BIOCONTROL OF PHYTOPHTHORA CAPSICI OF CAPSICUM ANNUUM BY USING FORMULATION OF AGERATUM CONYZOIDES AND PARTHENIUM HYSTEROPHORUS

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Botany

By

Rajeev Kumar

Registration Number: 12009930

Supervised By

Dr. Seweta Srivastava (21896)

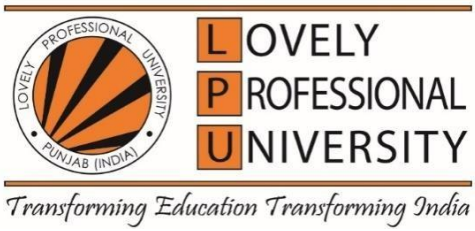
Department of Plant Pathology (Associate Professor)

Lovely Professional University

Co- Supervised By

Dr. Indu Sharma

Department of Biotechnology (Associate Professor) NIMS University Rajasthan

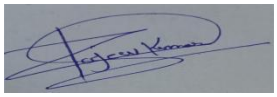


LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2024

DECLARATION

I, hereby declared that the presented work in the thesis entitled **"Biocontrol of *Phytophthora capsici* of *Capsicum annuum* by using formulation of *Ageratum conyzoides* and *Parthenium hysterophorus*"** in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of **Dr. Seweta Srivastava,** working as Associate Professor, in the Department of **Plant Pathology /School of Agriculture** of **Lovely Professional University**, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



(Signature of Scholar)

Name of the scholar: Rajeev Kumar

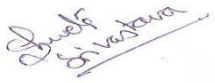
Registration No.: 12009930

Department/school: Botany/ Bioenginering and Biosciences

Lovely Professional University, Punjab, India

CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled **"Biocontrol of *Phytophthora capsici* of *Capsicum annuum* by using formulation of *Ageratum conyzoides* and *Parthenium hysterophorus***" submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Botany, School of Bioenginering and Biosciences, is a research work carried out by Rajeev kumar is 12009930 bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



(Signature of Supervisor)

Dr. Seweta Srivastava

Designation: Associate Professor

Department/school: Plant Pathology, Agriculture

University: Lovely Professional University.

(Signature of Co-Supervisor)

Dr. Indu Sharma

Designation: Associate Professor

Department/school: Biotechnology

University: NIMS, University, Rajasthan.

ACKNOWLEDGEMENT

With profound reverence and utmost humility, I bow my head in gratitude to **Radha Soami Baba Ji**. Your divine presence has been the guiding star in my life, illuminating my path through every challenge and uncertainty. Every step of this journey was made possible by Your boundless blessings, and I remain forever indebted to You for the strength, patience, and perseverance You instilled in me.

I extend my sincere gratitude to my supervisor, **Dr. Seweta Srivastava**, Associate Professor, Department of Agricultural Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab. Her invaluable guidance, scholarly advice, and unwavering support have been instrumental in the successful completion of my research. Her words of encouragement and constructive feedback have significantly contributed to my academic growth.

My heartfelt thanks go to **Dr. Joydeep Dutta**, Head of the Department of Botany/Zoology, School of Bioengineering and Biosciences, and to Assistant Professors **Dr. Meenakshi Rana** and **Dr. Vipul Kumar**, of the Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab. Their constant encouragement, insightful suggestions, and immense support have served as a great source of motivation throughout my academic journey.

I am deeply grateful to the Head of School, **Dr. Neeta Raj Sharma,** for their leadership and for fostering an excellent academic environment that has significantly contributed to my growth.

Special thanks to **Ms. Sandeep Kaur, Mr. Rahul Joshi, Mr. John,** and **Mr. Parvez**, Laboratory Technicians at the School of Bioengineering and Biosciences. Their technical expertise, unwavering support, and friendly cooperation made my research experience seamless and enjoyable.

I also express my heartfelt gratitude to my friends and colleagues, including **Sanofar Khokher, Rumana Rehman, Sapna Yadav, Manpreet Kaur, Ranjana Sharma, Lovepreet Kaur, Ritu Kumari**, and **Gourav Kumar**. Their constant encouragement, support, and companionship have been invaluable throughout my academic journey. The shared experiences and cherished memories will remain close to my heart.

My profound gratitude and affection go to my beloved parents, **Sh. Sher Singh** and **Smt. Usha Rani**, who have been my pillars of strength. Their encouragement has always uplifted me, and their faith in my abilities has been my constant motivation. Every achievement I celebrate is a reflection of their endless efforts and unconditional love. Their unwavering support, sacrifices, and unconditional love have given me the courage to persevere. They have instilled in me the values of honesty, perseverance, and compassion, which have guided me throughout my life.

To my Uncle Ji, **Mr. Bijender Kumar**, your wise counsel and constant encouragement have been a source of immense motivation. I will forever cherish the support and warmth you have shown me, serving as a guiding light in both my personal and academic pursuits.

I am deeply thankful to my brother, **Mr. Ashish Kumar**, and my Bhabhi Ji, **Mrs. Manjeet Kaur**, for

their steadfast belief in my abilities and their words of encouragement. Their support and pride in my accomplishments have meant the world to me.

To my adorable nephew, Master **Jiyansh Bhuryan**, and my lovely niece, **Pakhi Bhuryan**, your innocent laughter and unconditional love have brought immeasurable joy and light to my life. Your presence has been a constant reminder of the simple joys that make every effort worthwhile.

Lastly, I extend my gratitude to **Lovely Professional University** for providing me with the opportunity to study at this prestigious institution. The university's exceptional learning environment and state-of-the-art facilities have played a pivotal role in my academic growth.

To all who have contributed to my journey, I am forever grateful. Your kindness, support, and belief in me have left an everlasting imprint on my heart.

With deepest gratitude,

Rajeev Kumar

LIST OF ABBREVATION

a.i.	Active ingredient
BC	Before Christ
BOD	Biological Oxygen Demand
cm^2	Centimetre square
CRD	Completely Randomized Design
ºC	Degree Celsius
°E	Eastern latitude
EC	Emulsifiable Concentrate
G	Grams
i.e.	In other words
ITCC	Indian Type Culture Collection
Kg	Kilogram
kg/ha	Kilogram per hectare
KB	Kings B Media
L	Litre
m^2	Meter square
µg/ml	Microgram per milliliter
µm	Micrometer
ml	Milliliter
mm	Millimeter
viz.	Namely
°N	Northern latitude
Ppm	Parts per million
PDI	Percent Disease Index
±	Plus or Minus

PFT	Poison Food Technique
PDA	Potato Dextrose Agar
PSI	Pound-force per square inch
q/ha	Quintals per hectare
RWC	Relative Water Content
CAM	Carrot Agar Media
spp	Species
SC	Suspension Concentrates
TSM	Trichoderma Selective Media
USA	United State of America
WP	Wettable Powder

TABLE OF CONTENTS

Declaration		ii
Certificate		iii
Acknowledgement		iv-v
List of Abbreviations		vi-vii
List of Content		viii-ix
List of Table		x
List of Figure		xi-xiii
Abstract		xiv-xvi
1.	**INTRODUCTION**	**1-4**
	Objectives of the study	
2.	**REVIEW OF LITERATURE**	**5-24**
2.1.1	*Capsicum annuum* L	5
2.1.2	Classification of *Capsicum annuum* L	5
2.1.3	Origin and Distribution	6
2.1.4	History	6
2.1.5	Genetic Diversity	6
2.1.6	Biological Significance	6
2.2.1	*Phytophthora capsici*	7
2.3.1	Management	13
2.3.2	*Ageratum conyzoides* extract effect in different fungus	14
2.3.3	*Parthenium hysterophorus* extract effect in different fungus	16
2.4.1	Classification of *Ageratum conyzoides*	17
2.4.2	*Ageratum conyzoides* L	17
2.4.3	Synonyms	18
2.4.4	Vernacular name	18
2.4.5	Botanical Description	18

2.4.6	Geographical Distribution	18
2.4.7	Chemical constituents	19
2.4.8	Pharmacological Activities	19
2.5.1	Classification of *Parthenium hysterophorus* L	20
2.5.2	*Parthenium hysterophorus* L	20
2.5.3	Description of the plant	21
2.5.4	Chemical content	22
2.5.5	Pharmacological activity	22
2.6.1	Research Gap	23
3.	**MATERIALS AND METHODS**	**25-38**
3.1	Collection and isolation of *Phytophthora capsici* from the soil sample	25
3.1.1	Isolation of *Phytophthora capsici*	25
3.1.1.1	Method of Isolation of *P.capsici*	25
3.2	Identification of *P. capsici*	26
3.2.1	Scanning Electron Microscope Analysis	27
3.2.2	18s Molecular Sequencing	27
3.3	Preparation of formulation from *Ageratum conyzoides* and *Parthenium hysterophrous* for biological Control of *P. capsici*	28
3.3.1	Plant Material used in the study	28
3.3.2	Preparation of plant extract	29
3.3.3	Preparation of Weed Extracts	29
3.4	Evaluation of anti-fungal activity	30
3.4.1	Bioassay	30
3.4.1.1	Antifungal activity of *Ageratum* and *Parthenium* plant extracts on test pathogen	31
3.5	In vivo evaluation of plant extracts against the test pathogen	31
3.6	Inoculation of *P. capsici* in the field	34
3.6.1	Sterilization of Glasswares and tools	34

3.6.2	Culture Media preparation	34
3.7	Mass multiplication of *P. capsici*	34
3.8	Inoculation	35
3.8.1	Severity of P. capsici blight in *Capsicum annuum* L	35
3.8.2	Percent Disease Index	36
3.9	Biochemical analysis of Gas Chromatography Mass Spectra	37
3.9.1	FTIR Analysis	38
3.10	Statistical Analysis	38
4.	**RESULTS AND DISCUSSION**	**39-123**
4.1	Pathogen Identification	39
4.2	In-situ management of *Phycopthora capsici* with Biofungicides	39
4.2.1	Treatment with Acetone (A)	55
4.2.2	Treatment with Chloroform (C*)*	56
4.2.3	Treatment with Ethanol (E)	57
4.2.4	Treatment with Methanol (M)	58
4.3	Overall Interpretation Supported by Permanova Analysis and P-Values	58
4.4	GC-MS Analysis	59
5.	**SUMMARY & CONCLUSION**	**124-128**
	REFRENCES	**129-144**

LIST OF TABLES

S.NO.	Tables	Page No
1.	Field layout for testing the efficacy of Biofungicides	32
2.	Disease index scale	36
3.	Effect of different aqueous extract of *Ageratum* and *Parthenium* on percent disease index (PDI)	41
4.	Effect of *Ageratum* and *Parthenium* extract on percent germination of infected *Capsicum annuum* at different concentrations	44
5.	Effect of *Ageratum* and *Parthenium* extract on seedling fresh weight of infected *Capsicum annuum* at different concentrations	46
6.	Effect of *Ageratum* and *Parthenium* extract on seedling height at 35 days after sowing of infected Capsicum annuum at different concentrations	48
7.	*Ageratum* + Acetone compounds	61-66
8.	*Parthenium* + Acetone compounds	67-72
9.	*Ageratum* + Ethanol compounds	74-79
10.	*Parthenium* + Ethanol compounds	80-83
11.	*Ageratum* + Methanol compounds	85-88
12.	*Parthenium* + Methanol compounds	89-96
13.	*Ageratum* + Chloroform compounds	98-101
14.	*Parthenium* + Chloroform compounds	102-106
15.	Compound in methanolic extract of Fresh *Capsicum annuum*	108-114
16.	Compound in methanolic extract of Infected fruit of *Capsicum annuum*	116-121

LIST OF FIGURES

S.NO.	Figure	Page No
1.	Study Map	26
2.	Morphological structures of *Phytophthora capsici* : A) Colony morphology on carrot agar medium	27
3.	A and B *Ageratum conyzoides* L. Flower, Stem and Leaves, C *Parthenium hysterophorus* whole plant	28
4.	Method of Cold Mecerration of Extraction	30
5.	Prepared Field for Sowing	33
6.	*Capsicum annuum* L. at the Seedling stage	33
7.	Zoospore dispersal of *P. capsici*	35
8.	Severity of *P. capsici* blight in *Capsicum annuum* L. after inoculation	36
9.	Life cycle of the *Phytophthora capsici* organism that causes foliar blight, root, crown, and fruit rot in *Capsicum annuum* plants	37
10.	Morphological structures of *Phytophthora capsici*: A) Colony morphology on carrot agar medium; B) Coenocytic mycelium (CM) and papillate limoniform sporangia (PS) with branched sporangiophore	39
11.	Scanning electron microscopy: A. coenocytic mycelium, B. branched sporangiophore C. papillate sporangia	40
12.	18S gene sequence-inferred phylogenetic relationships of *Phytophthora capsici* identified in cultivated *capsicum* crop, the analysis also includes the 18s gene sequence of selected, well-studied *Phytophthora capsici* sps.	41
13.	Percentage inhibition of *Ageratum conyzoides* Extract fungus growth by the weed plant extracts by modified poison food assay	45

14.	In vitro inhibition percentage of acetone extract of A*geratum conzoides* and *Parthenium hysterophorus* against *Phytophthora capsici*	47
15.	In vitro inhibition percentage of chloroform extract of *Ageratum conzoides* and *Parthenium hysterophorus* against *Phytophthora capsici*	49
16.	In vitro inhibition percentage of ethanol extract of Ageratum conzoides and Parthenium hysterophorus against Phytophthora capsici	52
17.	In vitro inhibition percentage of methanol extract of *Ageratum conzoides* and *Parthenium hysterophorus* against *Phytophthora capsici*	54
18.	*Ageratum conyzoides* + Acetone compound peak with compound (A+A)	56
19.	*Parthenium hysterophorus* + Acetone compound peak with compound (P+A)	57
20.	*Ageratum conyzoides* + Ethanol compound peak with compound (A+E).	58
21.	*Parthenium hysterophorus* + ethanol compound peak with compound (P+E)	73
22.	*Ageratum conyzoides* + Methanol compound peak with compound (A+M).	84
23.	*Parthenium hysterophorus* + Methanol compound peak with compound (P+M)	97
24.	*Ageratum conyzoides* + Chloroform compound peak with compound (A+C).	107
25.	*Parthenium hysterophorus* + Chloroform compound peak with compound (P+C)	115
26.	FT-IR spectrum of leaf powder of *Ageratum conyzoides* and *Parthenium hysterophorous*	112
27.	GC-MS Chromatogram of methanolic Infected fruit extract of *Capsicum annuum*	112

28.	FT-IR Spectrumof leaf powder of *Ageratum conyzoides* and *Parthenium hysterophorus*	123

ABSTRACT

Capsicum annuum L. is a semi-perennial herbaceous plant typically grown as an annual crop, especially in India. *Capsicum annuum* L. is an important genus for the nutritional, economic and cultural values of its species. At the same time, the *Capsicum* species are affected by diseases caused by viruses, bacteria, fungi and pseudo fungi, in particular the oomycete *Phytophthora capsici.* The most detrimental disease to bell pepper production among allthose affecting bell pepper cultivation is blight disease, which is caused by *Phytophthora capsici*. It is a notorious oomycete pathogen, poses a significant threat to bell pepper production worldwide. Its devastating effects lead to substantial economic losses in the yield.

Phytophthora blight, caused by *Phytophthora capsic*i affects all parts of plants, causing rot, blight, and significant harm and losses in *Capsicum* species. During two years of soil collection, February-November 2021-2022, damping off and blight symptoms were observed. Seventy-two isolates were obtained from rhizosphere soil samples of the infected *Capsicum annuum* plants. *Phytophthora capsici* Leonian was identified based on morphological and cultural characteristics, with the culture being deposited in the Indian Type Culture Collection (ITCC) New Delhi, as ITCC No.11865.23 and 11871.23.18S conserved region gene sequencing of the ITS region was undertaken to confirm species identity. Aphylogenetic tree confirmed that the obtained ITS region sequences were identical to *Phytophthora capsici* isolates. This is the first report of *Phytophthora* blight on bell peppers from the Haryana region of India.

Recently, non-chemical approaches have received lots of attention from researchers due to their eco-friendly nature. The ultimate goal of this research is to present a comprehensive assessment of some bioformulations for preserving bell pepper quality during storage, alongside their mechanism of action on spoilage pathogen. The leaf extracts of *Ageratum conyzoides* and *Parthenium hysterophorus* showed promising anti-fungal activity to combat *Phytophthora capsici* in *Capsicum.* Alleopathic and pests resistance nature of these plants make them a better sustainable substitutes to synthetic fungicides. These extracts have been found to be effective in suppressing the growth of *Phytophthora capsici*, and illustrated their possible role as environmentally friendly solutions for disease management in *Capsicum annuum* L.

Agricultural plants produce phytotoxins known as “allelochemicals” through the natural and eco-friendly process of allelopathy, which can either stimulate or impede the plant growth. *Phytophthora capsici*-infected bell pepper plants seed germination and early seedling growth were examined in relation to the allelopathic effects of varying concentrations of aqueous extracts of two prevalent weed species, *Ageratum conyzoides* and *Parthenium hysterophorus*.

The objective of the current investigation was to determine the effect of different leaf extract concentrations of *Parthenium hysterophorus* and *Ageratum conyzoides* on (PDI), percent disease index, seed germination, seedling fresh weight and seedling height of blight of bell pepper. Various aqueous extracts of the two weed species showed percentage inhibitions ranging from 74.82% to 91.16%. Out of all four aqueous extracts of these two test weeds, ethanolic extract of leaves had shown the best antimicrobial effect.

The GC-MS analysis revealed that *Ageratum conyzoides* and *Parthenium hysterophorus* extracts exhibit promising anti-fungal properties and contain distinct groups of active compounds, suggesting potential differences in mode of action and potency their application in plant disease management. The chemical substances produced through allelopathy can be a potential tool to manage important plant diseases. Allelopathy can replace synthetic pesticides for crop disease management.

In vitro antifungal properties of *Ageratum conzoides* and *Parthenium hysterophorus* leaves with different solvents viz., ethanolic, methanolic, acetonic and chloroform have been also investigated against *Phytophthora capsici*. Acetone (A) and chloroform (C) treatments consistently demonstrated higher inhibition percentages, with the Permanova analysis likely affirming the statistical significance of these findings (p & lt; 0.05). These solvents, particularly at a 10% concentration, appear to be the most effective in inhibiting *Phytophthora capsici* when using biofungicides derived from *Parthenium* and *Ageratum*. The low standard deviations, especially in acetone and chloroform treatments, indicate result reliability and consistency, a characteristic likely supported by the Permanova analysis and associated p-values. The detection of bio molecule composition using FTIR spectroscopy has been shown to be accurate and sensitive.

Phytophthora capsici presents a worst-case scenario to growers due to its vast host

range, frequent production of long-lived latent sexual spores, substantial genotypic variability, and rapid asexual disease cycle. The necessity for sustainable management strategies to protect commodities from *P. capsici* and other oomycetes is becoming increasingly evident. *P. capsici* is an acceptable model for research since it is easy to culture in the lab and infect a wide range of plant species. This makes it useful for experiments on sexual reproduction, host range, and pathogenicity. Sexual reproduction, host range, and pathogenicity experiments can benefit from this. The *Phytophthora* blight disease of *Capsicum annuum*, which is caused by *Phytophthora capsici*, has never been reported in the Haryana region. The discovery of *Phytophthora capsici* poses a serious threat to the production of *Capsicum* in India and around the world, so surveillance needs to be enhanced to predict future outbreaks.

Keywords:- *Capsicum annuum,* Bicontrol, *Phytophthora capsici,* GC-MS, *Ageratum conyzoides*, *Parthenium hysterophorus,* Bacteria, Fungi.

Encouragement for Organic and Natural Farming

"Daan karan kaahu ko nahin, jey jaanai aapai koi. Amrit naam nidhaan hai, mil piavai bhojan soi."

"One should not claim ownership of nature's gifts. The real treasure is the divine nectar, and the best food is that which is naturally obtained."
(Guru Granth Sahib, Ang 1291)

CHAPTER I

INTRODUCTION

A semi-perennial herbaceous plant, *capsicum annum* L. is commonly grown as an annual crop, particularly in India and other countries (Bhatt et al., 2016). It is one of the most popular and nutrient-dense vegetables and a member of the Solanaceae family. It is widely cultivated worldwide, especially in India.

It is indigenous to tropical South America, particularly Brazil. In India, it is widely grown in Tamil Nadu, Karnataka, Haryana, and Himachal Pradesh, and a few locations in Uttar Pradesh. It is a summer vegetable widely grown in Himachal Pradesh's mid-hills and supplied to the plains. In North India, it is referred to as Shimla Mirch. The fruits have thick flesh with a mild flavour, are huge with a basal depression, inflated, and yellow or red. Vitamins, especially A, B6, and C, as well as calcium and folic acid, are abundant in bell peppers (Kumar et al., 2022). When compared to the United States, Holland, Italy, France, and other nations that cultivate *Capsicum,* India produces a very small amount of bell pepper (Costa et al., 2019).

The major fungal diseases of capsicum crops are damping off (*Pythium aphanidermatum* and *Phytophthora* spp.), leaf spots (*Cercospora capsica* and *Alternaria solani*), anthracnose and ripe rot (*Colletotrichum capsici*), fruit rot and leaf blight (*Phytophthora* spp.), powdery mildew (*Erysiphe cichoracearum* and *Leveillula taurica*), early blight (*A. solani*), wilt (*Fusarium oxysporum*), frog eye rot (*Phaeoramularia capsicicola*), leaf spot (*Septoria lycopersici*), fruit spot (*Phoma destructiva*), stem rot (*Macrophomina phaseoli*), dry rot (*Sclerotium rolfsii*), and fruit rot (*Phomopsis* spp.). The post-harvest rots are caused by *Aspergillus terreus*, *A. candidus*, *A.niger*, *F. moniliforme*, *F. sporotrichoides*, *Paecilomyces variotii*, and *Penicillium corylophilum* (Jadon et al., 2016).

It is a perishable vegetable with a short shelf life and a significant risk of illness, despite its significance (Tiamiyu et al., 2023). The primary cause of low production of bell pepper is that this crop is becoming contaminated with viruses, fungus, bacteria, and mycoplasmas, significantly reducing possible yields while being transported and stored (Jabeen et al., 2023). Among various biotic factors, diseases caused by fungi are critical because fungal spores are easily spread through wind, seed, infected debris, humans etc (Babasaheb Khaire Mahatma Phule Krishi Vidyapeeth et al., n.d.). Bell

pepper is vulnerable to a number of fungal illnesses, including as leaf blight and fruit rot. caused by *Phytophthora capsici.*

P. capsici can infect pepper plants at all growth stages, leading to seedling death, root rot, crown rot, stem blight, leaf spot, and fruit rot (Babadoost et al., 2015). *P. capsici* induces symptoms at various phenological stages of the plant (Lee et al., 2008), based on host resistance, affected tissue, and climatic conditions. The root system exhibits brown rot as the first tissue to be impacted (Saltos et al., 2021a). Later, brown lesions with a rough shape can be detected in the crown tissues (Steekelenburg, 1980). Other symptoms observed in adult plants are stunting and generalized wilting (Barchenger et al., 2018). Small, black, water-soaked lesions that eventually develop into necrotic lesions with a light brown core and dark rims are the first signs of leaf blight in the foliar region (Hyder et al., 2018). Fruits are also affected by the oomycete, initially, minute lesions with clear whitish centres are observed in the tissue (Naegele and Hausbeck, 2014). advancing rapidly until this organ completely rots (Granke et al., 2012). Young fruit often exhibits this last symptom, whereas mature fruits are more resistant to *P. capsici* (A. Moreira-Morrillo et al., 2023).

In the cultivation of peppers (*Capsicum* spp.), this disease has become a formidable foe since it has a severe negative impact on both yield and quality (Parada-Rojas et al., 2021). It is a damaging soilborne pathogen that infects more than fifty species of vegetable crops that are detrimental to agriculture and cause significant financial losses (Wang et al., 2022). Pepper plants are susceptible to *P. capsici* infection at any stage of growth, which may result in crown rot, stem blight, root rot, leaf spot, fruit rot and seedling death (Babadoost et al., 2015). *P. capsici* can cause losses in *Capsicum* up to 100% as a result of its rapid spread in field conditions and yields around $100 million in losses per year (Barchenger et al., 2018). Because of its polycyclic nature and aerial tissues reach, P. capsici is one of the most difficult phytopathogens to control (A. Moreira-Morrillo et al., 2023) (Sharma et al., 2023) (Rhouma et al., 2024).

It is undeniable that fungicides are effective in controlling these diseases. However, they have also been shown to cause negative side effects, such as environmental pollution, phytotoxicity, and fungicide resistance to plant pathogens. Consequently, it was suggested that biological control of *Phytophthora*-induced illnesses might be a

more environmentally friendly option than traditional chemical treatment (Volynchikova and Kim, 2022). The two plant species, *Ageratum conyzoides* and *Parthenium hysterophorus* are renowned for their vast range of phytochemicals contained in them. Based on the bio-activity of both plants, it has been demonstrated that they both possess antibacterial and anti-fungal characteristics (Motmainna et al., 2023). Allelopathy refers to the direct, indirect, stimulatory, or inhibitory impacts plants have on one another by chemical release into the surrounding environment (Choudhary et al., 2023). Allelochemicals are the compounds released during this process, which are typically secondary metabolites (Shan et al., 2023). The leaf extracts of *Parthenium hysterophorus* and *Ageratum conyzoides* include a wide variety of allelochemicals that have been discovered and recognized. The plant defense system uses these compounds (Banaras, 2021; Eridas et al., 2023; Motmainna et al., 2023). Depending on the organisms and allelochemical concentration, allelopathy may have both beneficial and detrimental consequences, according to recent study (Xu et al., 2023).

Ageratum conyzoides L., belonging to the Asteraceae family, is an allelopathic plant native to the tropical Americas but extensively found in tropical and sub-tropical regions. Thriving as an adventitious plant alongside both annual and perennial crops, it demonstrates a notable capability to resist pests and diseases attributed to its production and release of allelochemicals (Jayasundera et al., 2021) . *A. conyzoides* has a long agriculture history and is known for its broad variety of bioactive chemical components. It has proven to be an efficient treatment for phytopathogenic fungus (Kapeua Ndacnou et al., 2020) . There are roughly thirty species in the genus *Ageratum*, however, only a few have been studied phytochemically (Chahal et al., 2021). *Ageratum* leaf extract and essential oil have allelopathic effects on a range of cultivated crops (Javaid et al., 2020) (Ferdosi et al., 2021). It secretes phenolic chemicals that allelopathically affect agricultural plants, including gallic acid, coumaric acid, protocatechuic acid, p-coumaric acid, sinapic acid, and carboxylic acid (Jayasundera et al., 2021).

The Asteraceae family includes the invasive herbaceous plant *Parthenium* (*Parthenium hysterophorus* L.). It is presently found in 88 nations, with half of those locations believed to be outside of the plant's native habitat (Mao et al., 2021). It was

originally discovered in Nepal in 1982 and has been found in India (Devkota et al., n.d.). Even though the plant is quite infamous for its negative impact on biodiversity, agriculture, and human beings, it shows some positive results in the eradication of some bacteria and fungi, especially *Phytophthora capsici* (Tafese Bezuneh, 2015). In addition to exudates from their roots, it is believed that *Parthenium* weed plants release a considerable amount of allelochemicals from their leaves, stems, and flowers during the rainy season (Shi and Adkins, 2020). Studies have so far looked at how Parthenium weed extracts affect test plant germination, seedling growth, shoot and root growth, plant biomass generation, and the amounts of nitrogen and chlorophyll in the leaves (Bashar et al., 2023).

Recently, non-chemical approaches have received lots of attention from researchers due to their ecofriendly nature. This research presents a comprehensive assessment of some bioformulations for preserving bell pepper quality during storage. It also presents their mechanism of action against spoilage pathogens. The leaf extracts of *Ageratum conyzoides* and *Parthenium hysterophorus* showed promising anti-fungal activity to combat *Phytophthora capsici* in *Capsicum*. These plants have an alleopathic nature and are pest-resistant, making them a better and more sustainable alternative to synthetic fungicides. These extracts have been found to be effective in suppressing *Phytophthora capsici*. They illustrate their possible role as environmentally friendly solutions for disease management in *Capsicum annuum* L.

Objectives of the study

Therefore, keeping in the view of above facts, the present investigation aims to achieve the following objectives:

1. Isolation and identification of soil born pathogenic fungi *Phytophthora capsici*.
2. Collection and characterization of *Ageratum conyzoides* and *Parthenium hysterophorus* leaves for extraction.
3. Formulation for effective biocontrol against *Phytophthora capsici* pathogen in *Capsicum annuum* L.

CHAPTER II

REVIEW OF LITERATURE

2.1.1. Classification of *Capsicum annuum* L.

Division: Spermatophyta

Sub-division: Angiospermae

Class: Dicotyledones

Tribe: Solanales

Family: Solanaceae

Genus: *Capsicum*

Species: *annuum*

(Smith et al., 1987) (Devi et al., 2021)

2.1.2. *Capsicum annuum L*

Capsicum annuum L., commonly known as chili pepper or bell pepper, is a flowering plant in the Solanaceae family. Originating from the Americas, it has a rich history of cultivation and culinary use, contributing significantly to global cuisines and traditional medicine (Iqbal et al., 2023) . *Capsicum annuum* L. is a widely cultivated plant species valued for its culinary, medicinal, and ornamental properties. Belonging to the Solanaceae family, it includes a diverse range of cultivars, from mild bell peppers to fiery chili peppers. Understanding the origin and evolution of *Capsicum annuum* L. is crucial for elucidating its genetic diversity, adaptation mechanisms, and socio-economic importance (Perry and Flannery, 2007).

Thesis and Delelegn, (2011) claims that over the 20th century, pepper production and consumption increased globally. Similar to other members of the Solanaceae family like potatoes and tomatoes, peppers have gained prominence in international cuisines. As evidenced by the large areas of land dedicated to their cultivation in countries like Mexico, China, India, the United States, Korea, and Africa.

2.1.3. Origin and Distribution:

Capsicum annuum L.'s origin can be traced back to the Americas, with evidence of domestication dating back at least 6,000 years ago. Indigenous peoples of Central and South America cultivated various chili pepper varieties for food, medicine, and religious ceremonies. The plant's cultivation spread to other parts of the world through trade and exploration, leading to its widespread distribution in Asia, Africa, and Europe (Bosland et al., 2022).

2.1.4. History:

Phylogenetic studies have shed light on the evolutionary history of *Capsicum annuum* L. and its wild relatives. Molecular analyses suggest that the genus Capsicum diverged from its closest relatives around 16 million years ago. This divergence was driven by factors such as climate change, pollinator interactions, and human selection. Domestication of *Capsicum annuum* L. likely occurred independently in multiple regions of the Americas, resulting in the emergence of distinct cultivars adapted to local environmental conditions. (Aggarwal et al., 2023)

2.1.5. Genetic Diversity:

Capsicum annuum L. exhibits remarkable genetic diversity, encompassing a wide range of fruit shapes, sizes, colors, and pungency levels. Recent genomic studies have identified key genes associated with traits such as capsaicin biosynthesis, fruit development, and disease resistance. Understanding the genetic basis of these traits is essential for crop improvement efforts aimed at enhancing yield, quality, and resilience to biotic and abiotic stresses (Paran and van der Knaap, 2007).

2.1.6. Biological Significance:

Beyond its culinary uses, *Capsicum annuum* L. possesses various biological properties with potential applications in medicine, agriculture, and industry. Capsaicin, the compound responsible for the plant's pungency, exhibits analgesic, anti-inflammatory, and anti-cancer effects, making it a valuable pharmacological agent. Additionally, *Capsicum annuum* L. cultivars play a crucial role in pest management, as they resist certain insect pests and pathogens. (Kim et al., 2023)

The global disease of pepper (*Capsicum* sp.) that causes rots in the fruit, stem, and roots is called *Phytophthora capsici* Leonian (Ribeiro, 1978) (Stamps et al., 1990) . Farmers and technicians typically don't know the disease until the upper portion of the pepper vine exhibits symptoms including yellowing, wilting, and falling leaves. By

the time these symptoms appear, the infection has progressed to a severe level, with the roots mostly rotting and a brownish-black lesion visible on the underground stem. At this point, secondary microbes had taken over the contaminated collar. The disease spreads quickly, particularly in the rainy season, and plants die in two to three weeks. Given that the pathogen is soil-borne, soil and water are the primary channels for spreading (Saltos et al., 2021b).

According to Manohara, (2004), the initiation of *Phytophthora* diseases' epiphytotics is significantly influenced by the inoculum carried by the soil (Onesirosan, 1971). Due to its severity, *phytopthora* foot rot is considered the most significant illness. Known as the most destructive black pepper disease, *phytopthora* foot rot can result in an annual crop loss of 5–10% (Kueh, 1982) and up to 95% for individual farmers (Manohara et al., 2004). According to reports, the Keralan districts of Kozhikode and Kannur have an annual crop loss of over a thousand tonnes due to *Phytophthora* foot rot disease (Sibi et al., 2008). Losses from *Phytopthora capsici* can reach thirty percent.

According to Winters (2019), resistant and tolerant cultivars can be used to effectively treat root rot. Furthermore, it is essential to use biological control agents, chemical therapies, and cultural customs. Cultural practices can influence root infections caused by soil-borne pathogens, either directly or indirectly (Winter et al., 2019).

2.2.1. *Phytophthora capsici*:

Phytophthora, sometimes called the "plant destroyer," is one of the most studied taxa in the class Oomycetes. It is a pathogenically significant genus in the family Peronosporaceae and order Peronosporales, and it is a member of the kingdom Chromista (Sanogo et al., 2023).

Anandaraj, 2000 reported that foot rot disease in Kerala caused 30% losses. According to surveys carried out in the Karnataka districts of Chikkamagaluru, Shivamogga, and Sirsi, Jahagirdar and Siddaramaiah (2002) found that the average frequency of black pepper foot rot was 48.24% in 1997 compared to 44.64% in 1996. (Ashoka et al., 2021)

According to (Abdul Haq et al.,2022), the genus *Phytophthora* comprises severe plant infections that can be found in a variety of environments, including rain forests and

agricultural and non-agricultural systems (Anandaraj, 2012). Anton de Bary gave the term *Phytophthora* (a Greek word meaning "plant destroyer") in 1876. *Phytophthora*, as its name suggests, is one of the most damaging plant pathogens ever discovered. This can be partially explained by looking at its evolutionary history and basic biology (Abdul Haq et al.,2022).

Its life cycle requires free water because it resembles algae. Sporangia, the asexual reproductive structures of *Phytophthora*, discharge motile zoospores that can ignite explosive epidemics. Furthermore, *Phytophthora* contains a range of long-lived propagules that are difficult to eradicate and can endure for many years in a latent phase (Cooke, 2015). Clonal and sexual reproduction is made possible by the extraordinarily adaptable mating systems of *Phytopthora* species (Brasier et al., 2022).

Its genetic makeup allows for evolutionary flexibility that facilitates its adaptation to a variety of plant hosts and habitats. Plant parts can become infected with *Phytophthora*. The majority of *Phytophthora* species cause root disease and basal stem cankers when they infect below ground. In these situations, *Phytophthora's* existence and activity are overestimated (Cooke, 2015).

