A dissertation

On

Evaluation of Herbal Nano-biocide against *Alternaria alternata*

Submitted to

Department of bioscience

Integral University, Lucknow

For the partial fulfillment of degree in Master of Science in Microbiology

By

Ilma Khan

Under the supervision of

Dr. Aradhana Mishra

Principal Scientist

Division of Microbial Technology

CSIR-National Botanical Research Institute

सी एस आई आर – राष्ट्रीय वनस्पति अनुसंधान संस्थान
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद्)
राणा प्रताप मार्ग, पोस्ट बाक्स सं0 436, लखनऊ-226 001 (भारत)
CSIR - NATIONAL BOTANICAL RESEARCH INSTITUTE
(Council of Scientific & Industrial Research)
Rana Pratap Marg, P.B. No. 436, Lucknow-226 001 (India)

CSIR NBRI Estd. 1953 स्पॉट पोस्ट

सं0 / No. दिनांक / Date

विवेक श्रीवास्तव
सीनियर प्रिंसिपल साइंटिस्ट
तकनीकी हस्तान्तरण एवं वाणिज्य विकास
ईमेल – **vivek@nbri.res.in**

टी.टी.बी.डी. / ट्रेनिंग–22 / 1180
फरवरी 24, 2022

महोदय / महोदया,

कृपया अपने प्रशिक्षण हेतु दिये गये आवेदन का संदर्भ ग्रहण करें। इस संदर्भ में मुझे यह सूचित करने का निदेश हुआ है कि संस्थान में आपके प्रशिक्षण सम्बन्धित अनुरोध को स्वीकार कर लिया गया है। आपके प्रशिक्षण कार्यक्रम की अवधि दिनांक 02.03.2022 से दिनांक 30.08.2022 तक होगी। अतः आप प्रशिक्षण की जानकारी हेतु डा. आराधना मिश्रा, प्रिंसिपल साइंटिस्ट से यथाशीघ्र संपर्क करें। इस संबंध में आपको यह भी सूचित किया जाता है कि:–

1. संस्थान के अतिथि गृह (गेस्ट हाउस) में सीमित स्थान उपलब्ध होने के कारण प्रशिक्षण के दौरान रहने की व्यवस्था स्वयं करें।
2. कृपया उपरोक्त अवधि के लिए आप प्रशिक्षण शुल्क रू. 24000 / – + 18% जीएसटी (रू. 4320) = कुल रू. 28320 / – (रुपये अट्ठाइस हजार तीन सौ बीस मात्र) जमा करें। इस शुल्क का भुगतान दिनांक 02.03.2022 तक निदेशक, राष्ट्रीय वनस्पति अनुसंधान संस्थान, लखनऊ के पक्ष में देय डिमाण्ड ड्राफ्ट अथवा नगद द्वारा किया जा सकता है। कृपया ध्यान दें कि प्रशिक्षण शुल्क वापस नहीं होगा। **(The fee is non refundable)**
3. अस्थायी पहचान पत्र के लिए दो पासपोर्ट आकार के फोटो साथ में लाएं। आपको सूचित किया जाता है कि प्रशिक्षण की अवधि की समाप्ति पर यह अस्थायी पहचान पत्र प्रशिक्षण ईकाई को वापस करना होगा अन्यथा आपको प्रमाण पत्र प्राप्त नहीं होगा। अस्थाई पहचान पत्र प्राप्त करते समय अपने संस्थान / विश्वविद्यालय द्वारा जारी मूल पहचान पत्र अवश्य साथ लायें।
4. तत्कालीन परिस्थितियों (COVID-19) के अनुरूप सक्षम प्राधिकारी द्वारा बनाये गये नियमों के अन्तर्गत ही उक्त प्रशिक्षण दिया जा सकेगा।

भवदीय,

(विवेक श्रीवास्तव)

सेवा में,

सुश्री इल्मा खान
655 / 630 आदिल नगर
टेढ़ी पुलिया, रिंग रोड, लखनऊ–226022

प्रतिलिपि – डा. आराधना मिश्रा, प्रिंसिपल साइंटिस्ट।

INTEGRAL UNIVERSITY

Established Under U.P. Act No 09 of 2004 by state Ligation

Approved by University Grants Commission

Phone No; +91(0552) 2890812, 2890730, 3296117, 6451039

Kursi Road, Lucknow- 226026, Uttar Pradesh (INDIA)

TO WHOM IT MAY CONCERN

This is to certify that **Miss. Ilma khan** a student of M.Sc. Microbiology (2nd year/4th semester) Department of Bioscience, Integral University, has complete her four-month dissertation work entitled **"Evaluation of herbal nano-biocides against *Alternaria alternata*"** from Division of Microbial Technology, CSIR-National Botanical Research Institute, Lucknow under the supervision of Dr. Aradhana Mishra, Principal Scientist. The dissertation was a compulsory part of her M.Sc. Microbiology degree.

I wish her good luck and bright future.

HOD. Dr. Snober S Mir

Head of Department of Biosciences,

Integral University

Objects

Obj.1 Screening of potential antifungal activity of crude essential oil

Obj.2 synthesis and characterization of herbal nanoemulsion

Obj.3 evaluation of potential antifungal activity of synthesized herbal nanoemulsion against Alternaria alternata

DECLARATION

I, **Miss Ilma khan** hereby declare that the dissertation work entitled **"Evaluation of herbal nano-biocides against *Alternaria alternata*"** is carried out under the supervision and guidance of **Dr. Aradhana Mishra**, Principal Scientist, Division of Microbial Technology, CSIR-NBRI, Lucknow, for the partial fulfilment of the Master's degree in Microbiology, provided by Integral University, Lucknow.

I hereby declared that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reputed in this study are obtained.

Ilma khan

M.Sc. Microbiology

Integral University

ACKNOWLEDGEMENT

This dissertation, like all worthwhile pursuits has been a team effort. I wish to express my sincere gratitude to all those involved. I am extremely grateful to **Dr. S K Barik**, Director of National Botanical Research Institute, Lucknow, for granting me the permission to work in one of the India's premier Research Institute.

I would like to express my gratitude to **Dr. Aradhana Mishra**, Principal Scientist, Division of Microbial Technology, CSIR-NBRI for her invaluable guidance during the course of my dissertation. It was her support, advice and care that made the dissertation work possible. I have learnt a great deal about scientific quality which she always kept very high. I am heartily thankful to her for answering my questions and clearing my doubts in the subject in spite of her workloads.

I am thankful to **Prof. Sanobr** Head, Department of Bioscience and **Dr. Amita Dubey**, faculty of Microbiology, University of Lucknow, for kindly lending a hand and giving me the opportunity to work in this prestigious institute & make this training a successful endeavor. I am enthusiastically thankful for their support and encouragement to work on a field of my interest.

I express my heartfelt and deep sense of gratitude to **Dr. Ved Prakash Giri, Mr. Ashutosh Tripathi, Miss Pallavi Shukla and Mr. Navinit Kumar** for constantly guiding me**.** I owe a lot to them for their valuable suggestions, guidance, and moral boost up. Their valuable efforts enabled me to develop an understanding of the subjects and their friendly nature enabled me to work without any pressure.

I am also thankful to **Mrs. Priyanka Chauhan, Miss Pratibha Verma, Miss Priya Verma, Mr. Sanjeev, Miss Vinita, Mr. Udit Yadav, Mrs. Isha and Mrs. Anshu, Miss Shweta yadav, Mr. Pashupati Nath, Mr. Sumit Kushwaha, Mr. Abhishek Jain** for their support, guidance and cooperation in completing this project. Their valuable suggestions helped to shape this work.

Thanks to the non-scientific staff of NBRI for their kind assistance in providing me clean glassware and chemicals for my project work. I would also like to thank my co- trainees Preeti Madheshiya Akshita, Smriti, Faiza, Shalini, Shikha for their nice company and cooperation and for believing in me and helping me in all aspects as a person needs.

Above all, I owe everything to God and my parents. This project is the manifestation of their faith and confidence in me. Words are very short to express my gratitude towards my gratitude my parents and family whose love, blessings and care has always given me moral strength and kept me progressive on the tract of life. Finally, I thank all those whom I could not mention but have been helpful in any respect at any phase of this work.

INDEX

LIST OF TABLES

LIST OF FIGURES

LIST OF INSTRUMENTS

S. No.	Particulars	Company
1.	Horizontal autoclave	Equitron
2.	Vortex	GeNei
3.	Refrigerator Incubator shaker	Innova 4230
4.	Refrigerator	LG
5.	Weighing balance	Denver
6.	Refrigerator centrifuge	Sigma, Dynamico
7.	Table top centrifuge	GeNei
8.	Hot air oven	GeNei
9.	pH meter	Inolab
10.	Microwave	Samsung
11.	Laminar air flow	Yorko
12.	Ice machine	Icematic D101
13.	Freezer	Sanyo
14.	Ultrasonic homogenizer	Biochem
15.	Deep freezer (-20 ℃)	Vestfrost
16.	Particle size analyzer	Malvern Instruments

LIST OF ABBREVIATIONS/SYMBOLS

Conc.	Concentration
DW	Distilled water
%	Percentage
nm	Nanometer
DNA	Deoxyribonucleic acid
°C	Degree Celsius
HPH	High pressure homogenization
USH	Ultrasonic homogenization
pH	Potential of Hydrogen
g/L	Gram per liter
µL	Microliter
mL	Milliliter
T.80	Tween 80
PDA	Potato dextrose agar
PDB	Potato dextrose broth
DLS	Dynamic light scattering
EC	Electrical conductivity
NE	Nanoemulsion
O^3NE	Lab's oil code

1. INTRODUCTION

Country-level measures of the robustness of agrifood systems are provided in The State of Food and Agriculture 2021. The indicators assess the stability of primary production, the availability of food, and both physical and financial access to food As a result, they can aid in determining how well-equipped national agrifood systems are to withstand stressors and shocks, a crucial component of resilience. The report examines how rural households respond to risks and shocks as well as the weaknesses of food supply chains. It discusses options to minimize trade-offs that building resilience may have with efficiency and inclusivity. The aim is to offer guidance on policies to enhance food supply chain resilience, support livelihoods in the agrifood system and, in the face of disruption, ensure sustainable access to sufficient, safe and nutritious food to all (The world bank., 2021).

The Production food items through farming and forestry is called agriculture. Agriculture is an important component of the Indian economy, contributing roughly 17% of the country's total GDP and employing more than 60% of the population. (Kumar and Raghavendra., 2019). Agriculture is backbone of India which plays a pivotal role in Indian economy. In India agriculture is not just farming, but an art of living. The entire country depends upon agriculture. High crop output is the most important requirement for life due to growing population and limited natural resources. According to estimates, 54.6 percent of the population works in agriculture and related industries each year, which contributed 17 percent to the nation's Gross Value added in the 2017–2018 fiscal year (Khurana, and Kumar., 2020). For most Indian families, agriculture is the primary source of income for more than 58 percent of rural households (Mikosi., 2019). But it is very sad that the farmer who feeds the people is facing economic difficulty due to low productivity. Government of India, State governments, National Bank of Agriculture and Rural Development and other financial institutions continuously working to enhance the livelihood of farmers (Joshi., 2020).

1.1 Medicinal plants

According to F.A.O/W.H.O about 80% population in developing countries relive on plants derived drugs for their primary health care and essential to prevent the loss of medicinal crops(Luttrell., 2007). Since ancient times, people have found and employed therapeutic plants, often known as medicinal herbs, in traditional medical procedures. Plants are the backbone of a complex traditional system of medicine. Natural products from medicinal plants are an excellent source of active and lead compounds important for the development of new drugs. Plants synthesize hundreds of chemicals compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals (Ahn et al., 2017). Humanity has used medicinal plants for healing purposes for thousands of years. The fact that 30% of pharmaceuticals sold globally contain components derived from plant material and that approximately 80% of the population in underdeveloped nations rely on plant-based medicines for their therapeutic needs illustrates the significant global significance of medicinal plants use (Sharma., 2014).Two-thirds of the world's population are anticipated to still rely on plant-based goods for their essential medical needs. (Panet et al., 2014). *Withania somnifera*(L.) Dunal (Solanaceae), popularly known as Ashwagandha in Sanskrit, has been extensively implemented as herbal medicine. (Ahmed et al., 2018). An evergreen woody shrub of the Solanaceae family and grown widely in tropical and subtropical climates of Africa, the Middle East, and the Mediterranean region of the world. The roots of the plants contain several bioactive principal compounds which include withaferin-A, withanolides, sterols and phenols, etc (Singh *et al*., 2017). In Indian Pharmacopoeia-1985, W. somnifera is listed as an official drug (Mandlik and Namdeo., 2021). As it is believed to combat stress and enhance immunity, physical and mental health, its roots and leaves have been used to improve cognition and treat a variety of illnesses, such as arthritis, digestive issues, and thyroid dysfunction. (Mustefa et al.,2021). The demand for W. somnifera has greatly expanded in recent years as a result of the growing acceptance of herbal products on a global scale. But different pests and pathogens regularly infest this crop during production. One of these diseases that affects *W. somnifera* most frequently is leaf spot disease, which is brought on by *Alternaria alternata* and may be recognised by the presence of dots on the surfaces of the leaves that range in colour from black to brown (Singh et al., 2020). *Alternaria alternata* is a fungus which has been recorded causing leaf spot and other diseases on over 380 host species of plant. It is an opportunistic pathogen on numerous hosts causing leaf spots, rots and blights on many plant parts. It can also cause upper respiratory tract infections and asthma in humans with compromised immunity. *Alternaria alternata* growing on culture medium started as white-grayish airy mycelium at the margin with clear light to dark green inner zonation radiating from a common center. Dark brown conidia in chains were observed ranging in sizes from 5 to 35 μm.

