Study of Antifungal Activity of Geranium Oil Against *Colletotrichum* sp., *Fusarium* sp., and *Macrophomina phaseolina* by Poisoned Food Technique

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Abstract:
Geranium oil finds extensive application as a constituent in perfumes and cosmetic products. Its aromatic properties make it a popular choice for enhancing fragrances and adding a pleasant scent to various personal care items. The present study investigates the antifungal properties of geranium oil against three common fungal pathogens, *Colletotrichum* sp., *Fusarium* sp., and *Macrophomina phaseolina* isolated from ridge gourd (*Luffa acutangula*) seeds and watermelon (*Citrullus lanatus*) seeds by agar plate method by using the poisoned food technique. Geranium oil, known for its various biological activities, including antimicrobial properties, was tested for its efficacy in inhibiting the growth of these fungal species. The results suggest that geranium oil possesses significant antifungal activity against *Colletotrichum* sp., and *Fusarium* sp., and partial antifungal activity against *Macrophomina phaseolina*, providing valuable insights for its potential application in agricultural and pharmaceutical industries.

Keywords: Antifungal activity, Geranium oil, *Colletotrichum* sp., *Fusarium* sp., *Macrophomina phaseolina*, Poisoned food technique, Agar plate method

Introduction:
Fungal pathogens pose a significant threat to various industries, including agriculture, food production and pharmaceuticals.[1,2,3,4] Among them, *Colletotrichum* sp., *Fusarium* sp., and *Macrophomina phaseolina* are notorious for causing diseases in a wide range of crops, resulting in substantial economic losses worldwide. Traditional methods of fungal control often involve the use of synthetic fungicides, which may lead to environmental pollution and the development of resistant strains. Therefore, there is a growing interest in exploring natural alternatives, such as plant-derived essential oils, for their potential antifungal properties.
Colletotrichum is a genus of fungal pathogens known to infect a wide range of crops, including fruits, vegetables, and ornamental plants. It causes diseases such as anthracnose, which leads to significant economic losses in agriculture. Colletotrichum species infect plant tissues, causing lesions, rotting, and sometimes complete crop loss. These pathogens spread through spores produced on infected plant tissues or through contaminated soil, water, and farming equipment. Effective management strategies include crop rotation, use of disease-resistant cultivars, fungicide applications, and sanitation practices to minimize the spread of *Colletotrichum* infections and mitigate their impact on crop yields.[5,6]
Fusarium is a genus of fungal pathogens that pose significant threats to crops worldwide. These pathogens can infect a wide range of plants, including cereals, vegetables, and fruits, causing diseases such as *Fusarium* wilt, root rot, and head blight. Fusarium infections can result in yield losses, decreased crop quality, and economic impacts on farmers. Moreover, some *Fusarium* species produce mycotoxins, harmful compounds that can contaminate food and feed, posing risks to human and animal health. Effective management strategies, including crop rotation, resistant varieties, and fungicide applications, are crucial for mitigating *Fusarium*-related crop damage and safeguarding agricultural productivity.[7,8]
Macrophomina phaseolina is a fungal pathogen that poses a significant threat to crops worldwide. Commonly known as charcoal rot, it affects over 500 plant species, including soybeans, maize, cotton, and sunflowers. This soil-borne fungus survives in crop residues and soil, thriving in warm and dry conditions. It infects plants through roots, causing wilting, stem discoloration, and ultimately death. Charcoal rot reduces crop yield and quality, leading to economic losses for farmers. Management strategies include crop rotation, tillage practices, use of resistant varieties, and fungicidal treatments, emphasizing the importance of integrated disease management approaches to mitigate its impact.[9,10]
Geranium oil, derived from the *Pelargonium graveolens* plant, has been valued for centuries for its versatile properties. It's commonly used in aromatherapy, skincare, and holistic medicine. With its floral, sweet, and slightly fruity aroma, geranium oil is known for its balancing effects on emotions, making it a popular choice for reducing stress, anxiety, and depression symptoms. In skincare, geranium oil is prized for its ability to balance sebum production, making it beneficial for both oily and dry skin types. It's often used to treat acne, dermatitis, and other skin conditions due to its anti-

inflammatory and antimicrobial properties. Furthermore, geranium oil is believed to have various health benefits, including alleviating menstrual discomfort, improving circulation, and repelling insects. Some studies suggest that it may also have antioxidant properties, which could help protect cells from damage caused by free radicals. Several studies have highlighted the potential of geranium oil as an effective antimicrobial agent against bacteria, fungi, and even some viruses. However, its efficacy against specific fungal pathogens, such as *Colletotrichum* sp., *Fusarium* sp., and *Macrophomina phaseolina*, remains relatively unexplored. The primary objective of this study is to evaluate the antifungal activity of geranium oil against *Colletotrichum* sp., *Fusarium* sp., and *Macrophomina phaseolina* using the poisoned food technique. By elucidating the inhibitory effects of geranium oil on these fungal pathogens, this research aims to provide valuable insights into its potential application as a natural fungicide in agriculture and related industries.[11,12,13]