According to Sikora et al. (2012), Numerous plant pathogenic species have been identified since the identification and reporting of *P. infestans*, the infamous disease responsible for the Irish famine. In their book "*Phytophthora* worldwide," Erwin and Ribeiro (1996) evaluated the species of the genus *Phytophthora* and described 59 species with five variations. Afterwards, several newly discovered *Phytophthora* spp. A large number of species from around the globe were characterised; by 2012, over a hundred species had been identified (Sikora et al., 2012).

Hausbeck (2004) noted at a recent International Union of Forest Research Organisations (IUFRO) conference that the importance of plant pathogens in agriculture is based on their capacity to endure long enough to be dispersed as possible inoculum. *Phytophthora* species thrive in a variety of settings throughout the year. They create a variety of tiny structures that are highly effective at starting illnesses on their own. (Molnar et al., 2020)

Chlamydospores and oospores are the two types of spores *Phytophthora* generates intended for long-term survival. Asexual chlamydospores can form intercalary or

terminally at hyphae tips. They are spherical to oval in form and feature walls that are either thick or thin. An oospore is a sexual spore with strong walls that can survive desiccation, severe weather, and other environmental conditions. Without a live host, they can persist in soil for many years (Hausbeck, 2004).

When the right circumstances arise, these latent structures in the soil can come to life. They can lay dormant for years. The oospore wall can reach up to 3 μm in thickness, but the chlamydospore wall is typically between 0.5 and 1.5 μm thick. According to Erwin and Ribeiro (1996), chlamydospores can live up to six years and oospores up to thirteen years. In addition to these, hyphal swelling and zoospores are two further ways *Phytophthora* might persist. They have a few weeks to live at most. (Kasteel et al., 2023)

The encysted zoospores can live for several months, contrary to Tsao's 1991 publication. According to Erwin and Ribeiro (1996), *Phytopthora* species are often regarded as weak saprophytes, which restricts their ability to survive without a host. According to Anandaraj's (1997) research, *P. capsici* has poor competitive saprophytic ability. As a result, adding organic amendments to the soil encourages saprophytes, which lower *P. capsici* populations (Nysanth et al., 2022).

These zoospores had a 20-hour motile phase, which allowed them to disperse by irrigation. Under hunger, *P. parasitica* mycelia fragments undergo protoplasmic contraction; yet, they can survive in untreated waste water for up to 40 days. It has been noted that *Pythium ultimum* and other *Phytophthora* spp. survive similarly. (Pánek and Střížková, 2021)

Schick (1932) first noted *P. infestans* races when resistant hybrid potato cultivars were introduced. It has long been known that isolates within a species differ in their pathogenicity. The virulence of *phytophthora* varies greatly depending on the host. Plant pathologists and plant breeders consider this diversity significant. (Giachero et al., 2022) There may be variations in the pathogenic race type or aggression level. A pathogen's ability to infiltrate, proliferate, and reproduce on or within its host plant is known as its aggressiveness. (Maillot et al., 2022).

Pathogens' capacity to cause illness is called pathogenicity. However, the disease's severity is determined by the isolates' virulence. The Latin word "virulentus," which

means "full of poison," is where the word "virulence" originated (Wain, 1952). Pathogens can be distinguished from non-pathogens by their virulence.

(Silvar et al., 2006) reported virulent, moderately virulent and highly virulent isolates from pepper infected by *P. capsici* in Spain. Distinct pathogenic strains of *Phytophthora capsici* from eggplant, pepper, pumpkin, squash, tomato, and watermelon have been reported (Sanogo et al., 2023)

P. capsici isolates were categorised into 13 groups by Tamietti and Valentino (2001) based on their capacity to infect various plant types. According to their findings, every isolate was harmful to bell peppers, 95% to squash, 79% to tomatoes, 58% to nightshades, 38% to eggplants, 33% to peas, 20% to melon, and 8% to French beans. When (Lee et al., 2001) examined the virulence of *P. capsici* isolates from pepper and pumpkin on pumpkin cultivars, they discovered variations in aggressiveness. The variations in pumpkin plants' vulnerable and resistant reactions to *Phytophthora* blight were quantifiable instead of qualitative, as no cultivar of pumpkin that had received a *P. capsici* infection displayed hypersensitive reactions. (Sanogo et al., 2023).

Hantula et al. (2000) studied the pathogenicity of *P. cactorum* strains on Betula pendula and strawberries. The investigation demonstrated that the strains were more virulent on hosts than non-host plants and tended to specialise on particular hosts. Assessed the relative virulence of *P. capsici* isolates from cucumber and squash on pepper. Found that pumpkin isolates were more aggressive towards all tested cultivars than pepper isolates. Significant interspecific interactions were also observed. This implies that pumpkin isolates might have unique virulence factors for interactions with pumpkin compared to pepper. (Saltos et al., 2022)

(Suseela et al., 2011) used the stem inoculation technique to observe different levels of response among 491 cultivar accessions, 691 hybrids, 124 wild accessions, 182 Karimunda, and 60 Kottanadan selections of black pepper (*Piper nigrum*) against foot rot disease. The accessions were indexed using a 0–4 scale, and the depth of penetration of the infection was noted.

(Fry et al., 2015) Investigated how functionally asexual *P. infestans* gave rise to new and sophisticated races. She concluded that DNA fusion occurs when several races are raised together on a vulnerable host, creating distinct races with the combined

specificities of their parents. According to Goodwin (1997), there are several sources of genetic variation in the *Phytophthora* population, including mutation, parasexual recombination, mitotic recombination, and interspecific hybridization.

According to (Papavizas et al., 1981), certain combinations of *Phytophthora* isolates that harm pepper have produced both pathogenic and non-pathogenic strains of the fungus. In his investigation into pathogenicity variation causes, Thakur (1999) identified mutation, genetic drift, recombination, gene flow, and selection as potential causes. Additionally, he stated that the main goal of research on pathogenicity or virulence variation is to breed and utilise resistance for the purpose of managing disease.

Phytophthora spp. from a variety of hosts, including black pepper (*P. capsici*), rubber (*P. meadii*), cocoa (*P. palmivora*), cardamom (*P. meadii*), areca nut (*P. meadii*), betelvine (*P. parasitica* and *P. capsici*), and coconut (*P. palmivora*), were evaluated for their cross-infectivity by (Vijaya 2008). The *P. capsici* isolates were pathogenic to arecanut, rubber, black pepper, betel vine, small cardamom, cocoa, and coconut, and they exhibited a broad host range. While cocoa (*P. palmivora*), tiny cardamom (*P. meadii*), black pepper (*P. capsici*), and betel vine (*P. parasitica* and *P. capsici*) isolates could infect black pepper, rubber (*P. meadii*), arecanut (*P. meadii*), and coconut (*P. palmivora*) isolates were not pathogenic to black pepper (Jayalakshmi et al., 2021).

Islam et al., (2004) investigated the morphological, pathogenic, and genetic differences between *P. capsici* isolates from Illinois fields that processed cucurbita, moschata, and pumpkins. Using Random amplified polymorphic DNA (RAPD) markers, the genetic variation between the isolates was examined. The cluster analysis yielded six RAPD groups, or six pathogenicity groups, based on the separation of the 24 *P. capsici* isolates. (Samen et al., 2003) found no significant link between the virulence, RAPD, and AFLP groups of 32 *P. infestans* single zoospore isolates.

According to (Wang et al., 2006), *P. sojae's* pathogenicity varies constantly throughout asexual reproduction. Since acquired virulence was stable, it was determined that virulence variations were caused by mutations and gene expression variations. Based on RAPD analysis, DNA fingerprints were unchanged. Suppression Subtractive Hybridization (SSH) cDNA library enriched for sequences differentially expressed in the isolates was created using this approach. A total of 74 unigenes were

identified, and a majority of these were elevated from incompatible to compatible interactions with *P. sojae*. *P. sojae's* pathogenicity fluctuates continuously during asexual reproduction. It was concluded that mutations and differences in gene expression were the causes of the virulence changes because acquired virulence remained constant. The isolates' DNA fingerprints showed little variation, according to RAPD analysis. This method was used to construct a Suppression Subtractive Hybridization (SSH) cDNA library enriched for sequences differentially expressed in the isolates. In all, 74 unigene were identified; most of these genes were upgraded from incompatible to compatible interactions between P. sojae and its host.

Using microsatellite markers, (Ivors et al., 2006) investigated genetic variance among *Phytophthora ramorum* isolates in US nurseries. Allelic frequencies at 12 microsatellite loci showed modest variance within both US and EU populations, but significant variation between continents. The majority of microsatellite allelic variations were found in nurseries. These variations may have resulted from repeated exchanges of pathogen genotypes via infected plant material. They may also have resulted from strong selection pushing for the selection of novel genotypes produced by mutation or mitotic recombination. This may have resulted from both mechanisms combined. Such selection pressure in nurseries may also be caused by cultural practises and chemical treatments.

Using isozyme analysis, divided 84 *P. capsici* isolates into three categories. The isolates from cucurbit and annual solanaceous hosts, as well as from a few isolates of black pepper and cocoa *P. palmivora* MF4 isolates that have been previously characterised by Aragaki and Uchida's (2001) Hawaii isolates of *P. tropicalis* are included in the second category, CAP2, which is primarily composed of isolates from tropical crops such as black pepper, cocoa, papaya, macadamia, and rubber. Brazil's cocoa isolates belong to the third and least genetically varied category. Using 15 different enzymes, Mchau and Coffey's (1995) isozyme tests revealed two subgroups for 113 P capsici isolates. This validates the redaction by Tsao (1991) that unifies *P. capsici* kinds. (Rai, 2020) distinguished four groups from *P. capsici* pepper isolates based on mitochondrial (mt) DNA RFLP patterns.

2.3.1. Management:

In order to prevent, lessen, and maintain disease severity below the economic threshold of crop damage, the integrated management strategy for any plant disease, including those caused by *P. capsici*, is essentially based on the selection and application of genetic, cultural, physical, biological, and chemical measures. (Abrol, and Shankar, 2012).

Artemisia absinthium is an important medicinal plant with strong antioxidant activity. According to (Ali et al., 2015) in his study they use aqueous extract of *A. absinthium* to inhibit *Phytophthora* spp. Plant-based extract is always cost effective and environment friendly.

P. capsici at 0.1–0.2% concentration. Extracts from garlic and alamanda are also similarly efficient in preventing *P. capsici* from growing at varied concentrations, such as 1:2, 1:3, and 1:4. Therefore, plant extracts could be favored over chemical fungicides when considering costs and environmental effects. The cucurbit vegetable dhundal may be a preferable choice to plant because of its resistance to *P. capsici*. (Akter et al., 2017)

Secondary metabolites found in plants can be helpful in the postharvest management of phytopathogenic fungus. These substances can be extracted from plant tissue or administered as essential oils or plant extracts derived from various plant organs. (Villa et al., 2015) The exploration of novel, eco-friendly alternatives to chemical fungicides is crucial for effective plant fungal infection management. Study assesses botanical fungicides, revealing their potential in combating various phytopathogenic fungal species, including F. *oxysporum*, *F. solani*, *Goetrichum sp*., and *Phytophthora capsici*, isolated from chayote fruit. Notably, cinnamon essential oil demonstrates complete inhibition of fungal growth, suggesting its efficacy in curbing common fungal diseases during chayote fruit postharvest. Moreover, neem oil exhibits substantial inhibition against *F. solani* and *Phytophthora capsici,* while sapote extract displays efficacy against the genus *Geotrichum*. These findings underscore the promise of these natural extracts as viable alternatives for controlling specific fungal strains, thereby offering sustainable solutions to agricultural practices. (García-Ramírez et al., 2023)

According to La Torre In his study evaluated natural products for controlling *Phytophthora infestans* and *Phytophthora capsici*. Tagetes minuta, Lavandula

angustifolia, and ascorbic acid inhibited *P. infestans* in vitro with 91.1%, 91.3%, and 100% radial growth inhibition, respectively. In vivo, treatment with 1.0% *Tagetes minuta*, 0.5% *Lavandula angustifolia*, and 0.5% Adigor formulation reduced disease severity. Armicarb 85 and ascorbic acid showed efficacy against *P. capsici*, inhibiting mycelial growth by 100%. Results support using safe natural products for *Phytophthora* disease management. (La Torre et al., 2023)

According to (Luong et al., 2024) The study explored the antifungal potential of a methanolic extract from *Glycyrrhiza uralensis Fisch*. root against plant pathogens *Fusarium oxysporum* and *Phytophthora capsici*. The extract exhibited significant inhibition, with EC50 values of 113.4-191.4 µg/ml for *F. oxysporum* and 84.7-190.1 µg/ml for *P. capsici* over a nine-day period post-treatment, showing dose-dependent activity. Minimum inhibitory concentration (MIC) values ranged from 50-400 µg/ml for both pathogens. Observation revealed distinct effects: *F. oxysporum* hyphae showed abnormalities and atrophy, while *P. capsici* hyphae displayed swelling and increased branching after treatment. These findings shed light on *G. uralensis Fisch*. extract's antifungal properties, promising eco-friendly solutions for fungal control in agriculture.

2.3.2. *Ageratum conyzoides* extract effect in different fungus

Study assessed the effectiveness of *Ageratum conyzoides* extracts against *Fusarium solani* Mart. (Sacc.), a pathogenic fungus affecting eggplants. Aqueous methanolic and n-hexane extracts from different parts of *A. conyzoides* were tested at concentrations of 2%, 4%, and 6% w/v. All extracts significantly inhibited *F. solani* growth, with n-hexane extracts from leaves and inflorescence showing the highest reduction (84%), followed by stem (80%) and root (72%). Methanolic extracts from leaves were most effective, with up to 78% reduction, followed by inflorescence (74%), stem (63%), and root (59%). Aqueous extracts also showed significant reductions, with leaf extract leading (72%), followed by inflorescence, stem, and root, respectively. (Javed et. al., 2012)

The study investigated by Yusnawan, 2016, the efficacy of methanolic extracts from three weeds, namely ageratum (*Ageratum conyzoides* L.), spiny amaranth (*Amaranthus spinosus* L.), and coco-grass (*Cyperus rotundus* L.), in controlling

peanut rust disease caused by *Puccinia arachidis*. The extracts, obtained by macerating the weeds in methanol, were tested at various concentrations on uredospores and infected peanut plants. Results showed that 5% extracts of ageratum and coco-grass significantly inhibited spore germination by 78-80% and 76-80%, respectively. Disease intensity on treated peanut plants with 5% ageratum extract was notably lower compared to untreated plants and chemical applications. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids in the ageratum extract. Overall, the 5% *Ageratum* extract demonstrated promising potential as a botanical fungicide for managing peanut rust disease, offering a sustainable alternative to chemical control methods. (Yusnawan, and Inayati, 2016).

According to Banaras 2021 focused on identifying potential antifungal components in various parts of *Ageratum conyzoides* L. to combat *Macrophomina phaseolina*. Methanolic extracts from stems and leaves exhibited significant inhibition of fungal biomass, with stems showing the highest efficacy (20–83%). Further fractionation of stem extract using n-hexane, chloroform, ethyl acetate, and n-butanol yielded potent antifungal fractions. Chloroform sub-fraction displayed the highest activity (56–93% reduction), followed by n-butanol, ethyl acetate, and n-hexane sub-fractions. GC-MS analysis of the chloroform sub-fraction identified compounds such as 2H-1-benzopyran and hexadecanoic acid, suggesting their role in antifungal activity. (Banaras et al., 2021)

Study by Hazirah, 2023 found that different concentrations of plant parts function best. For example, *Ageratum conyzoides* leaf extract can exhibit maximum inhibition at 8% concentration, while stem and inflorescence extracts can both exhibit maximum inhibition at 10% concentration. It was also discovered that a larger concentration corresponds to a higher inhibition percentage. Consequently, a higher dosage might be employed in the next investigation to determine the maximal suppression of *Fusarium oxysporum* development or to eradicate the fungus entirely. (Hazirah et al.,2023).

2.3.3. *Parthenium hysterophorus* extract effect in different fungus

In the laboratory conditions of Devkota, and Sahu, 2017 a study was conducted to assess the phytochemical screening and antifungal properties of *Parthenium*

hysterophrous L (Asteraceae) leaves. Distilled water and methanol extracts was prepared and tested against five phytopathogenic fungi at various concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml). Phytochemical analysis revealed the presence of terpenoids, saponins, flavonoids, tannins, and alkaloids. Antifungal activity was evaluated using the poisoned food technique, with methanol crude leaf extract exhibiting superior effectiveness compared to the distilled water extract, as indicated by linear mycelium growth reduction percentages. (Devkota, and Sahu, 2017)

Parthenium hysterophorus, an invasive weed, competes aggressively, posing risks to health, livestock, the environment, soil, and agriculture. Despite these risks, its potential for antidiabetic, antioxidant, antitumor, herbicidal, pesticidal, and antimalarial therapies warrants further study. It's a viable fertilizer, herbicide, insecticide, and phyto-remedial mediator. Its morphology, reproduction, environmental impacts, and management has been outlined. Effects of methanol, ethanol, hexane, acetone, and aqueous extracts are discussed. Given its status among the world's seven worst weeds, control measures, including mechanical, chemical, cultural, and biological methods, are explored. Managing *Partheniums* allelopathy proves challenging, with both positive and negative interactions. (Bashar et al., 2021)

According to Bashar, 2023 study demonstrated that natural herbicides derived from *P. hysterophorus* are both cost-effective and eco-friendly alternatives to synthetic chemicals for weed control in crop fields. Methanol extracts from *P. hysterophorus* leaf, stem, and flower were tested at concentrations of 25, 50, 75, 100, and 150 g L-1, compared to a control of distilled water. As concentrations increased, seed germination and seedling growth of various crops decreased significantly, indicating allelopathic potential. Leaf extracts showed higher potency compared to stem and flower extracts, attributed to their higher phenolic compound content. This was confirmed by EC50 values, principal component analysis, and LC-MS analysis. This suggests that *P. hysterophorus* leaf phenolic compounds hold promise as natural herbicides for sustainable weed control in crop production. (Bashar et al., 2023)

A bioassay was conducted to assess the antifungal potential of organic solvents (ethanol and methanol) and an inorganic solvent (aqueous) using extracts from *Nerium indicum* (leaves, shoots, and flowers) against two fungal pathogens

(*Alternaria* and *Fusarium*) isolated from *Aegle marmelos*. Ethanol exhibited superior antifungal activity compared to methanol and aqueous, though all solvents showed efficacy against the fungal isolates. Aqueous demonstrated the least effect. Antifungal activity was evaluated by measuring the zone of inhibition, with the commercial fungicide fluconazole serving as a control for comparison. (Ojha, and Goyal, 2024

2.4.1. Classification of *Ageratum conyzoides*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Tracheophyta

Order: Magnoliopsida

Family: Asterales

Genus: *Ageratum*

Species: *conyzoides*

(Thorat et al., 2018)

2.4.2. *Ageratum conyzoides* L.

Ageratum conyzoides L. plants belong to the family Asteraceae and are commonly known as goat wildflower in English, Pumpillu in Tamil and Visadodi in Hindi. It's a polymorphic, sweet-smelling annual herb that grows in tropical rain forests. It is naturalized as a weed throughout India, and distinctive varieties of this spic are found in different parts of India (Baroroh, 2020). *Ageratum conyzoides* L. is also found in the lower subansiri region of Arunachal Pradesh. The class Ageratum is derived from the Greek term ‘a geras’ meaning non-maturing, which alludes to the long life-time of plants. Therefore, the species designation ‘konyz’ is derived from the Greek term elecampane, which appears to be a plant. The plant can be recognized by its light green color and blooms in corymbs are light blue or violet blue. It blooms from September to October. (Kaur et al., 2023)

2.4.3. Synonyms

Ageratum album, Ageratum cordifolium, Ageratum latifolium, Ageratum obtusifolium, Ageratum odoratum.

2.4.4. Vernacular name:

English: Goa Weed, Appa Grass

Hindi: Visadodi

Kannada: Uralgidda (Nayitulasi)

Malayalam: Muryampacha, (Kattappa, Appa, Muriyan)

Sanskrit: Visamustih

Tamil :Pumpillu, Sinnapoompillu, Vadaichedi

2.4.5. Botanical Description:

A. conyzoides is a straight, split basil with narrow, rubbery roots once a year. Depending on the ecological situation, it could range from 5 to 15 cm. Quite large in blossoms. The shoots, which originate somewhere in the center of the ground, but are soft on the top, are tube-shaped, solid, and mature in old age; little silver hairs surround the lumps and early parts of the stem. The leaves are about 2-5 cm long, 0.5-5 cm broad, on bushy petioles 0.5–7.5 cm elongated, broadly egg-shaped, and round. The leaf surfaces are finely hairy, irregular with protuberant veins and wrinkled leaves have a distinguishing odour evocative of the male goat. The branch, at the end of the inflorescence, allows 5–17 flower heads to be organized into popular and impressive flat-topped bunches. White, light blue and blueish-white blossom heads are carried in flower clusters of 50-150 mm with 56–70 tubular blossoms. The blossom nut is enclosed by two to three rows of rectangular bracts that are green and pale (Erida et al., 2023).

2.4.6. Geographical Distribution:

Ageratum conyzoides plants are found all over the world, and side by side different spices are also found. These are the countries where *Ageratum conyzoides* grows. L is abundant in India, Brazil, Cuba, Fiji, Gambia, Germany, Ghana, Indonesia, Italy, Japan, Malaysia, Netherlands, Nigeria, Philippines, Taiwan, Thailand Vietnam, Zambia, Australia and Zimbabwe (Kumar et al., 2023).

2.4.7. Chemical constituents:

According to reports, fresh leaves include flavones such as derivatives of chromenes, sesquiterpenes, and sinensetin, as well as flavones A, B, and C. An isoflavone glycoside is present in the stem, and the entire plant has sterols. Sterols, pyrrolizidine alkaloids, polymethoxylated flavonoids, flavones, and chromenes (Kaur, 2021).

2.4.8. Pharmacological Activities:

According to Garg and Grewal (2015), ageratum conyzoides has antiseptic effects outside of an artificial environment.

Ageratum conyzoides is a poisonous wildflower viewed as cutting allelopathy for yield. The current review has been directed at 4 distinctive dissolvable concentrates of *A.conyzoides*. Analyze the antimicrobial potential with regards to infectious microorganisms in an environment outside of the living organism in order to determine if they are effective. *Ageratum conyzoides* extract apply against five infectious organisms, including Gram positive *B. subtilis, S. aureus* as well Gram negative *E. coli, K. pneumoniae.* In the test thought, big restraint zones had been recorded on petroleum ether as well $(CH_3)_2CO$ remove in Mansa and Mandi populaces individually in contradiction of every one of the four infectious microscopic organisms that had similar to normal anti-infection drug i.e chloramphenicol. Direct hindrance development was seen in chloroform as well as methanol separate cultures of microbes. These results from the review showed that *Ageratum conyzoides* demonstrates occouring inconstancy in antiseptic properties. This might be due to the presence of natural components. These plants have antimicrobial properties.

(Odeleye et al., 2014) examined Ageratum's early plant biochemical and antibacterial activities against *A. conyzoides* (L) on a few medicinal microbial isolates. They assert that the Agar-well diffusion method was used to identify the bactericide action of *A.conyzoides* methanol extracts on Shigella dysenteries, *E. coli, P. aeruginosa,* and *S. aureus*. Using established procedures, the minimum bactericidal concentration and the phytochemical characteristics of the extract pertaining to the test isolates were also examined. In relation to the extracts, the majority of the trial microorganisms were oriented to more than and equal to 50 mg/ml. *A. conyzoides* extract with a methanolic concentration showed excellent action against all species at both minimum inhibitory and minimum bactericidal concentrations.

Sathyanathan et al., (2013) reported the laxative effect of *A. conyzoides* on rats as a model organism. The current evaluation was conducted to evaluate the purgative effects of an ethanolic concentrate made from the whole plant of *Ageratum conyzoides* Lon on exploratory pale-skinned rats. To illustrate the purgative effect, eight-hour and sixteen-hour faecal yield and check/recurrence were used. In albino rats, an ethanolic extract of *Ageratum conyzoides* at doses of 200 mg/kg and 400 mg/kg significantly increased faeces production. The results strengthen the viability and validate the historical narrative's promise as a diuretic. Furthermore, research is anticipated to fully understand *Ageratum conyzoides* L. purgative effect system (Kotta et al., 2020).

2.5.1. Classification of *Parthenium hysterophorus* L.

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Asterales

Family: Asteraceae

Genus: Parthenium

Species: hysterophorus

(Kaur et al., 2014)

2.5.2. *Parthenium hysterophorus* L.

The region around the Gulf of Mexico, Southern North America, the West Indies, and Central South America are *Parthenium's* native habitats. Now a days, the weed has spread to over 20 countries worldwide, encompassing five continents and several islands (Sarita et al., 2024). According to recent events, African nations are at considerable risk of invasion. It has now spread to eight Chinese provinces and is growing frighteningly. *Parthenium* most likely got into India around 1910 (through tainted cereal grains), but wasn't discovered until 1956. Reports have come from Australia, India, Nepal, Africa, China, Vietnam, and the United States, Central America, South America, West Indies, and Lesser Antilles in the new globe. When the weed was introduced to India in 1910 as an attractive plant, it was unable to take off. In the 1950s, it returned to infiltrate Australia and India through contaminated wheat and pasture seeds imported into the United States of America. Reports have come

from Australia, India, Nepal, Africa, China, Vietnam, and the United States, Central America, South America, West Indies, and Lesser Antilles in the new globe. When the weed was introduced to India in 1910 as an attractive plant, it failed to take off. In the 1950s, it returned to infiltrate Australia and India through contaminated wheat and pasture seeds imported into the United States of America. Wastelands, public lawns, orchards, forestlands, flood plains, agricultural and urban areas, overgrazed pastures, industrial sites, playgrounds, roadsides, railway tracks and residential plots are among the many places where *parthenium* thrives profusely (Kumar et al., 2023). Drought and pasture cover reduction provide the appropriate environment for *parthenium* weed growth. *Parthenium* weed grows in various types of soil, but is most common in alkaline clay loam soils (Khan; and Fahad et al., 2020).

2.5.3. Description of the plant:

The herbaceous plant *Parthenium hysterophorus* is highly branching, has a brief life (annual), and grows uprightly (erectly), forming a basal rosette of leaves in its early stages of growth. Its typical height is 0.5 to 1.5 m, but it occasionally reaches 2 m or higher. Mature stems have a greenish colour, longitudinal grooves, small, stiff hairs covering them, and many branches as they reach maturity. In the early stages of growth, the simple, alternately oriented leaves create a basal rosette with stalks (petioles) that can grow up to 2 cm in length. (Mao et al., 2021). The bottom leaves are deeply divided (bi-pinnatisect or bi-pinnatifid), and they are comparatively large (3–30 cm long and 2–12 cm broad). In comparison to the lower leaves, the leaves on the upper branches are smaller and less split. Short, stiff hairs that lie close to the surface cover the undersides of the leaves and, to a lesser extent, their upper surfaces (they are appressed pubescent). At the terminals of the branches, terminal panicles are clusters of numerous tiny flower-heads, or capitula. Every flower head, or capitulum, is carried on a 1–8 mm long stalk, or pedicel. There are five small 'petals' (ray florets) on these white or cream coloured flower-heads (4-5 mm across), which are 0.3-1 mm long. Additionally, they have two rows of tiny green bracts (an involucre) surrounding them, and a large number of tiny white flowers (tubular florets) in the centre. When seeds reach maturity, their colour shifts to light brown. Although it can happen at any time of year, the wet seasons are when it happens most frequently. Each flower head (capitulum) typically produces five tiny "seeds," or achenes. These achenes, which are 1.5–2.5 mm long, are made up of a flat bract, two straw-colored papery structures

(which are actually dead tubular florets), and a black seed with two or three tiny scales (a pappus), measuring approximately 0.5–1 mm long. (Tiawoun et al., 2024)

2.5.4. Chemical content:

Secondary metabolites of plants, or phytochemicals, have a variety of pharmacological and biochemical impacts on living things. The entire plant is composed of flavonoids, parthenin, stigmasterol, and 6-hydroxykaempferol-3,7-dimethyl ether. Leaves contain essential oil, flavonoids, parthenin, acids, campesterol, and stigmasterol. Flowers have ambrosanoli. It has been discovered that parthenin and its derivatives are soluble in acetone, ethyl acetate, alcohol, chloroform, and ether. However, they are nearly insoluble in water. *Parthenium hysterophorus* extract components have varying boiling points. The highest being 165ºC to 220ºC for *Parthenium hysterophorus* extract and the lowest being 64.7ºC for methanol. This means that the constituents do not form an azeotropic mixture and can be easily separated using basic distillation procedures. It was suggested to separate sesquiterpene lactones from *Parthenium hysterophorus* using chemical engineering procedures (Soxhlet Extraction and Packed Bed. Extraction), which might be employed as insecticides or pesticides with efficacy. (Roy; and Kumar, 2023)

2.5.5. Pharmacological activity:

Parthenium hysterophorus, a plant utilized in ancient remedies, alleviates fever, diarrhea, and various medical disorders (Surib-Fakim et al., 1996). Ethnobotanical practices reveal its usage in treating inflammation, skin ailments, and reproductive issues among the Trinidad and Tobago communities. Pharmacologically, it has shown promise as an analgesic for muscular rheumatism and neuralgia, and as a remedy for helminthic infections (Maishi et al., 1998). Parthenin, its primary constituent, exhibits notable medicinal properties, including antitumor effects (Venkataiah et al., 2003).

Studies have demonstrated the plant's potential for inhibiting cancer cell growth, particularly against lymphocytic leukemia, HL-60, and HeLa cancer cell lines (Das et al., 2007). Anticancer properties have been established both in vitro and in vivo, with positive outcomes in terms of tumor size reduction and overall survival rates (Ramos et al., 2002). Additionally, water-based extracts have shown hypoglycemic activity in diabetic rats (Patel et al., 2008), suggesting a potential role in developing treatments for Diabetes Mellitus.

Furthermore, (Parashar et al., 2009) discovered the production of silver nanoparticles using extracts of *P. hysterophorus*, opening avenues for its utilization in nanotechnology-based industries. Eco-friendly nanoparticles hold promise in various applications such as antiseptics, wound healing, and electronic devices, making this discovery significant for the synthesis of alternative nanomaterials on a large scale (Kumar et al., 2022).

Parthenium hysterophorus, renowned as one of the world's most invasive weeds, has significantly damaged biodiversity, agriculture, and human/animal health. Despite its notorious reputation, studies indicate potential agricultural uses. *Parthenium* can serve as biopesticides, green manure, compost, soil amendment, and vermicompost. Its nutrient-rich nature, especially before flowering, makes it ideal for organic manure production through uprooting and burial. Application of *Parthenium*-derived green manure and compost enhances soil properties and crop yields. Additionally, its insecticidal and pesticidal properties offer potential for pest management. Researchers have explored diverse agricultural applications, highlighting *Parthenium's* multifaceted role beyond nuisance weeds (Sarita et al., 2024).

2.6.1. Research Gap

Fungi are the second most impactful disease-causing organism, wreaking havoc on global crop yields annually. Agricultural productivity suffers significant losses due to fungal, bacterial, and viral diseases. While chemical interventions like fungicides and pesticides have historically proven effective in disease control, their prolonged use poses considerable threats. Synthetic fungicides often persist in the environment, exhibiting heavy toxicity to both humans and animals. Moreover, resistant fungal strains complicate disease management.

In nature, numerous secondary metabolites act as crucial defense mechanisms for plants, possessing antifungal, antiviral, antibacterial, and insecticidal properties. Although chemical fungicides such as carboxin, mancozeb, and carbendazim have been extensively utilized, their widespread application contributes to environmental pollution and elevated toxicity levels in crops, fruits, and plants. Transitioning to biofungicides offers a sustainable alternative, mitigating ecological impacts while effectively controlling fungal diseases in crops and plants.

Biofungicides, derived from plant and microbial sources, have garnered attention for their eco-friendly nature and targeted action against pathogens. Various weed and plant extracts demonstrate significant inhibitory effects on fungal growth, including *Alternaria spp*., *Fusarium solani*, *Candida albicans*, *Pythium spp*., *Phytophthora capsici,* and *Puccinia spp*. Notably, extracts from *Ageratum conyzoides* and *Parthenium hysterophorus* leaves exhibit promising antifungal properties against *Fusarium solani* and *Phytophthora capsici*, commonly known as soil-borne diseases.

Exploring the synergistic associations between microorganisms and plants offers further avenues for biological control of plant pathogens. Beneficial microbes, antagonistic to pathogens, present a viable strategy for disease management. The shift towards biofungicides aligns with selectivity and biodegradability, reducing disease control practices' ecological footprint.

This research endeavors to bridge existing gaps by evaluating the efficacy of *Ageratum* and *Parthenium* leaf extracts, previously untapped resources, against *Phytophthora capsici* in *Capsicum annum* L crops. By isolating fungal pathogens from infected plants and soil, conducting in vitro and in vivo trials, and assessing the antifungal properties of these extracts, the study aims to contribute to the arsenal of biocontrol measures in agriculture.

CHAPTER III

MATERIALS AND METHODS

During the Rabi season of 2021–2023, field testing was conducted on the farm of Lovely Professional University, which is situated in Punjab's Trans-Gangetic plain agro-climatic zone and has an elevation of 252 metres above sea level. Its coordinates are 25 degrees North latitude and 75 degrees 23 minutes and 3.02 seconds East longitude, while laboratory tests were conducted in the Central Instrumentation Laboratory.

3.1 Collection and isolation of *Phytophthora capsici* from the soil sample

Samples of rhizosphere soil were taken from the infected Capsicum annuum plants. Yamunanagar district of Haryana, India. After removing the top 3-5 cm of soil, soil samples were taken at a depth of 1-3 cm around the root system. These samples were collected in polythene bags, brought to the laboratory, and kept at 4°C for analysis.

