Original Research Article

Eco-friendly management of charcoal rot of sesame caused by *Macrophomina phaseolina* (Tassi.) Goid

ABSTRACT

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

Aim- Hence, the present investigation was undertaken to study the eco-friendly management of *M. phaseolina* through botanicals and bio-agents under *in vitro* and *in vivo* conditions in screen house of Department of Plant Pathology, CCSHAU, Hisar.

Methodology- The efficacy of botanicals and bio-agents was evaluated by dual culture and paired plate technique under in vitro conditions. The efficacy of both the botanicals and bio-agents was tested by seed treatment before sowing under screenhouse conditions.

Results- Among the botanicals evaluated for their efficacy *in vitro* against *M. phaseolina*, the phytoextract of *Lantana camara* inhibited maximum mycelial growth by 89.43 per cent at 20 per cent concentration followed by *Parthenium hysterophorus* and garlic (*Allium sativum*) extracts which inhibited upto 87.21 and 57.21 per cent, respectively at 20 per cent concentration. However, ginger (*Zingiber officinale*) was found to be least effective in inhibition of mycelial growth by 26.94% at 20% concentration. Among the combinations of phytoextracts and bio-agents tested under screen house conditions, seed soaking in solution of 20 per cent concentration of *L. camara* extract for 5-10 minutes followed by seed treatment with *T. harzianum* @ 10g/kg seed was found most effective in controlling the disease upto 36.43 per cent in HT-1 and 40.92 per cent in HT-2 variety followed by combination of *P. hysterophorus* + *T. harzianum* which controlled the disease up to 34.28 and 38.53 per cent in HT-1 and HT-2 varieties, respectively.

Keywords- Botanicals, Charcoal rot, Disease incidence, Growth inhibition, *Macrophomina phaseolina*

INTRODUCTION

Sesame (*Sesamum indicum* L.*)* commonly known as *til* is one of the important crop among edible oilseed crops having good nutritional, biomedical and religious value. It is also called as "Queen of oilseeds" among the oilseed crops. Sesame ranks first for its higher oil content with 6335 kcal/kg of dietary energy in seeds (Kumar and Goel, 1994). India contributes 2nd largest sesame acreage of above 17.77 million hectare with production and productivity of 8 million tonnes and 448 kg/ha, respectively (Anonymous, 2020). India accounts for 12-15% of oilseeds area, 7-8% of oilseed production. In Haryana, sesame is grown during *kharif* season in nearly 1600 hectares of area with 700 tonnes and 500 kg/ha production and productivity, respectively (Anonymous, 2020).

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Being rich in source of vitamins A, B_1, B_2, B_3, E and minerals including calcium and phosphorus, the seeds are rich source of oil (around 50%), proteins (18-20%) and oil contains of about 85 per cent unsaturated fatty acid having about 47% of oleic acid and 9% of linolenic acid (Shyu and Hwang, 2002).The important diseases of sesame include charcoal rot (*Macrophomina phaseolina*), Fusarium wilt (*Fusarium oxysporum*), Phytophthora blight (*Phytophthora parasitica*) and phyllody (phytoplasma). Charcoal rot caused by *Macrophomina phaseolina* has been a major threat to the successful cultivation of sesame in Haryana. The disease is reported to cause about 5-100% loss (Vyas, 1981), while the estimated yield loss of 57% and about 40% disease incidence had also been reported by Maiti *et al*., (1988).

The fungus has become a potential threat for the profitable cultivation especially in the changing warm climate and intensive farming situations (Saharan *et al.,* 2005). 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 (Kumar *et al*., 2011). The seed borne nature of the fungus has been reported (Javed *et al*., 1995; Verma *et al.,* 2002; Worastit *et al*., 2007) and it is responsible for seed rot. It also causes seedling decay, stem-discoloration and root rot (Zhang *et al*., 2001; Khare and Jharia 2002; El-Fikki *et al*., 2004). Infected seedlings show a brown discoloration at the soil line extending up the stem that may turn brown to black.

Charcoal rot disease in sesame used to appear every year in patches at farmers field at all growth stages in Haryana and usually go unnoticed and causes full amount loss to the crop at a time as the control of disease is not economical and feasible. The disease is inherited by minor genes, hence sources of resistance are not available to ulitilize in vertical resistance breeding programs. Moreover, the control of plant diseases using pesticides raises serious concerns about food and environmental safety and pesticide resistance, which have dictated the need for alternative disease management techniques. Keeping in view the importance of the disease in recent years in Haryana due to build up of high inoculum in soil and to avoid soil pollution through chemicals it has become necessary to test botanicals and bio-agents for their effectiveness against this mince under *in vitro* and *in vivo* conditions.

