

UTILIZATION OF PLANT LATEX FOR PLANT GROWTH AND OTHER BIOLOGICAL APPLICATIONS

A THESIS

Submitted by

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*in partial fulfillment for the award of the degree
of*

BACHELOR OF TECHNOLOGY



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL
SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
JEPPIAAR NAGAR, CHENNAI – 119**

MARCH 2021



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **S. SAIGEETHA (37230073)** who carried out the project entitled “**UTILIZATION OF PLANT LATEX FOR PLANT GROWTH AND DRUG DELIVERY**” under my supervision from December 2020 to March 2021.

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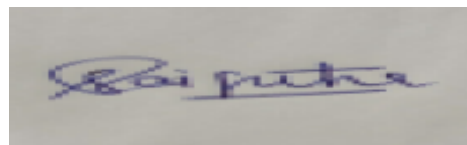
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DECLARATION

I, **S. Saigeetha (37230073)** hereby declare that the Project Report entitled “**UTILISATION OF PLANT LATEX FOR PLANT GROWTH AND DRUG DELIVERY**” was done by me under the guidance of **Dr. INBATHAMIZH**, Assistant Professor in the Department of Biotechnology, Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to **Board of Management of SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. Ramesh Kumar and Mr. Narendra Kumar, Head of the Department, Dept. of Biotechnology** for providing me necessary support and details at the right time during the progressive reviews. I would like to express my sincere gratitude to my Project Guide **Dr. Inbathamizh, Assistant Professor, Department of Biotechnology, Sathyabama Institute of Science and Technology**, for her encouragement which paved way for the successful completion of my project work.

I also express my sincere gratitude to **Dr. Antony V Samrot**, Senior lecturer, Department of Biomedical Sciences, MAHSA University, Malaysia for his encouragement, suggestions and unconditional help and without whom this project would not be successfully completed.

I would like to thank **Ms. Jenifer Selvarani, Ms. Jane Cypriyana P J, Ms. Shobana N, Ms. Kajal purohith** for their constant support in completing this project.

I also thank **Mr. Kumar C**, and **Dr. T. Stalin Dhas**, Scientist B, Ocean Research Centre, Sathyabama Institute of Science and Technology and my friends.

I wish to express my thanks to all Faculty members and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

I would also like to thank my parents for their constant support, encouragement and blessings.

S. SAIGEETHA

ABSTRACT

Plant exudes polymer like substance through a white sap known as Latex. Plants use latex in various ways, to protect themselves from insects and also to rapidly heal wounds caused by the insects. Due to these properties of the latex its efficiency was studied by subjecting it to the plant growth and its properties as drug delivery systems. In this study, In-vitro (Seed germination studies) studies was performed using latex at various concentration ranging from 10%- 100% respectively The best concentration obtained from seed germination was further subjected to In-vivo (pot studies) and the various factors influencing the growth was observed. Insecticidal and pest repulsion activity of latex was performed against *mealy bugs* at different concentration (10%- 100%). SPION's were synthesized, characterized and coated with papaya latex and further used for production of Drug loaded nanocarriers by adding curcumin as the drug. Papaya coated SPIONs as well as Drug loaded nanocarriers were characterized using UV-Vis, FTIR, SEM, XRD. Drug release kinetics for the drug loaded nanocarriers was observed. Adsorption studies were performed for SPIONs as well as papaya latex coated SPIONs and adsorption isotherm was calculated.

KEYWORDS: *Carica papaya*, Latex, Plant growth, Nanocarriers, Drug delivery

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CHAPTER 1

INTRODUCTION

Trees are most important to mankind not only environmentally, industrially, eco-friendly but also historically. They provide a wide range of products for prosperity and survival. Trees can be classified into several types based on their utility and end products. For example, “ornamental”, “fruit bearing”, “medicinal plants”, nitrogen fixing”, “drug yielding”, “Fuel yielding”, “Fiber yielding” etc. they play an important role in maintaining the oxygen cycle (Seth, 2013). Many industries including pharmaceutical and food industries are trying to develop new delivery systems for specific application from the polymeric compounds extracted from the trees. Either crude polymers obtained directly from various aerial parts of the tree or the mixing of different polymers in the form of gels attracted many researchers due to its wide range of application in pharmaceutical, technological, food and many other industries (Ribeiro et al., 2016). Among various defensive traits possessed by plants against herbivores most of these traits are multifunctional. It invites more complication in interpreting the evolutionary ecology. Some of the defensive traits like extrafloral nectar, alkaloids are known to have many other functions other than defense and it is not widespread. These plant substances exude polymer like substance known as latex when the tissues of the plants are damaged which indicates a defensive mechanism. Latex is generally an emulsion of aqueous fluid mostly containing rubber and other secondary metabolite (Agrawal & Hastings, 2019).

1.1. LATEX

Plant latex are saps that are exuded from the aerial parts of the plant during the damaged caused by the insects or due to mechanical damage. 20,000 species from more than 40 families of angiosperms are considered to exude latex. Latex contains various phytochemical compounds including alkaloids, terpenoids, flavonoids, rubbers and various other protein substances. Recently some of the findings show that latex contains proteins which also play a defensive role against trapping the insects (Konno, 2011). Usually, the color of the released latex is white and after release it has a coagulation like effect and becomes sticky at last. This milky white fluid is produced due to the metabolic activity of certain cells called as laticifers. According to various reports latex flows from petioles and leaves but some of the plants produce latex from fruits, trunk and also from the roots of the plant (Ramos et al., 2020).

1.2. DISTRIBUTION OF LATEX:

Latex is a loosely denoted term by the anatomist. It is a combination very little suspension dispersed in the water with entirely different refractive index. Most of the researcher reveal that the dispersed milky white fluid are hydrocarbons of terpene type consisting of balsam, essential oils, rubber, and resins. Latex are secreted from various plants and each latex consist of different varieties of phytochemical compounds. For example, *Carica papaya* latex contains a proteolytic enzyme called papain, latex of *papaveraceae* are the source of alkaloids and the latex from the euphorbia plants are rich in Vitamin B (Metcalf, 1967). The proportion of latex bearing families are similar in both widespread cosmopolitan family and tropical families. Latex bearing plants are mostly found in tropic areas than temperate or widespread areas. Initial survey of tropical forests revealed that the latex bearing plants are present more abundantly than the non-laticiferous plants (Lewinsohn, 1991).

1.3. COLOR

Most of the plants exude latex in white color but, there are some plants that exude latex in the color of yellow, orange and red. The color of the latex can be used to determine what ecological factors are linked with the latex to perform a comparative study (Ruxton, 2014; Bauer et al., 2014).

1.4. APPLICATIONS:

There are numerous medicines present based on plant origin. Among them latex is known to possess a wide range of medicinal values. Mostly, plant latex is used as analgesic, anthelmintic, to clear skin infections, antinociceptive, arrest bleeding from minor injuries and to enhance wound healing. It is also known that plant latex has been used in the management of gum bleeding, toothache etc. (Venkatesha et al., 2016). Plant latex are also used for biodiesel production by serving as an alternative for transesterification process (Mazou et al., 2016). Phenolic and alkaloids compounds present in the plant latex are used to treat neurodegenerative disease such as parkinsons and alzheimer's disease. Recent advancements in nanoscience offer advanced nanomaterials as nanocarriers of pharmacologically active latex natural agents. Conjugation of small natural latex compounds with nanoparticles allows to improve bioavailability and stability of these natural pharmacologically active latex compounds (Warowicka et al., 2020). Some of the plant latex are also used in cosmetic industries (Bruusgaard-Mouritsen et al., 2020). Plant latex are the source of various hydrolytic enzymes responsible for treating various ailments. Latex consisting of protease are used for wound healing purposes (Singh & Bindhu, 2020).

1.5. *CARICA PAPAYA*

Kingdom:	Plantae
Order:	Brassicales
Family:	Caricaceae
Genus:	<i>Carica</i>
Species:	<i>C. papaya</i>

India is mostly covered by tropic and sub-tropic regions that acts as a major source of medicinal and aromatic biodiversity which foundation for traditional medicine and the plant Papaya, which belongs to the family, Caricaceae, scientifically known as *Carica Papaya*. The term *Carica papaya* is commonly known as papaya in English, Erandarkarkati in Sanskrit and Papita in Hindi (Naria et al., 2017). All the parts of the papaya plant consist of different medicinal value and it is ethnomedicine (Roshan et al., 2014).

1.5.1. Seed

Seeds of papaya are black in color and are enclosed inside rice pulp. It can be used as an alternative for pepper. Seeds of papaya contains various compounds like papaya oil, crude proteins, crude fibers, caricin, tyrosine. Papaya seeds are used as a pharmacological agent in many ailments. It can be used as analgesic, contraceptive, antifertility and used for anti-inflammatory purpose (Agarwal et al., 2016).

1.5.2. Roots

Papaya roots consists of carposide, arposide, and myrosin. The roots of papaya plants can be used as a cure for urinary tract problems. It also helps in preventing the formation of kidney stones and kidney inflammation, it is also used in treatment of rheumatism and gastroenteritis (Chinonye et al., 2016).

1.5.3. Leaves

Leaves of papaya tree contain a large number of alkaloids, dehydrocarpaine I and II, carpain, pseudocarpain and carposide, choline, vitamin C and E. The leaves of Papaya consist of papain, phenolic compounds, and alkaloids and these acts as antioxidants

which, enhances the body immunity. The mixture of papain and other compounds helps in digesting the essential proteins which can cure disorders related to digestion. Freshly obtained papaya leaves are a rich source of essential fibres, papain, and chymopapain which when consumed within the sort of juice improves gastrointestinal system and control issues like heartburn, bloating, bowel movement and constipation. increases platelets and neutrophils counts in dengue patients can be done by consuming the Fresh papaya leaves extract (Imaga et al., 2010; Gadge et al., 2020).

1.5.4. Fruits

Fruit of papaya is the rich source of protein, amino acids, carbohydrates, fibre, fat, minerals, calcium, phosphorus, iron, thiamin, niacin, riboflavin, vitamin C, carotene, citric acid and malic acid (Gadge et al., 2020). The fruit of the papaya plant is 5-30 cm long, cylindric, yellow- orange in color and fleshy. An Average-sized papaya weighs about a pound. Papaya fruit act as antibacterial, antihelmintic, anti-inflammatory, antiprotozoal, neuroprotective, antihypertensive, diuretic, antitumour and antifertility properties (Maisarah et al., 2014).

1.5.5. Latex

The unripe papaya produces a milky sap like substance which contains papain, proteolytic enzymes, and chemopapain, chymopapain A, B and C, glutamine cyclotransferase, peptidase A and B (Nadkarni, 1954). The latex of the papaya plant is used to treat Dermatitis and psoriasis. Papain, a purified compound of papaya latex is a cysteine protease of 212 amino acids. It has a strong proteolytic capacity. It is widely used in several industries such as food applications for cheese-making, meat tenderization, baking industry, brewing/wine industry. It has various medicinal properties and it can be used to treat various ailments (Hafid et al., 2020).

1.6. BIOPESTICIDES

Biopesticides are developed from naturally occurring living organisms such as animals, plants, and microorganisms (e.g., bacteria, fungi, and viruses) that can control serious plant-damaging insect pests by their nontoxic eco-friendly mode of action on pests. They have gained more importance on pests as they pose less threats to the environment (Saxena & Pandey, 2001). When biopesticides are used as a constituent of pest management, its efficiency can be equal to that of the synthetic pesticides, particularly for crops like fruits, vegetables, nuts, and flowers. They also overcome the concern of residue problems especially for consumers of edible fruits and vegetables (Kumar, 2012).

1.6.1. Microbial biopesticides:

They contain a microorganism (bacterium, fungus, virus, protozoan or alga) as the active ingredient which are used for the biological control of plant pathogens such as pests, insects and weeds. Different microbial pesticides are responsible for control of different kinds of pests because each separate active ingredient is relatively specific for its target pests. For instance, certain type of fungi control weeds and other types that kill specific insects (Gupta & Dikshit, 2010). The most widely used organism in the development of microbial pesticides are varieties of insect pathogenic bacterium *Bacillus thuringiensis*, or Bt which provide control for insects present in cabbage, potatoes, and other crops. They produce a specific protein during the spore formation of the bacterium that is harmful to specific insects such as Lepidoptera, coleopteran and diptera (Chandler *et al*, 2011). However, drawbacks of microbial pesticide is they need to be monitored continuously to ensure they do not become capable of harming non-target organisms, including humans.

1.6.2. Biochemical pesticides

Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. These types of pesticides include substances that interfere with growth or mating, such as plant growth regulators, or substances that repel or attract pests, such as pheromones. Pheromones are certain chemicals produced by insects which is stimulated due to a certain behavioral reaction from other individuals. They can be known as sex pheromones, aggregation pheromones, alarm pheromones, etc., according to type of effects it displays. They have become essential tools in monitoring and managing agricultural pest populations (Witzgall *et al*, 2004).

1.6.3. Plant based pesticides:

Plant biopesticides or Botanical pesticides are pesticidal substances that use the natural compounds from plant to give security to insect pests. Exploring the potential to utilize the properties of plants has become a key focus in pest control. Some plants are known bioactive metabolites, which show repellent and toxic effects on a wide range of pests. These compounds were initially used in the nineteenth century and by the early period of the twentieth century, their use and benefits have been established worldwide (Morgan, 2004). Plants have been known to develop many strategies to defend themselves from predators due to the co-existence of plant and insects on the earth for almost three and a half million years. One such strategy is developing compounds that

are highly toxic to insects (Warthen & Morgan, 1985). Some plants also act as repellents and when effective, it causes the target pest to make an oriented movement away from the source of stimulus and in case of no escape, it leads to the death of the pest (Owusu, 2001).

1.7. MEALY BUGS

Taxonomic Tree

Domain	: Eukaryota
Kingdom	: Metazoa
Phylum	: Arthropoda Subphylum
	: Uniramia
Class	: Insecta
Order	: Hemiptera
Suborder	: Sternorrhyncha Unknown
	: Coccoidea
Family	: Pseudococcidae
Genus	: Phenacoccus
Species	: <i>Phenacoccus solenopsis</i>

1.8. NATURE OF MEALY BUGS

Mealybugs are a group of small (<5 mm) gregarious insects that are readily distinguishable due to the presence of a fringe of white filaments around their waxy covered bodies. Only at certain stages in their life cycle are wings produced. They pass through several nymph stages before reaching maturity, and frequently show several to multiple generations per year (Lu *et al*, 2008).

