

Study of Antifungal Activity of Different Concentrations of Clove Oil against *Corynespora cassiicola* by Poisoned Food Technique

Nalini T J.,¹ Prathibha K. Y.,² Harshitha B. R.,³ Sree padma K. V.,³ Marhoob Banu.,^{3*}

¹ Assistant Professor, ² Professor, ³ Research Scholars, Department of Botany, 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:

Fungal infections are a significant concern in various agricultural settings as they can cause substantial economic losses and reduce crop yields. *Corynespora cassiicola* is a common fungal pathogen affecting crops worldwide. To combat these infections, researchers have turned to natural remedies like essential oils, which possess potential antifungal properties.^{1,2,3} In the present study, we investigated the antifungal activity of clove oil against *Corynespora cassiicola* using the poisoned food technique. Different concentrations of clove oil (*Syzygium aromaticum*) viz., 20%, 40%, 60%, 80% and 100% were tested to determine the most effective concentration in inhibiting *Corynespora cassiicola* growth. Acetone was used as control. Our findings provide valuable insights into the potential application of clove oil as a natural antifungal agent in agriculture.

Keywords: Antifungal agent, *Corynespora cassiicola*, Crop yield, Essential Oils, Fungal Pathogens, *Syzygium aromaticum*

Introduction:

Fungal pathogens pose a significant threat to agriculture, causing substantial losses in crop yields and quality worldwide. These microscopic organisms attack various parts of plants, including leaves, stems, roots, and fruits, leading to devastating diseases that can decimate entire crops. The economic impact of such losses is profound. Fungal diseases reduce crop productivity through several mechanisms. They can inhibit photosynthesis, disturb nutrient uptake, and weaken plant structures, ultimately leading to stunted growth and lower yields. Additionally, infected crops may suffer from reduced market value due to quality deterioration, making them unsuitable for sale or processing. Farmers often resort to using fungicides to combat these pathogens, but this approach is not without its challenges. Prolonged fungicide use can lead to the development of resistant fungal strains, further exacerbating the problem. Moreover, climate change and global trade contribute to the spread of new and more virulent fungal pathogens into regions where they were previously absent. The emergence of new strains poses additional challenges for agricultural practices and necessitates constant monitoring and research for effective control strategies. Overall, fungal pathogens remain a persistent and formidable menace to agricultural systems, necessitating ongoing efforts in

research, disease management, and sustainable farming practices to minimize the losses they cause and ensure food security for a growing global population.^{1,2,3,4,5}

Clove oil, derived from the flower buds of the clove tree (*Syzygium aromaticum*), has demonstrated potent antifungal properties and has been used for centuries in traditional medicine to combat fungal infections. The primary active component responsible for its antifungal activity is eugenol, which constitutes a significant portion of clove oil. Studies have shown that clove oil exhibits broad-spectrum antifungal activity against various fungal species, including *Candida albicans*, *Aspergillus* spp., and dermatophytes. Eugenol works by disrupting the cell membranes of these fungi, leading to leakage of cellular contents and ultimately cell death. Additionally, it interferes with fungal enzyme systems, further weakening their ability to survive and proliferate. Clove oil's effectiveness as an antifungal agent has been explored in various applications, including topical treatments for skin and nail infections, oral health products, and in combination with other antifungal agents to enhance their efficacy. Its natural origin and generally safe profile make it an appealing alternative to synthetic antifungals. However, it is essential to note that while clove oil shows promise as an antifungal agent, more research is needed to determine its full potential and safety for various uses. As with any natural remedy, caution should be exercised, and consulting a healthcare professional is recommended before using clove oil as an antifungal treatment.^{6,7,8,9}

Corynespora cassiicola, also known as *Corynespora cassiicola* leaf spot, is a fungal pathogen that affects various crops and can lead to significant economic losses in agriculture. The pathogen primarily targets the foliage of plants and manifests as dark, circular or irregular spots on the leaves. These spots often have a distinct, chlorotic (yellowing) border, making them relatively easy to identify. Once *Corynespora cassiicola* infects a crop, it can spread rapidly under favorable environmental conditions, such as high humidity and moderate temperatures. The spores of the fungus can be easily disseminated through wind, rain, or irrigation, contributing to its wide distribution. The severity of the impact on crops depends on several factors, including the specific crop type, stage of growth, and prevailing weather conditions. Severe infections can result in defoliation, reduced photosynthetic capacity, and ultimately reduced yield and crop quality. In some cases, *Corynespora cassiicola* infection can cause premature ripening and fruit drop, further exacerbating the losses. To manage and control *Corynespora cassiicola* leaf spot, farmers employ various strategies such as crop rotation, use of resistant cultivars, sanitation practices, and the application of fungicides. Timely detection and early intervention are crucial to minimizing the damage caused by this fungal pathogen.^{15,16}

Fungal pathogens pose a significant threat to agricultural productivity, leading to substantial crop losses worldwide. *Corynespora cassiicola* is a common fungal genus known to infect numerous crops, including vegetables, fruits, and ornamental plants.^{15,16} Traditional fungicides have been widely used to control fungal infections; however, they have raised concerns about environmental pollution, toxicity, and development of resistance. Hence, there is a growing interest in exploring natural alternatives, such as essential oils, which have shown promising antifungal properties.^{2,3} Clove oil, derived from the *Syzygium aromaticum* plant, has been reported to possess significant antimicrobial activity, making it an attractive candidate for controlling fungal pathogens. The present study aims to evaluate the antifungal activity of clove oil against *Corynespora cassiicola* and determine the most effective concentration of clove oil in inhibiting *Corynespora cassiicola* growth.

