloroform. The filtrate obtained is treated with few drops of con.sulphuric acid. Shake well and allow it to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

h) Test for phenols

To 1ml of ethanol plant extract add 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

i) Test for flavonoids

To 2ml of plant extract add few drops of dil.NaoH. The intense yellow color formed become colorless, indicates the presence of flavonoids.

Screening of and optimization of plant extract for its anthelminthic activity

The primary screening of plant material was done by treating *Pheretima posthuma* in 5 mg/ml plant extract. The morphological changes were noted. This test helps to confirm whether the selected plant material possess anthelminthic property. After this in a different experiment the effect of concentration time and pH was analyzed. Plant extracts of different concentration (5 to 40 mg/ml) were used to understand the effect of concentration upon anthelminthic activity. Similarly effect of pH was studied with three different buffers; acetate (3.6-5.6) , phosphate(5.8-7.4) , bicarbonates(9.2-10.6) and time (1 to 60 minutes) .Test were carried out *in vitro* using adult earthworm as it is having anatomical and physiological resemblance with maintaining preferable conditions. All the test solutions were prepared freshly before starting the experiment. Observations were made. The time taken for paralysis was noted when no movement of any sort could be observed. The time for death of the worms were recorded after ascertaining that worms show no movement after shaking. Histopathologiacl analysis was done for this treated organism.

Results and Discussion

Phytochemical analysis of plant material

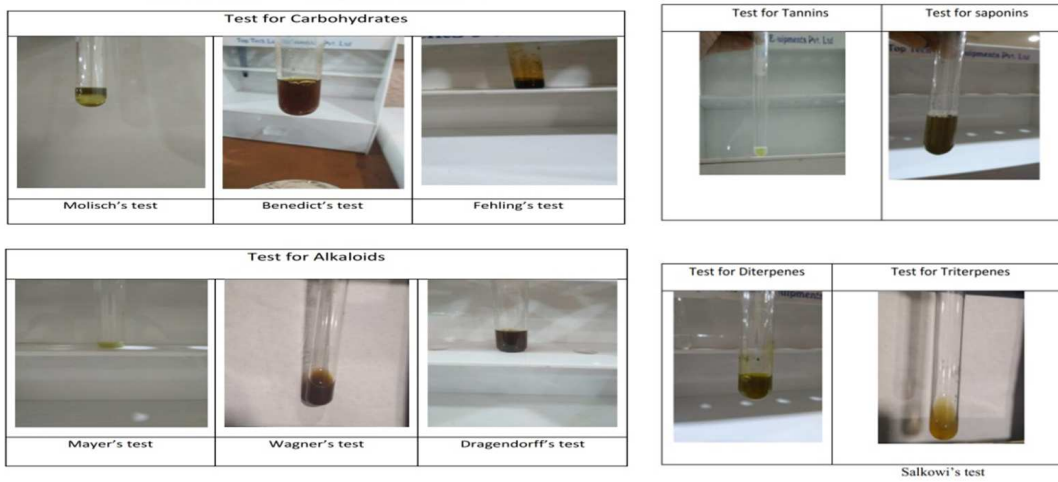
The qualitative phytochemical studies on leaf extract which was successfully extracted from *Psophocarpus tetragonolobus* shows the presence of primary and secondary metabolites such as carbohydrates, alkaloids, diterpenes, triterpenes; saponins and phenolic compounds and its positive results are shown in the figure.

Synthetic phenolic anthelmintics interfere with the energy generation in the helminthes parasites by uncoupling the oxidative phosphorylation another possible mechanism of action is that they bind to free proteins in the gastrointestinal tract of the host animal or to glycoprotein on the cuticle of the parasite and causes death. By uncoupling oxidative phosphorylation tannins interfere with generation of energy.

Tannins have capacity to bind with free proteins in the gastrointestinal tract of host animal or the glycoprotein on the cuticle of parasites and causes death (Patel et al., 2010).

