

SCREENING OF BIOACTIVE COMPOUND PRODUCING BACTERIA AGAINST PLANT PATHOGENS

Submitted in partial fulfillment of the requirements for the
award of Bachelor of Science degree in Biotechnology

by

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SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited with Grade “A” by NAAC
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **T.MUGILARASI** (Reg No.40770032) who carried out the project entitled "**SCREENING OF BIOACTIVE COMPOUND PRODUCING BACTERIA AGAINST PLANT PATHOGENS**" under our supervision from SEPTEMBER 2022 to MARCH 2023


Internal guide

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EXTERNAL GUIDE 2/05/23
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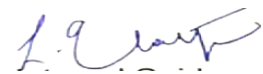

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Submitted for Viva voce Examination held on 12/5/23_____



INTERNAL EXAMINER

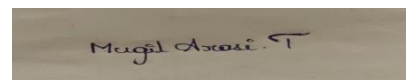

EXTERNAL EXAMINER

DECLARATION

I **T.MUGILARASI (Reg No.40770032)** here by declare that the project report entitled “**SCREENING OF BIOACTIVE COMPOUND PRODUCING BACTERIA AGAINST PLANT PATHOGENS**” done by me under the guidance of Dr. JAYSHREE NELLORE (Internal) and Dr. D. SARA VANAN (External) at Centre for Laboratory Animal Technology and Research, Sathyabama University is submitted in partial fulfillment of other requirements for the award of Bachelor of Science degree in **BIOTECHNOLOGY**.

DATE:12/5/23

PLACE:Chennai

A rectangular box containing a handwritten signature in black ink. The signature appears to be 'Mugil Arasi. T'.

SIGNATURE OF THE CANDIDATE

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ABSTRACT

Many plants are immune resistance to most of the pathogens, particularly fungus. Agricultural plants [Okara, Eggplant, and Tomato] are vulnerable to get attack by the plant pathogens so that to restrict the plant pathogens Bio pesticides are used as biological control agent against plant pathogens. Bioactive compounds were produced to cure the plant diseases by killing the harmful pathogens that are isolated from the diseased part of the plant. Such as *Fusarium oxysporum*, *Pythium sp.*, *Aspergillus sp.*, *Alternaria sp.*, which causes the diseases and affects the quality, quantity of agricultural plant production this includes lack of food availability. The aim of the study is screening of bioactive compound from isolated soil bacteria against the pathogens. The unknown fungal species were identified by guide lines like lacto phenol cotton blue by slide culture technique and bacterial species by the methods like staining, motility and biochemical tests. Antimicrobial activity test was carried out between the plant pathogens and soil bacteria by agar well diffusion method. Fungal isolates (TO1, TO2, TO3, BL4, LF1, and LF4) cultivated on PDA exhibited great antifungal activities. . There results were determined by the formation of zone of inhibitory concentration were identified. The extract from TP8 also exhibit antifungal activity was tested against test.

Keywords: Soil microbes, fungal pathogens, bioactive compound and antifungal activity,

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LIST OF ABBREVIATIONS

ABBREVIATIONS	EXPANSION
NA	Nutrient agar
PDA	Potato dextrose agar
CDA	Czapek dox agar
SDA	Sabouraud dextrose agar
MHA	Muller Hinton agar
μl	Microlitre
H ₂ SO ₄	Sulphuric acid
NaOH	Sodium hydroxide
HCL	Hydrogen chloride
ml	Millilitre

CHAPTER-1

INTRODUCTION

1. AGRICULTURAL PLANTS

Plants play a major role for human needs, plants provide us food, fibre, shelter, medicine, fuel etc...The basic food for all organisms is produced by green plants, especially agricultural plants which provide a major role in producing vegetables which is rich in nutrition [R.Thilagam *et al.*, 2019]. Some of the important agricultural plants that are highly cultivated in the regions of Tamil Nadu. Tomato, Lady's finger, Brinjal are the most essential and useful vegetable for humans

1. *Tomato [Solanum lycopersium]*



Fig 1.1 Infected Tomato Plant

In Tamil Nadu, Krishnagiri, Dharmapuri, Salem, Tirupur, Theni and Dindigul districts are major cultivation pockets for Tomatoes. Tomato is one of the popular vegetables consumed by a most people and enriched the nutrients and taste. An amount and the type of nutrients supplied for tomato will influence not only its yield but also a nutrient content, taste, and the post-harvest storage quality and some nutrients, such as N, P, K, Ca, Mg, and S, they needed in large amounts by tomato for a normal growth and the reproduction, others, such as Fe, Cu, Zn, Mn, B, Mo, and Cl, are needed in small amounts. The result of tomatoes is regularly fertilized with N, P, and K and they occasionally with Ca and Mg from the liming to adjust soil pH. Nutrients are not normally applied to the unless deficiency in plants occurs. Tomatoes grown in the greenhouse; the growth media other than soil is fortified with all the nutrients.

1.1.2. Brinjal [*Solanum melongena*]



Fig1.2 Infected Brinjal Plant

The situation for Brinjal is similar, which in Tamil Nadu majorly cultivated in Dharmapuri, Krishnagiri, Dindigul, Tiruvannamalai and Vellore. Vegetables are a rich source of vitamins that are essential for human health. Among the vegetables, the solanaceous crops including pepper (*Capsicum annuum*) and Brinjal (*Solanum melongena*) are of considerable economic importance. Brinjal, The King of Vegetables was the first cultivated in China in 5th century BC, and was introduced to Africa before a Middle Ages and then into the Italy. It subsequently spread throughout a Europe and the Middle East. Today, Italy, Greece, Turkey, France, Egypt, China and Japan are the leading growers of Brinjal and it is a very good source of a vitamin K, B1, B6, copper, dietary fibre, manganese, niacin, potassium, and folate. The nutritional value of Brinjal in 1 cup (or 82 Grams) consists of an 11% fibre, 10% manganese, 5.4% molybdenum, 5.3% potassium, 4.5% folate, 3.5% vitamin K, 3.5% copper, 63.5% vitamin B, 3.1% tryptophan, 3% vitamin C, and 2.8% magnesium

1.1.3. Lady's finger [*Ablmoschus esculentus*]



Fig 1.3 Infected lady's finger plant

In Tamil Nadu, major cultivation season for lady's finger is from February to April and June to August. It is largely grown in Salem, Karamadai, Periyannayakkanpalayam and Coimbatore. Ladyfinger is popularly known as bhindi in India which is rich in nutrients. It is considered as a good source of carbohydrates, proteins, vitamins, enzymes, calcium, potassium and many of other nutrients.

1.2 Major vegetable plants in Tamil Nadu:

Vegetables are an excellent source of vitamins, minerals, and proteins, making them an essential part of a healthy diet. With its diverse climate, Tamil Nadu is an ideal place for growing a wide range of vegetables, many of which have a high nutritional value and a low Glycaemic index. The most commonly grown vegetables in Tamil Nadu include Brinjal, Tapioca, Tomato, Onion, and Ladyfinger, which make up more than 70% of the state's total vegetable production. In an effort to increase vegetable cultivation, the government provides support for hybrid vegetable farming, including assistance with cultivation and subsidized seedlings from State Horticulture Farms. This initiative aims to expand the area and output of vegetable farming in the state. Vegetable crops grown are Onion, Tapioca, Tomato, Potato, Brinjal, Beans, Drumstick and Carrot in an area of 2,26,502 hector mainly in districts like Trichy, Namakkal, Dharmapuri, Salem, Erode, Tirupur, Dindigul, Villupuram , Krishnagiri, Nilgiris, Perambalur and Theni districts

Table 1.1 Productivity of vegetables in Tamil Nadu:

S. no.	Name of the crop	Area(hector)
1	Tapioca	89,609
2	Onion	28,357
3	Tomato	29,078
4	Brinjal	15,084
5	Ladies Finger	11,824
6	Beans	8,672
7	Carrot	3,742
8	Potato	3,506
9	Bitter gourd	2,922
10	Leafy Vegetable Plants	2,732

1.3 AREAS AND CROPS THAT COMMONLY AFFECTED BY PESTS IN INDIA:

Lately, there have been modifications in the cropping patterns of ecosystems, as well as changes in habitat, climate, and the introduction of high yielding varieties and hybrids that require intensive inputs. This has resulted in a shift in the pest status of various regions. Pests have widened their host range and developed resistance to pesticides, leading to secondary outbreaks. For instance, some areas in Tamil Nadu and Andhra Pradesh have experienced an increase in the incidence of the chilli gall mid. *Solenopsis* mealy bugs (*Phenacoccus solenopsis*) have been observed on crops such as Brinjal, tomato, okra, and cucurbits, while the Hadda beetle has been found on cowpea and bitter gourd. Additionally, plume moth has been detected in bottle gourd, among other examples.

1.4. PLANT DISEASES:

As humans get affected by disease, plants also get affected through fungal, virus, and bacteria. Among those fungi are the most dominant causal agents of plant diseases occurring in agriculture and horticulture. *Alternaria Spp.*, *Fusarium Spp.*, *Aspergillus Spp.*, and *Colletotrichum Spp.* These fungal pathogens commonly cause diseases to all parts of the plants such as fruit, root, leaf [R, Thilagam., 2019]. Phyto fungal pathogens pose serious problems worldwide in agriculture and food industry, especially in the subtropical and tropical regions. In addition, many also produce mycotoxins, which are harmful to humans and livestock and cause a number of diseases such as rusts, smuts, rots, downy mildew and may cause severe damage to crops. Detection of microbial pathogens refers to the process of establishing the consistent presence of a particular target organism(s) within the plant or in its environments, irrespective of the development of visible symptoms in the plant suspected to be infected by the pathogen. Pesticides are an essential input for preventing pre- and post-harvest crop loss. Fungal plant diseases are a major concern to worldwide agricultural production. It has been estimated that total losses from plant diseases range from 25 % of the yield in western countries to almost 50 % in developing countries (Gohel *et al.* 2006). Control of fungal diseases is imperative to ensure a constant food supply to an ever-increasing world population (Oskay 2009). However, extensive use of chemical fungicides in agriculture can produce resistant fungal strains and the accumulation of compounds potentially hazardous to humans and the environment. Therefore, control of fungal plant diseases using naturally occurring non-pathogenic microorganisms represents a promising approach for the control of plant disease (Heydary and Pessarakli 2010) and an attractive alternative to chemical fungicides (Talubnak and Soyong 2010). *Alternaria spp.* are particularly important wilt-producing fungi and attack important crops such as tomatoes, bananas, sweet potatoes, pears, and cereals can be responsible for considerable economic losses all over the world. In addition, some species of fungi may cause food and feed contamination, especially those fungi that are able to produce mycotoxins, which are known potent hepatocarcinogens in animals and humans.

5. BACTERIA:

Soil-borne bacteria that are antagonistic to plant pathogens could make a substantial contribution to prevention of plant diseases, and therefore represent an alternative to the use of chemical pesticides in agriculture [R.Thilagam *et al.*, 2019]. Due to their role in plant health and soil fertility, soil and the rhizosphere have frequently been used as a model environment for screening of putative agents for use in biological control of soil-borne plant pathogens. Among the 20 genera of bacteria *Bacillus spp.*, *Pseudomonas spp.*, and *Streptomyces spp.* are widely used as biocontrol agents and *Bacillus spp.* has been reported to produce several antibiotics. As they are bacterial antagonists, the assumption is that their antagonistic effects are due mainly to production of antifungal antibiotics, which appear to play a major role in biological control of plant pathogens

6. PESTICIDES:

Pesticides are mean to kill pests by chemical or biological agents such as disinfectant, antimicrobial, virus, bacteria fungi that kills the plant pathogens by producing secondary metabolites. Pesticide is also known as plant protection product. It is commonly used to control agricultural pests that can damage crops and livestock and reduce farm's yield.