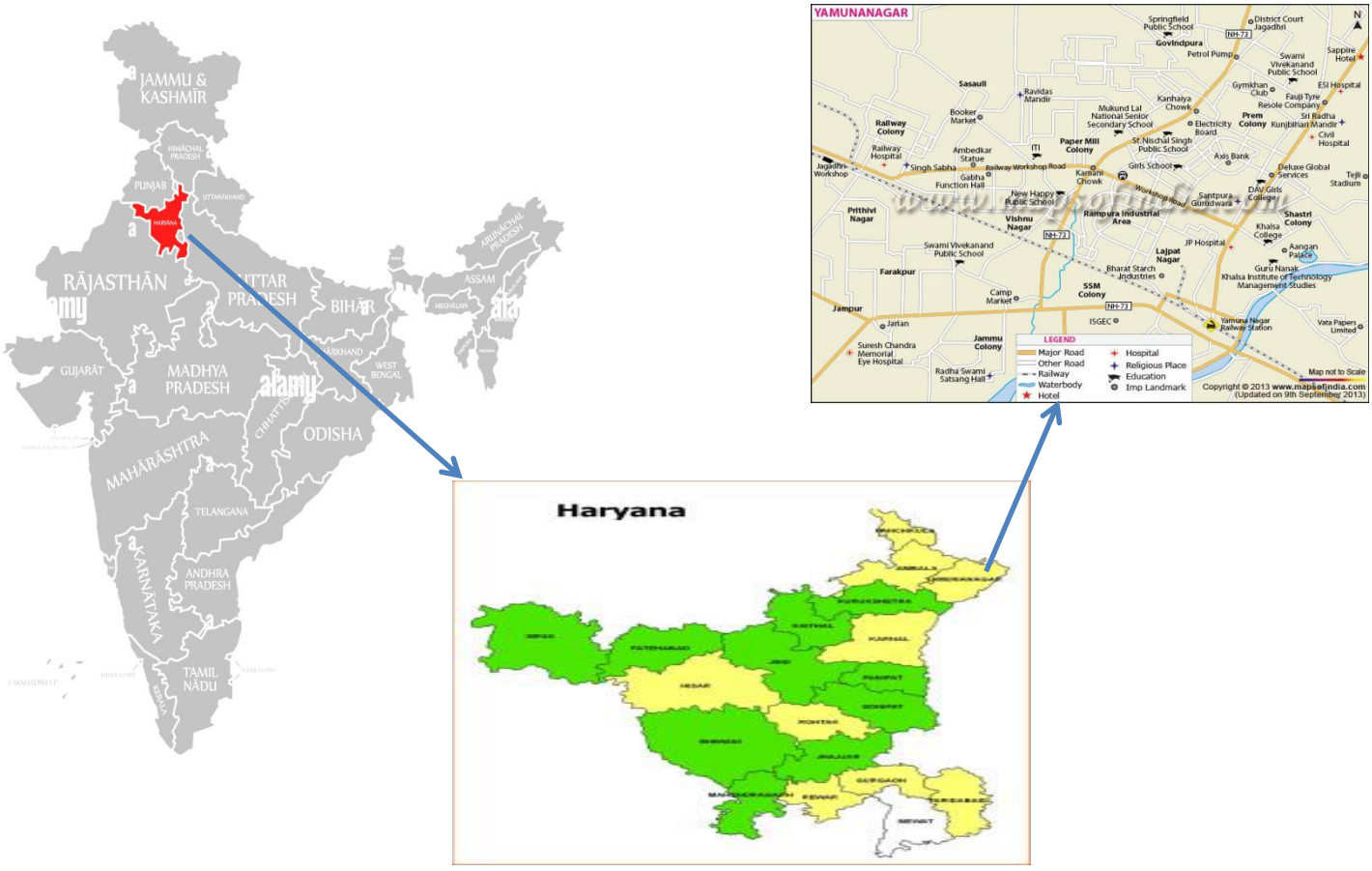


Figure 1:- Study Map

3.1.1 Isolation of *Phytophthora capsici*:

Carrot Agar Media, a basic element in both prior methods, was precisely prepared with minor modifications. Fresh carrots were peeled, cut into smaller pieces, and thoroughly washed with distilled water (DW). A mixture comprising 200 grams of carrots and 500 ml of distilled water underwent autoclaving at 15 Psi and 121°C for 30 minutes. Post- autoclaving, the carrots were crushed to extract carrot juice, which was then carefully filtered through muslin cloth and Whatman No. 1 Filter paper. The filtered juice was combined with 20 grams of agar and the remaining 500 ml of water to achieve a total volume of 1000 ml. This mixture was subjected to a second round of autoclaving under undistinguishable conditions, followed by cooling to a lukewarm temperature. Finally, Chloramphenicol ($C_{11}H_{12}C_{l2}N_2O_5$) was introduced at a concentration of 0.5 mg in 1000 ml of the carrot agar medium, and the resulting medium was poured into sterile petri plates, serving as a vital component for the isolation of *Phytophthora* sp. Publish patent Number (202311055757).

3.1.1.1 Method of Isolation of *P.capsici*:

Method 1: A 10-gram sample of infected soil, known to contain *Phytophthora capsici* was carefully dissolved in 100 ml of distilled water. Subsequently, fresh juvenile Kachnar (*Bauhinia variegata*) leaves were cleansed and then cut into smaller

pieces. These leaf fragments were introduced into the prepared solution, and the entire amalgamation was subjected to a 24-48 hour incubation period within a B.O.D incubator held at 27°C. As a result of this incubation, the Kachnar leaves underwent a noticeable transformation, turning a distinctive shade of brown. Post-incubation, the leaves were extracted from the solution, thoroughly washed with distilled water, and desiccated. The resultant infected dry leaves were then thoroughly transferred into Carrot Agar Media. Plates containing these samples were in turn placed into an incubator for a duration of 3-5 days, enabling the development of distinct colonies of *Phytophthora* spp. by this means sub-culture for further study.

Method 2: A sterile cork borer was used to precisely create two or three holes, each measuring 2 cm, within these fruits. The soil sample infected from *Phytophthora capsici* was then inoculate into these holes. These fruit specimens were subsequently placed within a B.O.D incubator for a span of 24-48 hours, held at 27°C. Following the incubation period, the fruits displayed a distinct brownish appearance. The next step involved carefully extracting the infected brown layers using a surgical blade, followed by a thorough wash with distilled water to eliminate any residual moisture. The resulting infected portions were then analytically transferred into Carrot Agar Media. Later, plates containing these samples were introduced into an incubator for a duration of 3-5 days, leading to the development of characteristic colonies of *Phytophthora capsici*, necessitating a sub-culture for further investigation. Publish patent Number (202311049913).

3.2. Identification of *P. capsici*

The fugal isolate was identified by using morphological studies and DNA sequencing. Using a compound microscope, the morphology of the mycelia and fungal spores that were cultured at 25±2°C for 7-8 days were examined. The fungus was identified by observing colony characters (Figure 1 A) such as linear growth, colour and sporulation following the available literature (Akter*et al.* 2007). The fungus was determined to be Phytophthora capsici Leonian based on physical and cultural traits, and the culture was deposited with the Indian Type Culture Collection (ITCC), New Delhi, under the ITCC Nos. 11865.23 and 11871.23.

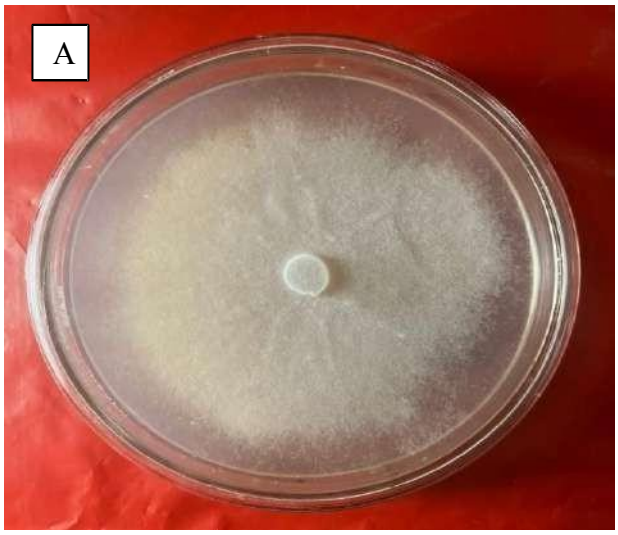


Figure 2: Morphological structures of *Phytophthora capsici* : A) Colony morphology on carrot agar medium

3.2.1. Scanning Electron Microscope Analysis:

The morphology of aseptate/coenocytic mycelium and branching sporangiophore carrying papillate sporangia were also examined using electron microscopy. The preparation of mycelial plugs for scanning electron microscopy (SEM). The samples were fixed five times for successive durations of 5, 10, 15, 20, 25, and 30 minutes in phosphate-buffered 3% glutaraldehyde dehydrate with a pH of 6.8 for the SEM preparation. The samples were then exposed to alcohol at increasing concentrations (30%, 50%, and 70%) for 15 minutes and for 20 minutes (80%, 90%, 95%, and 100%), respectively. When drying the graded aqueous series of acetone with CO_2 at the critical point, acetone was utilized as an intermediate fluid (Khan et al. 2011). The fungal hyphae of both the control and treatments were examined under a JSM-7610F Plus SEM (JEOL Ltd., made in Japan) and placed in the Central Instrumentation Facility of Lovely Professional University (LPU), India.

3.2.2. 18s Molecular Sequencing:

To verify species identification, sequencing of the 18S conserved region within the ITS region was performed. The obtained sequence matched *Phytophthora capsici* strains, as validated by NCBI nucleotide BLAST analysis and phylogenetic tree construction using MEGA11 software. This analysis was conducted on the FI1045 strain.

3.3. Preparation of formulation from *Ageratum conyzoides* and *Parthenium hysterophrous* for biological Control of *P. capsici*.

Different types of methods are used to cure different types of fungal, bacterial and as well as viral diseases in plants and crops. In our Research work we are using two weed plants such as *Ageratum conyzoides* and *Parthenium hysterophrous* leaves extract as a formulation we apply to cure *P. capsici* in from *Capsicum annum* crop. We use the two plant leaves extract, measure out the proper amount and mixes it with different solvents such as ethanol, methanol, acetone, and chloroform and aqueous.

3.3.1. Plant Material used in the study:

Ageratum conyzoids and *Parthenium hysterophorus* leaves were collected from local area near University campus. Identification of Plants was done by (FRI) Forest Research Institute. Collected leaves were washed with tap water to remove soil, dust and other contaminants. Then plant leaves will cut into small pieces, dried in air and sunlight to remove the moisture and dried in an oven at 45˚C until the constant weight were obtained. Finally plant leaves were ground separately using a laboratory blender to get uniform powder which is called as the "biomass". Prepared biomass were stored in an airtight container or zipped polythene bags to protect from moisture and used for further characterization.

Figure 3 A and B:- *Ageratum conyzoides* L. Flower, Stem and Leaves, C:- *Parthenium hysterophorus* whole plant (Rosette stage).

3.3.2. Preparation of plant extract

The dried powder of *A. conyzoides* and *P. hysterophorus* 50g in 250ml was sorted, extracted with Ethanol, Methanol, Acetone and Chloroform 90% by cold maceration method. The solvents filtrate was filtered, and the obtained residues were re-macerated in triplicates until colourless filtrate was obtained. Then, the filtrate was dried using a rotary evaporator at 50°C. (Widowati et al., 2018)

3.3.3. Preparation of Weed Extracts

Certified and Disease-free seeds of *Capsicum* were procured and sterilized with sodiumhypo-chloride. The seeds were treated with *Ageratum conyzoides* and *Parthenium hysterophrous* and various solvents at different concentrations. The physicochemical properties of the constituents in the extracts were used to determine the solubility of the *Ageratum conyzoides* and *Parthenium hysterophrous* extracts with the suitable solvents. The procedure suggested by Flanagan was used to determine the proper solvent. The procedure involves transferring 1.20 g of extract into a test tube and adding two or two parts by volume of suitable solvents up to a maximum of 10 ml of waterto it. Following each addition of two millilitres of solvent, the test tube was heated and agitated using a magnetic stirrer. By applying a variety of solvents, the best solvent was found to dissolve the extract, and the best formulation type was chosen in accordance. After considering the physical and chemical characteristics of the extract, the extract was mixed at 800 rpm in a vertical mixer with a suitable solvent and co-formulates.

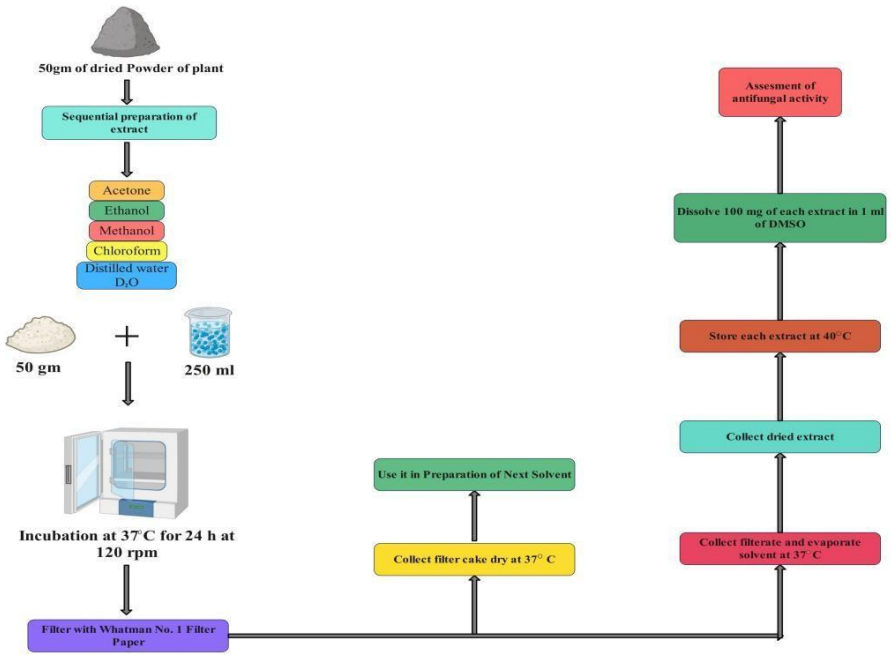


Figure 4 Method of Cold Mecerration of Extraction (Widowati et al., 2018)

After that, the mixture was stirred in a high-speed vertical mixer at 4500 rpm for 1.5 hours, till the particle size level reached 10–20 microns and an even distribution was attained. As a result, the insoluble components in the extract were distributed uniformly throughout. Following a 24-hour period, the items placed in the resting tank underwent quality control analysis. As a new fungicide against *P. capsici*, ethanolic and acetonic extracts of *Ageratum conyzoides* and *Parthenium hysterophrous* leaf powder were utilized. In order to formulate biofungicides on a big scale and for commercial manufacturing, it would be helpful to identify the active components of these extracts more thoroughly (Flanagan 1972; and Erdogan et al., 2019).

3.4. Evaluation of anti-fungal activity:

3.4.1. Bioassay

By using the poisoned food technique, the seeds were treated with varying concentrations of leaf leachates (Grover et. al., 1962) *Ageratum conyzoides* and *Parthenium hysterophrous* leaf leachates were generated in various concentrations for each solvent. Two layers of filter paper have been placed in Petri-dishes containing ten bell pepper seeds spaced equally apart. In accordance with the therapy, 15 ml of *A. conyzoides* and *P. hysterophrous* leachates of 2, 4, 6, 8, and 10% were applied to the

Petri plates. As a control, sterilized water was utilized. For every treatment and control, three replicates were kept. For five days, the Petri plates were kept in a laboratory setting. When the blotting paper's moisture content dropped, an equal volume of distilled water was added to each dish.

3.4.1.1. Antifungal activity of *Ageratum* and *Parthenium* plant extracts on test pathogen

Ageratum and *Parthenium* plant extracts were tested by using the poison food technique with slight modifications. 800 µl of sabouraud broth in the 2 ml (MCT) micro centrifuge tube, then 100 µl of the each solvent extract was taken with the help of micropipette. Separately, mix the plant extracts and Sabouraud broth thoroughly. 100 µl of test fungal microbe inoculums (McFarland standard) will be put in the Sabouraud broth. The micro centrifuge test tubes were incubated for one hour at 28±2°C. A micro centrifuge tube containing 800 µl of SD broth, 100 µl of plant extract, and 100 µl of fungal suspension culture was used to dip a sterile disc with a diameter of 0.5 mm. The sterilized discs are put on a Petriplate with Sabouraud dextrose agar medium. The incubation temperature for all Petriplates is 28±2°C. Sabouraud broth, the base medium, is devoid of phytoextracts. After 48 hours, mycelial growth of the test fungus was quantified and contrasted with the control. Vincent's formula was used to assess the proportion of mycelial growth inhibition (Vincent 1927)

$$\text{Growth Inhibition Percentage } \% = \frac{C-T}{C} \text{ x100}$$

Where, C : colony diameter in control, T : colony diameter in treatment.

3.5. In vivo evaluation of plant extracts against the test pathogen

The field trials for studying *Capsicum annuum* L. against *Phytophthora capsici* blight were carried out at the Plant Pathology Field, School of Agriculture, Lovely Professional University, Punjab. The location of the field was at latitude 31.2560°N, longitude 75.7051°E, and an elevation of 245 meters above sea level. This trial was conducted over the course of 2021 and 2023. The laboratory experiments, on the other hand, took place in the Project Lab (57A-503). The following section provides a detailed description of the experimental design and methodology used during the study.

Each plot - 5 X 4 = 20 m^2 – total plot 20 X 24 = 480 m^2

Total Area including water channels – Width 32 X Length 21 m^2

Table 1: Field layout for testing the efficacy of Biofungicides.

Border Space 1m	**Plot 5 m**	**Water Channel 1m**	**Plot 5 m**	**Water Channel 1m**	**Plot 5 m**	**1 m**
4 m	T_1R_1		T_2R_1		T_3R_1	4m
	T_4R_1		T_5R_1		T_6R_1	
4 m	T_6R_2		T_1R_2		T_2R_2	4m
	T_5R_2		T_3R_2		T_4R_2	
4 m	T_2R_3		T_6R_3		T_5R_3	4m
	T_3R_3		T_4R_3		T_1R_3	
4 m	T_5R_4		T_2R_4		T_6R_4	4m
	T_1R_4		T_3R_4		T_4R_4	
1 m	5 m	1 m	5 m	1 m	5 m	1 m

Design: Randomized Block Design (RBD)

Gross Area of Field: 480 m^2

Area of one plot: 5 x 4 = 20 m^2

Spacing plant to plant and row to row: 60x20 cm^2

Lines: 3

Plants in each line: 5

Seedling require for one plot: 125

Seedlings required for 25 plots: 3125

Treatments: 5

Replications: 3

Variety: Pusa Beej (California Wonder).

Figure 5: Prepared Field for Sowing

Figure 6: *Capsicum annuum* L. at the Seedling stage

3.6. Inoculation of *P. capsici* in the field

To facilitate the presence of *Phytophthora capsici* in field conditions, it is crucial to employ a mass-multiplied culture of *P. capsici*. In order to introduce the fungus into the field conditions, the following steps were implemented for inoculation.Using a set of methods, the glass wares were ready for use in laboratory investigations. The glasswares were first cleaned using ordinary water and then submerged in a cleaning solution made of 1000 ml of distilled water, 60 grams of potassium dichromate ($K_2Cr_2O_7$), and 60 ml of concentrated sulfuric acid (H_2SO_4). For a whole day, the glassware was subjected to its effects. The glassware was then thoroughly cleansed with tap water and cleaned with a detergent solution. To guarantee the sterility of the glassware before use, it was lastly washed with distilled water that had been disinfected. (Riker and Riker, 1936; Tuite, 1969).

3.6.1. Sterilization of Glasswares and tools:

The glassware, which had been dried in the air, underwent sterilization in a hot air oven at temperatures ranging from 150°C to 180°C for a duration of two hours. The inoculating needles were sterilized by immersing them in alcohol and heating them over a flame until they became red hot. Before sterilization, the handles of the needle holders were cleaned and the surfaces were sterilized with alcohol-soaked cotton. This 13 process was repeated 2-3 times. Similarly, the forceps were sterilized in the same manner as the inoculating needle.

3.6.2. Culture Media preparation:

The media were subjected to sterilization in an autoclave at a pressure of 15 psi for a duration of 30 minutes. (Sasaki & Imazato 2020)

3.7. Mass multiplication of *P. capsici:*

The *Phytophthora capsici* culture isolate from soil samples and Indian Type Culture Collection (ITCC), New Delhi as ITCC No.11865.23 and 11871.23. was acquired prior to planting the Pepper. Before undergoing mass multiplication, the culture was stored in a refrigerator. Since the culture was provided in a slant, it was transferred onto Petri plates containing carrot agar media using an inoculation needle in sterile conditions in Laminar Air Flow. These plates were then placed in an incubator at a temperature of 25±2°C for a duration of 7-10 days to facilitate the growth of the fungal pathogen.

3.8. Inoculation

A pure culture of *Phytophthora capsici* was utilized to cut 90 mm discs with a sterile cork borer, followed by placement of 3-4 discs onto a petri plate using a fungus inoculating loop. The plate was then covered and exposed to continuous light for 24-48 hours. Subsequently, the plate was subjected to freezing at -4°C for 1 hour before being transferred to an incubator set at 24°C for another hour. Zoospore dispersal due to stress physiology was observed under a microscope, and zoospores were counted using a hemocytometer. A hemacytometer was used to gather and count the zoospores. The concentration of the inoculum solution was set at 40,000 zoospores/ml.

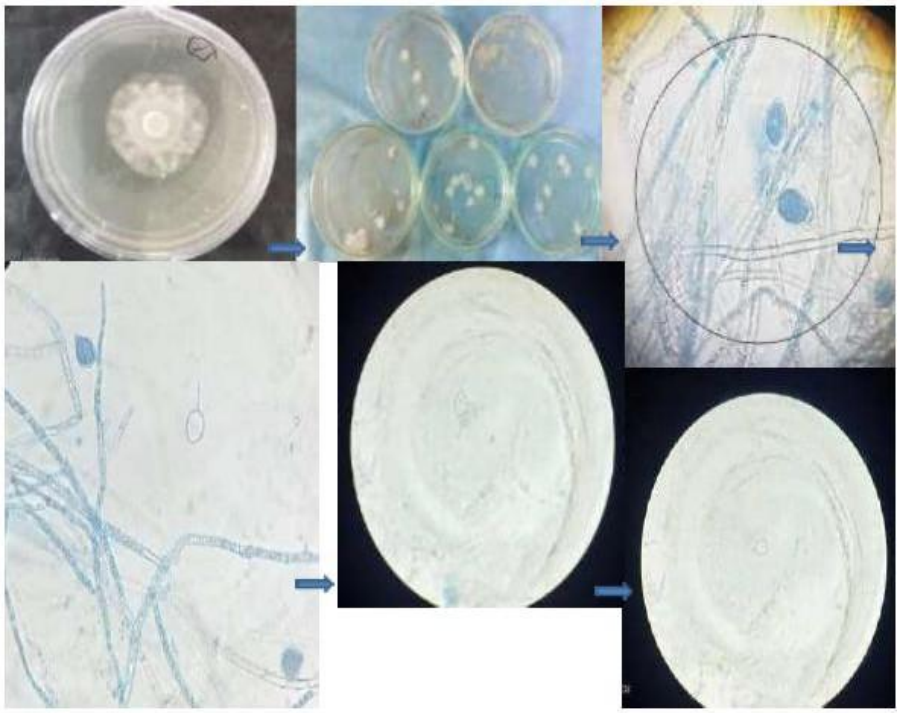


Figure 7: Zoospore dispersal of *P. capsici*

3.8.1 Severity of *P. capsici* blight in *Capsicum annuum* L:

Two sprays of each treatment were administered at 21 and 45 days after germination. Disease severity was assessed before spraying and again after 7 days of spraying. The disease assessment was conducted using the disease scale developed by Payak and Sharma (1983).

3.8.2.

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all disease ratings}}{\text{Total no.of leaves observed x Maximum grade}} \times 100$$

Using the formula given by Wheeler (1969), the Percent Disease Index was computed.

Table 2: Disease index scale (Sunwoo et al., 1996)

Disease Scale	Percent Infection (%)
0	No infection
1	0-10%
2	11-20%
3	21-40%
4	41-60%
5	More than 60%

Figure 8 : Severity of *P. capsici* blight in *Capsicum annuum* L. after inoculation. Life cycle of the *Phytophthora capsici*

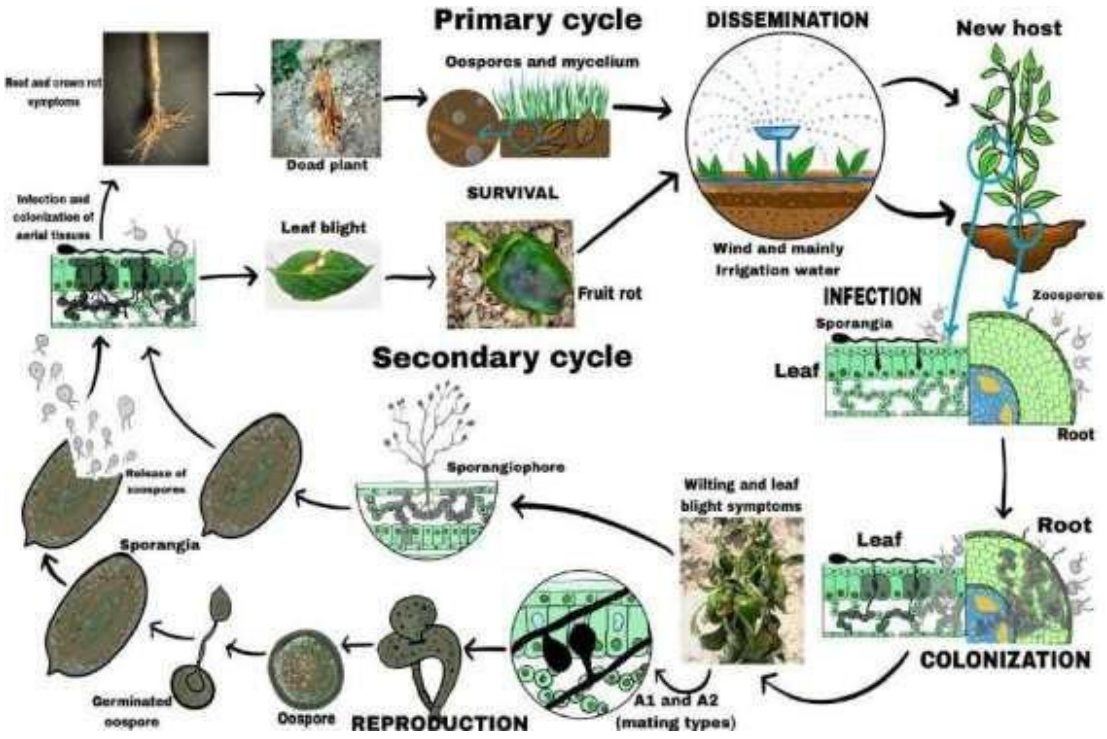


Figure 9: Life cycle of the *Phytophthora capsici* organism that causes foliar blight, root, crown, and fruit rot in *Capsicum annuum* plants

3.9. Biochemical analysis of Gas Chromatography Mass Spectra.

Freshly harvested *Ageratum* and *Parthenium* leaves underwent a thorough wash with double distilled water to eliminate any traces of dust and pollutants. Subsequently, the leaves were subjected to a drying process in a hot air oven set at 30°C to remove excess water content. Once dried, the leaves were finely ground into powder using a mechanical grinder, which served as the base material for extract preparation. In a nutshell, a 40 g portion of the powder was dispersed in 400 ml of absolute ethanol, methanol, acetone, and chloroform (in a 1:10 w/v ratio) within a conical flask. This mixture was then placed on an orbital shaker for 72 hours. Following this, the sample underwent filtration using Whatman Filter Paper No. 1, after which it was refrigerated (maintained at 4–7°C) for an additional 72 h to allow for the evaporation of solvents. The resulting extract were then available for further analysis via GC-MS (Najda et al., 2021). Gas Chromatography Mass Spectra (GC-MS) Analysis 17 The components contained in the ethanolic extract of *Ageratum conzoides* and *Parthenium hysterphorus* leaf sections were identified using GC-MS analysis was performed at Lovely Professional University, Phagwara, Punjab India. GC analysis of the ethanolic extract was done using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a Rxi-5MS fused silica capillary column (5% diphenyl/95% dimethyl polysiloxane) and AOC_{20}i+s (autosampler) of 0.25 mm diameter, 30 m length, and 0.25 µm film thickness. The sample size of 2 µl was supplied through using an injector. Helium, an inert gas, used as the carrier gas. The MS was obtained at an ionisation energy of 70 eV. The overall flow was 16.3 ml/min, while the column flow was 1.21 ml/min. Flow control with linear velocity was 39.9 cm/s. Oven temperature initialization was 50°C, followed by 250°C for 5 minutes, a 22-minute ramp to 280°C, a 69.98-minute hold, and ACQ mode. Scan range: 40 m/z to 700 m/z, 0.50 s scan period, 260°C, and 10:0 split ratio. The GC-MS took 65 minutes to complete its run. The relative % amount of each component was expressed as a percentage with peak area (Yamuna et al., 2017).

3.9.1. FTIR Analysis

Each sample comprised 5 grams of finely powdered plant materials, individually mixed with 100 mg of KBr (FT-IR grade). This blend was then compacted to form a salt disc with a diameter of 3 millimeters. Analysis was conducted using a Perkin-

Elmer Range on FT-IR-84005 Shimadzu, Japan, within the absorption spectrum ranging from 4000 to 500 cm-1 (Raut et al., 2019)

3.10. Statistical Analysis

In this study, we comprehensively analyzed the inhibitory effects of bio fungicides derived from *Parthenium* and *Ageratum* on the growth of *Phytophthora capsici*. To bolster the significance of our findings, we performed statistical analysis using the Permutational Multivariate Analysis of Variance (PERMANOVA) method, which allows us to derive further insights from the data. Our results align with the Permanova analysis, including the associated p-values (Anderson 2017).

CHAPTER IV

RESULTS AND DISCUSSION

The findings of the experiments conducted during the present investigation are presented in this chapter and the results obtained during the present investigation have been discussed and interpreted in the light of the research work done in India and abroad.

4.1 Pathogen Identification

With the use of DNA sequencing and morphological features, we identified the strains of the test pathogen. Using a compound microscope, we examined the morphology of the test fungi's mycelium and spores after they were grown for 7 to 8 days at 25±2°C. As per the extant literature (Akter et al. 2007), the fungus was identified by the examination of colony features, including color, growth pattern, and sporulation (Figure 9 A). The fungus was determined to be *Phytophthora capsici* based on its shape and culture development pattern (Figure 9 B). The culture was submitted to the Indian Type Culture Collection (ITCC), New Delhi, under the ITCC Nos. 11865.23 and 11871.23.

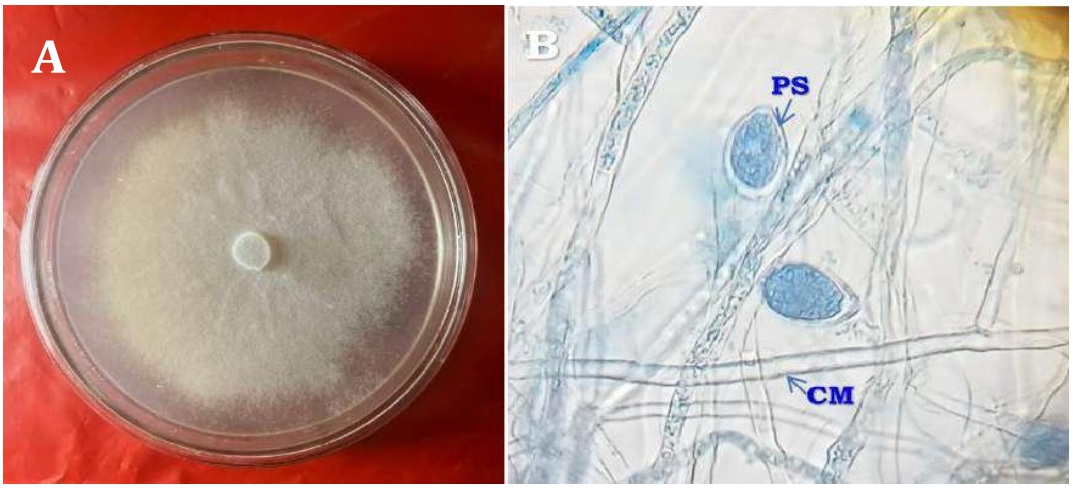


Figure 10: Morphological structures of *Phytophthora capsici*: A) Colony morphology on carrot agar medium; B) Coenocytic mycelium (CM) and papillate limoniform sporangia (PS) with branched sporangiophore

Electron microscopy was also used to observe the morphology of aseptate/coenocytic mycelium and branched sporangiophore bearing papillate sporangia (Fig 10).

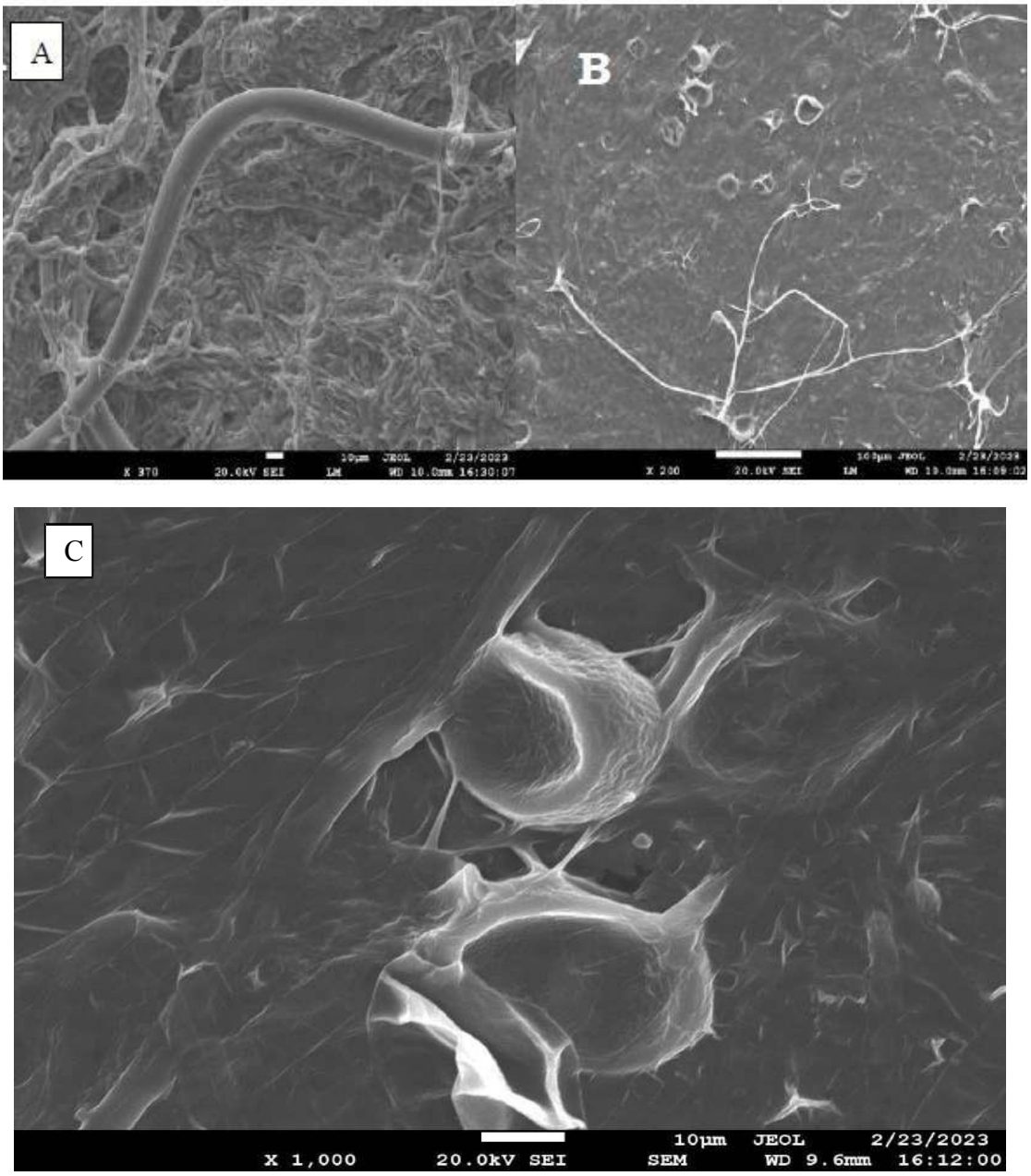


Figure 11: Scanning electron microscopy: A. coenocytic mycelium, B. branched sporangiophore C. papillate sporangia

18S conserved region gene sequencing of the ITS region was confirmed by NCBI nucleotide BLAST and a phylogenetic tree made with the sequences applying the MEGA11 programme (Figure 11). The final sequence of the ITS region was identical to *Phytophthora capsici* strains.