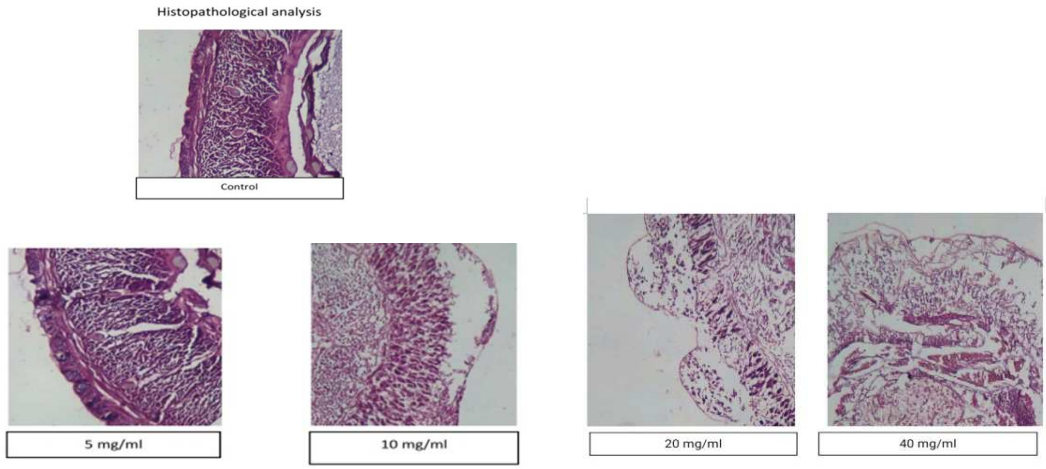
Figure-5.1.b

Phytochemical screening of plant material



Analysis of Antihelminthic activity

The primary screening by treating *Pheretima postuma* in 5 mg/ml plant extract showed Antihelminthic activity, such as wriggling movements in 6-7 minutes. Treating in 10 mg/ml plant extract showed curling movements after 3-4 minutes. Elongation of body was clearly observed in earth worm treated with 40 mg/ml plant extract solution. Paralysis was observed based on concentration of plant extract and time. As concentration increases time taken for paralysis decreases. The histopathological analysis shows more clear idea about the study.



Bibliography

- Akhtar, M.S., Z. Iqbal, M.N. Khan and M. Lateef. (2000). Anthelmintic activity of medicinal plants with particular reference to their use in animals in Indo–Pakistan subcontinent. Res, 38: 99-107.
- Apri Dey and Laxmipriya Nampoothir. (2019). Neutraceuticals as therapeutic agents for management of endocrine disorders-Bioavailability and mechanisms underlying their bioactivities. Scientific nutritional health 397-109.
- Mehlhorn. (2014). *In-Vitro* Anthelmintic Activity of Luffa cylindrical Leaves in Indian Adult Earthworm.Encyclopaedia for reference of parasitology(vi, ed)(vi);Springer press. ISSN 2278-4136 ZDB-Number: 2668735-5 IC Journal No: 8192
- K.D Jones and Berkley. (2014). Severe acute malnutrition and infection. Paediatr.int.child health 34(1). PP.S1-S29.
- Award, N E (2004). Bioactive brominated diterpenes from the marine red alga Jania rubens (L.) Lamx. Phytother Research: Vol. 18 (4); Pp. 275 - 279, doi; 10.1002/ptr.1273
- Achal K, Nandan R, Kole P C (2018). Agronomic performance for tuber characters in winged bean (Psophocarpus tetragonolobus) (L.) DC. International Journal of Economic Plants. Vol. 5 (2) Pp. 86-89. Puspa Publishing House.
- Sumanthi S, Sonia K R, Banupriya S K, Poornima A and Padma P R (2019). Reverse screeing bioinformatics: Approach to identify potential antibreast cancer target using Tynoquinone from neutraceutical, black iol alum oil. Anti Cancer Agents Medicinal Chemistry. Vol. 19 (5). Pp. 120 - 127. doi; 102174/1871520619666190124155359

STUDIES ON THE BIO CONTROL POTENTIAL OF *AMBLYSEIUS DIOSCOREAE* TO MANAGE THE TWO SPOTTED SPIDER MITE - *Tetranychus urticae* (C. L. Koch) - INFESTATION IN LAC INSECT HOST PLANT - *Flemingia macrophylla* (Willd.) Merr.

MARIYA A. JOSEPH [1], SAJEEV T.V. [2], SAJANA HANEEF [3], SOUMYA K.C.[2] DIXY B.A[1]

[1] Department of Zoology, Vimala College, Thrissur, [2] Kerala Forest Research Institute, Thrissur.
[3] Govt. Higher Secondary School, Calicut.

Abstract

Though India is the leader producer of lac in the world, selection of lac insect host plant according to the topographic and climatic condition of an area is very important in lac cultivation. *Flemingia macrophylla* is an Angiosperm plant in the family Fabaceae which is one of the most suitable plants for lac cultivation in Kerala. *Amblyseius dioscoreae* – a member of the Phytoseiidae family which comes under genus Amblyseius noted to be an effective bio control agent against *Tetranychus urticae* (two spotted spider mite) which causes serious threat to *F.macrophylla* by sucking the plant fluid from the foliage. This study attempted to elucidate the bio control potential of *A.dioscoreae* in controlling the pest *T.urticae* so as to avoid the use of chemical pesticide to manage the pest and would ensure the quality of host plant which in turn would help the production of qualitative and quantitative increase in the lac production.

Key words: Angiosperm, Bio control agent, Chemical pesticide.

