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# Study of Antifungal Activity of Different Concentrations of Clove Oil against *Fusarium graminearum* by Poisoned Food Technique

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Abstract:

Fungal infections pose a significant threat to human health, agriculture, and the environment. The emergence of drug-resistant strains has further emphasized the need for novel antifungal agents. The present study aims to investigates the antifungal activity of clove oil, a natural product derived from *Syzygium aromaticum*, against *Fusarium graminearum* isolated from ridge gourd (*Luffa acutangula*) using the Poisoned Food Technique. Different concentrations of clove oil were tested to determine their inhibitory effects on *Fusarium graminearum* growth. The results demonstrated that clove oil exhibits promising antifungal properties, inhibiting the growth of *Fusarium graminearum* in a dose-dependent manner. These findings contribute to our understanding of the potential use of clove oil as a natural alternative to conventional antifungal agents.

Keywords: Antifungal activity, Clove oil, *Fusarium graminearum*, *Luffa acutangula*, Poisoned Food Technique, *Syzygium aromaticum*

## Introduction:

Fungal pathogens, including *Fusarium* species, are responsible for devastating diseases in plants, animals, and humans.[1,2] The rise of drug-resistant strains and the associated side effects of synthetic antifungal drugs have created an urgent need for the discovery of novel antifungal agents. Natural products derived from plants have long been recognized for their therapeutic potential due to their diverse chemical composition and bioactive properties. Clove oil, obtained from the flower buds of *Syzygium aromaticum*, has been traditionally used for its antimicrobial and antifungal properties. It has been used for centuries for its various medicinal and culinary properties. Clove oil is highly aromatic and contains a compound called eugenol, which gives it its distinctive fragrance and flavor. It is commonly used as a flavoring agent in foods and beverages, as well as a natural remedy for dental and oral health issues. Additionally, clove oil has antimicrobial, analgesic, and anti-inflammatory properties, making it a popular ingredient in topical creams, aromatherapy, and traditional medicine.[3,4] Considering the abundant properties of clove oil present study aims to evaluate the antifungal activity of clove oil against *Fusarium graminearum* using the Poisoned Food Technique.

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Ridge gourd, scientifically known as *Luffa acutangula*, is a versatile and nutritious vegetable widely cultivated in many parts of the world. This elongated green vegetable belongs to the cucumber family and has a distinctive ridged skin, hence its name. Ridge gourd is highly valued for its culinary and medicinal properties. It is rich in dietary fiber, essential vitamins, and minerals, making it a healthy addition to one's diet. The tender, young ridge gourd is commonly used in various cuisines, including stir-fries, curries, and soups. Additionally, ridge gourd is known for its cooling properties and is often used in traditional medicine to alleviate various ailments. With its unique flavor and numerous health benefits, ridge gourd continues to be a popular choice for both culinary and therapeutic purposes.[5,6] Fungal infections in ridge gourd, caused by *Fusarium* species, pose significant threats to crop yield and quality. *Fusarium* species are notorious plant pathogens that can invade the root system and spread to the stem, leaves, and fruits. The infection often starts with discoloration and wilting of leaves, followed by the appearance of brown or dark lesions on the stem. As the disease progresses, the ridge gourd fruits may develop rot, leading to severe economic losses. Effective management strategies involve practicing good crop rotation, maintaining proper sanitation, and utilizing resistant varieties. Fungicides and biocontrol agents can also be employed to control *Fusarium* infections, ensuring healthy ridge gourd production.[7,8]

Materials and Methods:

2.1 Collection of Seeds Sample:

Ridge gourd (*Luffa acutangula*) seeds were collected from Indian Institute of Horticultural Research, Bengaluru. Seeds were packed in a sterile bag and brought to laboratory and were stored in sterile condition till further use.

2.2 Isolation of *Fusarium gramenearum:*

Seeds were surface sterilised with 0.2% sodium hypochlorite followed by rinsing in distilled water and drying in the hood of laminar air flow. 10 surface sterilised seeds were arranged on potato dextrose agar plate with the help of sterilised forceps and plates were incubated at 28°C for 48 hours. Seeds were inoculated in triplicates. After 48 hours fungus was isolated, followed by mounting using lactophenol cotton blue method and identified as *Fusarium gramenearum* using direct microscopy.

2.3 Preparation of Different Concentration of Clove Oil:

Commercially available clove oil was obtained from a reliable source and stored in amber glass bottles at room temperature. Prior to testing, the clove oil was diluted in acetone to obtain a range of concentrations (e.g., 20%, 40%, 60% and 80%).

