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Antifungal Activity of Clove Oil against *Colletotrichum orbiculare* by Poisoned Food Technique

Nalini T J.,[1a] Manjula A C.,[1b] Prathibha K. Y.,[1c] Sree padma K. V.,[2] Harshita B. R.,[2] Marhoob Banu.,[2]*

[1a] Assistant Professor, [1c] Professor, [2] Research Scholars, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India, 560001

[1b] Professor, Department of Sericulture, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India, 560001

***Corresponding author: Marhoob Banu**

Research Scholar, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, 560001

Email: marhoobbanu@gmail.com

Abstract:

Colletotrichum orbiculare is a phytopathogenic fungus that causes devastating diseases in various crops, leading to significant agricultural losses worldwide. The increasing concerns about the environmental and health risks associated with synthetic fungicides have sparked interest in exploring natural alternatives. Clove oil, derived from the aromatic spice *Syzygium aromaticum*, has shown promising antifungal properties in previous studies. In this research, we investigated the potential antifungal activity of clove oil against *Colletotrichum orbiculare* using the Poisoned Food Technique. The research involved preparing different concentrations of clove oil and incorporating them into potato dextrose agar media to evaluate their impact on *Colletotrichum orbiculare* growth. The experiment was conducted in a controlled environment, and the results were recorded by measuring the radial growth of the fungus. Statistical analyses were performed to assess the significance of the inhibitory effects of clove oil. Our findings revealed that clove oil exhibited significant antifungal activity against *Colletotrichum orbiculare*. The most potent inhibitory effect was observed at the highest concentration of clove oil tested viz., 40%, 60%, 80% and 100%. These results suggest that clove oil has the potential to be used as a natural and eco-friendly alternative to synthetic fungicides for controlling *Colletotrichum orbiculare* infections in crops.

Keywords: Antifungal activity, Clove oil, *Colletotrichum orbiculare*, Poisoned Food Technique, Natural fungicide, *Syzygium aromaticum*, phytopathogenic fungus, agricultural crop diseases.

Introduction

Colletotrichum orbiculare is a genus of fungal pathogens that significantly impacts various plant species, causing devastating diseases known as anthracnose. These fungi are classified as ascomycetes and are particularly notorious for their ability to infect a wide range of hosts,

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including fruits, vegetables, ornamental plants, and crops. The infection process typically begins when *Colletotrichum orbiculare* spores come into contact with susceptible plant tissues, often through wounds or natural openings. Upon entry, the fungi establish themselves and start proliferating, leading to the characteristic symptoms of anthracnose, such as dark, sunken lesions on leaves, stems, or fruits. *Colletotrichum orbiculare* species employ a diverse array of enzymes and toxins to facilitate their infection and colonization. These factors weaken the host's defense mechanisms, allowing the pathogen to thrive and spread rapidly. Additionally, environmental factors like temperature and humidity play a crucial role in disease development. Anthracnose outbreaks can result in significant economic losses in agriculture, affecting crop yield and quality. Management strategies involve cultural practices, chemical control, and the use of resistant cultivars to mitigate the impact of these fungal pathogens on plants. Researchers continue to study *Colletotrichum orbiculare* to better understand its biology, genetics, and interactions with host plants, aiming to develop sustainable and effective methods for disease prevention and control in the future.[1,2,3,4,5]

Fungi can develop resistance to conventional fungicides due to their high reproductive rate and genetic variability. Frequent and indiscriminate use of fungicides selects for resistant strains, leading to reduced effectiveness of these chemicals over time. Resistant fungi carry specific genetic mutations that prevent the fungicide from targeting their vital cellular processes, allowing them to survive and multiply. To combat resistance, integrated pest management strategies, rotation of different fungicides, and proper application techniques are essential. Additional research of biocontrols and natural products and its modes of action is crucial to stay ahead of evolving fungal populations and maintain effective disease control in agriculture and other fields.[6,7]

Clove oil is a potent natural antifungal agent known for its ability to combat fungal infections. It contains compounds like eugenol, which exhibit strong antifungal properties. When applied topically or used in aromatherapy, clove oil can inhibit the growth and spread of various fungal species, including *Candida* and *Aspergillus*. Its effectiveness makes it a popular choice for treating conditions like athlete's foot, ringworm, and nail fungus. Clove oil exhibits antifungal properties through several mechanisms. Its main active compound, eugenol, disrupts fungal cell membranes, leading to leakage of essential molecules and cell death. Additionally, eugenol inhibits fungal enzymes and interferes with their metabolic processes. The oil also disrupts biofilm formation, preventing fungi from establishing resilient communities. Furthermore, clove oil generates reactive oxygen species, causing oxidative stress in fungal cells. These combined actions make clove oil an effective natural antifungal agent, providing an alternative approach to combat fungal infections and growth in various applications.[8,9,10]

