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Assessment of plant-derived essential oils as sustainable antifungal agents for managing *Macrophomina phaseolina* causing root and stem rot in sesame

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Abstract
Root and stem rot caused by *Macrophomina phaseolina* represents a significant agricultural challenge, spreading through contaminated seeds and soil-borne residual materials that persist in cropping systems. This soil borne pathogen poses a substantial economic threat to Indian agriculture, with yield losses ranging from 5-100% under epidemic conditions, making it one of the most destructive diseases affecting multiple crop species. The growing emphasis on eco-friendly pest management has driven researchers to explore sustainable alternatives to synthetic fungicides. Among various plant-based control strategies, essential oils have emerged as promising bioactive compounds with significant antifungal potential. These volatile, odoriferous organic compounds represent complex mixtures of secondary plant metabolites extracted from leaves, bark, stems, and other plant tissues. Essential oils demonstrate broad-spectrum biological activities, including antibacterial, insecticidal, and antimicrobial properties, positioning them as valuable tools for integrated disease management. The multifaceted nature of essential oils makes them particularly effective against phytopathogenic fungi like *M. phaseolina* (synonym: *Rhizoctonia bataticola*). Their volatile compounds can disrupt fungal cell membranes, interfere with enzymatic processes, and inhibit spore germination and mycelial growth. Unlike synthetic fungicides, essential oils typically exhibit lower environmental persistence and reduced risk of resistance development. This study focuses on evaluating various essential oils for their efficacy against *M. phaseolina* under controlled *in vitro* conditions. This research contributes to the broader goal of developing environmentally compatible disease management strategies that reduce dependence on synthetic chemicals while maintaining effective pathogen control.

Keywords: *Macrophomina phaseolina*, essential oils, antifungal, bio-control, sustainable agriculture, pathogen management

Introduction
Sesame (*Seasmum indicum* L.) is commonly known as ‘Til’ also called as ‘Queen of oil seeds’. Sesame seeds contain a high amount of edible oil (46-52%), mainly oleic acid (47%) and linoleic acid (39%), which helps to lower cholesterol (Shyu and Hwang, 2002) [1]. In India, sesame is cultivated over an area of 15.23 lakh ha^{-1} in India, with a total production of 527 kg/ha during 2022-23 (Vishwakarma *et al*., 2024) [2]. The major sesame growing districts in India are primarily located in the states of Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, West Bengal, Tamil Nadu, and Andhra Pradesh. The primary reason for the low productivity of Sesame is the influence of several biotic and abiotic factors. Biotic factors include various pests and diseases *viz*., root & stem rot, *Alternaria* leaf spot, Bacterial blight, Powdery mildew, *Cercospora leaf* spot, and Sesame phyllody (Gupta *et al*., 2018) [3]. Among these are root & stem rot of Sesame, which is caused by *Macrophomina phaseolina* (Tassi.) Goid. (Sclertoial stage-*Rhizoctonia bataticola*), is one of the most devastating diseases. It affects the crop at almost all stages of its growth, particularly during the flowering to capsule initiation stage which drastically reduce germination and seedling stand (Indra, 2020) [4]. *M. phaseolina* was considered as very destructive pathogen in all sesame growing areas and causes 15-100% yield loss (Thirunarayanan *et al*., 2017) [5]. *Macrophomina phaseolina*