1. *Types of Pesticides:*

Based upon their killing, the pesticides are insecticides, herbicides, rodenticides, fungicides, bactericides, larvicides. some pesticides are degradable more are persistent

7. CHEMICAL PESTICIDES:

Chemical pesticides are substances that are used to eradicate pests such as weeds, rodents, fungi, and insects. Throughout the world, more than a thousand different pesticides are utilised. Pesticides are used in agricultural and public health to eradicate pests that harm crops and serve as disease vectors, such as mosquitoes.

1. *Dis advantages of chemical pesticides:*

Chemical pesticides do not always stay in the area where they are applied. They are highly mobile in their surroundings, moving frequently through water, air,

and soil. The issue with pesticide mobility is that when pesticides migrate, they come into contact with other organisms and potentially harm them. Chemical pesticides have also been demonstrated to disturb an ecosystem's balance. When a pesticide is used, it often kills non-pest creatures as well.

1.8 NEED OF PESTICIDES:

In 1845, Ireland's vast potato fields were struck by an invasive fungal disease that rapidly infested this staple crop. The effect was devastating. One million people died of famine, and over a million more were forced to leave Ireland. Nowadays, we avoid such agricultural catastrophes with the help of pesticides. Those are a range of manmade chemicals that control insects, unwanted weeds, funguses, rodents, and bacteria that may threaten our food supply. They've become an essential part of our food system. As populations have grown, monoculture, single crop farming, has helped us feed people efficiently but it's also left our food vulnerable to extensive attack by pests. In turn, we've become more dependent on pesticides. Today, we annually shower over 5 billion pounds of pesticides across the Earth to control these unwanted visitors.

Later, as large-scale farming spread, we began sprinkling arsenic, lead, and copper treatments on crops. But these were incredibly toxic to humans as well. As our demand for more, safer produce increased, so did the need for effective chemicals that could control pests on a grander scale. As pesticides destroy pest populations, they leave behind only the most resistant individuals. They then pass on their pesticide-resisting genes to the next generation.

Pesticides have improved over time and are currently regulated by strict safety standards, but they still have the potential to pollute soil and water, impact wildlife and even harm us.

Although they're imperfect because they currently may be our best outcome against major agricultural disasters, not to mention mosquito-borne diseases. Today, scientists are on a quest for alternative pest control strategies that balance the demands of food production with environmental concerns. Nature has become a major source of inspiration, from natural plant and fungal chemicals that can repel or attract insects, to recruiting other insects as crop bodyguards.

We're also turning to high-tech solutions, like drones Programmed to fly over crops, these machines can use their sensors and GPS to carry out more targeted sprays that limit a pesticide's wider environmental impact with a combination of biological understanding, environmental awareness, and improved technologies, we have a better chance of finding a holistic solution to pests. Chemical pesticides may never shake their controversial reputation, but with their help, we can ensure that agricultural catastrophes stay firmly in our past.

1.9 EFFECTS OF PESTICIDES:

Pesticides are used to protect crops from disease and infestation. They are considered important for protecting harvests and ensuring our food supply. All pesticides contain active substances. These are the essential ingredients that enable them to function. This can be a chemical or it can be a microorganism, such as a bacterium or a virus. In some cases, the chemical works by confusing insects, in other cases they make the crop less palatable for pests but commonly they work by killing the damaging insects, weeds or fungi. Pesticides can be widely distributed in the environment, where they could be harmful to plants and animals. In some cases, small amounts called residues can find their way into the food we eat. These residues could be harmful if they exceed certain levels. There are many ways in which pesticide residues can get into our food.

With this view the present study searched by bioactive compound producing bacteria against fungal pathogens from Tomato, Brinjal, and Lady's finger.

CHAPTER-2

REVIEW OF LITERATURE

- The goal of this study was to find potential plant growth-promoting rhizobacterial (PGPR) actinobacterial isolates that were effective in inhibiting the fungus pathogen *Colletotrichum capsici*, which causes anthracnose. In order to conduct this study, actinobacterial were isolated from soil in the rhizosphere using the dilution plate method, and their antagonistic potential towards the pathogenic fungus *C. capsicum* was evaluated. Both primary and secondary screening tests conducted on the actinobacterial isolate BS26 revealed significant antagonistic activity against the fungus pathogen. *Streptomyces violaceoruber* was identified in isolate BS26 by 16S rDNA sequencing. Furthermore, it has been demonstrated that *S. violaceoruber* produces indole acetic acid, phosphate solubilization, and ammonia, all of which point to their potential for usage as PGPR bacteria. It was revealed in a greenhouse experiment that utilising *S. violaceoruber* fermentation broth boosted germination rates, total plant height, fresh weight, and chlorophyll content relative to controls, and lowered the incidence of chilli anthracnose. Considering this research, *Streptomyces Violaceoruber* can be utilised for growing chillies as a bio fertilizer and a biocontrol agent to prevent the growth of fungi (R Thilagam et al., 2019)
- The *Chaetomium globosum* strain F0142, which was isolated from barnyard grass, showed significant disease control effectiveness against both rice blast and wheat leaf rust (*Puccinia recondita*). From the broth of this bacteria, chaetoviridins A and B were identified as two antifungal substances. Chaetoviridins A demonstrated higher antifungal activity than chaetoviridins B in both in vitro and in vivo studies against plant pathogenic fungi. Strong antifungal activity against *M. griseas* and *P. recondita* on rice and wheat plants was shown by a liquid culture of *C. globosum* F0142. The culture continued to prevent the emergence of these diseases by more than 80% even after being diluted nine times. Additionally, it demonstrated some in vivo antifungal effectiveness against tomato plant *P. infestans*. However, the

culture showed little to no activity against the other plant pathogenic fungi that were studied. Based on the results of this study, the effectiveness of Chaetoviridins A and B in treating wheat leaf rust, barley powdery mildew, tomato grey mould, tomato late blight, rice blast, and rice sheath blight was investigated in growth chambers. Of the six plant diseases, *M. grisea*'s rice blast and *P.recondita*'s wheat leaf rust were two that both compounds most effectively stopped the spread of. (JH Park et al., 2005)

- Numerous kinds of fungi can cause plant illnesses. Different strategies are used by pathogenic fungi to colonise plants and infect them with illness. The demise of certain fungi while biotrophs occupy living tissue, necrotrophs live on and eat dead stuff. For a pathogen to successfully invade plant organs, pathogenic growth must be tightly regulated and particular infection structures must emerge. To spread disease and colonise new hosts, fungal infections make use of a number of virulence factors. Depending on the method of infection, various virulence factors fulfil various functions. Although necrophages release toxins to destruct plant tissue, most illnesses compromise the main plant defence. Contrarily, biotrophs can decrease plant cell death and change plant metabolism to their advantage by using effector chemicals. The *B. cinerea* ailment most commonly observed by people is grey mould. Everywhere in the world, this illness is widespread and has a substantial negative economic impact. It is especially damaging to dicotyledonous hosts' senescent or mature tissues. The fungus usually infects these tissues early in the growth of the crop and does not become active until the environment is conducive and the host physiology has changed. Because of this, storage sustains serious harm after harvesting crops that seem to be in good shape. Prior to harvest, *B. cinerea* also significantly damages horticultural crops grown in greenhouses and fields, especially decorative flowers, fruits, and vegetables. In light of this research in this article, fungi are characterised as a widely varied group of plant diseases that have a serious detrimental effect of agriculture. Pathogens can swiftly infiltrate new hosts, develop resistance to fungicide treatments, or get beyond plant resistance caused by R-genes since fungi have a variety of life modes and significant genetic plasticity. (G Doehlemann et al., 2017)

- A bacterium coupled to an EPN and the rhabditid EPNRhabditis (*Oscheius*) sp. shown strong antibacterial activity in the cell-free culture filtrate. After the ethyl acetate extract of the bacterial culture filtrate was purified, silica gel column chromatography was used to create three diketopiperazines (DKPs). The structure and absolute stereochemistry of this chemical were established using extensive spectroscopic experiments (FABMS, ¹H NMR, ¹³C NMR, ¹H¹H COSY, and ¹H¹³C HMBC), as well as Marfey's method. It was determined that the chemicals were cyclo (LProLLeu), cyclo (DProLLeu), and, consequently, cyclo (DProLTyr). Three DKPs were effective against each of the five fungi examined (*Aspergillus flavus*, *Candida albicans*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Penicillium expansum*) in compared to the widely used fungicide bavistin. Based on this research, isolated DKPs showed strong antibacterial action against bacteria and fungus, particularly against fungi that cause plant diseases. Welcome to the conclusion that the bacterium linked to EPN is a prospective source of naturally occurring, bioactive secondary metabolites that could be very useful for agriculture. (S Nishanth Kumar et al., 2012)
- This study's objective was to characterise and look into the endophytic fungus that live on specific Western Himalayan plants to see whether they would have any biological activity. A total of 72 strains of endophytic fungi were able to be isolated and morphologically characterised thanks to the acquisition and analysis of the ITS1-5.8S-ITS2 ribosomal gene sequence. The isolates were from 27 different fungi genera, with two of those genera (Basidiomycota) each representing one species of fungus. Ascomycetous fungus made comprised the remaining isolates. The highest sequence similarity of ten of the isolated strains with taxonomically described species was s 95% or less, making it impossible to place them in a genus. Different taxonomic affiliations among the isolated endophytes were discovered by collecting and analysing ITS1-5.8S-ITS2 sequencing data. Out of a total of 72 endophytic strains found in this experiment, only two isolates of Basidiomycete (CN2 and FEF4) were obtained, as control to 70 isolates of Ascomycetes. In this study, eleven plants that were indigenous to the

designated Himalayan regions were evaluated for their phylogenetic relationships and bioactive potential. The lengthy lifespans of conifers and *Platanus* species, as well as the medicinal properties of other plants, led to the selection of these species. (M Qadri et al., 2013)

- Soil samples were collected from three separate saline agricultural areas in the hamlet of Kassoki in the Gujrat region of Pakistan, at latitudes 32.658080 N and 74.303055 E, respectively. In the clayey soil, wheat and fodder were typically planted. We collected soil samples from the top and bottom of each sampling site in an effort to isolate the greatest number of bacteria with the necessary characteristics. Surface samples were obtained by carefully removing any loose dirt from the field's top, and subsurface samples were obtained by excavating the sampling site up to one foot below the surface. Using a clean spatula, 300 g of soil were roughly collected, and they were then placed in a clean, suitable-sized zip-lock bag. Samples were stored at 4 °C for as long as practicable. With the use of the selective isolation approach, a bioactive bacterial colony that was exhibiting distant hues (i.e., coloured colonies) and forming clear zones against the antibiotic-sensitive *B. subtilis* was selected and purified for further study. Colonies of this type were chosen in purple, light green, and dark green colours. Research indicates that bioactive pigmented bacteria are used in biocontrol techniques that are mostly unexplored. Our research—one of the few on the biocontrol potential of pigmented bacteria against fungi and bacteria that cause plant diseases—is one of the few studies that has been done. It was an exploratory study with promising results that demanded further research to identify, identify, and assess the active components responsible for bioactivity. In the current work, the bioactivity of two unusual actinobacterial species—*Nonomurae salmonae* and *Actinocorallia libanotica*—against bacterial plant diseases is also described for the first time. Our team is now doing extensive research to identify the best circumstances for the activation of the synthesis of active metabolites by unusual isolates and the characterization of active components in the crude pigmented extract. (Samina Bashir et al., 2022)

