

Poisoned Food Assay for Study of the Antifungal Activity of Camphor Oil on *Colletotrichum orbiculare*

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Abstract

Colletotrichum orbiculare is a widespread plant pathogen responsible for causing anthracnose disease in various crops. The excessive use of synthetic fungicides has led to environmental concerns and the emergence of resistant strains. Therefore, the search for eco-friendly alternatives has become imperative. This research paper investigates the antifungal activity of camphor oil as a potential biocontrol agent against *Colletotrichum orbiculare*. The oil which was extracted from *Cinnamomum camphora* was collected from local market and different concentrations of oil viz., 20%, 40%, 60%, 80% and 100% was prepared by dissolving the oil in acetone. The in vitro antifungal activity of camphor oil was assessed through poisoned food technique. The results demonstrate that different concentrations of camphor oil were not effective in inhibiting the growth of *Colletotrichum orbiculare*.

Keywords: Camphor oil, *Cinnamomum camphora*, *Colletotrichum orbiculare*, anthracnose disease, antifungal activity, biocontrol agent, natural fungicide, eco-friendly alternative, plant disease management.

Introduction

Colletotrichum orbiculare is a fungal pathogen known to cause anthracnose disease in cucurbits, affecting crops such as pumpkins, cucumbers, and melons. This plant pathogen spreads through spores and infects various plant tissues, leading to characteristic dark, sunken lesions on leaves, stems, and fruits. The disease can result in reduced yields, economic losses, and even complete crop failure in severe cases. Management strategies for controlling *Colletotrichum orbiculare* involve a combination of cultural practices, such as crop rotation, removal of infected plant material, and the use of disease-resistant cultivars. Additionally, fungicides can be applied preventively to minimize disease spread. Regular monitoring and early detection are crucial to implementing effective control measures. Research efforts continue to explore sustainable and environmentally friendly approaches to combat this pathogen, emphasizing the importance of integrated pest management techniques. By employing these strategies, farmers can mitigate the impact of *Colletotrichum orbiculare* on cucurbit crops and ensure a more resilient agricultural system.^{1,2,3}

Colletotrichum can infect a wide range of crops, including cereals, legumes, fruits, and vegetables, leading to devastating economic losses for farmers and food shortages for communities. The infection typically starts as small, circular lesions on leaves, stems, or fruits, eventually spreading and causing tissue decay. Factors such as environmental conditions, host susceptibility, and farming practices influence the severity of crop losses. Fungal spores can survive in crop debris or soil, leading to recurring infections in subsequent growing seasons. Farmers try to manage *Colletotrichum* infections through crop rotation, fungicide application, and the use of resistant cultivars. However, resistance-breaking strains and limited fungicide efficacy pose ongoing challenges. *Colletotrichum*-induced losses continue to be a major concern for agriculture, and concerted efforts in research, disease management strategies, and sustainable farming practices are crucial to mitigate its impact on global food security.^{4,5,6}

Camphor oil is a natural essential oil derived from the wood of the camphor tree, scientifically known as *Cinnamomum camphora*. It has been utilized for centuries due to its various therapeutic properties. The oil is known for its distinct, uplifting aroma and is often used in aromatherapy to promote relaxation and alleviate stress. Topical application of diluted camphor oil is believed to relieve minor skin irritations and muscle aches. Additionally, it has been used as an insect repellent and for respiratory relief when added to steam inhalation. However, it's essential to use camphor oil with caution, as it can be toxic when ingested or applied directly to the skin in concentrated forms.^{7,8,9}

The present study aims to investigate the antifungal activity of camphor oil specifically targeting *Colletotrichum orbiculare*. The objectives include determining the effectiveness of camphor oil in inhibiting the growth of *Colletotrichum orbiculare*, evaluating its potential as a natural fungicide, understanding the mechanism of action, and exploring its potential application in plant disease management.

Materials and Methods

➤ Sample collection

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

➤ Antifungal assay

The antifungal activity of camphor oil was studied by Poisoned Food Technique. Potato dextrose agar media was prepared, autoclaved and amoxicillin was added to avoid bacterial contamination. Different concentrations of camphor oil were prepared by dissolving the oil in acetone. Different concentrations of camphor oil were incorporated in the potato dextrose agar media with the help of micropipette at 1000 ppm. The media was mixed thoroughly and agar plates were prepared. *Colletotrichum orbiculare* was inoculated and plates were incubated at 27°C for seven days. Experiments were performed in triplicates and the results obtained in the present study is the average of the triplicates. After seven days the plates were observed for zone of inhibition and recorded

Results

Results revealed that Camphor Oil didn't possessed antifungal activity against *Colletotrichum orbiculare*.