Introduction

India is the largest producer of lac in the world. Lac is produced by the phytophagus insect, *Laccifera lacca* which comes under Kerriidae family (Rahul et al. 2017). It secretes true lac, the only natural resin of animal origin with immense industrial applications. Nearly 70% of the lac produced in India is exported. According to the difference in the climatic and topographic conditions of an area, selection of host tree is very important in lac cultivation. The quality of lac mainly depends on the variety of the host tree on which they feeds. *Flemingia macrophylla* commonly known as Kamatteri is an Angiosperm plant in the family Fabaceae which is one of the most suitable host plants for lac cultivation in Kerala. *Tetranychus urticae* is known as the pest of *F.macrophylla* which causes serious threat to *F.macrophylla* by sucking the plant fluid from the foliage. Heavy infestation causes discolouration, reduce the photosynthetic ability and premature drop of foliage. Under this circumstances *Amblyseius dioscoreae* – a member of the Phytoseiidae family which comes under

genus Amblyseius noted to be an effective bio control agent against *Tetranychus urticae* (two spotted spider mite). This study attempted to elucidate the bio control potential of *A.dioscoreae* in controlling the pest *Tetranychus urticae* so as to avoid the use of chemical pesticides in managing the pest.

Materials and Method

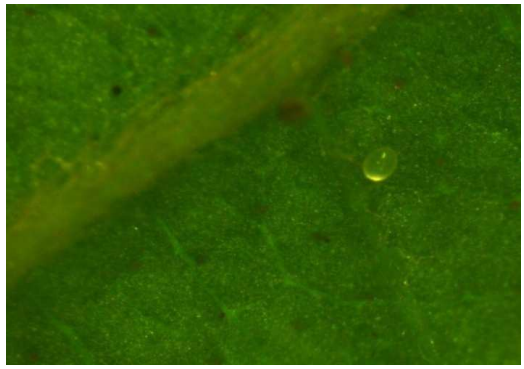
Samples of predatory mites (*Amblyseius dioscoreae*) and their target pest (*Tetranychus urticae*) were collected from the Lac Insect Germplasm Centre of Kerala Forest Research Institute (KFRI) located at Peechi, in Thrissur District. Samples of infested leaves having mites were put in self sealing plastic bags for subsequent screening of predatory and pest mites in the laboratory. At the laboratory individual leaves was thoroughly examined under a Leica stereo zoom microscope (magnification ranging from 10x to 40x) for the recovery of the predatory as well as the pest mites. Successful rearing and maintenance of sufficient stock cultures of both mites in the laboratory were carried out by following the leaf flotation technique (Chandra et al. 2008). Prey mites were provided to the predatory mites during the rearing period. Renewal of leaf disc was carried out at an interval of 2 – 3 days and regular supply of prey mites was ensured. Dehydrated speciemen of mites were slide mounted in Hoyer's medium for identification. Studies were conducted by placing the cultures of *A.dioscoreae* at room temperature (33^0c) and experimental temperature (20^0c) for analysing the influence of temperature in feeding rate of the predatory mite.

Heavily infested leaves of *F.macrophylla* by *T.urticae*.

Culturing of *A.dioscoreae* by leaf flotation technique.

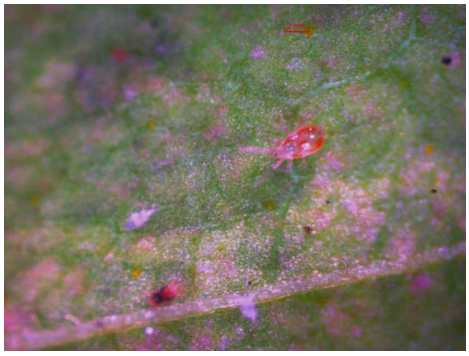
Result and Discussion

Amblyseius dioscoreae belongs to Phytoseiidae family characterised by four pairs of long legs. Adults are pear shaped, transparent to slightly yellowish orange with an unsegmented body, completes its life cycle in five stages, which were identified based on their metabolic and physical activities, body size and frequency of their resting period. Different behavioural characters of *A.dioscoreae* were observed during the study. High intensity of searching behaviour (zig – zag motion) was shown by adults compare to immature stages. Presence of trichomes and tiny water drops on the leaf blade act as barrier which deflects them from their searching direction of interest. The activity and locomotion of *A.dioscoreae* was found to be reduced under lower and higher temperatures. *A.dioscoreae* feeds on all stages of the two spotted spider mite including eggs, larva, nymphal and adult stages. Majority of them feeds on the juvenile stages of *T.urticae* which were easy to cut the cuticle and also to locate them due to poor web production of pest mites as compared to adult mites. Hence the level of pest mites can be controlled to an extent by application of *A.dioscoreae* in early stages of pest infestation. Adult males of *A.dioscoreae* are slightly smaller than females. As they attain maturity the male predatory mites compete among themselves to find healthy females for mating. Duration of copulation ranges from 30 to 70 minutes during which the sperms were transferred to spermatheca of the female through insemination. Within 2 -3 days after internal fertilization, eggs are laid on trichomes (adaptation to avoid egg predators) by severe contraction of their body parts. Mature eggs were seen in the body of adult females by using stereo zoom microscope. Temperature plays an important role in metabolic activities of predatory mites. Decrease in temperature results in decrease of hunger level and the reduction of searching behaviour in *A.dioscoreae*. This result had been observed from the less feeding rate of *Amblyseius dioscoreae* on *Tetranychus urticae* under controlled condition (20^0c).



