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Eco-friendly Management of Charcoal Rot of Sesame Caused by *Macrophomina phaseolina* (Tassi) Goid

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Authors' contributions

This work was carried out in collaboration among all authors. Author PV designed the study, performed the statistical analysis, and wrote the protocol. Authors HSS and ASR managed the analysis of the study and helped in writing the protocol. All authors read and approved the final manuscript.

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ABSTRACT

The most damaging soil and seed-borne disease of sesame (*Sesamum indicum* L.), which appears every year in Haryana and causes significant output losses, is charcoal rot disease, which is caused by *Macrophomina phaseolina* (Tassi) Goid.

Aim: The current experiment was conducted to learn more about the environmentally friendly management of *M. phaseolina* using botanicals and bio-agents, under in vitro and in vivo conditions in the Department of Plant Pathology, CCSHAU, Hisar.

Methodology: Under in vitro circumstances, the effectiveness of botanicals was assessed using the poison food technique. Seed treatment prior to sowing under screenhouse conditions was used to examine the effectiveness of both the botanicals and bio-agents.

Results: The phytoextract of Lantana camara, among the botanicals tested for its effectiveness under in vitro conditions against *M. phaseolina*, inhibited maximum mycelial growth by 89.43% at a 20% concentration, followed by extract of *Parthenium hysterophorus* and garlic (*Allium sativum*), which inhibited up to 87.21 and 57.21%, respectively, at the same concentration. But ginger (*Zingiber officinale*), which only inhibited mycelial development by 26.94% at a 20% concentration, was shown to be the least effective. The most effective combination of phytoextracts and bio-agents for controlling the disease up to 36.43 percent in the HT-1 variety and 40.92 percent in the HT-2 variety was *P. hysterophorus* + *T. harzianum*, which controlled the disease up to 34.28 and

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38.53 per cent, respectively, among the combinations of phytoextracts and bioagents tested under screen house conditions.

Keywords: Botanicals; charcoal rot; disease incidence; growth inhibition; Macrophomina phaseolina.

1. INTRODUCTION

Sesame (*Sesamum indicum* L.), one of the most significant edible oilseed crops, sometimes referred to as til, has significant religious, biomedical, and nutritional value. Among oilseed crops, it is also referred to as the "Queen of Oilseeds." The highest oil content is found in sesame, which has 6335 kcal/kg of nutritional energy in its seeds [1]. With a contribution of around 17.77 million hectares and production and productivity of 8 million tonnes and 448 kg/ha, respectively, India contributes the second-largest sesame acreage [2]. A total of 12–15% of the world's oilseeds are grown in India, and 8% are produced there. Sesame is farmed in Haryana during the kharif season on roughly 1600 hectares, with yields of 700 tonnes and 500 kg/ha, respectively [2].

In addition to being a good source of the vitamins A, B_1, B_2, B_3 and E as well as minerals like calcium and phosphorus, seeds are also a good supply of oil (about 50%), proteins (18–20%), and around 85% unsaturated fatty acids, with about 47% of the oil being oleic acid and 9% being linolenic acid [3]. Charcoal rot (*Macrophomina phaseolina*), fusarium wilt (*Fusarium oxysporum*), phytophthora blight (*Phytophthora parasitica*), and phyllody (phytoplasma) are some of the significant diseases of sesame. Haryana's successful sesame farming has been seriously threatened by the charcoal rot disease *Macrophomina phaseolina*. According to Vyas [4], the disease can result in losses ranging from 5 to 100 percent. Maiti *et al*., [5] found a 57 percent yield loss and a 40 percent disease incidence.

The fungus has become a potential threat for the profitable cultivation especially in the changing warm climate and intensive farming situations [6]. The most common symptoms of the disease are the sudden wilting of the plants throughout the crop growth mainly after the flowering phase. The pathogen attacks mostly at the basal region of the plant [7]. The seed borne nature of the fungus has been reported [8 9,10] and it is responsible for seed rot. It also causes seedling decay, stem-discoloration and root rot [11,12,13]. Infected seedlings show a brown discoloration at the soil line extending up the stem that may turn brown to black.

In Haryana, areas of the sesame disease known as charcoal rot used to occur each year in farmers fields at various stages of growth. Usually going unreported, the disease results in a full crop loss at once because it is not affordable nor practical to control it. Since the disease is carried through minor genes, vertical resistance breeding efforts cannot utilise sources of resistance. Additionally, employing pesticides to address plant diseases creates severe issues with food safety, environmental safety, and pesticide resistance, necessitating the use of alternate disease management methods. It has become vital to investigate botanicals and bioagents for their efficiency against this infection under in vitro and in vivo circumstances due to the importance of the illness in recent years in Haryana as a result of the buildup of high inoculum in soil and to avoid soil pollution through pesticides.