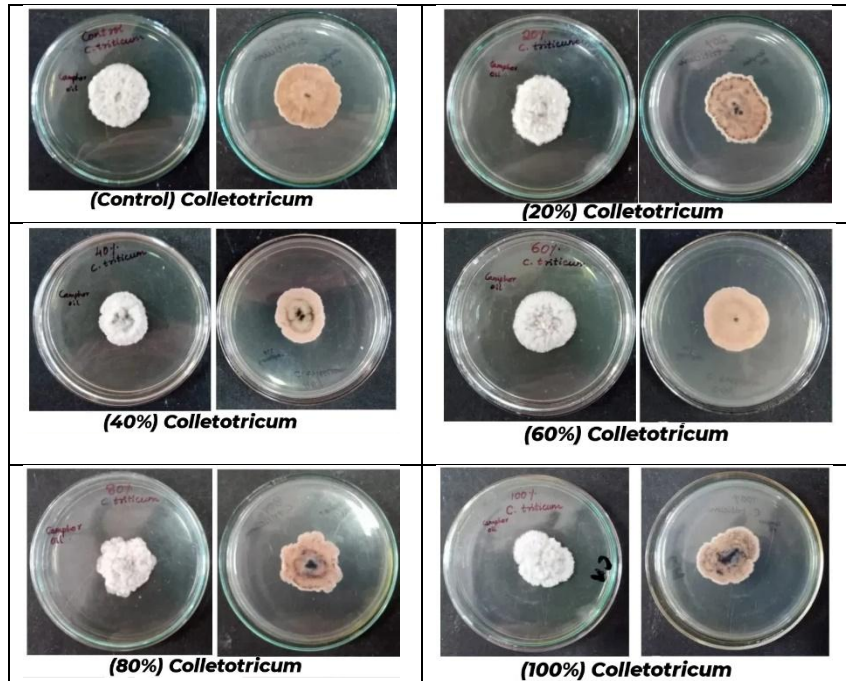


Fig 1: Antifungal activity of Camphor oil against *Colletotrichum orbiculare*

Table 1: Antifungal activity of Camphor oil against *Colletotrichum orbiculare*

| Concentration | Mean colony diameter (mm) |
|---------------|---------------------------|
| 0% | 30 mm |
| 20% | 30 mm |
| 40% | 30 mm |
| 60% | 30 mm |
| 80% | 30 mm |
| 100% | 30 mm |

Discussion

The present study investigates the potential of camphor oil as an antifungal agent against *Colletotrichum orbiculare*, a pathogenic fungus affecting various crops and plants. The study aimed to explore natural alternatives to chemical fungicides, considering their environmental impact and potential health hazards. We conducted in vitro experiments to assess the inhibitory effects of camphor oil on the growth and development of *Colletotrichum orbiculare*. Different concentrations of camphor oil were used to measure its impact on fungal growth, spore germination, and mycelial development. Results demonstrated that camphor oil didn't exhibit significant antifungal activity against *Colletotrichum orbiculare* at all concentrations tested viz., 20%, 40%, 60%, 80% and 100%. Furthermore, studies should be carried out to investigate the antifungal activity of different natural products and their mode of action.

Ivanov M et al., had studied the antifungal activity of camphor oil against *Candida* sp., and the results revealed that camphor oil exhibited antifungal activity against *Candida* sp.,¹⁰ This result is in contrast to our result where camphor oil didn't exhibit antifungal activity against *Colletotrichum*. Mahilrajan S et al., had studied antifungal activity of camphor oil against

Aspergillus flavus, *Aspergillus niger* and *Penicillium* sp., and camphor oil possessed significant antifungal activity against these fungal pathogens.¹¹ These results are also in contrast to our result where camphor oil didn't exhibited antifungal activity against *Colletotrichum*.

Conclusion

In conclusion, the present study aimed to investigate the antifungal properties of camphor oil on *Colletotrichum orbiculare* by Poisoned Food Technique and the results revealed that Camphor Oil was not effective in inhibiting the growth of *Colletotrichum orbiculare*. Furthermore, studies should be carried out to investigate the antifungal activity of different natural products and their mode of action and their practical application in crop protection and disease management.

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