- Numerous novel small molecule chemical substances that are introduced as medicines are either derived from or inspired by natural substances. Actinomycetes is the genus of microorganisms that produces the most antibiotics throughout recorded history. The annual reported number of antibacterial compounds from the diverse species of Actinomycetes has increased virtually exponentially since about 20 years ago. Because of the differences between the marine environment and the terrestrial environment, it is thought that marine Actinomycetes may produce a variety of distinctive bioactive compounds and potent antibiotics. Actinomycetes were isolated from salt pans from five different Marakanam locations. The size, shape, and colour of the colonies were used to identify a total of 30 Actinomycetes. 130 isolates were obtained from soil samples and grown in a variety of medium sizes, shapes, and colours. There were small to medium sized colonies. Two isolates came from ISP-5 medium, three from SCA and ISP-3, four from ISP-4 and ISP-6, five from ISP-2, and finally seven from Kuster Agar. Antibacterial effectiveness against a specific bacterial and fungal infection was tested on each isolate. Considering this research Modern medical research is dependent on the study of actinomycetes. These are present in almost all situations. To find the first Actinomycetes from a maritime environment, salt was utilised. St. Padenbur moulds from Nadson (1903). Actinomycetes have been the main source of novel bioactive compounds from the ocean's microbiota, which has been exploited since the early 1960s. The bulk of Actinomycetes species found in the maritime environment and described to date are from novel genera. As a result, over the past 50 years, more than 10,000 bioactive chemicals from oceanic Actinomycetes have been isolated and described. (K.Roshan et al., 2013)
- The human race depends on bioactive substances originating from microbial and plant sources, and ground-breaking research in this area must continue. The primary sources of naturally occurring bioactive chemicals for several biotechnological uses are plants and microorganisms. Recent advancements in the study of bioactive substances and soil chemistry in agriculture have since provided man with a lead in the search for powerful medications that treat both human and plant ailments. The soil serves as the growing medium

for medicinal plants, but its deterioration has a significant impact on the quality of medicines, food crops, and other crucial components found in plants that strengthen the body. Scientists and the pharmaceutical industry have focused their attention on this area in an effort to create stronger medications from medicinal plants cultivated in various types of soil. A measure of the quality dependence of the soil producing secondary metabolites and soil containing microbes has been provided by studies of the effect of various parameters and the qualities of soil, such as; effect of heavy metals, pH, soil organic matter, and phytoremediation process. The knowledge offered will be helpful in figuring out how microorganisms' function and how they interact with soil and all real plants that are creating. Based on the results of this study, scientists should continue to pursue their work in the area of isolating new medications from medicinal plants as well as identifying and improving the quality of bioactive chemicals from microbial sources. The need for the discovery of novel chemicals from plants and microorganisms, including a new class of compounds to combat diseases, is driven by the rapid spread of ailments such novel coronavirus (COVID-19), for which there is now no recognised therapy. New medications are typically—if not always— designed to be effective against a variety of diseases. (Abdurrashid Haruna 2021)

- One of the top 10 fungal diseases that endanger the security of the world's food supply is the catastrophic *Pyricularia oryzae*-caused fungal leaf blast, which annually causes major production losses throughout the world. So, it's crucial to develop a solid plan of action to combat this illness. The antagonistic activity of native photosphere Actinomycetes against *P. oryzae* was clarified in this study both in vitro and in planta in order to create a practical, effective, and environmentally friendly method of protecting rice plants from *P. oryzae*. *P. oryzae* was inhibited by >45% by 18 out of 75 isolates of Actinomycetes that were taken from the rice phyllo sphere. The majority of the 18 isolates were found to belong to the *Streptomyces* genera, as determined by analysis of their 16 S rRNA gene sequences; some were found to be members of the *Saccharothrix*, *Gordonia*, or *Lentzea* genera. Among the 18 isolates, isolates that might have produced a bioactive

substance or compounds were found. Of these, 17 (94.44%) contained a domain marker for the gene for non-ribosomal peptide synthetase (NRPS), and 12 (66.67%) had the gene for type-I polyketide synthase (PKS) in their corresponding genome. It's interesting to note that isolates JSN1.9, SKB2.14, and SKB2.3 decreased illness by about 88%. To our knowledge, this is the first report on the use of rice-phylllo sphere Actinomycetes that produce bioactive chemicals to prevent leaf blast disease in Indonesia. These discoveries have therefore increased the potential use of phyllo sphere Actinomycetes as a top biocontrol agent against fungal leaf blast disease.⁷⁵ Actinomycetes isolates were taken from the rice phyllo sphere and tested in vitro for antifungal activity against *P. oryzae*. A halo zone developed between

P. oryzae and the isolate when the isolate impeded *P. oryzae* mycelial growth. In the initial screening of the 75 isolates, 38 (56%) were inhibitory against *P. oryzae*; these isolates were then further tested in haemolytic, and hypersensitivity, pathogenicity assays; 11 isolates were pathogenic and 27 Actinomycetes, were nonpathogenic. As a result of this study, Actinomycetes in the phyllo sphere are being used as a source of BCAs for potential biotechnological uses as a bio pesticide to suppress

- Phytopathogenic bacteria. The ability of Actinomycetes from the rice phyllo sphere to lessen the *P. oryzae*-caused rice blast disease is first revealed in this work. (Wiwiek Harsonowati 2017)
- Environmental change has made it more challenging to detect new plant leaf diseases. Identification of the variables affecting the emergence and rising prevalence of these diseases is necessary. Here, we discuss newly discovered fungal plant leaf diseases and their environmental speciation. We took into account the elements influencing local speciation brought on by the environment. We found a strong correlation between environmental diversification and the emergence of emergent fungal leaf diseases. The entire plant body may be negatively impacted by fungi that attack the leaves. It is essential to be able to recognise and recognise these pathogens early in the infection process in order to lessen the harm they cause. They could be lessened in terms of dispersion, virulence, incidence, and severity in this way. As a result of environmental change, finding novel plant leaf diseases is

becoming more and more challenging. The causes driving the emergence and rising prevalence of these diseases must be determined. Here, we list new fungal plant leaf diseases and detail how they differ depending on their habitat. We took into account the variables influencing local speciation brought on by environmental adaptability. We found a strong correlation between environmental diversification and the emergence of emergent fungal leaf diseases. The entire plant body may suffer if fungi infections that attack the leaves do so. It is essential to be able to recognise and recognise these pathogens early on in the infection process in order to lessen the harm they cause. They could be lessened in terms of dispersion, virulence, incidence, and severity in this way. About 30% of crop diseases are caused by pathogenic fungus. It is necessary to record fungal pathogen epidemics and the changes they bring about to ecosystems. Chest nut trees have experienced nearly 100% defoliation as a result of well-reported outbreaks of *Cryphonectria parasitica*. *Phytophthora cinnamomic*, which causes root rot or dieback in many crops, was the cause of another more recent blight. Devastating fungal diseases can indirectly damage beetles, birds, and other animals while also affecting a variety of host plant species. Potato blight in Ireland was brought on by *Phytophthora infestans*. Since the 1980s, a fungal blast has infected wheat in Brazil and has spread to other South American countries. The cause of wheat stem rust is the fungus *Puccinia graminis*. In the near future, accurate diagnostic techniques that can be applied in situ to identify plant disease must be created, examined, and perfected. DNA sequencing may be used in new fungal leaf disease identification methods intended for field use. It may be logistically difficult to sample and apply this technology in the form of portable biosensors. Furthermore, because the genomes of plant leaves are big and complicated, it can be challenging to find on-site DNA sequencing expertise. High-throughput computational biology and informatics are crucial for data mining and analysis. There are currently no plans to develop portable DNA sequencing biosensors that can accurately identify fungal plant diseases. According to the research the accuracy of fungal crop disease detection will be improved by the integration of bio sensing mechanisms with DNA sequencing technologies. In this article, we covered a few of the main plant foliar fungal infections as well as the

triggers for environmental speciation and host alterations in these pathogens. In commercially significant crops all throughout the world, fungal leaf infections cause significant yield losses. It is vital to provide methods that allow for the quick identification of these illnesses in addition to analysing the agricultural impact of fungal leaf diseases. Identification of environmental speciation in these diseases is one strategy that could be used. (Archana Jain et.al., 2019)

- The vegetable crop known as Brinjal, or *Solanum melongena* L., is very important in South East Asia and South Asia. Some of the regional names for Brinjal in various indigenous languages around the world include aubergine, baingan, eggplant, garden egg, guinea squash, and melongene. Brinjal has been grown for the past 40 centuries on the Indian subcontinent. The goal of the current study was to ascertain the biochemical alterations in epilachna beetle (*Epilachna vigintioctopunctata*)-infected Brinjal plant leaves. Changes in biological components like chlorophyll content, proteins, carbohydrates, phenols, carotenoids, and anthocyanins were examined. The amounts of chlorophyll a, b, and total chlorophyll in uninfected leaves were 0.082mg/g, 0.136 mg/g, and 0.536 mg/g, respectively. In contrast, *Epilachna*-infected Brinjal leaves had lower levels of these chlorophyll substances, which were 0.012 mg/g, 0.01 mg/g, and 0.025mg/g, respectively. Uninfected Brinjal leaves had 0.174 mg/g of carbohydrates, but infected Brinjal leaves contained 0.148 mg/g. The protein content of *Epilachna* beetle-infected Brinjal leaves was lower (0.20 mg/g) than that of uninfected Brinjal leaves (0.22 mg/g). The amount of total phenols in the *Epilachna*-infected leaves is lower (0.028 mg/g) than it is in the unaffected leaves (0.07 mg/g). It was kept track of the harm the *Epilachna* beetle did... When it feeds on the epidermal tissues of leaves, flowers, and fruits by scraping the chlorophyll content, it is extremely damaging at both the adult and larval stages and results in a significant yield loss. Based on this research the identified biochemical indicators demonstrate that the ladybird beetle *Epilachna vigintio ctopunctata* causes biochemical modifications in the biochemical status of the infected plants, which are discernible through visible symptoms. (C Sundareswari et.al., 2019)