2. MATERIALS AND METHODS

2.1 Glasswares and Cleaning

Sterilized glassware was utilised to conduct the tests in the lab, including test tubes, conical flasks, and petri plates. Prior to use, they were soaked for 24 hours in a cleaning solution made of 1000 ml of water, 60 g of potassium dichromate, and 60 ml of strong sulfuric acid. After that, detergent, tap water, and lastly distilled water were used to wash the glasses. The glassware was then sanitized by being kept in a hot air oven for two hours at 180^0 C.

2.2 Preparation of Media

2.2.1 Potato dextrose agar (PDA)

In all experiments studies, the standard potato dextrose agar medium was used with the following composition:

Peeled potato	200 g
Dextrose	20 g
Agar-Agar	20 g
Distilled water	1000 ml (to make up the volume)

(In case of Double strength medium, the above composition was doubled in 1000 ml of distilled water).

Potato tubers weighing 200 gm that had been cleaned, washed, and peeled were cut into tiny pieces. Later, the extract from these pieces was extracted by boiling them in distilled water and shifting it through muslin cloth. 20 g of agar-agar was dissolved in approximately 400 ml of distilled water. A total 20 g of dextrose were dissolved in the mixture, which was continuously stirred throughout the solution's boiling process to prevent the formation of clots. It was combined with the potato extract, and then distilled water was used to increase the volume to 1000 ml. The ideal amount of medium was added to a conical flask, which was then sealed with non-absorbent cotton. Rubber bands were used to wrap brown paper around the flask. The autoclave was used to sterilise the flasks containing the dispensed media for 20 minutes at 15 psi.

2.2.2 Isolation, purification and multiplication of culture

Sesame (*Sesamum indicum* L.) plants showing typical charcoal rot symptoms were collected from Oilseed Research Area of CCSHAU, Hisar. The isolation of fungus was done by following the standard isolation technique. The parts of root and stem which were showing the symptoms were washed in running tap water and cut into small bits. The surface sterilization of bits was done with the help of 0.1 per cent mercuric chloride solution or sodium hypochlorite solution for 30 seconds and were washed thoroughly in sterilized distilled water for three times to remove traces of mercuric chloride and then aseptically transferred to sterilized potato dextrose agar (PDA) plates and were incubated at 27^0 C for three days for fungal growth. Later, the bit of mycelium was transferred on PDA slants. The pure culture of fungus was also obtained by following the hyphal tip method [14]. After 7 days, pure culture was obtained and it was maintained at 4^0 C for further studies.

2.3 Evaluation of Botanicals

The effect of ten botanicals on the growth of *M. phaseolina* was studied using poison food technique (Mayer, 1962). The botanicals, given in table were used at different concentrations viz., 5%, 10%, 15%, 20%. The double strength potato dextrose agar (PDA) medium was prepared and sterilized at 15 psi for 20 minutes. An equal volume of double strength phytoextracts solution and double strength PDA were mixed in a sterilized conical flask to achieve the final concentration and 20 ml of the solution was poured aseptically in to 90 mm sterilized petri plates. Upon solidification, each plate was centrally inoculated with five mm disc of mycelium obtained from seven days old culture of *M. phaseolina* and incubated at 27^0 C till the plate was filled with mycelial growth in control. Four replications were maintained for each treatment in completely randomized design (CRD). Potato dextrose agar medium without any of the botanicals served as control.

2.4 Observations Recorded

2.4.1 Colony diameter

By measuring the size of the fungal colony both horizontally and vertically, and using the mean of these two measurements, the colony diameter of fungus was reported in metric scale (mm). Radial growth of the pathogen was determined by the colony diameter divided in half.

2.4.2 Per cent growth inhibition

Colony diameter of the fungus of each treatment along with control was measured (mm) and recorded after every 24 hours, till the test fungus occupied the full petri plate in the control. The per cent inhibition of mycelial growth over control was calculated by Vincent formula [15].

2.5 Evaluation of Combined Effect of Effective Bio-agents and Botanicals under Screen House Conditions

The integrated effect of bio-agents and botanicals was evaluated to formulate the suitable eco-friendly management strategy to control charcoal rot disease *in vivo* under screen house conditions. Earthen pots were filled with sterilized sandy loam soils @ of 3 kg soil/ pot. Upper one cm layer of soil in pot was inoculated with 30 ml of mycelial suspension (15 mg/L water). Seeds of cultivars (HT-1 and HT-2) were soaked in 20% concentration of each plant extract for 5-10 minutes and after drying in shade seeds were treated with bio-agents @ 10 g/kg seed. Five plants per pot were grown in artificially inoculated soil. Four replications of the below mentioned treatments were maintained as CRD and un-inoculated pots were also maintained as control. Then per cent disease incidence was recorded after 15 days interval.