1.9. INTRODUCTION TO NANOCARRIERS

Nanoparticles in recent trends have found its prominence in various fields of Medical nanotechnology and other important fields of Nanotechnology. They have been used due to their small size and their ability to penetrate through many pores in cells. The initial research on nanoparticles was principally focused on the synthesis of metal based nanoparticles that were basically non-biodegradable and commonly get accumulated in the system leading to high toxicity in the systems (Martin *et. al.*, 2008). Eventually focusing on the synthesis of nanoparticles that are more biodegradable in

nature. One of the finest sources for synthesis of biodegradable nanoparticles are commonly polymers (Fiorenza et al.,2016). Later studies began focusing more on natural sources of polymers or the so called 'Biopolymers'. There have been reports stating the various sources of obtaining biopolymer such as Microorganisms (Tan et al, 1997), animal sources (Lei et.al.,2017) etc. One of most commonly available and important of these belong to plant sources (Shivakumar et.al.,2011). The plants are made up of many polymeric compounds and conventional of them is that which are exuded by the plants. Some species of plant exude a white sap that contains gum, resins etc. and are known to be a vital source of plant polymers (Pratik et.al.,2013).

1.10 HISTORY OF NANOPARTICLES

The history of nanoparticles dates to the golden era when the first soluble gold was discovered in parts of Egypt and China around 1200-1300BC. The soluble gold was used as piece of art (Giljohann, D.A. et al.,2010). The first nanotechnology was used in the manufacturing of Lycurgus Cup in Alexandria the specialty of which was its capacity of changing colour from opaque green to glowing translucent red when held to light (Pereira L.,2015).

The first book on drinkable gold that contains metallic gold in neutral media was published in the 1676 by J.von Lowenstern-Kunckel in Germany which led to the special interest in the study of nanoparticles. This was followed by many Laws and theories. like the Moore's law (Fashola et.al.,2016), the Kubo effect (Kubo et al.,1986) etc. and the new inventions like Scanning Electron Microscope(SEM) in 1937, Transmission Electron Microscope(TEM) in 1931 etc.

1.11. SPIONS

All iron oxide nanoparticles are ferromagnetic or ferrimagnetic nanoparticles whose atomic dipoles are arranged mannerly or in opposite alignment expressing multi-domains. In superparamagnetic nanoparticles, all the atoms of individual nanoparticle exist as a single domain unlike ferromagnetic nanoparticles where the atoms of single nanoparticle exist in multiple domains. In both cases, the magnetic moment of the domain(s) gets aligned according to the directions of the external magnetic field (Martella et al., 2018; Malhotra et al., 2019). Iron oxide is biocompatible to some extent with the human system since the chelator of haemoglobin consists of Fe(II) atoms. However, the high concentration exposure can disturb the normal physiological functioning of cells (Patil et al., 2018). Due to the smaller size, easy absorption and biocompatibility they are regarded as adaptable nanoparticle to the *in-vivo* system.

1.12. TYPES OF NANOCARRIERS

1.12.1 JANUS NANOPARTICLE

These are simple oblate to dumbbell shaped nanoparticles that are anisotropic in nature they have the unique property of self- assembly. They are formed by simple polymerization. They are normally 25nm in size and thus can be used for various surface-based activities. Due to their anisotropic property the Janus nanoparticle can be used to interact with both hydrophilic and hydrophobic surfaces [Samrot et al., 2017].

1.12.2 METAL ION NANOCARRIERS

Some of the preliminary studies was done in these types of nanoparticles. Metal nanoparticles were the first to be synthesized in the field of nanotechnology [Samrot et al., 2021].

1.12.3 POLYMER COATED NANOPARTICLES

The conventional metal nanoparticles sometimes can be toxic in nature also they may not be environmentally friendly. In order to overcome this the nanocarriers were synthesized using polymers or have been coated with polymers. Some important examples being the synthesis of Chitosan-carboxymethylcellulose nanoparticles., Solid lipid nanoparticles (SLN) coated with a folate-grafted chitosan, Polyhydroxy[R]alkanoate based nanoparticles,etc.[Senthilkumar et al., 2018; Samrot et al., 2016]

1.13 CHARACTERISTICS OF A NANOCARRIER

1.13.1 PHYSICAL CHARACTERISTICS

According to Girdhar et al. 2018, nanocarriers are known to possess the following physical characters:

- They normally range from 1 to 100nm
- They normally have a large surface area.
- Some nanoparticles have been found to have superior UV blocking properties
- Nanoparticles, due to their small size confine their electrons and produce quantum effects.
- Nanoparticles are capable of their suspension in a bulk media even at high temperature
- Their strength may be hard, soft or semi-solid

1.13.2 CHEMICAL CHARACTERISTICS

According to Tiwari & Amiji, (2006), nanocarriers are known to possess the following physical characters:

- They have complex surface
- liquid interfaces and polyelectrolytes have the ability to modify surface properties
- The particles are environment sensitive
- They have good adhesion properties
- They act as lubricants
- Nanocarriers are generally stable and have controlled flocculation of colloidal dispersions.

1.14 APPLICATIONS

1.14.1 FOOD TECHNOLOGY

Due to their hydrophobic nature, polymeric nanoparticles have a close affinity to full cream milk powder. Thus, nanoparticles can be used to stabilize milk powders and many such dehydrated dairy products. Using Hybrid nanocarriers consisting of a hydrophilic compound show an added advantage. Weiss et al 2006, has reported that amphiphilicity of nanoparticles help food additives blend well with a solution. This property assist in enhancing their mixture by providing high stability and prevent particle aggregation.

1.14.2 DETERGENTS

One of the most common application of using detergent is the removal of oil stains. Samrot et al (2018), reported that nanoparticles are some of the best sources for increasing the efficiency of a detergent. Nanoparticles due to their small size actively interact with the dirt particles and can be eliminated as foam using active surfactant molecules.

1.14.3 NANO DRUG DELIVERY

There have been many prodigious advancements in modern medicine and drugs for every possible disease on earth has a cure. A recent study done by the FDA shows that almost 2 out of every 3 new molecules discovered have been disapproved due to their ineffective nature of action during their *in-vivo* screening. Based on the theory of Surface Plasmon Resonance there arouse a new field of Medical Nanotechnology where Nanoparticles have been used to deliver the drugs to their targeted site. Biopolymers have been proved to be one of the finest sources through which nanocarriers can be

synthesized and delivered without any compatibility and degradability issues [Samrot et al., 2021].

1.15. DYES

Dyes are chromophores and auxochromes combined in an organic compound. Auxochromes improve the colour intensity of dyes for the substrate to which they are applied. Chromophores are responsible for imparting colour to dyes, and auxochromes are responsible for raising the colour intensity of dyes for the substrate to which they are applied (Allen *et al.*, 2004). Dyes are generally water soluble and produce a light colour in water, but some dyes can produce a very dark colour even at very low concentrations. Dyes can be natural, meaning they come from plants, animals, insects, and natural mineral sources, or synthetic, meaning they come from non-biodegradable complex molecular structures and pose a significant risk to the water supply (Sharma *et al.*, 2000; Felipie *et al.*, 2017). Acidic and simple dyes are two types of dyes. Acidic dyes are water-soluble salts of sulphuric, carboxylic, or phenolic groups that can be partially or completely attached to cationic substrates like linen, silk, nylon, and acrylic fibres. Basic dyes, on the other hand, are cationic dyes that contain monoazoic, diazoic, and azine compounds (Natarajan *et al.*, 2017). MB is commonly used in printing, paper colouring, wool, cotton, tannin, and clothing dyeing, as a temporary hair colourant, and as an oxidation-reduction process indicator. It is commonly used as an antiseptic (Lenglet *et al.*, 2011). Methyl violet or Crystal violet which is a green powder. The methyl groups attached to the dye determine the colour of Methyl violet dye. The term methyl violet (MV) refers to a group of three compounds with varying numbers of methyl groups attached to the amine functional group. Water, ethanol, diethylene glycol, and dipropylene glycol are all soluble in these. MV is commonly used as a purple dye for textiles, as well as a deep violet pigment in paint and ink (Fayazi *et al.*, 2016; Keyhanian *et al.*, 2016).

1.15.1 Toxicity of Dyes

Because of their complex structures and non-biodegradable existence, dyes like CR, RB, MB, MV, and CV are harmful to living organisms and pose a significant threat to society (Ma *et al.*, 2017). These dyes have an effect on the environment and are often carcinogenic. These colored compounds not only degrade the aesthetic quality of water, but they also prevent sunlight from reaching living organisms (Siddiqui *et al.*, 2018; Ayad *et al.*, 2010). Methylene Blue and Crystal violet are the most harmful to living beings of all the dyes. Acidic dyes have negative effects on the eyes, respiratory system, and skin, as well as the potential to cause cancer and mutagenicity in humans (Cai *et al.*, 2010).

Basic dyes are also poisonous, and they can cause allergic reactions, skin inflammation, mutations, and even skin cancer in humans. They can also cause shock, vomiting, cyanosis, jaundice, quadriplegia, and tissue necrosis (Chung *et al.*, 2008; Hameed *et al.*, 2002).

1.15.2. Environmental remediation

Nanomaterials have the ability to eliminate toxins and biological contaminants effectively in the field of environmental remediation. Nanomaterials come in a range of shapes and morphologies, including nanoparticles, tubes, cables, and fibres. As adsorbents and catalysts, they are used to detect and remove gases, contaminated chemicals, organic contaminants, and biological substances such as bacteria, viruses, fungi, and antibiotics, as well as their composites with polymers (Khin *et al.*, 2012). Various factories emit large amounts of wastewater containing toxic and dangerous dyes, polluting water in both direct and indirect ways. The majority of the dyes are a harmful class of water pollutants that have had a significant impact on the environment. Congo red, rhodamine B, methylene blue, methyl violet, and crystal violet are among the dyes that pose a significant danger to humans.

CHAPTER 2

LITERATURE SURVEY

Latex is a sticky sap that flows from the plants due to any cuts or wounds caused by the external agents. Latex is mostly stored in specialized chain like cells (tube like regions) known as laticifers. These white sap seals the wound once it is discharged. Besides sealing wounds latex has various function like defensive properties, restoration of mechanical properties in plants that are injured (Hunter, 1994; Dussourd & Eisner, 1987; Agrawal & Konno, 2009; Bauer & Speck, 2012). Latex differs not only in their chemical properties and color but it also differs in laticifers found. The laticifers found can be connected continuously, may be unbranched, may be connected in like nets and are arranged together. Most of the times the laticifers found are branched (Bauer & Speck, 2012). Latex is differentiated as gums, resins and mucilage which also exude due to damages in tissue. Resins usually consist of phenolics and terpenoids which are sometimes called as ducts. Compared to all these compounds latex consist of various phytochemical compounds like alkaloids, terpenoids, proteins, phenols etc. laticifers usually present as two forms namely non- articulating form. These are usually formed from single cells and branched they are interconnected but not a formation of loop. Articulation form are usually formed by the interconnection of neighboring laticifers. It is formed by perforating cell wall of the laticifers (Agrawa & Konno, 2009).

Agriculture has faced the abundant destructive activities of pests like weeds, insects and other plant pathogens leading to a drastic decrease in crop yields (Salma & Jogen, 2011). Due to the resistance, the number of pests exhibiting pesticide resistance has increased. One of the most noted resistance was witnessed in cotton, for which American bollworm is a serious pest (Ghayur & Alam, 2000). Therefore, it had become imperative to find an eco-friendly alternative to overcome the drawbacks associated with synthetic pesticides. The use of biopesticides has offered an ecologically sound and effective solution to pest problems and provide a better solution than chemical pesticides (Salma & Jogen, 2011).

Among all nanoparticles, SPIONs is the most researched metal oxide nanoparticle due to its fantastic properties and application. One special characteristic of SPIONs is its superparamagnetic behavior. The particles exhibit magnetism under external applied

field where all each particle act as a single domain to align to the direction of the field (Dadfar et al.,2020).

Nanocarriers are nanomaterials which are used to carry a drug to the site of action, where in these nanocarriers protect the drug from degradative actions of the body and deliver the drug to the site of action even to the region of its low solubility, permeability and stability thereby enhancing the Pharmacokinetic aspects of the drug. These Nanocarriers further enhance the safety, pharmacokinetic profiles and bioavailability of the administered drugs which leads to improved therapeutic effects when compared to that of conventional therapies. The accepted size for the nanocarrier is from 10-100nm, which facilitates its transport in circulation or prevention of its leakage into the blood capillaries, clearance by reticuloendothelial system, uptake of cells (Champion et al.,2007). There are many drugs being developed for brain diseases and other neurodegenerative disorders, but they have many limitations conferred the blood brain barrier. This blood brain barrier allows molecules less than 1nm of size to pass through it(Begle.,2004). This limitation is overcome by the nanocarrier, which is a few nanometers in diameter and therefore can easily pass through the blood brain barrier and thus the nanocarrier acts as a very effective vehicle to carry the drug to the targeted site. Nanocarriers are also being replaced for viral vectors in the gene delivery in order to overcome the immunogenicity (Xu et al.,2014).

CHAPTER 3

AIM AND SCOPE

3.1. AIM:

The identify the effect of latex on influencing growth of plants, to synthesize nanocarriers using plant latex for biomedical application.

3.2. OBJECTIVE:

- To collect and characterize latex.
- To study the effect of latex on influencing growth of *Vigna radiata* seeds (In- vitro and In-vivo).
- To evaluate the pesticidal and pesticide repulsion activity of Latex
- To synthesis and characterize SPIONs
- To coat SPIONs with papaya latex and load drug
- To characterize drug loaded nanocarrier
- To perform adsorption studies for SPIONs and papaya latex coated SPIONs

3.3. SCOPE

The biopolymers are chemically inert, biocompatible, biodegradable and highly sustainable in nature, Thus, it is widely been used for various applications. They also act as good surface stabilizing agents, when coated on the SPIONs, gives stability for the SPIONs.

CHAPTER 4

MATERIALS AND METHODS

4.1. MATERIALS

Carica papaya latex, Sodium hypochlorite, *Vigna radiata* seeds, Distilled water, DPPH, Iodine resublimates, Chloroform, Ethanol, TLC plate, Ferrous chloride, Ferric chloride, Sodium hydroxide pellets, Ammonia, Hydrochloric acid, pH paper, Methanol, Curcumin, Dialysis Bag, Methylene Blue, Crystal violet.

4.2. METHODS

4.2.1. COLLECTION OF LATEX

Carica papaya latex was purchased from Sisco research Laboratories, India. The obtained latex was in the form of fine powder.

4.2.2. CHARACTERIZATION OF *Carica papaya* latex

4.2.2.1. UV- Vis spectroscopy

The characterization of plant Latex was carried out using UV-Visible spectroscopy (Shimadzu UV-1800, Japan). The latex was dissolved in distilled water (1mg/mL) and was centrifuged. Supernatant was collected and the sample was exposed to light range between 200 and 800 nm in UV – Vis spectrophotometer. A spectrum of Latex was plotted with Wavelength on X – axis and Absorbance on Y – axis.