Table: 1 A list of selected medicinal plants with their ethno-medicinal uses and bioactive compound.

Name	Bioactive compounds	Role	Reference

Withania somnifera	alkaloids, steroidal lactones	Taken for treating neurological disorder cold and coughs, anxiety , diabetes, Parkinson's disease a suppressant in HIV/AIDS	Saleem, S et al,.(2020)
Ageratum conyzoides L. (Asteraceae)	Flavonoids Phenol	Treatment of leprosy, diarrhea, dysentery, intestinal colic, rheumatism, fever	Yadav, N et al,.(2019)
Asparagus curillus	steroidal saponins, arginine, tyrosine, flavonoids	Used to treat dysuria, diabetes and dysentery	Singh, L et al,.(2018)
Drimia indica (Asparagaceae)	alkaloids, flavonoids, phenols,	Juice of bulb is used in cough, bronchitis, nematode infection, pyrexia	Dwivedi.,et al .,(2019)
Cordia myxa L. (Boraginaceae)	proteins, glycosides, alkaloids, flavonoids	Taking for treating fever, ringworm, ulcers, prolapsed of uterus/vagina,	Madhubala and Santhi., (2019)

		headache, infection of urinary passage, diseases of lungs	
Phyllanthus emblica L. (Phyllanthaceae)	alkaloids, glycosides, flavonoids,	Fruit are rich in polyphenols, minerals and regarded as one of the richest source of Vit. C	Akbar.,(2020).
Urena *lobata L. (Malvaceae*)	flavonoids glycoside, alkanes,	Tonic and paste of whole plant is administered orally with milk to control urinary problems and sexual transmitted diseases	Maity and Patra., et al,.(2018)
Vigna mungo (L.) Hepper (Fabaceae)	flavonoids, isoflavonoids, phytoestrogens, phenolic acids,	Paste of fruits applied over fractured bone to join	Dwivedi et al .,(2019)
Piper longum L. (Piperaceae)	alkaloids, flavonoids, tannins,	Powder of fruits is administered orally to cure cough,	Asadi .,(2022).

	saponins	respiratory tract asthma, analgesic, muscular pains, inflammation.	
Acorus calamus L. (Acoraceae)	flavonoid, monoterpene, quinone, sesquiterpene**,**	Used to treat Fever, asthma, bronchitis, cough, digestive problems (gas, bloating, colic)	Kumar et al,.(2022).

Every year major loss in a crop productivity occurred due to abiotic and biotic stresses which are mainly categorized as:

Table 2: Various abiotic and biotic stress

Abiotic stress	**Biotic stress**
Environmental	Insects
High salinity	Herbivores
deficient or excessive water	Other plants
Chemicals	Fungi, Bacteria
Ultraviolet Radiation	Viruses
Temperature (high / low)	Caused by living organism
Floods	Weeds

1.2 Abiotic and Biotic stress

Due to abiotic and biotic stress, as well as other variables, leads to significant crop losses every year. which includes parasites, nematodes, fungi, and bacteria that cause severe crop loss and pose a threat to agricultural output. The current pressing need is to develop a technique to reduce the losses caused by biotic and abiotic stress. It has a considerable negative impact on agriculture, resulting in production, quality, and profit losses. (Calanca et al.,2017). Plants are sessile and unable to escape stressful conditions resulting from interactions with insects and microorganisms such as fungi and bacteria, as well as the physical environment that is abiotic and biotic stress. (Peters et al., 2011)

1.3 During abiotic stress:

Abiotic stress is a potential threat to agriculture and poses serious agronomical loss worldwide. The abiotic factors are considered as an essential component of environment and involves in crop distribution and productivity. Adverse environmental factors such as drought, salinity, extreme temperature, presence of toxic metal and nutrient deficiency leads to alteration in crop productivity and loss in availability of cultivable land (Zhang et al., 2021).

1.4 During biotic stresses

Biotic stresses cause extensive losses in agri-sector worldwide and raises the risk of hunger in several areas. Every year, approximately 20–40% of crop yield is lost due to plant diseases caused by several pests and pathogens (Moustafa-Farag et al., 2019). Plants are constantly exposed to biotic stresses including viral, bacterial, and fungal pathogens, insect and pests which have drastic economic and ecological impact. Some major phytopathogen and their detrimental effect on host are listed as follows:

1.5 Major fungal diseases

Table3: Major fungal diseases in medicinal crops

Pathogen	Host plant	Effective part	Reference
Sclerotium rolfsii	Azadirachta indica	Root, Stem, Flowers, Leaves	Paparu et al.,(2020)
Fusarium oxysporum	Adhatoda vasica	Leaves	Han Y et al., (2019)
Rhizoctonia solani	Nigella sativa L.	Fruit, Leaves	Zhao et al.,(2020)
Alternaria alternata	Withania somifera	Leaves	El-Gazzar et al.,(2020)
Cercospora beticola	Swiss chard	Leaves	Rangel et al.,(2020)

Nanotechnology has emerged as a potential tool to combat such deteriorative effect and develops cop up mechanism in response to the detrimental factors. Nanotechnology is a new field that developing a new technique to fight disease and control pathogens. Nanotechnology is concerned with the study of matter structures with dimensions on the order of a billionth of a meter (Charles P et al., 2015). Nanotechnology has made a significant contribution to agriculture through the use of nano-fertilizers and nano insecticides (Polyakov et al., 2019). Green nanotechnology has emerged as a viable subject for the biological creation of nanoparticles. (Judy et al., 2010).Nanotechnology is mostly used in contemporary fields of agriculture, food processing, and food protection , dairy industry, packaging, transportation and quality control of agricultural products.(Pandey.,2018). Nanoemulsion, a new facet of nanotechnology, is already gaining traction in associated industries. It is a more advanced and stable emulsion. . In its most basic form, a nanoemulsion is a mixture of oil, water, surfactant, and co-surfactant (Baker et al., 2000). A nanoemulsion is made up of droplets with dimensions ranging from 20 to 200 nanometers. (El-Aasser et al., 2004) 40nm-400nm (Yuan et al., 2008), and <500 nm (Aboofazeli, 2010; Singh et al., 2017). A narrow droplet size distribution can be obtained for nanoemulsions depending on the formation methods (Sheth et al., 2020). Nanoemulsions have been used in the pharmaceutical and drug delivery system, cosmetic industries for decades due to their lipophilic properties, but there is still a need to investigate the hidden side of the nanotized emulsion in agriculture. Due to their unusual qualities such as robust stability, transparency, high surface area, and, they have gained a lot of attention in recent decades. Oil is a plant's secondary metabolite with powerful antibacterial properties due to the presence of volatile and aromatic compounds. (Diao et al.,2013). The use of high-energy technologies to create microscopic droplets uses a lot of energy (around 1010 W/kg). Low-energy approaches, on the other hand, are an elegant emulsification technique for ultra-small droplet generation that takes advantage of system features (103 W/kg) (Kumar, N et al,.2021). Nanoemulsions are chosen over unstable emulsions and suspensions because of their increased solubilization capacity and thermodynamic stability. Nanoemulsions' physicochemical features are appealing for practical applications due to their small droplet size and long-term stability. In emulsions, the best nanoemulsion droplets are made with the best hydrophilic-lipophilic balance (HLB) and surfactant content. The right HLB value of the surfactants is critical for emulsion formation. Because of the large chain length distribution, nanoemulsions are frequently prepared with a mixed surfactant to improve stability. Vitamin E-enriched nanoemulsions have 16 been reported by adjusting the HLB values of the surfactants with Tween 20, 40, 60, 80, and 85 (Lu et al,.2018).

1.6 Advantages of nanoemulsion

- **i.** It may be used as a substitute for liposomes and vesicles.
- **ii.** It improves the bioavailability of the drug.
- **iii.** It is non-toxic and non-irritant in nature.
- **iv.** It has improved physical stability.
- **v.** Nanoemulsions have small-sized droplets having greater surface area providing greater absorption.
- **vi.** It can be formulated in a variety of formulations such as foams, creams, liquids, and sprays.
- **vii.** It provides better uptake of oil-soluble supplements in cell culture technology.
- **viii.** It helps to solubilize lipophilic drugs.
- **ix.** Helpful in taste masking.
- **x.** Less amount of energy is required.

1.7 Difference between Macroemulsion, Microemulsion and Nanoemulsion:-

Table4: Difference between Macroemulsion, Microemulsion and Nanoemulsion

Characteristics	Macroemulsion	Microemulsion	Nanoemulsion
Size	1–100µM	10–100 nm	10-200nm

Thermodynamic Stability	Meta stable	Stable	Meta stable
Kinetic stability	Stable	Unstable	Stable
Optical property	Turbid	Transparent	Transparent/slightly translucent
Polydispersity	High (>40%)	Low (<10%)	Low (<10–20%)
Preparation method	High and low energy methods	Low energy methods	High and Low energy methods