Materials and Methods:

1. Fungal Strains: *Colletotrichum* sp., and *Fusarium* sp., were isolated from ridge gourd seeds by agar plate method and *Macrophomina phaseolina* was isolated from watermelon seeds by agar plate method.
2. Geranium Oil: Commercially available geranium oil was used for the experiments. Different concentrations of geranium oil viz., 20%, 40%, 60% and 80% were prepared by diluting with acetone.
3. Poisoned Food Technique: Potato dextrose agar (PDA) media was prepared according to manufacture's instructions on product label. Amoxicillin was added to prevent bacterial contamination. Petri plates were sterilized and agar plates were prepared and inoculated with fungal spores and incubated at optimal conditions for fungal growth. The experiment was performed in triplicates.
4. Antifungal Assay: The diameter of fungal growth inhibition zones around each geranium oil concentration was measured after incubation and compared to control plates without geranium oil.
5. Statistical Analysis: Data were analyzed using appropriate statistical methods to determine the significance of antifungal activity.

Results:

The results of the antifungal assay revealed that geranium oil exhibited concentration-dependent inhibitory effects against *Macrophomina phaseolina* and it inhibited the growth of *Colletotrichum* sp., and *Fusarium* sp., at all the concentratios. The zones of fungal growth inhibition increased with higher concentrations of geranium oil in *Macrophomina phaseolina*. Statistical analysis confirmed the significant antifungal activity of geranium oil compared to the control groups.