4.2.2.2. Fourier Transform Infrared Spectroscopy

FT-IR spectroscopy allows valuable insight into the different functional groups that are present in the nanoparticles by measuring the vibrational frequencies of the chemical bonds involved in its formation. The infrared spectra are recorded on Fourier Transform Spectrometer in the mid-infrared region (MIR) within the range (400-4000 cm^{-1}). FTIR spectroscopy can serve as a wonderful complement to UV-Vis and NMR spectroscopy. FTIR analysis for Crude latex was performed (Shimadzu, Japan).

4.2.2.3. GC- MS analysis

GC- MS analysis was done to identify the bioactive compounds present in the Latex.

4.2.2.4. Thin Layer Chromatography

Thin layer Chromatography was performed for the latex (TLC Silica gel 60 F₂₅₄). The TLC analyses of samples were performed with ethanol: water (2:1) as mobile phase. R_f values of different spots were determined.

4.2.2.5. TLC Bio- Autography

Thin layer Chromatography was performed for the latex. The TLC analyses of samples were performed as mentioned above. DPPH solution was prepared by taking (0.004 % (w/v) in 90 % of methanol). The prepared DPPH solution was sprayed completely on TLC plates and fluorescence was observed for identifying the antioxidant properties of the latex (Samrot et al., 2018).

4.2.2.6. Antibacterial activity

Plant Latex was tested for antibacterial activity through well diffusion method (Bouaziz et al; 2016). In this method, Nutrient agar plates were swabbed all over with the bacterium (Gram positive – *Staphylococcus sp.*; Gram negative – *Klebsiella sp.*) using a sterile swab. 1 mg of extracted latex was dissolved in distilled water. Wells were bored and four concentration of prepared sample (20µl, 40µl, 60µl, 30µl, 100 µl) was added to the wells along with the Control. After 24 hours the zone of clearance were measured and recorded.

4.2.3. SEED GERMINATION STUDIES

The seeds of *Vigna radiata* were purchased from market near Tambaram. Purchased seeds were surface sterilized using 2 % sodium hypochlorite solution then soaked in water for 6 h. The seeds were subjected for surface sterilization again and 3 seeds / petri plate were taken and 10 different concentration ranging from 10 % to 10 0% of latex was sprayed in each petriplate containing seeds. Seed germination and its growth was observed on 3rd and 7th day respectively (Samrot et al., 2020). Best concentration of latex influencing the growth of the seeds was identified and used for pot studies

4.2.4. POT STUDIES

Garden soil enriched with natural manure was taken as positive control, poor quality soil collected from the construction sites near tambaram was used as negative control, the best concentration of latex identified from the seed germination was used as test sample. 10 seeds were sowed on each pots and triplicates were maintained. Biomass, shoot length, root length and soil analysis were observed on 10th, 20th and 30th day.

4.2.5. SOIL POROSITY

A 500 mL conical flask was taken and soil was filled till it reaches 200 mL. measuring cylinder was filled with 100mL of water. The water is slowly poured into the

conical flask until all the water is completely absorbed by the soil. Volume of water poured into soil was noted. Soil porosity is calculated using the formula (Matko *et al.*, 2003):

$$\text{Porosity} = \frac{\text{Amount of water added to the samples}}{\text{Total amount of sample}} \times 100$$

4.2.6. SOIL ANALYSIS

Soil Analysis was performed in order to identify the amount of macro and micro nutrients, pH, conductivity, Saturation% of nutrients, bacterial count etc.. present in the soil.

4.2.7. SCANNING ELECTRON MICROSCOPY

Nature of the soil and its surface morphology was characterized using Scanning electron microscopy. Scanning electron microscopy is a technique to determine the size, shape and surface morphology with direct visualization of the nanoparticles. It is usually used for high-determination imaging of surfaces of nanoparticles.

4.2.8. PERCENTAGE REPULSION ACTIVITY

Filter paper disc method was done to determine the repellence property of the crude pesticides. 10 petri plates were taken with different concentration ranging from 10 % to 100 %. Each filter papers were cut by scissors into half from the middle horizontally where one half was used as control (distilled water) and the other was used as pesticide treated part (latex at different concentration). The filter papers were air-dried and both the treated and control part was placed along the corners of the petri dishes with a gap in the middle. Ten adult mealy bugs were applied at the center of the filter papers present in the petri dish. This process was continued for each petri dishes (Ulubelen *et al.*, 2001). Triplicates were performed and the percentage repulsion was calculated by means of mean $\pm$ standard deviation.

$$\text{Percentage repulsion} = \frac{\text{A Number of pest on control} - \text{number of pest on treated}}{\text{Number of pest on control}} \times 100$$

Where, positive value indicates repellency and negative value indicates attractancy.

4.2.9. BIOPESTICIDAL ACTIVITY

Matured mealy bugs were collected from the plant *Hibiscus rosasinensis* along with some of the host leaf which served as a feed for mealy bug. Latex was taken in different concentration (dispersed in water) ranging from 10 % to 100 % in a petriplate and was allowed to air dry for 3 h. A host leaf was kept in each petriplate and different concentration of latex was sprayed on top of the leaf. 10 Matured mealy bugs were kept on each petriplate and it was observed for 48 hours. After 48 hours the pest from each

concentration was put inside 10 % formalin solution and the effect of latex on the pest was identified by histopathological staining (Khan et al., 2014).

4.2.10. Synthesis of SPIONS

Synthesis of Super Paramagnetic Iron Oxide Nanoparticles (SPIONS) was carried out by chemical co-precipitation method (Justin et al; 2017). 100mL of 1 M FeCl_3 [Fe^{3+}] and 100mL of 1 M FeCl_2 [Fe^{2+}]. The solution was homogenized together and subjected to magnetic stirring for 30 min. Reducing agent was prepared by adding 1 M sodium hydroxide solution in 150mL Millipore water and mixed well. Ammonia was also added in the volume of 50mL Sodium hydroxide solution and ammonia was added drop wise to the precursor ion solution under stirring. Upon continuous addition of NaOH, the solution turns brown black, at that instance the addition of sodium hydroxide was stopped. In the presence of magnetic field the nanoparticles were allowed to settle down and the excess NaOH was pipetted out. Particles were washed with Millipore water and then lyophilized (Justin et al., 2017).

4.2.11. CHARACTERIZATION OF SPIONS

4.2.11.1. UV- Vis Spectroscopy

The characterization of SPIONS was carried out using UV-Visible spectroscopy (Shimadzu UV-1800, Japan). The sample was exposed to light range between 200 and 800 nm. A spectrum of synthesized SPIONS was plotted with wavelength on X – axis and absorbance on Y – axis.

4.2.11.2. Fourier Transform Infrared Spectroscopy

FT-IR helps in surface characterization of nanoparticles (Baraton and Merhari, 2007). FT-IR measures the absorbance of electromagnetic radiation at a particular wavelength within the mid-infrared region ($4000\text{--}400\text{ cm}^{-1}$). The absorbance of incident infrared rays by the surface atoms creates energy excitation which causes the dipole of the atoms engaged in bonding to flip via bending, stretching and vibration. The synthesized SPIONS was subjected for FT-IR Spectroscopy (Shimadzu, Japan) analysis.

4.2.11.3. Scanning Electron Microscopy

This characterization method was done to analyse the surface morphology. Scanning electron microscopy was performed by preparing the sample as a thin film. The

samples were dried and then observed. They are then analyzed for energy dispersive X-Ray analysis (EDX). SPIONs was characterized using SEM (JEOL- JSM- IT 200). analysis to identify its morphology

4.2.11.4. X-RAY diffraction spectroscopy (XRD)

X-ray diffraction (XRD) is one of the most important non-destructive tools to analyze all kinds of matter ranging from fluids, to powders and crystals. X-ray diffraction techniques are used for the identification of crystalline phases of various materials and the quantitative phase analysis subsequent to the identification. X-ray diffraction techniques are superior in elucidating the three-dimensional atomic structure of crystalline solids. This analysis is used to predict the nature of the sample i.e., either crystalline or amorphous. XRD (PANalytical, Netherlands) analysis of synthesized SPIONs was performed

4.2.12. SYNTHESIS OF PAPAYA LATEX COATED SPIONS

Papaya latex coated SPIONs were synthesized with the slight modification of Zhang et al. (2009). 10 mg of synthesized 1 M SPION was taken and it was dissolved in 1 mL of nitrogen purged Milli Q water and was sonicated in a water bath sonicator for 10 min. 1 g of *Carica papaya* latex was dissolved in nitrogen purged Milli Q water and stirred for 15 min until it dissolved completely. Both the prepared solutions were mixed together under mechanical stirring for about 30 min. Further the solutions were sonicated for 15 min. It was washed for 5 times and dried for further purpose.

4.2.13. SYNTHESIS OF DRUG LOADED NANOCARRIERS

0.02 g of latex coated with SPION was taken and 10 mL of distilled water was added and stirred for 15 min. 1 mL of methanol containing 0.001 g of curcumin was added to latex coated SPIONS and stirred for 2 h, sonicated for 10 min and kept undisturbed for overnight. Further, the SPIONS were sonicated for 15 min in a water bath sonicator and the solution was centrifuged at 4000 rpm for 15 min. Pellet was collected and dispersed in Nitrogen purged Milli Q water and dialysed in dialysis bag for 24 h. After 24 h, the sample was dried and collected for further use (Akbarian et al., 2020).

4.2.14. CHARACTERIZATION OF PAPAYA LATEX COATED SPIONS AND DRUG LOADED NANOCARRIER

4.2.14.1. UV- Vis Spectroscopy

The characterization was carried out using UV-Visible spectroscopy (Shimadzu UV-1800, Japan). The sample was exposed to light range between 200 and 800 nm. A

spectrum of synthesized SPIONs was plotted with Wavelength on X – axis and Absorbance on Y – axis.

4.2.14.2. Fourier Transform Infrared Spectroscopy

FT–IR was used to study the presence of functional groups to determine the chemical bonds present in the sample. The synthesized papaya latex coated SPIONs and Drug loaded nanoarrier was subjected for FT-IR Spectroscopy (Shimadzu,Japan) analysis at the frequency range of 4000 – 500 cm⁻¹. The spectrum was obtained with Wave number (cm-1) on X – axis and Transmittance (%) on Y – axis.

4.2.14.3. Scanning Electron Microscope

Before subjecting the nanoparticles for imaging under SEM, it should be totally dried or lyophilised. The dry powder is then further mounted on a carbon strip fitted on a sample holder (stub) which is then followed by coating with a conductive metal (e.g. gold) using a sputter coater. The sample is then analyzed by scanning with a focused beam of electrons. Secondary electrons are emitted from the sample surface determining the surface characteristics of the sample. Papaya latex coated SPIONs and Nanocarrier was characterized using SEM (JEOL- JSM- IT 200). analysis to identify its morphology

4.2.14.4. X-Ray Diffraction Spectroscopy (XRD)

This analysis is used to predict the nature of the sample i.e., either crystalline or amorphous.

4.2.15. DRUG RELEASE EFFICIENCY

10 mg of synthesized drug loaded nanocarriers were dissolved in 1mL of Nitrogen purged Milli Q water. Phosphate buffer solution was prepared. The nanocarriers were put in dialysis bag and dialyzed against phosphate buffer solution (Sodium chloride -8g, Potassium chloride - 200 mg, Disodium hydrogen phosphate - 1.44 g, Potassium dihydrogen phosphate - 245 mg) of pH 7.4. Absorbance was measured by taking the OD values at 30th, 60th, 90th, 120th, 150th and 180th minute respectively. Graph was plotted by taking time in the X- axis and OD values in Y axis (Samrot et al., 2016).

4.2.16. ANTIBACTERIAL ACTIVITY

Papaya Latex coated SPIONs and drug loaded nanocarrier was tested for antibacterial activity through well diffusion method (Bouaziz et al; 2016). In this method, Nutrient agar plates were swabbed all over with the bacterium (Gram positive – *Bacillus subtilis*.; Gram negative – *Pseudomonas aeruginosa*) using a sterile swab. Wells were bored and four concentration of prepared sample (25µl, 50µl, 75µl, 100 µl) was added to

the wells along with the Control (Azithromycin). After 24 hours the zone of clearance was measured and recorded.

4.2.17. ANTICANCER ACTIVITY

4.2.17.1. MTT Assay

Cell viability assay, L929 and MDA-MB-231 viable cells were harvested and counted using haemocytometer diluted in DMEM medium to a density of 1×10^4 cells/ml was seeded in 96 well plates for each well and incubated for 24 h to allow attachment. After L929 cells treated with control and the containing different concentrations of sample 2.5 to 15 $\mu\text{g/ml}$ were applied to each well. L929 and MDA-MB-231 cells were incubated at 37°C in a humidified 95% air and 5% CO_2 incubator for 24 h. After incubation, the drug-containing cells wash with fresh culture medium and the MTT (5 mg/ml in PBS) dye was added to each well, followed by incubated for another 4 h at 37°C . The purple precipitated formazan formed was dissolved in 100 μl of concentrated DMSO and the cell viability was absorbance and measured 540 nm using a multi-well plate reader. The results were expressed at the percentage of stable cells with respect to the control. The half maximal inhibitory concentration (IC_{50}) values were calculated and the optimum doses were analysed at different time period.

$$\begin{aligned} &\text{Inhibitory of cell proliferation (\%)} \\ &= \frac{\text{Mean absorbance of the control} - \text{Mean absorbance of the sample}}{\text{Mean absorbance of the control}} \times 10 \end{aligned}$$

4.2.17.2. Acridine Orange/Ethidium Bromide (AO/EB) Dual Staining:

MDA-MMB-231 cells were seeded at 5×10^4 cells/well in a 6 well plate and incubated for 24 hours. After treatment with sample (Papaya latex coated SPIONs and Nanocarrier) (12.5 and 15 $\mu\text{g/ml}$) for 24 h, the cells were detached, washed with cold PBS and then stained with a mixture of AO ($100\mu\text{g ml}^{-1}$)/ EB ($100\mu\text{g ml}^{-1}$) ratio (1:1) at room temperature for 5 min. The stained cells were observed by a fluorescence microscope at 40x magnifications. At the end of treatment, the cells were collected and washed three times with PBS. The plates were stained with acridine orange/ethidium bromide (AO/EB 1:1 ratio; 100 $\mu\text{g/ml}$) for 5 minutes and examined immediately under fluorescent microscope 40x magnification.

4.2.18. PREPARATION OF DYE SOLUTION

For the study, different concentrations of Dyes (Methylene Blue, Crystal Violet) in parts per million (ppm) were prepared in 100 ml of MilliQ water corresponds to 50 ppm, further dilution was done to obtain 5 ppm of Dye solution. The prepared different concentrations of Dye solutions were tested against different dosages of adsorbents, on different time intervals and pH in order to fix the optimal conditions.

4.2.18.1. Standard Curve for methylene blue and crystal violet

Different concentrations of methylene blue and crystal violet solution such as 0.01, ...0.1, ppm was prepared by diluting the stock solution. Then, it was spectrophotometrically read at 663nm and 590 nm respectively. A graph was plotted in ppm Vs OD_{590nm} and OD_{663nm}.

