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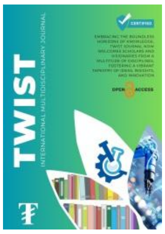

Antifungal Activity of Eight Plant Extracts against *Macrophomina phaseolina* by Agar Well Diffusion Method

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Abstract
Macrophomina phaseolina is a devastating fungal pathogen that causes charcoal rot disease in a wide range of crops, leading to significant yield losses. In the present study, we investigated the antifungal activity of eight plant extracts viz., *Coriandrum sativum*, *Cymbopogon citratus*, *Eucalyptus camaldulensis*, *Mangifera indica*, *Mentha piperita L*, *Moringa oleifera*, *Psidium guajava L*, *Trigonella foenum* against *Macrophomina phaseolina* by agar well diffusion method. *Macrophomina phaseolina* was isolated from watermelon seeds (*Citrullus lanatus*) by agar plate method. Plant extracts were prepared by drying the washed plant samples followed by grinding it to fine powder, sieving and soaking in 50% ethanol with periodic shaking for 48 hours. After 48 hours plant extracts were purified and kept in fumigator for evaporating the solvent. 50% ethanol and itraconazole were used as control. The aim of this study is to identify potential natural sources of antifungal compounds that could be used in the development of eco-friendly and sustainable strategies for the management of this pathogen. The plant extracts were evaluated for their inhibitory effects on the mycelial growth and spore germination of *Macrophomina phaseolina*. Our results demonstrate that *Macrophomina phaseolina* was resistant to the plant extracts and the commercial antifungal agent tested.

Keywords
Macrophomina phaseolina, Charcoal rot, Antifungal activity, Plant extracts, Natural fungicides, Disease management

INTRODUCTION

Macrophomina phaseolina, commonly known as charcoal rot fungus, is a devastating plant pathogen with a wide host range encompassing over 500 plant species, including important crops like soybeans, maize, sorghum, and cotton. It thrives in warm, arid climates and is particularly troublesome in regions with drought stress. This fungus infects plants through their roots, where it colonizes and eventually obstructs the vascular system, leading to wilting, stunted growth, and often death. The pathogen survives in soil and plant debris for extended periods, making crop rotation less effective as a control measure. Furthermore, *M. phaseolina* produces numerous resistant structures called microsclerotia, enabling it to persist in adverse conditions. Effective management strategies include optimizing irrigation practices, using resistant plant varieties, and employing biological control agents. Additionally, integrated approaches involving cultural, chemical, and genetic methods are crucial for mitigating the impact of this destructive plant pathogen on global agriculture [1-4].

Fungi play a significant role as plant pathogens, causing a wide array of diseases that impact agricultural productivity and natural ecosystems. These microscopic organisms have evolved intricate mechanisms to infiltrate, colonize, and extract nutrients from plant tissues. Common fungal pathogens include species like *Fusarium*, *Phytophthora*, and powdery mildews. One key characteristic of fungal plant pathogens is their ability to produce specialized structures, such as spores or mycelium, which facilitate their dispersal and infection. They often enter plants through natural openings, wounds, or by secreting enzymes that break down the plant's protective barriers. Once inside, fungi can rapidly multiply and spread, leading to the development of visible symptoms like wilting, lesions, or the characteristic powdery growth seen in powdery mildew infections. Fungal diseases can have devastating effects on crops, leading to reduced yields, economic losses, and in severe cases, crop failure. They can also disrupt natural ecosystems by affecting wild plant species. Managing fungal plant pathogens requires integrated approaches, including cultural practices (crop rotation, sanitation), biological control methods (introducing beneficial fungi or bacteria), and the judicious use of fungicides. Continuous research is essential to understand the biology and ecology of fungal plant pathogens, as well as to develop new and sustainable methods for disease control. Additionally, breeding for disease-resistant plant varieties is a crucial strategy in mitigating the impact of fungal pathogens on agriculture and natural ecosystems. Overall, fungi as plant pathogens represent a significant challenge, but with proper management strategies, their detrimental effects can be minimized [16-18].