2.6 Treatments

T_1 : *Trichoderma harzianum* @10 g/ kg seed – ST
T_2 : *Trichoderma viridae* @ 10 g/ kg seed - ST
T_3 : *Pseudomonas fluorescence* @10 g/ kg seed - ST
T_4 : Seeds soaking in first most effective plant extract (20%) before sowing
T_5 : Seeds soaking in second most effective plant extract (20%) before sowing
T_6 : Seeds soaking in third most effective plant extract (20%) before sowing
T_7 : *Trichoderma harzianum* + First most effective botanical
T_8 : *Trichoderma viridae* + Second most effective botanical
T_9 : *Pseudomonas fluorescence* + Third most effective botanical
T_{10} : Control

2.7 Observations

Per cent disease incidence was recorded by using the following formula.

$$\text{Percent disease incidence} = \frac{\text{Number of diseased plants X } 100}{\text{Total number of plants}}$$

3. RESULTS AND DISCUSSION

3.1 Efficacy of Botanicals *in vitro*

Efficacy of botanicals was tested *in vitro* for the per cent inhibition of mycelial growth of *M. phaseolina*. The data in the Table 2 clearly revealed that *L. camara* and *P. hysterophorus* phytoextracts inhibited the mycelial growth up to 80.92 and 78.32%, respectively even at 5% concentration. Maximum inhibition of mycelial growth was shown by *L. camara* (89.43%) followed by *P. hysterophorus* (87.21%) at highest concentration (20%) of phytoextract used. Ginger (26.94%) and *Boungianvillea* (28.60%) exhibited least and statistically equal inhibition of mycelial growth even at 20% concentration. Rest of phytoextracts used, showed intermediate effect on mycelial growth inhibition of *M. phaseolina* under laboratory conditions. (Plate 1, 2, 3

In vivo evaluation of combined effect of botanicals and bio-agents against *Macrophomina phaseolina.*

In screen house conditions the integrated effect of bio-agents and botanicals was evaluated to formulate the suitable eco-friendly management strategy to control charcoal rot disease. Seeds of susceptible cultivars HT-1 and HT-2 were sown in artificial inoculated soil in pots under screen house conditions. Among the bio-agents, seed treatment with *Trichodema harzianum* alone was found most effective as charcoal rot incidence found least as 60.33% in HT-1 and 59.66% in HT-2, whereas among the botanicals, seed soaking with the phytoextract of *Lantana camara was found most effective* where disease incidence was 63.14% for HT-1 and 60.33% for HT-2 varieties. Combined treatment of *T. harzianum* and *L. camara* was found most effective, as disease incidence in both the varieties were observed less i.e. 57.1% and 45.74% in cultivar HT-1 and HT-2, respectively, followed by combination of *T. harzianum* and *P. hysterophorus* where disease incidence was 59.25% for HT-1 and 48.16% for HT-2 as compared to the control. Likewise, the combination of *T. harzianum* + *L. camara* was also found most effective in reducing the charcoal rot disease, as disease control was 36.43% and 40.92% in HT-1 and HT-2 cultivars respectively, followed by combination of *T. harzianum* + *P. hysterophorus* where disease control was 34.28% in HT-1 and 38.53% in HT-2 cultivar.

Table 1. List of botanicals

Sr. No.	Common Name	Scientific Name
1.	Neem	*Azadirachta indica*
2.	Garlic	*Allium sativum*
3.	Mehandi	*Lawsonia inermis*
4.	Ginger	*Zingiber officinale*
5.	*Parthenium*	*Parthenium hysterophorus*
6.	Datura	*Datura stramonium*
7.	Turmeric	*Curcuma longa*
8.	*Bougainvillea*	*Bougainvillea glabra*
9.	Aloevera	*Aloe vera*
10.	*Lantana*	*Lantana camara*

Table 2. Efficacy of different botanicals against *Macrophomina phaseolina* under *in vitro* conditions