4.2.19. ADSORPTION STUDIES

Dye removal by different adsorbents like SPIONs and Papaya latex coated SPIONs were studied. The parameters like concentration, pH and Contact time were optimized.

4.2.19.1 Optimization of Adsorbate concentration

Dye solution (Methylene blue, Crystal Violet) was prepared in different ppm ranging from 0.01ppm to 0.1ppm. 0.01g of adsorbent material, SPIONs and Papaya latex coated SPIONs were added to each concentration of the dyes. It was allowed to react and the samples were centrifuged and the supernatants were subjected for obtaining the OD values at OD_{590nm} and OD_{663nm} respectively. Maximum absorbance corresponding to the ppm was fixed for further studies.

4.2.19.2. Optimization of Adsorbent-Adsorbate Concentration

Fixed concentration of adsorbate was taken from previous study and now the adsorbent concentration is varied from 0.01g to 0.1g. It was allowed to react and the samples were centrifuged and the supernatants were subjected for obtaining the OD values at OD_{590nm} and OD_{663nm} respectively. Maximum absorbance corresponding to the gram of adsorbent was fixed for further studies.

4.2.19.3. pH Optimization

To identify the activity of adsorbents against dye solutions at different pH, the adsorption was checked at a pH range of 5,6,7,8,9. 1N HCl and 1M NaOH prepared in Millipore water was used to adjust the pH of the dye solution. Fixed adsorbent

and adsorbate concentration was taken and pH is varied. After incubation, the supernatant from each set up was spectrophotometrically read at OD_{590nm} and OD_{663nm} for crystal violet and methylene blue respectively. The final concentration of dye in the solution were calibrated from the standard curve.

4.2.19.4. Contact hour Optimization

Adsorbent, adsorbates at fixed pH was allowed to react for different time intervals ranging from 1 hour to 5 hours. After incubation, the supernatant from each set up was spectrophotometrically read at OD_{590nm} and OD_{663nm} for crystal violet and methylene blue respectively.

4.2.20. ADSORPTION ISOTHERM

The mechanism of adsorption of adsorbate and the adsorbent can be understood from adsorption isotherms. After optimizing all the parameters, the resultant data was used to bring up the following isotherm models namely Langmuir ($1/q_e$ vs $1/C_e$), Freundlich ($\ln q_e$ vs $\ln C_e$) and Temkin isotherms (q_e vs $\ln C_e$). The isotherms were plotted by calculating C_e , q_e , $1/C_e$, $\ln C_e$, $1/q_e$, and $\ln q_e$ values (Ayawei et al., 2017).

Where $q_e = (C_i - C_e) V/m$;

q_e is the adsorption capacity at equilibrium;

C_i is the initial concentration;

C_e is the final concentration;

V = volume of the adsorbate and

m = mass of the adsorbent.

CHAPTER 5

RESULTS AND DISCUSSION

5.1. CHARACTERIZATION OF *Carica papaya* LATEX

5.1.1. UV- Vis SPECTROSCOPY

The Absorbance maxima was found at 200- 230nm and the absorbance and further no absorbance was found. It indicates the presence of Alkyl group and also flavonoid content (Maisarah et al., 2013). Similarly maximum absorbance of 228nm was obtained by Samrot et al., (2018) for the latex of *Calotropis gigantea*.

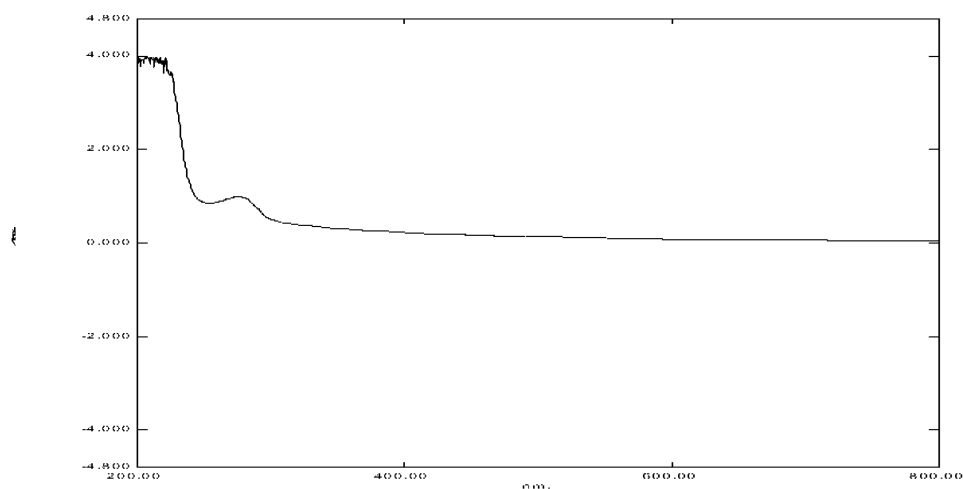


Fig. 5.1.1. UV- Vis of Latex

5.1.2. Fourier Infrared Transmission Spectroscopy

The crude latex showed band near 3378cm^{-1} and 3314cm^{-1} indicates the -CH, -OH and -NH band stretching. The band near 2939cm^{-1} indicates the presence of $-\text{CH}_3$ group. The bands near 1237cm^{-1} indicates the presence of OH group. The band near 830-860 indicated the presence of C- H bending (Samrot et al., 2018; Spanò et al., 2012).

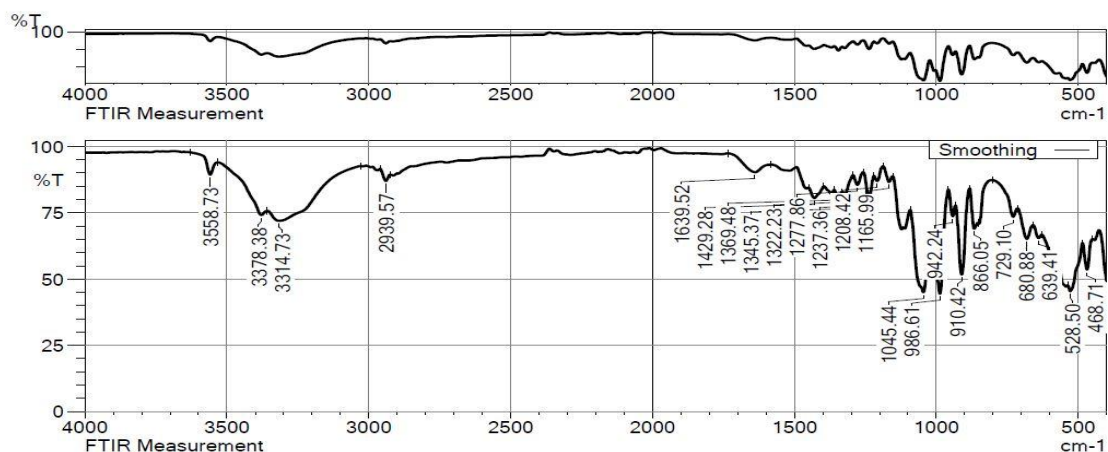


Fig. 5.1.2. FTIR of Latex

5.1.3. GC- MS ANALYSIS

GC- MS analysis of papaya latex revealed the presence of various bioactive compounds like 2-Hydroxy-gamma-butyrolactone, 1,3-propanediamine, thymine, hexadecanoic acid, methyl ester octadecanoic acid, methyl ester (Tharini et al., 2018).

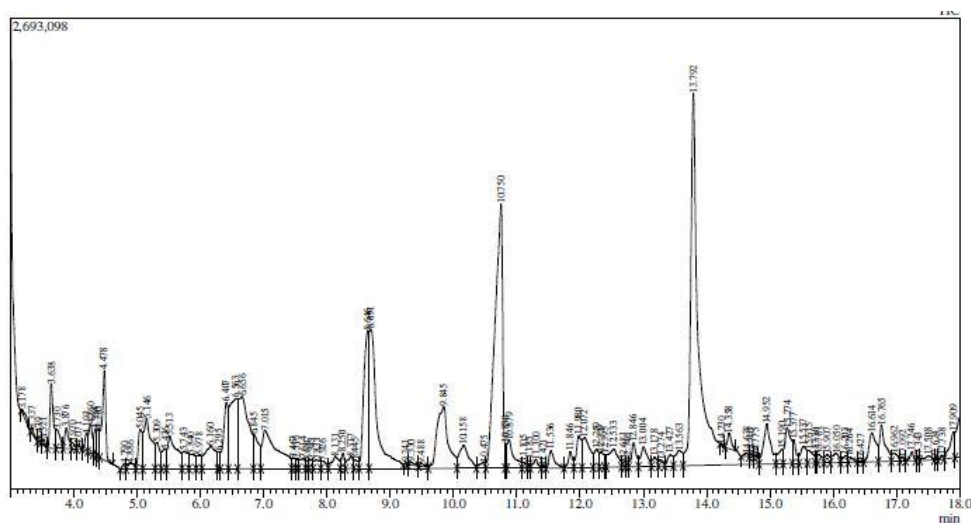


Fig. 5.1.3. GC- MS of Latex

5.1.4. THIN LAYER CHROMATOGRAPHY

R_f value for ethanol: water (2:1) ratio was found to be 0.836. Similarly, Shambharkar et al., (2020) got same R_f value which indicated the presence of coumarin a benzene like compound.

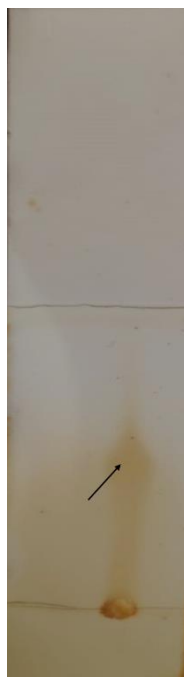


Fig. 5.1.4 –TLC of latex (Ethanol: Water (2:1))

5.1.5. TLC- BIO AUTOGRAPHY

Fluorescence was observed when this solvent system was used. This indicated the presence of antioxidant activity of *Carica papaya* latex.



Fig. 5.1.5 –TLC DPPH bio- autography of latex (Ethanol: Water (2:1))

5.1.6. Antibacterial Activity

The crude *Carica papaya* latex was tested for antibacterial activity against a gram positive bacterium (*Staphylococcus*) and a gram negative bacterium (*Klebsiella*). The antibacterial activity of the particles was evaluated against a Control Azithromycin. The latex showed inhibition against the gram-negative bacterium at higher concentration of 100µg and no zone of inhibition was formed against the gram positive bacterium.

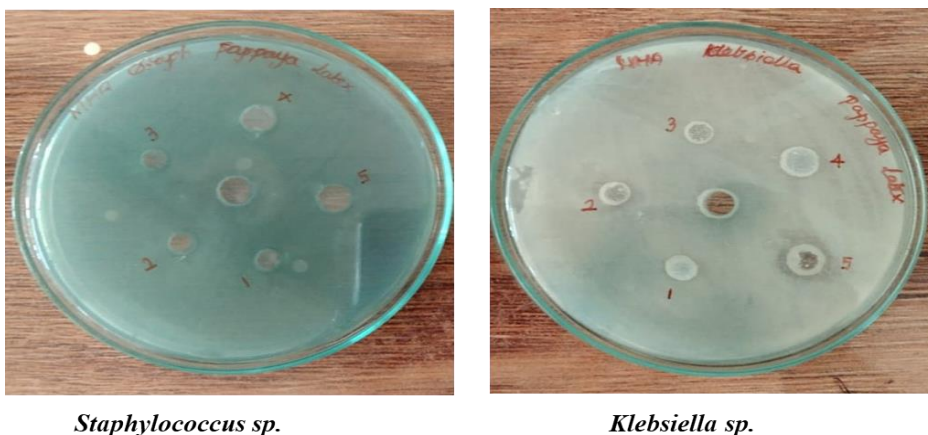


Fig. 5.1.6 –Antibacterial activity

Table.5.1.6. Antibacterial activity

Name of the Organism	Zone of Inhibition(mm)					ZOI (mm) control
	20 µg	40 µg	60 µg	80 µg	100 µg	
<i>Staphylococcus sp.</i>	-	-	-	-	-	
<i>Klebsiella sp.</i>	-	-	-	-	0.2cm	

5.2. SEED GERMINATION STUDIES

Seed germination studies revealed at minimum concentration (10 % latex) the latex found to increase the rate of germination, biomass, shoot and root length and at increased concentration due to accumulation of latex in the seeds, germination did not take place when observed at both 3rd and 7th day.



Fig. 5.2A Day 3 – Observation for seed germination study

A- control; b- 10 % latex; c- 20 % latex; d- 30 % latex; e- 40 % latex; f-50 %- latex; g- 60 %- latex; h- 70 %- latex; i- 80 %- latex; j- 90 %- latex; k- 100 % latex

Table.5.2A. Day 3 – Observation for seed germination study

Concentration	Biomass (g)	Shoot length (cm)
Control	0.04± 0.005	0.5±0.6
10% Latex	0.05± 0.005	0.56 ± 0.15
20% Latex	0.05±0.02	0.4± 0.4
30% Latex	0.1 ± 0.03	0.6± 0.26
40% Latex	0.05±0.02	0.23± 0.32
50% Latex	0.04 ± 0.02	0.13± 0.23
60% Latex	0.03±0.005	-
70% Latex	0.01 ± 0	-
80% Latex	0.07±0.04	0.03± 0.05
90% Latex	0.06 ± 0.01	-
100% Latex	0.04±0.03	-



Fig. 5.2B. Day 7 – Observation for seed germination study

A- control; b- 10 % latex; c- 20 % latex; d- 30 % latex; e- 40 % latex; f-50 %- latex; g- 60 %- latex; h- 70 %- latex; i- 80 %- latex; j- 90 %- latex; k- 100 % latex

Table.5.2B. Day 7 – Observation for seed germination study

Seed	Biomass (g)	Shoot length (cm)	Root length (cm)
Control	0.02±0.02	2.56 ± 2.97	2.16± 3.32
10% Latex	0.12 ± 0.05	1± 1	2± 2.29
20% Latex	0.02±0.005	0.66± 0.57	-
30% Latex	0.01 ± 0.01	0.4± 0.1	-
40% Latex	0.04±0.01	0.06± 0.1	-
50% Latex	0.01 ± 0	0.13± 0.15	-
60% Latex	0.02±0.02	-	-
70% Latex	0.03 ± 0.01	-	-
80% Latex	0.02±0.005	-	-
90% Latex	0.03 ± 0.01	0.03± 0.05	-
100% Latex	0.02±0.02	-	-

5.3. POT STUDIES

Pot studies was performed. Garden soil with natural manure was taken as positive control which showed maximum growth on all the day of observation (10th day; 20th day; 30th day). Next maximum growth was showed by the test soil (10% latex mixed with poor quality soil) followed by the poor-quality soil (negative control). Biomass, Root length, shoot length were observed.