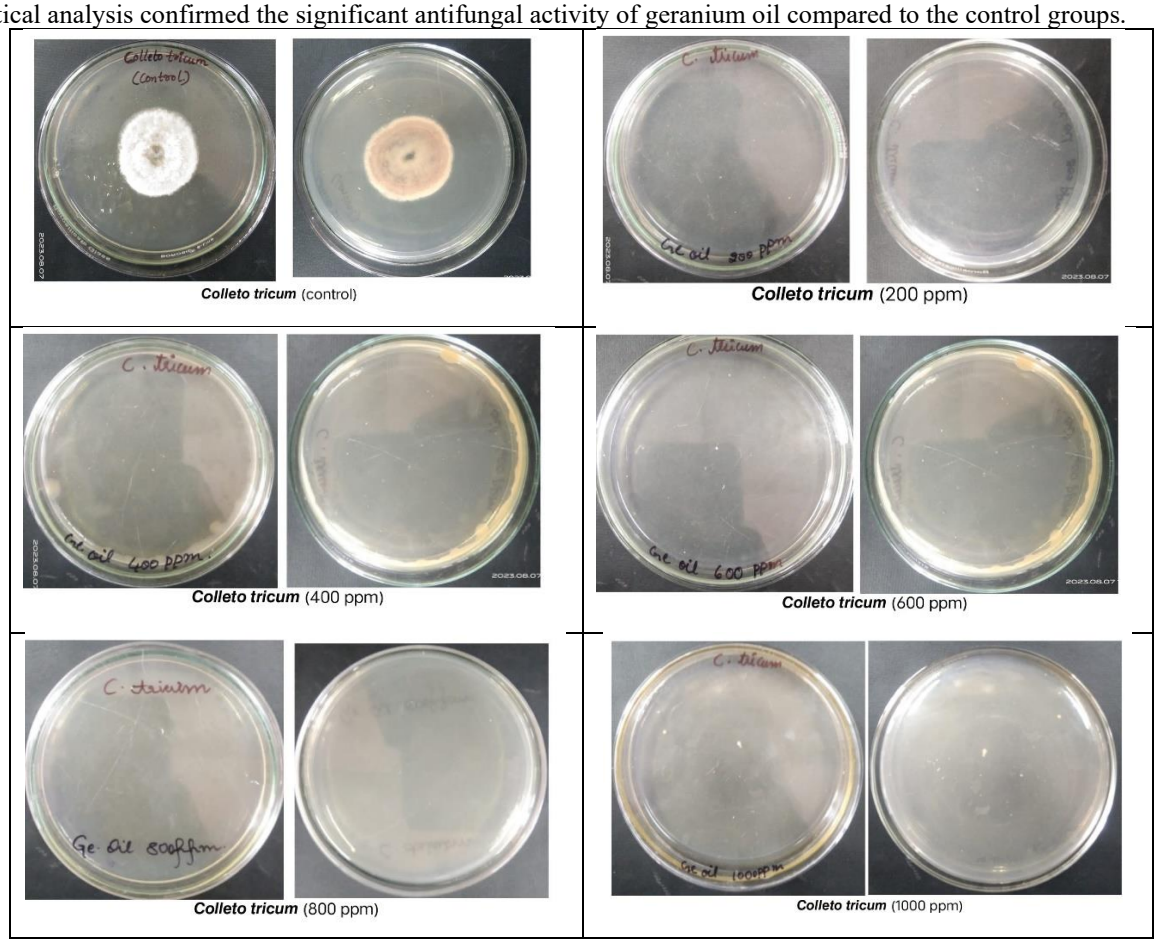

Fig 1: Antifungal activity of geranium oil against *Colletotrichum* sp.,

Table 1: Antifungal activity of geranium oil against *Colletotrichum* sp.,

Concentration	Zone of inhibition
Control	-
20%	Complete inhibition
40%	Complete inhibition
60%	Complete inhibition
80%	Complete inhibition
100%	Complete inhibition

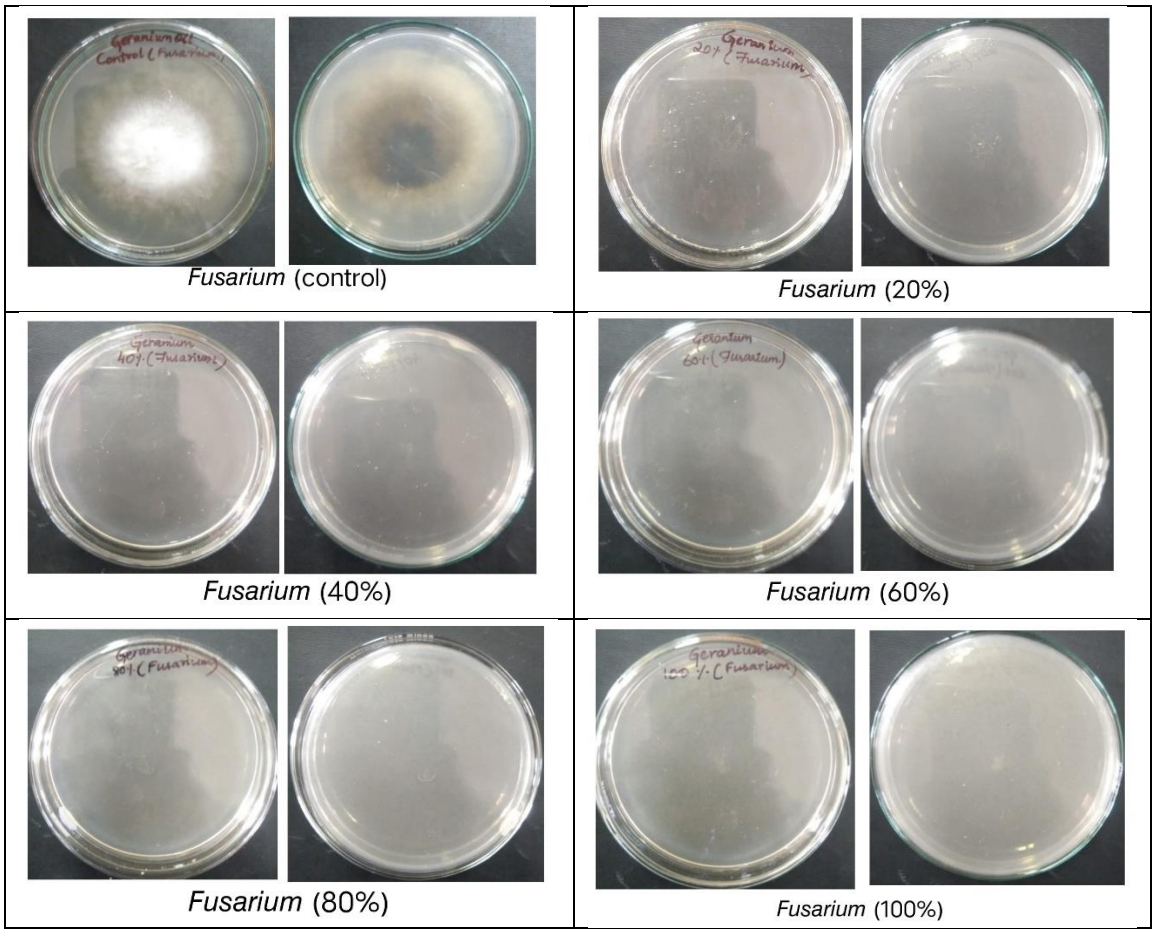

Fig 2: Antifungal activity of geranium oil against *Fusarium* sp.,

Table 2: Antifungal activity of geranium oil against *Fusarium* sp.,

Concentration	Zone of inhibition
Control	-
20%	Complete inhibition
40%	Complete inhibition
60%	Complete inhibition
80%	Complete inhibition
100%	Complete inhibition