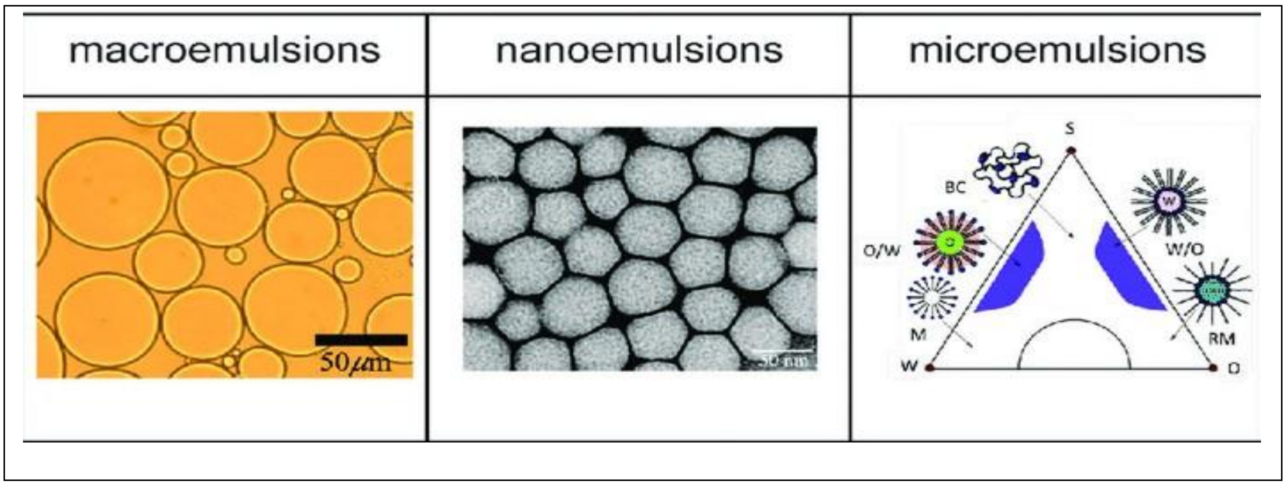

Figure 1: Macro, micro and nanoemulsion

2. REVIEW OF LITERATURE

Alternaria alternata is a fungal pathogen causing leaf spot, rots and blights on many plant parts. More than 300 *Alternaria* species have been described to cause diseases in more than 400 plant species (Rychlik *et al.*, 2018). This caused serious loss in yield. For example, tomato from Canada recorded yield loss of up to 79% due to early harvest damage. (Abbo et al., 2014) and has potential to cause 30% yield loss and postharvest losses of up to 10% (Dube., 2014). *A. alternata* is an economically significant food and feed contaminating fungus because it has already infected more than 100 plant species. (Lee *et al.*, 2015). Even while *A. alternata* may often colonies a variety of plants, some species-specific pathotypes can infect a host and release poisons that are particular to that host. (Cho,.2015). In addition to causing crop loss through plant infections, A. alternata also seriously harms agriculture by contaminating food and feed with mycotoxins and causing postharvest deterioration (Garganese *et al.*, 2016). Since the fungus is mostly airborne and has a long survival time, disease management techniques including rotation with non-host crops and cleanliness are not totally effective. The best method for bringing the disease under control and preventing damage is fungicide therapy. Fungicides are often applied beginning two weeks after transplanting and ending two weeks before harvesting every two to three weeks. Such heavy use of chemicals is not economically feasible for the generally resources-limited growers (Abbo et al., 2014). Using pesticides improperly results in a number of environmental harms, including the buildup of hazardous substances found in the water, soil, and living things through the food chains. It could also result in the pathogens that have developed resistance, using chemicals that are getting stronger necessary, making the situation even worse effects on the environment (Franca et al., 2018). To achieve sustainability in agroecosystems, it became crucial to utilize less hazardous plant pathogen management agents. These goods must be effective, affordable, environmentally and human health-safe. The essential oils produced from aromatic plants, whose raw materials are accessible, manufacturing is affordable, and many of which exhibit antifungal activity (Camargo et al,.2011) For many years, the peppermint essential oil (Mentha x piperita L.) has been studied as an antibacterial agent. Studies reported that in vitro condition Peppermint essential oil showed positive results against various phytopathogens such as *Macrophomina phaseolina*, *Colletotrichum gloeosporioides, Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii, Aspergillus niger and A. flavus* (Sousa et al., 2012;Ugulino et al.,2018). This study reported to provide a healthy and environmentally safe product to fight against leaf spot diseases caused by *A. alternata*. This study assesses the antifungal effect of peppermint essential oil to control *A. alternata* during *in-vitro* condition (Franca et al,. 2018).

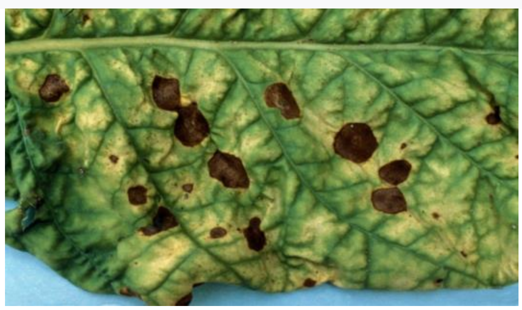

Figure 2: Leaf spot disease caused by *Alternaria alternata*

It is evident from the data presented in table no 5. That different type of essential oil is effective for the antimicrobial activity.

Table5: Antimicrobial activity of essential oil

S. no.	Name of oil	Activity against pathogen	Part used	References
1-	Peppermint	*Aspergillus niger*	Leaves	Hu et al.,(2019)
2.	*Cynara scolymus* (globe artichoke)	*Alternaria spp*.	Seeds flower	Ghareeb, H.S.,(2018).
3.	*Eucalyptus globulus* (Eucalyptus oil)	*Pseudomonas aeruginosa*	leaves	Quatrin et al.,(2017)
4.	*Calabash nutmeg* (Monodora myristica)	*Pseudomonas aeruginosa*	Seed	Sylvester, C. et al.,(2018)
5.	*Linumusitatissimum (Linseed)*	S. *aureus, B. subtilis, E.coli, Shigella boyedi*	Seeds	Hussien, Z.G, et al.,(2021)
6	*Cinnamomum verum* (Cinnamon oil)	*A. flavus*	Bark, seed.	Lee, J.E, et al.,(2020)
7.	*Syzygium aromaticum* (Clove oil)	*A. flavus, C. Albicans*	Bud	Latifah-Munirah et. al.,(2015)

8.	*Cymbogogancitrs* (Lemongrass)	*S.auerus, E.coli*	leaves	Naik et.al.,(2010)
9.	*Coriandru msativum* (coriander)	*C. albicans*	leaves	Matasyoh,et. al.,(2009), Begnami et al.,(2010)
10.	*Nigella sativa* (black cumin)	*S. aureus, B. cereus, E. coli, P. aeruginosa*	seed	Abdullah, S.A., et al.,(2021)
11.	*M. insularis*	*Staphylococcus xylosus*	stems leaves	Venturi et al.,(2015)
12.	*Eucalyptus citriodora* (Lemon-scented gum)	*Phytophthora cactorum,*	leaves green branchlets	Tolba, H et al.,(2018)
13.	*Ricinus communis* (Castor oil)	S. *aureus and E. Coli*	Derivatives of castor oil	Mohammed, M.A.Y.A., (2021)
14.	*Fortunella margarita Lour* (Oval Kumquat)	*Influenza (H5N1)*	Fruit and seed	Ibrahim,et.al.,(2015)

15.	*Commiphora wightii(*Indian bdellium-tree)	Gram positive and negative bacteria	Gum extract	Singh and Siddiqui, .,(2015)
16	*Origanum vulgare* (Oregano)	*Listeria monocytogens*	leaves and shoots	Maggio, F et al.,(2022).

2.1 Nanoemulsion

The development of nanoemulsion-based technology allowed for the promotion of green nanotechnology by increasing the antibacterial activity of essential oils. When used as a therapeutic tool, nanoemulsions have powerful antibacterial properties against harmful microbes. Nanoemulsions, which range in size from 100 to 200 nm and are thermodynamically stable, are created when two immiscible liquids are combined into a single phase with the aid of the proper co-surfactant and surfactant. Oil-in-water (O/W) and water-in-oil (W/O) nanoemulsions are the two main varieties. Because of their unique qualities, including their high surface area per unit volume, prolonged shelf life, translucent appearance, and tuneable rheology, over the past ten years, there has been a lot of research on nanoemulsions (Gupta et al., 2016). The development of small-sized droplets depends on the use of an emulsifier because it lowers the interfacial tension, or the floor strength per unit area, between the oil and water phases of the emulsion. Through repellent electrostatic interactions and steric hindrance, the emulsifier also functions to stabilise nanoemulsions (Kumar et al., 2021). The emulsifier used is commonly a surfactant; however proteins and lipids have also been superb in the education of nanoemulsions (Delmas et al., 2011).

2.2 Types of Nanoemulsion

On the basis of composition there are three types of nanoemulsion:

a) **Oil in water nanoemulsion (O/W)-**Oil droplets are dispersed in the continuous aqueous phase that is water.

b) **Water in oil nanoemulsions (W/O) -**Water droplets are dispersed in the continuous oil phase.

c) **Bi-continuous nanoemulsions-**Micro domains of oil and water are interspersed within the System.

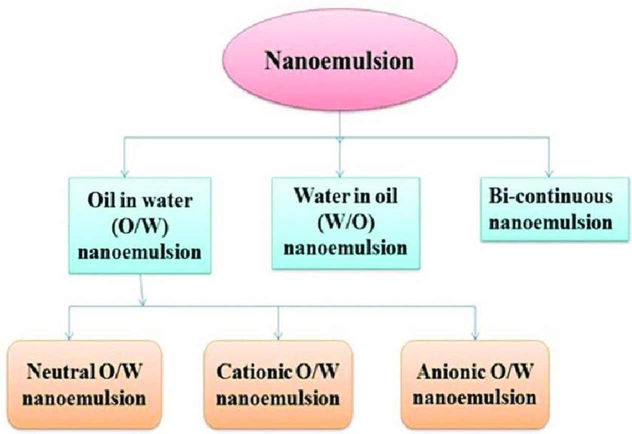

Figure3: Types of Nanoemulsion

2.3 Components of Nanoemulsion

Oil, emulsifying agents, and aqueous phases make up the majority of a nanoemulsion. Any type of oil can be used, including castor oil, peanut oil, coconut oil, evening primrose oil, linseed oil, mineral oil, and olive oil. A mixture of oil and water may yield a crude temporary emulsion, which upon standing, will separate in two distinct phases due to the coalescence of the dispersed globules. Such systems can gain stability via emulsifiers or emulsifying agents. Emulgents fall into three categories: hydrophilic colloids like acacia, surfactants like spans and tweens, and finely divided solids like bentonite and veegum. In addition to its ability to emulsify, an emulgent should be safe and compatible with the product in terms of taste, odour, and chemical stability.

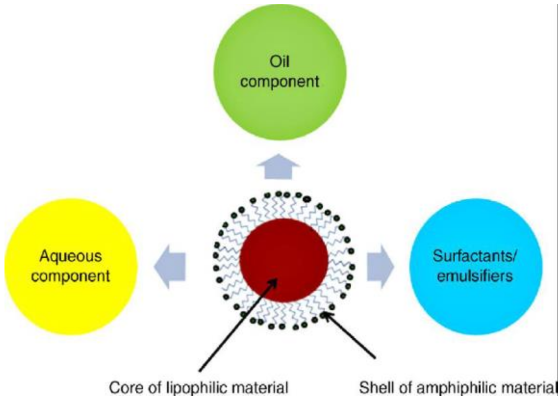

Figure4: components of nanoemulsions

Some of the desirable properties of a surfactant are

1. It must be capable of lowering surface tension to less than 10 dynes/cm.
2. To prevent coalescence, it should be quickly adsorbed around the dispersed phase globule to form a complete and coherent film.
3. It should contribute to increasing the system's zeta potential and viscosity to ensure maximum stability.
4. It ought to function well at relatively low concentrations. Around the distributed globules, surfactant creates monomolecular, multimolecular, or particulate films.

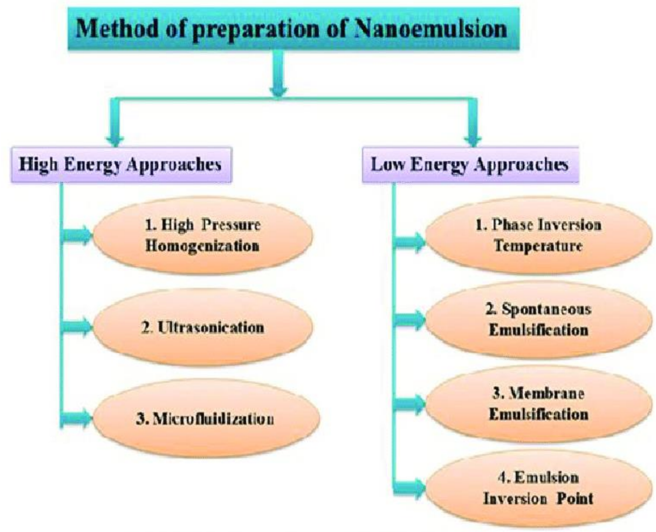

Figure5: Methods for synthesis of nanoemulsion

2.4 Formulation and method of preparation of nanoemulsion

Nanoemulsions are made up of an emulsifier, an additive, and an active substance. Two techniques are among the several ways to make a nanoemulsion.

(a) High-energy emulsification

(b) Low-energy emulsification.

A. High energy method-

- **Ultrasonic emulsification:** By using high-intensity ultrasonic waves to provide the powerful disruptive forces required to break up oil and water phases into very small droplets, ultrasonic emulsification is very effective at lowering droplet size (Leong et al.,2009). In ultrasonic emulsification, sonotrodes referred to as sonicator probes deliver the energy. It is mostly used in laboratories, where emulsion droplet sizes as small as 0.2 micrometer can be achieved, where ultrasonic homogenizers are employed.

Figure6: An instrument used for ultra-sonication process during nanoemulsion synthesis

- **High pressure homogenization:** High-pressure homogenization is necessary for the creation of nanoemulsion. This method creates nanoemulsion with incredibly small particle sizes using a high-pressure homogenizer or piston homogenizer (up to 1 nm) (Anton et al.,2008).

Figure7: An instrument used for the high pressure homogenization process during nanoemulsion synthesis.

- **Micro fluidization:** A unique mixing technique known as micro fluidization uses a device known as a micro fluidizer**.** This device uses high pressure which forces the drug product through the interaction chamber resulting in a very fine particle of submicron range. The processes repeated several times to obtain a desired particle size to produce uniform nanoemulsion.

B. Low energy method-

- **Spontaneous emulsification**

It involves three steps:

1) The creation of a homogenous organic solution containing water-soluble solvent, hydrophilic surfactant, and oil with a lipophilic surfactant,
2) Under continuous magnetic stirring, the organic phase is pumped into the aqueous phase to create an emulsion.
3) Evaporation under lower pressure is used to remove the aqueous phase pressure (Tadros et al.,2004).

