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Antifungal Activity of Clove (*Syzygium aromaticum*) Essential Oil against *Aspergillus parasitus* isolated from Okra (*Abelmoschus esculentus*)

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Abstract

Aspergillus parasitus is a ubiquitous and opportunistic fungal pathogen that poses a significant threat to both human health and various agricultural products and has shown resistance to conventional antifungal agents. The increasing incidence of fungal infections and the emergence of drug-resistant strains have prompted the search for novel and effective antifungal agents. The present study aims to investigates the antifungal activity of clove essential oil against *Aspergillus parasitus*, aiming to explore its potential as an alternative or adjunct therapeutic agent. The antifungal activity of the essential oil was evaluated using Poisoned Food Technique. *Aspergillus parasitus* was isolated from okra seeds. The results demonstrated significant antifungal activity of different concentrations of clove essential oil viz., 20%, 40%, 60%, 80% and 100% at 1000 ppm against *Aspergillus parasitus*. The findings of this study demonstrate the promising antifungal activity of clove essential oil against *Aspergillus parasitus.* The presence of eugenol and β-caryophyllene may contribute significantly to the observed efficacy. Clove essential oil could serve as a natural and accessible therapeutic option for the treatment of *Aspergillus parasitus* infections.

Keywords: Antifungal activity, *Aspergillus parasitus*, Clove essential oil, Eugenol, β-caryophyllene, Fungal infections, *Syzygium aromaticum*, Natural antifungal agent

Introduction

Aspergillus parasitus is a fungal pathogen that poses a significant threat to various plant species. As a member of the *Aspergillus* genus, this pathogen can infect a wide range of plants, including crops, ornamental plants, and trees, causing devastating plant diseases. Its prevalence is particularly concerning in warm and humid climates, where conditions favour its growth and spread. Once the fungus gains entry into the host plant, it proliferates rapidly, colonizing and damaging plant tissues. The most common symptoms of *Aspergillus parasitus* infection include wilting, yellowing of leaves, stunted growth, and the appearance of brownish lesions. In severe cases, the entire plant may succumb to the infection, leading to significant economic losses for farmers and horticulturists. The spread of *Aspergillus parasitus* is facilitated by airborne spores,

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contaminated soil, and plant debris. Preventive measures such as maintaining proper plant hygiene, avoiding overcrowding, and practicing crop rotation can help reduce its impact. Fungicides can also be used for control, although their effectiveness may vary, and resistance can develop over time.[1,2,3,4,5] Fungal infections caused by *Aspergillus* species are a significant public health concern worldwide. *Aspergillus parasitus*, a member of the *Aspergillus* genus, is known to cause respiratory and cutaneous infections, particularly in immunocompromised individuals. Conventional antifungal agents, such as azoles and polyenes, have been the primary treatment option. However, the emergence of drug-resistant strains has limited their effectiveness. Hence, exploring natural alternatives with potent antifungal properties is crucial to overcoming these challenges.[7,8]

Clove oil, derived from the dried flower buds of the clove tree (*Syzygium aromaticum*), has long been recognized for its potent medicinal properties. One of its notable attributes is its significant antifungal activity, making it an effective natural remedy for combating fungal infections. The antifungal properties of clove oil can be attributed to its active components, particularly eugenol, which constitutes a substantial portion of the oil. Eugenol exhibits strong antimicrobial effects, including antifungal activity against various fungal species. Studies have shown that clove oil demonstrates inhibitory effects against a wide range of fungi, including *Candida albicans, Aspergillus* species, and dermatophytes that cause skin infections. The mode of action of clove oil's antifungal activity involves damaging the fungal cell membrane, disrupting cellular functions, and inhibiting the growth and reproduction of the fungi. This mechanism sets it apart from conventional antifungal drugs and contributes to its effectiveness against drug-resistant strains. Due to its natural origin, clove oil is generally considered safe and well-tolerated, although it should be used with caution, especially in concentrated forms, as it can cause skin irritation. As with any alternative treatment, it is advisable to consult with a healthcare professional before using clove oil for antifungal purposes. The present research aims to evaluate the antifungal activity of clove essential oil against *Aspergillus parasitus* and elucidate the underlying mechanism of action.[9,10,11,12,13]

Materials and Methods

- Sample collection

Seeds sample:

Untreated seeds of okra were collected from IIHR, Bengaluru

Fungal Strain:

Aspergillus parasitus was isolated from okra seeds by agar plate method

Clove essential oil:

Clove essential oil was obtained from the local markets, Bengaluru. The essential oil was diluted with acetone to obtain various concentrations for antifungal testing viz., 20%, 40%, 60%, 80% and 100%.