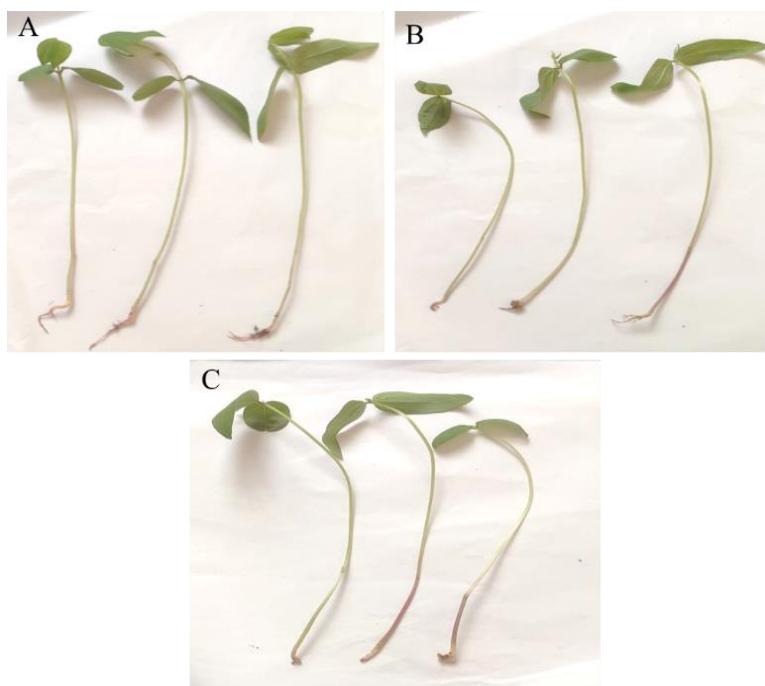


Fig. 5.3.A Day 10th – Observation for Pot study

A- Positive control; B- Negative control; C- Test

Table.5.3A. Day 10th – Observation for Pot study

Seed	Biomass (g)	Shoot length (cm)	Root length (cm)
Positive control	0.51± 0.14	18.1±2.18	2.5± 0.65
Negative control	0.32± 0.09	16.3± 0.7	1.3±0.75
Test	0.24± 0.05	14.9±1.25	0.6±0.25

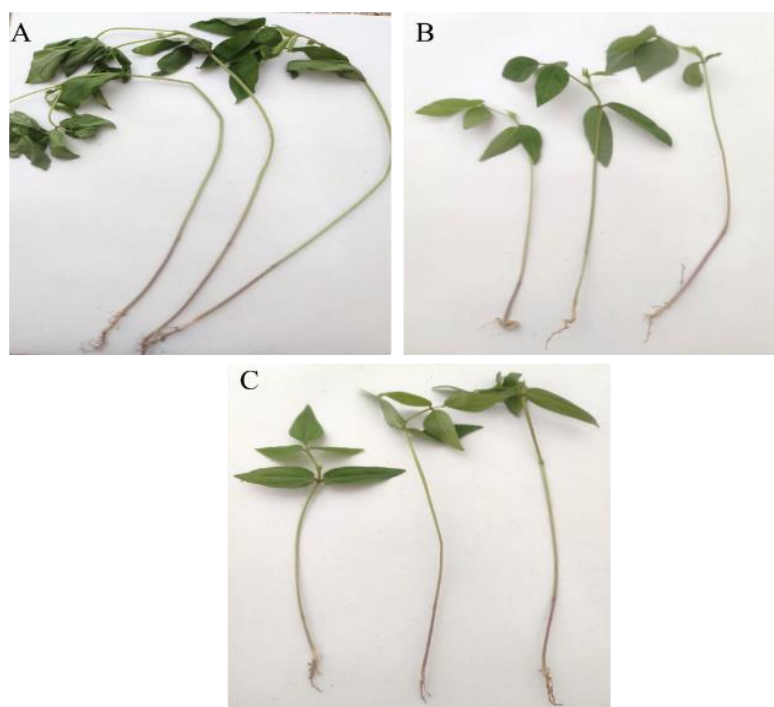


Fig. 5.3 B. 20th day: – Observation for Pot study

A- Positive control; B- Negative control; C- Test

Table.5.3B. Day 20th – Observation for Pot study

Seed	Biomass (g)	Shoot length (cm)	Root length (cm)
Positive control	1.10± 0.05	30.3± 2.54	3.6± 1.16
Negative control	0.38± 0.08	17.0± 1.51	2.26± 0.15
Test	0.54± 0.13	16.4± 1.51	2.06± 1.16

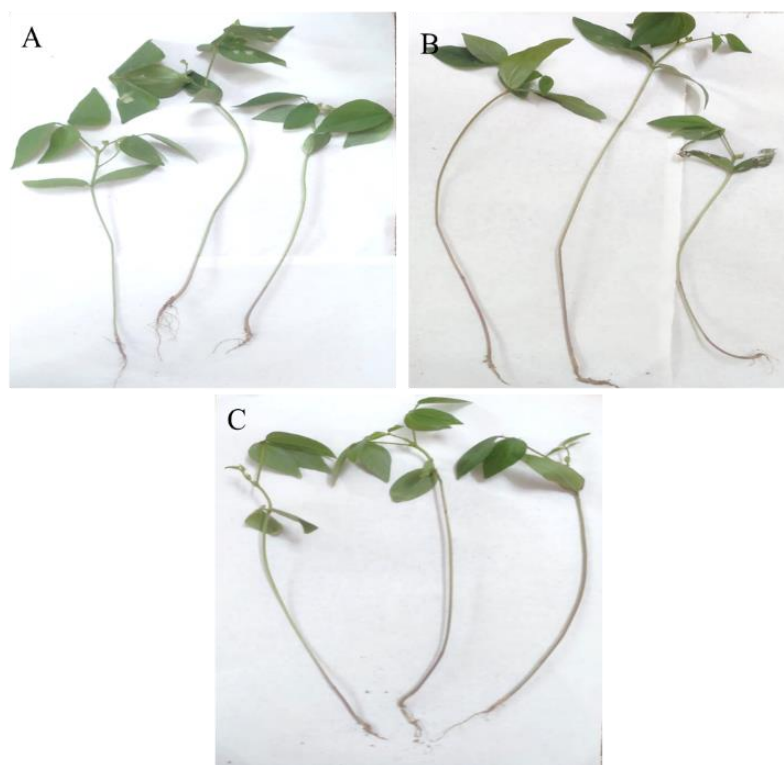


Fig. 5.3C. 30th day: – Observation for Pot study
A- Positive control; B- Negative control; C- Test

Table.5.3C. Day 30th – Observation for Pot study

Seed	Biomass (g)	Shoot length (cm)	Root length (cm)
Positive control	3.2± 1.03	30.6± 3.20	5.36± 1.53
Negative control	0.59± 0.18	21.8± 5.0	2.03± 0.40
Test	0.68± 0.09	20.8± 0.4	2.23± 0,40

5.4. SOIL POROSITY

Soil porosity studies revealed that positive control found to have high porosity of 47.5% which complemented the highest growth rate due to circulation of more oxygen content. Followed by the test soil having 40% of porosity and 32.5% of porosity was exhibited by poor quality soil (negative control).

5.5 SOIL ANALYSIS

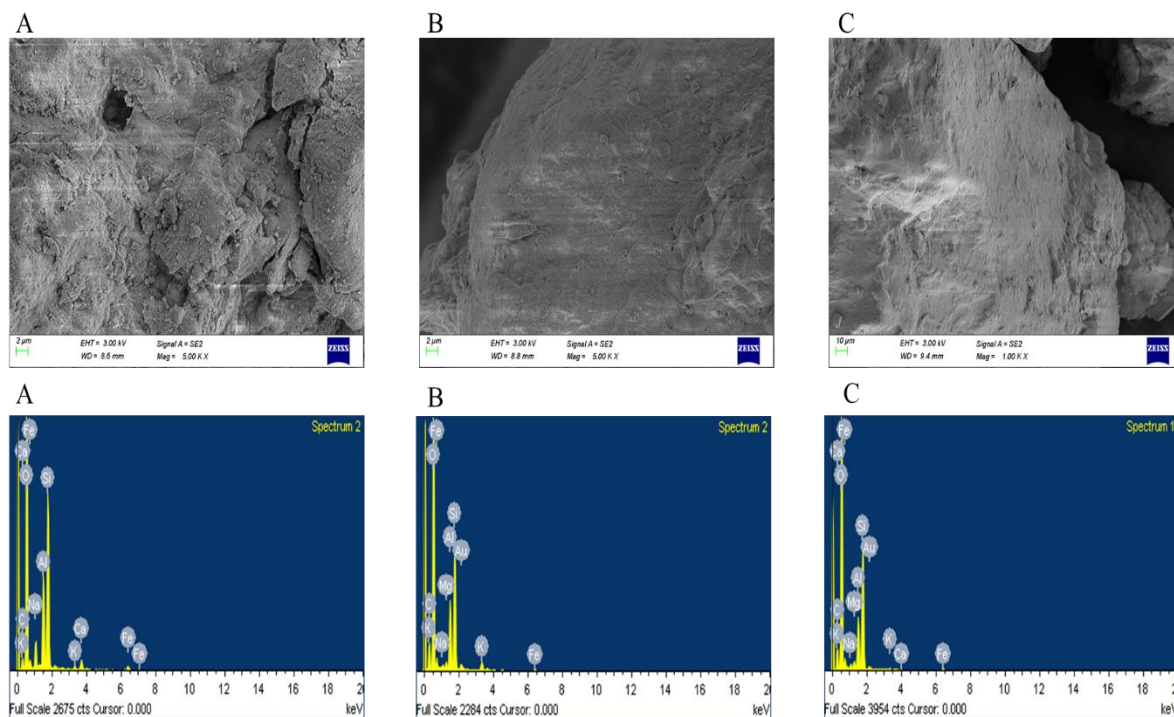
Soil analysis results revealed that the maximum nutrients content is present in positive control, followed by test soil and at last by the poor-quality soil.

Table.5.5. Soil analysis

S. No	Parameters	Units	P. C. Results	N. C. Results	Test Results
1	pH	-	7.00	7.65	7.42
2	Electrical conductivity	mS/cm	0.625	0.530	0.463
3	Organic matter	%	4.24	0.13	0.10
4	Nitrate nitrogen	Mg/kg	32.6	71.1	38.9
5	Available phosphorous	Mg/kg	30.64	10.36	6.43
6	Potassium exchangeable K	Mg/kg	583	104	27
7	Calcium exchangeable Ca	Mg/kg	926	1238	647
8	Magnesium exchangeable Mg	Mg/kg	437	246	274
9	Sulfur available S	Mg/kg	64.4	204.8	242.4
10	Zinc available Zn	Mg/kg	2.34	2.15	2.61
11	Manganese available Mn	Mg/kg	43.49	1.27	3.14
12	Iron available Fe	Mg/kg	4.37	4.23	9.11
13	Copper available Cu	Mg/kg	1.05	1.37	0.68
14	Boron available B	Mg/kg	0.9	0.6	0.6
15	Sodium exchangeable Na	Mg/kg	310	272	346
16	Cation exchange capacity (by addition)	Meq/ 100g	11.1	9.69	7.09
17	K saturation	%	13.45	2.75	0.98
18	Ca saturation	%	41.66	63.89	45.62
19	Mg saturation	%	32.77	21.16	32.20
20	Na saturation	%	12.13	12.21	21.21
21	Soil Porosity	%	47.5%	32.5%	40%
22	Total Bacterial count	CFU/g	12×10^5	48×10^5	35×10^5

5.6. SCANNING ELECTRON MICROSCOPY

Scanning Electron Microscopy revealed surface morphology of the soil and the Energy dispersive X-Ray analysis revealed the elements present in the soil. The energy dispersive X-ray analysis revealed the presence of nutrients like Calcium, potassium, Magnesium, Iron and sodium.



A- Positive control; B- Negative control; C- Test

Fig. 5.6. Scanning Electron Microscopy- Energy dispersive X-Ray analysis

5.7. BIOPESTICIDAL ACTIVITY

Bio pesticidal activity revealed that as the concentration of latex increased from 10 % to 100 % deterioration of cells in gut region of the mealy bugs were observed. The changes were compared with the control. From 10 % to 40 % there were mild changes but as the concentration increased from 50 % complete degradation of muscles (myolysis), oedema, increase in the intestinal spaces was observed and at maximum concentration gut region was completely degraded. This proved that latex has excellent pesticidal activity.

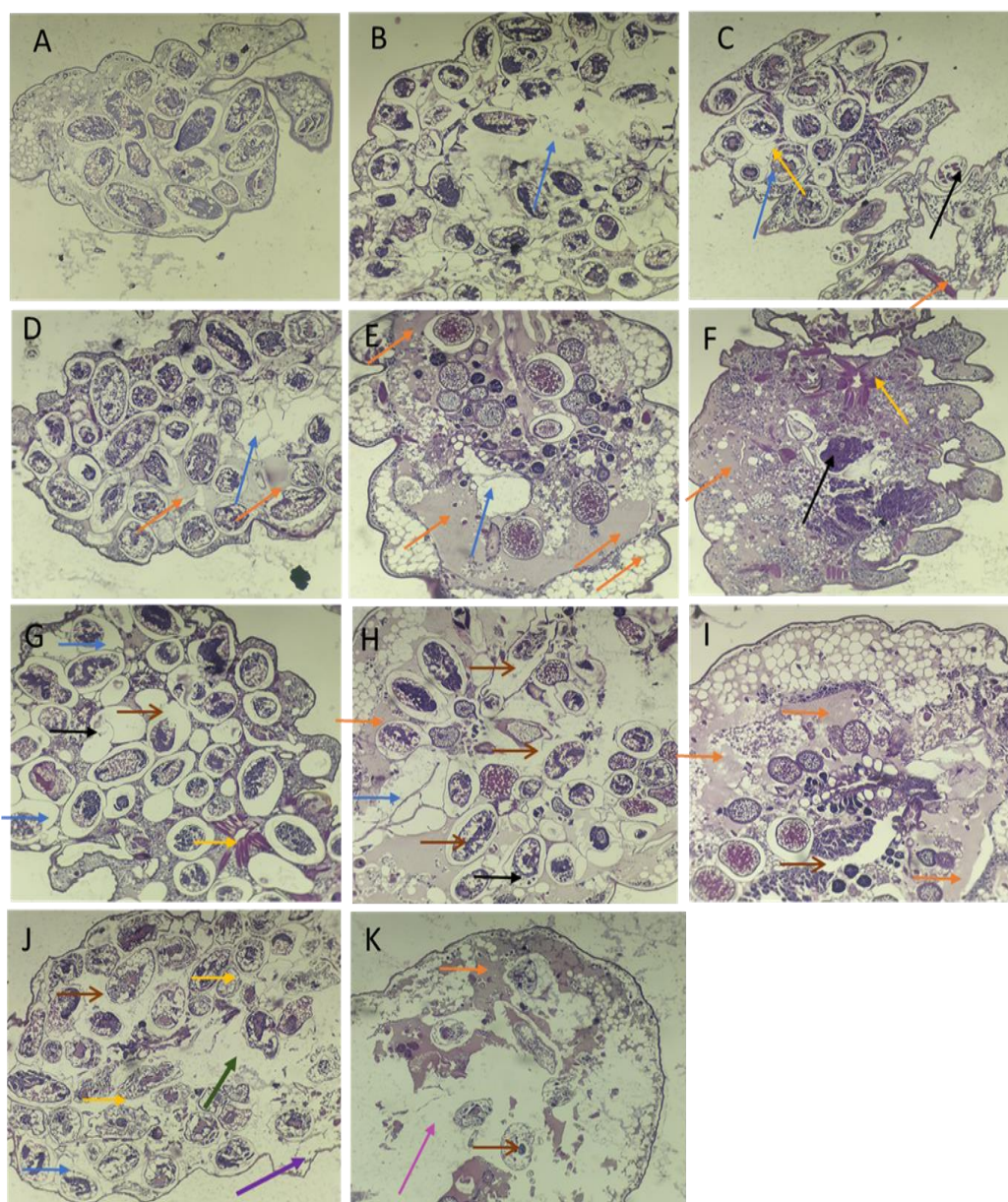


Fig. 5.7. Bio pesticidal activity

A- control; b- 10% latex; c- 20% latex; d- 30% latex; e- 40% latex; f-50%- latex; g- 60%- latex; h- 70%- latex; i- 80%- latex; j- 90%- latex; k- 100% latex