2.4 Poisoned Food Technique:

The Poisoned Food Technique was employed to evaluate the antifungal activity of clove oil against *Fusarium gramenearum*. Potato dextrose agar (PDA) plates were prepared with various concentrations of clove oil at 1000 ppm, and a standardized inoculum of *Fusarium gramenearum* was inoculated on each plate. Acetone was used as control. Control plates without clove oil were also included. The plates were then incubated at 28°C for 7 days and after 7 days zone of inhibition was calculated and recorded.

 

2.5 Measurement of Antifungal Activity:

The antifungal activity of clove oil was evaluated by measuring the growth inhibition zone (mm) around the fungal colonies. The diameter of the zone of inhibition was measured using a calibrated ruler. Each experiment was performed in triplicate, and the average value was calculated.

Results:

Table 1: Inhibitory activity of clove oil against Fusarium graminearum

| Control | 20% | 40% | 60% | 80% | 100% |
|---|---|---|---|---|---|
| Complete growth | Complete inhibition | Complete inhibition | Complete inhibition | Complete inhibition | Complete inhibition |

The results demonstrated a clear dose-dependent effect of clove oil on growth inhibition of *Fusarium gramenearum*. It was observed that control plates showed the maximum growth and complete inhibition was seen at all the concentrations of clove oil (20%, 40%, 60%, 80% and 100%) at 1000ppm.

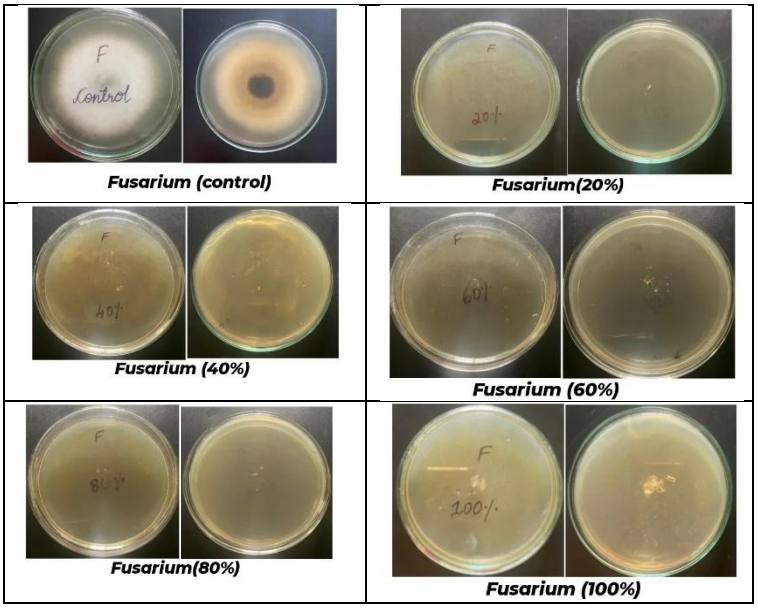


Fig 1: Inhibitory activity of clove oil against Fusarium graminearum

Discussion:

Clove oil has been reported to possess a variety of bioactive compounds, including eugenol, eugenyl acetate, and β-caryophyllene,[3,4] which are known for their antimicrobial properties. These compounds are believed to interfere with the cell membrane integrity, disrupt vital cellular processes, and inhibit fungal growth. The observed dose-dependent effect suggests that increasing concentrations of clove oil lead to enhanced antifungal activity against *Fusarium gramenearum*.[12,13] Laila Muñoz Castellanos et al. had studied the antifungal activity of clove oil against *Aspergillus niger* and *Fusarium oxysporum*. Results revealed that clove oil inhibited the growth of *Aspergillus niger* and *Fusarium oxysporum*.[9] Similar to that study, in the present study clove oil exerted strong antifungal property against *Fusarium*

*graminearum* isolated from ridge gourd seeds. The study by Rana Inder Singh et al revealed the antifungal property of clove oil against fungal species such as *Fusarium moniliforme*, *Fusarium oxysporum*, *Aspergillus* sp., *Mucor* sp., *Trichophyton rubrum* and *Microsporum gypseum*.[10] Similarly, strong antifungal activity of different concentrations clove oil against *Fusarium graminearum* was revealed in the present study. Eugénia Pinto et al., had studied the antifungal activity of clove oil against *Candida* and *Aspergillus* sp., Results revealed that clove oil demonstrated antifungal activity against these fungi.[11] Similarly, in the present study strong antifungal activity of different concentrations of clove oil against *Fusarium graminearum* was observed.

Conclusion:

The study demonstrates the potential antifungal activity of clove oil against *Fusarium gramenearum*. The Poisoned Food Technique allowed for the evaluation of different concentrations of clove oil, and the results showed a clear inhibitory effect on fungal growth. Clove oil may serve as a natural alternative to conventional antifungal agents, offering potential benefits such as reduced toxicity and decreased likelihood of drug resistance. Further research is warranted to explore the underlying mechanisms of action and optimize the application of clove oil as an antifungal agent.

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