- **Phase inversion temperature**

By using a higher temperature on a micro emulsion, this technique entails a shift in phase (Komaiko & McClements (2015). At room temperature, the components are combined; as the temperature rises, the surfactant is mixed with the oil phase. The O/W is changed into a W/O emulsion after cooling (Forgiarini et al., 2001). Earlier nanoemulsions can be produced by methods requiring high-energy. Now, recent research has focused on developing a variety of reproducible low-energy emulsification methods, which can be divided into two main groups:

(a) Spontaneous emulsification methods and

(b) Phase inversion temperature methods (Anton et al., 2008).

Natural, primarily herbal, bioactive substances are currently gaining popularity. Size and stability of an object depend on its physio-chemical characteristics. Several elements, including the ultrasonic duty cycle and intensity of ultrasonic irradiation, have a role in the high energy method's ability to synthesise stable nanoemulsions. While pulsed ultrasound applied at regular intervals was more effective than continuous ultrasonication, the average droplet size of oil nanoemulsion dropped as duty cycle increased. While continuous ultrasonication was less effective than pulsed ultrasound administered at regular intervals, the average size of the oil nanoemulsion droplets decreased with increasing duty cycle (McClements and Rao. 2011). The pharmaceutical industry has paid close attention to nanoemulsions in drug delivery studies as a means of transporting hydrophobic medicines dissolved in the oil phase. (Ghotbi et al., 2014). In order to produce a nanoemulsion with a smaller droplet size and improved stability, system parameters including emulsifier type, emulsifier concentration, and emulsification procedures were tuned. The high-energy approach of ultrasonication was used to reduce the droplet diameter.

Table6: Comparison of thermodynamic stability and physicochemical properties of emulsion, nanoemulsion, microemulsion **(**McClements & Rao, 2011**)**

System	Droplet Radius	Stability	Surface-to-Mass Ratio (m2/g particles)
Emulsion	100 nm–100 µm	Unstable	0.07-70
Nanoemulsion	10-100nm	Unstable	70-330

Micro emulsion	2-100nm	Stable	330-1300

Table7 : Use of nanoemulsion in agriculture: (Pandey et al, 2019).

Source of Oil	**Method of preparation**	**Properties/ Application**	**Reference**
Phyllanthus emblica	Hot high pressure homogenization	Drug delivery	Chaiittianan and Sripanidkulchai.,(2014)
Boswellia Serrata	high speed homogenizer	Encapsulate drug for inflammatory disorder	Gohel et al., (2014).
Ani seed, peppermint and lemongrass	High Intensity Ultrasonic	Pesticide	Pascual-Villalobosa et al., (2017).
Thymol	Sonication method	Antibacterial against pustules disease	Kumari et al., (2018).
Eucalyptus oil	Low energy emulsification method	Antibacterial against *Listeria monocytogenes*	Sugumar et al., (2014).

Eucalyptus globulus	Ultrasonicator	Insecticidal activity of wheat weevil	Mossa et al., (2017).
Ocimumbasilicum L	Sonicator	Larvicidal activity against *Aedesaegypti*	Ghosh et al., (2014).
Azadirachta indica	high-energy sonication	Larvicidal against *Culex quinquefasciatus*	Anjali et al., (2012).
Thymus daenensis	High intensity ultrasound	*Escherichia coli*	Moghimi et al., (2016).
Jojoba oil	Sonication	Insecticidal Activity against *Sitophilus oryzae*	Sh et al., (2015).
Capsicum annuum L.	High speed homogenizer	Encapsulation of drug	Pascual-Pineda et al., (2015).
Vitexagnus-castus	Ultra sonication	Drug delivery	Piazzini et al., (2017).
Olive oil	Phase inversion temperature	Lipstick	Munawiroh et al., (2017).

Eucalyptus globulus; Pterodonemarginatus	Sonication; Low energy method	Larvicidal activity against *Culex quinquefasciatus*	Sugumar et al., 2014; Oliveira et al., (2017).
Orange oil	Iso-thermal low energy method	Delivery system in food industries	Chang and MaClements, (2014).
Ani seed	Homogenization	Antimicrobial activity against food borne pathogens	Topuz et al., (2016).
Soybean oil	Boiling	Sporicidal activity against *Bacillus cereus* and *B. anthracis*	Hamouda et al., (1999).
Luteolin loaded emulsion	Phase inversion composition and sonication	Hair growth promotion activity	Shin et al., (2018)
Carapaguianensis (seeds); Rosmarinus officinalis; Baccharis reticularia	Low energy method	Larvicidal activity against *Aedesaegypti*	Jesus et al., (2017).

Various reports are available for the antibacterial activity of nanoemulsions (Dávila-Rodríguez et al.,2019; Badr et al.,2021; Liang et al 2012;) have created a peppermint oil nanoemulsion that has antibacterial properties against the gram positive pathogens *Staphylococcus aureus* and Listeria monocytogenes Scott A. These innovative uses of essential oils in nanoemulsions have been found to increase the shelf life of aqueous food products. *Escherichia coli,* a food-borne pathogen, was successfully eradicated by a stable nanoemulsion of cinnamaldehyde and thyme oil. (Tian et al 2016). In the recent years, nanoparticles have received much attention for controlling pathogens in agriculture (Elek et al., 2010). Nanoemulsions of neem oil showed that the LC50 (the concentration required to achieve 50% mortality (Anjali et al., 2012) decreased with droplet size, which was interpreted as indicating an increased uptake of smaller droplets. In this second study, the effects on non-target organisms (i.e., soil bacteria and plants) were reduced (Kumar et al., 2013), but the reasons for the different effects on target and non-target organisms have yet to be elucidated. Unfortunately, no comparisons were carried out with commercial formulations. Nanoemulsions for water soluble (i.e., glyphosate) have recently been presented in a series of papers from a research group based in Malaysia. Their nanoformulations were designed to boost the herbicide's absorption while eliminating some adjuvants included in conventional glyphosate formulations that have been linked to toxicity to creatures other than the intended targets. (Piola et al.,2013). The glyphosate nanoemulsions – prepared with various proportions of fatty acid methyl esters, organosilicones, and alkyl glucosides – showed an efficacy similar to, or slightly greater than the commercial round up formulation (Jiang et al., 2012). In the present study, bioassay was followed for assessing the insecticidal activities of Ag and Ag-Zn nanoparticles on the *Aspidiotusnerii*. Results showed that these nanoparticles could be an effective pest control approach for A. nerii. (Rouhani et al.,2012). The investigation that followed demonstrated that as pesticide concentrations rose, insect mortality also rose noticeably. Low likelihood of resistance developing with prolonged use is one benefit of utilising them. As a nanopesticide, a stable nanoemulsion of clove oil in water with the appropriate droplet size was created (Shahavi et al., 2015).

2.5 Antimicrobial activity of nanoemulsion-

Nanoemulsions are efficient anti-biofilm agents and have antibacterial qualities. Antimicrobial nanoemulsions are a detergent, oil, and water mixture that have been emulsified. They have been demonstrated to exhibit wide antimicrobial activity against bacteria, enveloped viruses, and fungi at doses that are harmless to animals (Hamouda *et al*., 2001). Contrary to antibiotics, nanoemulsion's antibacterial activity is nonspecific, allowing for broad-spectrum activity while reducing the potential for resistance development. These characteristics make nanoemulsion an excellent choice for surface cleaning and the treatment of wounds. (Hemmila *et al*., 2010). By disrupting their outer membranes, nanoemulsions show broad biocide effectiveness against bacteria, enveloped viruses, and fungi (Premanathan et al., 2011). The nanoemulsion particles are thermodynamically driven to fuse with lipid containing organisms. When enough nanoparticles fuse with the pathogens, they release part of the energy trapped within the emulsion. Both the active ingredient and the energy released destabilize the pathogen lipid membrane, resulting in cell lysis and death Neem (*Azadirachta indica*) has been used in traditional medicine over centuries and it showed different biological activities which makes it a good target for antibacterial studies. Broad-spectrum antibacterial efficacy against both gram positive and gram negative bacteria is demonstrated by antimicrobial nanoemulsions *(S.aureus, E.coli, B.cereus, P.aeruginosa)* (Majeed *et al*., 2015). The opposing charge of the nanoemulsion attracts pathogens, causing lipid membrane ruptures, cell lysis, and ultimately cell death. Due to the numerous negative side effects of using antibiotics, it can be utilised long-term.

PLACE OF WORK

The present study entitled **"Evaluation of herbal nano-biocides against *Alternaria alternata*"** was conducted at the Division of Microbial Technology, National Botanical Research Institute, Lucknow, under the supervision of Dr. Aradhana Mishra, from 1st March to 31st August 2022

3. Procurements of materials

3.1 Fungal isolates: Fungal isolate *Alternaria alternata* (AA) used in this work was procured from the lab repository of Division of Microbial Technology, CSIR-NBRI, Lucknow. Media and other chemicals were obtained from Sigma, Himedia, Qualigens, CDH and Merck Chemicals.

3.2 Media a**nd other chemical**- were obtained from Sigma, Himedia, Qualigens, CDH, and Merck chemicals.

3.3 Composition of potato dextrose agar

S. No.	**Composition**	**Amount (g/L)**
1)	Potato infusion	200
2)	Dextrose	20
3)	Agar	15

3.4 Composition of potato dextrose broth

S. No.	Composition	Amount (g/L)
1)	Potato infusion	200
2)	Dextrose	20

3.5 Essential oils and chemicals:

Five crude essential oils *viz*; GO, TO, PO, CO and EO (oils name could not be disclosed) were taken to be screened for antimicrobial activity against AA. All the chemicals used in this study were purchased from Sigma Aldrich, India. Essential oil were purchased from HiMedia Laboratories, Mumbai, India. All other reagents used were of analytical grade. Double distilled ultrapure water (18.2M Ω; Millipore Co.) was used in the experiments.

4. Methods

4.1 Screening of oils

- The antimicrobial activity of essential oils was enumerated against the phytopathogen *Alternaria alternata* that causes leaf spot in the host plant.
- PDA was prepared in a flask. After autoclave PDA plates were prepared.
- After this a fungal bid was placed in the corner of the plate.
- In the same plate, sterilized cotton piece were kept at the corner opposite to the fungal bid and treated with 150μL of essential oil. These plates were incubated for seven days.

The plates were observed for the clear zone of inhibition.

4.2 Synthesis of Nanoemulsion

- Crude Oil of different plants is used to synthesize and stabilized antimicrobial nanoemulsion, for oil-in-water (O/W) nanoemulsion synthesis, proper concentration of oil Emulsifier/surfactant and dispersant is required. For the synthesis of 10% O^3NE (20 ml) nanoemulsion we take 2000µl oil, 1000µl surfactant (T.80) and rest volume of dispersant (distilled water) and for 5% (20ml) nanoemulsions, we take 2000µl oil, 1000µl surfactant (T.80) and rest volume of dispersant (distilled water).
- Mixed the given volume of crude oil and surfactant (T.80) after that add dispersant slowly to the oil phase with a constant rate of agitation.
- Before sonication, vortex the sample to mix the content for 2 minutes.
- After vortexing the mixture, sonicate the sample for 20 minutes with 10 seconds on/off interval at 25% amplitude.

Figure8: Ultrasonic homogenizer

4.3Characterization of synthesized nanoemulsion

- **Particle size distribution**

The particle size distribution of the emulsions was determined by particle size analyzer. All measurements were made at a fixed scattering angle of 90° and a temperature of 25.0± 0.1 °C. The light source of the particle size analyzer is a solid-state laser operating at 658 nm with 30 mW power, and the signals were detected by a high-sensitivity avalanche photodiode detector. To avoid multiple scattering effects, emulsions were first diluted 100 times with deionized water and stirred continuously before the measurements to ensure the samples were homogeneous. The mean diameter of each emulsion was determined by cumulant analysis of the intensity−intensity autocorrelation function.

Figure 9: Particle size analyzer

- **Polydispersity index -** It indicates the uniformity of droplet size in nanoemulsion. The higher the value of polydispersity, the lower will be uniformity of the droplet size of the nanoemulsion. It can be defined as the ratio of standard deviation to mean droplet size. It is measured by a spectrophotometer.
- **Measurement of pH-** The pH value of the nanoemulsions was measured by immersing the electrode directly into the emulsion using a calibrated pH meter at 25°C ±1°C.

Figure10: pH meter

- **Measurement of Transmittance-** Turbidity of all the synthesized nanoemulsions was analyzed by measuring the transmittance of undiluted emulsions at a wavelength of 600 nm ultraviolet.
- **Conductance measurement-** The conductance of nanoemulsion is measured by a conductometer. In this test, a pair of electrodes connected to a lamp and an electric source is dipped into an emulsion. If the emulsion is o/w type, water conducts the current and the lamp gets lit due to the passage of current between the electrodes. The lamp does not glow when the emulsion is w/o: oil being in the external phase does not conduct the current.