Figure 12. 18S gene sequence-inferred phylogenetic relationships of *Phytophthora capsici* identified in cultivated *capsicum* crop, the analysis also includes the 18s gene sequence of selected, well-studied *Phytophthora capsici* spp.

Table 3: Effect of different aqueous extract of *Ageratum* and *Parthenium* on percent disease index (PDI)

***Ageratum* Extract**	**Inhibition Percent (%)**	***Parthenium* Extract**	**Inhibition Percent (%)**
T0	0	T0	0

AE	85.09	PE	87.37
AM	88.57	PM	89.16
AC	91.16	PC	74.82
AA	78.39	PA	90.01
SEm±	1.0	SEm±	2.1
CD or LSD	3.1	CD or LSD	6.7
F-Test (p=0.05)	S	F-Test (p=0.05)	S
CV%	4.0	CV%	7.9

T_0 = Control; AE = *A. conzoides* + Ethanol; AM = *A. conzoides* + Methanol; AC = *A. conzoides* + Chloroform; AA = *A. conzoides* + Acetone; PE = *P. hysterophorus* + Ethanol; PM = *P. hysterophorus* + Methanol; PC = *P. hysterophorus* + Chloroform; PA = *P. hysterophorus* + Acetone

By measuring the pathogen's radial growth, several aqueous extracts of *Ageratum conzoides* and *Parthenium hysterophorus* were evaluated for their anti-fungal effectiveness against *Phytophthora capsici* under *in vitro* condition. There was significant ($p<0.005$) interaction effect between different extracts of *Ageratum* and *Parthenium* used in inhibiting radial growth of *P. capsici*. Generally, all different aqueous extracts of *Ageratum* and *Parthenium* inhibited the mycelia growth of *P. capsici* compared to the untreated control (Table 3). Various aqueous extracts of the two weed species had inhibitory effects ranging from 74.82% to 91.16%. The highest inhibition of test pathogen was recorded on Petri dishes treated with *A. conzoides* + Chloroform extract (91.16%) followed by aqueous extract of *P. hysterophorus* + Acetone (90.01%). Least inhibitory effect was shown by *P. hysterophorus* + Chloroform extract (74.82%) followed by aqueous extract of *A. conzoides* + Acetone (78.39%).

It has been demonstrated that *Ageratum conyzoides* possesses antimicrobial properties over a variety of diseases, including those belonging to the *Phytophthora* genus (Ndacnou et al., 2020). Due to its abundance of bioactive chemical constituents, *Aspergillus fumigatus, Phytophthora citrophthora, Pythium aphanidermadum, Fusarium solani, Phytophthora infestans, Cercospora musae,* and *Cercospora capsici* are just a few of the phytopathogenic fungi that *A. conyzoides* has long been used to

combat in agriculture (Nguyen et al., 2021). Amongst the various sections of the examined weed, leaf extract was found to be particularly effective in controlling target fungal species. *n*- Hexane and methanolic extracts of *A. conyzoides* demonstrated antifungal activity against

Fusarium solani (Kotta et al., 2020) and *Macrophomina phaseolina* (Banaras et al., 2021). *A. conyzoides* was the most successful weed species in terms of its ability to impede the growth of the mycelium of *Rhizoctonia solani, Aspergillus niger,* and *Pestalotiopsis theae* (Kaur et al., 2023 &Paul et al., 2022). High flavonoid concentration in the leaves is closely connected with *Ageratum* leaf extract's capacity to suppress plant disease (Rianosa et al., 2020). Flavonoids are more efficient at slowing the growth of fungal mycelium at greater concentrations (Aboody & Mickymaray 2020 &Jones et al., 2020). Additionally, *Ageratum* leaf extract can increase crop yield while substituting synthetic fungicides (Rianosa et al., 2020).

Parthenium is known to be allelopathic, and research has shown that its roots, stem, leaves, inflorescence, pollen, and seeds all contain water-soluble phenolics and sesquiterpene lactones (Kostina-Bednarz et al., 2023 &Oli et al., 2024). *Parthenium hysterophorus* leaf powder ethanolic and acetonic extracts demonstrated strong antifungal efficacy against *Phytophthora capsici,* however methanolic extract demonstrated less antifungal activity (Kumar et al., 2022). *Parthenium hysterophorus* aqueous botanical extracts showed up to 100% suppression against the test pathogen and showed encouraging antifungal efficacy (Dilshad & Gupta 2023). *P. hysterophorus* releases phytotoxic chemicals like ambrosin, coronopilin, p-hydroxybenzoic acids, coumaric and parthenin, ferulic, vanillic, and caffeic, which contribute to its allelopathic and antifungal properties (Bashar et al., 2022). *Ageratum conyzoides* and *P. hysterophorus*, two asteraceous plant species, were found to have antifungal properties against *Macrophomina phaseolina* (Tassi) Goid, which causes the disease known as charcoal rot in *Helianthus annus* L. A measurable decrease in *M. phaseolina* biomass was noted as a result of various number of aqueous extracts (Parveen et al., 2023).

Table 4 Effect of *Ageratum* and *Parthenium* extract on percent germination of infected *Capsicum annuum* at different concentrations

Treatments	Germination Percentage (%)					
	T0	T1	T2	T3	T4	T5
AE	67.96d*	85.1b	89.53a	91.49a	87.18a	75.61c
AM	67.56d	79.76b	88.57a	90.47a	87.72a	78.02c
AC	68.45d	74.74c	71.63d	78.39c	72.07d	71.16d
AA	69.94d	81.36b	88.67a	90.59a	88.61a	83.27b
PE	68.39d	87.34a	90.64a	90.75a	88.4a	77.69c
PM	67.31d	78.89c	89.16a	90.63a	89.07a	78.55c
PC	69.33d	75.7c	69.42d	74.82c	70.46d	70.44d
PA	67.92d	80.9a	89.11a	89.84a	90.01a	83.32b
SEm±	2.01					
LSD 0.05	5.65					
CV (%)	4.33					
F-Test (0.05)	S					

AE = *A. conzoides* + Ethanol; AM = *A. conzoides* + Methanol; AC = *A. conzoides* + Chloroform; AA = *A. conzoides* + Acetone; PE = *P. hysterophorus* + Ethanol; PM = *P. hysterophorus* + Methanol; PC = *P. hysterophorus* + Chloroform; PA = *P. hysterophorus* + Acetone; T0 = Control; T1 = 2%; T2 = 4%; T3 = 6%; T4 = 8% and T5 = 10% concentration of extracts

The results of the statistical analysis demonstrated that, in comparison to the untreated control, the aqueous extracts of *Ageratum* and *Parthenium* species at varying concentrations significantly affected the germination of the seeds in infected bell pepper plants; however, the interaction between the means of the concentrations was not statistically significant (Table 4). The concentration means indicated that each of the several aqueous extracts of *parthenium* and ageratum had a minor stimulatory effect on seed germination as compared to the untreated control. The stimulatory effect of different queous extracts of both the weed plants on percent germination of infected bell pepper plants ranged from 69.42% to 91.49%. The highest stimulatory effect was recorded on plants treated with 6% *A. conzoides* + Ethanol extract (91.49%) followed by 6% aqueous extract of *P. hysterophorus* + Ethanol (90.75%) under *in vitro*

conditions.

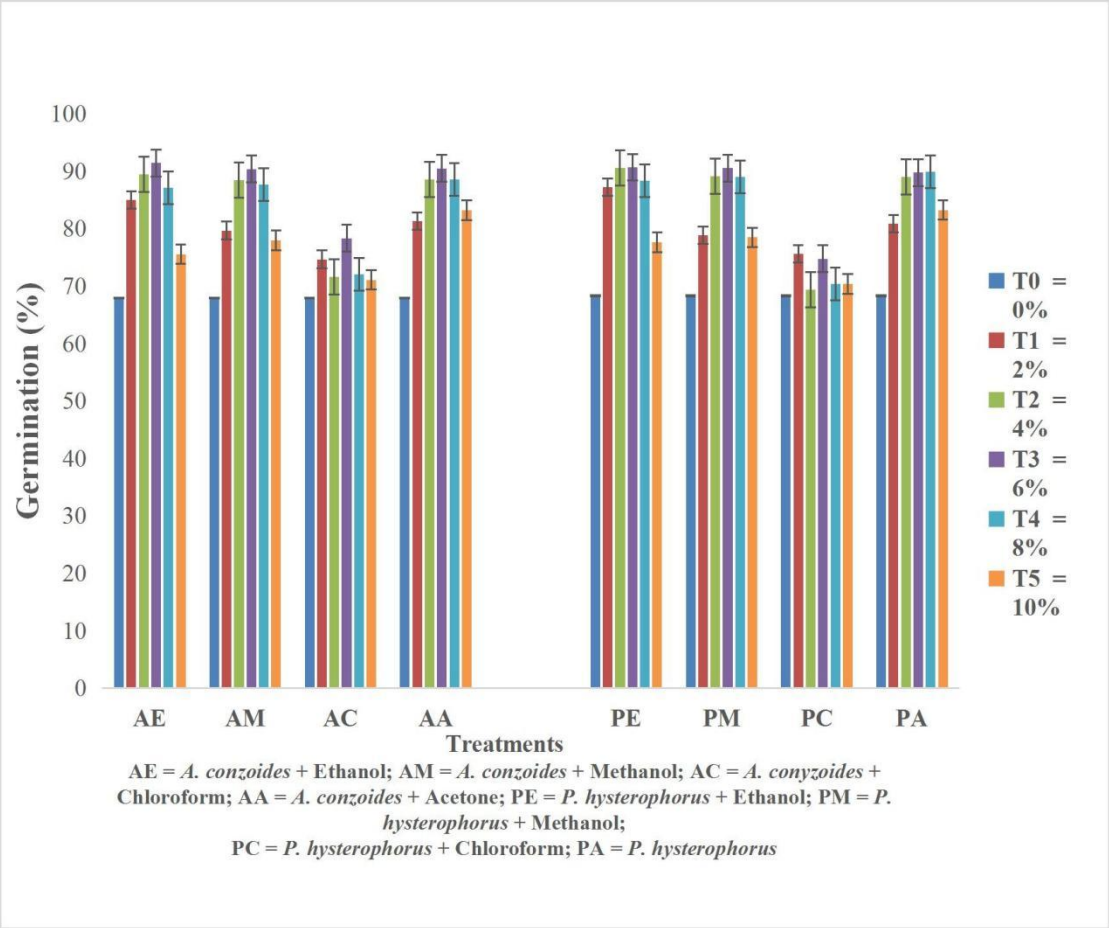


Figure 13. Stimulatory effect of *Ageratum* and *Parthenium* extract on percentage germination of infected *Capsicum annuum* at different concentrations

Table 5: Effect of *Ageratum* and *Parthenium* extract on seedling fresh weight of infected *Capsicum annuum* at different concentrations

Treatments	Seedling Fresh Weight (g)					
	T0	**T1**	**T2**	**T3**	**T4**	**T5**
AE	63.16j*	129.05b	131.8b	137.78a	127.44c	97.12e
AM	63.90j	97.66e	131.99b	133.83b	130.67b	93.95f
AC	65.36i	88.07g	66.90i	72.60h	69.95i	69.92i
AA	68.08i	120.03d	129.15b	132.22b	128.73b	126.82c
PE	66.58i	131.45b	133.88b	139.86a	129.28b	101.29e
PM	68.14i	99.81e	135.28a	136.39a	133.05b	95.86f
PC	69.13i	88.82g	67.97i	75.33h	72.40h	73.38h
PA	71.6h	123.27c	130.98b	132.93b	131.24b	128.49c
SEm±	1.89					
LSD 0.05	5.32					
CV (%)	3.13					
F-Test (0.05)	S					

AE = *A. conzoides* + Ethanol; AM = *A. conzoides* + Methanol; AC = *A. conzoides* + Chloroform; AA = *A. conzoides* + Acetone; PE = *P. hysterophorus* + Ethanol; PM = *P. hysterophorus* + Methanol; PC = *P. hysterophorus* + Chloroform; PA = *P. hysterophorus* + Acetone; T0 = Control; T1 = 2%; T2 = 4%; T3 = 6%; T4 = 8% and T5 = 10% concentration of extracts.

Statistical analysis of the data presented in Table revealed that different concentrations of all the four extracts of *Ageratum* and *Parthenium* species had a stimulating effect on seedling fresh weight of infected bell pepper plants as compared to the untreated control.

The stimulatory effect of different aqueous extracts of both the weed plants on seedling fresh weight of infected bell pepper plants ranged from 66.33 g to 139.33 g. The highest fresh weight of seedling was recorded on plants treated with 6% solution of *P. hysterophorus* + Ethanol (139.33 g) followed by 6% aqueous extract of *A. conzoides* + Ethanol (137.33 g) under *in vitro* conditions.

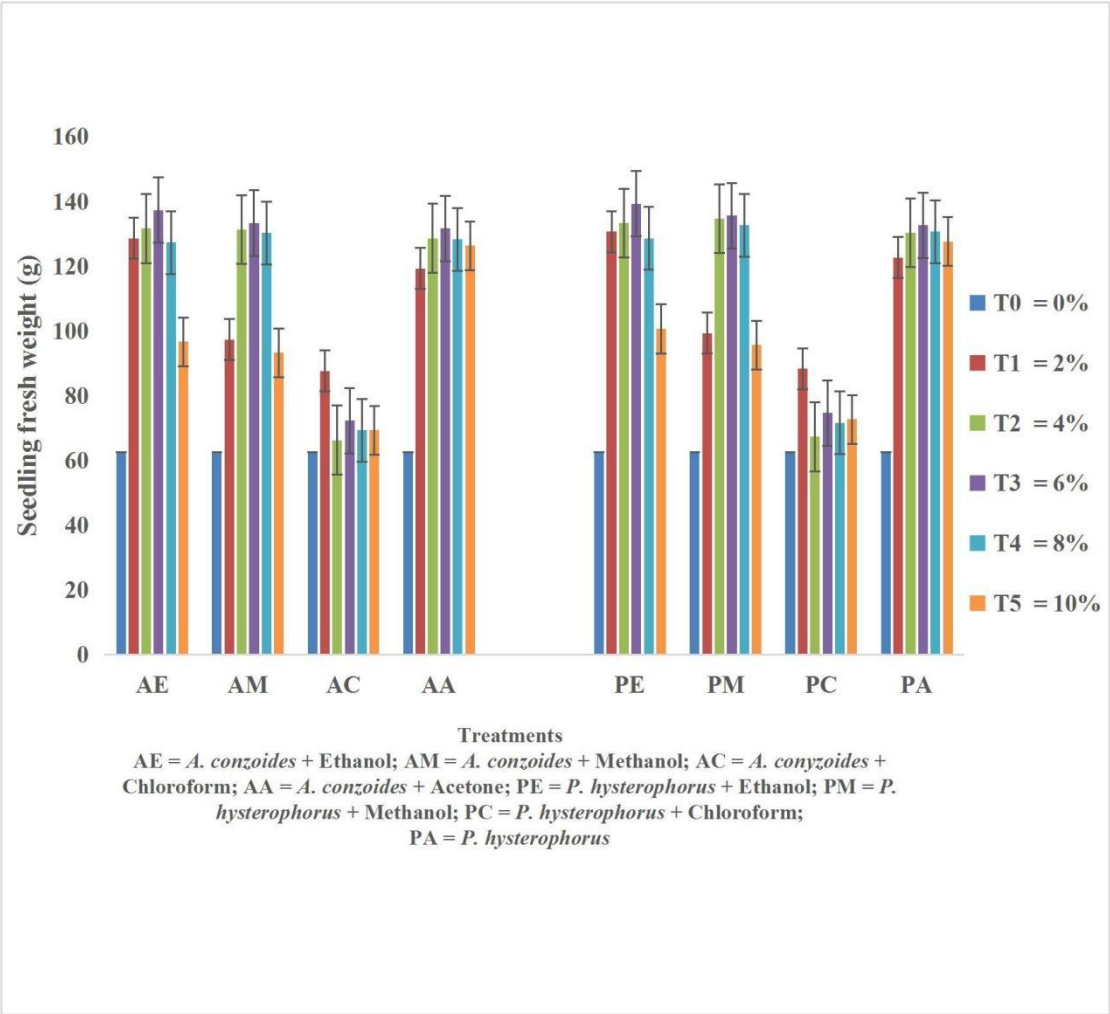


Figure 14. Stimulatory effect of *Ageratum* and *Parthenium* extract on seedling fresh weight of infected *Capsicum annuum* at different concentrations

Table 6: Effect of *Ageratum* and *Parthenium* extract on seedling height at 35 days after sowing of infected *Capsicum annuum* at different concentrations

Treatments	Seedling Height at 35 DAS (cm)					
	T0	**T1**	**T2**	**T3**	**T4**	**T5**
AE	5.37e*	8.23b	8.43a	8.73a	8.53a	6.13d
AM	6.13d	7.07c	8.47a	8.47a	8.43a	6.93c
AC	5.53e	6.43d	6.13d	7.23c	7.13c	7.07c
AA	6.03d	7.83b	8.47a	8.83a	8.53a	8.13b
PE	6.63d	8.37a	8.63a	8.97a	8.83a	6.57d
PM	6.97c	7.37c	8.23b	8.37a	8.73a	7.07c
PC	6.5d	6.87c	6.03d	7.33c	7.37c	7.33c
PA	6.77c	7.87b	8.47a	8.77a	8.93a	8.53a
SEm±	0.22					
LSD 0.05	0.63					
CV (%)	5.09					
F-Test (0.05)	S					

AE = *A. conzoides* + Ethanol; AM = *A. conzoides* + Methanol; AC = *A. conzoides* + Chloroform; AA = *A. conzoides* + Acetone; PE = *P. hysterophorus* + Ethanol; PM = *P. hysterophorus* + Methanol; PC = *P. hysterophorus* + Chloroform; PA = *P. hysterophorus* + Acetone; T0 = Control; T1 = 2%; T2 = 4%; T3 = 6%; T4 = 8% and T5 = 10% concentration of extracts.

When compared to the untreated control, the seedling height of infected bell pepper plants at 35 days after sowing was significantly affected by the various concentrations of aqueous extracts from *Ageratum* and *Parthenium* species, but the statistical analysis revealed that the interaction between the concentration means was not significant (Table 6). The concentration means showed that there was a slightly stimulatory effect of all different aqueous extracts of *Ageratum* and *Parthenium* on seedling height at 35 DAS as compared to the untreated control. The stimulatory effect of different aqueous extracts of both the weed plants on seedling height at 35 DAS of infected bell pepper plants ranged from 6.03cm to 8.97cm. The maximum seedling height at 35 DAS was recorded on plants treated with 6% *P. hysterophorus* + Ethanol extract (8.97cm) followed by 8% aqueous extract of *P. hysterophorus* +

Acetone (8.93cm), 6% *A. conzoides* + Acetone (8.83cm) and 8% *P. hysterophorus* + Ethanol (8.83 cm) under *in vitro* conditions.

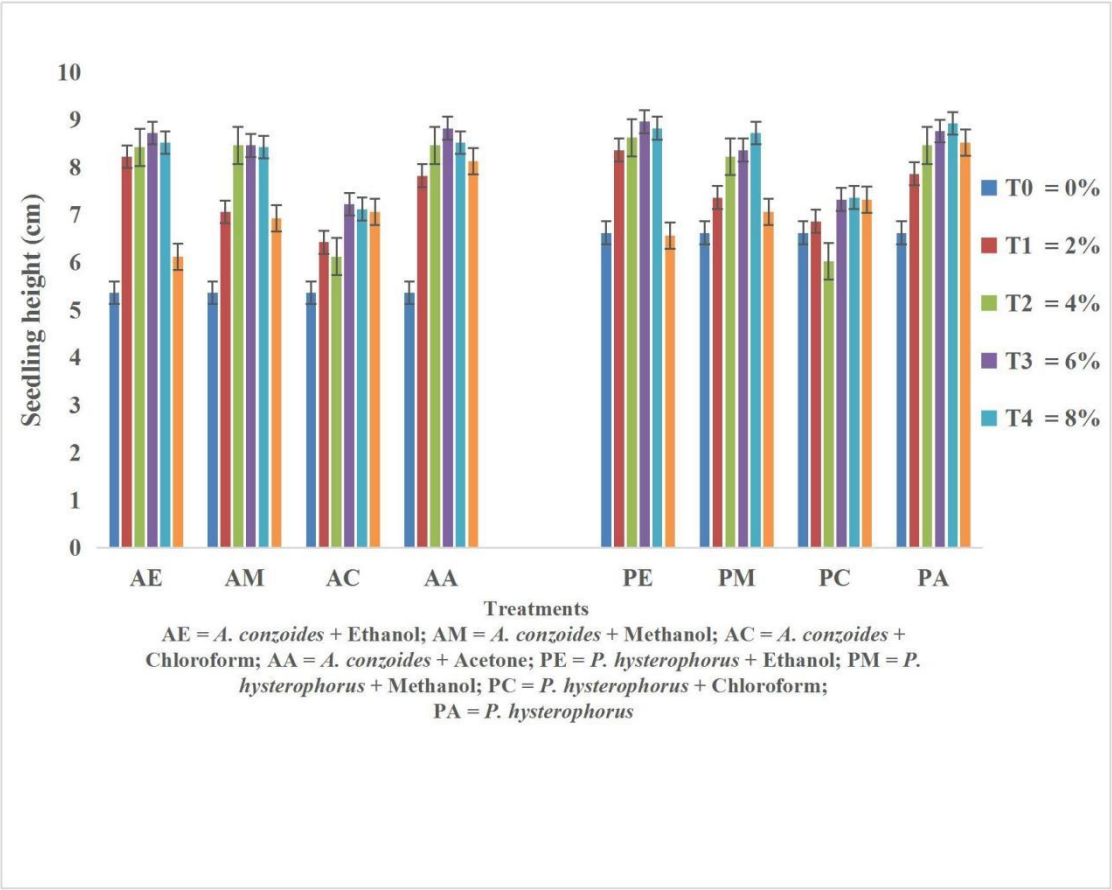


Figure 15. Stimulatory effect of *Ageratum* and *Parthenium* extract on seedling height at 35 days after sowing of infected *Capsicum annuum* at different concentrations

As the need for sustainable agriculture grows, so does worry over the overuse of synthetic chemicals. Many studies have demonstrated the significance of allelopathy in the relationship between weeds and crops (Choudhary et al., 2023). Because the diverse phytochemical elements of *Ageratum* and *Parthenium* plants are valuable as insecticides in agriculture and have medicinal uses in human civilization, these weed plants have a positive as well as negative impact on agriculture, ecosystems, and human health (Jones et al., 2020). According to preliminary screening, ethanolic leaf extract had the strongest antifungal activity, which is consistent with the findings of the present research (Saleh & Abu-Dieyeh 2021).

Raj and Jha 2016 was observed no inhibitory effect of leaf extract of *Parthenium* on seed germination of *Phaseolus mungo* (Raj and Jha 2016). In the rice field, applying dried *A. conyzoides* leaves boosted grain yield by 14% (Negi et al., 2020). In a different investigation, *A. conyzoides* extracts demonstrated encouraging outcomes in weed management while simultaneously preserving and enhancing the soil microbiota (Paul et al., 2022), suggesting its advantageous application. *A. conyzoides* exhibits potential as a valuable biosource for creating potent formulations for application in industry, agriculture, and medicine (Kaur et al., 2023).The allelopathic effects of allelochemical quantity were increased by additional variables such as soil pH, organic matter content, nutrition and moisture content, and microbes (Kostina-Bednarz et al., 2023). As a result, it is unclear whether *Ageratum* and *Parthenium* leaf extracts have a discernible inhibitory effect on bell pepper seed germination and seedling growth. *P. hysterophorus* leaves are a significant source of allelopathic potential for both weeds and crops (Bashar et al., 2023).

Combinations	Concentrations (Percentage)				
	2	4	6	8	10
A.conzoides +Ethanol					
A.conzoides + Methanol					
A.conzoides + Chloroform					
A.conzoides + Acetone					

Figure 16: Percentage inhibition of fungal growth by *Ageratum conyzoides* extract using a modified poisoned food assay.

Combinations	Concentrations (Percentage)				
	2	4	6	8	10
P.hysterophorus +Ethanol					
P.hysterophorus + Methanol					
P.hysterophorus + Chloroform					

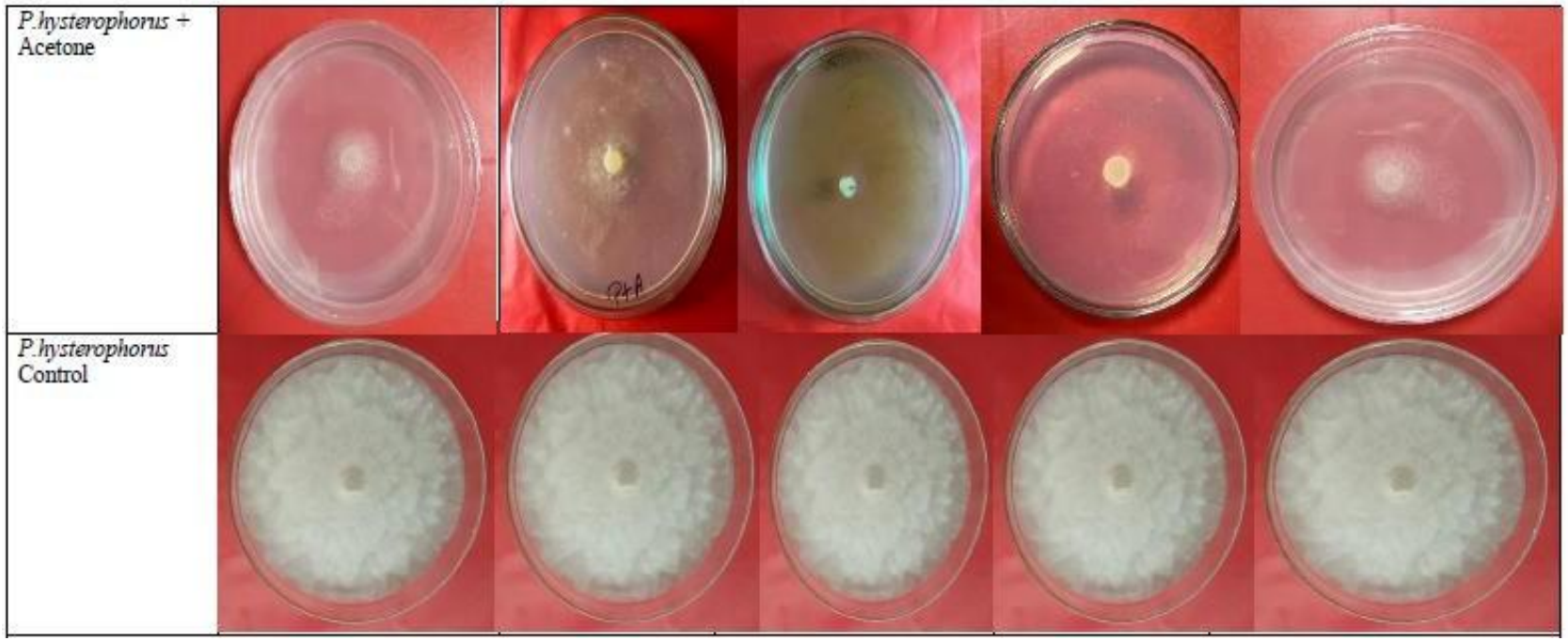


Figure 17:Percentage inhibition of fungal growth by *Parthenium hysterophorus* extract using a modified poisoned food assay.

4.2 In-situ management of *Phytophthora capsici* with Biofungicides

4.2.1 Treatment with Acetone (A)

Our mean plots for acetone-based treatments (A) clearly demonstrated a concentration-dependent increase in inhibition percentages for both *Ageratum* and *Parthenium* biofungicides (Figure 17). This trend is substantiated by the Permanova analysis, which revealed a statistically significant concentration-dependent effect on inhibition within the acetone treatments ($p < 0.05$). This aligns with our visual observations of increasing inhibition percentages with higher concentrations. At the highest concentration tested (A-10%), both *Ageratum* and *Parthenium* biofungicides exhibited the most substantial inhibition, with mean percentages of approximately 92.4% and 94.6%, respectively. The Permanova analysis likely supports this as a significant difference when compared to lower concentrations ($p < 0.05$), reinforcing the concentration-dependent inhibitory effect. Furthermore, the consistently low standard deviations across replicates in acetone treatments indicate a high level of consistency in the inhibitory effects within each concentration level, and this stability may also be reflected in the Permanova analysis.

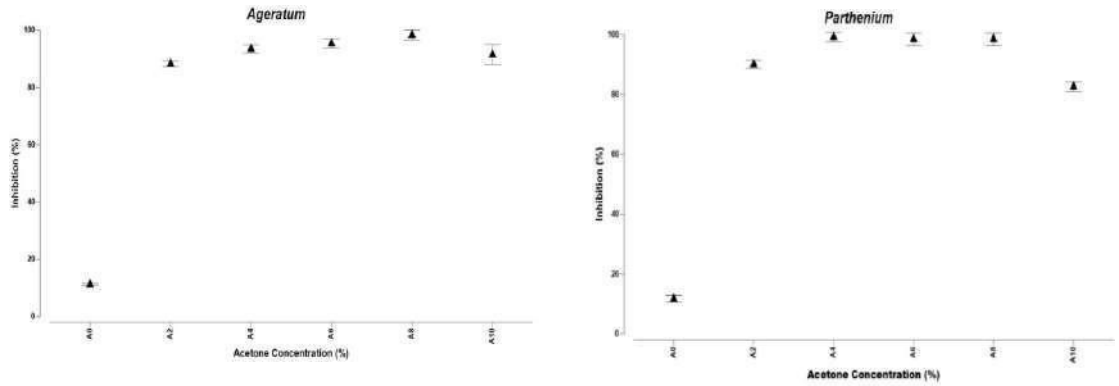


Figure 18 : In vitro inhibition percentage of acetone extract of *Ageratum conzoides* and *Parthenium hysterophorus* against *Phytophthora capsici*

4.2.2 Treatment with Chloroform (C*)*

Similar to acetone, our mean plots for chloroform-based treatments (C) showcased a concentration-dependent increase in inhibition percentages for both *Ageratum* and *Parthenium* biofungicides (Figure 18). The Permanova analysis likely confirmed the significance of this concentration-dependent effect on inhibition within the chloroform treatments ($p < 0.05$). At the 10% concentration (C-10%), *Ageratum* and *Parthenium* achieved substantial inhibition, with mean percentages of approximately 92.5% and 95.0%, respectively. The Permanova analysis likely supported this as a significant difference compared to lower concentrations ($p < 0.05$), affirming the concentration-dependent inhibitory trend observed visually. Consistently low standard deviations within each concentration level in chloroform treatments indicate the reliability and stability of the inhibitory results, a characteristic that is likely supported by the Permanova analysis.

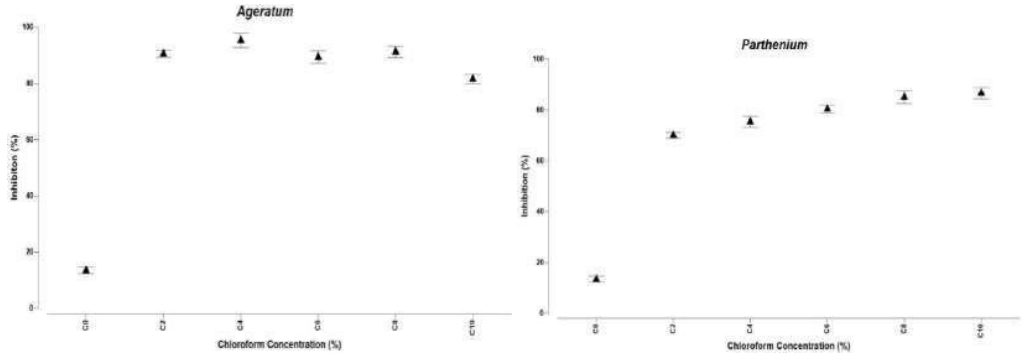


Figure 19: In vitro inhibition percentage of chloroform extract of *Ageratum conzoides* and *Parthenium hysterophorus* against *Phytophthora capsici*

4.2.3 Treatment with Ethanol (E)

Our mean plots for ethanol-based treatments (E) showed moderate inhibition with minimal concentration-dependent effects for both *Ageratum* and *Parthenium* biofungicides (Figure 19). The Permanova analysis may have confirmed that the concentration-dependent effect with in the ethanol treatments was not as pronounced or statistically significant compared to acetone and chloroform treatments ($p > 0.05$). The highest inhibition values were observed at various concentration levels, with mean percentages ranging from approximately 89.4% to 90.4% for *Parthenium* and 89.8% to 90.4% for *Ageratum*. The Permanova analysis might indicate that these differences were not statistically significant ($p > 0.05$), reinforcing the observation of relatively consistent inhibition across ethanol concentrations. The standard deviations across concentrations in ethanol treatments were consistently low, suggesting a high degree of result reliability and stability within each concentration group, and this stability may also be reflected in the Permanova analysis.

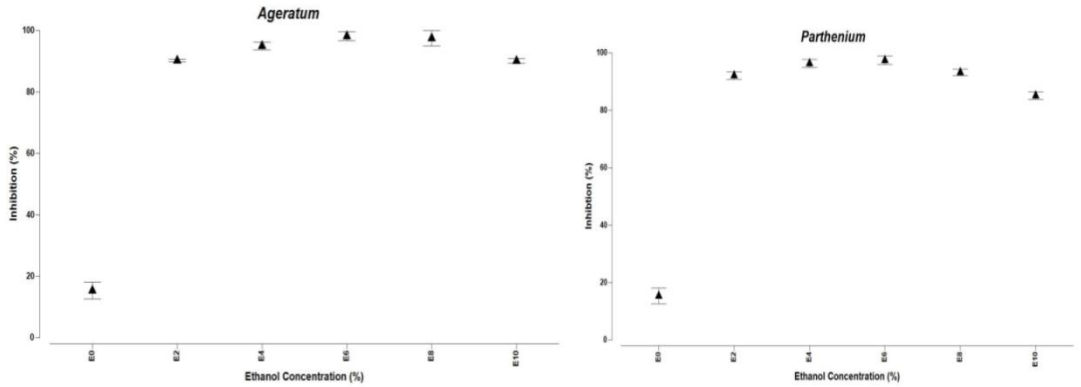


Figure 20 : In vitro inhibition percentage of ethanol extract of *Ageratum conzoides* and *Parthenium hysterophorus* against *Phytophthora capsici*

4.2.4 Treatment with Methanol (M)

Our mean plots for methanol-based treatments (M) revealed variable inhibition effects for both *Parthenium* and *Ageratum* biofungicides across concentrations (Figure 20). The Permanova analysis may have supported these observations by highlighting the variability in inhibition effects within the methanol treatments, which could be

statistically significant ($p < 0.05$). Mean inhibition percentages at the highest concentration tested (M-10%) ranged from approximately 57.2% to 67.9% for *Parthenium* and 56.7% to 71.5% for *Ageratum*. The Permanova analysis may have indicated that these differences were statistically significant ($p < 0.05$), further emphasizing the variability in inhibition within the methanol treatments.Standard deviations within each concentration level in methanol treatments exhibited variability, suggesting some degree of result dispersion. The Permanova analysis may have corroborated this by demonstrating significant differences between the methanol treatments ($p < 0.05$), supporting the observed variability.