(Tassi). Goid. soil inhabiting pathogen, attacks many host plants including oilseeds, pulses, vegetables and ornamentals (Bandopadhyay *et al*., 2022) [6]. The most common symptoms of the disease is sudden wilting of growing plant mainly after the flowering stage, stem portion near the ground level show dark brown and dark black lesion at the collar region show shredding and to destroy the vascular bundles leading to plant death. Stem portion can be easily pulled out leaving the rotten rot portion in the soil which helps pathogen to survive in debris over long period of time (Avila *et al*., 1999) [7]. The pathogen is usually soil borne but can also be found in seed testa, where microscopic sclerotia bodies adhere to it (Mishra *et al*., 2024) [8]. Management strategies used against stem and root rot of sesame is challenging for farmers due to its soil borne nature thus production of sclerotia and difficult to apply control measures (Nasari, 2008) [9]. The conventional synthetic chemicals have raised ecological problems due to their high cost as well as adverse effect on environment and may induce resistance in the pathogen (Rathmell, 1984) [10]. Keeping these facts in mind attempts have been made to control this fungus by natural extracts especially by essential oils namely Peppermint, tea tree, rosemary, geranium, basil, clove, thyme, lemongrass, eucalyptus. Many volatile oils of many plants species possess significant biological activity against agriculturally important microbes and insect pests (Singh and Pant, 2001) [11]. Essential oils represent very complex mixture of compounds mainly monoterpenes and sesquiterpenes (Letessier *et al*., 2001) [12]. Essential oils are known to possess a variety of biological properties including antimicrobial activity (Dubey *et al*., 2000) [13]. There are reports on the screening of essential oils against many phytopathogenic fungi (Dubey, 2001) [14]. In the present study an attempt study has been made to evaluate fungitoxic properties of some oils for the successful, safe and ecofriendly control of sore shin pathogen under *in vitro* conditions.

Material and Methods

Evaluation of essential oils against *Macrophomina phaseolina*

The experiment was laid out as per details given below-
Design-CRD, Replication-3, Treatments-10, Method-Poisoned food Technique, Dose (µl)-100,250

Methodology

For the management of *Macrophomina phaseolina*, nine different essential oils *viz*., Peppermint, Tea tree, Rosemary, Geranium, Basil, Clove, Thyme, Lemon grass & Eucalyptus were evaluated. All essential oils used in this study were purchased from Natural Biotech Solutions, India and listed in Table-3.18. For the *in vitro* antifungal activity test, essential oils were diluted in acetone and 100 & 250 µl of each dilution was mixed into the PDA and was poured into 90 mm Perti Plate. The culture agar disc of actively growing test fungus was placed in the centre of the Petri Plate and plates were incubated at 28±2 °C for 7 days till full plate growth in control plate.

Observations to be recorded

Mean colony diameter at 3, 5 & 7 days, sclerotial production & percent inhibition over control was calculated as suggested by Vincent (1947).

$$I = C\text{-}T/C \times 100$$

I = percent inhibition
C= radial growth measurement of the pathogen in control plate
T = radial growth measurement of the pathogen in treatment plate

Results and Discussion

A total of nine essential oils were tested for antifungal activities as volatile compound against *M. phaseolina* at 100 and 250 µl concentration. Result shows that all nine essential oils showed inhibitory activities against mycelial growth of *M. phaseolina* at both concentrations. At 100 µl concentration treatment T_8 (Lemongrass) was found most effective cent percent fungal growth inhibition over control after 7 days with no sclerotia formation (-). After lemongrass, T_6 (Clove) found to be effective having 93.33 percent fungal growth inhibition over control with poor sclerotia formation (+), followed by T_1 (87.77%), T_2 (80.00%), T_3 (73.88%), T_5 (33.52%), T9 (25.37%), and minimum percent fungal growth inhibition over control in T_4 (12.77%). Fair sclerotia produced in case of T_2, T_3 and T_7 (++). Treatment T_5 and T_9 produces good sclerotia (+++). Treatment T_4 produces excellent sclerotia (++++). At 250 µl concentration treatment T_8 (Lemongrass) and T_6 (Clove) were found to be most effective having cent percent fungal growth inhibition over control after 7 days with no sclerotia formation (-), followed by T_2 (93.52%), T_1 (91.48%), T_3 (84.07%), T_7 (81.66%), T_5 (49.07%), T_9 (39.44%), and minimum fungal growth inhibition was found in T_4 (28.88%). Fair sclerotia produced in case of T_3 and T_7 (++). Treatment T_5 and T_9 produce good sclerotia (+++). Treatment T_4 produces excellent sclerotia (++++).