- The Tomato leaf curl virus, a member of the genus Begomovirus, affects tomato and other plants by causing upward leaf curling, a defining disease sign. We assessed the functions of miRNAs in tomato leaf curl New Delhi virus (TOLCNDV) induced leaf curling because microRNAs (miRs) are known to regulate plant developmental processes. Micro array investigations of miRNAs, extracted from the leaves of healthy and TOLCNDV-agro infected tomato, showed that infection with TOLCNDV dramatically downregulated a number of miRNAs from 13 different conserved families (e.g: miR319, miR172 etc.). These miRNAs' precursors displayed comparable deregulated patterns, suggesting that the transcription regulation of the corresponding miRNA genes may have been the root of the deregulation. When compared to the quantity of the matching miRNA, the expression levels of the miRNA-targeted genes were antagonistic. Since no comparable expression was discovered in flowers, this deregulation was tissue-specific in nature. In tomato cultivar, accumulation of miR159/319 and miR172 was seen to rise with the number of days after inoculation with TOLCNDV agro infection. Similar to how they were induced in tomato cv. JK Asha and chilli plants that had been infected with TOLCNDV and were showing symptoms of leaf curl. According to our findings, the symptoms of leaf curl may be related to miR159/319 and miR172. This study suggests that miRNAs could serve as possible hallmark molecules for TOLCNDV infection. Based on this research Viral infection influences the expression of a number of host miRNAs. Additionally deregulated were the concentrations of the expected targets and the related pre-miRs. This shift in miRNA expression levels was exclusive to leaf tissues and was found to be related to the development of the illness. As a result, a number of hosts miRs are probably indicators of viral infection and may be used to create viral resistance mechanisms. (Afar R Naqvi et.al., 2010)
- Ladies finger (*Abelmoschus esculentus*) seeds were planted in soil supplemented with salvinia vermicomposting at four levels: 0 (V0), 2.5 (V1), 3.75 (V2), and 5 (V3) t/ha in outdoor tests that mimicked how farmers grow vegetables. In addition to evaluating plant germination success and

subsequent growth, yield, and biochemical makeup, the effect of pest attacks on the plants was investigated. Vermicomposting made from *Salvinia* dramatically improved plant germination, growth, and yield. Maximum growth was seen in V4 (5t/ha) in terms of shoot length (96.2 cm) root length (48.2 cm), shoot and root dry weight (23.31, 7.96 g), stem diameter (14.04 mm), and number of leaves and branches (26.8, 4.8). Vermicomposting-treated plants had much higher mineral and biochemical contents than untreated ones. Plants become more resistant to pests and disease as a result of the vermicomposting process. Vermicomposting significantly decreased fruit borer infection in V1, V2, and V3 as compared to the controls by 65, 78, and 82%, respectively. According to this study, when *salvinia* is vermicompost, its toxicity

- Is largely removed and the result develops the properties of a high-quality organic fertiliser. The current work has the potential to result in the creation of a low-cost, environmentally friendly, and sustainable means of utilising the billions of tonnes of *Phyto* mass produced annually by *salvinia* that are currently wasted. (Naseer Hussain et.al., 2017)
- Fungi called *Alternaria* and *Fusarium* are well-known plant diseases that seriously reduce the productivity of agricultural crops everywhere. The globe. Early tomato blight and wilt are being caused by *A. alternate* and *F. oxysporum*. Synthetic fungicides are typically used to control infections. In addition to being poisonous to humans, it is thought to be the most polluting substance for the environment. In place of synthetic fungicides, plant extracts offer a safe, healthful, and environmentally responsible option. Asteraceae, Boraginaceae, Convolvulaceae, and other groups of plants were gathered throughout the state of Oaxaca. Orchidaceae, Rubiaceae, Tiliaceae, Verbenaceae, and Crssulaceae are some examples of plant families. The detection of mycelium radial growth was used to quantify the antifungal action of various doses of plant extracts (50-100 mg/ml) on *A. Alternaria* and *F. solani*, and to determine the tested fungi's minimum inhibitory concentration (MIC). Gas Chromatography-Mass Spectroscopy (GC- MS) Phytochemical study of 10 plant extracts revealed the existence of physiologically active Alkaloids, flavonoids, saponins, tannins, and Terpenoids are important

chemicals . A. *Alternaria* and *F. oxysporum*, two plant pathogens, were inhibited by these chemicals. The importance of natural products in drug discovery and antibacterial activity has increased research on them in recent years. Numerous plant compounds with antifungal action against various pathogenic fungus have been discovered and are crucial to humans. These plant molecules may be used directly or as building blocks for the creation of antifungal chemicals . Alkaloids, flavonoids, tannins, Terpenoids, saponins, and other bioactive secondary metabolites are only a few examples of the diverse types of secondary metabolites that are abundant in plants. Based on this study in an effort to reduce the use of dangerous synthetic fungicides, numerous investigations were carried out for the potential using plant-based ingredients as natural remedies against fungi. The antifungal activity of 10 plant extracts was investigated in this article against

- *Alternaria* and *Fusarium oxysporum*, two tomato phytopathogens. Therefore, these plant extracts have a potential for the control of plant diseases in agricultural crops (Haytham M. Daradka et.al. 2021)
- Numerous pests, such as bacteria, fungus, weeds, and insects, have a negative impact on agriculture, reducing productivity and degrading the quality of the produce [1]. Since the 1960s, the predominant technique for pest management synthetic insecticides have been used often. Dichloro- diphenyl-trichloroethane (DDT) was first used as a pesticide in the 1940s, and thereafter additional organophosphate and carbamate insecticides [2]. After that, using a lot of inputs like chemical fertilisers and pesticides, among other things, Green Revolution agricultural production technology might enhance food output in underdeveloped countries. Although the use of agrochemicals greatly increased agricultural output, they also had negative effects on soil health, water quality, produce quality, and the development of issues like insect resistance, genetic variety in plants, toxic residues in food, and other difficulties. The naturally occurring formulations of compounds that manage pests through non-toxic and environmentally beneficial means, such as bio pesticides and bio fertilizers, are not newer technology. Since the dawn of human history, they have been utilised in numerous ways Bio pesticides gaining popularity as a safer way to control pest populations, including weeds, plant diseases, and insects, while posing less of a threat to people

and the environment. Bio pesticides are regulated by the Environmental Protection Agency in the US, which supports their registration based on the conclusion that they have "no unreasonable adverse effects" on humans and the environment. This allows for their sale and distribution under the Federal Insecticide, Fungicide, and Rodenticide Act. Based on this research Bio pesticide use and manufacturing are growing quickly on a global scale. Increased use of bio pesticides by farmers is undoubtedly justified by the interest in organic farming and produce free of pesticide residues. For bio pesticides to be more widely used, manufacturers may need to undergo production and quality control training, as well as organisational training for extension agents and farmers. (Suresh Kumar et al., 2015)

- This research was done to examine possible antimicrobial generating microorganisms in soil samples that were obtained. The forest of Nanga Merit. After primary and secondary screening, *Staphylococcus aureus*, *Salmonella typhi*, and four fungal isolates (F10, F11, F14, and F26) all displayed antibacterial activity. Bacterium *Enterobacter aerogenes* and *Escherichia coli*. By using the disk-diffusion and agar well diffusion procedures, the minimal inhibitory concentration (MIC) of the methanol extract from isolate B17 against the four tested bacteria was identified. When tested against *S. aureus*, *S. typhi*, and *E. coli*, the MIC values were 25 g/l, however when tested against *E. aerogenes*, the MIC value was 12.5 g/l. The relative MIC values in the agar well diffusion method was 1.5625 g/l for *S. aureus*, 12.5 g/l for *S. typhi* and *E. coli*, and 9.375 g/l for *E. aerogenes*. Besides Extracts from F26 grown on PDA, CDA, and V8 juice agar displayed mediocre anti-*E. Aerogenes* activity and were not further examined for MIC values. When tested against the test fungus (*Fusarium sp.*), the extract from B17 also exhibited antifungal activity. On PDA, cultured fungal isolates (F10, F11, F14, and F26) had strong antifungal properties. The potential of the presence of local microorganisms that haven't been investigated by other researchers led researchers to choose soil samples from a distant area (Nanga Merit Forest, site 2) for investigation in this study. There is a larger likelihood that these natural soil bacteria may develop new antibiotic compounds. In Using preliminary screening and selection, a total of 30

bacterial and 38 fungal isolates from various depths were successfully isolated for this investigation. Only 26 fungal isolates and 23 bacterial isolates were chosen out of these to proceed through secondary screening. Due to similarities in several isolates' physical characteristics and growth morphologies, it was determined that the total number of isolates had decreased. In conclusion, it was discovered that Nanga Merit Forest has a high potential for discovering beneficial antibiotic-producing microorganisms, including some potentially novel strains that other researchers have not previously explored. Therefore, more research may be done on this distant region to investigate novel antibiotics and the bacteria that are living there that produce them. (Samuel Lihan.2014)

- The goal of this work was to describe a bioactive chemical produced by endophytic fungus on the aquatic plant *Nelumbo nucifera*. To isolate endophyte fungi, all sections of this plant were surface sterilised, cleaned, and cultured on potato dextrose agar. The label read "character-"molecular and morphological techniques. By using the disc diffusion method, fungal isolates were tested for antibacterial activity against the MRSA strain of *Staphylococcus aureus* DMST20651. These crude fungal extracts' MIC and MBC values were established. Finally, gas chromatography and mass spectrometry were used to determine the chemical profile of the crude extract. From the sections of *N. nucifera* that had their surfaces satirised, six endophytic fungi were recovered. Disc diffusion assay revealed that isolate ST9.1, which was later determined to be *Aspergillus cejpaii*, had the highest antibacterial activity against MRSA. According to the findings, the ethyl acetate extraction produced higher active fractions with MIC of 2.5 mg/ml and MBC concentration. Is 50.0 mg/ml. By using mass spectrometry and gas chromatography, the crude extracts were created to pinpoint the chemical components. It was 5-(1H- Indol-3-yl)-4 5-dihydro- [1, 2, 4] triazin-3-ylamine (C₁₁H₁₁N₅) that made up the majority of the crude extract of endophytic fungus. The plant may therefore be utilised to treat infectious disorders brought on by bacterial pathogens. *Nelumbo nucifera* was found to have six endophytic fungi in its roots, seeds, petals, leaf veins, leaf inter-veins, pollen, stolon's, stems, and receptacles (Fig. 1). The outcome revealed that theroot

of *N. nucifera* has the highest percentage of endophytic fungus (3/6 or 50.0%) compared to other components. Eight of the twenty endophytic fungi were identified from the leaves of the aquatic plant *Nymphaea nuchal* in Sri Lanka, which is similar to the findings of the researcher there. To determine their antibacterial activity, all fungal isolates were tested. According to the findings of the *Aspergillus cejpaii* was the endophytic fungal isolate ST9.1 that was taken from the lotus stolon. The ethyl acetate extracts of the fungi demonstrated antibacterial active has a MIC and MBC of 2.5 mg/ml and 50.00 mg/ml, respectively, it against methicillin-resistant *S. aureus*. (Surachai Techaoei2020)

- Plant growth-promoting rhizobacterial (PGPR) are a class of bacteria that are present in the rhizosphere, near the roots, and which can either directly or indirectly promote plant growth. There are many different types of bacteria, including *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, and *Bacillus* species. According to reports, *Serratia*
- And *Rhizobium* promote plant growth. Six different French bean rhizospheric soil samples were used to collect a total of thirty bacterial isolates. In accordance with Table No. 1, each isolate was given a designation. Every isolate had PGPR activity that was significant. On modified PVK agar, a total of 30 bacterial isolates were tested for their ability to solubilize phosphate; 12 of these isolates developed distinct phosphate solubilisation zones with dimensions between 4 and 20 mm. The emergence of hazy zones was visible in other isolates' increase plant development, but the precise process by which they do so is unclear. However, many mechanisms, including the synthesis of Plant growth promotion is typically thought to involve phytohormones, the inhibition of harmful organisms, activation of phosphate solubilization, and enhancement of the mineral nutrient intake. (Ajay Kumar 2012)
- The antibacterial and antioxidant properties of a few plant-derived bioactive substances generated by fungal endophytes have been established. In this investigation, endophytic fungi were found in 20 orchid samples that were

gathered in northern Thailand from 12 orchid genus names. The orchid samples' leaves (44.3%), stems (40.2%), and flowers (15.5%) yielded a total of 97 isolates. The antifungal activity of the endophytic isolates against the plant pathogenic fungi was investigated. According to the findings, 13 endophytic isolates have antifungal properties against *Fusarium* sp., *Colletotrichum* sp., and *Curvularia* sp. Even the non-sporulating endophytic fungi can be challenging to distinguish using morphological characteristics (Guo et al., 2000). Therefore, molecular methods have been employed. For the purpose allocating and identifying endophytes in the current study, endophytic fungus colonisation of 20 orchid samples from 12 different orchid taxa that were collected in northern Thailand was described. According to the results of the experiment, the endophytes CK F05-5 (also known as *Fusarium oxysporum* KU527806) may have chemical components that have antifungal, antioxidant, and ant mutagenic properties. (Nattawadee Bungtongdee 2018)