Materials and Methods

Fungal strain:

Colletotrichum orbiculare was isolated from ridge gourd seeds by agar plate method

Clove oil:

Clove oil was collected from local market, Bengaluru and different concentrations of clove oil was prepared by dissolving in acetone

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Poisoned Food Technique:

Potato dextrose agar was prepared, autoclaved and amoxicillin was added to avoid bacterial contamination. Different concentrations of clove oil viz., 20%, 40%, 60%, 80% and 100% was incorporated in PDA at 1000 ppm. After preparing agar plates, *Colletotrichum orbiculare* was inoculated, sealed and incubated at 27°C for seven days.

Results

Table 1: Antifungal activity of clove oil against *Colletotrichum*

Concentration (%)	**Mean colony diameter (mm)**	**Zone of inhibition (mm)**
Control	30 mm	60 mm
20%	26 mm	64 mm
40%	0 mm	90 mm
60%	0 mm	90 mm
80%	0 mm	90 mm
100%	0 mm	90 mm

The colony diameter of 30 mm was seen in control plates followed by 26 mm colony in plates treated with 20% clove oil. Complete inhibition of *Colletotrichum orbiculare* was seen at all other concentrations of clove oil viz., 40%, 60%, 80% and 100%.

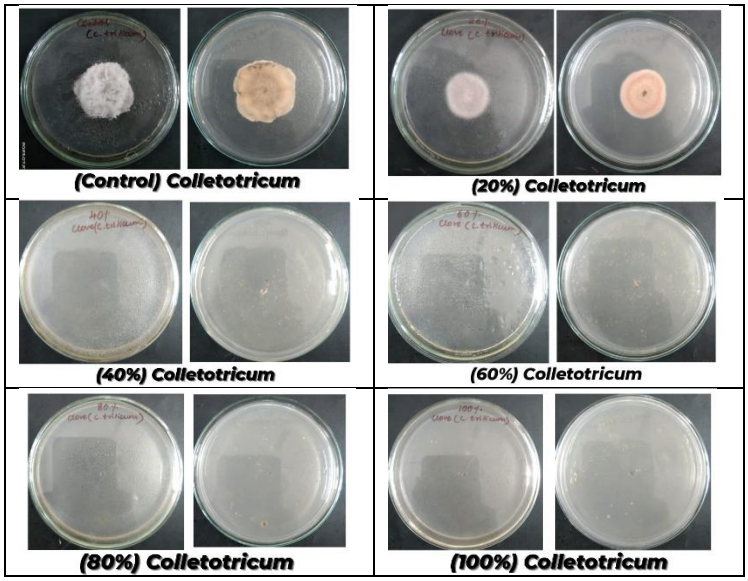

Fig 1: Antifungal activity of Clove oil against *Colletotrichum*

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Discussion

The research paper investigates the antifungal activity of clove oil against *Colletotrichum*, using the poisoned food technique. *Colletotrichum orbiculare* is a common plant pathogen causing various diseases in crops. The study aims to explore the potential of clove oil as a natural alternative to synthetic fungicides in managing this pathogen. The poisoned food technique involves preparing agar media infused with different concentrations of clove oil and inoculating it with *Colletotrichum orbiculare* spores. The growth inhibition and effectiveness of clove oil in controlling the fungal growth are then evaluated and compared to standard fungicides. The findings suggest that clove oil exhibits significant antifungal properties against *Colletotrichum*. Its inhibitory effect on fungal growth is dose-dependent, indicating that higher concentrations yield better results. The research also sheds light on the potential mechanism of action of clove oil against the pathogen.

Chee HY et al., had studied the antifungal activity of clove oil against dermatophytic fungi. Results revealed that clove oil exhibited strong antifungal effect against those fungi.[9] Similarly, in the present study strong antifungal activity of clove oil against *Colletotrichum orbiculare* was observed. From the study Rana IS et al., strong antifungal activity of clove oil against *Fusarium moniliforme NCIM 1100, Fusarium oxysporum MTCC 284, Aspergillus sp., Mucor sp., Trichophyton rubrum* and *Microsporum gypseum* was revealed.[10] Similarly in the present study clove oil exhibited strong antifungal activity against *Colletotrichum*. From the study of Wang D et al., strong antifungal activity of clove oil against *Colletotrichum orbiculare* was revealed.[11] Similarly, in the present study strong antifungal activity of clove oil against *Colletotrichum orbiculare* was observed.