Fig 1: Symptoms of root & stem rot of sesame

Table 2: Evaluation of different essential oils against *M. phaseolina* at different concentration under *in vitro* condition

Treatment	Treatment Details	100 µl			250 µl		
		*Colony diameter (mm)	% inhibition over control	Sclerotia density	*Colony diameter (mm)	% inhibition over control	Sclerotia density
T_1	Peppermint	11.00 (19.34)	87.77	+	7.66 (16.04)	91.48	+
T_2	Tea tree	18.00 (25.08)	80.00	++	5.83 (13.96)	93.52	+
T_3	Rosemary	23.50 (28.98)	73.88	++	14.33 (22.22)	84.07	++
T_4	Geranium	48.16 (43.93)	48.33	+++	36.83 (53.11)	59.07	+++
T_5	Basil	59.83 (50.65)	33.52	++++	45.83 (42.59)	49.07	++++
T_6	Clove	6.00 (14.16)	93.33	+	0.00 (0.00)	100.00	-
T_7	Thyme	25.16 (30.09)	72.04	++	16.50 (23.94)	81.66	++
T_8	Lemon grass	0.00 (0.00)	100	-	0.00 (0.00)	100.00	-
T_9	Eucalyptus	67.16 (55.01)	25.37	+++	54.50 (47.56)	39.44	+++
T_{10}	Control	90.00 (71.53)	-	++++	90.00 (71.53)	-	++++
SE (m)±		1.240			1.337		
CD (p=0.05)		0.417			0.450		

*Average of three replication; Parenthesis are angular transformed value

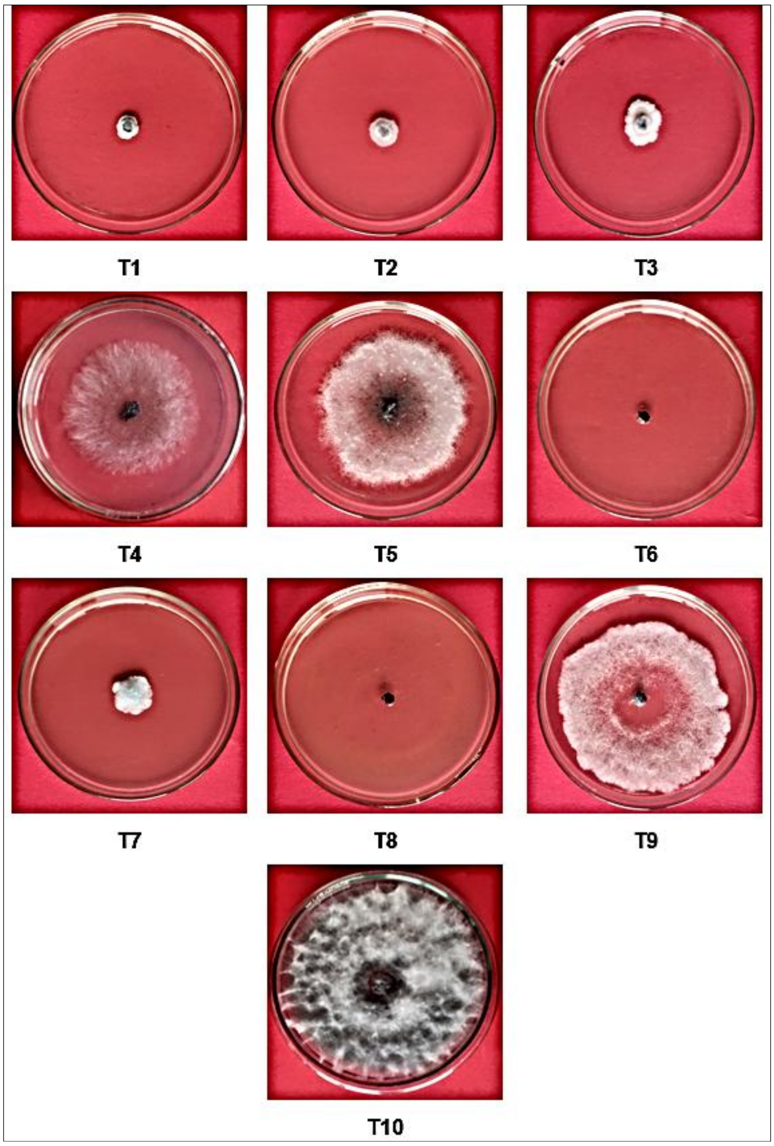

Fig 2: Evaluation of different essential oils against *M. phaseolina* at 100 µl concentration