- By providing nutrients, vitamins, and minerals, vegetables are essential to a healthy human diet and the main force behind achieving global nutritional security. Vegetable production has expanded dramatically, and this was made possible by the creation of superior varieties and hybrids. Technologies for productivity and protection through methodical study and widespread use by farmers. However, just a few significant vegetative sources contributed to this amazing yield. Although India's unique agro climatic conditions allow for the cultivation of more than 60 farmed and approximately 30 lesser-known vegetable crops, underutilised vegetables have not received much attention. Vegetable crops that are neither farmed economically on a large scale nor marketed widely may be referred to as underused vegetable crops. Despite their acknowledged value, underutilised vegetables are not used as often as they may be for a variety of reasons, including a lack of planting materials, a lack of knowledge of the crops' nutritional and therapeutic benefits, and a lack of knowledge about their production methods. Utilising underutilised veggies more frequently and successfully is helping to combat economic prosperity , poverty, and malnutrition. They are a vital part of the rural poor's biological resources and can help the millions of tribal people in the world live happier, healthier lives. Underutilised veggies have strong antioxidant activity and are

a good source of vitamins, minerals, and other nutrients that are good for health. Vegetables that are underused yet have high nutritional potential and can withstand unfavourable weather conditions could be beneficial to all parties involved, including growers, Consumers and environmentalists, as long as they are adequately controlled (Anil Kumar Jena 2018)

- A shift in pest status has been observed in recent years due to changes in cropping patterns, ecosystems and habitat, climate, and the introduction of input-intensive, high-yielding cultivars and hybrids. Many pests have widened their range of potential hosts, become resistant to insecticides, and frequently have subsequent outbreaks. Prevalence of chillies some examples include hadda beetles (*Henosepilachna vigintioctopunctata* and *Epilachnadodecastigma*) on cowpea and bitter gourd, and plume moth (*Sphenaeches caffer*) in bottle gourd. Gall midge (*Asphondylia capparidis*) is found in parts of Tamil Nadu and Andhra Pradesh. This research considers these newly developing insect pests in the vegetable environment, their effective control techniques, and certain problems or obstacles in their management. Due to changes in the environment, some insect pests of vegetable crops are gradually becoming serious pests in various parts of the country. Habitats and the ecosystem. A few examples include the tomato hornworm *Helicoverpa armigera*, the whitefly *Bemisia tabaci*, the serpentine leaf miner *Liriomyza trifolii* on tomato and cucurbitaceous crops, fruit flies on fruits and vegetables, mealybugs on several horticultural crops, the gall midge on Brinjal, the okra stem fly, and the bitter gourd leafhopper, the red spider mite on okra, Brinjal, cow (A. B. RAI, JAYDEEP HALDER et.al., 2014)
- In Tamil Nadu's Kanchipuram and Tiruvallur Districts, natural enemies of insect pests in vegetable crops (Brinjal, okra, and tomato) were identified, and the diversity of these enemies was investigated. Since January, natural enemies have been harvested by hand picking, sweeping nets, and pitfall traps. 2011 to December 2013: twice per month. It was discovered 129 different species of parasitic and predatory insects. Under 50 families and eight separate orders, all the natural foes were categorised. The most families and species were found in the hymenopteran order. Natural pest

population reduction can occur as a result of diverse agroecosystems, which can sustain a variety of natural enemies [4]. Natural enemies will have access to alternate food sources including nectar, pollen, and honeydew when border or intercrop crops are grown alongside the primary crop. In addition, it will naturally occurring adversaries with a haven, a favourable microclimate, and breeding grounds. Biodiversity studies can be used to evaluate the effect of intercropping on helpful insects. Diversity indices serve as indicators in biological diversity investigations. The quantity of different species in a community is known as its species richness. In this study, the variety of natural enemies was evaluated separately in vegetable fields with monoculture and intercropped crops. Seasonal variations as well as crop patterns had asignificantimpact on natural enemy diversity. Different species of natural enemies were supported by intercrops and border crops, and mixed cropping outperformed monoculture in terms of the overall number of individuals belonging to various families of natural enemies. Produced vegetables. (Veeramuthu Anbalagan 2016)

- Sustainable farming practises have grown in prominence over the past several years to rank among the most crucial agricultural challenges. The output of agricultural goods is also still significantly constrained by plant diseases. The use of traditional pesticides to control plant diseases has led to severe concerns about food safety, environmental quality, and pesticide resistance. As a result, new pest management methods have become necessary. Tactics for management. The susceptibility of plants to illness or their resistance to pathogens may be specifically impacted by nutrients. The impact of nutrients on plant diseases, however, has been the subject of conflicting reports, and many of the elements that affect this response are poorly understood. The nutritional status of the plant and environmental factors are two ways that fertiliser application affects the development of plant disease under field conditions. Factors affect the disease's growth, including crop stands that are wet, have dense stands, or change how light is intercepted. In the majority of the studies discussed here, fertiliser treatment or nutrient addition has reduced the prevalence of disease in crop plants. This is likely as a result of these nutrients, play a role in the host plant's

tolerance and resistance systems. When the plants were at deficiency levels, nutrient administration had a far stronger impact on lowering disease. The incidence of disease can also be lowered by providing nutrients at above-optimal levels. (Christos Dordas2008)

- Plant infections brought on by phytopathogenic fungi can result in significant losses in agricultural fields and hence continue to pose a risk to the safety of the world's food supply. Fungicides made of chemicals made a big contribution to protecting agricultural productivity. However, the uncontrolled use of fungicides has promoted chemical resistance and raised concerns about the environment and human health. Find the development of pathogens with numerous fungicide resistances as a result of the careless use of these fungicides has made treating the diseases more difficult.in new bioactive natural compounds and turning them into new bio pesticides are thus urgently needed. It is generally known that fungal endophytes, which are common in plants and symbiotic with their hosts, produce a variety of antimicrobial compounds and increase the resistance of plants to infections and pests. (Kuo Xu et.al., 2021)
- Pesticides that are used to control illnesses and pests are also linked to risks to, the ecosystem, and human health. Certain antagonistic bacteria from the rhizosphere of various agricultural plants have been identified to lessen the harmful effects of these agrochemicals by suppressing certain plant diseases and reducing the need for pesticides. In the past 20 years, there has been a lot of research done on the use of these specialised antagonistic microorganisms in the biological control of soil borne diseases. By creating antibiotics, bacteriocins, siderophores, hydrolytic enzymes, and other secondary metabolites, these helpful rhizosphere microorganisms inhibit the harmful bacteria and fungus. The environment can be changed, beneficial organisms can be mixed together, biocontrol mechanisms can be physiologically and genetically strengthened, formulations can be changed, and biocontrol can be combined with other complementary techniques to achieve additional benefits. These biocontrol agents could be used to increase growth in sustainable agriculture. The interplay between the

biocontrol agent, rhizosphere microbial population, plant, and environment are to blame for the variation in disease that is seen. Encouragement of plant growth and repression. One of the main obstacles to these biocontrol strains' widespread usage in commercial agriculture is their inconsistent performance. (Satyavir -2016)

CHAPTER-3

AIM AND OBJECTIVES

AIM:

- To identify bioactive compounds producing soil bacteria against selected agricultural plant pathogens

OBJECTIVES:

- Collection of infected leaf from selected plants.
- Isolation of morphologically different fungus from infected leaves.
- Isolation and selection of bacteria from soil.
- Screening and selection of bioactive compound producing bacteria against plant pathogens.
- Production and purification of bioactive compounds from potent isolate.
- Characterization of fungi and potent bacteria.

CHAPTER-4

MATERIALS AND METHODS

4.1 COLLECTION OF INFECTED PLANT LEAF AND ISOLATION OF FUNGI:

The infected part of leaf sample was collected from different agricultural form land. Infected leaves from Tomato, Brinjal and Lady's finger were taken and shifted to lab in sterile zip lock bags then processed within 24 hours. Fungal pathogens from infected leave are isolated by following guide lines. After collecting the leaf sample affected part was washed through sterile hot water to remove soil particles and cut into small pieces 2-6mm square with the help of sterilization forceps and scissors[3].The cut leaf parts are shifted into sterile Petri dish and washed through 0.1 sodium hypo chloride solution which is helped as surface sterilization agent then to remove the agent with distilled water by washing it again [R.Thilagam *et al.*,2019].Now the affected part of leaves were shifted to PDA plates and kept for incubation for 5-7 days at 27. C to observe the growth of fungi.

4.2. COLLECTION OF SOIL AND ISOLATION OF BACTERIA:

The Soil sample were collected from different places and those samples were taken and shifted to lab in sterile zip lock bags then processed within 24 hours. Microorganisms were isolated from soil sample through the process of serial dilution method on the NA media [Kannan *et al.*, 2018]. Soil sample were collected and taken from 5cm under the ground of different areas and sampled into zip lock cover then it processed into lab. From collected soil Sample one gram of soil were taken and suspended in 10ml of distilled water in each test tubes and they were diluted from 10⁻¹ to 10⁻⁹ and performed spread plate method was carried out to isolate the bacteria from the sample [5].Now 0.1 ml was taken by using of pipette from the test tubes of 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸. Put through on NA plates and Spreader around the plates with the help of L shaped glass rod and inoculated at 37°C for 24 hours. The specific colonies were Isolated and Sub cultured on Nutrient agar slants [chan *et al.*,2003]

4.3 SCREENING OF ANTIMICROBIAL ACTIVITY OF SELECTED BACTERIA:

To Check Inhibition of soil bacteria against plant fungal pathogens. Antimicrobial activity followed and also mostly performed through agar well diffusion method. Observe the sub cultured fungus Potato dextrose agar slants were grown completely 27°C for 7 days. Bacterial isolates were screened for their antifungal

activity it is using dual culture technique against plant pathogenic fungi consisting of *Aspergillus*, *Alternaria salani*, *Fusarium*, *Albugo candida*, *Plasmopara*. All those fungal pathogens were obtained from infected parts of collected agricultural plants by following protocol.

1. Preparation Nutrient broth:

Isolated bacteria strains from soil were produce bioactive Compound to inhibit the plant pathogens. Now different morphological soil bacterial strains are inoculated on nutrient broth and kept for incubation at 37°C for 72 hours to produce secondary metabolites such as antifungal, antibiotic, antitumor drugs etc...

2. Antimicrobial screening [well diffusion method]:

The Agar well diffusion method is a commonly used technique for testing the antimicrobial properties of microbial extracts. The MHA plates were prepared for antimicrobial activity in this method, the surface of an MHA agar plate is inoculated with fungi strain inoculum, and a hole with a diameter of 6 to 8 mm is then created using a sterile tip. Next, a volume (20–100 µL) of the antimicrobial solution is introduced into the well. The agar plates are then incubated under 4-6 days at 27C as the antimicrobial agent diffuses into the agar medium, after incubation, zones are observed around the discs or wells. This denotes the antimicrobial activity results are positive. If there is no zone formation, the result is negative.