Conclusion

In conclusion, the study highlights the promising antifungal activity of clove oil, making it a potential eco-friendly and cost-effective alternative to synthetic fungicides in agricultural practices. Further research is warranted to understand the precise mode of action and assess its practical application in crop protection strategies.

References

1. Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD. The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol. 2012 May;13(4):414-30. Doi: 10.1111/j.1364-3703.2011.00783.x. Erratum in: Mol Plant Pathol. 2012 Sep;13(7):804. PMID: 22471698; PMCID: PMC6638784.
2. Jain A, Sarsaiya S, Wu Q, Lu Y, Shi J. A review of plant leaf fungal diseases and its environment speciation. Bioengineered. 2019 Dec;10(1):409-424. Doi: 10.1080/21655979.2019.1649520. PMID: 31502497; PMCID: PMC6779379.
3. Weir BS, Johnston PR, Damm U. The Colletotrichum gloeosporioides species complex. Stud Mycol. 2012 Sep 15;73(1):115-80. Doi: 10.3114/sim0011. Epub 2012 Aug 27. PMID: 23136459; PMCID: PMC3458417.
4. Talhinhas P, Sreenivasaprasad S, Neves-Martins J, Oliveira H. Genetic and Morphological Characterization of Colletotrichum acutatum Causing Anthracnose of

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, Nov 2022

Lupins. Phytopathology. 2002 Sep;92(9):986-96. Doi: 10.1094/PHYTO.2002.92.9.986. PMID: 18944024.

5. Gan P, Ikeda K, Irieda H, Narusaka M, O'Connell RJ, Narusaka Y, Takano Y, Kubo Y, Shirasu K. Comparative genomic and transcriptomic analyses reveal the hemibiotrophic stage shift of Colletotrichum fungi. New Phytol. 2013 Mar;197(4):1236-1249. Doi: 10.1111/nph.12085. Epub 2012 Dec 17. PMID: 23252678.
6. Yin Y, Miao J, Shao W, Liu X, Zhao Y, Ma Z. Fungicide Resistance: Progress in Understanding Mechanism, Monitoring, and Management. Phytopathology. 2023 Apr;113(4):707-718. Doi: 10.1094/PHYTO-10-22-0370-KD. Epub 2023 May 4. PMID: 36624725.
7. Lucas JA, Hawkins NJ, Fraaije BA. The evolution of fungicide resistance. Adv Appl Microbiol. 2015;90:29-92. Doi: 10.1016/bs.aambs.2014.09.001. Epub 2014 Nov 12. PMID: 25596029.
8. Haro-González JN, Castillo-Herrera GA, Martínez-Velázquez M, Espinosa-Andrews H. Clove Essential Oil (Syzygium aromaticum L. Myrtaceae): Extraction, Chemical Composition, Food Applications, and Essential Bioactivity for Human Health. Molecules. 2021 Oct 22;26(21):6387. Doi: 10.3390/molecules26216387. PMID: 34770801; PMCID: PMC8588428.
9. Chee HY, Lee MH. Antifungal activity of clove essential oil and its volatile vapour against dermatophytic fungi. Mycobiology. 2007 Dec;35(4):241-3. Doi: 10.4489/MYCO.2007.35.4.241. Epub 2007 Dec 31. PMID: 24015106; PMCID: PMC3763181.
10. Rana IS, Rana AS, Rajak RC. Evaluation of antifungal activity in essential oil of the Syzygium aromaticum (L.) by extraction, purification and analysis of its main component eugenol. Braz J Microbiol. 2011 Oct;42(4):1269-77. Doi: 10.1590/S1517-83822011000400004. Epub 2011 Dec 1. PMID: 24031751; PMCID: PMC3768706.
11. Wang D, Zhang J, Jia X, Xin L, Zhai H. Antifungal Effects and Potential Mechanism of Essential Oils on Collelotrichum gloeosporioides In Vitro and In Vivo. Molecules. 2019 Sep 18;24(18):3386. Doi: 10.3390/molecules24183386. PMID: 31540346; PMCID: PMC6766912.