Plant extracts have been utilized for their antifungal properties throughout history. Ancient civilizations, like the Egyptians and Greeks, employed substances such as garlic and onion extracts to combat fungal infections. The renowned Greek physician Hippocrates documented the use of vinegar, another plant-based product, for treating fungal skin conditions. In traditional Chinese medicine, herbs like *Sophora flavescens* were used for their antifungal effects. Similarly, indigenous cultures in various parts of the world utilized plants like tea tree oil (from the Melaleuca tree) and neem (*Azadirachta indica*) for their potent antifungal properties. These practices were often based on empirical knowledge passed down through generations. Over time, scientific research has validated many of these traditional uses, leading to the development of modern antifungal medications derived from or inspired by plant compounds. Today, plant-based extracts continue to be a valuable source for antifungal agents in both traditional and modern medicine [5-8]. In the present study, we investigated the antifungal activity of eight plant extracts viz., *Coriandrum sativum, Cymbopogon citratus, Eucalyptus camaldulensis, Mangifera indica, Mentha piperita L, Moringa oleifera, Psidium guajava L, Trigonella foenum* against *Macrophomina phaseolina* by agar well diffusion method.

MATERIALS AND METHODS

Collection and Preparation of Plant Extracts

Leaf samples of eight plants viz., *Coriandrum sativum, Cymbopogon citratus, Eucalyptus teriticornis, Mangifera indica, Mentha piperita L, Moringa oleifera, Psidium guajava L, Trigonella foenum* were collected from local market, Bengaluru. Plant extracts were prepared by drying the washed plant samples followed by grinding it to fine powder, sieving and soaking in 50% ethanol with periodic shaking for 48 hours. After 48 hours plant extracts were purified and kept in fumigator for evaporating the solvent.

Fungal Isolate

Macrophomina phaseolina was isolated from watermelon seeds by agar plate method and identified with the help of sporulation characteristics using direct microscopy.

In vitro Antifungal Assays

Potato dextrose agar media was prepared and autoclaved at 250°F (121°C) temperature and 15 pounds per square inch pressure for 30–60 minutes. Agar plates were prepared in sterilized glass petri plates and 3 wells of 10 mm each were prepared using sterile cork borer. Plant extract, ethanol and itraconazole were loaded in the wells using the sterile micropipette. Spores of *Macrophomina phaseolina* were inoculated in the centre and sealed with cling wrap. Plates were incubated at 20-25 °C for seven days. The plates were observed daily and the observations were recorded till seven days.

RESULTS AND DISCUSSION

Results demonstrate that *Macrophomina phaseolina* was resistant to all the eight plant extracts viz., *Coriandrum sativum, Cymbopogon citratus, Eucalyptus camaldulensis, Mangifera indica, Mentha piperita L, Moringa oleifera, Psidium guajava L, Trigonella foenum* and the commercial antifungal agent (itraconazole) tested.

Table 1 Antifumgal activity 8 plant extracts against *Macrophomina phaseolina*

Plant samples	Zone of inhibition exhibited by plant samples	Zone of inhibition exhibited by 50% ethanol	Zone of inhibition exhibited by itraconazole
Coriandrum sativum	0 mm	0 mm	0 mm
Cymbopogon citratus	0 mm	0 mm	0 mm
Eucalyptus camaldulensis	0 mm	0 mm	0 mm
Mangifera indica	0 mm	0 mm	0 mm
Mentha piperita L	0 mm	0 mm	0 mm
Moringa oleifera	0 mm	0 mm	0 mm
Psidium guajava L	0 mm	0 mm	0 mm
Trigonella foenum	0 mm	0 mm	0 mm