Egg of *A.dioscoreae*

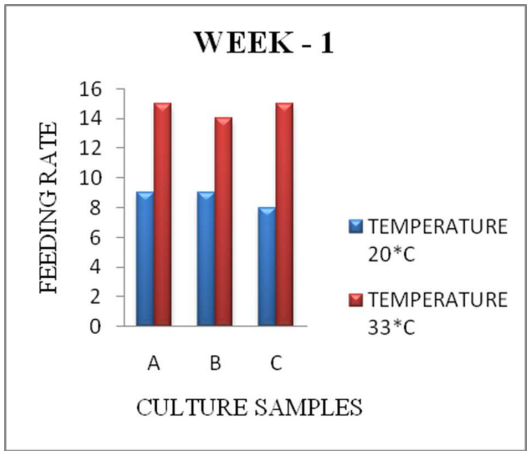
Amblyseius dioscoreae

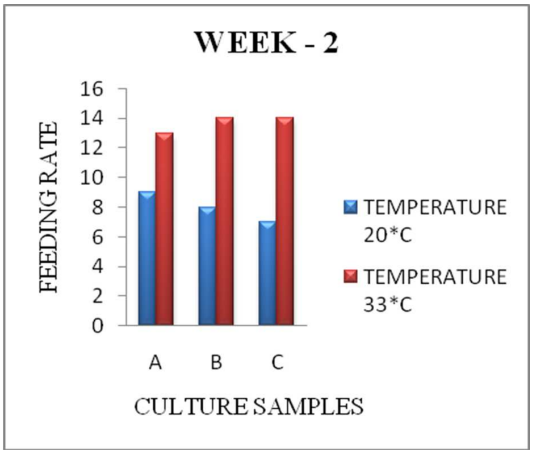


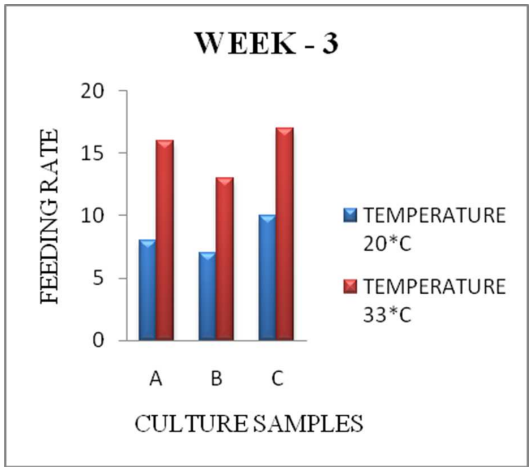
A.dioscoreae finding its prey (*Tetranychus urticae*).

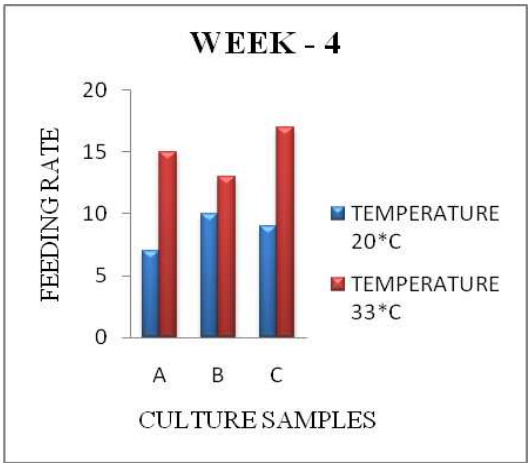
T.urticae colony with their shedded exoskeleton.

Influence of temperature on feeding rate of *Amblyseius dioscoreae*









Conclusion

The study shows the *Amblyseius dioscoreae* is an effective control against the *Tetranychus urticae* on the plant *Flemingia macrophylla*. But temperature plays an important role in the effectiveness of predatory mites against the prey. The feeding rate of *A.dioscoreae* is directly influenced by ambient temperature. Thus in high altitudes where temperature is low, results the effectiveness of this bio control agent may decline. Level of pest mites can be controlled by the application of *A.dioscoreae* in the early stages of pest infestation since majority of them prefers to feeds on juvenile stages of *T.urticae*. This study has shown that the use of *A. dioscoreae* during the early stages of the two spotted spider mites infestation can prevent the loss of foliage of the lac insect host plant - *F.macrophylla*. The result would help in avoiding the use of chemical pesticides to manage the pest and would ensure quality of the host plant which in turn would help the production of qualitative and quantitative increase in lac production.