4. SECONDARY SCREENING [AGAR PLUG METHOD]:

The process is the same as that employed in the disk-diffusion method, and it is frequently used to highlight the antagonistic relationships between microorganisms. By making tight streaks on the plate surface, it includes creating an agar culture of the desired strain using the proper culture media. Molecular secretions produced by microbial cells during growth cause the agar medium to saturate with the molecules. After the test microorganism has been incubated, an agar-plot or cylinder is sliced aseptically using a sterile corn borer and placed on the agar surface of another plate. To the agar media, the chemicals diffuse from the plug. When the inhibition zone appears around the agar plug the antimicrobial activity of the chemicals released by the microbes is then discovered.

5. PRODUCTION OF POTENT ISOLATE:

The isolated sample was inoculated in the 5ml nutrient broth and it was incubated with shaker at 29C for 2 days then the pre inoculated broth was inoculated

into 100ml of production media and incubated at 29C with periodical shaking for 5 days [Ganesh Moorthy *et al.*, 2020].

6. EXTRACTION OF ETHYL ACETATE FOR BIOACTIVE METABOLITES:

The extraction was expert as per the procedure done by [Tanvir *et al.* (2016)]. The TP8 production media were centrifuged at 15 minutes for 7000 rpm and supernatant was collected. The filtrate of TP8 culture was mixed with the ethyl acetate solvent at 1:1 proportion in the separating funnel and agitated overnight. Then the separation was done by separating funnel and the upper layer containing bioactive compounds was collected for ethyl acetate. Finally, the collected upper layer was concentrated in 100ml beaker and allow it to dry to separate bioactive compound from the ethyl acetate. [Ganesh Moorthy *et al.*, 2020]. Now add 100ul of ethyl acetate in dried extraction which was poured in 100 ml beaker using pipette and mix it well. Filtered extraction then pour it in a collection vial tube.

7. EXTRACTION OF CHLOROFORM FOR BIOACTIVE METABOLITES:

The extraction was expert as per the procedure done by [Tanvir *et al.* (2016)]. The production media were centrifuged at 15 minutes for 7000 rpm and supernatant was collected. The filtrate of TP8 culture was mixed with the chloroform solvent at 1:1 proportion in the separating funnel and agitated overnight. Then the separation was done by separating funnel and the lower layer containing bioactive compounds was collected for chloroform. Finally, the collected lower layer was concentrated in 100ml beaker and allow it to dry to separate bioactive compound from chloroform. [Ganesh Moorthy *et al.*, 2020]. Add 100ul of chloroform in dried extraction which was poured in 100 ml beaker using pipette and mix it well. Filtered extraction then pour it in a collection vial tube.

8. PHYTOCHEMICAL SCREENING:

Qualitative tests for alkaline reagent test for flavonoids, Lead acetate test for tannins, and Ammonia test for anthraquinonoid were performed according to the procedure described by (Harborne *et al.*, 1973). Salkowski's test for sterols and terpenoids, Ferric chloride test for flavonoids, Wagner's test for Alkaloids, Froth test for saponins.

Materials required: Extraction of ethyl acetate compound

1. Test for Flavonoid's [Alkaline Reagent Test] (a):

Add 100ul of extract and add few drops of 20% of NaOH solution and adding HCL. A change to yellow colour to colourless solution presence of Alkaloids

2. Test for Tannis (b):

Take some extract add few drops of Ferric Chloride, pale yellow appears in chloroform extract, and yellow colour appears in methanol extract.

3. Test for Anthraquinones (c):

Add 100ul of extract and add 2ml of Ammonia solution. Separated Aqueous layers were observed for any colour change, delicate rose pink colour shows the presence of Anthraquinones [Tulika Tyagi and mala Agarwal].

4. Test for Terpenoids (d):

Add 100ul of extract and add 2ml of Chloroform, 3ml of conc.H₂SO₄. Reddish brown colour indicates the presence of Terpenoids.

5. Test for Sterols (e):

Take some extract add few drops of conc.H₂SO₄ and shake well. Reddish brown colour indicates the presence of Sterols.

6. Ferric Chloride Test for Flavonoid's (f):

Take 2ml of extract add few drops of Ferric Chloride. Green-Blue or violet colour indicates the presence of Phenolic Hydroxyl group.

7. Test for Alkaloids [Wagner's Reagent Test] (g):

Add 100ul of extract and add 1ml of Wagner's reagent (Iodine Potassium Iodine) were added. Presence of brown/reddish colour indicates the presence of Alkaloids.

8. Test for saponins:

Take 2ml of aqueous extract and 2ml of distilled water are shaken for 15 minutes in a test tube. A 1cm of foam layer is a positive response to the presence of saponins [B. Ramesh *et al.*, 2013].

4.9 ANTIMICROBIAL ACTIVITY [DISC DIFFUSION METHOD]:

In this notable technique, agar plates are inoculated with a normalized inoculum of the test microorganism. Then, at that point, Whatman filter paper disc (around 6 mm in width), containing the test compound at an ideal focus, are put on the agar surface. The Petri dishes are brooded under reasonable circumstances. By and large, antimicrobial specialist diffuses into the agar and represses germination and development of the test fungal microorganism and afterward the breadths of restraint development zones are measured. It shows the development media, temperature, time of hatching and inoculum size expected by CLSI guidelines. (Baskaran *etal.*, 2012)

10. CHARACTERIZATION OF FUNGAL PATHOGENS:

Pure fungal isolates were analysed through macroscopic and microscopic examinations to establish their characterization as fungal pathogens. These isolates were grown in various media, namely PDA, SDA, and CDA, and their growth morphologies were noted, including top colour, reverse colour, perimeter, mycelium mat, mycelium end, and medium colour changes. The identification of these fungal isolates was determined through the slide culture technique.

1. **Slide culture technique:**

Place a sheet of sterile wet cotton in a Petri dish using forceps in an aseptic manner. Place a clean glass rod in the shape of a U. Pole can be disinfected by blazing, pour enough sterile water onto the cotton to completely moisten it. Place a sterilized slide on the U-shaped rod with forceps, then cut a 5 mm square block of the medium from the plate of SDA agar with a scalpel. Get the block of agar by embedding the surgical blade and cautiously move this block aseptically to the focal point of the slide. Four sides of the agar square should be inoculated with the fungus's spores or mycelium. Make certain to fire and cool the circle before getting spores. Aseptically, put a sterile cover glass on the upper surface of the agar 3D square. After covering the Petri dish, incubate it for 48 hours at room temperature. Following 48 hours, analyse the slide under low power. Hyphae will expand and spores will be produced if growth has taken place. Allow the mold to grow for an additional 24 to 48 hours if growth is insufficient and spores are not visible. After the spore add lacto phenol cotton blue to the slide and view under microscope

11.CHARACTERIZATION OF PURE ANTIBIOTICS PRODUCING BACTERIA ISOLATES:

1.Gram staining: Gram staining was performed for bacteria stain as indicated by the normal technique. A smear of bacterial cells was ready on a spotless glass slide by a delicate intensity obsession. The intensity fixed smear was overflowed with gem violet arrangement briefly. Smear was washed with water followed by adding severe Gram's iodine. The smear was decolorized with 95% ethyl liquor and flushed with water. At long last safranin was utilized as counter stains for 60-80 sec and washed with water. Cells were then inspected under microscope. (Kohinoor Begum *etal.*,2017).

2. Motility test [Hanging drop method]: soak the four edges of the coverslip with water to keep the coverslip solidly in place. A ring of petroleum jelly might be utilized instead of water to keep the coverslip immovably set up on a magnifying instrument slide on the off chance that magnifying instrument slides with focal gloom are not accessible. The Vaseline-fixed gloom or some of the time utilizing plasticise likewise dials back the drying-out process, so the living beings can be noticed for longer periods. place a little drop of fluid bacterial culture in the focal point of a coverslip rearrange a slide with a focal despondency over the coverslip the coverslip will adhere to the slide and when the slide is rearranged the drop of bacterial culture will be suspended in the well inspect minutely (x400)

Positive outcome: A shooting, crisscross, tumbling or other coordinated development.

Negative outcome: No development or Brownian movement as it were

3. Biochemical test:

Catalase test: It was performed by taking a drop of 3% hydrogen peroxide was added to 48 hr old bacterial province on a perfect glass slide and blended utilizing a sterile tooth-pick. The foam shown catalase action.

Oxidase test: It was performed with 1% arrangement of Tetramethyl-phenylamine-dihydrochloride which was soaked in a piece of filter paper. A culture was placed on filter paper by using sterile glass rod furthermore, addressed to the paper with impregnated Reagent. A dim purple variety improvement inside 5-10 second was viewed as positive and no difference as negative.

4. Endospore staining:

Take a spotless oil free slide and make smear utilizing sterile method. Air dry and intensity fix the creature on a glass slide and cover with a square of smudging paper or drying slice to fit the slide. Immerse the smudging paper with malachite green stain arrangement and steam for 5 minutes, keeping the paper soggy and adding more colour as required. On the other hand, Wash the slide in regular water. Counterstain with 0.5% safranin for 30 seconds. Wash with regular water; smudge dry. Observe the slide under microscope for the presence of endospores. Endospores are dazzling green and vegetative cells are caramel red to pink.

5. Capsule staining:

On the slide, apply a small amount of a negative stain, such as Nigrosine stain. Add a loop full of bacterial culture to the slide using a sterile method, coating

it with the dye. Utilize the other slide to drag the ink-cell blend into a slender film along the principal slide and let represent 5-7 minutes. Air dry the item (do not heat fix). For approximately one minute, coat the smear with crystal violet stain, which will only stain the capsules but not the cells. Tip the slide at a 45-degree angle to drain the crystal violet, then let the stain run off until it dries in the air. Microscopically (100X) examine the smear for the presence of encapsulated cells, as indicated by the presence of clear zones around the cells.