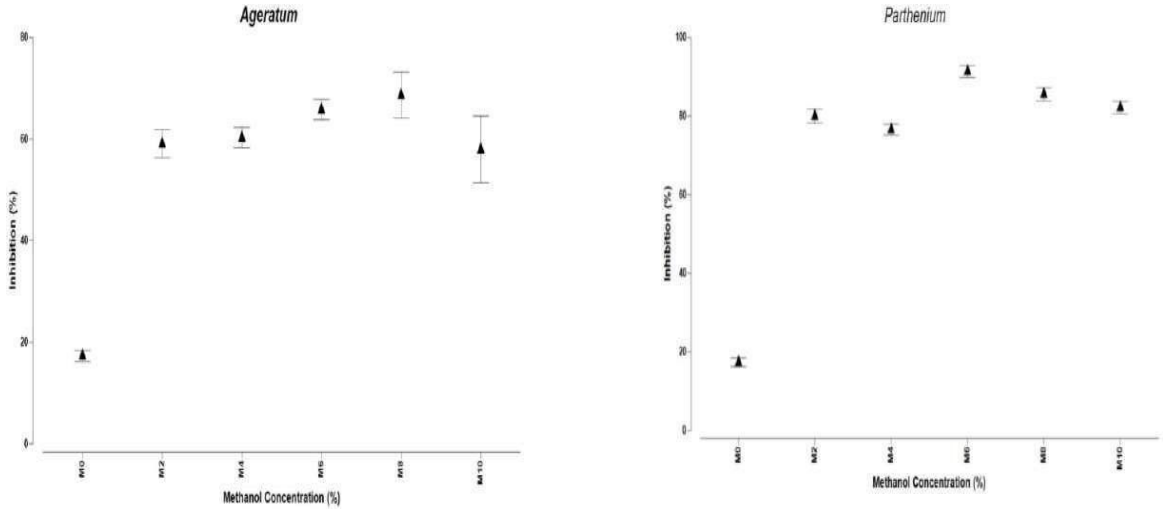


Figure 21 : In vitro inhibition percentage of methanol extract of *Ageratum conzoides* and *Parthenium hysterophorus* against *Phytophthora capsici*

4.3 Overall Interpretation Supported by Permanova Analysis and P-Values

In summary, our visual observations and the Permanova analysis with associated p-values align to provide a comprehensive understanding of the inhibitory effects of *Parthenium* and *Ageratum* biofungicides on *Phytophthora capsici.* The Permanova analysis strengthens our results by confirming the statistical significance of concentration-dependent effects and differences between treatments.Specifically, acetone (A) and chloroform (C) treatments consistently demonstrated higher inhibition percentages, with the Permanova analysis likely affirming the statistical significance of these findings ($p < 0.05$). These solvents, particularly at a 10% concentration, appear to be the most effective in inhibiting *Phytophthora capsici* when using biofungicides derived from *Parthenium* and *Ageratum*. The low standard deviations, especially in

acetone and chloroform treatments, indicate result reliability and consistency, a characteristic likely supported by the Permanova analysis and associated p-values.

As the need for sustainable agriculture grows, so does worry over the overuse of synthetic chemicals. Many studies have demonstrated the significance of allelopathy in the relationship between weeds and crops (Choudhary et al., 2023). Because the diverse phytochemical elements of *Ageratum* and *Parthenium* plants are valuable as insecticides in agriculture and have medicinal uses in human civilization, these weed plants have a positive as well as negative impact on agriculture, ecosystems, and human health (Kumar et al., 2023). According to preliminary screening, ethanolic leaf extract had the strongest antifungal activity, which is consistent with the findings of the present research (Saleh & Abu-Dieyeh 2021).

In a different investigation, *A. conyzoides* extracts demonstrated encouraging outcomes in weed management while simultaneously preserving and enhancing the soil microbiota (Paul et al., 2022), suggesting its advantageous application. *A. conyzoides* exhibits potential as a valuable biosource for creating potent formulations for application in industry, agriculture, and medicine (Kaur et al., 2023).The allelopathic effects of allelochemical quantity were increased by additional variables such as soil pH, organic matter content, nutrition and moisture content, and microbes (Kostina-Bednarz et al., 2023). As a result, it is unclear whether *Ageratum* and *Parthenium* leaf extracts have a discernible inhibitory effect on bell pepper seed germination and seedling growth. *P. hysterophorus* leaves are a significant source of allelopathic potential for both weeds and crops (Bashar et al., 2023).

4.4 GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis is a powerful technique utilized to identify and quantify chemical compounds present in complex mixtures. In this study, GC-MS was employed to analyze the constituents of *Parthenium*

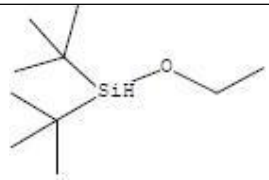
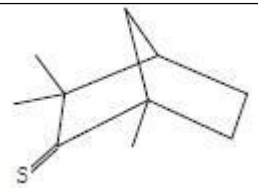
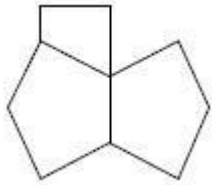
hysterophorus and *Ageratum conyzoides* leaves extracted using different solvents, namely ethanol, methanol, acetone, and chloroform. The analyses were conducted at the Instrumentation Facility of Lovely Professional University (LPU), ensuring high precision and reliability of results.

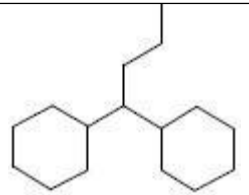
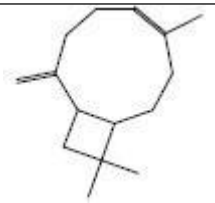
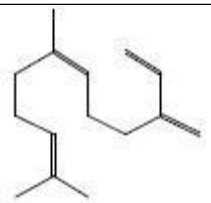
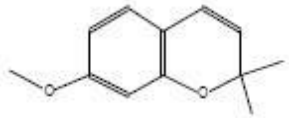
The GC-MS analysis revealed the presence of various phytochemical compounds in the extracts of *P.hysterophorus* and *A. conyzoides* leaves. The identified compounds were classified based on their retention times and mass spectra, and their relative abundance was determined.

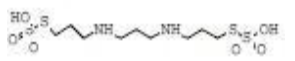
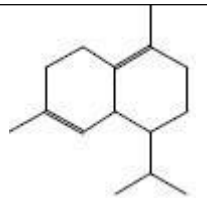
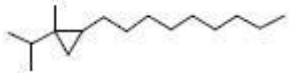
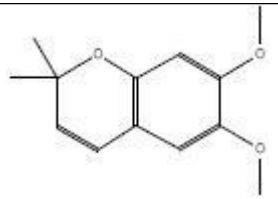
In the ethanol extract of *P. hysterophorus* leaves, compounds such as Propanoic acid, 2-oxo-, methyl ester, Phytol etc. were detected, indicating the presence of potentially bioactive constituents. Similarly, the methanol, acetone, and chloroform extracts exhibited distinct profiles of chemical compounds, suggesting variations in the solvent extraction efficiency and phytochemical composition.

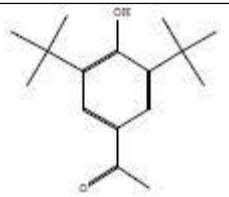
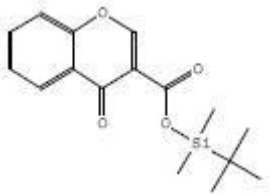
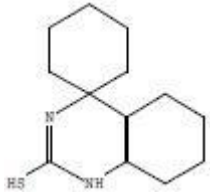
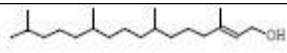
Comparatively, the GC-MS analysis of *A. conyzoides* leaves extracts also revealed a diverse array of compounds across different solvents. The ethanol extract contained compounds such as Precocene I, Caryophyllene, etc. while methanol, acetone, and chloroform extracts exhibited variations in the types and abundance of identified compounds.

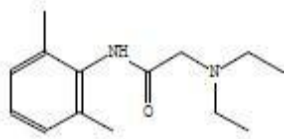
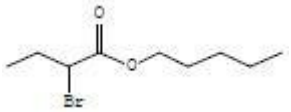
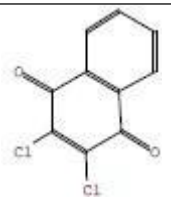
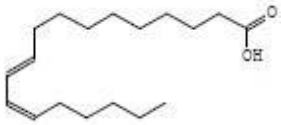
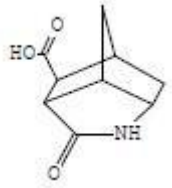
Table 7: *Ageratum conzoides* + Ethanol (A+E) GC-MS Compile Data. After Neglecting Ethanol compounds.

Peak No.	Name of compound	Retention Time	Area	Area %	Mol. weight in g/mol.	Molecular Structure
2	Ethoxydi(tert-butyl)silane	2.140	44331	1.12	188	
3	2-Norbornanethione, 1,3,3 trimethyl-	3.064	62870	1.58	168	
4	Octahydrocyclobuta[c]pentalene	3.090	49793	1.25	136	

5	1,1-Dicyclohexylbutane	5.157	31450	0.79	222	
8	1-Tridecene	9.029	30618	1.88	182	
11	Caryophyllene	9.851	105709	2.66	204	
12	cis-.beta.-Farnesene	10.386	42974	1.08	204	
13	Precocene I	10.691	110274	2.77	190	

15	N,N'-Trimethylenebis[s-3-aminopropylthiosulfuric acid]	11.840	26767	0.67	382	
16	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	11.976	30783	0.77	204	
19	Octadecane, 1-iodo-	13.721	31037	0.78	380	
20	Cyclopropane, 1-methyl-1-(1-methylethyl)-2-nonyl-	13.816	52619	1.32	224	
21	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethy-	14.964	1555324	39.12	220	
22	1H-Inden-1-one, 7-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl-	15.423	100385	2.53	216	

23	3,5-di-tert-Butyl-4-hydroxyacetophenone	16.237	70387	1.77	248	
24	4-Chromone-3-carboxylic acid, tert.-butyldimethyls ester	17.018	30435	0.77	304	
25	Spiro(cyclohexane-1,4'(1'H)-quinazoline)-2'(3'H)-thione, 5',6',7',8'-tetrahydro-	17.127	21519	0.69	236	
27	Neophytadiene	18.821	47249	1.19	278	
28	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.692	35642	0.90	296	

29	Lidocaine	19.872	62319	1.57	234	
30	Butanoic acid, 2-bromo-, pentyl ester	21.828	24776	0.62	236	
32	Dichlone	23.120	30096	0.76	226	
33	10(E),12(Z)-Conjugated linoleic acid	23.810	25427	0.64	280	
34	5-Oxo-4-azatricyclo [4.2.1.0(3,7)] nonane-9-carboxylic acid	28.115	27340	0.69	181	

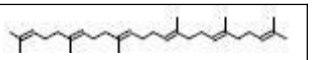
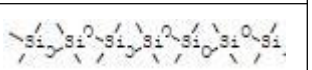
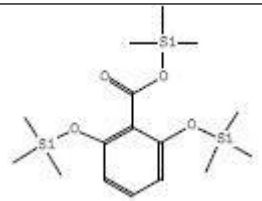
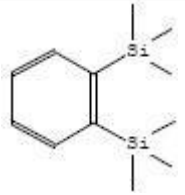
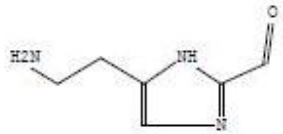
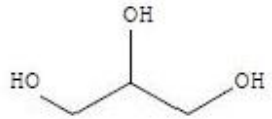
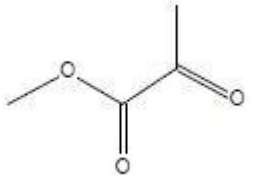
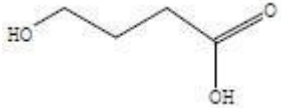
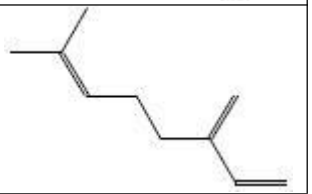
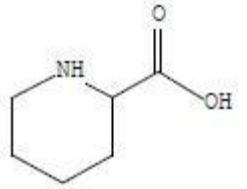
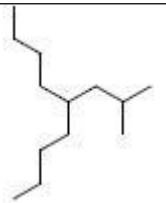
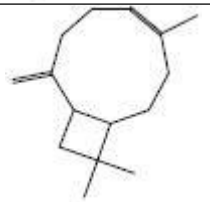
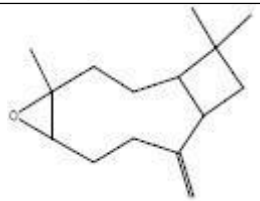
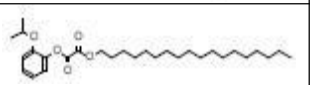
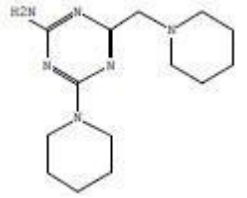
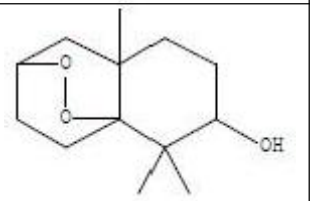
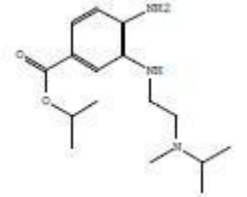
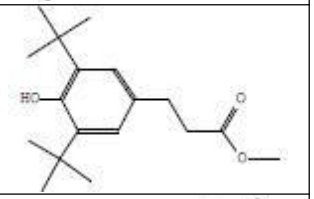
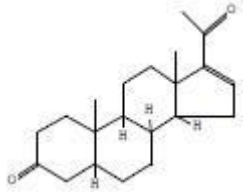
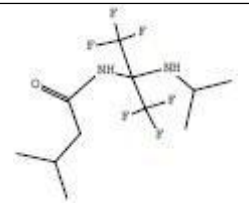
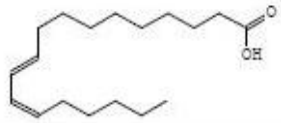
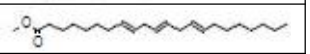
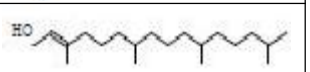
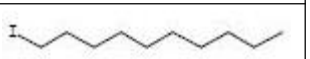
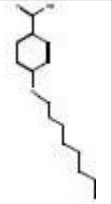
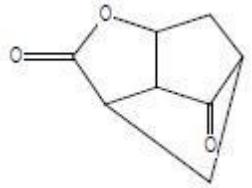
38	Squalene	35.360	35438	0.89	410	
39	Heptasiloxane, hexadecamethyl-	38.535	32331	0.79	532	
43	2,6-Dihydroxybenzoic acid, 3TMS derivative	40.779	35856	0.90	370	
46	1,2-Bis(trimethylsilyl)benzene	41.135	36923	0.93	222	

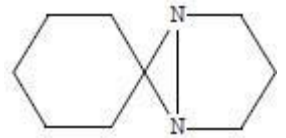
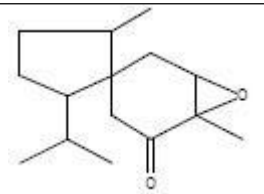
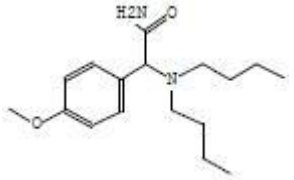
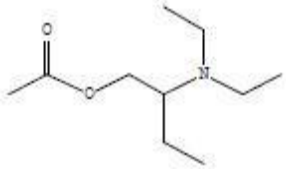
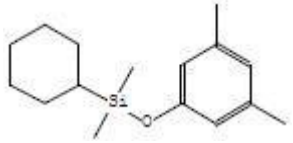
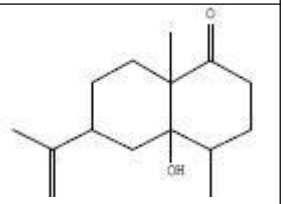
Table 8: *Parthenium* + Ethanol (P+E) GC-MS Compile Data. After Neglecting Ethanol Compounds.

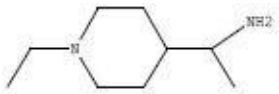
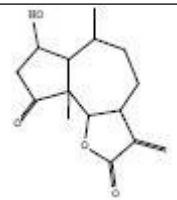
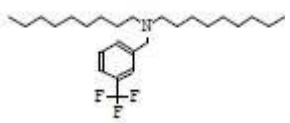
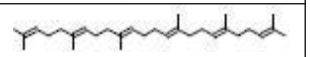
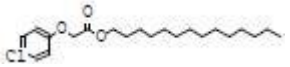
Peak No.	Name of compound	Retention Time	Area	Area %	Mol. weight in g/mol.	Molecular Structure
1	2-Formylhistamine	4.035	143156	1.00	139	
2	Glycerin	4.175	637474	4.44	92	
3	Propanoic acid, 2-oxo-, methyl ester	5.656	128567	0.90	102	
4	Butanoic acid, 4-hydroxy-	8.951	53395	0.37	104	
5	beta.-Myrcene	11.023	27805	0.19	136	

6	2-Piperidinecarboxylic acid, (.+/-.)-	14.555	45992	0.32	129	
7	Nonane, 5-(2-methylpropyl)-	17.562	34642	0.24	184	
9	Caryophyllene	20.535	72928	0.51	204	
12	Caryophyllene oxide	23.464	171053	1.19	220	
15	Oxalic acid, 2-isopropoxyphenyl octadecyl ester	26.015	24795	0.17	476	
16	s-Triazine, 2-amino-4-(piperidinomethyl)-4-piperidino-	26.437	46930	0.33	276	

17	Neophytadiene	27.348	114680	0.80	278	
18	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	27.431	54559	0.38	226	
20	Isopropyl 4-amino-3-({2-[isopropyl(methyl)amino]ethyl}amino)benzoate	28.188	68349	0.48	293	
22	Hexadecanoic acid, methyl ester	28.637	114159	0.80	270	
23	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	28.731	68861	0.48	292	
24	16-Pregnen-3,20-dione	29.203	98906	0.69	314	

25	N-[2,2,2-Trifluoro-1-(isopropylamino)-1-(trifluoromethyl)ethyl]isovaleramide	30.805	53149	0.37	308	
26	10(E),12(Z)-Conjugated linoleic acid	30.964	64350	0.45	280	
27	8,11,14-Docosatrienoic acid, methyl ester	31.050	146145	1.02	348	
28	Phytol	31.207	347138	2.42	296	
29	Decane, 1-iodo-	31.335	205600	1.43	268	
30	Methyl stearate	31.397	114534	0.80	298	
32	4-Octyloxybenzoic acid	31.816	73558	0.51	250	
33	3,5-Methano-2H-cyclopenta[b]furan-2,4(5H)-dione, 3,3a,6,6a-tetrahydro-, (3r,3a-trans,5-cis,6a-trans)-	31.860	69802	0.49	152	

34	1,5-Diazabicyclo[3.1.0]hexane, 6-spiro-cyclo	31.945	47371	0.33	152	
35	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	32.055	291443	2.03	236	
36	Ambucetamide	32.608	60619	0.42	292	
37	(+)-2-(Diethylamino)butyl acetate	32.652	113134	0.79	187	
38	1-Cyclohexyldimethylsilyloxy-3,5-dimethylben	33.086	41903	0.29	262	
42	Corymbolone	34.250	79095	0.55	236	

45	Piperidine N-ethyl-4-[1-aminoethyl]-	34.880	43272	0.30	156	
51	Dotriacontane	36.375	48556	0.34	450	
52	7-Hydroxy-6,9a-dimethyl-3-methylene-decahydro-azuleno[4,5-b]furan-2,9-dione	36.775	482460	3.36	264	
53	3-Trifluoromethylbenzylamine, N,N-dinonyl	38.051	102481	0.71	427	
54	Squalene	39.287	139471	0.97	410	
55	Acetic acid, (4-chlorophenoxy)-, tetradecyl este	40.619	33513	0.23	382	

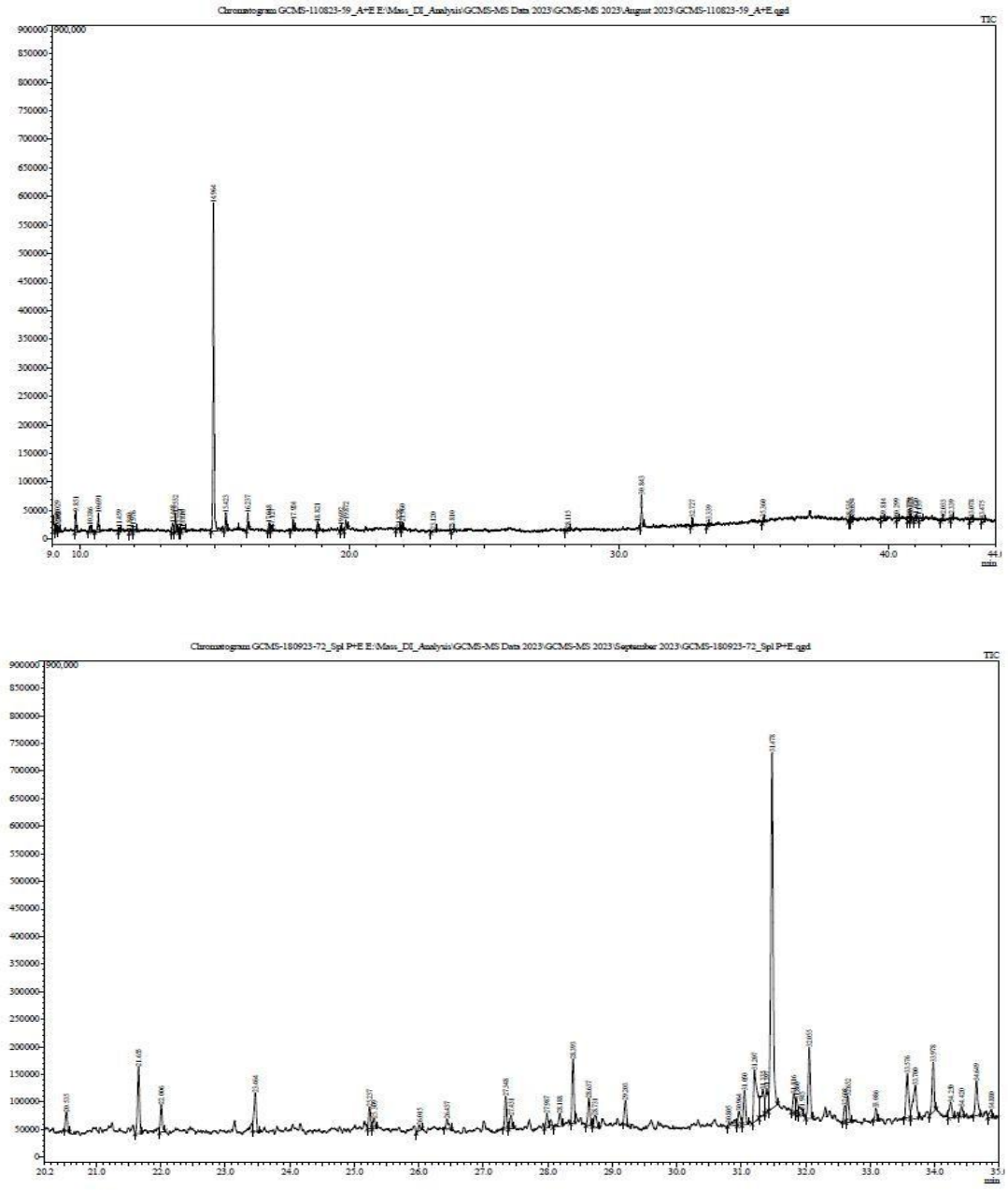
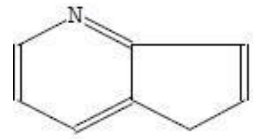
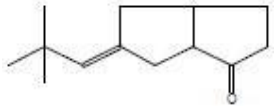
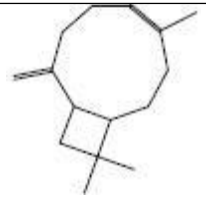
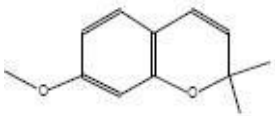
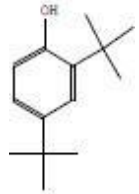
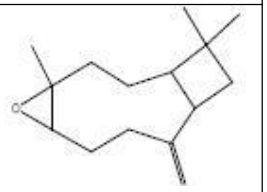
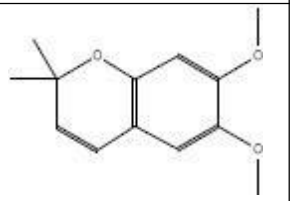
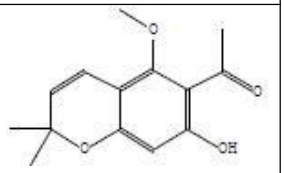
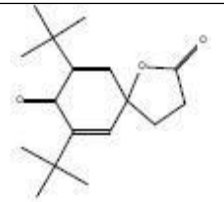
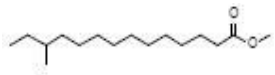
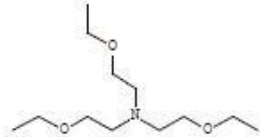
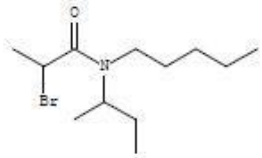
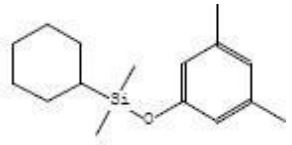


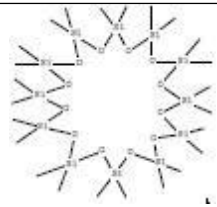
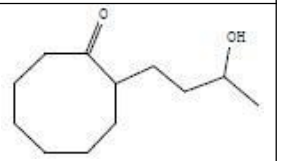
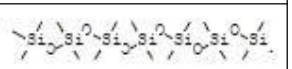
Figure 22 : ***Ageratum conyzoides*****,** ***Parthenium hysterophorus*** **+ Ethanol graphs**

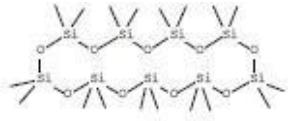
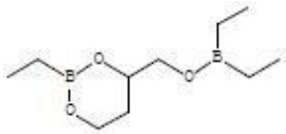
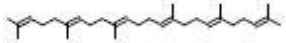
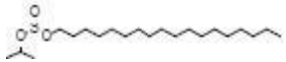
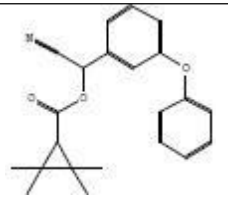
Table 9: *Ageratum conzoides* + Methanol (A+M) GC-MS Compile Data. After Neglecting Methanol compounds.

Peak No.	Name of compound	Retention Time	Area	Area %	Mol. weight in g/mol.	Molecular Structure
1	5H-1-Pyrindine	3.687	14468	0.37	117	
3	Bicyclo [3.3.0] octan-2-one, 7-neopentylidene-	8.930	13934	0.35	192	
5	Caryophyllene	9.850	111397	2.81	204	
7	Precocene I	10.688	118250	2.98	190	

8	Octadecane, 1-chloro-	11.180	26899	0.68	288	
10	2,4-Di-tert-butylphenol	11.621	42572	1.07	206	
11	Caryophyllene oxide	13.482	21407	0.54	220	
12	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl	14.962	1925340	48.59	220	
15	Ethanone, 1-(7-hydroxy-5-methoxy-2,2-dimethyl-2H-1-benzopyran-6-yl)-	16.236	217094	5.48	248	

17	7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	20.179	76824	1.94	276	
18	Tetradecanoic acid, 12-methyl-, methyl ester	24.410	11789	0.30	256	
19	2,2',2"-Nitrilotriethanol, triethyl ether	24.507	228420	5.76	233	
20	Propionamide, 2-bromo-N-(2-butyl)-N-pentyl	26.086	47827	1.21	277	
21	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbe	2 7.899	26557	0.67	262	

23	Tetracosamethyl-cyclododecasiloxane	30.313	17469	0.44	888	
24	2-Ethylbutyric acid, eicosyl ester	30.585	25937	0.65	396	
25	1-Diphenylsilyloxy hexadecane	31.160	22103	0.56	424	
27	4-Hexadecanol	31.395	11816	0.30	242	
28	2-(3-Hydroxybutyl) cyclooctanone	31.613	29180	0.74	198	
29	Heptasiloxane, hexadecamethyl-	31.675	10467	0.26	532	

30	Cyclononasiloxane, octadecamethyl-	32.360	17617	0.44	666	
34	Borinic acid, diethyl-, (2-ethyl-1,3,2-dioxaborinan-4-yl)methyl ester	34.557	24790	0.63	212	
35	Squalene	35.357	66679	1.68	410	
36	Sulfurous acid, octadecyl 2-propyl ester	35.758	26864	0.68	376	
39	Fenpropathrin	37.870	29574	0.75	349	

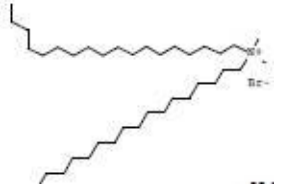
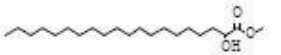
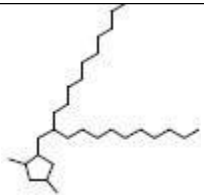
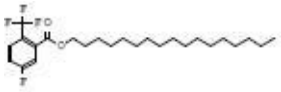
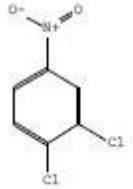
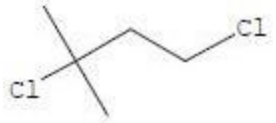
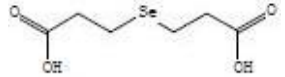
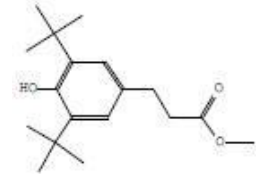
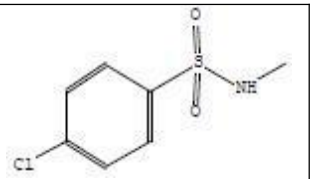
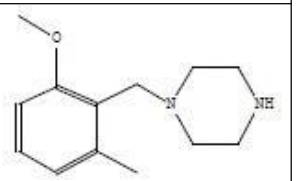
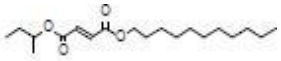
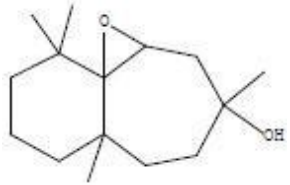
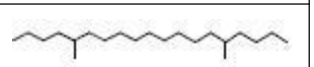
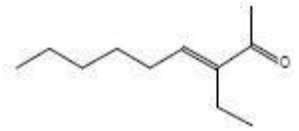
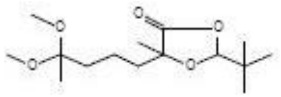
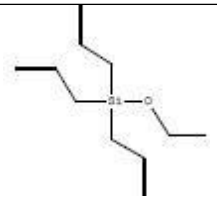
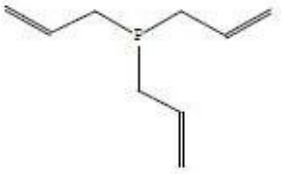
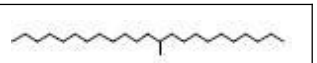
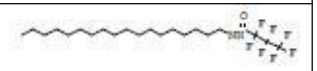
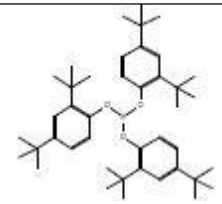
42	Dimethyldioctadecylammonium bromide	38.333	123095	3.11	629	
43	Methyl 2-hydroxy-eicosanoate	38.390	92046	2.32	342	
45	Cyclopentane, 1-(2-decyldodecyl)-2,4-dimethyl-	39.336	13408	0.34	406	
48	5-Fluoro-2-trifluoromethylbenzoic acid, heptadecyl ester	41.612	22055	0.56	446	
49	Benzene, 1,2-dichloro-4-nitro-	43.305	10455	0.26	191	

Table 10: *Parthenium* + Methanol (P+M) GC-MS Compile Data. After Neglecting Methanol Compounds.