Materials and Methods:

1. Sample collection
 - Essential oil

Essential oil of clove (*Syzygium aromaticum*) was collected from local market.

- Seeds sample:

Watermelon (*Citrullus lanatus*) seeds were collected from IIHR, Bengaluru.

- Fungal isolate

A virulent strain of *Corynespora cassiicola* was isolated from watermelon (*Citrullus lanatus*) seeds by agar plate method and maintained on Potato Dextrose Agar (PDA) plates.

2. Preparation of different concentrations of clove oil

Different concentrations of clove oil viz., 20%, 40%, 60%, 80% and 100% were prepared using acetone.

3. Poisoned Food Technique:

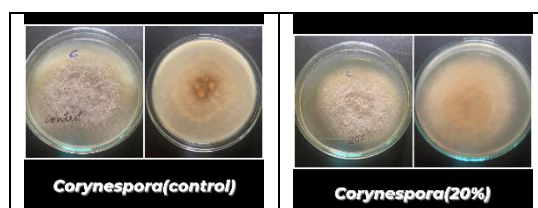
The poisoned food technique was employed to evaluate the antifungal activity of different concentrations of clove oil. Different concentrations of clove oil viz., 20%, 40%, 60%, 80% and 100% were incorporated into PDA medium at 1000 ppm using micropipette by poisoned food technique. Control plates using acetone without clove oil were also prepared. Fungal mycelium was inoculated on the centre of each PDA plate. Petriplates were sealed and incubated at 27°C for 7 days. After 7 days of incubation, zone of inhibition was measured using a ruler and recorded. Experiments were performed in triplicates and results produced in this study is the mean of triplicates.

Results:

Table 1: Inhibitory activity of clove oil against *Corynespora cassiicola*

Control	20%	40%	60%	80%	100%
Complete growth	10 mm	Complete inhibition	Complete inhibition	Complete inhibition	Complete inhibition

The results of the experiment indicated that clove oil exhibited significant antifungal activity against *Corynespora cassiicola*. Clove oil at 40%, 60%, 80% and 100% inhibited the mycelial growth of *Corynespora cassiicola* completely while Clove oil at 20% showed zone of inhibition of 10 mm. Maximum growth was seen in control plates.



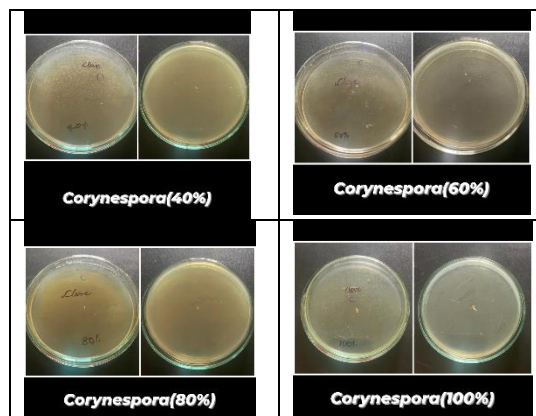


Fig 1: Inhibitory activity of clove oil against *Corynespora cassiicola*

Discussion:

The findings of this study suggest that clove oil possesses potent antifungal properties against *Corynespora cassiicola*. The inhibition of fungal growth could be attributed to the presence of active compounds such as eugenol, which has demonstrated strong antimicrobial effects in previous studies.^{8,13} The mode of action of clove oil on the fungal cells needs further investigation to understand the underlying mechanisms.

Chee HY, Lee MH et al., had studied the antifungal activity of clove essential oil and its volatile vapour against dermatophytic fungi such as *Candida albicans*, *Epidermophyton floccosum*, *Microsporum audouinii*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*. Results revealed that clove essential oil and its volatile vapour exhibited strong antifungal activity against these fungal pathogens.¹⁰ Similarly, in the present study strong antifungal activity of clove oil against *Corynespora cassiicola* was seen. Rana IS, Rana AS, Rajak RC et al., evaluated the antifungal activity of essential oil of the *Syzygium aromaticum* (L.) against *Fusarium moniliforme* NCIM 1100, *Fusarium oxysporum* MTCC 284, *Aspergillus* sp., *Mucor* sp., *Trichophyton rubrum* and *Microsporum gypseum*. Results revealed that clove essential oil exhibited strong antifungal activity against these fungal pathogens.¹¹ Similarly, in the present study strong antifungal activity of clove oil against *Corynespora cassiicola* was seen. The study by Eugénia Pinto et al., revealed the antifungal activity of the clove essential oil against *Candida*, *Aspergillus* and dermatophyte species.¹² Similar to this study, in the present study antifungal activity of different concentrations of clove oil against *Corynespora cassiicola* was seen. Haiyang Jiang et al., had studied the antifungal activity of clove oil against different fungi and the results revealed the strong antifungal activity of clove oil against those fungi.¹⁴ Similarly, in the present study strong antifungal activity of clove oil against *Corynespora cassiicola* was seen.

Conclusion:

Clove oil showed significant antifungal activity against *Corynespora cassiicola* and its effectiveness increased with higher concentrations. The results indicate that clove oil has the potential to be used as a natural alternative to synthetic fungicides for controlling *Corynespora cassiicola* infections in agriculture. However, additional research is required to determine its

practical application, including studies on its efficacy under field conditions and potential side effects on non-target organisms.

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