BIBLIOGRAPHY

- M.P. Rahul and Mary Anithalatha Sadanandam (2017). Two new species of Amblyseius from Kerala, India". Biological forum – An International Journal 9(2), p224 – 226.
- D Chandra Sekar, Jagdishwar Reddy, S.J. Rahman, A. Ranga Reddy, and V.V. Narendranath (2008). Ecology and management of red spider mite, *Tetranychus urticae* Koch on grape".Acta Horticulture 785.

A REVIEW ON THE GREEN SYNTHESIS OF SILVER NANOPARTICLES AND ITS APPLICATIONS

M S SHILPA[1], INDU M S[2], SR. FRENY JACOB[3]

[1, 2, 3] Department of Zoology, Vimala College, Thrissur.

Abstract

Nanoparticles were earlier synthesized using chemical and physical methods. Although the chemical methods are faster, it leads to the production of toxic and non-eco-friendly by-products. The need for environmentally non-toxic protocols for the synthesis of nanoparticles has lead to develop interest in biological approaches which are free from the production of toxic chemicals as by-products. This review mainly focuses on the green synthesis of silver nanoparticles using various plant sources and their applications in various fields.

Key words: Green synthesis, silver nanoparticles.

Introduction

Nanotechnology is a rapidly growing science of producing and utilizing nano-sized (1-100nm) particles. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution, and morphology. Nanoparticles can be synthesized using various approaches which include physical, chemical and biological methods. Many adverse effects have been associated with chemical methods of synthesis due to the presence of some toxic chemicals absorbed on the surface.

Eco-friendly alternatives to chemical and physical methods are the biological ways of nanoparticle synthesis using microorganisms, enzymes, fungi, and plants or plant extracts (Hasan, 2015). The use of plant materials in nanoparticle synthesis could be more advantageous than microbes, as it does not require intra or extra cellular synthesis, purification and maintenance of microbial culture. Green synthesis approaches utilize mild experimental conditions (such as ambient temperature and pressure), require careful considerations in selecting non-toxic, environmentally benign solvents, reducing agents as well as capping agents.

Silver nanoparticles (AgNPs) are one of the most widely used engineered nanoparticles because of their unique properties in various fields like catalysis, chemical sensing, biosensing, photonics, electronics, pharmaceuticals, etc.

Green Synthesis and Applications of AgNPs

Sinha *et al.*, (2009) and Goodsell (2004) have reported that the biosynthesis of

nanoparticles is advantageous over other chemical and physical methods as it is cost effective and environment friendly method. Rajan *et al*., 2015 reported a simple, versatile and ecofriendly protocol for the synthesis of silver nanoparticles using the aqueous extract of *Areca catechu* nut and its application in catalysis and antioxidant activity. The synthesized AgNPs were stable for two months.

Banerjee *et al*., (2014) reported that the leaf extracts of three plants, *Musa balbisiana* (banana), *Azadirachta indica* (neem), and *Ocimum tenuiflorum* (black tulsi), were used as biological reducing agents to produce AgNPs. The suitability of coffee and green tea extracts in green synthesis of silver nanoparticles was shown by Ronavari *et al*., (2017). They showed that GT-AgNPs exhibited antimicrobial activity but these particles were proved to be highly toxic to mammalian cells, whereas C-AgNPs exhibited antimicrobial activity and were also proved to be non-toxic to human and mouse cells.

Silver nanoparticles have also reported to possess anti-fungal (Kim *et al*., 2009), anti-inflammatory (Nadworny *et al*., 2008), anti-viral activity (Rogers *et al*., 2008), and antibacterial activity (Hamouda *et al*., 1999). The silver nanoparticles synthesized from leaf extracts of *Clitoria ternatea* and *Solanum nigrum* showed anti-bacterial effect against common nosocomial pathogens (Krithiga *et al*., 2015). From the study of Jones and Hoek, (2010) we can assume that silver nanoparticles directly damage bacterial cell membranes whereas silver nanomaterials appear to exert bactericidal activities. Marimuthu *et al*., 2011 reported that the AgNPs synthesized from *Mimosa pudica* plant extract possessed anti-parasitic activity against the larvae of malaria and filariasis vectors. Jiang *et al*., 2004 showed that silver has an inhibitory effect on many bacteria and micro-organisms commonly present in medical and industrial fields. The anti-bacterial effects of silver nanoparticles mainly arise from silver ions.

Priyadarshini *et al*., 2012 used silver nanoparticles synthesized using plant extracts as reducing, stabilizing and capping agents as an alternative to botanic larvicides. The microbicidal properties of silver, the insecticidal activity of the selected plant and a favourable surface area to volume ratio due to the small size of particles (1-100nm), are used for finding an application through this technology (Borase *et al*., 2013), (Muthukumaran *et al*., 2015).