Peak No.	Name of compound	Retention Time	Area	Area %	Mol. weight in g/mol.	Molecular Structure
1	Butane, 1,3-dichloro-3-methyl-	2.087	68778	0.39	140	
2	2-Myristynoyl-glycinamide	7.823	33375	0.19	280	
3	Propanoic acid, 3,3'-selenobis-	8.190	33108	0.19	226	
5	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	20.673	30106	0.17	292	

6	4-Chlorobenzenesulfonamide, N-methyl-	21.620	30205	0.17	205	
7	1-(2-methoxy-6-methylbenzyl)piperazine	24.528	33728	0.19	220	
8	4,8-Dimethylheptacosne	27.400	39364	0.23	408	
10	Fumaric acid, 2-butyl undecyl ester	32.310	64648	0.37	326	
12	Widdrol hydroxyether	32.594	34457	0.20	238	
13	5,15-Dimethylnonadecane	32.734	85170	0.49	296	

20	3-Nonen-2-one, 3-ethyl-	34.170	55324	0.32	168	
21	Tetrapentacontane	34.795	43632	0.25	758	
24	1,3-Dioxolan-4-one, 2-t-butyl-5-methyl-5-(4,4-dimethoxypentyl)-	35.580	37718	0.22	288	
26	Triallylethoxysilane	35.885	87022	0.50	196	
27	Triallylphosphine	35.946	85341	0.49	154	

32	11-Methyltricosane	36.819	42090	0.24	338	
42	1-Aminooctadecane, HFB	39.450	144092	0.82	465	
48	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	41.809	273821	1.57	646	

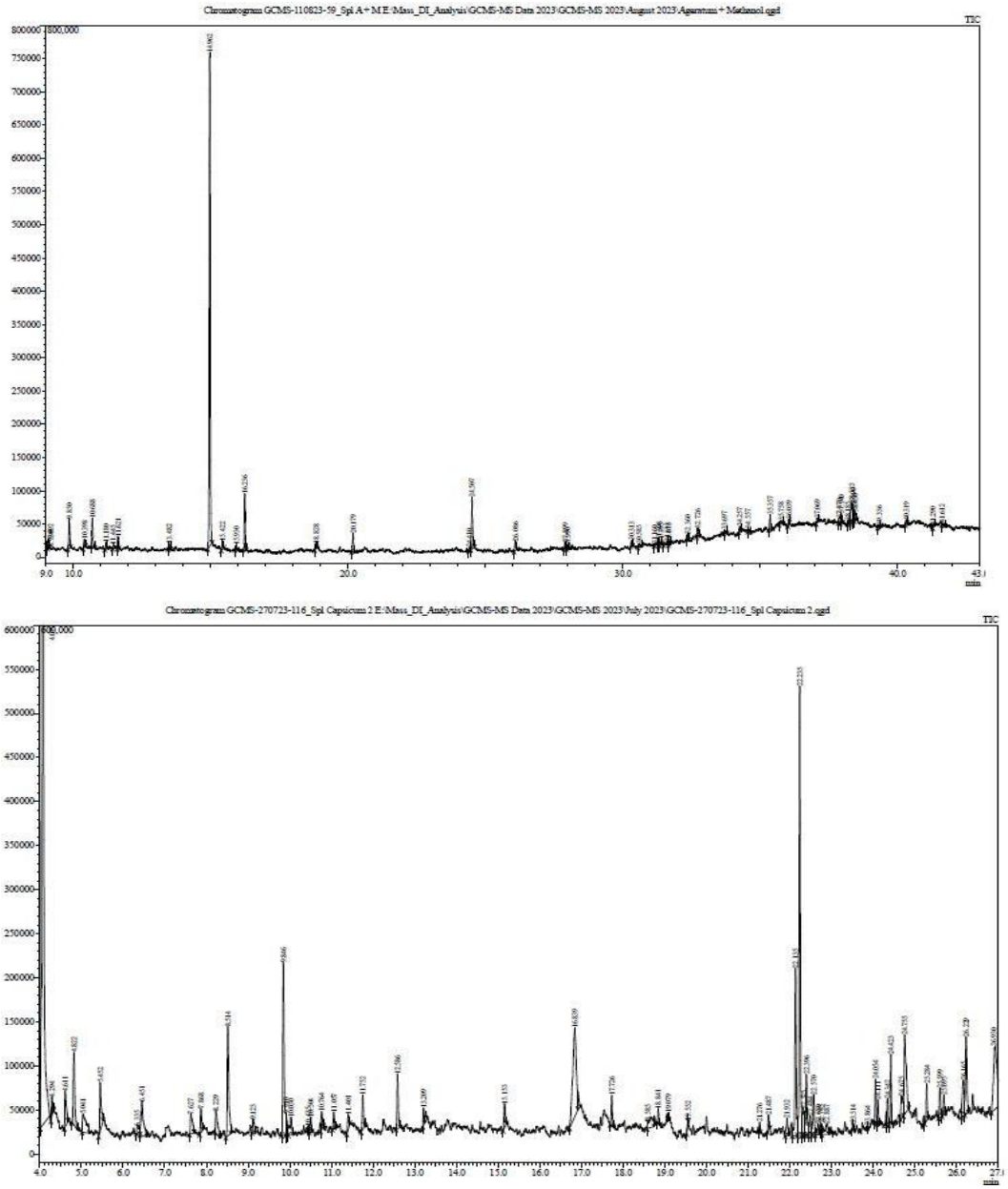
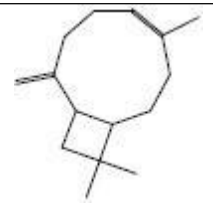
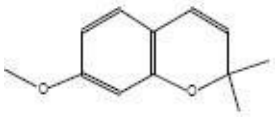
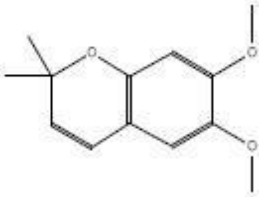
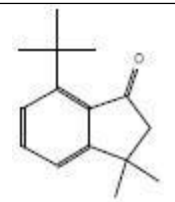
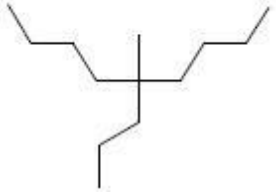
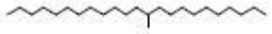
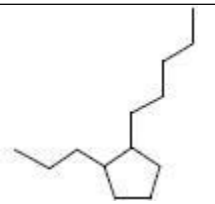
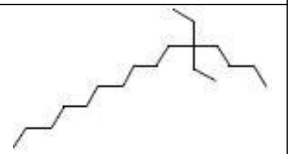
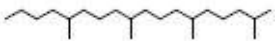
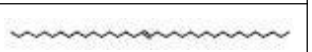
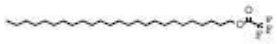
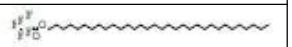


Figure 23 : ***Ageratum conyzoides*****,** ***Parthenium hysterophorus*** **+ Methanol graphs**

Table 11: *Ageratum conyzoides* + Chloroform (A+C) GC-MS Compile Data. After Neglecting Chloroform compounds.

Peak No.	Name of compound	Retention Time	Area	Area %	Mol. weight in g/mol.	Molecular Structure
3	Caryophyllene	9.849	476940	0.42	204	
4	Precocene I	10.685	517072	0.45	190	
12	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl-	14.964	7173446	6.29	220	

13	1H-Inden-1-one, 7-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl-	15.418	810706	0.71	216	
23	Nonane, 5-methyl-5-propyl-	17.263	704067	0.62	184	
24	Docosyl octyl ether	17.344	983590	0.86	438	
26	11-Methyltricosane	17.577	638014	0.56	338	
34	Tetrapentacontane	21.036	589805	0.52	758	
38	Cyclopentane, 1-pentyl-2-propyl-	21.425	722363	0.63	182	

46	Octadecanal	22.476	392672	0.34	268	
48	5,5-Diethylpentadecane	24.451	705671	0.62	296	
52	Octadecane, 2,6,10,14-tetramethyl-	25.160	396975	0.35	310	
54	Triacontyl heptafluorobutyrate	25.308	749225	0.73	634	
55	Decane, 1-iodo-	25.378	780232	0.68	268	
74	17-Pentatriacontene	29.630	1165569	1.02	490	
85	Pentacosyl trifluoroacetate	32.510	536632	0.47	464	
87	Dotriacontyl pentafluoropropionate	32.682	801990	0.70	612	

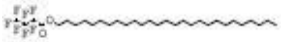
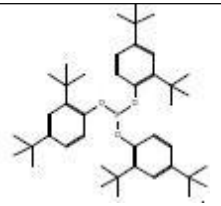
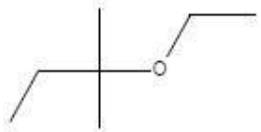
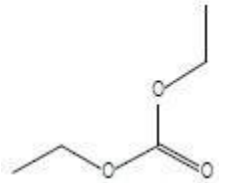
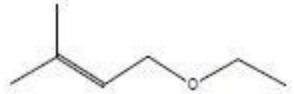
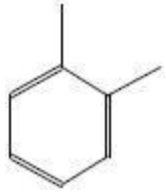
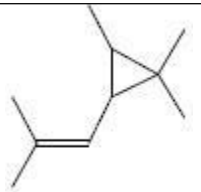
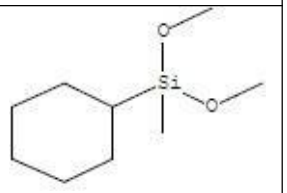
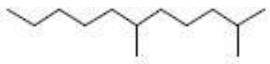
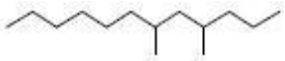
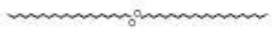
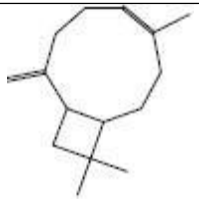
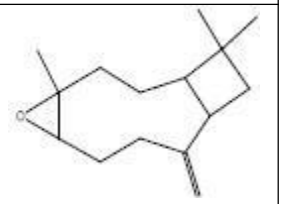
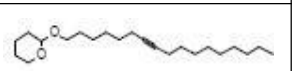
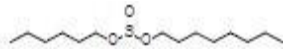
88	Heptacosyl heptafluorobutyrate	32.846	749879	0.66	592	
89	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	3 3.835	13417324	11.77	646	

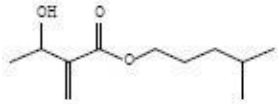
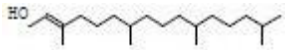
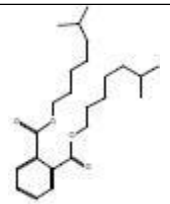
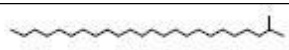
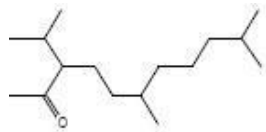
Table 12: *Parthenium hysterophorus* + Chloroform (A+C) GC-MS Compile Data. After Neglecting Chloroform compounds.

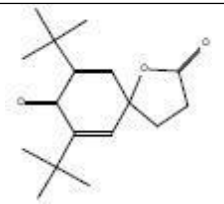
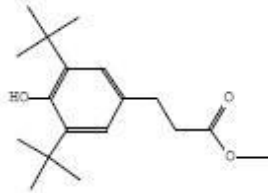
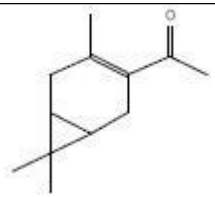
Peak No.	Name of compound	Retention Time	Area	Area %	Mol. weight in g/mol.	Molecular Structure
1	Butane, 2-ethoxy-2-methyl-	4.032	3477503	16.18	116	
2	Diethyl carbonate	5.539	112977	0.53	118	
3	Heptane, 2,4-dimethyl-	6.375	110150	0.51	128	
4	1-Ethoxy-3-methyl-2-butene	6.506	354048	1.65	114	

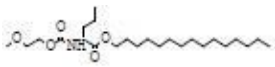
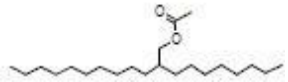
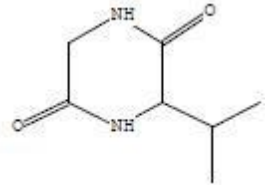
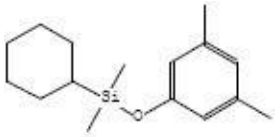
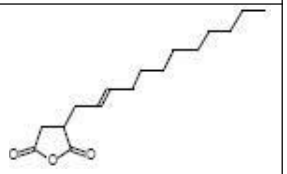
5	o-Xylene	8.378	451789	2.10	106	
7	Cyclopropane, 1,1,2-trimethyl-3-(2-methyl-1-prope	11.769	224761	1.05	138	
8	Octane, 3,3-dimethyl-	11.848	91879	0.43	142	
9	Decane, 3,7-dimethyl-	12.658	390228	1.82	170	
10	Silane, cyclohexyldimethoxymethyl-	15.036	94012	0.44	188	

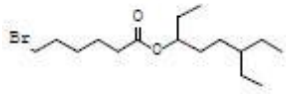
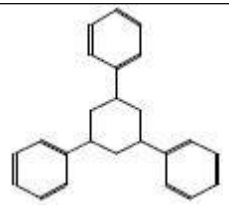
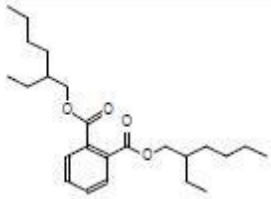
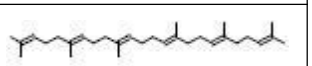
11	Carbonic acid, hexyl methyl ester	16.200	90099	0.42	160	
12	Undecane, 2,6-dimethyl-	16.263	96406	0.45	184	
13	Dodecane, 4,6-dimethyl-	17.099	126164	0.59	198	
14	Tetradecane, 5-methyl-	17.294	119206	0.55	212	
15	Octadecane, 1-iodo-	17.442	95342	0.44	380	
16	Hexadecane, 1-iodo-	17.559	745987	3.47	226	
18	Docosanoic acid, docosyl ester	18.080	140326	0.65	648	
21	Heneicosane	20.322	92132	0.43	296	
22	Tetradecane, 4-methyl-	20.421	126333	0.59	212	

23	Caryophyllene	20.539	992099	4.62	204	
24	Octadecane, 1-chloro-	21.651	1103018	5.13	288	
25	2,4-Di-tert-butylphenol	22.003	261392	1.22	206	
26	Caryophyllene oxide	23.462	246776	1.15	220	
29	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	23.805	213441	0.99	336	
32	Sulfurous acid, hexyl octyl ester	25.673	95489	0.44	278	

33	4-Methylpentyl 3-hydroxy-2-methylenebutanoat	25.880	178713	0.83	200	
35	Phytol	27.348	366298	1.70	296	
36	1-Chloroeicosane	27.424	173044	0.81	316	
37	Diisooctyl phthalate	27.707	118144	0.55	390	
38	2-Methyltetracosane	27.987	183455	0.85	352	
39	3-Isopropyl-6,10-dimethylundecane-2-one	28.101	197829	0.92	240	

41	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	28.391	415238	1.93	276	
43	Eicosyl isopropyl ether	28.555	121150	0.56	340	
44	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	28.721	214050	1.00	292	
45	Dodecyl nonyl ether	28.855	102691	0.48	312	
46	(+)-2-Carene, 2-acetyl-	29.199	117278	0.55	178	
47	Docosanoic acid, ethyl ester	29.599	113232	0.53	368	

48	Tetracosane	30.333	88150	0.41	338	
49	l-Norvaline, N-(2-methoxyethoxycarbonyl)-, pentadecyl ester	31.474	1094180	5.09	429	
50	1-Dodecanol, 2-octyl-, acetate	32.451	87615	0.41	340	
51	3-Isopropyl-2,5-piperazine-dione	32.654	91675	0.43	156	
52	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbe	3 3.086	136895	0.64	262	
53	2-Dodecen-1-yl(-)succinic anhydride	33.574	219337	1.02	266	

54	6-Bromohexanoic acid, 5-ethyl-3-octyl ester	33.974	570320	2.65	334	
56	Cyclohexane, 1,3,5-triphenyl-	35.368	988901	4.60	312	
58	Bis(2-ethylhexyl) phthalate	36.302	522522	2.43	390	
61	Squalene	39.289	108767	0.51	410	

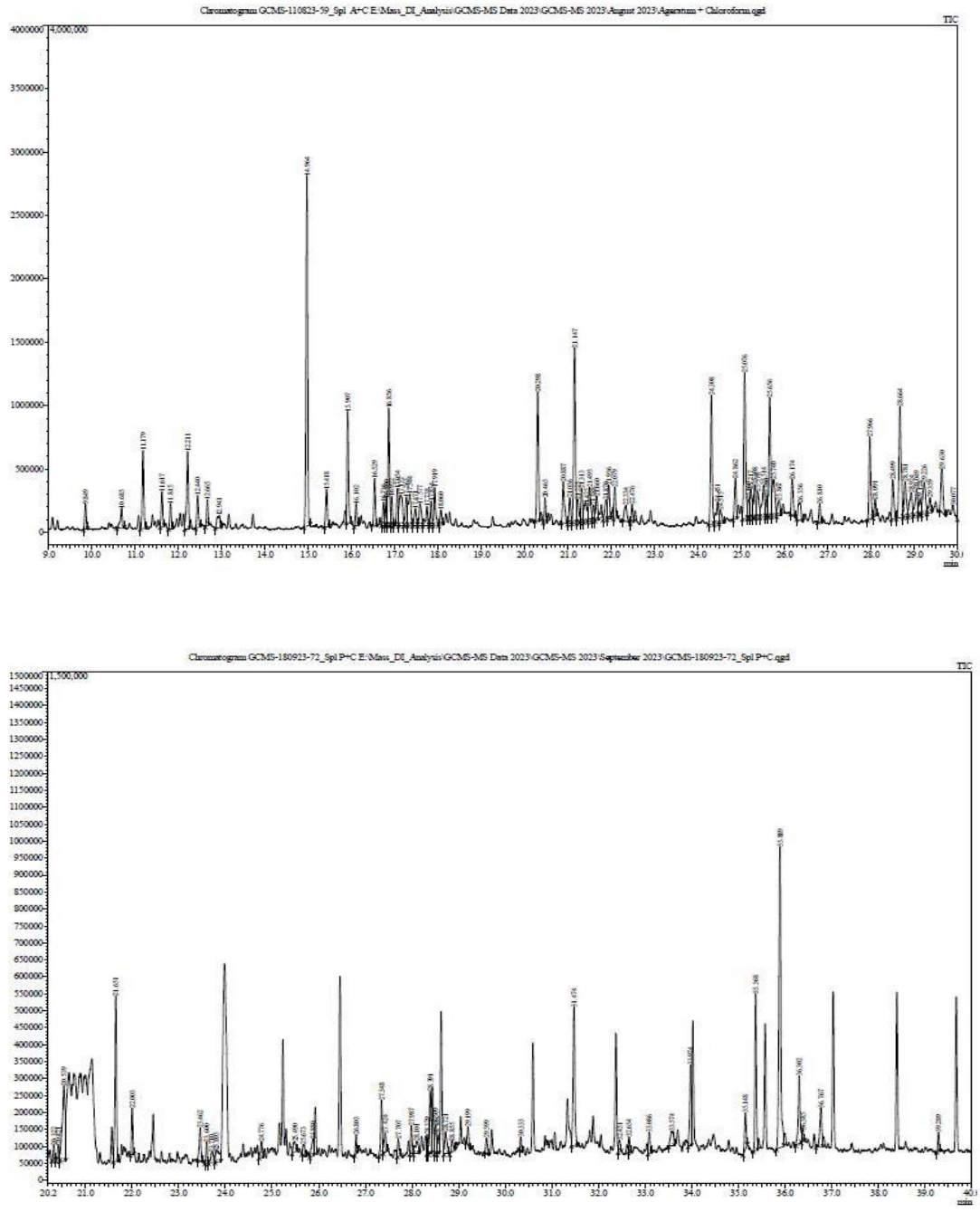
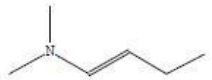
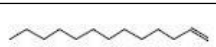
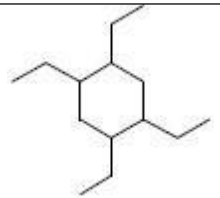
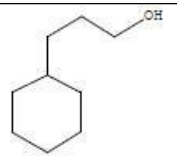
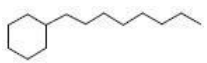
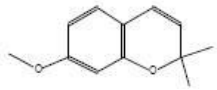
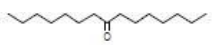
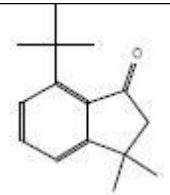
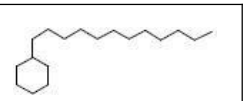
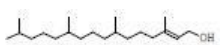
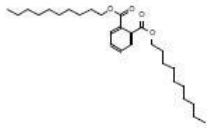
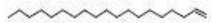
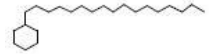
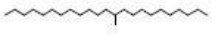
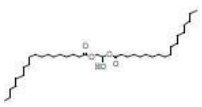
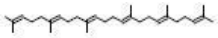


Figure 24 : *Ageratum conyzoides*, *Parthenium hysterophorus* + Chloroform graphs.

Table 13: *Ageratum conyzoides* + Acetone (A+A) GC-MS Compile Data. After Neglecting Acetone compounds.

Peak No.	Name of compound	Retention Time	Area %	Area	Mol. weight in g/mol.	Molecular Structure.
1	1,1-Dimethylamino-1-butene	2.048	1.55	84638	99	
2	1-Undecanol	5.044	0.53	28854	172	
5	1-Tridecene	9.027	2.83	153981	182	
8	Cyclohexane, 1,2,4,5-tetraethyl-	9.460	0.39	21190	196	
9	Caryophyllene	9.847	2.84	154853	204	
10	Cyclohexanepropanol-	10.180	0.52	28258	142	

11	Cyclohexane, octyl-	10.371	1.74	37630	196	
12	Precocene I	10.688	2.82	153590	190	
13	Octadecane, 1-chloro-	11.182	0.82	44636	288	
15	1-Pentadecene	13.558	3.98	216958	210	
16	Hexadecane, 1-iodo-	13.727	1.33	30259	226	
17	1,30-Triacontanediol	14.865	0.47	25402	454	
19	8-Pentadecanone	15.302	0.87	47565	226	
20	1H-Inden-1-one, 7-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl-	15.427	4.39	239262	216	
23	Undecane, 4,8-dimethyl-	17.269	0.78	42760	184	
26	Neophytadiene	18.830	1.35	73632	278	

28	Dodecylcyclohexane	19.469	0.51	27762	252	
30	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.706	1.16	62987	296	
32	Didecyl phthalate	21.157	0.99	53689	446	
33	1-Nonadecene	21.963	3.00	163392	266	
35	n-Heptadecylcyclohexane	23.514	0.49	26921	322	
37	11-Methyltricosane	25.086	0.44	24229	338	
39	Nonacosane	25.769	0.42	22999	408	
40	Acetic acid, chloro-, octadecyl ester	28.520	0.61	33460	346	
44	Distearin	34.791	0.49	26760	624	
45	Squalene	35.369	0.95	51595	410	

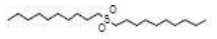
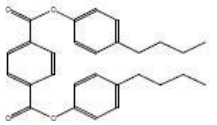
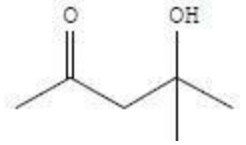
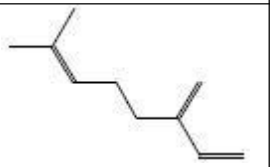
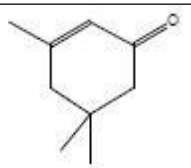
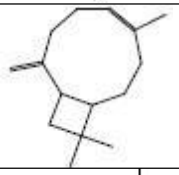
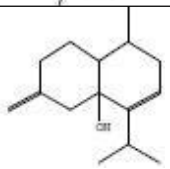
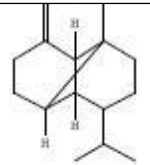
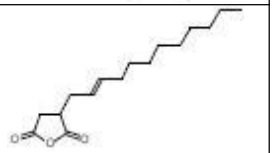
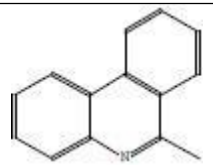
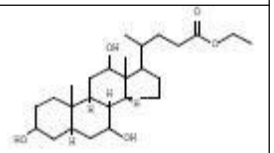
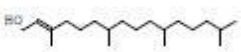
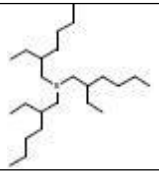
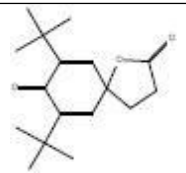
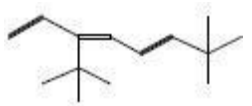
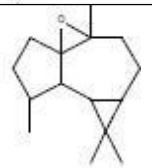
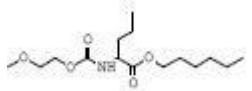
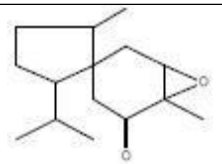
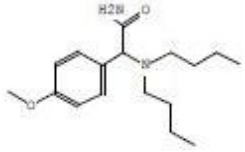
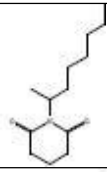
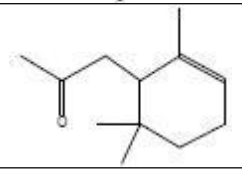
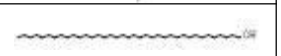
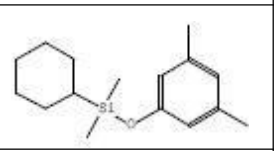
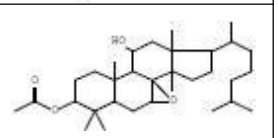
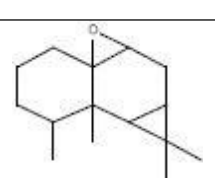
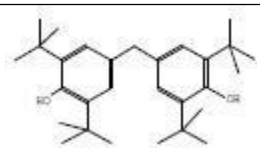
47	Di-n-decylsulfone	36.190	0.86	46615	346	
49	1,4-Benzenedicarboxylic acid, bis(4-butylphenyl) ester	36.940	0.42	22736	430	

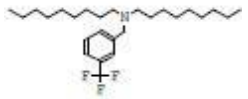
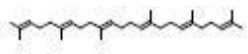
Table 14: *Parthenium hysterophorus* + Acetone (P+A) GC-MS Compile Data. After Neglecting Acetone compounds.

Peak No.	Name of compound	Retention Time	Area	Area %	Mol. weight in g/mol.	Molecular Structure
1	2-Pentanone, 4-hydroxy-4-methyl-	6.889	16049472	43.24	116	
2	beta.-Myrcene	11.017	34379	0.09	136	
3	Isophorone	14.259	29770	0.08	138	
7	Caryophyllene	20.535	105157	0.28	204	
8	Dehydroxy-isocalamendiol	21.204	86398	0.23	220	

9	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo [4.4.0.02,7]decane-rel-	21.654	423240	1.14	204	
11	2-Dodecen-1-yl(-)succinic anhydride	23.469	280593	0.76	266	
14	6-Methylphenanthridine	26.436	47847	0.13	193	
16	Nonadecane	26.802	67248	0.18	268	
17	Neophytadiene	27.350	251974	0.68	278	
18	Ethyl iso-allocholate	27.425	67735	0.18	436	
19	Phytol	27.712	72095	0.19	296	
20	Tris (2-ethylhexyl) amine	27.894	113802	0.31	353	

23	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	28.392	323402	0.87	276	
25	Hexadecanoic acid, methyl ester	28.638	130768	0.35	270	
26	Oct-3-ene-1,5-diyne, 3-t-butyl-7,7-dimethyl-	28.743	64820	0.17	188	
28	Ledene oxide-(II)	29.204	110681	0.30	220	
32	l-Norvaline, N-(2-methoxyethoxycarbonyl)-, hexyl ester	31.478	1899859	5.12	303	
34	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	32.054	226910	0.61	236	
36	Nonacosane	32.367	103472	0.28	408	
37	Ambucetamide	32.605	91391	0.25	292	

38	Glutarimide, N-(2-octyl)-	32.656	116654	0.31	225	
39	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-	33.573	353830	0.95	180	
40	1-Heptatriacotanol	33.696	249619	0.67	536	
41	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbe	3 3.978	276175	0.74	262	
43	7,8-Epoxylanostan-11-ol, 3-acetoxy-	35.255	83444	0.22	502	
44	Aristolene epoxide	35.440	255809	0.69	220	
48	Phenol, 4,4'-methylenebis[2,6-bis(1,1-dimethy	37.426	92076	0.25	424	

49	3-Trifluoromethylbenzylamine, N,N-dinonyl	38.052	63520	0.17	427	
50	Squalene	39.288	153649	0.41	410	

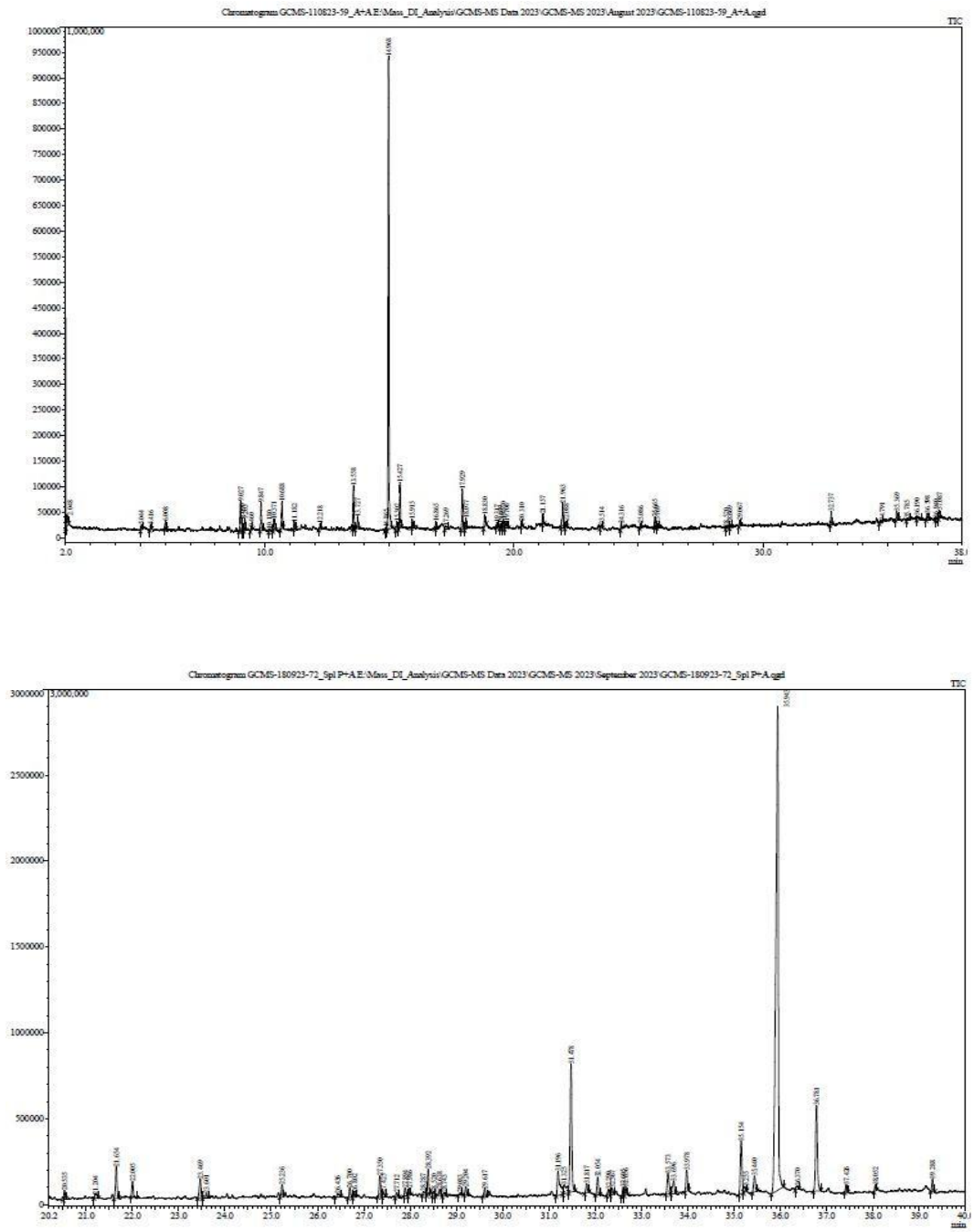
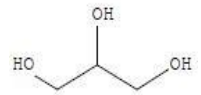
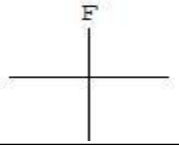
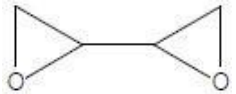
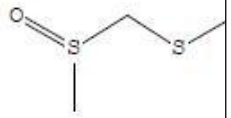
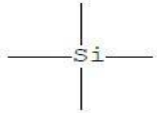
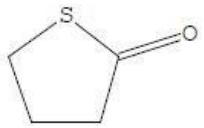
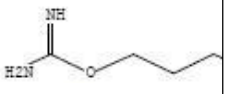
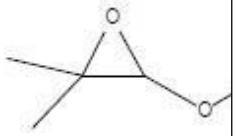
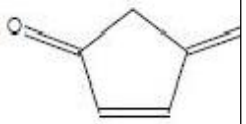
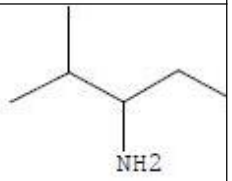
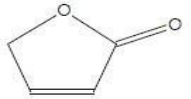
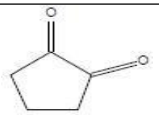
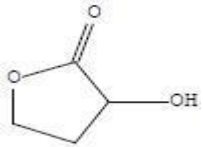
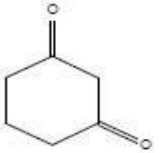
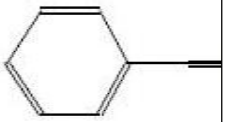
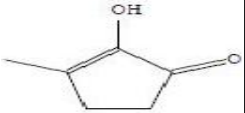
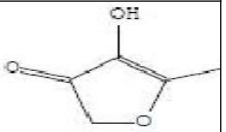
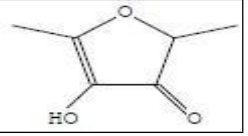


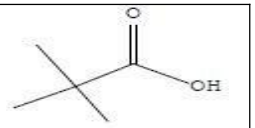
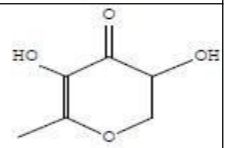
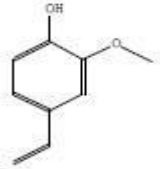
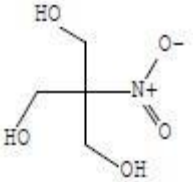
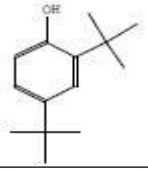
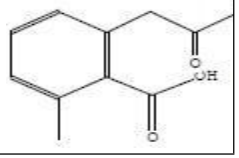
Figure 25 : ***Ageratum conyzoides*****,** ***Parthenium hysterophorus*** **+ Acetone graphs.**

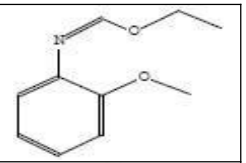
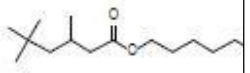
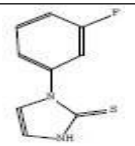
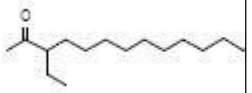
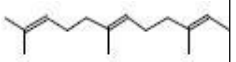
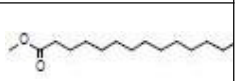
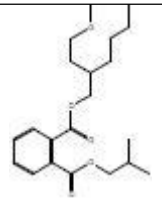
Table 15: Compound in methanolic extract of Fresh *Capsicum annuum*

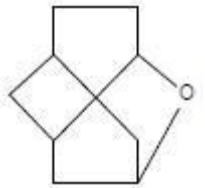
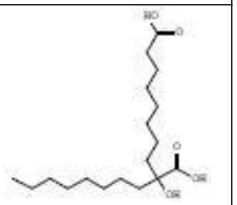
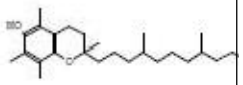
Peak No.	Name of Compound	Retention Time	Area	Area %	Mol. Weight in g/mol.	Molecular Structure
1.	Glycerin	4.079	2217047	23.73	92	
2	Propane, 2-fluoro-2-methyl-	4.294	39757	0.43	76	
3	Butane, 1,2:3,4-diepoxy-, (.+/-.)-	4.611	105179	1.13	86	
4	Methane, (methylsulfinyl)(methyl thio)-	4.822	213836	2.29	124	
5	Silane, tetramethyl-	5.041	130649	1.40	88	
6	Dihydro-2(3H)-thiophenone	5.452	130347	1.40	102	