4.4 Antifungal Test

1) To examine the antifungal activity, the plant pathogenic fungus *Alternaria alternata* (AA) was procured from laboratory and was cultured under specific environment. The culture of the fungus was maintained at 4°C throughout the study and was used as stock cultures.

2) PDA was prepared in different flasks.

3) Different concentrations of O^3NE were added in the PDA flasks before pouring.

4) 500 µL, 1000 µL, 1500 µL and 2000 µLO^3NE treatments were put in the sterile PDA flasks before pouring and treatment plates were prepared.

5) Control was left as it is without any treatment.

6) Culture bids of AA were placed in the middle of the plates.

7) After this, the fungal plates were left for the incubation for 5-6 days at 28°C.

5. Results and Discussion

Nanoemulsions are emulsions with nano-size range, Oil in water (O/W) emulsion is widely used in various applications such as pharmaceuticals, cosmetics, detergent etc. Nanoemulsions has good stability, rapid digestibility, protection against degradation, controlled release, and high capability of enhancing drugs' bioavailability. Crude essential oils in the form of nanoemulsion are diluted and more persistent. The use of some fine emulsions as antimicrobials agent, is a new and promising application. During our work we find the following results;

5.1 Screening of essential oils-

The crude oil extracts of some plants have potential antimicrobial activity against various microbial pathogens which is a good alternative of chemically generated antimicrobial agents. Antimicrobial activity of some essential oils was screened by cotton immobilization assay.

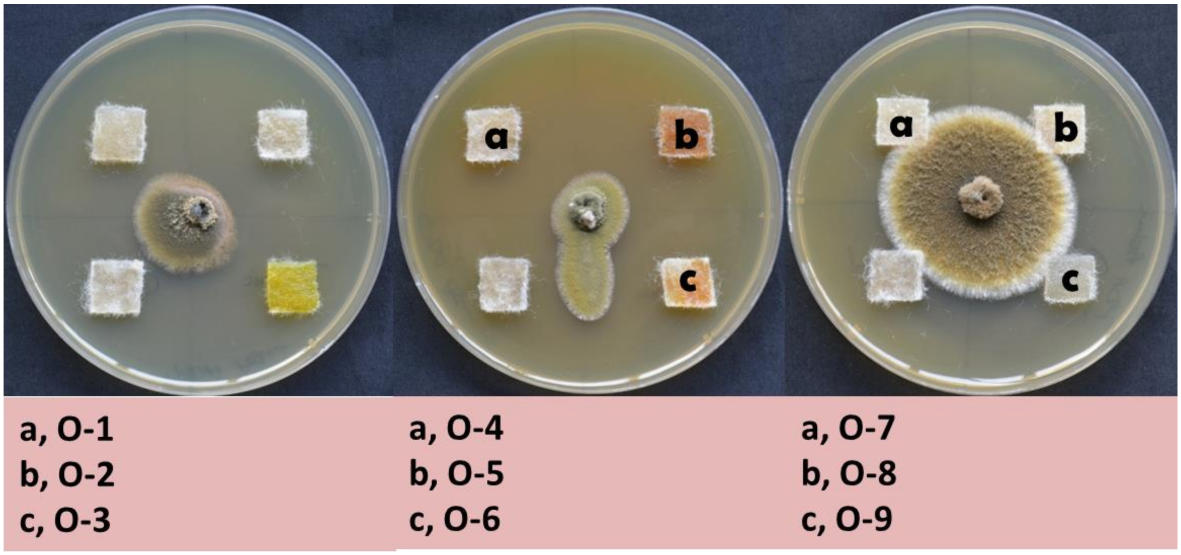

Figure 11: Screening of different essential oils for antimicrobial activity

Among all the 9 crude oils we found that only O-2 shows clear fungicidal activity against selected phytopathogen *Alternaria alternata.* Based on this screening test, nanoemulsion has been synthesized by potent crude oil O-2 that may be revealed a better substitute of chemically synthesized antimicrobial agents.

5.2 Preparation of Nanoemulsion-

In our study the nanoemulsion O-2NE (Oil-2 nanoemulsion) has been synthesized by using high energy sonication method, the basic component of this instrument is a probe that imposes high mechanical energy that disrupts and combine the immiscible phase of oil and water and forms small droplets with increased surface area (Kumar et al., 2019). The homogenous mixture was initially prepared by mixing 2000 µL of crude oil, 1000 µL of surfactant (Tween 80) and rest volume (20 mL) was made up by adding 17ml of dispersant (distilled water). After sonication, the synthesized O-2NE was stable at room temperature.

Table 8: Ingredients of O-2NE

Nanoemulsion	**Oil (v/v)**	**Surfactant (Tween80)**	**Dispersant (DW)**
O-2 NE (10%)	200 µl/ml	75 µl/ml	Rest volume

5.3 Characterization of Nanoemulsion-

5.4 Particle size distribution and zeta potential- The particle size distribution of prepared emulsions was determined by particle size analyzer (Malvern, nanoseries zetasizer). Dynamic light scattering is a reliable technique which provides valuable information on the size distribution of nanoemulsions. In our study the size of droplets present in homogenized nanoemulsion (O-2 NE) were 130.9 nm. Moreover, poly dispersity index (PDI) of nanoemulsion was 0.200, showing the homogeneity nature of nanoemulsion (Kumar et al., 2021). Zeta potential of prepared O-2 NE was -15.2. The zeta potential is a method for measuring surface charge of particles present in the solution; it can be used for predicting the stability of the nanoemulsion. The greater zeta potential values correspond to the greater stability of the nanoemulsion (Midekessa *et al.,* 2020). Zeta potential can be used to evaluate the potential stability of the nanoemulsion system as it provides the electrostatic repulsion between the similarly charged dispersed droplets (Sharif *et al.,* 2017).

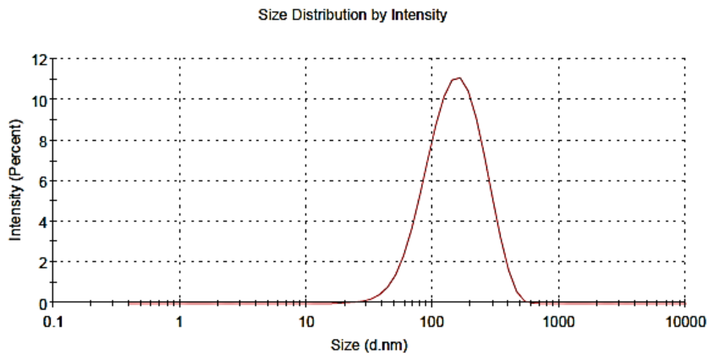

Figure 12: Droplet size of O-2 NE

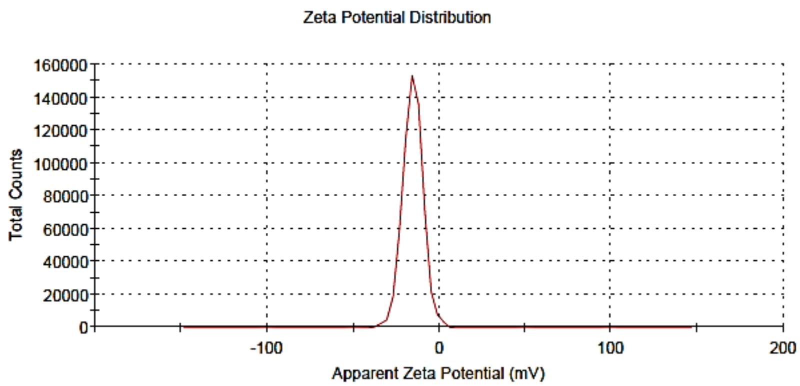

Figure 13: Zeta potential of O-2 NE

5.5 Physiochemical properties-

The pH value of synthesized nanoemulsion was 4.151. Electrical conductivity determines the nature of the continuous phase and helps in detecting phase inversion phenomenon (Hasssanzadeh et al., 2018). However, the refractive index shows the transparency of nanoemulsion if the refractive index is equal or near to water then the prepared nanoemulsion is considers being transparent. (Gurpreet, and Singh., 2018). In our study the electrical conductivity of O-2 NE was 55.63µS/cm and refractive index was 1.358 which is near to water, hence the prepared nanoemulsion is stable and an effective formulation to combat pathogenic fungi *A. alternata.*

Table 9: Physiochemical properties of O-2 NE

Colour	The prepared O-2 NE was milky white in appearance
Measurement of pH	The pH value of O-2 NE was found to be 4.151
Electrical conductivity	The EC of O-2 NE was observed as 55.33 μS/cm
Refractive index	The refractive index of prepared O-2 NE was 1.358

5.6 Antimicrobial activity-

Most of the microbes developed resistance against different antimicrobial agents such as antibiotics, therefore investigations for new alternatives and novel strategies are gaining importance in today's world. The use of antimicrobial nanoemulsions may exert synergistic antimicrobial effects and would be a novel approach. Antimicrobial activity of O-2 NE has been observed against phyto pathogenic fungal strain *Alternaria alternata*, which is a major causative agent for leaf spot disease in Ashwagandha. The minimum inhibitory concentration of synthesized nanoemulsion was evaluated through agar diffusion assay which shows effective result that as follows:

5.7 Minimum inhibitory concentration of O-2 NE against *Alternaria alternata*-

Antifungal activity of O-2 NE against *A. alternata* was observed by minimum inhibitory concentration through agar diffusion assay at different concentrations 0.5%, 1%, 1.5%, 2% of O-

2 NE. The MIC value is expressed in terms of % growth inhibition. Among these concentrations 1.5% O-2 NE shows maximum growth inhibition 90.13% against pathogen *A. alternata*.

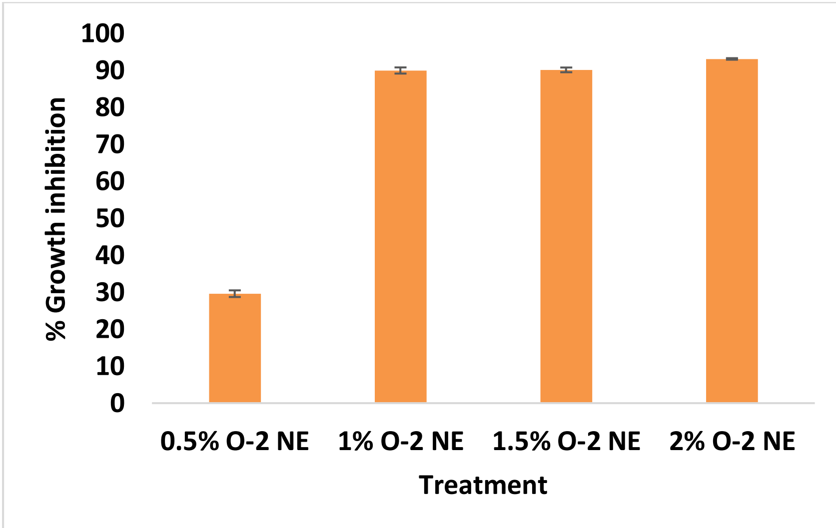

Figure 14: Minimum inhibitory concentration of O-2 NE against *Alternaria alternata*.

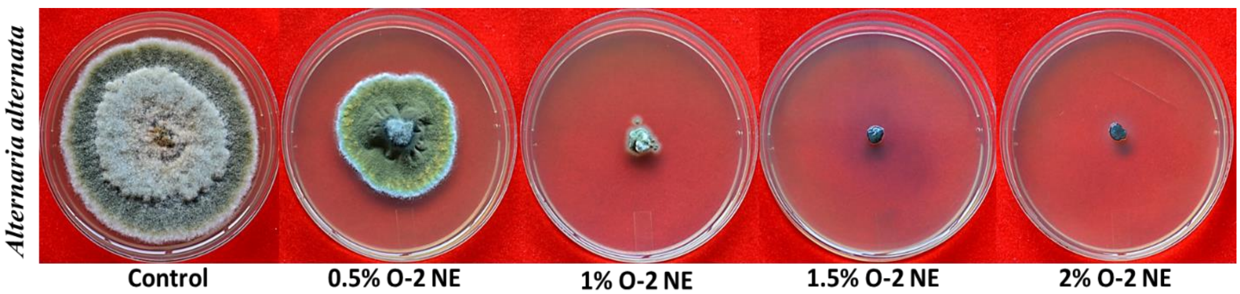

Figure 15: Antifungal activity of O-2 NE against *Alternaria alternata*, through agar diffusion assay.