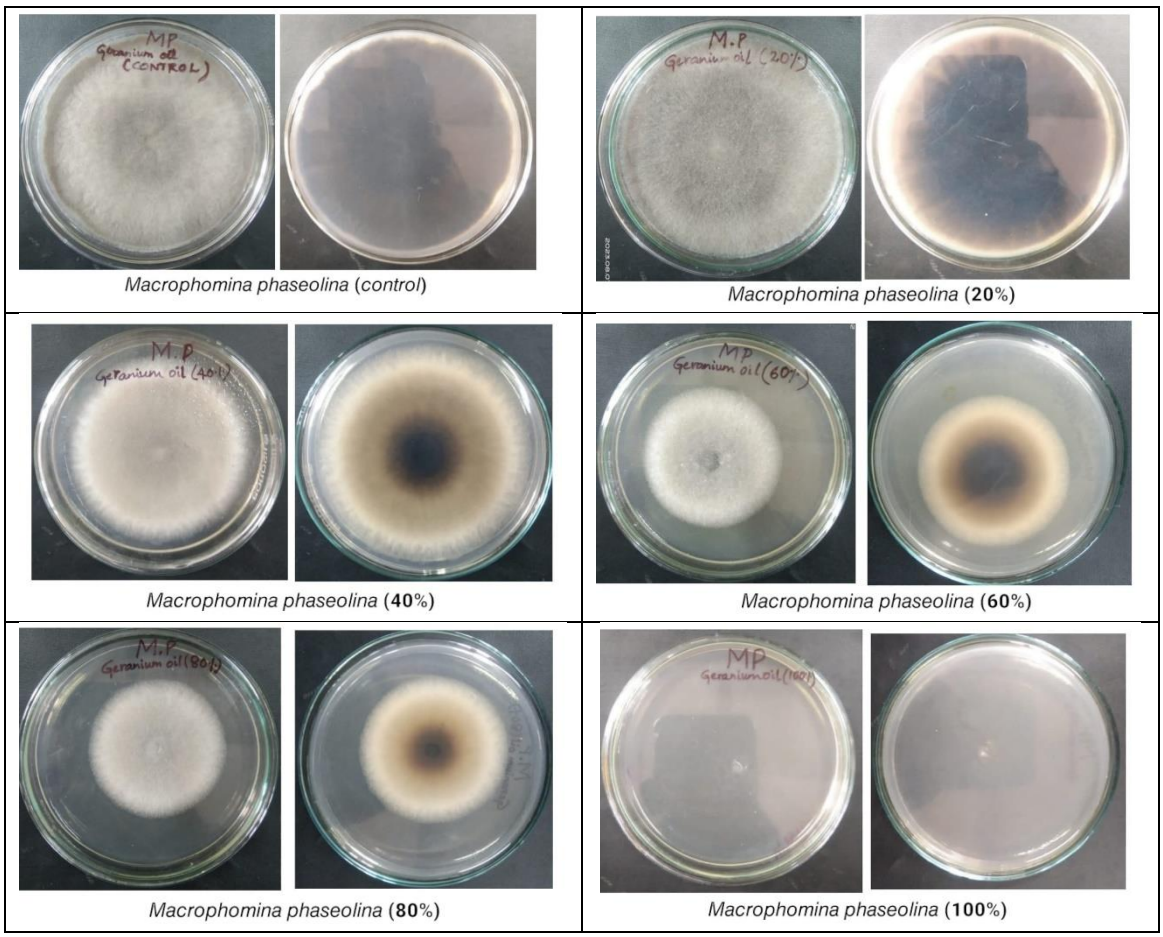

Fig 2: Antifungal activity of geranium oil against *Macrophomina phaseolina*

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Concentration	Zone of inhibition
Control	0 mm
20%	0 mm
40%	20 mm
60%	30 mm
80%	35 mm
100%	90 mm

Discussion:

Essential oils (Eos) are derived from plants and consist of various chemical compounds. These volatile substances possess diverse properties, one of which is their antifungal activity. Fungi can proliferate in agricultural settings, such as on wheat or corn crops and others, and produce mycotoxins that can harm both livestock and human health. The findings of this study demonstrate the potential of geranium oil as a natural alternative to synthetic fungicides for controlling fungal pathogens such as *Colletotrichum* sp., *Fusarium* sp., and *Macrophomina phaseolina*. The observed antifungal activity of geranium oil can be attributed to its complex chemical composition, including various terpenes, alcohols, and phenolic compounds, (Gallardo A et al.,)[14] which have been reported to possess antimicrobial properties. Moreover, the concentration-dependent nature of the antifungal effects suggests the importance of optimizing geranium oil formulations for maximum efficacy in practical applications.