- Preparation of Potato Dextrose Agar media

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Potato Dextrose Agar media was prepared by adding 39.5 grams of readymade media in distilled water and was autoclaved at 121 °C (250 °F) for around 30-60 minutes at a pressure of 15 pounds per square inch. Amoxicillin was added to avoid bacterial contamination.

- Antifungal Susceptibility Testing:

The antifungal activity of clove essential oil was evaluated using poisoned food technique. Different concentrations of clove oil was incorporated in potato dextrose agar media at 1000 ppm with the help of micropipette and was mixed thoroughly. After mixing, media was poured in the sterilised petriplates and agar plates treated with clove oil were prepared. Briefly, *Aspergillus parasitus* spores were suspended in sterile distilled water, and the inoculum density was adjusted to 106 colony-forming units per milli litre (CFU/mL). The spore suspension was used to inoculate the plates. The plates were sealed and incubated at 27°C for seven days. After seven days, the plates were observed for the antifungal activity and recorded.

Results

Table 1: Inhibitory activity of clove oil against *Aspergillus parasitus*

Concentration	Control	20%	40%	60%	80%	100%
Mean colony diameter (mm)	Complete growth	10 mm	15 mm	Complete inhibition	Complete inhibition	Complete inhibition
Zone of inhibition (mm)	0	80 mm	75 mm	90 mm	90 mm	90 mm

Clove essential oil at 60%, 80% and 100% completely inhibited the growth of *Aspergillus parasitus* while clove oil at 20% and 40% showed zone of inhibition.

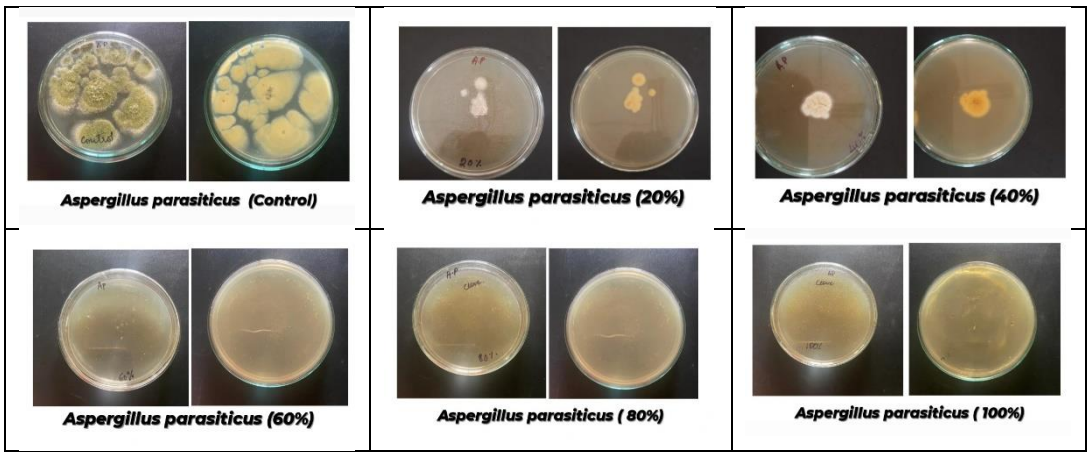

Fig 1: Antifungal activity of clove oil against *Aspergillus parasitus*

Discussion

The results of this study demonstrate that clove essential oil exhibits potent antifungal activity against *Aspergillus parasitus*. Clove essential oil at 60%, 80% and 100% completely inhibited