Bio active compounds present in essential oil show antifungal activity. There are several factors involve during antagonistic activity of nanoemulsion with fungus (Fani *et al*., 2015) 1.5% O-2 NE shows complete inhibition against *Alternaria alternata*.

5.8 Effect of O-2 NE on fungal mycelia:

To evaluate the antifungal activity of prepared O-2 NE against *A. alternata*, histological analysis was performed through lactophenol cotton blue assay.

5.9 Lactophenol cotton blue assay:

Upon observation, 1 % and 1.5 % O-2 NE shows marked deformation is fungal spores.

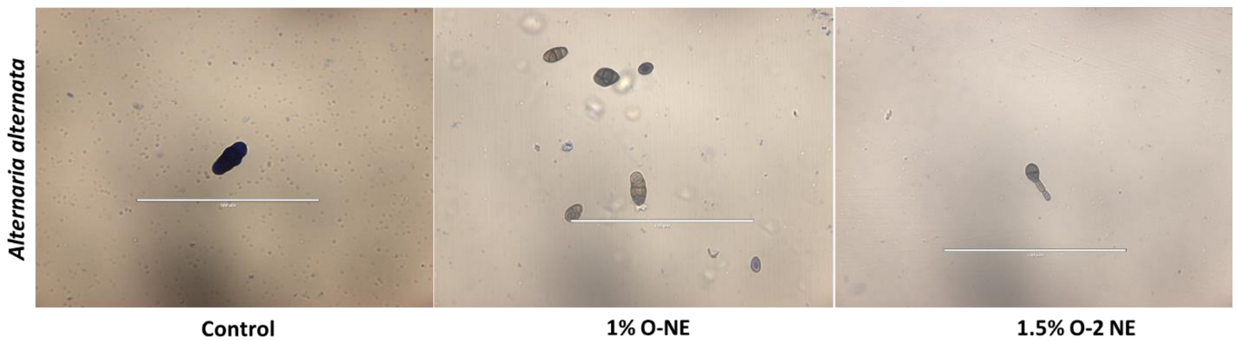

Figure16: Structural changes in *A. alternata* after O-2 NE treatment.

Spore deformations with swollen margins and less stained spores were observed due to degradation of chitin present in the cell wall of fungi, whereas the control sample showed normal stained morphology.

The above results indicate the antifungal potential of prepared herbal O-2 Nanoemulsion against *A. alternata*.

6. Conclusion

The most cutting-edge nanoemulsion formulation is used in many different sectors. An effective antibacterial agent against a variety of phytopathogens, O-2 NE nanoemulsion was found in the study to be a viable tool for agriculture. The concentration of the crude oil, the choice of surfactant, and the duration of the sonication process are all factors that can be optimised to create a stable nanoemulsion. 10 percent O-2 nanoemulsion has the potential to be antibacterial and can slow the growth of plant pathogens, according to a concentration-dependent study. High energy sonication was used to create O-2 NE. An effective, stable, and potent formulation, the prepared nanoemulsion comprises 10% O-2 and has droplet sizes of 130.09 nm, PDI 0.200, and zeta potential of -15.2 mV. A. alternata was able to be controlled at 1.5 percent O-2 NE dosage, which is a powerful antifungal drug that is effective against the phytopathogen *A. alternata* and can be utilised as herbal and environmentally friendly formulation. Different doses of nanoemulsion were examined. Nanoemulsion's non-specific antimicrobial action allows for broad-spectrum activity, and 1.5% of it can be used in place of chemical fungicides.

7. Future Perspectives

Nanoemulsion is becoming a revolutionary benchmark for all those high costs and less safe chemical agents that are used in various industrial fields. The scope for this technology is increasing with new researches. In agriculture, it will prove as a green revolution with a low-cost product to control the growth of phytopathogens. Various agrochemical industries synthesized nano pesticides that can encapsulate chemical pesticides and herbicides but nano pesticides that are less harmful to the environment than conventional formulation. However, there is controversy about the fate of nano pesticides and nano fertilizer, including a lack of consensus about a definition of 'nanopesticide' that satisfies international regulations. To overcome with this, nanoemulsion would be used as a delivery system for delivery of a targeted gene to plant cells and itself it acts as an antimicrobial agent for the future. Apart from agriculture, nanoemulsion will establish milestones in pharmaceuticals, cosmetics, biomedical, pollutant degradation etc.

7.1 In antimicrobial agent preparation

Antimicrobial drugs that can be incorporated in nanoemulsions are O/W droplets that range from 200-600 nm. They are made of oil and water and are stabilized by surfactants. The antimicrobial nanoemulsions have a broad spectrum of activity against bacteria like *E. coli*, *Salmonella, S. aureus;* enveloped viruses like HIV, herpes simplex; fungi like Candida, dermatophytes and spores like Anthrax. Nanoemulsions are much safer than other chemical agents because of the low amount of detergent in each droplet.

7.2 In Biomedical

The proficiency of any therapeutic agent is highly dependent on the extent of the drug reaches the systemic circulation by voyage number of barriers, smaller the particle size of the drug maximum the absorption of any therapeutic agent. The attractiveness of nanotechnology is 43 majorly due to the smallest particle size at the nanoscale. The nanoemulsion is the latent outcome of nanotechnology.

7.3 In vaccine delivery

The nanoemulsion can be used as an effective vaccine delivery system to deliver attenuated viruses. This medication delivery system uses nanotechnology to vaccinate against Human Immunodeficiency Virus (HIV). There is recent evidence that HIV can infect the mucosal immune system. Therefore, developing mucosal immunity through the use of nanoemulsions may become very important in the future fight against HIV. The oil-based emulsion can be administered in the nose, unlike traditional vaccine routes.

However recent researches indicate that the genital mucosa immunity may be attained with vaccines that are administered to the nasal mucosa. Nanoemulsions are being used to transport inactivated organisms to a mucosal surface to produce an immune response. The first application as a vaccine, influenza vaccine, and HIV vaccine can proceed to clinical trials.

7.4 In cell culture technology

Cell cultures are used for in-vitro assays or to produce biological compounds like antibodies and other recombinant proteins. For standard cell growth, the culture medium must be supplemented with a large number of molecules. It has been very difficult to provide the media with oil-soluble substances that are easily available to the cells, and only a few amounts of the lipophilic compounds could be absorbed by the cells. Nanoemulsions are a new method for the delivery of oil-soluble substances to human cell cultures. The system is based on a nanoemulsion that is stabilized by phospholipids. This nanoemulsion is transparent and can be passed through 0.1 mm filters for sterilization. Nanoemulsions oil droplets are very easily taken up by the cells. Therefore, nanoemulsions have high bioavailability to cells in culture. It also improves the growth and vitality of cultured cells.

7.5 In Ocular and pulmonary drug delivery

Ocular administration of O/W nanoemulsions has been attempted to deliver water incompatible, environmentally sensitive, poorly absorbed drugs. Special consideration is accorded to transparency, viscosity and refractive index of nanoemulsions for ophthalmic applications. Any nanoemulsion intended for ocular administration should always be titrated against different doses to evaluate its tolerability.

Some drugs like antisense oligonucleotides which have shown great therapeutic potential at in vitro level in a variety of ailments, but are limited by their dispositional susceptibility in vivo, are good candidates for localized delivery via nanoemulsions.

7.6 As a non-toxic disinfectant cleaner

Nanoemulsions have been employed as a disinfectant cleaner. A non-toxic disinfectant cleaner for use in routine markets that include healthcare, travel, food processing and military applications has been developed by Enviro Systems. They have been found to kill tuberculosis and a large spectrum of viruses, bacteria, and fungi within 5 to 10 min without any of the hazards posed by other categories of disinfectants.

7.7 In cosmetics

Nowadays, nanotechnology is also focusing on the colloidal system and nanotized emulsion. These colloidal form have acted as penetration enhancers, and enormous properties for the designing of a novel, low-dose and cost-effective for drug delivery. These products are easily valued in skincare products with their good biophysical and sensorial benefits various studies prove that the nanoemulsion penetrated significantly quicker than other chemical agents. These have given with formulation for reducing aging effect, antimicrobial activity, Sun-blocking agents. Along with these, colloidal forms are also for drug delivery into deer layers of skin; therefore, nanoemulsion can be a powerful tool for a safe and economic alternative of chemical agents that are costly and less safe than it.

8. References

- Abdel-Kader, M., El-Mougy, N. and Lashin, S., 2011. Essential oils and Trichoderma harzianum as an integrated control measure against faba bean root rot pathogens. *Journal of Plant Protection Research*, *51*(3).
- Abdullah, S.A., Salih, T.F.M., Hama, A.A., Ali, S.I. and Hamaamin, H.H., 2021. The Antibacterial Property of Nigella sativa (Black seed) Oil Against Gram-positive and Gram-negative Bacteria. *Kurdistan Journal of Applied Research*, pp.156-165.
- Adnan, M., 2019. Bioactive potential of essential oil extracted from the leaves of Eucalyptus globulus (Myrtaceae). *J. Pharmacogn. Phytochem*, *8*(1), pp.213-216.
- Ahmed, W., Mofed, D., Zekri, A.R., El-Sayed, N., Rahouma, M. and Sabet, S., 2018. Antioxidant activity and apoptotic induction as mechanisms of action of Withania somnifera (Ashwagandha) against a hepatocellular carcinoma cell line. *Journal of International Medical Research*, *46*(4), pp.1358-1369.
- Akbar, S., 2020. Phyllanthus emblica L.(Phyllanthaceae). In *Handbook of 200 Medicinal Plants* (pp. 1391-1408). Springer, Cham.
- Anjali, C. H., Sharma, Y., Mukherjee, A., & Chandrasekaran, N. (2012). Neem oil (*Azadirachta indica*) nanoemulsion—a potent larvicidal agent against *Culex quinquefasciatus*. *Pest Management Science*, *68*(2), 158-163

- Anjali, C. H., Sharma, Y., Mukherjee, A., & Chandrasekaran, N. (2012). Neem oil (*Azadirachta indica*) nanoemulsion—a potent larvicidal agent against *Culex quinquefasciatus*. *Pest Management Science*, *68*(2), 158-163

- Anton, N., Benoit, J. P., & Saulnier, P. (2008). Design and production of nanoparticles formulated from nano-emulsion templates—a review. *Journal of Controlled Release*, *128*(3), 185-199.
- Asadi, M., 2022. Chemical constituents of the essential oil isolated from seed of black pepper, Piper nigrum L.,(Piperaceae). *International Journal of Plant Based Pharmaceuticals*, *2*(1), pp.25-29
- Badr, M.M., Badawy, M.E. and Taktak, N.E., 2021. Characterization, antimicrobial activity, and antioxidant activity of the nanoemulsions of Lavandula spica essential oil and its main monoterpenes. *Journal of Drug Delivery Science and Technology*, *65*, p.102732..
- Baker Jr, J. R., Wright, D. C., Hayes, M. M., Hamouda, T., & Brisker, J. (2000). *U.S. Patent No. 6,015,832*. Washington, DC: U.S. Patent and Trademark Office.
- Bayat, M., Pakina, E., Astarkhanova, T., Sediqi, A.N., Zargar, M. and Vvedenskiy, V., 2019. Review on agro-nanotechnology for ameliorating strawberry cultivation. *Research on Crops*, *20*(4).
- Bhushan, B., 2017. Introduction to nanotechnology. In *Springer handbook of nanotechnology* (pp. 1-19). Springer, Berlin, Heidelberg.
- Boyno, G., Demir, S. and Danesh, Y.R., 2022. Effects of some biological agents on the growth and biochemical parameters of tomato plants infected with Alternaria solani (Ellis & Martin) Sorauer. *European Journal of Plant Pathology*, *162*(1), pp.19-29.
- Camargo, R.B., Peixoto, A.R., Terao, D., Ono, E.O. and Cavalcanti, L.S., 2011. Fungos causadores de podridões pós-colheita em uvas apirênicas no pólo agrícola de Juazeiro-BA e Petrolina-PE. *Revista caatinga*, *24*(1), pp.15-19.