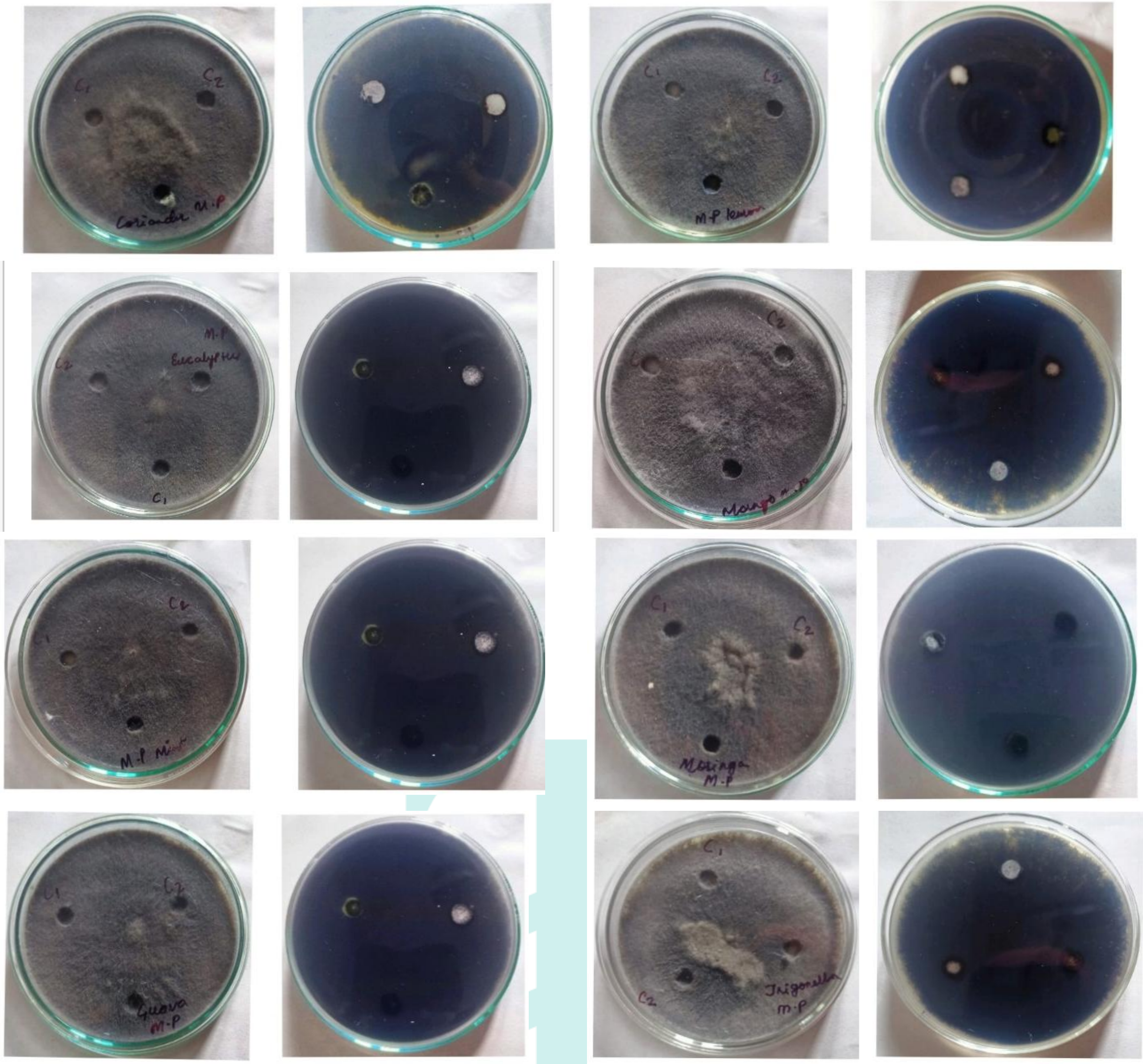

Fig. 1 Antifumgal activity 8 plant extracts against *Macrophomina phaseolina*

Silva et al. [9] had studied the antifungal activity of *Coriandrum sativum* against *Candida* species. Results demonstrated very good antifungal potential against *Macrophomina phaseolina*. This result is in contrast with our result where *Coriandrum sativum* was not potent in inhibiting the mycelial growth of *Macrophomina phaseolina*. From the study of Bily Nebie et al. [10] antifungal activity of *Cymbopogon citratus* and *Eucalyptus camaldulensis* was revealed. This result is in contrast with our result where *Cymbopogon citratus* and *Eucalyptus camaldulensis* were was not successful in inhibiting the mycelial growth of *Macrophomina phaseolina*. Kanwal et al. [11] had evaluated antifungal activity against five fungal species, namely *Alternaria alternata (Fr.)* Keissler, *Aspergillus fumigatus Fresenius, Aspergillus niger van Tieghem, Macrophomina phaseolina* (Tassi) Goid. And *Penicillium citrii*. Results revealed the strong antifungal potential of *Mangifera indica* against these fungal pathogens. These results are also in contrast with our results were *Mangifera indica* was not able to inhibit the mycelial growth of *Macrophomina phaseolina*. From the results of Mohammad Moghaddam et al. [12] antifungal activity of *Mentha piperita* against *Macrophomina phaseolina* was revealed. This result is also in contrast with our results were *Mentha piperita* was not potent in inhibiting the mycelial growth of *Macrophomina phaseolina*. Riad et al. [13] had studied the antifungal activities of *Moringa oleifera* oil and seed extract against seven plant pathogenic fungi i.e., *Fusarium oxysporum, Fusarium solani, Alternaria solani, Alternaria alternate, Rhizoctonia solani , Sclerotium rolfsii* and *Macrophomina phaseolina* . Results clearly showed that seed extracts and oil of *Moringa oleifera* significantly reduced linear growth, spore germination and dry growth weight of all tested pathogens. This result is also in contrast with our results were *Moringa oleifera* was not potent in inhibiting the mycelial growth of *Macrophomina phaseolina*. In the study of Beatriz et al. [14] *Psidium guajava* leaves were studied for their antifungal properties against *Trichophyton rubrum, Trichophyton tonsurans, Sporotrix schenckii, Microsporum canis, Cryptococcus neoformans, Candida parapsilosis* and *Candida albicans* by using the agar disk diffusion technique. Results demonstrated strong antifungal activity of *Psidium guajava* against these fungal pathogens. This result is also in contrast with our results were *Psidium guajava* was not potent in inhibiting the mycelial growth of *Macrophomina phaseolina*. From the study of Omezzine et al. [15] antifungal activity of *Trigonella foenum* against *Fusarium oxysporum*. This result is also in contrast with our results were *Trigonella foenum* was not potent in inhibiting the mycelial growth of *Macrophomina phaseolina*.