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the growth of *Aspergillus parasitus* while clove oil at 20% and 40% showed zone of inhibition, indicating its effectiveness in inhibiting the growth of the fungus. The observed antifungal efficacy of clove essential oil can be attributed to its major component, eugenol,[13] which has been reported to disrupt fungal cell membranes, interfere with intracellular processes, and inhibit fungal growth. Moreover, the use of essential oils like clove oil as antifungal agents offers several advantages over conventional drugs. Essential oils are generally regarded as safe and are less likely to induce drug resistance in fungi due to their complex composition, making it challenging for fungi to develop resistance mechanisms against them. Clove essential oil's efficacy against *Aspergillus parasitus* highlights its potential as a promising alternative or adjunct therapy for the management of fungal infections. However, further studies are warranted to explore its safety profile, pharmacokinetics, and in vivo efficacy before clinical application.

Conclusion

In conclusion, this research provides valuable insights into the antifungal activity of clove essential oil against *Aspergillus parasitus*, shedding light on its potential as a natural therapeutic agent to combat fungal infections. As the global burden of antifungal resistance continues to rise, investigating and harnessing the therapeutic potential of natural compounds like clove essential oil becomes crucial in the battle against fungal pathogens.

References

1. Kirsten Foley, GÃ©raldine Fazio, Annette B. Jensen, William O.H. Hughes, The distribution of Aspergillus spp. Opportunistic parasites in hives and their pathogenicity to honey bees, Veterinary Microbiology, Volume 169, Issues 3â€“4, 2014, Pages 203-210,ISSN 0378-1135, https://doi.org/10.1016/j.vetmic.2013.11.029., (https://www.sciencedirect.com/science/article/pii/S0378113513005531)
2. https://en.wikipedia.org/wiki/Aspergillus_parasiticus
3. Pitt, J.I.; Hocking, A.D. (1999). Fungi and food spoilage (2nd ed.). Gaithersburg, Md.: Aspen Publications. ISBN 978-0834213067.
4. Sobel, Lanette. "Aspergillus parasiticus". Bugwood Wiki. Retrieved 21 November 2017
5. Horn, Bruce W.; Ramirez-Prado, Jorge H.; Carbone, Ignazio (20 January 2017). "The sexual state of". Mycologia. 101 (2): 275–280. Doi:10.3852/08-205. JSTOR 20619175. PMID 19397202. S2CID 28375699.
6. Fosses Vuong M, Hollingshead CM, Waymack JR. Aspergillosis. [Updated 2023 Feb 22]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482241/
7.
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
9. https://www.pennmedicine.org/for-patients-and-visitors/patient-information/conditions-treated-a-to-z/aspergillosis 22;26(21):6387. Doi: 10.3390/molecules26216387. PMID: 34770801; PMCID: PMC8588428.

ISSN PRINT 2319 1775 Online 2320 7876

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10. Cortés-Rojas DF, de Souza CR, Oliveira WP. Clove (Syzygium aromaticum): a precious spice. Asian Pac J Trop Biomed. 2014 Feb;4(2):90-6. Doi: 10.1016/S2221-1691(14)60215-X. PMID: 25182278; PMCID: PMC3819475.
11. Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. Syzygium aromaticum L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. Biomolecules. 2020 Jan 30;10(2):202. Doi: 10.3390/biom10020202. PMID: 32019140; PMCID: PMC7072209.
12. Ouadi S, Sierro N, Goepfert S, Bovet L, Glauser G, Vallat A, Peitsch MC, Kessler F, Ivanov NV. The clove (Syzygium aromaticum) genome provides insights into the eugenol biosynthesis pathway. Commun Biol. 2022 Jul 9;5(1):684. Doi: 10.1038/s42003-022-03618-z. PMID: 35810198; PMCID: PMC9271057.
13. Zari AT, Zari TA, Hakeem KR. Anticancer Properties of Eugenol: A Review. Molecules. 2021 Dec 6;26(23):7407. Doi: 10.3390/molecules26237407. PMID: 34885992; PMCID: PMC8659182.