- Chaerani R and Voorrips R E. 2006. Tomato early blight (Alternaria solani): the pathogen, genetics, and breeding for resistance. Journal of Genertic Plant Pathology 72: 335–47.
- Chaiittianan, R., & Sripanidkulchai, B. (2014). Development of a nanoemulsion of *Phyllanthus emblica* L. branch extract. *Drug Development and Industrial Pharmacy*, *40*(12), 1597-1606.
- Chang, Y., & McClements, D. J. (2014). Optimization of orange oil nanoemulsion formation by isothermal low-energy methods: influence of the oil phase, surfactant, and temperature. *Journal of Agricultural and Food Chemistry*, *62*(10), 2306-2312
- Charles P. Poole Jr. and Frank J. Owens. ISBN 0471-07935-9. Copyright 0 2003 John Wiley & Sons, Inc
- Cho, Y., 2015. How the necrotrophic fungus Alternaria brassicicola kills plant cells remains an enigma. *Eukaryotic cell*, *14*(4), pp.335-344.
- Dandona, P., Aljada, A., Mohanty, P., Ghanim, H., Hamouda, W., Assian, E. and Ahmad, S., 2001. Insulin inhibits intranuclear nuclear factor κB and stimulates IκB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect?. *The Journal of Clinical Endocrinology & Metabolism*, *86*(7), pp.3257-3265.
- Dávila-Rodríguez, M., López-Malo, A., Palou, E., Ramírez-Corona, N. and Jiménez-Munguía, M.T., 2019. Antimicrobial activity of nanoemulsions of cinnamon, rosemary, and oregano essential oils on fresh celery. *Lwt*, *112*, p.108247.

- Dávila-Rodríguez, M., López-Malo, A., Palou, E., Ramírez-Corona, N. and Jiménez-Munguía, M.T., 2019. Antimicrobial activity of nanoemulsions of cinnamon, rosemary, and oregano essential oils on fresh celery. *Lwt*, *112*, p.108247.

- Dávila-Rodríguez, M., López-Malo, A., Palou, E., Ramírez-Corona, N. and Jiménez-Munguía, M.T., 2019. Antimicrobial activity of nanoemulsions of cinnamon, rosemary, and oregano essential oils on fresh celery. *Lwt*, *112*, p.108247
- Degani, O. and Kalman, B., 2021. Assessment of commercial fungicides against Onion (Allium cepa) basal rot disease caused by Fusarium oxysporum f. sp. cepae and Fusarium acutatum. *Journal of Fungi*, *7*(3), p.235.
- Delmas, T., Piraux, H., Couffin, A. C., Texier, I., Vinet, F., Poulin, P., ... & Bibette, J. (2011). How to prepare and stabilize very small nanoemulsions. *Langmuir*, *27*(5), 1683-1692
- Elek, N., Hoffman, R., Raviv, U., Resh, R., Ishaaya, I., & Magdassi, S. (2010). Novaluron nanoparticles: Formation and potential use in controlling agricultural insect pests. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *372*(1-3), 66-72
- El-Gazzar, N. and Ismail, A.M., 2020. The potential use of Titanium, Silver and Selenium nanoparticles in controlling leaf blight of tomato caused by *Alternaria alternata*. *Biocatalysis and Agricultural Biotechnology*, *27*, p.101708.
- Forgiarini, A., Esquena, J., Gonzalez, C., & Solans, C. (2001). Formation of nano-emulsions by low-energy emulsification methods at constant temperature. *Langmuir*, *17*(7), 2076-2083
- França, K.R.S., Silva, T.L., Cardoso, T.A.L., Ugulino, A.L.N., Rodrigues, A.P.M. and de Mendonça Júnior, A.F., 2018. In vitro effect of essential oil of peppermint (Mentha x piperita L.) on the mycelial growth of *Alternaria alternata*. Journal of Experimental Agriculture International, 26(5), pp.1-7.

- França, K.R.S., Silva, T.L., Cardoso, T.A.L., Ugulino, A.L.N., Rodrigues, A.P.M. and de Mendonça Júnior, A.F., 2018. In vitro effect of essential oil of peppermint (Mentha x piperita L.) on the mycelial growth of Alternaria alternata. *Journal of Experimental Agriculture International*, *26*(5), pp.1-7.
- Garganese, F., Schena, L., Siciliano, I., Prigigallo, M.I., Spadaro, D., De Grassi, A., Ippolito, A. and Sanzani, S.M., 2016. Characterization of citrus-associated Alternaria species in Mediterranean areas. *PloS one*, *11*(9), p.e0163255.
- Ghareeb, H.S., 2018. Antimicrobial Activity of Artemisia herba-alba Extract against Pathogenic Fungi of Pigeon Droppings. *Journal of Plant Protection and Pathology*, *9*(9), pp.567-571.

- Ghareeb, H.S., 2018. Antimicrobial Activity of Artemisia herba-alba Extract against Pathogenic Fungi of Pigeon Droppings. *Journal of Plant Protection and Pathology*, *9*(9), pp.567-571.

- Ghotbi, R. S., Khatibzadeh, M., & Kordbacheh, S. (2014, August). Preparation of neem seed oil nanoemulsion. In *Proceedings of the 5th International Conference on Nanotechnology: Fundamentals and Applications* (pp. 11-13).
- Gohel, M., Soni, T., Hingorani, L., Patel, A., & Patel, N. (2014). Development and optimization of plant extract loaded nanoemulsion mixtures for the treatment of inflammatory disorder. *Curr Res Drug Discov*, *1*(2), 29-38.
- Gohel, M., Soni, T., Hingorani, L., Patel, A., & Patel, N. (2014). Development and optimization of plant extract loaded nanoemulsion mixtures for the treatment of inflammatory disorder. *Curr Res Drug Discov*, *1*(2), 29-38

- Gupta, A., Eral, H.B., Hatton, T.A. and Doyle, P.S., 2016. Nanoemulsions: formation, properties and applications. *Soft matter*, *12*(11), pp.2826-2841.
- Hamouda, T., Hayes, M. M., Cao, Z., Tonda, R., Johnson, K., Wright, D. C., ... & Baker Jr, J. R. (1999). A novel surfactant nanoemulsion with broad-spectrum sporicidal activity against Bacillus species. *Journal of Infectious Diseases*, *180*(6), 1939-1949
- Han, Y., Zhao, J., Zhang, B., Shen, Q., Shang, Q. and Li, P., 2019. Effect of a novel antifungal peptide P852 on cell morphology and membrane permeability of Fusarium oxysporum. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1861*(2), pp.532-539..
- Hemmila, M.R., Nathens, A.B., Shafi, S., Calland, J.F., Clark, D.E., Cryer, H.G., Goble, S., Hoeft, C.J., Meredith, J.W., Neal, M.L. and Pasquale, M.D., 2010. The Trauma Quality Improvement Program: pilot study and initial demonstration of feasibility. *Journal of Trauma and Acute Care Surgery*, *68*(2), pp.253-262.
- Hu, F., Tu, X.F., Thakur, K., Hu, F., Li, X.L., Zhang, Y.S., Zhang, J.G. and Wei, Z.J., 2019. Comparison of antifungal activity of essential oils from different plants against three fungi. *Food and Chemical Toxicology*, *134*, p.110821.
- Jesus, F. L., de Almeida, F. B., Duarte, J. L., Oliveira, A. E., Cruz, R. A., Souto, R. N., ... & Guedes, M. (2017). Preparation of a nanoemulsion with *Carapa guianensis aublet* (Meliaceae) oil by a low-energy/solvent-free method and evaluation of its preliminary residual larvicidal activity. *Evidence-Based Complementary and Alternative Medicine*, *2017*
- Jiang, K., Lepak, D.P., Hu, J. and Baer, J.C., 2012. How does human resource management influence organizational outcomes? A meta-analytic investigation of mediating mechanisms. *Academy of management Journal*, *55*(6), pp.1264-1294.

- Joshi, S., 2020. Importance of Agriculture Financing with Special Reference to India. *International Journal of Modern Agriculture*, *9*(3), pp.319-323.
- Judy, J. D., Unrine, J. M., & Bertsch, P. M. (2010). Evidence for biomagnification of gold nanoparticles within a terrestrial food chain. *Environmental Science & Technology*, *45*(2), 776-781.
- Khoshru, B., Mitra, D., Khoshmanzar, E., Myo, E.M., Uniyal, N., Mahakur, B., Mohapatra, P.K.D., Panneerselvam, P., Boutaj, H., Alizadeh, M. and Cely, M.V.T., 2020. Current scenario and future prospects of plant growth-promoting rhizobacteria: An economic valuable resource for the agriculture revival under stressful conditions. *Journal of Plant Nutrition*, *43*(20), pp.3062-3092.
- Khurana, A. and Kumar, V., 2020. State of Organic and Natural Farming: Challenges and Possibilities. *New Delhi: Centre for Sc*
- Komaiko, J., & McClements, D. J. (2015). Low-energy formation of edible nanoemulsions by spontaneous emulsification: Factors influencing particle size. *Journal of Food Engineering*, *146*, 122-128
- Kumar, C., Akhter, S., Satti, N.K., Gupta, V.K., Meena, S.R., Vishwakarma, R., Hassan, Q.P. and Verma, M.K., 2022. Dereplication approach for the first time isolation of tatarinowin a and pentadecanoic acid from Acorus calamus L. by using GC-MS. *Natural Product Research*, pp.1-6..
- Kumar, N., Verma, A. and Mandal, A., 2021. Formation, characteristics and oil industry applications of nanoemulsions: a review. *Journal of Petroleum Science and Engineering*, *206*, p.109042.

- Kumar, N., Verma, A. and Mandal, A., 2021. Formation, characteristics and oil industry applications of nanoemulsions: a review. *Journal of Petroleum Science and Engineering*, *206*, p.109042.
- Kumar, R. S., Shiny, P. J., Anjali, C. H., Jerobin, J., Goshen, K. M., Magdassi, S., ... & Chandrasekaran, N. (2013). Distinctive effects of nano-sized permethrin in the environment. *Environmental Science and Pollution Research*, *20*(4), 2593-2602.
- Kumar, S.S. and Raghavendra, B.K., 2019, March. Diseases detection of various plant leaf using image processing techniques: a review. In *2019 5th International Conference on Advanced Computing & Communication Systems (ICACCS)* (pp. 313-316). IEEE.
- Kumari, S., Kumaraswamy, R. V., Choudhary, R. C., Sharma, S. S., Pal, A., Raliya, R., ... & Saharan, V. (2018). Thymol nanoemulsion exhibits potential antibacterial activity against bacterial pustule disease and growth promotory effect on soybean. *Scientific reports*, *8*.
- Lee, H.B., Patriarca, A. and Magan, N., 2015. Alternaria in food: ecophysiology, mycotoxin production and toxicology. *Mycobiology*, *43*(2), pp.93-106.
- Leong, T.S.H., Wooster, T.J., Kentish, S.E. and Ashokkumar, M., 2009. Minimising oil droplet size using ultrasonic emulsification. *Ultrasonics sonochemistry*, *16*(6), pp.721-727.
- Liang, R., Xu, S., Shoemaker, C. F., Li, Y., Zhong, F., & Huang, Q. (2012). Physical and antimicrobial properties of peppermint oil nanoemulsions. *Journal of agricultural and food chemistry*, *60*(30), 7548-7555

- Lu, W.C., Huang, D.W., Wang, C.C., Yeh, C.H., Tsai, J.C., Huang, Y.T. and Li, P.H., 2018. Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. *Journal of food and drug analysis*, *26*(1), pp.82-89.
- Luttrell, R.G., 2007. 6 Ecological Effects of Chemical Control Practices: The Environmental Perspective. In *Ecologically Based Integrated Pest Management* (p. 111). CAB International Wallingford
- Madhubala, M. and Santhi, G., 2019. Phytochemical and GC-MS analysis on leaves of selected medicinal plants in Boraginaceae family Cordia dichotoma L. *Pramana Res. J*, *9*, pp.2249-2276
- Maggio, F., Rossi, C., Chaves-López, C., Valbonetti, L., Desideri, G., Paparella, A. and Serio, A., 2022. A single exposure to a sublethal concentration of Origanum vulgare essential oil initiates response against food stressors and restoration of antibiotic susceptibility in Listeria monocytogenes. *Food Control*, *132*, p.108562.

- Maggio, F., Rossi, C., Chaves-López, C., Valbonetti, L., Desideri, G., Paparella, A. and Serio, A., 2022. A single exposure to a sublethal concentration of Origanum vulgare essential oil initiates response against food stressors and restoration of antibiotic susceptibility in Listeria monocytogenes. *Food Control*, *132*, p.108562.

- Maity, S.K., Patra, A., Pal, B. and Malakar, A., 2018. Palyno-taxonomic study of some members of Malvaceae in Purba Medinipur, West Bengal, India. *Annals of Plant Sciences*, *7*(2), pp.2069-2072
- Majeed, A., Abbasi, M.K., Hameed, S., Imran, A. and Rahim, N., 2015. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Frontiers in microbiology*, *6*, p.198.