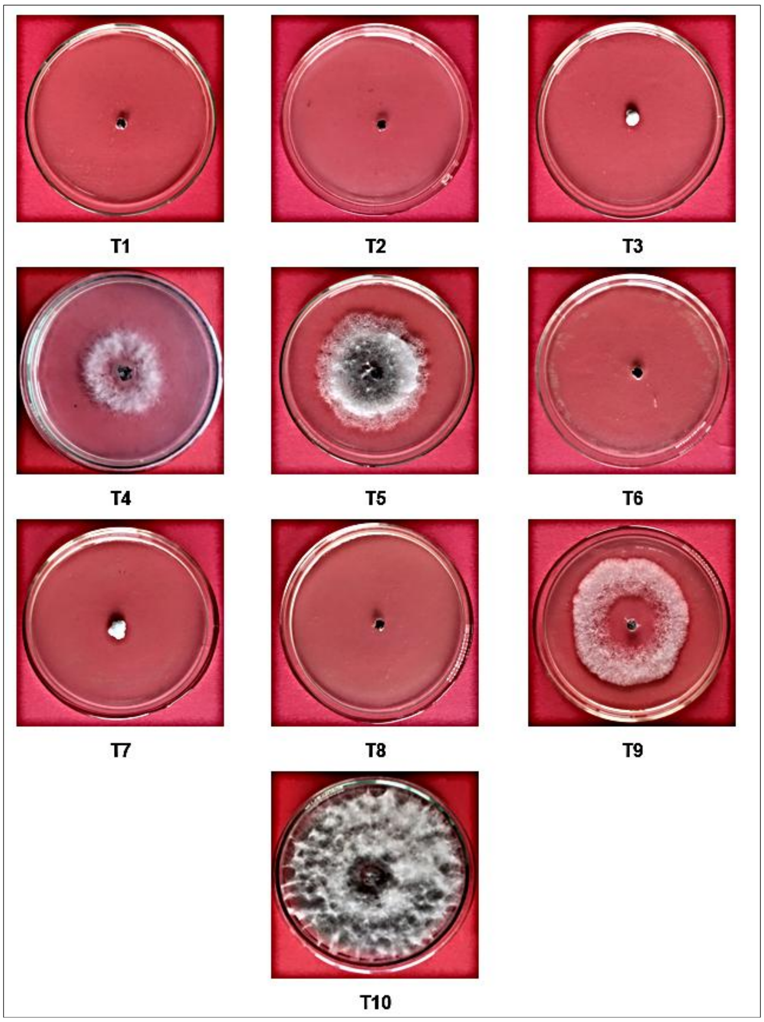

Fig 3: Evaluation of different essential oils against *M. phaseolina* at 250 µl concentration

Conclusion

The current work depicts the antifungal activities of the essential oils obtained from the plants. Essential oils are regarded as GRAS (generally regarded as safe) grade chemicals. Additionally, because of their pleasant fragrance, they can even be used in food applications. But mere laboratory analysis and formulations cannot make up the demand for controlling pathogenic fungi in an eco-friendly way. The results of this study show that all the EOs and major components of EOs tested possess antifungal activity, being able to inhibit radial growth of *M. phaseolina* (*R. btaticola*). Nine essential oils were assessed against *M. phaseolina* under laboratory and different treatments found significantly superior over control. The lemon grass essential oil was found best among all treatments with mycelia growth inhibition of cent percent at 100 and 250µl concentration, followed by clove essential oil with mycelia growth inhibition of 93.33 and cent percent at 100 and 250 µl concentration.

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