CHAPTER-5

RESULT AND DISCUSSION

5.1 COLLECTION OF PLANTS AND ISOLATION OF FUNGAL PATHOGENS:



Fig 5.1 Infected Tomato Leaf



Fig 5.2 Infected Brinjal Leaf



Fig 5.3 Infected Lady's finger Leaf

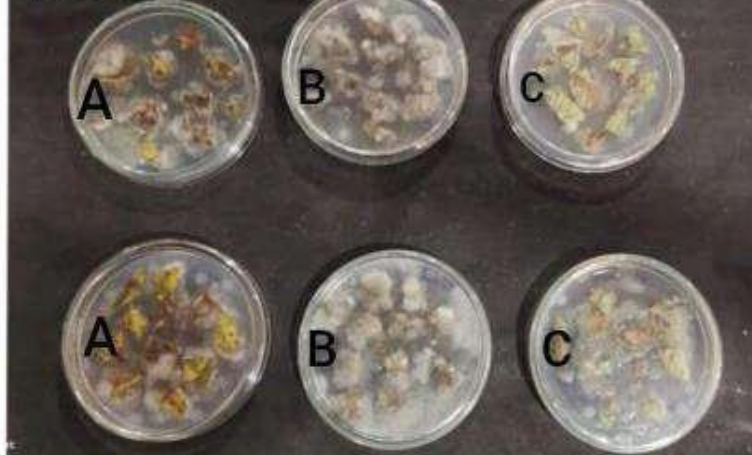
In this study infected part of leaves from three different plants were collected and isolated fungi namely

- Tomato plant leaf
- Brinjal plant leaf
- Lady's finger plant leaf

DAY 1:



DAY 2:



DAY 3:

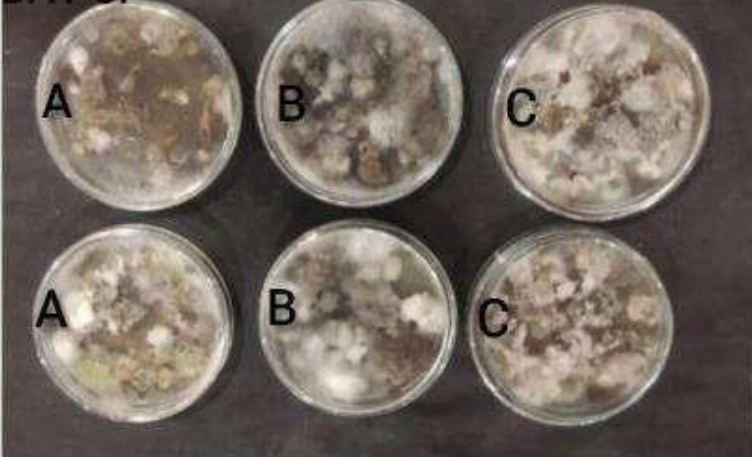


Fig 5.4 Isolation of fungal pathogens from infected Tomato, Brinjal, Lady's finger leaf's

Table 5.1 Isolated infected leaf samples

S.no.	Plant Name	Scientific Name	Sample code	No.of. isolates
1	Tomato Plant	<i>Solanum lycopersium</i>	TO	03
2	Brinjal Plant	<i>Solanum melongena</i>	BL	01
3	Lady's Finger Plant	<i>Abelmoschus esculentus</i>	LF	02

Totally 6 morphologically different fungi were isolated from 3 plants

5.2 COLLECTION OF SOIL SAMPLES AND ISOLATION OF BACTERIA:



Fig 5.5 Collected soil samples

In this study, the soil samples are collected from different places are tabulated below

Table 5.2 Isolated soil sample

S.no.	Soil samples	Sample Code	No. of. isolates
1	Thiruporur Agricultural land	TP	09
2	Gummidipoondi Agricultural Land	GA	05
3	Vittaiah palem Forest	VP	07
4	Tada Hills	TH	04
5	Tada Falls	TF	02

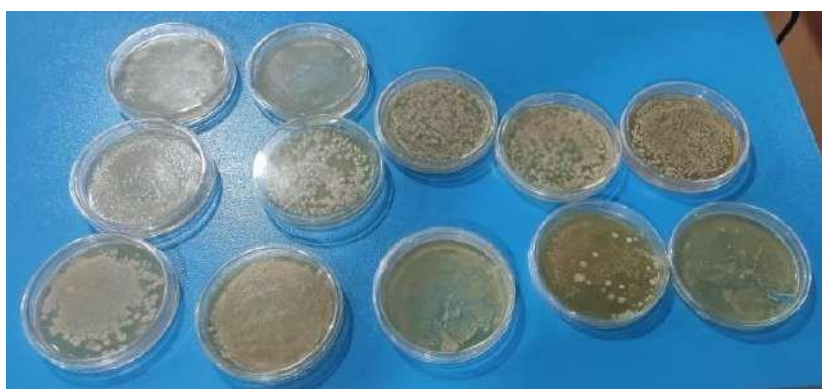


Fig 5.6 isolation of soil samples

Totally 27 morphologically different bacteria were isolated from the soil sample

Those 27 bacteria isolates were studied for bioactivity

5.3 ANTIMICROBIAL ACTIVITY [WELL DIFFUSION]:

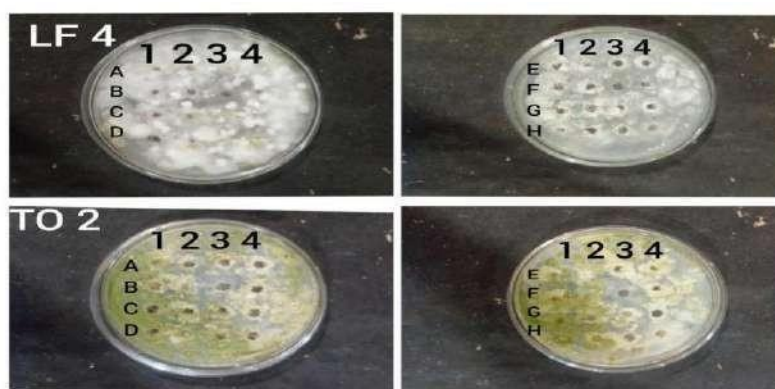


Fig 5.7 Antimicrobial activity of TO2 and LF4

Out of 27 isolates, 16 bacteria showed bioactivity against any one of the fungal pathogens. The bioactivity produced from bacteria is inhibited against 6 plant

pathogen. Presence of zone of inhibition indicates that the compounds stop the growth of fungus . Reffer table 5.3 and table 5.4

Table 5.3 Antimicrobial activity of TO2 and LF4

S.no.	Isolation of Staining	Screening code	
		TO 2	LF 4
A1	TP1	+	+
A2	TP4	-	+
A3	TP5	-	+
B1	TP5.1	-	+
B2	TP6	+	-
B3	TP6.1	+	-
C1	TP7	-	-
C2	TP7.2	-	-
C3	TP8	+	+
D1	GA1	-	+
D2	GA2	-	-
D3	GA3	-	-
E1	GA4	-	-
E2	GA5	-	-
E3	VP1	-	-
E4	VP2	+	+
F1	VP3	+	+
F2	VP4	+	-
F3	VP5	-	+
F4	VP7	-	-
G1	VP10	-	-
G2	TH1	-	+
G3	TH2	-	-
G4	TH3	-	-
H1	TH4	-	-
H2	TF1	+	-
H3	TF2	-	-

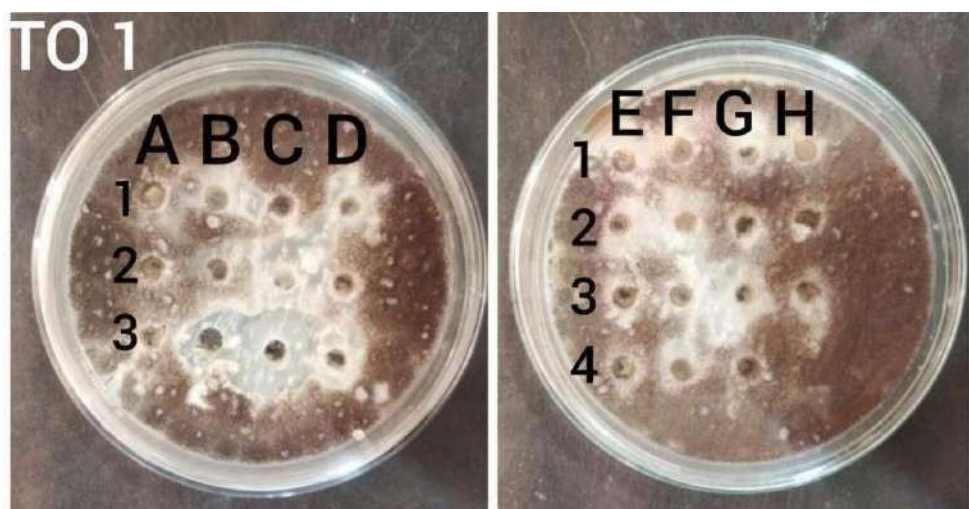


Fig 5.8 Antimicrobial screening of TO1

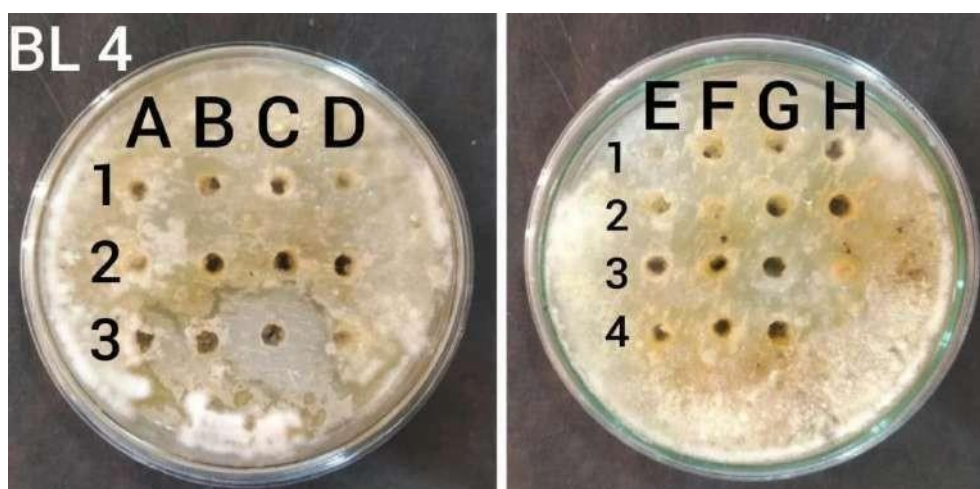


Fig 5.9 Antimicrobial screening of BL4

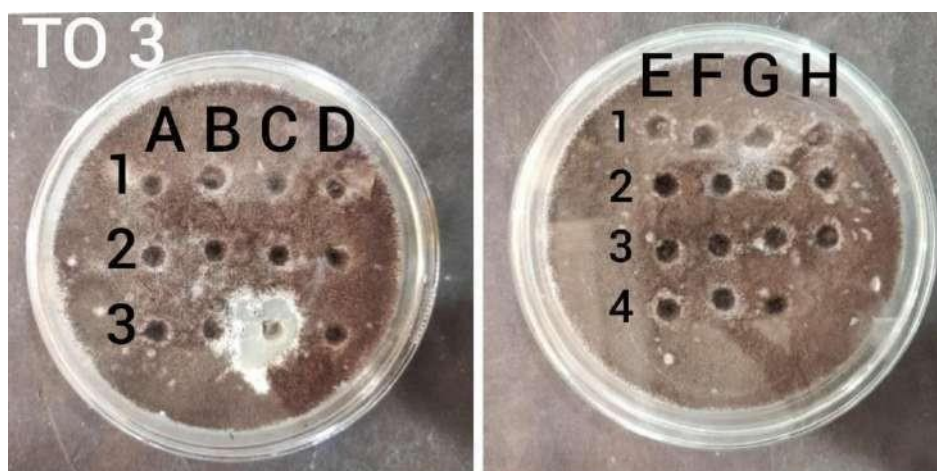


Fig 5.10 Antimicrobial screening of TO3

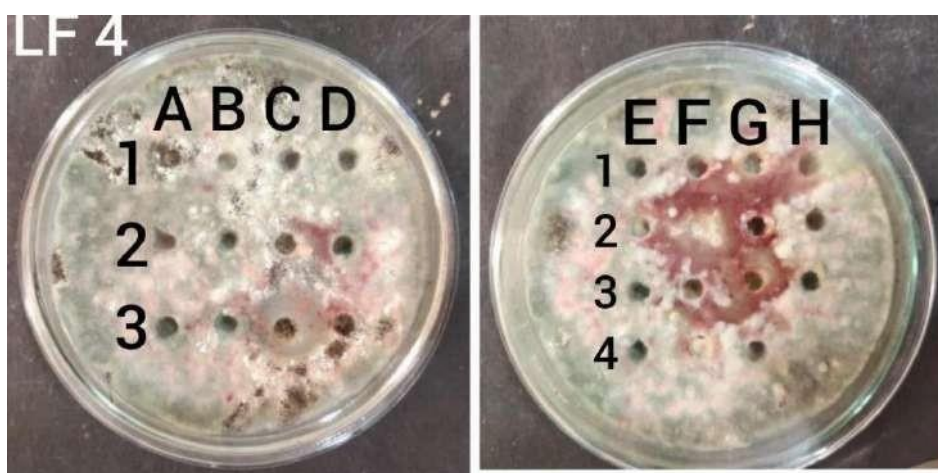


Fig 5.11 Antimicrobial screening of LF4

Table 5.4 Antimicrobial activity of TO1, BL4, TO3, LF4

S.no.	Isolation of staining	Screening Code			
		TO 1	BL 4	TO3	LF4
A1	TP1	-	-	-	-
A2	TP4	-	-	-	-
A3	TP5	-	-	-	+
B1	TP5.1	-	-	-	-
B2	TP6	-	-	-	-