- Mandlik, D.S. and Namdeo, A.G., 2021. Pharmacological evaluation of Ashwagandha highlighting its healthcare claims, safety, and toxicity aspects. *Journal of Dietary Supplements*, *18*(2), pp.183-226.
- McClements, D. J., & Rao, J. (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Critical Reviews in Food Science and Nutrition*, *51*(4), 285-330.
- Mikosi, K., 2019. *Exploring the contribution of alternative food systems towards food security: a case study of the siyazenzela food garden project* (Master's thesis, Faculty of Science).*ience and Environment. https://www. cseindia. org/state-of-organic-and-natural-farming-in-india-10346.*
- Moghimi, R., Ghaderi, L., Rafati, H., Aliahmadi, A., & McClements, D. J. (2016). Superior antibacterial activity of nanoemulsion of *Thymus daenensis* essential oil against *E. coli*. *Food Chemistry*, *194*, 410-415
- Mohammed, M.A.Y.A., 2021. *Antibacterial Activity of Raw Castor (Ricinus communis) Oil Against Escherichia coli and Staphylococcus aureus* (Doctoral dissertation, University of Gezira).
- Moosavi-Nasab, M., Jamalian, J., Heshmati, H. and Haghighi-Manesh, S., 2018. The inhibitory potential of Zataria multiflora and Syzygium aromaticum essential oil on growth and aflatoxin production by Aspergillus flavus in culture media and Iranian white cheese. *Food Science & Nutrition*, *6*(2), pp.318-324..
- Mossa, A. T. H., Abdelfattah, N. A. H., & Mohafrash, S. M. M. (2017). Nanoemulsion of camphor (*Eucalyptus globulus*) essential oil, formulation, characterization and insecticidal

activity against wheat weevil, *Sitophilus granarius*. *Asian Journal of Crop Science*, *9*(3), 50-62

- Moustafa-Farag, M., Almoneafy, A., Mahmoud, A., Elkelish, A., Arnao, M.B., Li, L. and Ai, S., 2019. Melatonin and its protective role against biotic stress impacts on plants. Biomolecules, 10(1), p.54.
- Munawiroh, S. Z., Nabila, A. N., & Chabib, L. (2017). Development of Water in Olive Oil (W/O) Nanoemulsions as Lipstick Base Formulation. *Int J Pharma Med Biol Sci [Internet]*.
- Mustefa, A., Belayhun, T., Melak, A., Hayelom, M., Tadesse, D., Hailu, A. and Assefa, A., 2021. Phenotypic characterization of Raya cattle in northern Ethiopia. *Tropical Animal Health and Production*, *53*(1), pp.1-11.
- Oliveira, A. E., Duarte, J. L., Cruz, R. A., Souto, R. N., Ferreira, R. M., Peniche, T., ... & Carvalho, J. C. (2017). Pterodon emarginatus oleoresin-based nanoemulsion as a promising tool for *Culex quinquefasciatus* (*Diptera: Culicidae*) control. *Journal of Nanobiotechnology*, *15*(1), 2.
- Pandey, G., 2018. Challenges and future prospects of agri-nanotechnology for sustainable agriculture in India. *Environmental Technology & Innovation*, *11*, pp.299-307
- Paparu, P., Acur, A., Kato, F., Acam, C., Nakibuule, J., Nkuboye, A., Musoke, S. and Mukankusi, C., 2020. Morphological and pathogenic characterization of Sclerotium rolfsii, the causal agent of southern blight disease on common bean in Uganda. *Plant Disease*, *104*(8), pp.2130-2137

- Pascual-Pineda, L. A., Flores-Andrade, E., Jiménez-Fernández, M., & Beristain, C. I. (2015). Kinetic and thermodynamic stability of paprika nanoemulsions. *International Journal of Food Science & Technology*, *50*(5), 1174-1181
- Pascual-Villalobos, M. J., Cantó-Tejero, M., Vallejo, R., Guirao, P., Rodríguez-Rojo, S., & Cocero, M. J. (2017). Use of nanoemulsions of plant essential oils as aphid repellents. *Industrial Crops and Products*, *110*, 45-57.
- Piazzini, V., Monteforte, E., Luceri, C., Bigagli, E., Bilia, A. R., & Bergonzi, M. C. (2017). Nanoemulsion for improving solubility and permeability of *Vitex agnus-castus* extract: formulation and in vitro evaluation using PAMPA and Caco-2 approaches. *Drug Delivery*, *24*(1), 380-390.
- Piola, L., Fuchs, J., Oneto, M.L., Basack, S., Kesten, E. and Casabé, N., 2013. Comparative toxicity of two glyphosate-based formulations to Eisenia andrei under laboratory conditions. *Chemosphere*, *91*(4), pp.545-551.
- Premanathan, M., Karthikeyan, K., Jeyasubramanian, K. and Manivannan, G., 2011. Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. *Nanomedicine: Nanotechnology, Biology and Medicine*, *7*(2), pp.184-192.
- Quatrin, P.M., Verdi, C.M., de Souza, M.E., de Godoi, S.N., Klein, B., Gundel, A., Wagner, R., de Almeida Vaucher, R., Ourique, A.F. and Santos, R.C.V., 2017. Antimicrobial and antibiofilm activities of nanoemulsions containing Eucalyptus globulus oil against Pseudomonas aeruginosa and Candida spp. *Microbial pathogenesis*, *112*, pp.230-242.

- Quatrin, P.M., Verdi, C.M., de Souza, M.E., de Godoi, S.N., Klein, B., Gundel, A., Wagner, R., de Almeida Vaucher, R., Ourique, A.F. and Santos, R.C.V., 2017. Antimicrobial and antibiofilm activities of nanoemulsions containing Eucalyptus globulus oil against Pseudomonas aeruginosa and Candida spp. *Microbial pathogenesis*, *112*, pp.230-242.
- Rangel, L.I., Spanner, R.E., Ebert, M.K., Pethybridge, S.J., Stukenbrock, E.H., de Jonge, R., Secor, G.A. and Bolton, M.D., 2020. Cercospora beticola: The intoxicating lifestyle of the leaf spot pathogen of sugar beet. *Molecular Plant Pathology*, *21*(8), pp.1020-1041
- Rege, N.N., Thatte, U.M. and Dahanukar, S.A., 1999. Adaptogenic properties of six rasayana herbs used in Ayurvedic medicine. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *13*(4), pp.275-2
- Rouhani, S., Ghazanfari, M. and Jafari, M., 2012. Evaluation model of business intelligence for enterprise systems using fuzzy TOPSIS. *Expert Systems with Applications*, *39*(3), pp.3764-3771.
- Rychlik, M., Zappa, G., Añorga, L., Belc, N., Castanheira, I., Donard, O.F., Kouřimská, L., Ogrinc, N., Ocké, M.C., Presser, K. and Zoani, C., 2018. Ensuring food integrity by metrology and FAIR data principles. *Frontiers in chemistry*, *6*, p.49.
- Saleem, S., Muhammad, G., Hussain, M.A., Altaf, M. and Bukhari, S.N.A., 2020. Withania somnifera L.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective. *Iranian Journal of Basic Medical Sciences*, *23*(12), p.1501
- Sh, A., Abdelrazeik, A. B., & Rakha, O. M. (2015). Nanoemulsion of jojoba oil, preparation, characterization and insecticidal activity against *sitophilus oryzae* (coleoptera:

Curculionidae) on wheat. *International Journal of Agriculture Innovations and Research*, *4*(1), 72-75.

- Shahavi, M.H., Hosseini, M., Jahanshahi, M. and Darzi, G.N., 2015. Optimization of encapsulated clove oil particle size with biodegradable shell using design expert methodology. *Pakistan Journal of Biotechnology*, *12*(2), pp.149-160.
- Shakeel, F., Baboota, S., Ahuja, A., Ali, J., Aqil, M., & Shafiq, S. (2007). Nanoemulsions as vehicles for transdermal delivery of aceclofenac. *Aaps Pharmscitech*, *8*(4), 191.
- Shin, K., Choi, H., Song, S. K., Yu, J. W., Lee, J. Y., Choi, E. J., ... & Kim, J. W. (2018). Nanoemulsion vehicles as carriers for follicular delivery of l*uteolin*. *ACS Biomaterials Science & Engineering*, *4*(5), 1723-1729.
- Sindhu, K.M., Saravanan, K.S. and Mohanakumar, K.P., 2005. Behavioral differences in a rotenone-induced hemiparkinsonian rat model developed following intranigral or median forebrain bundle infusion. *Brain research*, *1051*(1-2), pp.25-34.
- Singh, B., Kaur, N., Kumar, P., Hallan, V. and Pati, P.K., 2020. Reactive oxygen species generating and scavenging systems play critical role in conferring leaf spot disease resistance in Withania somnifera (L.) Dunal. *Industrial Crops and Products*, *157*, p.112889.
- Singh, L., Kumar, A., Choudhary, A. and Singh, G., 2018. Asparagus racemosus: The plant with immense medicinal potential. *Journal of Pharmacognosy and Phytochemistry*, *7*(3), pp.2199-2203.
- Singh, L., Kumar, A., Choudhary, A. and Singh, G., 2018. Asparagus racemosus: The plant with immense medicinal potential. *Journal of Pharmacognosy and Phytochemistry*, *7*(3), pp.2199-2203.

- Singh, V., 2017. Biotechnological Interventions Against Leaf Spot Disease of *Withania somnifera* (L.) Dunal. PhD Thesis. Guru Nanak Dev University, Amritar, Punjab, India [Dataset].
- Sousa, R.M.S.D., Serra, I.M.R.D.S. and Melo, T.A.D., 2012. Effect of essential oils as an alternative in the control of Colletotrichum gloesporioides in pepper. *Summa Phytopathologica*, *38*, pp.42-47.
- Sugumar, S., Clarke, S. K., Nirmala, M. J., Tyagi, B. K., Mukherjee, A., & Chandrasekaran, N. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against *Culex quinquefasciatus*. *Bulletin of entomological research*, *104*(3), 393-402.
- Sugumar, S., Clarke, S. K., Nirmala, M. J., Tyagi, B. K., Mukherjee, A., & Chandrasekaran, N. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against *Culex quinquefasciatus*. *Bulletin of Entomological Research*, *104*(3), 393-402.
- Tadros, T., Izquierdo, P., Esquena, J. and Solans, C., 2004. Formation and stability of nano-emulsions. *Advances in colloid and interface science*, *108*, pp.303-318.
- Tian, W. L., Lei, L. L., Zhang, Q., & Li, Y. (2016). Physical stability and antimicrobial activity of encapsulated cinnamaldehyde by self-emulsifying nanoemulsion. *Journal of Food Process Engineering*, *39*(5), 462-471.
- Tolba, H., Moghrani, H., Aboun, A. and Maachi, R., 2018. Essential oil of Algerian Eucalyptus citriodora: Chemical composition, antioxidant and antimicrobial activities. *Nature & Technology*,*(18)*, pp.19-27.
- Topuz, O. K., Özvural, E. B., Zhao, Q., Huang, Q., Chikindas, M., & Gölükçü, M. (2016). Physical and antimicrobial properties of anise oil loaded nanoemulsions on the survival of foodborne pathogens. *Food chemistry*, *203*, 117-123.

- Ugulino, A.L.N., Mendonça Júnior, A.F., Rodrigues, A.P.M.S., Santos, A.B., França, K.S., Cardoso, T.A.L. and Prado Júnior, L.D., 2018. Inhibition effect of vegetable oils on the mycelial growth of Macrophomina phaseolina (Tassi.). Goid. *Journal of Agricultural Science*, *10*(6), pp.49-56.
- Yadav, N., Ganie, S.A., Singh, B., Chhillar, A.K. and Yadav, S.S., 2019. Phytochemical constituents and ethnopharmacological properties of Ageratum conyzoides L. *Phytotherapy Research*, *33*(9), pp.2163-2178..
- Yadav, N., Ganie, S.A., Singh, B., Chhillar, A.K. and Yadav, S.S., 2019. Phytochemical constituents and ethnopharmacological properties of Ageratum conyzoides L. *Phytotherapy Research*, *33*(9), pp.2163-2178..
- Zhang, H., Zhu, J., Gong, Z. and Zhu, J.K., 2021. Abiotic stress responses in plants. Nature Reviews Genetics, pp.1-16.
- Zhao, Y., Yang, N., Deng, Y., Tao, K., Jin, H. and Hou, T., 2020. Mechanism of action of novel pyrazole carboxamide containing a diarylamine scaffold against Rhizoctonia solani. *Journal of Agricultural and Food Chemistry*, *68*(40), pp.11068-11076.