→ Indicates increase in the interstitial spaces; → degradation of intestinal region; → indicates oedema; → indicates degradation of t. princeps cells; → degradation of gamma proteobacterial cell; → complete erosion of gamma proteobacterial cell; → complete degradation of gut region; → myolysis

5.8. PERCENTAGE REPULSION ACTIVITY

Percentage repulsion activity revealed that at for first two hours percentage repulsion of the mealy bugs was increasing gradually as the concentration of latex was increasing. At the end of 5th hour complete repulsion was observed at higher concentration. As the time increase the repulsion activity also increased. 100% repulsion activity was observed when 90% and 100% of latex concentration was used.

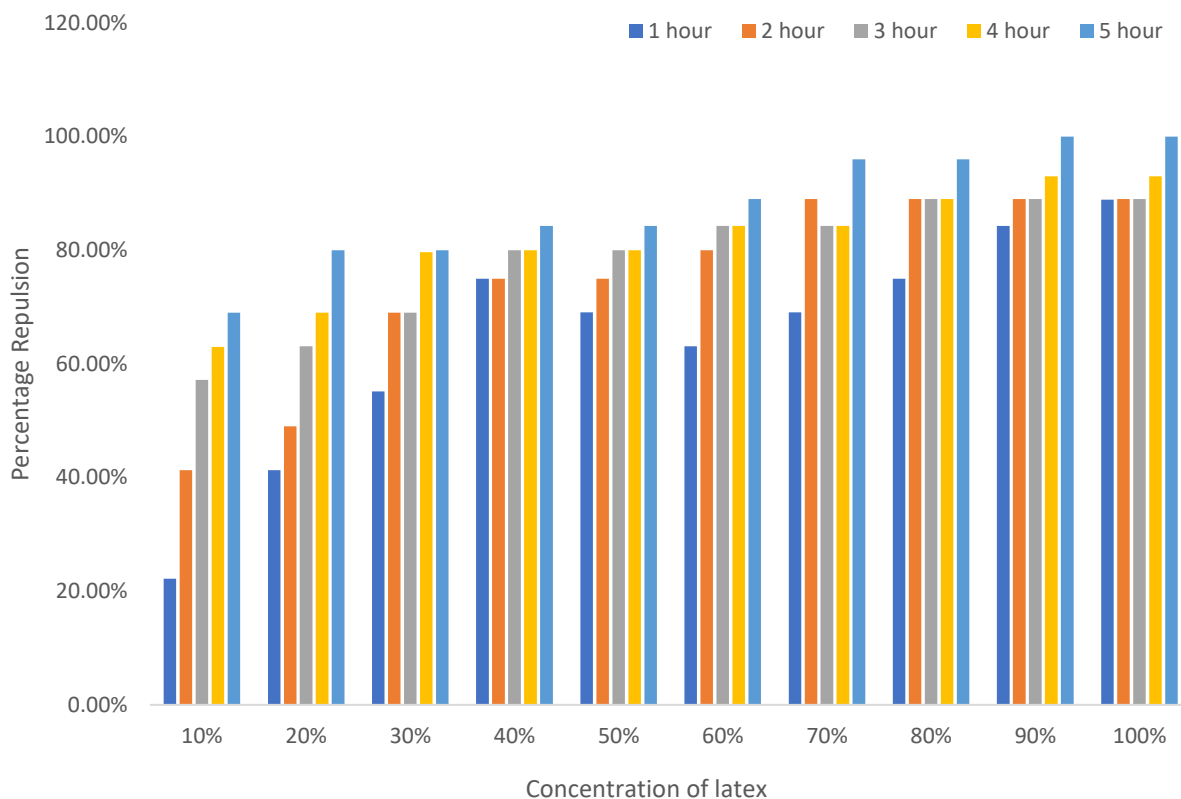


Fig. 5.8. Percentage repulsion activity of mealy bugs against Papaya latex

5.9. CHARACTERIZATION OF SPIONS, PAPAYA COATED SPIONS AND PAPAYA COATED SPIONS- DRUG LOADED

5.9.1. UV- Vis SPECTROSCOPY

The synthesized SPIONS, Papaya latex coated SPIONS, drug loaded nanocarrier were characterized using UV-Vis Spectroscopy. In this analysis the maximum absorbance was seen at 225 nm; 222nm and 200nm respectively. As the wavelength increased there was gradual decrease in the absorbance. The absorbance range at 225 nm confirmed the presence of SPIONS (Samrot et al., 2018).

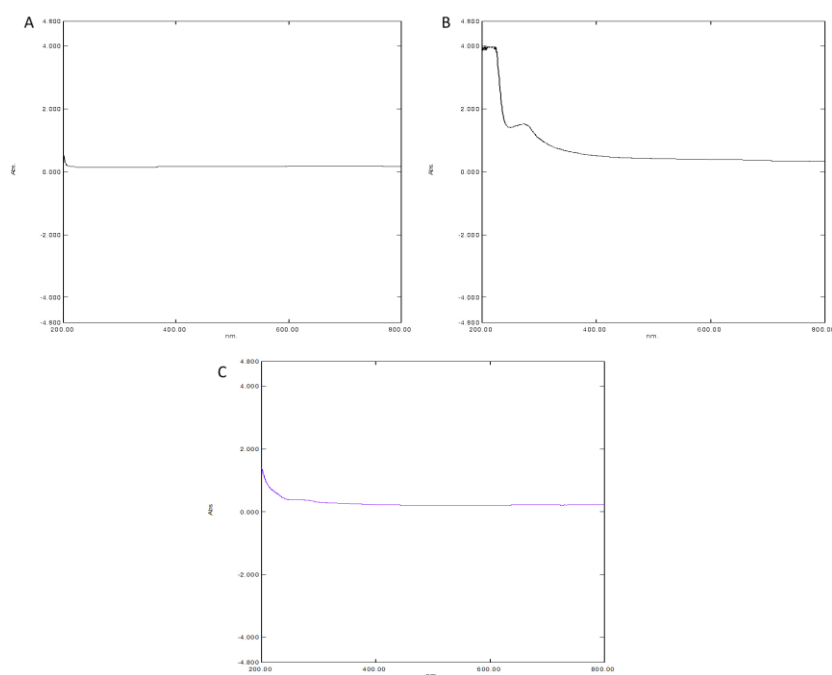


Fig. 5.9.1. UV- Vis of A) SPIONs B) papaya coated SPIONs C) papaya coated SPIONs- drug loaded

5.9.2. FOURIER TRANSFORM INFRARED SPECTROSCOPY

In metal nanoparticles like SPIONs, the core of each particle consists of Fe_3O_4 . FT-IR helps in identifying the organic or inorganic bonds that are associated with the nanoparticles. The spectra of SPION clearly show IR adsorption bands in the low frequency regions in the range of 569 cm^{-1} . This represents the stretching vibrations of Fe-O bond from Fe_3O_4 as reported elsewhere (Samrot et al., 2018). The bands near 2921 cm^{-1} , 1640 cm^{-1} , 1458 cm^{-1} of papaya latex coated SPIONs indicated the presence of -CH, N- H, C- N- vibration of amino group. The band at 2925 cm^{-1} indicated the vibration of C- H stretching. (Samrot et al., 2018; Seoudi t al., 2010). The band at 3450 confirmed the presence of Curcumin in Drug loaded nanocarrier (Samrot et al., 2016). The peak at 1628 indicated the enol-carbonyl stretching vibration of curcumin. Band at 1505 attributes to the benzene ring stretching (Mahmood et al., 2016).

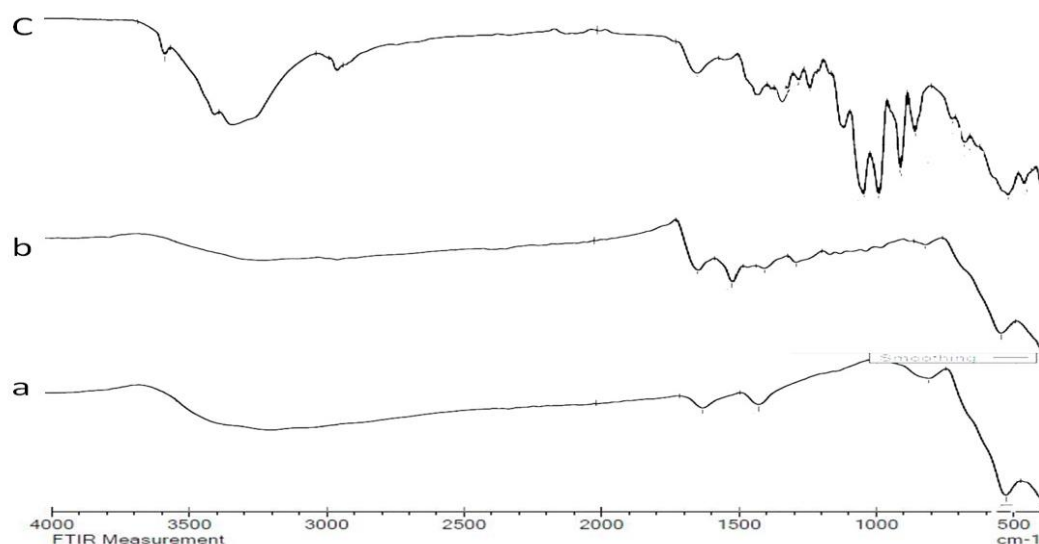


Fig. 5.9.2. FTIR of A) SPIONs B) papaya coated SPIONs C) papaya coated SPIONs- drug loaded

5.9.3. SCANNING ELECTRON MICROSCOPY

The Scanning Electron microscopy images are used to identify the size of the nanoparticles. The SEM image shows that the synthesized SPIONs are in the size ranging between 33nm and 90 nm. The SEM images of papaya latex coated SPIONs are in the size ranging between 44.6nm to 111nm. The SEM images of drug loaded nanocarrier 65nm to 89nm. This increased size confirms the papaya latex coating on the SPIONs. The images of SPIONs, Papaya latex coated SPIONs and Nanocarrier further shows that the particles are spherical and are slightly aggregated. Further, the elemental composition of SPIONs is analyzed using EDX (Justin *et al*; 2017). The EDX image shows the presence of Fe and O which corresponds to the iron oxide, Fe_3O_4 for all the three samples which again confirms the nature of SPIONs.

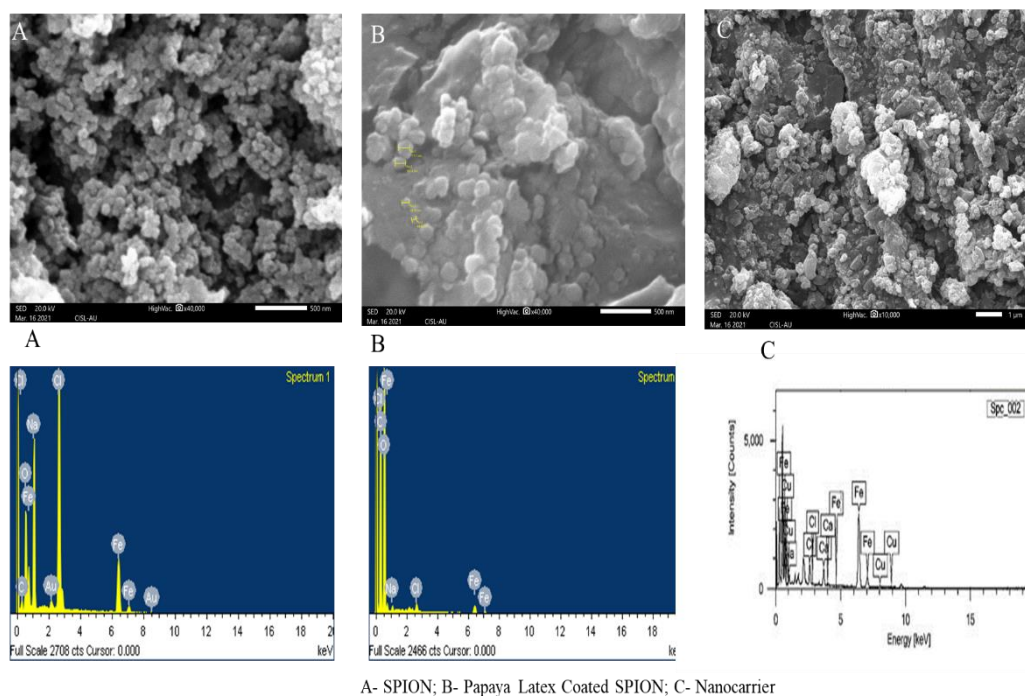


Fig.5.9.3 Scanning Electron Microscopy – Energy Dispersive X-ray Analysis of A) SPIONs B) papaya coated SPIONs C) papaya coated SPIONs- drug loaded

5.9.4. X- Ray Diffraction Spectroscopy

XRD analysis of SPIONs revealed that the peaks formed are close to each other the peaks at $2\theta = 30.1^\circ$, 35.45° , 45.5° , 56.9° and 62.6° were observed. The sharp and intense peaks indicate the crystallite size of the particles (Layek et al., 2010). This XRD spectrum shows a high degree of crystallinity. Papaya latex coated SPIONs also showed high degree of crystallinity and similar 2θ values as SPIONs which confirmed that the papaya latex coated SPIONs was coated smoothly on SPIONs and did not mask the effect of SPIONs. Drug loaded nanocarriers showed sharp peaks at $2\theta = 35.45^\circ$, 56.9° and 62.6° , confirmed the presence of SPIONs.