Mondal *et al*., 2014 showed that the silver nanoparticle synthesized from aqueous root extract of *Parthenium hysterophorus* has the potential to be used as a larvicidal agent against *Culex quinquefasciatus.* The larvicidal activity of silver nanoparticles (AgNPs) using *Belosynapsis kewensis* (*B. kewensis*) leaf extract against the *Anopheles stephensi* (*A. stephensi*) and *Aedes aegypti* (*A. aegypti*) *in vitro* study ($LC_{50}$and LC_{90}) was analyzed by Bhuvaneshwari *et al*., 2016. Their results proved that the green synthesized AgNPs can be used for the control of *A. stephensi* and *A. aegypti* through eco-friendly and cost effective approach.

Morones *et al*., 2005 proved that nanoparticles play an important role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants and tissue engineering. Lee *et al*., 2003 showed that silver nanoparticles can be used in various areas including clothes, cosmetic materials, toothpaste and washing machines.

Conclusion

The biological method of nanoparticle synthesis has drawn attention due to its rapid, economical and ecofriendly protocol. It also provides a single step technique for the biosynthesis process. Thus, it has paved the way for the "greener synthesis" of silver nanoparticles, which is a safer alternative when compared to the conventional chemical and physical methods.

BIBLIOGRAPHY

- Priyadarshini, K. Murugan, C. Panneerselvam, S. Ponarulselvam, J. Hwang, and M. Nicoletti. (2012). Biolarvicidal and Pupicidal potential of silver nanoparticles synthesized using *Euphorbia hirta* against *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitolology Research*, vol. 111, no. 3, pp. 997-1006.
- Andrea Ronavari, Kovacs, D., Igaz, N., Boros, I.M. and Pfeiffer, I. (2017). Biological activity of green synthesized silver nanoparticles depends on the applied natural extracts. *International Journal of Nanomedicine*, vol. 12, pp. 871-883.
- Anish Rajan, Vidya Vilas and Daizy Philip. (2015). Catalytic and antioxidant properties of biogenic silver nanoparticles synthesized using *Areca catechu* nut. *Journal of Molecular Liquids*, vol. 207, pp. 231-236.
- Byung, H. Kim, Young-ki Jo and Geunhwa Jung. (2009). Antifungal activity of Silver ions and nanoparticles on phytopathogenic fungi. *Plant disease*, 93 (10), 1037-1043.
- Catalina Marambio-Jones and Eric, M.V. Hoek. (2010). A review of the antibacterial effects of silver nanomaterials and potential complications for human health and the environment. *Journal of Nanoparticles Research*, 12(5), 1531-1551.
- David, S. Goodsell. (2004). Bionanotechnology: Lessons from Nature. *Wiley-Liss*, doi:10.1002.
- Hemant, P. Borase, C.D. Patil, R.B. Salunkhe, C.P. Narkhede, B.K. Salunke and S.V. Patil. (2013). Phyto-synthesized Silver Nanoparticles: A Potent Mosquito Biolarvicidal Agent. *Journal of Nanomedicine & Biotherapeutic Discovery*, vol. 3, no.1.
- Hamouda, T., Hayes, M.M., Cao, Z., Tonda, R., Johnson, K., Wright, D.C., Brisker, J. and Baker, J.R. Jr. (1999). A novel surfactant nanoemulsion with broad-spectrum sporicidal activity against Bacillus species. *Journal of Infectious Diseases*, 180(6) 1939-49.
- Hoon Joo Lee, S.Y. Yeo, S.H. Jeong. (2003). Antibacterial effect of nano-sized colloidal solution on textile fabrics. *Journal of Material Science*, 38(10), 2199-2201.
- Hongquan Jiang, Sorin Manolache, Amy C. Lee Wong, Ferencz S. Denes. (2004). Plasma-enhanced deposition of silver nanoparticles on to polymer and metal surfaces for the generation of antimicrobial characteristics. *Journal of Applied Polymer Science*, 93(3), 1411-1422.
- James V. Rogers, Christopher V. Parkinson, Young W. Choi, Janice L. Speshock and Saber M. Hussain. (2008). A preliminary assessment of silver nanoparticle inhibition of monkeypox

virus plaque formation. *Nanoscale Research Letters* 3 (4), 129.