Macrophomina phaseolina, commonly known as charcoal rot fungus, is a destructive plant pathogen that affects a wide range of crops. It exhibits high resistance to natural antifungal agents, posing a significant challenge for agricultural practices. This resistance is attributed to the fungus's ability to rapidly adapt to various environmental conditions and its capacity to produce secondary metabolites that counteract antifungal compounds. To combat this issue, a multi-faceted approach is required. Firstly, crop rotation strategies can be employed to break the pathogen's life cycle and reduce its buildup in the soil. Additionally, utilizing resistant crop varieties and employing biological control agents, such as beneficial fungi or bacteria, can help suppress *M. phaseolina*. Implementing good agricultural practices like proper irrigation management and optimizing plant nutrition can also enhance plant vigor and resilience. Furthermore, integrated disease management (IDM) practices should be adopted, combining cultural, biological, and chemical control methods. This may involve the use of biocontrol agents, organic amendments, and targeted fungicides in a balanced and sustainable manner. Research into novel antifungal compounds or genetic approaches to enhance plant resistance could also offer promising avenues for combating the challenges posed by *M. phaseolina*. Overall, a holistic and adaptable strategy is essential to effectively manage this resilient fungal pathogen.

CONCLUSION

In conclusion, *Macrophomina phaseolina*, the charcoal rot fungus, presents a significant threat to a wide range of crops, leading to substantial yield losses. This study investigated the antifungal potential of eight plant extracts against *M. phaseolina*, aiming to identify natural sources of antifungal compounds for sustainable pathogen management. However, the results showed resistance of *M. phaseolina* to all tested plant extracts and the commercial antifungal agent, itraconazole. These findings highlight the formidable challenge posed by *M. phaseolina*'s resistance to natural antifungal agents. To combat this issue, a comprehensive approach is crucial. Strategies such as crop rotation, utilizing resistant varieties, and employing biological control agents should be employed. Additionally, integrating cultural, biological, and chemical control methods is essential for effective disease management. While this study did not yield the desired outcomes, it underscores the complexity of combatting resilient pathogens like *M. phaseolina*. Further research into alternative antifungal compounds and innovative approaches to enhance plant resistance remains imperative. By adopting a holistic and adaptable strategy, we can strive towards more effective management of this destructive fungal pathogen and safeguard global agriculture.

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