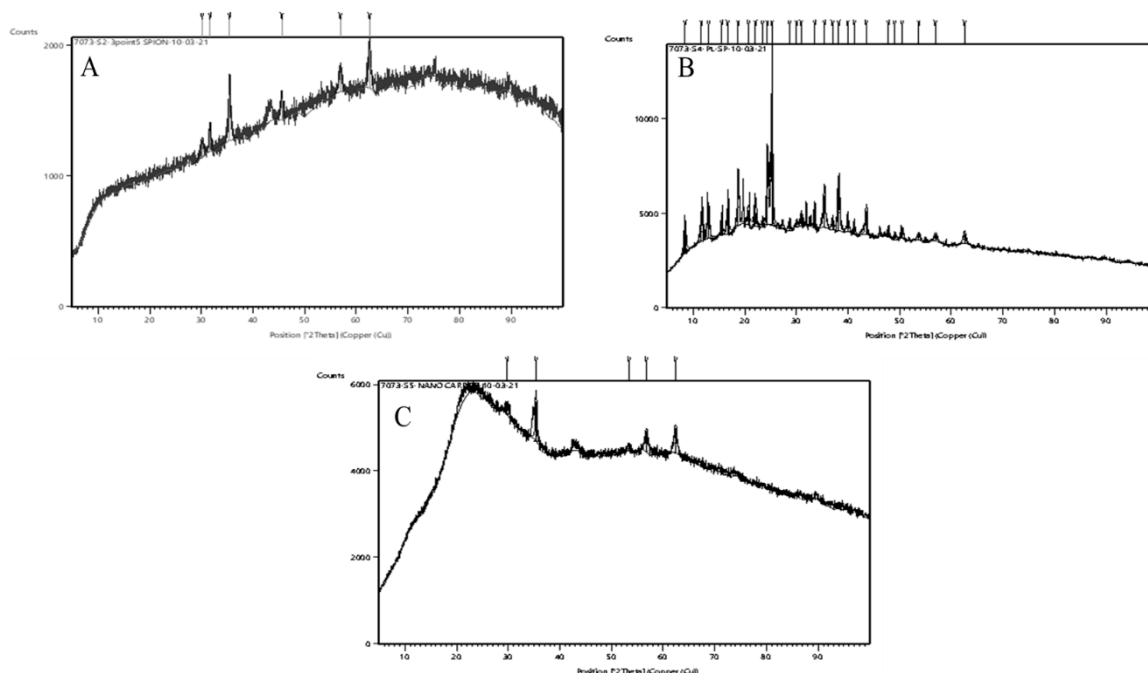


Fig.5.9.4 Scanning Electron Microscopy – Energy Dispersive X-ray Analysis of A) SPIONs B) papaya coated SPIONs C) papaya coated SPIONs- drug loaded

5.9.5. Drug release efficiency

Drug release efficiency was calculated by observing the absorbance at 425 nm. UV readings were taken at every 30th minute starting from 0th minute to 180th minute. It was seen that from 0th minute to 90th min there was gradual increase in drug release and at 90th min maximum drug release was observed. After 90th min it started to decline.

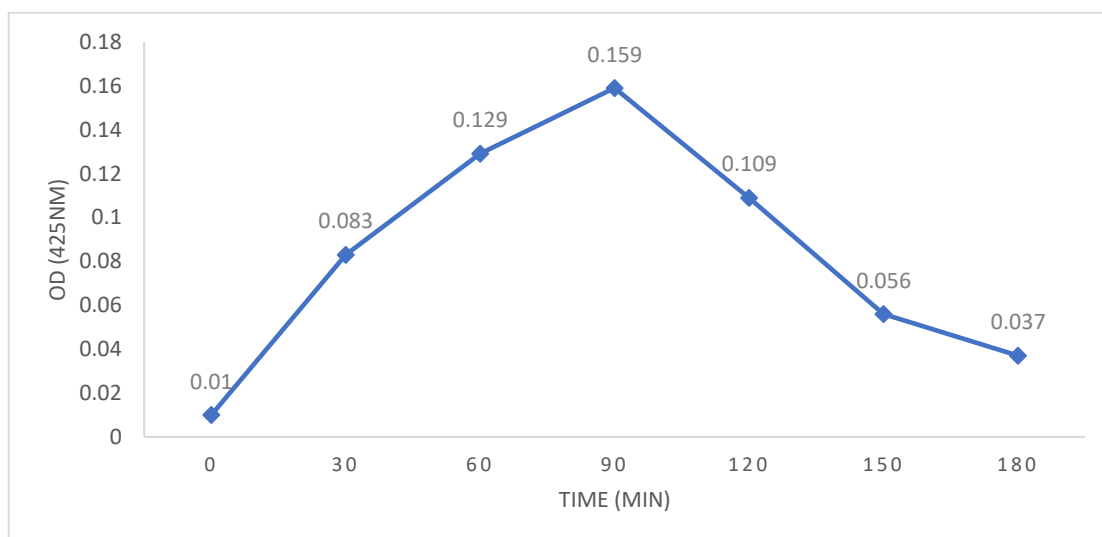


Fig. 5.9.5 DRUG RELEASE EFFICIENCY OF DRUG LOADED NANOCARRIER

5.10. ANTIBACTERIAL ACTIVITY

The *papaya* latex coated SPIONs and Nanocarrier was tested for antibacterial activity against a gram positive bacterium (*Bacillus subtilis*) and a gram negative bacterium (*Pseudomonas aeruginosa*). The antibacterial activity of the was evaluated against a Control Azithromycin. Both the *papaya* latex coated SPIONs and Nanocarrier showed inhibition against the gram positive and gram-negative bacterium at all the concentration. Zone of inhibition of nanocarrier was found to be higher than the *papaya* latex coated SPIONs. This can be due to the addition of drug Curcumin.

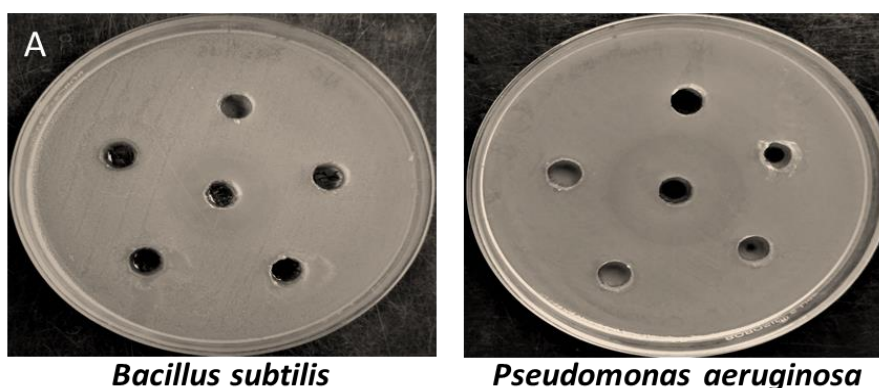


Fig. 5.10A –Antibacterial activity of PAPAYA LATEX COATED SPIONs

Table. 5.10A –Antibacterial activity of PAPAYA LATEX COATED SPIONs

Name of the Organism	Zone of Inhibition(mm)				ZOI (mm) Control
	25 µg	50 µg	75 µg	100 µg	30µg/well
<i>Bacillus subtilis</i>	9	11	14	18	21
<i>Pseudomonas aeruginosa</i>	7	8	9	10	28

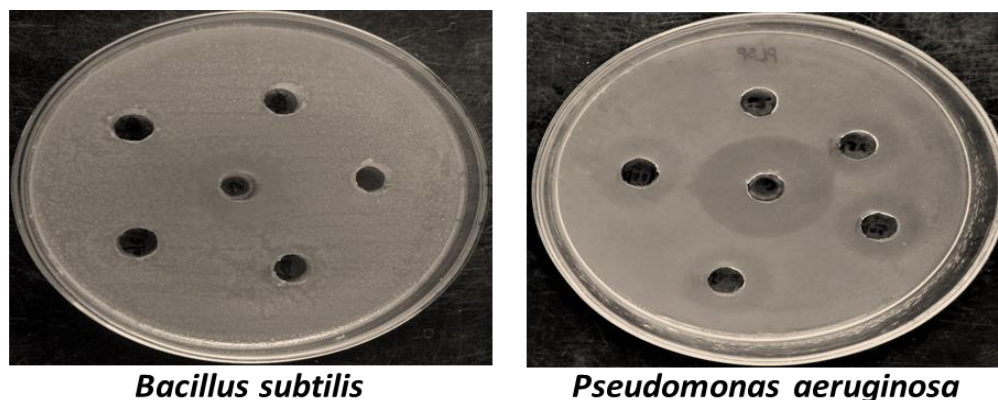


Fig. 5.10B –Antibacterial activity of DRUG LOADED NANOCARRIERS

Table. 5.10B –Antibacterial activity of DRUG LOADED NANOCARRIERS

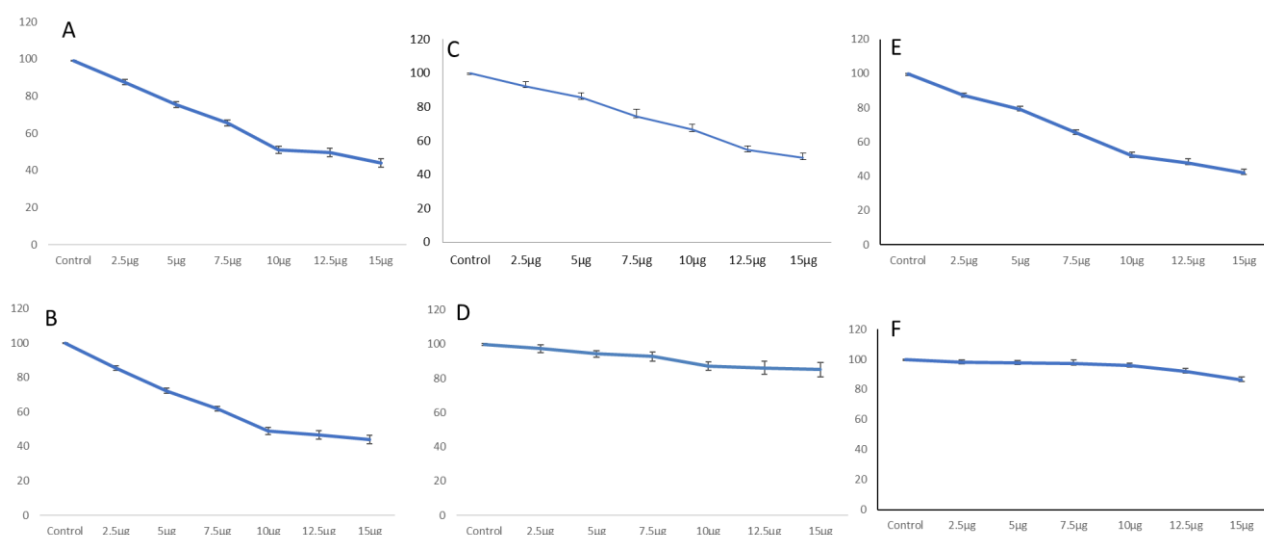
Name of the Organism	Zone of Inhibition(mm)				ZOI (mm) Control
	25 µg	50 µg	75 µg	100 µg	30µg/well
<i>Bacillus subtilis</i>	7	10	13	14	20
<i>Pseudomonas aeruginosa</i>	15	17	19	20	28

A- PAPAYA LATEX COATED SPION; B- NANOCARRIER

5.11. ANTICANCER ACTIVITY

5.11.1. MTT Assay

MTT assay is generally one of the most popular viability assays. The lethal dose concentration of 50% of the SPIONs, Papaya latex coated SPIONs and Nanocarrier (LC_{50}) was observed at the third dilution (i.e., at 7.5µg of the Sample was found as the lethal dose). Among the three samples nanocarrier showed high anticancer activity. This may be due to the addition of curcumin. Followed by papaya latex coated SPIONs and SPIONs showed no anticancer activity.

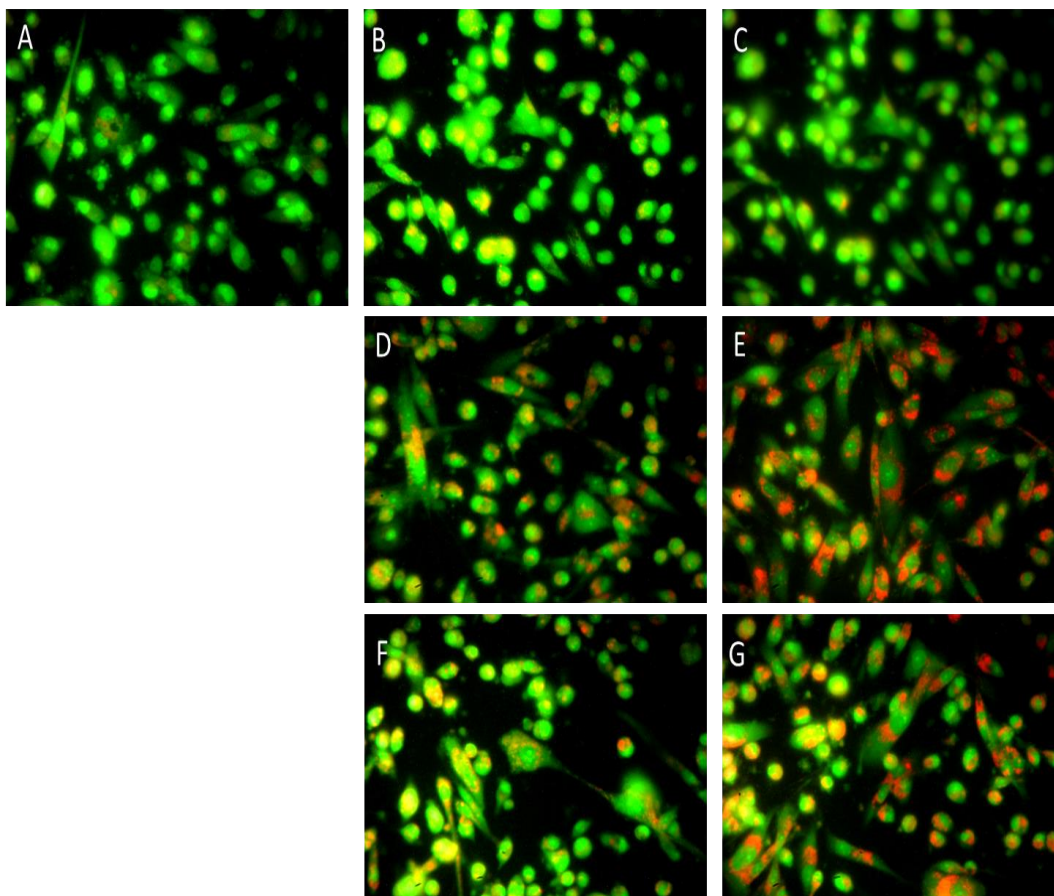


A- Control (Spion); B- Breast Cancer Cell (Spion); C- Control(papaya Latex Coated Spion); D- Breast Cancer Cell (Papaya Latex Coated Spion); E- Control (Nanocarrier); F- Breast Cancer Cell (Nanocarrier).