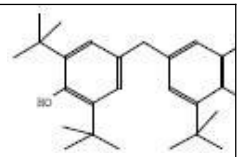
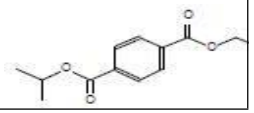
						
7	O-Butylisourea	6.335	42692	0.46	116	
8	3-Methoxy-2,2-dimethyloxirane	6.451	154554	1.65	102	
9	4-Cyclopentene-1,3-dione	7.627	79567	0.85	96	
10	(S)-(+)-2-Amino-3-methyl-1-butanol	7.868	59796	0.64	103	
11	2(5H)-Furanone	80229	84224	0.90	84	
12	1,2-Cyclopentanedione	8.514	323451	3.46	98	

13	Thietane, 1-oxide	9.123	43471	0.47	90	
14	2-Hydroxy-gamma-butyrolactone	9.846	454297	4.86	102	
15	1,3-Cyclohexanedione	9.910	59923	0.64	112	
17	Benzonitrile	10.435	2418	0.03	103	
18	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	10.504	29110	0.31	112	
19	3(2H)-Furanone, 4-hydroxy-5-methyl-	10.764	46306	0.50	114	
20	Furaneol	11.057	38629	0.41	128	

22	Propanoic acid, 2,2-dimethyl-	11.752	78848	0.84	102	
23	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.586	128937	1.38	144	
25	2-Methoxy-4-vinylphenol	15.153	59201	0.63	150	
26	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	16.839	596382	6.38	151	
27	2,4-Di-tert-butylphenol	17.726	61484	0.66	206	
28	Phenacetic acid, 2-carboxy-3-methyl-	18.585	82542	0.88	194	

29	Ethyl N-(o-anisyl)formimidate	18.841	28277	0.30	179	
30	Hexanoic acid, 3,5,5-trimethyl-, heptyl ester	19.552	7204	0.08	256	
32	1-(3-Fluorophenyl)imidazoline-2-thione	21.276	13418	0.14	194	
33	3-Ethyl-2-pentadecanone	21.487	27621	0.30	254	
37	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	22.325	80137	0.86	222	
38	Hexadecanoic acid, methyl ester	22.396	139711	1.50	270	
42	Phthalic acid, isobutyl 2-(2-methoxyethyl)hexyl ester	22.745	9797	0.10	364	

43	2-Oxatricyclo[4.3.1.0(3,8)]decane	22.887	67979	0.73	138	
44	2-Hydroxy-2-octylsebacic acid	23.514	5430	0.06	330	
48	Methyl stearate	24.342	50945	0.55	298	
53	Silane, dimethyldimethyl((dodec-9-ynyloxy)silyloxy)ethoxy-	25.599	57601	0.62	358	
57	Vitamin E	26.930	441020	4.72	430	
58	Cyclononasiloxane, octadecamethyl-	27.234	14685	0.16	666	

60	Phenol, 4,4'-methylenebis[2,6-bis(1,1-dimethylethyl)-	28.688	40115	0.43	424	
61	Terephthalic acid, isobutyl isopropyl ester	29.480	43517	0.47	264	

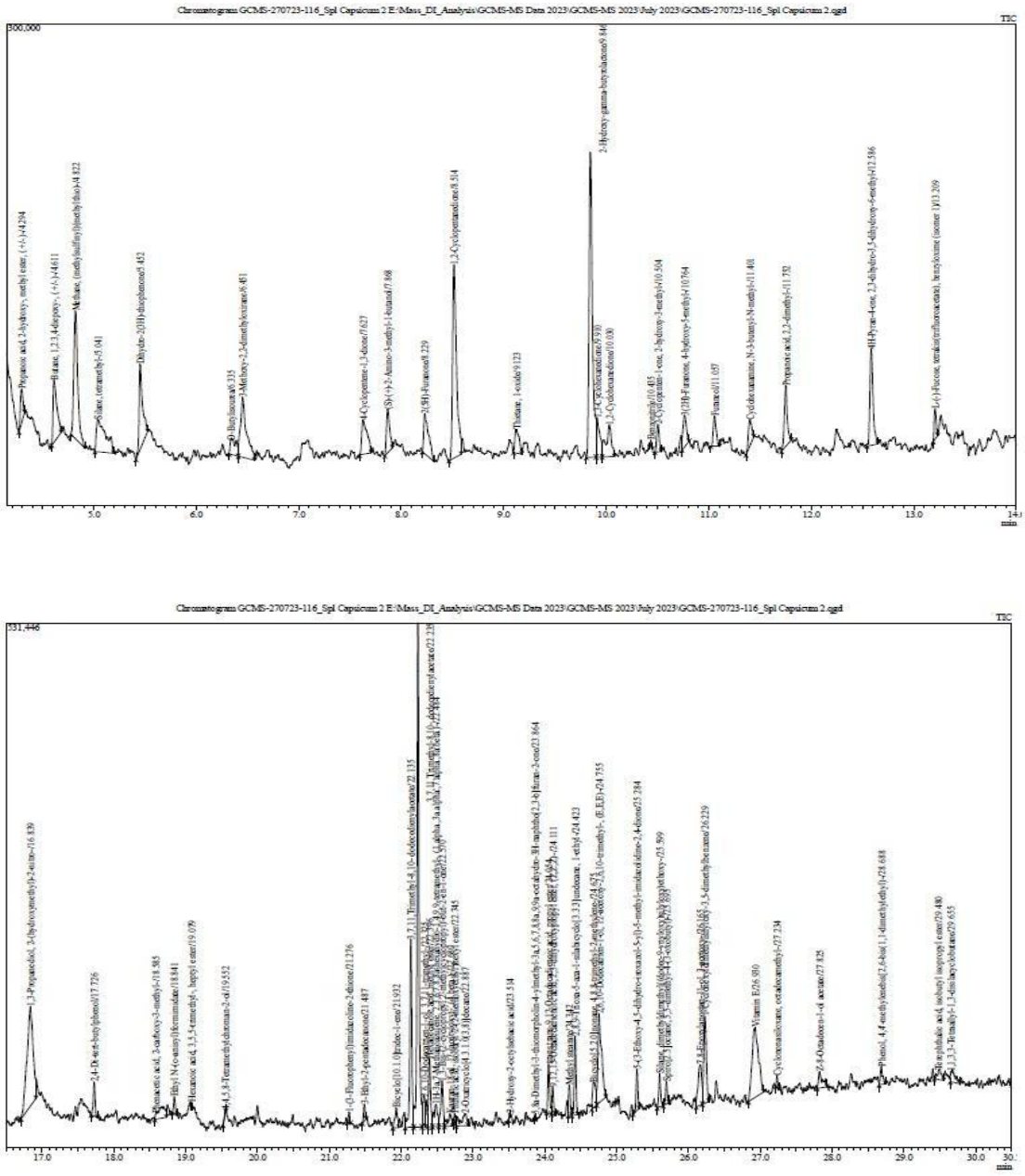
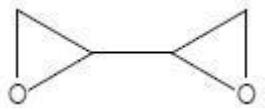
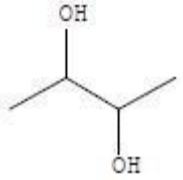
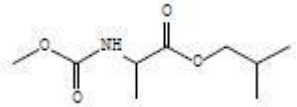
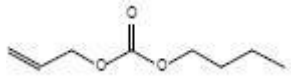
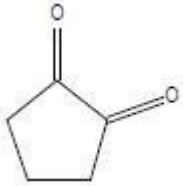
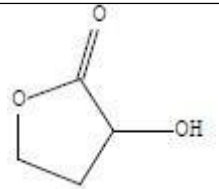
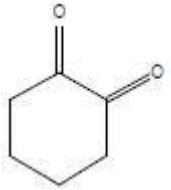
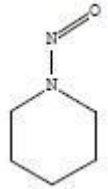
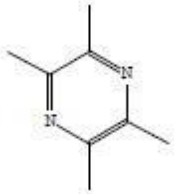
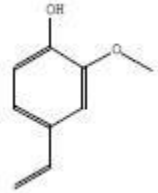
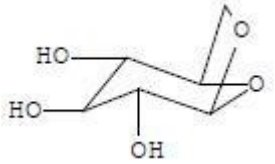
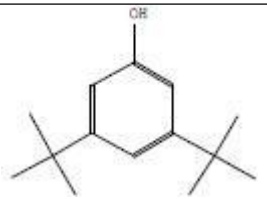
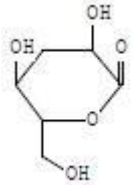
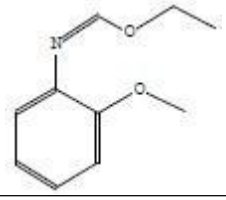


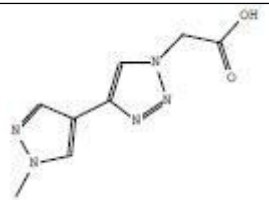
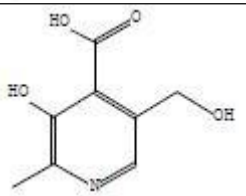
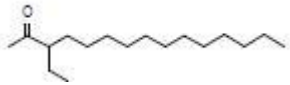
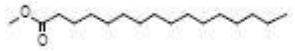
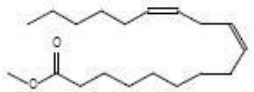
Figure 26 : GC-MS Chromatogram of methanolic fresh fruit extract of *Capsicum annuum*

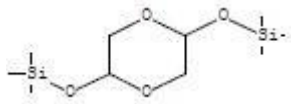
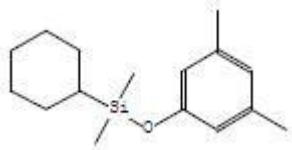
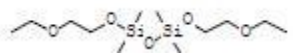
Table 16: Compound in methanolic extract of Infected fruit of *Capsicum annuum*

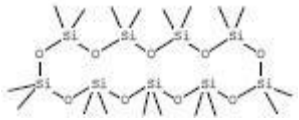
Peak No.	Name of Compound	Retention Time	Area	Area %	Mol. Weight in g/mol.	Molecular Structure
2.	1,2:3,4-diepoxy-, (.+/-.)-	4.640	95729	1.38	86	
4	2,3-Butanediol, [R-(R*,R*)]	5.407	1539043	22.22	90	
5	l-Alanine, N-methoxycarbonyl-, isobutyl ester	5.475	245012	3.54	203	
6	Carbonic acid, allyl butyl ester	5.635	865478	12.50	158	
10	1,2-Cyclopentanedione	8.521	369981	5.34	98	

11	2-Hydroxy-gamma-butyrolactone	9.849	305820	4.42	102	
13	1,3-Cyclohexanedione	10.037	41693	0.60	112	
14	Piperidine, 1-nitroso-	10.767	45442	0.28	114	
15	Pyrazine, tetramethyl-	11.592	45442	0.66	136	
18	2-Methoxy-4-vinylphenol	15.155	72892	1.05	150	

20	3,7,11,Trimethyl-8,10-dodecedienylacetate	16.986	40997	0.59	266	
21	beta.-D-Glucopyranose, 1,6-anhydro-	17.543	39704	0.57	162	
22	Phenol, 3,5-bis(1,1-dimethylethyl)-	17.725	43876	0.63	206	
23	3-Deoxy-d-mannoic lactone	18.678	95842	1.38	162	
24	Ethyl N-(o-anisyl)formimidate	18.840	55971	0.81	179	

26	[4-(1-Methylpyrazol-4-yl)-1,2,3-triazol-1-yl]acetic acid	20.348	40084	0.58	207	
27	4-Pyridinecarboxylic acid, 3-hydroxy-5-(hydroxymethyl)-2-methyl-	21.125	56273	0.81	183	
28	3-Ethyl-2-pentadecanone	21.488	33966	0.49	254	
29	3,7,11,Trimethyl-8,10-dodecedienylacetate	22.152	66961	0.97	266	
31	Hexadecanoic acid, methyl ester	22.397	134928	1.95	270	
32	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	24.050	105560	1.52	294	

33	Methyl 8,11,14-heptadecatrienoate	24.110	67789	0.98	278	
36	Heptadecanoic acid, 16-methyl-, methyl ester	24.342	55068	0.80	298	
39	1,4-Dioxane-2,5-diol, 2TMS derivative	24.875	34189	0.49	264	
43	Methyl 12,13-octadecadienoate	26.125	66659	0.96	294	
45	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	26.229	188922	2.73	262	
47	7,7,9,9-Tetramethyl-3,6,8,10,13-pentaoxa-7,9-disilapentadecane	30.490	41736	0.60	310	

48	Cyclononasiloxane, octadecamethyl-	30.765	34139	0.49	666	

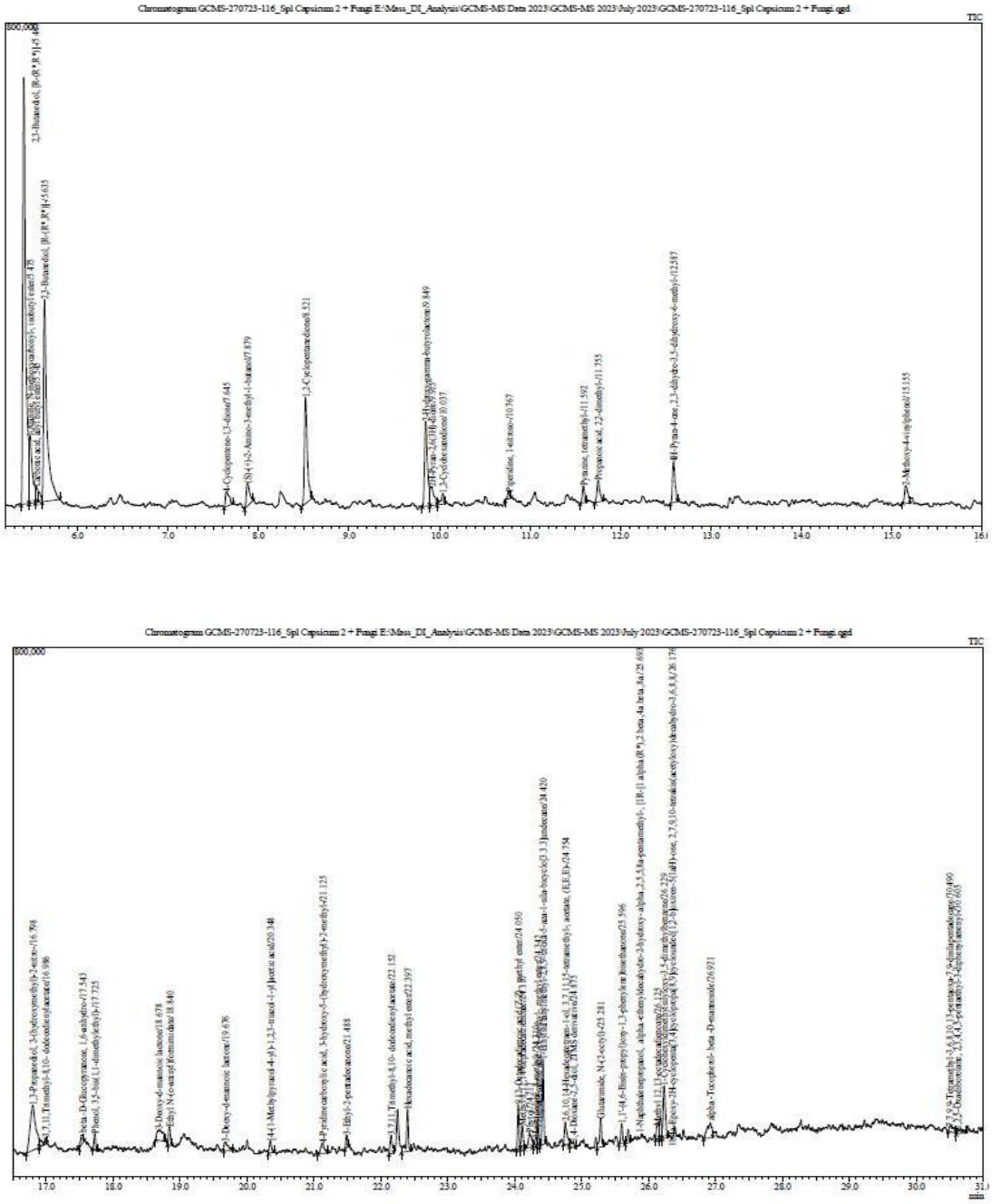


Figure 27 : GC-MS Chromatogram of methanolic Infected fruit extract of *Capsicum annuum*

4.5 FTIR Analysis

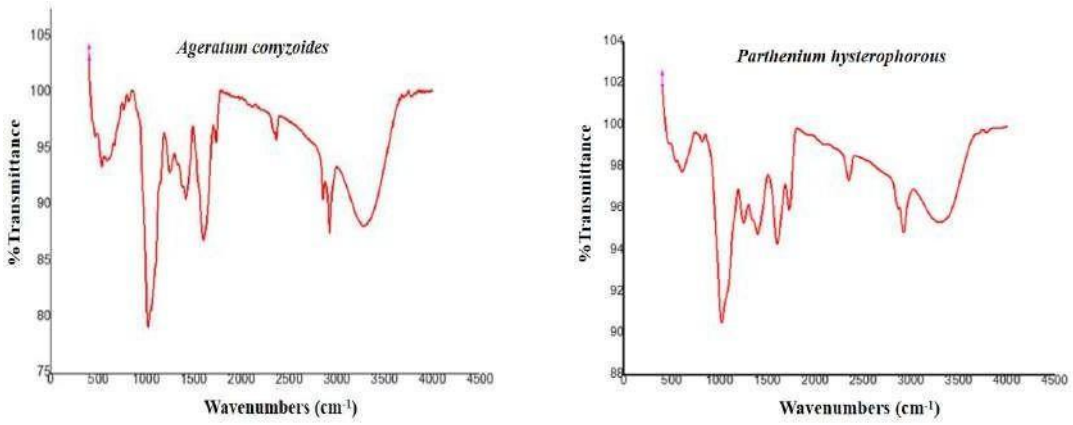


Figure 28: FT-IR Spectrum of leaf powder of *Ageratum conyzoides* and *Parthenium hysterophorus*

FTIR spectroscopy was employed to identify the functional groups present in (Figure 28) the bioactive compounds of *Ageratum conyzoides* and *Parthenium hysterophorus* leaf extracts. The infrared (IR) spectrum provides insights into the molecular composition of the extracts by analyzing the characteristic absorption peaks corresponding to different functional groups. The obtained FTIR spectra indicate the presence of various functional groups such as N-H, O-H, C=C, C-H, C-O, and CH_3 , which are crucial in defining the biochemical properties of the extracts.

For *Ageratum conyzoides*, the identified absorption peaks were at 3280.94 cm^{-1} (N-H stretching), 2918.75 cm^{-1} (O-H stretching), 1727.35 cm^{-1} (C=C stretching), 1408.12 cm^{-1} (C-H bending), 1240.25 cm^{-1} (C-O stretching), and 1016.15 cm^{-1} (CH_3 bending). Similarly, the FTIR spectrum of *Parthenium hysterophorus* revealed absorption peaks at 3281.43 cm^{-1} (N-H stretching), 2918.83 cm^{-1} (O-H stretching), 1729.93 cm^{-1} (C=C stretching), 1413.01 cm^{-1} (C-H bending), 1244.59 cm^{-1} (C-O stretching), and 1019.07 cm^{-1} (CH_3 bending). These spectral signatures confirm the presence of significant phytochemical constituents, which may contribute to the biological activity of these plant extracts.

FTIR spectroscopy has proven to be a highly sensitive and accurate tool for detecting

biomolecular compositions in plant extracts (Pakkirisamy et al., 2017). This analysis provides valuable information regarding the chemical composition of *Ageratum conyzoides* and *Parthenium hysterophorus*, which can further support their applications in agricultural and pharmaceutical research.

CHAPTER V

SUMMARY & CONCLUSION

SUMMARY

A growing concern over the environmental impact of chemical pesticides and the resilience of pathogens has made the pursuit of sustainable and eco-friendly methods for the eradication of plant diseases an increasingly important field in agricultural science due to the declining use of chemical pesticides and the resilient nature of pathogens. Specifically, soil-borne pathogens pose a significant threat to global food security by reducing crop yields and resulting in reduced food production. One of these pathogens is *Phytophthora capsici*, which is widely recognized for its devastating impact on *Capsicum annuum* L., commonly referred to as chili peppers or bell peppers. In spite of the fact that chemical control methods have been widely used, they have proven to be not only expensive but also hazardous to the environment. A further limitation of such treatments is the rapid development of resistance among pathogens, which further decreases their effectiveness. It is this increasing need for alternatives that has led to the development of biocontrol strategies, which offer a promising solution to reducing environmental impact while managing a wide range of plant diseases.

This study aimed to investigate the efficacy of *Phytophthora capsici* biocontrol formulations derived from two plant species, *Ageratum conyzoides* and *Parthenium hysterophorus*, for the management of *Phytophthora capsici* in *Capsicum annuum* by using biocontrol formulations derived from these two plants species. The research journey began with the careful isolation and identification of the target pathogen, *P. capsici*, an essential step in understanding the nature of the pathogen and its role in the transmission of diseases in chili plants. In order to formulate effective biocontrol strategies, it was important to ensure that the identification was correct in order to provide the right information. As a result of the use of traditional and molecular techniques for pathogen identification, effective biocontrol solutions were developed that were capable of targeting *P. capsici* with precision, there by paving the way for the development of efficient biocontrol methods.

A conyzoides and *A hysterophorus* plant species were collected and characterized after identification of *P. capsici*. Various bioactive compounds present in these plants possess antimicrobial properties, making them suitable candidates for the development of biocontrol agents. In order to identify the active compounds responsible for the antimicrobial effects of both plants, a detailed chemical analysis of their leaves was conducted. Bioactive compounds were found to be present in both extracts of the two species, and these compounds were then used to formulate biocontrol agents capable of suppressing the growth of *P. capsici*.

In the formulation process, bioactive compounds were extracted using appropriate solvents, then biocontrol formulations were created for use both in the laboratory and in the field. To maximize the yield of bioactive compounds, a variety of extraction techniques were employed in order to ensure the formulations' efficacy. As a result of the development of these biocontrol agents, significant inhibitory effects were observed on *P. capsici*, both in vitro and in vivo. In addition to inhibiting pathogen growth, the formulations also activated the plants' own defense mechanisms, increasing their resistance to infection in the future. Consequently, the biocontrol agents proved particularly successful in controlling *P. capsici* due to their dual action.

Among the primary outcomes of this research was the elucidation of the mechanisms through which the biocontrol formulations exerted their effects. Molecular and biochemical analyses revealed that the bioactive compounds produced by *A conyzoides* and *P hysterophorus* act through multiple mechanisms. It includes disruption of the pathogen's cell membrane, inhibition of spore germination, and modulation of plant defense mechanisms. In combination with multiple modes of action, the biocontrol agents were able to provide a comprehensive and robust defense against *P. capsici*. In addition to their efficacy, the eco-friendly nature of the biocontrol formulations was a significant advantage over conventional chemical pesticides. Traditional pesticides often leave harmful environmental residues, affecting non-target organisms and contributing to ecological imbalances. In contrast, the formulations derived from *A. conyzoides* and *P. hysterophorus* were found to have minimal impact on non-target organisms, including beneficial microbes and insects. This made them ideal candidates for integration into sustainable farming practices, particularly in the context of integrated pest management (IPM) programs.

The practical applicability of these biocontrol agents was further tested through field trials conducted in *Capsicum annuum* cultivation areas. These trials were designed to assess the real-world efficacy of the formulations under natural growing conditions, where environmental factors and farming practices could influence the outcome. The field trials yielded promising results, showing that the biocontrol agents were not only effective in reducing the incidence of *P. capsici* infections but also compatible with existing agricultural practices. Farmers who participated in the trials reported improved crop yield and quality, along with a reduction in their reliance on chemical inputs. The positive reception from farmers highlighted the potential for these biocontrol strategies to be widely adopted in commercial agriculture.

Moreover, this research emphasized the scalability and broader applicability of the biocontrol formulations. While the study focused on *P. capsici* in *Capsicum annuum*, the underlying principles could be extended to other crops and pathogens. The bioactive compounds from *A. conyzoides* and *P. hysterophorus* could potentially be adapted to control a range of soil-borne pathogens, offering a versatile tool for pest management across different agricultural systems. This scalability is crucial for addressing the global challenge of soil-borne diseases and enhancing food security in a sustainable manner.

In conclusion, this research has made significant strides towards the development of sustainable biocontrol solutions for managing plant diseases in agriculture. By harnessing the bioactive potential of indigenous plant species, such as *Ageratum conyzoides* and *Parthenium hysterophorus*, we have developed biocontrol agents that are not only effective against *Phytophthora capsici* but also environmentally friendly and compatible with modern agricultural practices. The journey from pathogen isolation to formulation development and field application has expanded our scientific understanding of biocontrol mechanisms and provided practical solutions for sustainable pest management. Moving forward, it is essential to focus on the technology transfer and capacity building required to ensure that these biocontrol strategies are adopted by farmers and integrated into pest management programs globally. In an era of escalating environmental challenges, this research serves as a beacon of hope for the future of sustainable agriculture, demonstrating the power of nature-based solutions to combat plant diseases and protect our food systems.

CONCLUSION

The main goal of this research was to explore sustainable and environmentally friendly alternatives for managing soil-borne pathogens, with a particular focus on *Phytophthora capsici* in *Capsicum annuum*. In a world where chemical control methods are becoming increasingly problematic due to their environmental impact and the development of pathogen resistance, biocontrol strategies offer a promising solution. This study sought to harness the bioactive potential of two indigenous plant species, *Ageratum conyzoides* and *Parthenium hysterophorus*, to develop biocontrol formulations that could effectively suppress *P. capsici* while enhancing plant resilience.

The research began with the isolation and identification of *P. capsici*, a crucial step in understanding the pathogen responsible for causing devastating losses in chili pepper crops. Accurate identification of the pathogen was essential for formulating targeted biocontrol strategies. The use of molecular and biochemical tools ensured that the pathogen was properly characterized, laying the foundation for the subsequent development of biocontrol agents. Following pathogen identification, the study focused on the collection and characterization of *A. conyzoides* and *P. hysterophorus*, both of which are known to contain bioactive compounds with antimicrobial properties. Through extensive chemical analysis, it was confirmed that the leaves of these plants harbored compounds capable of inhibiting the growth of *P. capsici*. The extraction and formulation processes were carefully optimized to ensure that the bioactive compounds were preserved and concentrated for maximum efficacy. The biocontrol agents developed from these plant extracts demonstrated significant inhibitory effects on *P. capsici* in both laboratory and field settings. Not only did the formulations suppress the growth of the pathogen, but they also stimulated the plants' natural defense mechanisms, providing a dual layer of protection. This dual action was a key factor in the success of the biocontrol agents, as it addressed both the immediate threat posed by the pathogen and the long-term resilience of the plants.

One of the most significant findings of this research was the discovery of the multiple mechanisms through which the biocontrol agents exerted their effects. By disrupting the pathogen's cell membranes, inhibiting spore germination, and modulating plant defense pathways, the bioactive compounds provided a comprehensive defense

against *P. capsici*. This multi-faceted approach is particularly important in the context of pathogen resistance, as it reduces the likelihood of the pathogen developing resistance to the biocontrol agents.

In addition to their efficacy, the eco-friendly nature of the biocontrol formulations was a major advantage. Unlike chemical pesticides, which can have harmful effects on non-target organisms and leave toxic residues in the environment, the formulations derived from *A. conyzoides* and *P. hysterophorus* were found to be safe for non-target species and posed minimal risk to the environment. This aligns with the principles of integrated pest management (IPM), which seeks to minimize the use of harmful chemicals in agriculture.

The field trials conducted as part of this research demonstrated the practical applicability of the biocontrol agents in real-world agricultural settings. The formulations were not only effective in reducing the incidence of *P. capsici* infections but were also compatible with existing agricultural practices. Farmers who participated in the trials reported positive outcomes, including improved crop yield and reduced reliance on chemical inputs. This practical validation is crucial for the widespread adoption of biocontrol strategies, as it demonstrates their feasibility in commercial farming operations. However, the research also highlighted some challenges and limitations. While the biocontrol agents were effective in the conditions studied, their scalability and adaptability to different crops and pathogens require further exploration. The economic feasibility of producing these formulations at a large scale must also be considered, particularly in regions where farmers have limited access to resources. Additionally, while the biocontrol agents were found to have minimal impact

REFRENCES

Abdul Haq, M., Shahzad, S., Lodhi, A. M., & Rajput, A. Q. (2024). Morphological and molecular characterization of four *Phytophthora* species with the first report of *Phytophthora lacustris* from Pakistan. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, 1-7. https://doi.org/ 10.1080/1126 3504. 2024.2326821

Aboody, M.S.A. and Mickymaray, S. (2020). Anti-Fungal Efficacy and Mechanisms of Flavonoids. Antibiotics (Basel) 9 (2) :45. https://doi.org/10.3390 %2 Fantibiotics 9020045

Abrol, Dp; Shankar, U. 2012. History, overview and principles of ecologically based pest management. In: Abrol, Dp; Shankar U (eds). Integrated Pest Management: Principles and practice. London, UK: CABI International. p. 1-26. https://doi.org/10.1079/9781845938086.0001

Aggarwal, S., Kumar, A., & Singh, S. (2023). Genomic insights into the origin and domestication of Capsicum species. Molecular Plant, 16(4), 567-578.

Akter MK, Hossain MD, Nahar K, Meah MB, Hossain MA (2007) Isolation and identification of *Phytophthora capsici* and its mating type determination. J. Agro for. Environ. 1(2): 89-92.

Akter, M. K., Hossain, M. D., Nahar, K., Meah, M. B., & Hossain, M. A. (2017). Pathogenicity of *Phytophthora capsici* and possibilities of its biological and chemical control. J. Agrofor. Environ, 1(4).

Ali M, Kim B, Belfield KD, Norman D, Brennan M, Ali GS. Inhibition of *Phytophthora parasitica* and *P. capsici* by Silver Nanoparticles Synthesized Using Aqueous Extract of *Artemisia absinthium*. Phytopathology. 2015 Sep;105(9):1183- 90. https://doi.org/10.1094/phyto-01-15-0006-r

Anandaraj, M. (2012). Diversity of *Phytophthora* affecting Horticultural crops in India. Indian Phytopathology, 65, 317–327. https://epubs.icar.org.in/index. php/IPPJ/article/view/25408

Anandaraj, M., and Sarma, Y. R. (2000). Analysis of spread of *Phytophthora* foot rot disease of black pepper by STCLASS–Software. Indian Phytopathology, 53, 428—432. https://epubs.icar.org.in/index.php/IPPJ/article /view/19355

Aragaki, M., & Uchida, J. Y. (2001). Morphological distinctions between *Phytophthora capsici* and P. tropicalis sp. nov. Mycologia, 93(1), 137-145. https://doi.org/10.1080/00275514.2001.12061285

Ashoka, N., Hongal, S., Raju, R., Harshavardhan, M., Venkatesha, K. T., & Vishwanatha, S. (2021). Comparative study of black pepper (*Piper nigrum* L.) nursery raising in Karnataka: traditional variety Sigandhini versus popular variety Panniyur-I. Current Science, 1201-1207. http://dx.doi.org/10.18520/cs/v121/i9/ 1201-1207

Babadoost, M., Pavon, C., Islam, S. Z., & Tian, D. (2015). *Phytophthora* blight (*Phytophthora capsici*) of pepper and its management. Acta Horticulturae, 1105, 61–66. https://doi.org/10.17660/ActaHortic.2015.1105.9

Babasaheb Khaire Mahatma Phule Krishi Vidyapeeth, P., V, P. S., B, K. P., & S, M. S. (n.d.). Management Strategies Used against Fungal Diseases of *Capsicum* AgriCos e-Newsletter. www.agricosemagazine.com

Banaras, S. (2021). Bioassays Guided Fractionation of *Ageratum conyzoides* Extract for the Identification of Natural Antifungal Compounds against Macrophomina phaseolina. International Journal of Agriculture and Biology, 25(04), 761–767. https://doi.org/10.17957/IJAB/15.1727

Barchenger, D. W., Lamour, K. H., & Bosland, P. W. (2018). Challenges and Strategies for Breeding Resistance in *Capsicum annuum* to the Multifarious Pathogen, *Phytophthora capsici*. Frontiers in Plant Science, 9. https:// doi.org /10.3389/fpls.2018.00628

Baroroh, H. N. (2020). Pharmacognostic Profile of *Ageratum conyzoides* L Plant and Simplicia. Pharmacognosy Journal, 12(5). http://dx.doi.org/10.5530/pj. 2020 .12.151

Bashar, H. K., Juraimi, A. S., Ahmad-Hamdani, M. S., Uddin, M. K., Asib, N., Anwar, M. P., & Rahaman, F. (2021). A mystic weed, *Parthenium hysterophorus*: threats, potentials and management. Agronomy, 11(8), 1514.https://doi.org/ 10. 3390/agronomy11081514

Bashar, H. K., Juraimi, A. S., Ahmad-Hamdani, M. S., Uddin, M. K., Asib, N., Anwar, M. P., ... & Hossain, A. (2023). Evaluation of allelopathic effects of *Parthenium hysterophorus* L. methanolic extracts on some selected plants and weeds. Plos one, 18(1), e0280159. https://doi.org/10.1371/journal.pone.0280159

Bashar, H. M. K., Juraimi, A. S., Ahmad-Hamdani, M. S., Uddin, Md. K., Asib, N., Anwar, Md. P., Rahaman, F., Haque, M. A., & Hossain, A. (2023). Evaluation of allelopathic effects of *Parthenium hysterophorus* L. methanolic extracts on some selected plants and weeds. PLOS ONE, 18(1), e0280159. https://doi. org/10.1371/journal.pone.0280159

Bezuneh, T.T. (2015). Phytochemistry and antimicrobial activity of *Parthenium hysterophorus* L.: A review. Science Journal of Analytical Chemistry, 3(3), 30-38. https://doi.org/10.11648/j.sjac.20150303.11

Bhatt BS, Chahwala FD, Rathod S, Singh AK (2016) Identification and molecular characterization of a new recombinant begomovirus and associated betasatellite DNA infecting *Capsicum annuum* in India. Arch. Virol. 161: 1389–1394. https://doi.org/10.1007/s00705-016-2769-z

Bosland, P. W., & Baral, J. B. (2022). Evolution, diversity, and ecology of *Capsicum*. In The *Capsicum* Genome (pp. 1-23). Springer, Cham.