Botanicals	*Per cent mycelial growth inhibition at different Concentrations (%)				
	5	10	15	20	Mean
Neem	28.32 (32.13)	30.55 (33.53)	33.88 (35.57)	45.27 (42.26)	34.50 (35.87)
Garlic	46.1 (42.74)	48.32 (44.02)	51.38 (45.77)	57.21 (49.13)	50.75 (45.42)
Mehandi	29.99 (33.18)	34.16 (35.74)	37.77 (37.9)	44.16 (41.62)	36.52 (37.11)
Ginger	15.27 (22.96)	16.38 (23.86)	21.66 (27.72)	26.94 (31.17)	20.06 (26.43)
Parthenium	78.32 (62.26)	80.83 (64.04)	83.88 (66.36)	87.21 (69.04)	82.56 (65.42)
Datura	39.25 (38.77)	49.16 (44.50)	51.38 (45.77)	55.27 (48.00)	48.76 (44.26)
Turmeric	40.83 (39.69)	48.60 (44.18)	51.66 (45.93)	58.88 (50.10)	49.99 (44.98)
Bougainvillea	19.16 (25.93)	23.6 (29.05)	26.38 (30.86)	28.6 (32.29)	24.43 (29.53)
Aloe vera	23.85 (29.23)	28.6 (32.31)	31.66 (34.22)	36.1 (36.89)	30.05 (33.16)
Lantana	80.92 (64.10)	83.6 (66.13)	86.38 (68.35)	89.43 (71.06)	85.0 (67.41)
Mean	40.20 (39.10)	41.52 (41.73)	47.60 (43.84)	52.90(47.16)	
	C.D. (p=0.5)		SE(m)±		
Treatment(T)	1.03		0.37		
Concentration (C)	0.65		0.23		

**Mean of four replications. The figure in parenthesis is angular transformed value*

Table 3. *In vivo* evaluation of combined effect of botanicals and bio-agents against *Macrophomina phaseolina*

	Disease Incidence in %			
	HT-1		HT-2	
T1- *T. harzianum* (ST)	60.33(51.05)	33.2	59.66 (50.66)	27.00
T2 - *T. viride*(ST)	63.92(53.40)	29.61	61.86 (52.04)	24.8
T3 - *P. fluorescens*(ST)	88.12(73.50)	5.41	85.45(68.05)	1.21
T4 - *L. camara* (Seed soaking)	63.14(52.78)	30.39	60.33 (51.05)	26.33
T5 - *P. hysterophorus* (Seed soaking)	68.69(56.14)	24.84	63.92 (53.40)	22.74
T6 - Garlic (Seed soaking)	67.1(55.05)	26.43	67.1 (55.05)	19.56
T7 - *T. harzianum* + *L. camara*	57.1 (49.18)	36.43	45.74 (42.51)	40.92
T8 - *T. harzianum* + *P. hysterophorus*	59.25(50.50)	34.28	48.16 (43.92)	38.53
T9 - *T. harzianum* + *Garlic*	62.33(52.19)	31.2	60.7 (51.65)	25.96
T10 - Control	93.53(77.64)	-	86.66 (71.70)	-

**Mean of three replications.The figure in parenthesis are angular transformed values*

Plate 1

Plate 2

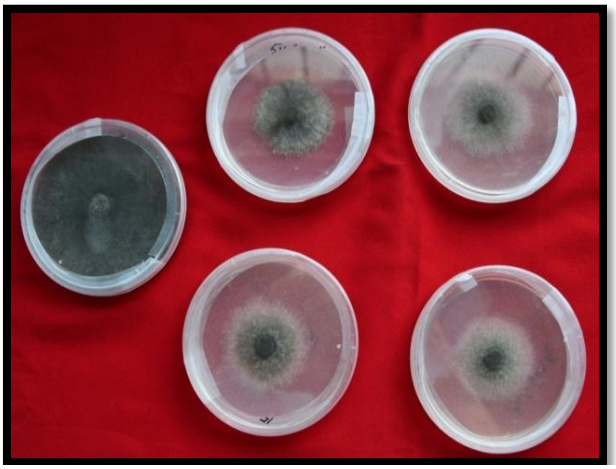

Plate 3

Sesame charcoal rot caused by *Macrophomina phaseolina* can affect all growth stages and can result in pre- and post-emergence seed rot. The disease significantly reduces the output of all cultivars in Haryana. Since the pathogen is soil-borne, it can persist and grow in soil for a long time and is challenging to control, especially in farmer fields with small land holdings where they are unable to implement crop rotation and preventative measures at the appropriate times. A study was done on this illness in order to evaluate the effectiveness of botanicals and bioagents *in vitro* and *in vivo* due to the disease's difficulty in management and lack of genetic resistance in sesame cultivars [16].

4. CONCLUSION

Overuse of pesticides pollutes the environment and raises public awareness of a health risk that affects not only humans and other animals but also all valuable organisms on Earth. This led to the employment of botanicals and bioagents in the present study which is eco-friendly management of *M. phaseolina* in sesame under *in vitro* and *in vivo* circumstances in a screen house. Among the botanicals evaluated the phytoextract of *Lantana camara* inhibited maximum mycelial growth followed by *Parthenium hysterophorus* and garlic (*Allium sativum*) extracts. The combination of seed soaking in *L. camara* extract followed by seed treatment with *T. harzianum* was found most effective in reducing the disease.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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