- Morones, J.R., Elechiguerra J.L., Camacho, A., Holt, K., Kouri, J.B., Ramirez, J.T. and Yacaman, M.J. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16(10): 2346-53.
- Naba Kumar Mondal, Arnab Chowdhury, Uttiya Dey, Priyanka Mukhopadhya, Soumendranath Chatterjee, Kousik Das and Jayanta Kumar Datta. (2014). Green synthesis of silver nanoparticles and its application for mosquito control. *Asian Pacific Journal of Tropical Disease*, 4 (Suppl. 1): S204-S210.
- Nadworny, P.L., Wang, J., Tredget, E.E. and Burrell, R.E. (2008). Anti-inflammatory activity of Nanocrystalline silver in a porcine contact dermatitis model. *Nanomedicine*, 4(3): 241-251.
- Narayanaswamy Krithiga, Athimoolam Rajalakshmi and Ayyavoo Jayachitra. (2015). Green synthesis of silver nanoparticles using leaf extracts of *Clitoria ternatea* and *Solanum nigrum* and study of its antibacterial effects against common nosocomial pathogens. *Journal of Nanoscience*, Volume 2015, Article ID 928204, 8 pages.
- Priya Banerjee, Mantosh Satapathy, Aniruddha Mukhopahayay and Papita Das. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: Synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, 1:3.
- Rajamani Bhuvaneswari, Raju John Xavier and Manickam Arumugam. (2016). Larvicidal property of green synthesized silver nanoparticles against vector mosquitoes (*Anopheles stephensi* and *Aedes aegypti*). vol. 28, pp. 318-323.
- Saba Hasan. (2015). A review on nanoparticles: their synthesis and types. *Research Journal of Recent Sciences,* vol. 4, pp.1-3.
- Sampath Marimuthu, Abdul Abdul Rahuman, Govindasamy Rajakumar, Thirunavukkarasu Santhoshkumar, Arivarasan Vishnu Kirthy, Chidambaram Jayaseelan, Asokan Bagavan, Abdul Abduz Zahir, Gandhi Elango and Chinnaperumal Kamaraj. (2011). Evaluation of green synthesized silver nanoparticles against parasites. *Parasitol*, 108:1541-1549.
- Sinha, S., Pan, I., Chanda, P. and Sen, S.K. (2009). Nanoparticle fabrication using ambient biological resources. *Journal of Applied Biosciences*, 19: 1113- 1130.
- Udaiyan Muthukumaran, M. Govindarajan, M. Rajeswary, and S. Hoti. (2015). Synthesis and characterization of silver nanoparticles using *Gmelina asiatica* leaf extract against filariasis, dengue, and malaria vector mosquitoes. *Parasitology Research*, vol. 114, no. 5, pp. 1817–1827.

EFFECT OF METHYL ORANGE UPON Danio *rerio* and AQUAMICROBIOTIC IMPLICATION BY THE EFFLUENT

SANA PARVEEN.C[1], KAYEEN VADAKKAN[2], DIXY B A[3]

[1, 3] Department of Zoology, Vimala College, Thrissur.

[2] Department of Biotechnology, St. Mary's College, Thrissur.

Abstract

The effect of pollutants on the aquatic fauna cannot be studied in their habitat since they are contaminated by a combination of pollutants. So a single organism was collected and the effect of single pollutant was studied in invitro condition. The present study shows the effect of textile dye methyl orange on fish *Danio rerio* and microbial population. Increase in the concentration of methyl orange reduced the bacterial concentration. High concentrations of dye contamination could probably inhibit the microbial growth in water which indirectly affects the potability of water. The effect of methyl orange on aquatic animals was analyzed by taking *Danio rerio* as a model. The textile dye methyl orange is observed to have depleted microbial concentration in water and adversely effects the survival of fish *Danio rerio*.

Introduction

Water pollution occurs when harmful substances often chemicals or microorganisms contaminate a stream, river, lake, ocean and other body of water, degrading water quality and rendering it toxic to humans or the environment. Synthetic dyes have been used increasingly in the textile and dyeing industries. Since dyes are stable, recalcitrant, colorant, and even potentially carcinogenic and toxic, their release into the environment poses serious environmental, aesthetical and health problems. Weilgart, L. S. (2008). Thus, industrial dye-laden effluents are an increasingly major concern and need to be effectively treated before being discharged into the environment in order to prevent these potential hazards. Methyl orange was used to study the effect of dye on aquatic fauna. It is a main component of textile effluents. Methyl orange is an orange, azoic dye. It has a transition range from 3.1 to 4.4. Methyl orange does not have a full spectrum of color change, but has a sharper end point. Methyl orange shows red color in acidic medium (pH < 3.1) and yellow color in basic medium (pH > 4.4). It is used as a pH-indicator. Hassan, S. A. et al (2012). So the effect of dye upon microbes and fish Danio rerio was studied as a part of studying aquatic pollution.

Materials and Method

Collection of water

Water was collected from the pond area to the frontage of St. Mary's college

Thrissur, Kerala during April 2019.The pond consists of different types of vegetation and aquatic organism. The site was selected on the basis of availability of water microbes and fishes. A sufficient amount of water sample was taken from the sampling site.

Analysis and enumeration of aquatic microbes

Among water microorganisms, bacteria are particularly suitable for quantification by counting. It should be considered, however, that bacteria are never uniformly distributed in the water and that their spatial arrangement varies even in neighboring microsites. Counting of aquatic bacteria can be achieved by direct microscopy as well as by cultural methods, the plate count technique. This technique records the only viable bacteria that are able to proliferate on nutrient media, such count is called viable counts.