B3	TP6.1	+	-	-	-
C1	TP7	-	-	-	-
C2	TP7.2	+	-	-	-
C3	TP8	+	+	+	+
D1	GA1	-	-	-	-
D2	GA2	-	-	-	-
D3	GA3	-	-	-	-
E1	GA4	-	-	-	-
E2	GA5	-	-	-	-
E3	VP1	-	-	-	-
E4	VP2	-	-	-	-
F1	VP3	-	-	-	-
F2	VP4	+	-	-	-
F3	VP5	-	-	-	-
F4	VP7	-	-	-	-
G1	VP10	-	-	-	-
G2	TH1	-	-	-	-
G3	TH2	+	-	-	-
G4	TH3	-	-	-	-
H1	TH4	-	-	-	-
H2	TF1	-	-	-	-
H3	TF2	-	-	-	-

5.4 SECONDARY SCREENING:

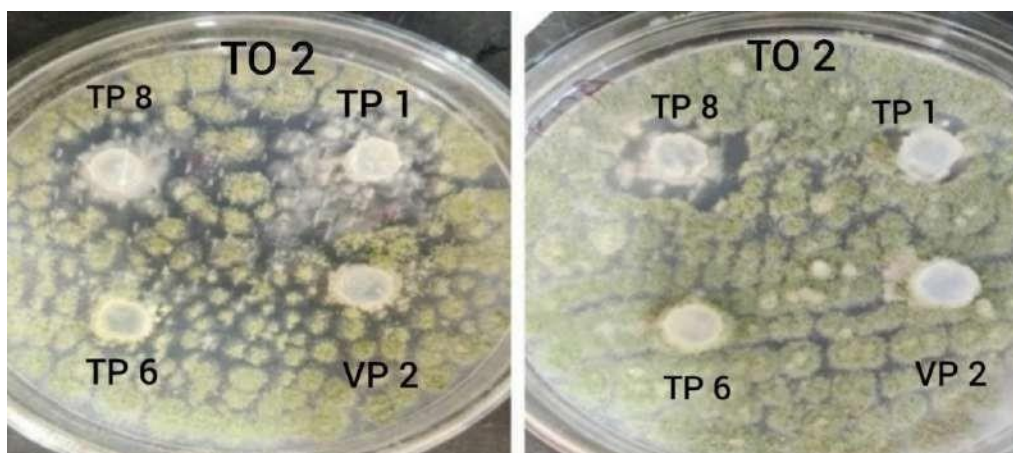


Fig 5.12 Secondary screening of TO2 and LF4

In this study Out of 16 bacteria stains, 4 different stains were taken for screening from that TP1 and TP8 stains shown bioactivity against different major fungal pathogens.

Table 5.5 Secondary Screening of TO2, LF4

Isolation of staining	Screening code			
	TO 2	TO 2.1	LF 4	LF 4.1
TP1	22 mm	-	18 mm	20 mm
TP8	20 mm	20 mm	18 mm	12 mm
TP6	-	-	-	-
VP2	-	-	-	-

5.5 PRODUCTION OF POTENT ISOLATE TP8 STAIN:



Fig 5.13 Production of Potent isolate

Among two positive isolates TP8 showed bioactivity against 8 major different morphological types of fungal pathogens, hence TP8 selected as potent isolate
Production of bioactive compounds from potent isolate (TP8) by shake flask method

5.6 EXTRACTION OF BIOACTIVE COMPOUND FROM POTENT ISOLATE:



Fig 5.14 Ethyl acetate extract



Fig 5.15 Chloroform extract

Ethyl acetate and chloroform is added separately to the solution and the mixture is shaken or stirred to allow the ethyl acetate and chloroform to extract the compound from the solution. The mixture is then allowed to settle, and the ethyl acetate layer is separated from the aqueous layer using a separating funnel. The ethyl acetate layer is then evaporated to obtain the purified compound



Fig 5.16 Confirmation test for TP8 potent isolate against TO1 and TO2

5.7 PHYTOCHEMICAL SCREENING:

The preliminary phytochemical study's findings, which were showed certain secondary metabolites present in the ethyl acetate fungal extract. Glycosides are crucial in the fight against microbial and insect predation. Likewise, herbivores. It is well known that steroids have antibacterial effects. In particular, steroidal chemicals interact with membrane lipid and cause liposome leakages.

Terpenoids, a group of phytochemicals with antibacterial activities that disrupt membranes, are also cell wall inhibitors. Eventually, saponins may have an impact on bacterial development. In this study the, qualitative assessment of ethyl acetate extract indicates presence of alkaloids and Tannis

In this study, tannis and alkaloids compounds are present in ethyl exctract compound

Table 5.7. Results of Phytochemical screening

Chemical screening test	Results
Flavonoids [Alkaline reagenttest]:	Absence
Flavonoids with ferric chloride test	Absence
Saponins	Absence
Alkaloids	Presence
Tannis	Presence
Terpenoids	Absence
Sterols	Absence
Anthraquinones	Absence

5.8 ANTIMICROBIAL SCREENING WITH EXTRACT:

In previous study. Antifungal was evaluated against *Aspergillus fumigates* and *Aspergillus flavus*. The ethyl acetate extract showed maximum zone of inhibition as compared to other extracts. Chloroform extract showed mild antifungal activity. Furthermore, the highest percentage of phenolic and flavonoid compounds was estimated in ethyl acetate extract [Kundan Singh Bora *et al.*, 2018]

In this study, antimicrobial activity of the compounds extracted with ethyl acetate and chloroform was performed. The ethyl acetate extract showed highest zone of inhibition as compared to chloroform. Chloroform showed mild antimicrobial activity.

Furthermore, the percentage of alkaloids and tannins compounds was present in ethyl acetate extract.

Table 5.7 Result of extracted solvents

Extracts with solvent	Screening code				
	TO 1	TO 2	BL4	LF1	LF4
Ethyl acetate	-	+	+	+	+
Chloroform	-	+	-	-	-
TP8 Stain	+	+	+	+	+

5.9 CHARACTERIZATION OF FUNGI

In this study, Observing and identifying the different morphological fungal pathogens by slide culture technique and lacto phenol cotton blue

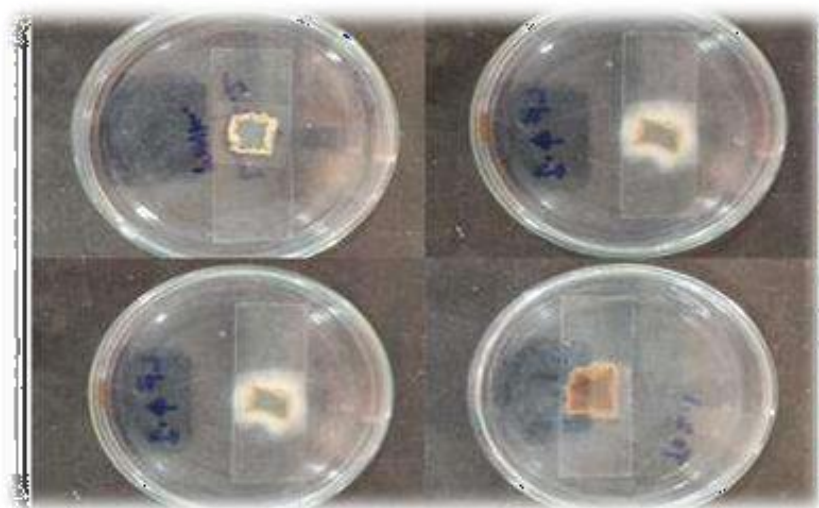
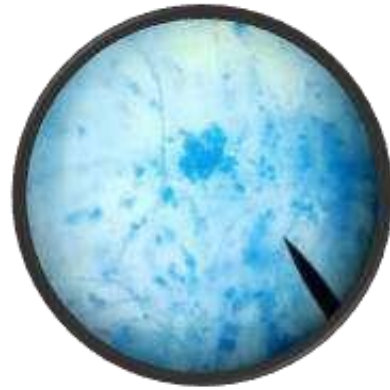


Fig 5.17 Slide culture technique



TO1 - Aspergillus



TO2 - Alternaria



BL4-Basidioboul



LF4- Fusarium



LF1- Microsporium nanum

Fig 5.18 LPC Microscopic view

5.10 CHARACTERIZATION OF PURE BACTERIA ISOLATE:

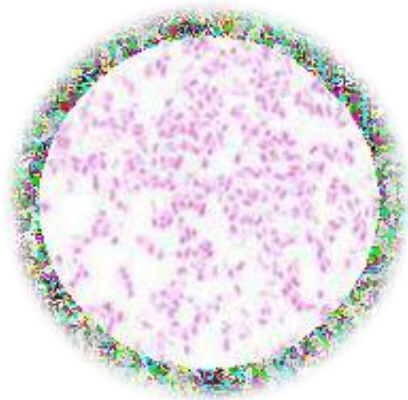


Fig 5.19 microscopic view of potent isolate

In this study, gram staining and biochemical tests were used to analyse the characteristics of bacterial isolates. In contrast, the bacteria isolates were gram negative.

Table 5.8 Characterization of potent isolate

Characterization	Result
Gram staining	Gram negative [Short rod]
Motility	Non motile bacteria
Catalase	Negative
Oxidase	Negative
Endospore staining	Non sporing bacteria
Capsule staining	Negative

CHAPTER-6

SUMMARY AND CONCLUSION

The infected three different plants namely Tomato, Brinjal, Lady's finger was collected from different agricultural lands and isolated fungus from the leaf's. Totally 6 fungal pathogens were obtained. The soil samples are collected from different aeras and isolated the bacteria from soil. Totally 27 bacteria stains were isolated. Antimicrobial activity was done by well diffusion method for 6 fungal pathogens. Out of 27 different isolated, TP8 stain were selected for potent isolate .TP8 stain Production were performed and then extraction was done by the chemical compound ethyl acetate and chloroform. The extracts obtained from extraction were carried out for antimicrobial by the using disc diffusion method and results were noted. Phytochemical screening was performed. The potent isolate stain was subjected to various identification techniques. They were identified by considering their morphological characteristics Gram staining, motility test, endospore staining, capsule staining and Bio chemical test (catalase, oxidase). The different 6 fungal pathogens were identified by slide culture technique and lactophenol cotton blue staining. Finally in this study we found that bioactivity activity of TP8 stain ethyl acetate extract showed maximum zone of inhibition as compared to chloroform extract against fungal pathogens (TO1, TO2, TO3, BL4, LF1, LF4) whereas Chloroform extract showed mild bioactivity.

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