Brasier, C., Scanu, B., Cooke, D., & Jung, T. (2022). *Phytophthora*: An ancient, historic, biologically and structurally cohesive and evolutionarily successful generic

concept in need of preservation. IMA fungus, 13 (1), 12. https://doi.org/ 10.1186/s43008-022-00097-z

Chahal, R., Nanda, A., Akkol, E. K., Sobarzo-Sánchez, E., Arya, A., Kaushik, D., Dutt, R., Bhardwaj, R., Rahman, Md. H., & Mittal, V. (2021). *Ageratum conyzoides* L. and Its Secondary Metabolites in the Management of Different Fungal Pathogens. Molecules, 26(10), 2933. https://doi.org/10.3390/ molecules 26102933

Choudhary, C. S., Behera, B., Raza, M. B., Mrunalini, K., Bhoi, T. K., Lal, M. K., Nongmaithem, D., Pradhan, S., Song, B., & Das, T. K. (2023). Mechanisms of allelopathic interactions for sustainable weed management. Rhizosphere, 25, 100667. https://doi.org/10.1016/j.rhisph.2023.100667

Cooke, D. E. (2015). Threats posed by *Phytophthora* to Scottish plant health; a review of previous findings, pathways of entry and further spread and the status of diagnostic techniques. pp 22

Costa, J., Rodríguez, R., Garcia-Cela, E., Medina, A., Magan, N., Lima, N., Battilani, P., & Santos,C. (2019). Overview of fungi and mycotoxin contamination in capsicum pepper and in its derivatives. In Toxins (Vol.11, Issue 1). MDPI AG. https://doi.org/10.3390/toxins11010027

Das B, Reddy VS, Krishnaiah M, Sharma AVS, Ravi Kumar K, Rao JV, Sridhar V. Acetylated pseudoguaianolides from Parthenium hysterophorusand theircytotoxicactivity. Phytochemistry. 2007;68:2029–2034. https://doi.org/ 10. 1016/j.phytochem.2007.05.002

Delelegn, S. (2011). Evaluation of Elite Hot Pepper Varieties (*Capsicum* species) for growth, dry pod yield and quality under Jimma condition, South West Ethiopia (Doctoral dissertation, Jimma University).

Devi, J., Sagar, V., Kaswan, V., Ranjan, J. K., Kumar, R., Mishra, G. P., Dubey, R. K., & Verma, R. K. (2021). Advances in Breeding Strategies of Bell Pepper (*Capsicum annuum* L. var. grossum Sendt.). In Advances in Plant Breeding Strategies: Vegetable

Crops (pp. 3–58). Springer International Publishing. https://doi.org/10.1007/978-3-030-66961-4_1

Devkota, A., & Sahu, A. (2017). Assessment of Phytochemical screening and Antifungal Activity of *Parthenium hysterophrous* L. In Biological Forum–An International Journal (Vol. 9, No. 1, pp. 14-19).

Devkota, A., Devkota, A., & Sahu, A. (n.d.). Assessment of Phytochemical screening and Antifungal Activity of *Parthenium hysterophrous* L. Assessment of Phytochemical screening and Antifungal Activity of *Parthenium hysterophrous* L. Biological Forum–An International Journal,9(1), 14-19. https://www. researchg ate.net/publication/327719560

Dilshad, M. and Gupta, C. (2023). Inhibition of Plant Pathogens by *Parthenium hysterophorus*: An Investigation into Antimicrobial Properties. Mathews Journal of Nutrition & Dietetics6(3): 28. https://doi.org/10.30654/MJND.10028

Erida, G., Ichsan, C.N., Syamsuddin, Kurniawanc, T., Khan, I.H., & Javaid, A. (2023). Potential of secondary metabolites of *Ageratum conyzoides* L. in weed management: A review. Allelopathy Journal,58(1),23-40. https://doi.org/ 10.26651 /allelo.j/2023-58-1-1417

Erida, G., Syamsuddin, C. N. I., Kurniawan, T., Khan, I. H., & Javaid, A. (2023). Potential of Secondary metabolites of *Ageratum conyzoides* L. in weed management : A review. Allelopathy Journal, 58(1), 23–40. https://doi.org/ 10. 26651/allelo.j/2023-58-1-1417

Erwin, D.C., and Ribeiro, O.K. (1996). *Phytophthora* diseases worldwide. St. Paul, Minnesota: American Phytopathological society. Press. 562 pp.

Ferdosi, M. F. H., Javaid, A., Khan, I. H., Ferdosi, M. F. A., & Munir, A. (2021). Bioactive Components In Methanolic Flower Extract of *Ageratum Conyzoides*. Journal Of Weed Science Research, 27(2), 181–190. Https://Doi.Org/10.28941/Pjwsr.v27i2.954

Fry, W. E., Birch, P. R. J., Judelson, H. S., Grünwald, N. J., Danies, G., Everts, K. L., ... & Smart, C. D. (2015). Five reasons to consider *Phytophthora infestans* a reemerging pathogen. Phytopathology, 105(7), 966-981. https://doi.org/ 10.1094 /PHYTO-01-15-0005-FI

García-Ramírez, E., Contreras-Oliva, A., Salinas-Ruiz, J., Hernández-Ramírez, G., Spinoso-Castillo, J. L., & Colmenares Cuevas, S. I. (2023). Plant extracts control in vitro growth of disease-causing fungi in chayote. Plants, 12(9), 1800. https://doi.org/10.3390/plants12091800

Garg, P., & Grewal, A. (2015). In vitro antibacterial activity of *Ageratum conyzoides* L. (Asteraceae). World Journal of Pharmacy and Pharmaceutical Sciences, 4(7), 893-897.

Giachero, M. L., Declerck, S., & Marquez, N. (2022). *Phytophthora* root rot: Importance of the disease, current and novel methods of control. Agronomy, 12(3), 610. https://doi.org/10.3390/agronomy12030610

Goodwin, S. B. (1997). The population genetics of *Phytophthora*. Phytopathology, 87, 462–473. 33

Granke, L. L., Quesada-Ocampo, L., Lamour, K., & Hausbeck, M. K. (2012). Advances in Research on *Phytophthora capsici* on Vegetable Crops in The United States. Plant Disease, 96(11), 1588–1600. https://doi.org/10.1094/PDIS-02-12-0211-FE

Hantula, J., Lilja, A., Nuorteva, H., Parikka, P., and Werres, S. (2000). Pathogenicity, morphology and genetic variation of *Phytophthora cactorum* from strawberry, apple, rhododendron, and silver birch. Mycological Research, 104, 1062–1068. https://doi.org/10.1017/S0953756200002999

Hausbeck, M. (2004). *Phytophthora* lessens learned: Irrigation water and snap beans. The Vegetable Growers News, 38, 2829.

Hazirah, M. N., Hamizah, O., & Natasya, W. W. (2023, June). Antifungal activity of *Ageratum conyzoides* extract against *Fusarium oxysporum* in *Musa* spp. In IOP Conference Series: Earth and Environmental Science (Vol. 1182, No. 1, p. 012074). IOP Publishing.

Hyder, S., Inam-ul-Haq, M., Ahmed, R., Gondal, A. S., Fatima, N., Hanan, A., & Zhao, Y. F. (2018). First Report of *Phytophthora capsici* Infection on Bell Peppers (*Capsicum annuum* L.) from Punjab, Pakistan. International Journal of Phytopathology, 7(1), 51–51. https://doi.org/10.33687/phytopath.007.01.2543

Iqbal, B., Hussain, F., khan, M. S., Iqbal, T., Shah, W., Ali, B., Al Syaad, K. M., & Ercisli, S. (2023). Physiology of gamma-aminobutyric acid treated *Capsicum annuum* L. (Sweet pepper) under induced drought stress. PLOS ONE, 18(8), e0289900. https://doi.org/10.1371/journal.pone.0289900

Islam, S.Z., Babadoost, M., Lambert, K., Ndeme, A., and Fouly, H. M. (2004). Characterization of *Phytophthora capsici* isolates from processing pumpkin in Illinois. Plant Disease, 88, 191–197.). https://doi.org/10.1094/PD-89-0191

Ivors, K., Garbelotto, M., Vries, I. D. E., Ruyter‐Spira, C., Hekkert, B. T., Rosenzweig, N., & Bonants, P. (2006). Microsatellite markers identify three lineages of *Phytophthora ramorum* in US nurseries, yet single lineages in US forest and European nursery populations. Molecular Ecology, 15(6), 1493-1505. https://doi.org/10.1111/j.1365-294x.2006.02864.x

Jabeen, S., Ali, M. F., Mohi ud Din, A., Javed, T., Mohammed, N. S., Chaudhari, S. K., Javed, M. A., Ali, B., Zhang, L., & Rahimi, M. (2023). Phytochemical screening and allelopathic potential of phytoextracts of three invasive grass species. Scientific Reports, 13(1), 8080. https://doi.org/10.1038/s41598-023-35253-x

Jadon, K. S., Shah, R., Gour, H. N., & Sharma, P. (2016). Management of blight of bell pepper (*Capsicum annuum* var. grossum) caused by Drechslera bicolor. Brazilian

Journal of Microbiology, 47(4), 1020–1029. https://doi.org/10.1016 /j.bjm.2016.04.032

Jahagirdar, S., & Siddaramaiah, A. L. (2002). Prevalence of foot rot of black pepper in Karnataka. Agricultural Science Digest, 22(1), 45-47.

Javaid, N., Javaid, A., Shah, M. H., Khan, I. H., & Waleed, S. M. (2020). Herbicidal Activity of *Ageratum Conyzoides* Against *Parthenium*. Journal Of Weed Science Research, 27(2), 137–146. Https://Doi.Org/ 10.28941/Pjwsr.v26i2.823

Javed, S., & Bashir, U. (2012). Antifungal activity of different extracts of *Ageratum conyzoides* for the management of *Fusarium solani*. African Journal of Biotechnology, 11(49), 11022-11029. https://doi.org/10.5897/AJB12.366

Jayasundera, M., Florentine, S., Tennakoon, K. U., & Chauhan, B. S. (2021). Medicinal Value of Three Agricultural Weed Species of the Asteraceae Family: A Review. Pharmacognosy Journal, 13(1), 264–277. https://doi.org/10.5530/ pj.2021.13.36

Jones, M., Mautner, A., Luenco, S., Bismarck A. and John, S. (2020). Engineered mycelium composite construction materials from fungal biorefineries: A critical review. Materials & Design187: 108397 https://doi.org/10.1016/ j.matdes. 20 19.108397

Kapeua Ndacnou, M., Pantaleon, A., Saha Tchinda, J., Ngonkeu Mangapche, E. L., Keumedjio, F., & Begoude Boyoguemo, D. (2020). Phytochemical study and anti-oomycete activity of *Ageratum conyzoides* Linnaeus. Industrial Crops and Products, 153, 112589. https://doi.org/10.1016/j.indcrop.2020.112589

Kasteel, M., Ketelaar, T., & Govers, F. (2023, October). Fatal attraction: How *Phytophthora* zoospores find their host. In Seminars in Cell & Developmental Biology (Vol. 148, pp. 13-21). Academic Press. https://doi.org/10.1016/ j.semcdb.2023.01.014

Kaur, A., Kaur, S., Singh, H. P., Datta, A., Chauhan, B. S., Ullah, H., ... & Batish, Ecology, Biology, Environmental Impacts, and Management of an Agro-Environmental Weed *Ageratum conyzoides*. Plants, 12(12), 2329. http://dx.doi.org/10.3390/plants12122329

Khan, N., & Fahad, S. (2020). Economic Review of *Parthenium Hysterophorus* L. Plant in the World. Plant in the World (January 12, 2020). https://dx. doi.org/10.2139/ssrn.3518020

Kim, S., Park, M., & Kim, S. (2023). Recent advances in understanding the genetics of capsaicin biosynthesis in *Capsicum*. Trends in Plant Science, 28(3), 216-225.

Kostina-Bednarz, M., Płonka, J. and Barchanska, H. (2023). Allelopathy as a source of bioherbicides: challenges and prospects for sustainable agriculture. Reviews in Environmental Science and Bio/Technology22: 471–504. http://dx. doi.org/10.1007/s11157-023-09656-1

Kotta, J. C., Lestari, A. B., Candrasari, D. S., & Hariono, M. (2020). Medicinal effect, in silico bioactivity prediction, and pharmaceutical formulation of *Ageratum conyzoides* L.: a review. Scientifica, 2020, 1-12.

Kueh, T.K., and Kew, K.L. (1982). Survival of *Phytophthora palmivora* in soil and after passing through alimentary canals of snails. Plant Disease, 66, 887–899.

Kumar, M., Kumar, R., & Singh, R. (2022). *Parthenium* a Noxious Weed: A Review on the Allelopathic Impact on Crop Plants and Their Management. Bio Science Research Bulletin, 38(2), 106-112. https://doi.org/10.48165/

Kumar, R., Kapoor, D., Sharma, I.,&Bhadarwaj, R. (2022). Biofungicide activity of *Parthenium hysterophorus* leaves extract against phytopathogenic, *Phytopthora capsici* fungi. International Journal of Innovative Science and Research Technology, 7(4), 790-793. https://doi.org/10.5281/zenodo.6535983

Kumar, R., Kumar, M., Srivastva, S., Singh, R., & Sharma, I. (2023). Role of *Parthenium hysterophorus* in Human Health, Agriculture and Sustainability of Ecosystem. Bio Science Research Bulletin-Biological Sciences, 39(1). https://doi.org/10.48165/bpas.2023.39.1.2%20

La Torre, A., De Santis, Y., Battaglia, V., Costantini, E., Pulcini, P., & Polito, A. (2023). Natural products for the control of *Phytophthora infestans* and *Phytophthora capsici*. Journal of Plant Pathology, 105(3), 1001-1012. http://dx.doi.org/10.1007/s42161-023-01421-1

Lamour, K.H., Stam, R., Jupe, J.,& Huitema, E. (2011) The Oomycete broad-host-range pathogen *Phytophthora capsici*. Molecular Plant Pathology, 13(4), 329-337.

Lee, B. K., Kim, B. S., Chang, S. W., and Hwang, B. K. (2001). Aggressiveness to pumpkin cultivars of isolates of *Phytophthora capsici* from pumpkin and pepper. Plant Disease, 85, 497–500. http://dx.doi.org/10.1094/PDIS.2001.85.5.497

Lee, K. J., Kamala-Kannan, S., Sub, H. S., Seong, C. K., & Lee, G. W. (2008). Biological control of *Phytophthora* blight in red pepper (*Capsicum annuum* L.) using Bacillus subtilis. World Journal of Microbiology and Biotechnology, 24(7), 1139–1145. https://doi.org/10.1007/s11274-007-9585-2

Luong, T. H., Nguyen, V. V., & Jung, W. J. (2024). Antifungal activity of a methanolic extract of Glycyrrhiza uralensis Fisch. root against *Fusarium oxysporum* and *Phytophthora capsici*. Vietnam Journal of Science, Technology and Engineering, 66(1), 46-52. https://doi.org/10.31276/VJSTE.66(1).46-52

Maillot, G., Szadkowski, E., Massire, A., Brunaud, V., Rigaill, G., Caromel, B.,.....& Lefebvre, V. (2022). Strive or thrive: Trends in *Phytophthora capsici* gene expression in partially resistant pepper. Frontiers in Plant Science, 13, 980587. https://doi.org/10.3389/fpls.2022.980587

Maishi AI, Ali PKS, Chaghtai SA, Khan G. A proving of *Parthenium hysterophorus*, L. Brit Homoeopath J. 1998;87:17–21. https://doi.org/10.1016/ S0007-0785(98)80005-7

Manohara D, Mulya K, Purwantara A, Wahyuno D, 2004a. *Phytophthora capsici* on black pepper in Indonesia. In: Drenth A, Guest DI (eds), Diversity and Management of *Phytophthora* in Southeast Asia Australian Centre for International Agricultural Research, Canberra, pp. 132–142.

Mao, R., Shabbir, A., & Adkins, S. (2021). *Parthenium hysterophorus*: A tale of global invasion over two centuries, spread and prevention measures. Journal of Environmental Management, 279, 111751. https://doi.org/10.1016/j.jenvman. 2020.111751

Mao, R., Shabbir, A., &Adkins, S. (2021). *Parthenium hysterophorus*: A tale of global invasion over two centuries, spread and prevention measures. Journal of Environmental Management,279,111751 https://doi.org/10.1016 /j.jenvman. 20 20.111751

Mchau, G.R.A., and Coffey, M.D. (1995). Evidences for the existence of two distinct sub populations in *Phytophthora capsici* and a redescription of the species. Mycological Research, 99, 89–102.https://doi.org/10.1016/S0953-7562(09)80321-3

Molnar, C., Nikolaeva, E., Kim, S., Olson, T., Bily, D., Kim, J. E., & Kang, S. (2020). *Phytophthora* diversity in Pennsylvania nurseries and greenhouses inferred from clinical samples collected over four decades. Microorganisms, 8(7), 1056. https://doi.org/10.3390/microorganisms8071056

Moreira-Morrillo, A., Monteros-Altamirano, Á., Reis, A., & R. Garcés-Fiallos, F. (2023). *Phytophthora capsici* on *Capsicum* Plants: A Destructive Pathogen in Chili and Pepper Crops. In *Capsicum* - Current Trends and Perspectives. Intech Open. https://doi.org/10.5772/intechopen.104726

Motmainna, Mst., Juraimi, A. S., Ahmad-Hamdani, M. S., Hasan, M., Yeasmin, S., Anwar, Md. P., & Islam, A. K. M. M. (2023). Allelopathic Potential of Tropical Plants—A Review. Agronomy, 13(8), 2063. https://doi.org/10.3390/ agronomy 13082063

Naegele, R. P., & Hausbeck, M. K. (2014). Evaluation of Pepper Fruit for Resistance to *Phytophthora capsici* in a Recombinant Inbred Line Population, and the Correlation with Fruit Shape. Plant Disease, 98(7), 885–890. https://doi.org /10.1094/PDIS-03-13-0295-RE

Ndacnou, M.K., Pantaleon, A., Tchinda, J.S., Mangapche, E.L.N., Keumedjio, F., & Boyoguemo, D.B. (2020). Phytochemical study and anti-Oomycete activity of *Ageratum conyzoides* Linnaeus. Industrial Crops and Products, 153, 112589. http://dx.doi.org/10.1016/j.indcrop.2020.112589

Negi, B., Bargali, S.S., Bargali, K. and Khatri, K. (2020). Allelopathic interference of *Ageratum conyzoides* L. Against Rice Varieties. Current Agriculture Research Journal 8: 69–76. http://dx.doi.org/10.12944/CARJ.8.2.01

Nguyen, C.C., Nguyen, T.Q.C., Kanaori, K., Binh, T.D., Dao, X.H.T., Vang, L.V. and Kamei, K. (2021). Antifungal activities of *Ageratum conyzoides* L. extract against rice pathogens *Pyricularia oryzae* Cavara and *Rhizoctonia solani* Kühn. Agriculture11: 1169. https://doi.org/10.3390/agriculture11111169

Nysanth, N. S., Divya, S., Nair, C. B., Anju, A. B., Praveena, R., & Anith, K. N. (2022). Biological control of foot rot (*Phytophthora capsici* Leonian) disease in black pepper (*Piper nigrum* L.) with rhizospheric microorganisms. Rhizosphere, 23, 100578. https://doi.org/10.1016/j.rhisph.2022. 100578

Odeleye, O. P., Oluyege, J. O., Aregbesola, O. A., & Odeleye, P. O. (2014). Evaluation of preliminary phytochemical and antibacterial activity of *Ageratum conyzoides* (L.) on some clinical bacterial isolates. Int. J. Eng. Sci, 3(6), 1-5.

Ojha, S., & Goyal, M. (2024). Efficacy of organic and inorganic extracts of *Nerium indicum* against pathogenic fungi of *Aegle marmelos*.
Oli, S., Joshi, R., Bohara, B. (2024). A Review on *Parthenium hysterophorus* L. and its application in Agriculture. Journal of Agricultural Sciences and Engineering 6(1): 16-31. http://dx.doi.org/10.48309/jrws.2023.409381.1198

Onesirosan, P. T. (1971). The survival of *Phytophthora palmivora* in a cacao plantation during the dry season. Phytopathology, 61, 975-977. https://doi. org/10.1094/Phyto-61-975

P. K., ... & Dutta, R. (2021). Isolation, characterization and biomanagement of Villosiclava virens, a unique flower-infecting fungus causing rice false smut disease in India. https://doi.org/10.1111%2Fmpp.12362

Pánek, M., & Střížková, I. (2021). A comparison of the virulence of selected *Pythium*, *Globisporangium*, *Phytopythium* and *Phytophthora* species against strawberry plants. Journal of Plant Diseases and Protection, 128(6), 1447-1458. https://doi.org/10.1007/s41348-021-00531-1

Papavizas, G.C., Bowers, J.H., and Johnston, S.A (1981) Selective isolation of *Phytophthora capsici* from soils. Phytopathology, 71, 129–133.

Parada-Rojas, C. H., Granke, L. L., Naegele, R. P., Hansen, Z., Hausbeck, M. K., Kousik, C. S., McGrath, M. T., D. Smart, C., & Quesada-Ocampo, L. M. (2021). A Diagnostic Guide for *Phytophthora capsici* Infecting Vegetable Crops. Plant Health Progress, 22(3), 404–414. https://doi.org/10.1094/PHP-02-21-0027-FI

Paran, I., & van der Knaap, E. (2007). Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. Journal of Experimental Botany, 58(14), 3841–3852. https://doi.org/10.1093/jxb/erm257

Parashar, V., Parashar, R., Sharma, B., & Pandey, A. C. (2009). *Parthenium* leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization. Digest Journal of Nanomaterials & Biostructures (DJNB), 4(1).

Parveen, J., Sultana, T., Kazmi, A., Malik, K., Ullah, A., Ali, A., Qayyum, B., Raja, N.I., Mashwani, Z.R. and Rehman, S.U. (2023). Phytosynthesized Nanoparticles as Novel Antifungal Agent for Sustainable Agriculture: A Mechanistic Approach, Current Advances, and Future Directions. Journal of Nanotechnology 2023: 801189. http://dx.doi.org/10.1155/2023/8011189

Patel VS, Chitra VP, Prasanna L, Krishnaraju V. Hypoglycemic effect of aqueous extract of *Parthenium hysterophorus* L. in normal and alloxan induced diabetic rats. Ind J Pharmacol. 2008;40:183–185. doi:10.4103/0253- 7613.43167. https://doi.org/10.4103%2F0253-7613.43167

Paul, S., Datta, B.K., Ratnaparkhe, M.B. and Dholakia, B.B. (2022). Turning Waste into Beneficial Resource: Implication of *Ageratum conyzoides* L. in Sustainable Agriculture, Environment and Biopharma Sectors. Molecular Biotechnology64: 221–244. https://doi.org/10.1007/s12033-021-00409-5

Pawar, S.V., Khaire, P.B., &Mane, S.S. (2020). Management strategies used against fungal diseases of Capsicum. AgriCos e-Newsletter, 01(05), 22-26.

Perry, L., & Flannery, K. V. (2007). Precolumbian use of chili peppers in the Valley of Oaxaca, Mexico. Proceedings of the National Academy of Sciences, 104(29), 11905–11909. https://doi.org/10.1073/pnas.0704936104

Perry, L., & Flannery, K. V. (2021). Precolumbian use of chili peppers in the Americas: Reassessing the evidence. Science, 352(6284), 812-815.

Rai, G. S. (2020). Aetiology and integrated management of *Phytophthora* wilt of chilli (*Capsicum annuum* L.) in Bhutan (Doctoral dissertation, University of Sydney).

Raj Shikha and Jha, A.K. (2016). Evaluation of effect of leaf extract of *Parthenium hysterophorus* L. on seed germination, seedling growth and fresh weight of *Phaseolus mungo.* American Journal of Research Communication 4(2): 86-103.

Ramos, A., Rivero, R., Visozo, A., Piloto, J., & Garcıa, A. (2002). Parthenin, a sesquiterpene lactone of Parthenium hysterophorus L. is a high toxicity clastogen. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 514(1-2), 19-27. https://doi.org/10.1016/S1383-5718(01)00321-7

Rhouma, A., Hajji-Hedfi, L., & Khrieba, M. I. (2024). *Phytophthora capsici* the Causal Agent of *Phytophthora* Blight of *Capsicum* spp.: From Its Taxonomy to Disease Management. Egyptian Journal of Phytopathology, 52(1), 1–10. https://doi.org/10.21608/ejp.2024.342742

Rianosa, R., Hartal, H. and Setyowati, N. (2020). Effectiveness of the *Ageratum* (*Ageratum conyzoides*) Leaf Extract as Botanical Fungicide Against Twisted Disease of Shallot. Journal of Suboptimal Lands 9 (1): 1-10.

Riberio, O.K. 1978. A sourcebook of the genus PhytophJhora, 417 pp. l. Kramer, Lehre

Roy, A., & Kumar, P. (2023). A Review on The Pharmacological Properties and Other Aspects of *Parthenium Hysterophorus* (L.). Journal of Survey in Fisheries Sciences, 10(2S), 2073-2089. https://doi.org/10.17762/sfs.v10i2S.1107

Saleh, I. and Abu-Dieyeh, M.H. (2021). Novel Prosopis juliflora leaf ethanolic extract as natural antimicrobial agent against food spoiling microorganisms. Sci Rep11(1):7871. https://doi.org/10.1038/s41598-021-86509-3

Saltos, L. A., Corozo-Quiñones, L., Pacheco-Coello, R., Santos-Ordóñez, E., Monteros-Altamirano, Á., & Garcés-Fiallos, F. R. (2021a). Tissue specific colonization of *Phytophthora capsici* in *Capsicum* spp.: molecular insights over plant-pathogen interaction. Phytoparasitica, 49(1), 113–122. https://doi.org/10.1007/s12600-020-00864-x

Saltos, L. A., Monteros-Altamirano, Á., Reis, A., & Garcés-Fiallos, F. R. (2022). *Phytophthora capsici*: the diseases it causes and management strategies to produce

healthier vegetable crops. Horticultura Brasileira, 40, 5-17. https://doi.org/ 10.1590/s0102-0536-20220101

Saltos, L.A., Corozo-Quiñones, L., Pacheco-Coello, R., Santos-Ordóñez, E., Monteros-Altamirano, L.,& Garcés-Fiallos, F.R. (2020). Tissue specific colonization of *Phytophthora capsici* in *Capsicum* spp.: Molecular insights over plant-pathogen interaction. Phytoparasitica, 49(1), 113-122. https://link. springer. com/article/10.1007/s12600-020-00864-x

Samen, F. M. A., Secor, G. A., and Gudmestad, N. C. (2003). Variability in virulence among asexual progenies of *Phytophthora infestans*, 93, 293–304. https://doi.org/10.1094/phyto.2003.93.3.293

Sanogo, S., Lamour, K., Kousik, C. S., Lozada, D. N., Parada-Rojas, C. H., Quesada-Ocampo, L. M., ... & Miller, S. A. (2023). *Phytophthora capsici*, 100 years later: Research mile markers from 1922 to 2022. Phytopathology®, 113(6), 921- 930. https://doi.org/10.1094/phyto-08-22-0297-rvw

Sarita, O., Rajeev, J., & Bhuwan, B. (2024). A Review on *Parthenium hysterophorus* L. and its application in Agriculture. Journal of Agricultural Sciences and Engineering, 6(1), 16-31. https://doi.org/10.48309/jase.2024.183986

Sathyanathan V, Gunda S, Kumar A E, Mantry S and Thilothama L R. 2013. Pharmacological Evaluation of laxative effect of *Ageratum conyzoides* L. on experimental albino rats. International Journal of Research in Pharmacology and Pharmacotherapeutics 2: 274–8

Schick, R. (1932). Uber das Verhalten von *Solanum dessimum*, *Solanum tuberosum* and Herkunften von *Phytophthora infestans*. (Behaviour of *Solanum dessimum, Solanum tuberosum* and their hybrids to different isolates of *Phytophthora infestans*). Zuchter. 4, 233– 237.

Shan, Z., Zhou, S., Shah, A., Arafat, Y., Arif Hussain Rizvi, S., & Shao, H. (2023). Plant Allelopathy in Response to Biotic and Abiotic Factors. Agronomy, 13(9), 2358. https://doi.org/10.3390/agronomy13092358

Sharma, M., Gaviyappanavar, R., & Tarafdar, A. (2023). Evaluation of Fungicides and Fungicide Application Methods to Manage Phytophthora Blight of Pigeonpea. Agriculture, 13(3), 633. https://doi.org/10.3390/agriculture13030633

Shi, B., & Adkins, S. (2020). The phytotoxic activity of *Parthenium hysterophorus* L. seedlings on a range of pasture species. Crop Protection, 137, 105211. https://doi.org/10.1016/j.cropro.2020.105211

Sibi, M. C., Anandaraj, M., Eapen, S. J., & Devasahayam, S. (2008). Effect of carrier media on population fluctuation of Trichoderma harzianum (MTCC5179) in black pepper (*Piper nigrum* L.) rhizosphere and their interaction with soil microflora and fauna. Journal of Biological Control, 25-32. https://doi. org/10.18311/jbc/2008/3794

Sikora, K., Verstappen, E., Mendes, O., Schoen, C., Ristaino, J., and Bonants, P. (2012). A universal microarray detection method for identification of multiple *Phytophthora* spp. using padlock probes. Phytopathology, 102, 635–645. https://doi.org/10.1094/phyto-11-11-0309

Silvar, C., Merino, F., and Diaz, J. (2006). Diversity of *Phytophthora capsici* in Northwest Spain: analysis of virulence, metalaxyl response, and molecular characterization. Plant Disease, 90, 1135–1142. https://doi.org/10.1094/pd-90-1135

Smith, P. G., Villalon, B., & Villa, P. L. (1987). Horticultural Classification of Peppers Grown in the United States. HortScience, 22(1), 11–13. https://doi.org /10.21273/HORTSCI.22.1.11

Stamps, D. J., Waterhouse, G. M., Newhook, F. J., & Hall, G. S. (1990). Revised tabular key to the species of *Phytophthora*. Mycological Papers, CAB International, Wallingford Oxon, 162. http://www.cabdirect.org/abstracts/ 19902 300161.html

Steekelenburg, N. A. M. (1980). *Phytophthora* root rot of sweet pepper. Netherlands Journal of Plant Pathology, 86(5), 259–264. https://doi.org/10.1007 /BF01977301

Surib-Fakim A, Swerab MD, Gueho J, Dullo E. Medicinal plants of Rodrigues. Int J Pharmacogn. 1996;34:2–14. https://doi.org/10.1076/phbi. 34.1.2.13177

Suseela Bhai, R., Anandaraj, M., Sarma, Y. R., Veena, S. S., and Saji, K. V. (2011). Screening of black pepper (*Piper nigrum* L.) germplasm for resistance to foot rot disease caused by *Phytophthora capsici* Leonian. Journal of Spices and Aromatic Crops, 16: 115-117. https://updatepublishing.com/journal/index. php/josac/article/view/4876

Tafese Bezuneh, T. (2015). Phytochemistry and Antimicrobial Activity of *Parthenium hysterophorus* L.: A Review. Science Journal of Analytical Chemistry, 3(3), 30. https://doi.org/10.11648/j.sjac.20150303.11

Tamietti, G., and Valentino, D. (2001). Physiological characterisation of a population of *Phytophthora capsici* Leon. from Northern Italy. Journal of Plant Pathology, 199–205. https://doi.org/10.4454/JPP.V83I3.1129 3

Thakur, R. P. (1999). Pathogen diversity and plant disease management. Indian Phytopathology, 52, 1–9. https://epubs.icar.org.in/index.php/IPPJ/article/ view/19439

Thesis, M. S., & Delelegn, S. (2011). Evaluation Of Elite Hot Pepper Varieties (*Capsicum* Species) For Growth, Dry Pod Yield And Quality Under Jimma Condition, South West Ethiopia. Https://Hdl.Handle.Net/10568/3613

Thorat, V. H., Ghorpade, S. S., & Patole, T. (2018). *Ageratum conyzoides* Linn.: A review. Int. J. Pharmacogn, 5, 213-221. http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(4).213-18

Tiamiyu, Q. O., Adebayo, S. E., & Ibrahim, N. (2023). Recent advances on postharvest technologies of bell pepper: A review. Heliyon, 9(4), e15302. https://doi.org/10.1016/j.heliyon.2023.e15302

Tiawoun, M. A., Malan, P. W., Moshobane, M. C., Ramarumo, L. J., Comole, A. A., & Naidoo, K. K. (2024). Ecological Traits and Socio-Economic Impacts of the Alien Invader Weed *Parthenium hysterophorus* L. in South Africa's Rangeland Ecosystems: A Review. Diversity, 16(4), 205. https://doi.org/10.3390/d16040205

Tsao, P.H. (1991). The identities nomenclature and taxonomy of *Phytophthora* isolates from black pepper. Pages 185–211 in: Diseases of Black pepper. Proc. Int. Pepper. Comm. Workshop on Black pepper Diseases. Goa. India. Eds.YR Sarma and Premkumar. 209 pp.

Venkataiah B, Ramesh C, Ravindranath N, Das B. Charminarone, a seco-pseudoguaianolide from *Parthenium hysterophorus*. Phytochemistry. 2003;63:383–386. https://doi.org/10.1016/S0031-9422(03)00147-X

Villa-Martínez, A.; Pérez-Leal, R.; Morales-Morales, H.A.; Basurto-Sotelo, M.; Soto-Parra, J.M.; Martínez-Escudero, E. Situación actual en el control de *Fusarium* spp. y evaluación de la actividad antifúngica de extractos vegetales. Acta Agron. 2015, 64, 194–205. http://dx.doi.org/10.15446/acag.v64n2.43358

Volynchikova, E., & Kim, K. D. (2022). Biological Control of Oomycete Soilborne Diseases Caused by *Phytophthora capsici*, *Phytophthora infestans*, and *Phytophthora nicotianae* in *Solanaceous* Crops. Mycobiology, 50(5), 269–293. https://doi.org/10.1080/12298093.2022.2136333

Wain, H. (1952). The story behind the word; some interesting origins of medical terms. Ohio medicine: Journal of the Ohio State Medical Association, 48, 236–236.

Wang, B., Li, P., Yang, J., Yong, X., Yin, M., Chen, Y., Feng, X.,& Wang, Q. (2022). Inhibition efficacy of *Tetradium glabrifolium* fruit essential oil against *Phytophthora capsici* and potential mechanism. Industrial Crops and Products,176,114310. https://doi.org/10.1016/j.indcrop.2021.114310

Wang, Z., Wang, Y., Chen, X., Shen, G., Zhang, Z., & Zheng, X. (2006). Differential screening reveals genes differentially expressed in low-and high- virulence near-isogenic *Phytophthora sojae* lines. Fungal Genetics and Biology, 43(12), 826-839. https://doi.org/10.1016/j.fgb.2006.06.001

Winter, M., Samuels, P. L., Otto-Hanson, L. K., Dill-Macky, R., & Kinkel, L. L. (2019). Biological control of *Fusarium* crown and root rot of wheat by Streptomyces isolates–it's complicated. Phytobiomes journal, 3(1), 52-60. https://doi.org/10.1094/PBIOMES-11-18-0052-R

Xu, Y., Chen, X., Ding, L., & Kong, C.-H. (2023). Allelopathy and Allelochemicals in Grasslands and Forests. Forests, 14(3), 562. https://doi.org/ 10.3390/f14030562

Yusnawan, E., & Inayati, A. (2016). Methanolic extracts of three weeds as botanical fungicides to controlpeanut rust disease. Nusantara Bioscience, 8 (1). http://dx.doi.org/10.13057/nusbiosci/n080120