Serial Dilution of soil sample

Shake up the water suspension (dilution 10-1) by hand, and transfer by means of a sterile pipette tip attached to a piston- stroke pipette 1 ml into a 9 ml water blank (dilution 10-2) and shake vigorously by hand. This process can be repeated until we reach a considerable dilution (dilution 10-7).

Pouring Plates and Incubation

Melt solidified nutrient agar by heating, and keep the medium liquid in a water bath at 500C until use. Select a range of 3 dilutions 10-1, 10-4, 10-7. Set up a laminar air flow chamber to create sterilized condition. Starting with the most diluted suspension, transfer with a sterile pipette tip 0.1 ml aliquots into the first petri dish. Make similar transfers from the next lower dilutions. Subsequently, pour about 10 ml of sterile nutrient agar into plates while lifting the lid as little as possible. Immediately thereafter rotate the plates five times clockwise and counter clockwise by hand so that the inoculum is dispersed in the medium. Pile the plates carefully with the still liquid medium to minimize condensation of water on the lids. After solidification of agar, incubate the plates upside down in a pile in an incubator at 28^0C for 24 hours.

Counting of Colonies

After the incubation of 24 hours, inspect the plates at low magnification using a colony counter.

The number of colonies in each petridishes was counted and recorded. A positive reading indicates that at least one organism, capable of proliferating, was present in the transferred inoculum. From the three dilutions, having one or more colonies, the colony forming unit (CFU) of bacteria is calculated using the formula:

CFU = Number of colonies x Dilution factor

Preparation of stock solution for analyzing its effect on terrestrial environment

1 g (10mg/ml) of methyl orange dye was accurately weighed. Make up this into 100 ml of distilled water. Different aliquots of working solution were made for further studies.

Analysis of effect of methyl orange

The collected water samples were incorporated with varying concentrations of methyl orange (0, 1, 2.5, 5 and 10 mg/ml). The sample was kept for 24 hrs to investigate the effect of methyl orange upon aquatic ecosystem.

Exsitu analysis of effect of methyl orange upon aquatic microbiome

The stock solution was administrated in the study sample after which the water samples was collected and analyzed for microbial population. Different treatment groups varying from (0, 1, 2.5, 5 and 10 mg/ml) was plated (10-1, 10-4, 10-6) in nutrient agar for the purpose. Danio rerio fingerlings, the study animal was procured from local supply stores in Thrissur, Kerala. Subjects were acclimated in aerated recirculating tank containing dechlorinated tap water and air pumps under laboratory circumstances. The fish were fed commercial fish food twice daily and were kept at approximately 28 °C with 12 h/12 h light/dark cycle. The water was changed daily to reduce the impurities from the metabolic wastes. The stock solution (Methyl orange10mg/ml) was prepared freshly before starting the experiments. The solution was used perform mortality assay and tissue level impacts. Varying concentration of test solutions were prepared ranging from (0, 1, 2.5, 5 and 10 mg/ml). Observations were made such as morphological changes, time taken for death etc.

Result and Discussion

It was observed that natural water remained enriched with aquatic micro-biome as the bacterial concentration was normal which indicates that the sampling site selected for the study is having good quality and microbial quantity. In untreated sample the culture plates showed 39 x 10^{-6}CFU per ml which indicates the microbial abundance. A significant effect was seen upon aquatic microbes when treated with dye. The increase in the concentration of methyl orange reduced the bacterial concentration. In higher concentration of pollutant, the presence of microbes was negligible which suggests that high concentrations of dye contamination could probably inhibit the microbial growth in water which indirectly effect the potability of water. Apparently the microbial concentration observed in the concentration 10 mg/ml was $6x10^{-6.}$ There was a reduction in microbial concentration even in lower concentration; however microbes could survive in lower concentration (Hassan et al. 2012). The effect of methyl orange on aquatic animals was analyzed by taking *Danio rerio* as a model organism at varying concentrations that of 0 to 10 mg/ ml. It was observed that subjects showed different morphological and behavioral changes in response to the treatment. In higher concentration that of 10mg/ml the subject was found dead on 61^{tst} hour however the administration of low concentration eventually killed the subject. It took 107th hour to cause death when the dosage was 1mg/ml.

BIBLIOGRAPHY

- Weilgart, L. S. (2008). The impact of ocean noise pollution on marine biodiversity. *International Ocean Noise Coalition*.
- Hassan, S. A., Moussa, E. A., & Abbott, L. C. (2012). The effect of methyl mercury exposure on early central nervous system development in the zebra fish (Danio rerio) embryo. *Journal of Applied Toxicology*, *32*(9), 707-713.

ISBN 978-81-7255-135-3

PG Department of Zoology
Vimala College (Autonomous) Thrissur

Vimala College Publications
Thrissur, Kerala, India 680 009

Distributors
Sooryagatha (Publishers), Kochi-682035