Khaledi N et al., had studied the antifungal activity of various essential oils viz, *Mentha piperita, Bunium persicum* and *Thymus vulgaris* against *Macrophomina phaseolina*. Analysis demonstrated that EO's of *Bunium persicum* and *Thymus vulgaris* exhibited maximum inhibition of growth of *Macrophomina phaseolina* while *Mentha piperita* exhibited minimum inhibition.[15] In our study, we observed concentration-dependent inhibition of *Macrophomina phaseolina* by geranium oil, consistent with previous findings. Abdel-Kader M. M. et al., investigated the efficacy of essential oils and *Trichoderma harzianum* as an integrated control measure against faba bean root rot pathogens. Their findings demonstrated that geranium oil, when applied at a concentration of 4%, effectively inhibited the growth of *Macrophomina phaseolina*.[16] Consistent with their findings, our study also observed concentration-dependent inhibition of *Macrophomina phaseolina* by geranium oil. Azza A Ghazi et al. worked on management of charcoal rot (*Macrophomina phaseolina*) infection in geranium (*Pelargonium graveolens* L.) using biocontrol agents and essential oils, their findings demonstrated

that Peppermint oil recorded the highest reduction in radial growth of pathogen followed by Basil oil while Marjoram oil had no effect on the growth of *Macrophomina phaseolina*.[17] Aligned with their discoveries, our investigation also corroborated the hindrance of *Macrophomina phaseolina* facilitated by the essence of geranium oil.
In the meticulous exploration conducted by Lipi Parikh et al., the potent antifungal prowess of Palmarosa, oregano, clove, cinnamon, lemongrass, citronella, and thyme oils against a spectrum of fungal adversaries, including *Aphanomyces euteiches, Botrytis cinerea, Colletotrichum lentis, Didymella pisi, D. rabiei, D. lentis, Fusarium avenaceum, Stemphylium beticola, Sclerotinia sclerotiorum*, and *Pythium sylvaticum*, was meticulously scrutinized. The findings unveiled a resounding triumph as all seven essential oils inhibited the mycelial proliferation of the pathogens by an impressive 50 to 100%, save for *B. cinerea* and *S. sclerotiorum*.[18] Echoing the resonance of their study, our own investigation corroborated this phenomenon, showcasing the formidable inhibition of *Colletotrichum* by the sublime essence of geranium oil. Bhardwaj Jyoti et al., studied the antifungal activity of fifteen essential oils against *Colletotrichum lindemuthianum*. They found that lemongrass and peppermint oils were the most effective, completely inhibiting the fungus. Wintergreen and geranium oils also showed significant inhibition.[19] Our results corroborate their findings, demonstrating the inhibitory effect of geranium oil on *Colletotrichum*. Krzyśko-Łupicka T et al. studied the antifungal activity of certain essential oils against *Fusarium graminearum*. They found that geranium and rosewood oils completely stopped the growth of the tested isolates at all concentrations.[20] Our results also showed that geranium oil inhibited *Fusarium* growth, similar to theirs. The antifungal activity of selected essential oils was studied by Perczak A et al. These oils included cinnamon bark from Indonesia, oregano herb from Mediterranean countries, palmarosa leaves from India, orange peel from Brazil, verbena leaves and flowers from Spain, spearmint leaves from China, fennel seeds from Russia/Bulgaria, and rosewood from India. The results showed that these essential oils significantly inhibited the growth of *Fusarium culmorum* and *Fusarium graminearum*.[21] Our results also demonstrated similar inhibition of *Fusarium* by geranium oil.

Conclusion:
Geranium oil exhibits significant antifungal activity against *Colletotrichum* sp., *Fusarium* sp., and *Macrophomina phaseolina*, as demonstrated by the poisoned food technique. These findings underscore the potential of geranium oil as a natural fungicide for controlling fungal diseases in agriculture and related industries. Further research is warranted to explore its mode of action, formulation optimization, and field efficacy to facilitate its practical implementation.

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