Fig. 5.11.1 –MTT Assay

5.11.2. Acridine Orange- Ethidium Bromide Dual Staining

MDA-MB-231 cells treated within control and sample SPIONs, Papaya latex coated SPIONs and Nanocarrier (12.5 & 15 µg/ml) at 24 h, stained with dual dye AO/EB and then analysed by fluorescence microscopy. Living cells normal green nucleus appeared, early apoptotic condensed or fragmented form of yellow colour nucleus with chromatin, late apoptotic chromatin condensation or fragmentation orange-stained nuclei and necrotic cells (uniformly red-stained cell nuclei) (Liu et al., 2015). In case of SPIONs no apoptosis was found.



A- Control; B- 12.5 µg/ml SPION; C- 15 µg/ml SPION; D- 12.5 µg/ml PAPAYA LATEX COATED SPION; E- 15 µg/ml PAPAYA LATEX COATED SPION; F- 12.5 µg/ml NANOCARRIER; G- 15 µg/ml NANOCARRIER.

Fig. 5.11.2 – Acridine Orange- Ethidium Bromide Dual Staining

5.12 OPTIMIZATION OF ADSORBATE CONCENTRATION

Here, a fixed concentration of adsorbent (0.01g of SPIONs and Papaya latex coated SPIONs) is allowed to react with varied concentration of adsorbate (0.01ppm- 0.1ppm of Methylene blue and Crystal violet dyes). Doing so, the maximum adsorbate required for complete removal is known. The adsorption result is represented as a graph with X-axis as adsorbate concentration (ppm), Y-axis as % removal. For SPIONs the percentage removal increased as the ppm increased till 0.09ppm and 0.06ppm for methylene blue and crystal violet respectively. Later that %removal decreased gradually. For papaya latex coated SPIONs percentage removal increased as the ppm increased till 0.08ppm and 0.06ppm for methylene blue and crystal violet respectively. Later that %removal decreased gradually.

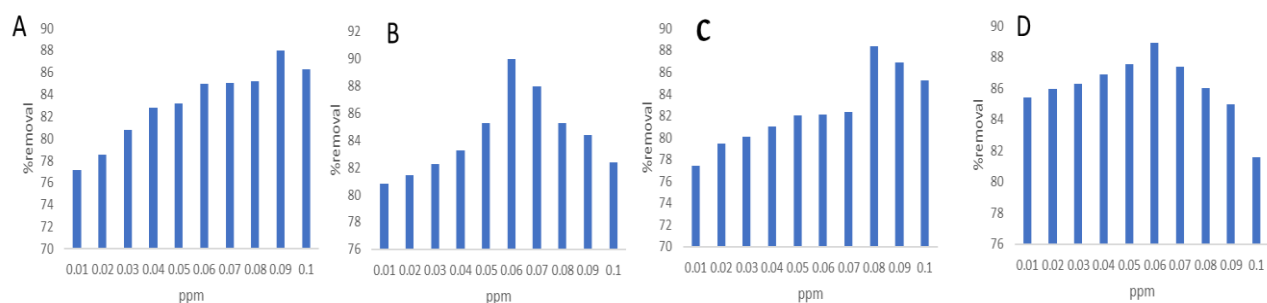


Fig. 5.12.1 Optimization of Adsorbate concentration

5.13. OPTIMIZATION OF ADSORBENT CONCENTRATION

Here, a fixed concentration of adsorbate is allowed to react with varied concentration of adsorbent –0.01g to 0.1g of SPIONs and papaya latex coated SPIONs. through this trial, the maximum efficiency of the adsorbent against the dyes (Methylene blue and Crystal violet) is identified. The adsorption result is represented as a graph with X-axis as adsorbent concentration (g/L), Y-axis as % removal. For SPIONs the percentage removal increased as the adsorbent concentration increased till 0.06g/L and 0.08g/L for methylene blue and crystal violet respectively. Later that %removal decreased gradually. For papaya latex coated SPIONs percentage removal increased as the ppm increased till 0.05ppm and 0.04ppm for methylene blue and crystal violet respectively. Later that %removal decreased gradually.

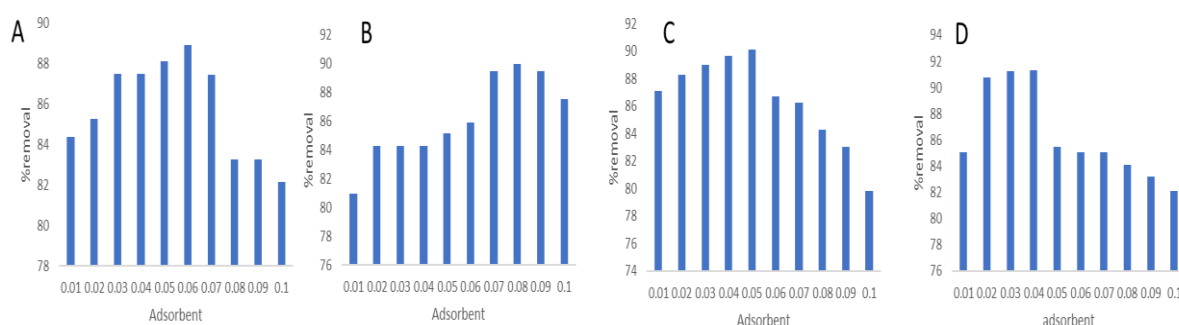


Fig. 5.13.1 Optimization of Adsorbent concentration

5.14 PH OPTIMIZATION

Fixed adsorbent and adsorbate concentration was taken and pH is varied. The adsorption result is represented as a graph with X-axis as pH, Y-axis as % removal. For SPIONs the percentage removal was high at pH 5 for both methylene

blue and crystal violet. Later that %removal decreased gradually. For papaya latex coated SPIONs percentage removal was high at pH8 and pH7 for methylene blue and crystal violet respectively.

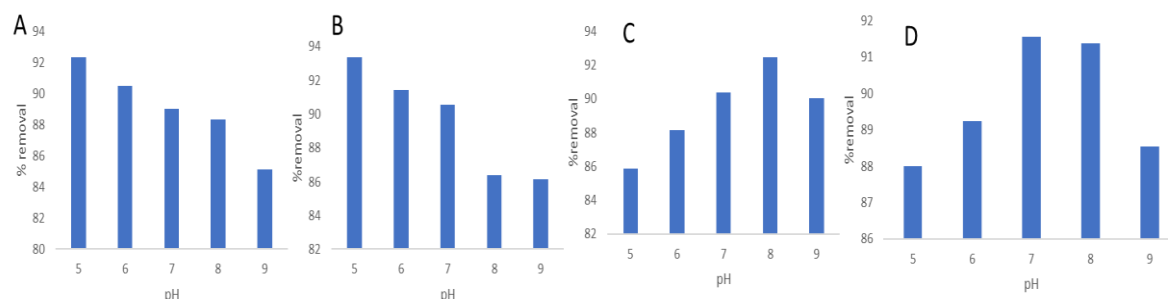


Fig. 5.14.1 Optimization of pH

5.15. CONTACT HOUR OPTIMIZATION

Fixed adsorbent- adsorbate concentration and pH was taken and contact hour is varied. The adsorption result is represented as a graph with X-axis as Contact hour, Y-axis as % removal. For SPIONs the percentage removal was high at 3rd and 2nd hour for methylene blue and crystal violet respectively.. For papaya latex coated SPIONs percentage removal was high at 3rd and 4th hour for methylene blue and crystal violet respectively.

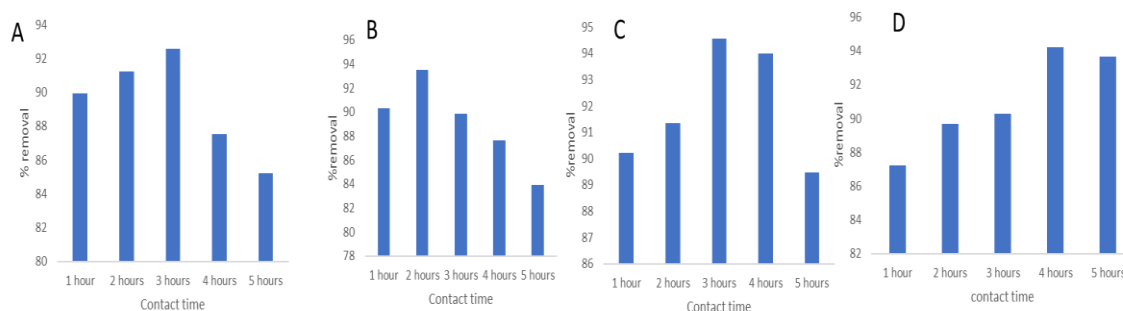


Fig. 5.15.1 Optimization of Contact hour

5.16. ADSORPTION ISOTHERM

The adsorption process was evaluated using the following isotherms namely Langmuir, Freundlich and Temkin isotherms. The correlation coefficient (R^2) from each case were compared and the R^2 with highest value was considered as the best fit model. The SPIONs followed Freundlich model with highest R^2 value of 0.83 and

0.77 for both the dyes respectively. Whereas, papaya latex coated SPIONs followed Langmuir model for methylene blue dye with highest R^2 value of 0.92 and Freundlich model for crystal violet with highest R^2 value of 0.35.

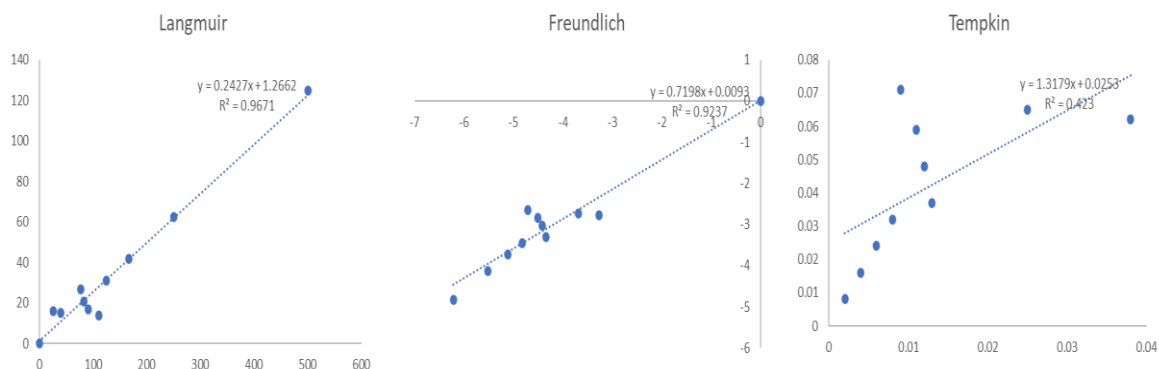


Fig. 5.16.1. Adsorption Isotherms of Papaya latex coated SPIONs- Methylene Blue

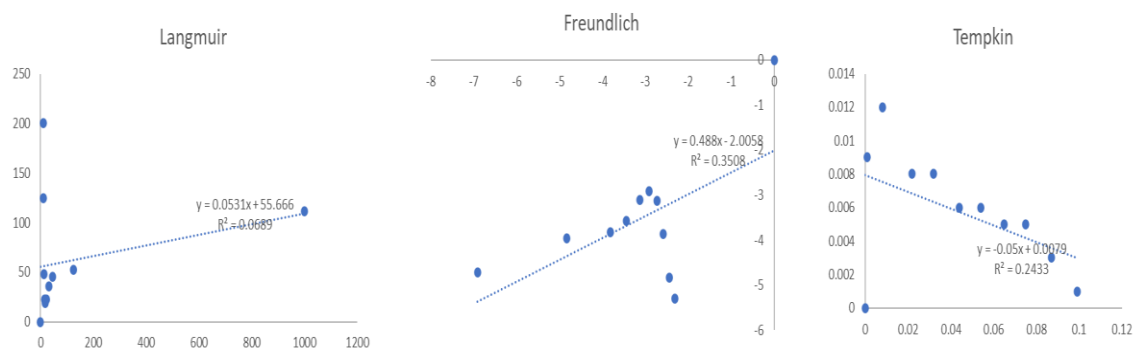


Fig. 5.16.2. Adsorption Isotherms of Papaya latex coated SPIONs- Crystal Violet

CHAPTER 6

SUMMARY AND CONCLUSION

The latex was collected and it is further used for plant growth, drug delivery and other biological applications. The latex was characterized using UV- Vis, FTIR and GC- MS. TLC was performed for the latex and TLC Bio autography showed the presence of antioxidant activity for the latex. Antibacterial activity was performed for the latex and it was found to inhibit the growth of *Klebsiella sp.* at highest concentration.

The latex was further subjected for seed germination and pot studies to analyze its efficiency in inducing the plant growth. It was observed that at lowest concentration it increased the growth whereas, at highest concentration it caused programmed cell death or apoptosis. 10% of latex was promoting the growth so it was taken as the best concentration and further subjected to pot studies. The root length, shoot length and biomass was calculated. It was observed that garden soil contributed to the maximum growth rate followed by the latex mixed soil and followed by the poor-quality soil.

Super paramagnetic iron oxide nanoparticles (SPIONs) were synthesized and characterized using UV- Vis, FTIR, XRD and SEM. Anticancer activity was evaluated for the SPIONs and it was observed that SPIONs did not possess anticancer activity. The synthesized SPIONs were coated with the papaya latex and further characterized using UV- VIS, FTIR, XRD and SEM which revealed the presence of coating of SPIONs with latex. Antibacterial and anticancer activity was performed which showed that papaya latex coated SPIONs inhibits the growth of both *Bacillus subtilis* and *Pseudomonas aeruginosa*. Anticancer activity was also observed.

This SPIONs coated with latex was subjected to the synthesis of drug loaded nanocarrier. Drug curcumin was added and it was seen that the nanocarrier was in small size and were characterized using UV- Vis, FTIR, XRD and SEM. And it was seen that the maximum drug release efficiency was found at 90th minute. Antibacterial and anticancer activity was performed which showed that papaya latex coated SPIONs inhibits the growth of both *Bacillus subtilis* and *Pseudomonas aeruginosa*. Anticancer activity was also observed.

Adsorption studies were performed for dye removal (Methylene blue, Crystal violet). Both SPIONs and papaya latex coated SPIONs was used for the adsorption studies and it was seen that freunlich model fits for dye removal of SPIONS and Langmuir model fits for methylene blue dye removal of papaya latex coated SPIONS and Freundlich model fits for crystal violet dye removal of papaya latex coated SPIONS.

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