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Materials and methods

Glasswares and cleaning

To conduct the experiments in laboratory, sterilized glasswares were used as test tubes, conical flasks, petri plates. Before using them they were kept in a cleaning solution for 24 hours containing 60 g of potassium dichromate and 60 ml of concentrated sulphuric acid in 1000 ml of water. The glasswares were then washed with detergent followed by tap water and finally with distilled water. Then the glasswares were sterilized by keeping them in hot air oven at 180^0C for two hours.

Preparation of media

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).

Two hundred grams of cleaned, washed and peeled potato tubers were sliced into thin pieces. Later these pieces were boiled in distilled water and the extract was collected by filtering it through muslin cloth. Roughly 400 ml of distilled water was taken and 20 g agar-agar was dissolved in it. The mixture was stirred continuously while boiling the solution to avoid the formation of clots and 20 g dextrose was dissolved in it. The potato extract was mixed in it and then volume was made up to 1000 ml by adding distilled water. The Optimum quantity of medium was dispensed into conical flask and plugged with non-absorbent cotton. The flask was rapped with brown paper with the help of rubber bands. The flasks containing dispensed medium was sterilized in the autoclave at 15 psi for 20 minutes.

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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 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 27^0C 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 (Rangaswami, 1972). After 7 days, pure culture was obtained and it was maintained at 4^0C for further studies.

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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 27^0C 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.

Comment [TR23]: Detailed procedure of botanical extraction along with reference for phytoextracts is to be included

Observations recorded

Colony diameter

The colony diameter of fungus was recorded in metric scale (mm) by taking measurement horizontally and another vertically of the size of the fungal colony and mean of these two was

the colony diameter. Half of the measurement of the colony diameter gave radial growth of the pathogen.

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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 (1927).

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.

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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

Comment [TR28]: Seed priming/hydropriming

T_5 : Seeds soaking in second most effective plant extract (20%) before sowing

Comment [TR29]: Seed priming/hydropriming

T_6 : Seeds soaking in third most effective plant extract (20%) before sowing

Comment [TR30]: Seed priming/hydropriming

T_7 : *Trichoderma harzianum* + First most effective botanical

T_8 : *Trichoderma viridae* + Second most effective botanical

T_9 : *Trichoderma harzianum* + Third most effective botanical

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T_{10} : Control

Observations:

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

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

Results and Discussion

Efficacy of botanicals *in vitro*

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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)

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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.

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Macrophomina phaseolina caused charcoal rot in sesame causes pre-emergence seed rot as well as post emergence and also can appear at all growth stages. In Haryana, the disease causes substantial losses in yield in all available varieties. As the pathogen is soil borne, it survives and multiplies in soil for many years and difficult to manage, particularly at farmers field having small land holdings , as they could not go crop rotation and protective measures at right time. Difficulty in managing and lack of genetic resistance in sesame cultivars, a study was conducted on this disease in relation to test the efficacy of botanicals and bio-agents *in vitro* and *in vivo*.

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Conclusion

The use of excessive pesticides causes environmental pollution and has become concern for public awareness for health hazard not only for human beings and animals but for all useful organisms on earth. Hence, in the present study eco-friendly approaches to manage *M. phaseolina* in sesame were undertaken through use of botanicals and bio-agents under *in vitro* and *in vivo* conditions in 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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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	28.6	31.66	36.1	30.05

	(29.23)	(32.31)	(34.22)	(36.89)	(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

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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

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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**

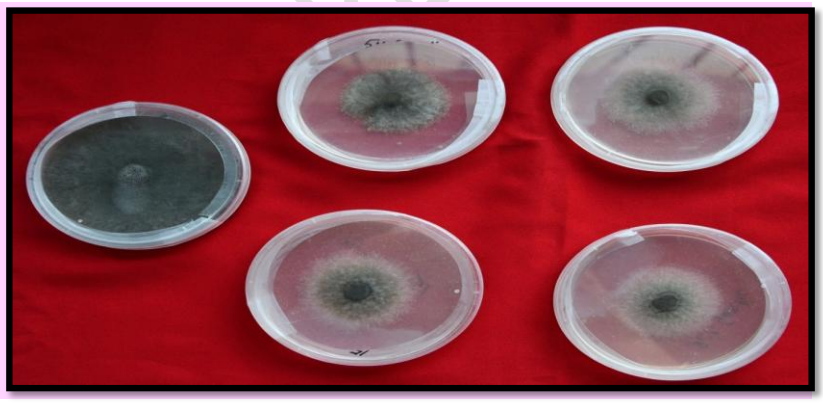

Plate 3

Plate 1-3. Effect on mycelial growth inhibition of M. phaseolina under laboratory conditions

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References-

Anonymous. Area, Production and Productivity of Sesamum in India. *Ministry of Agriculture and Farmers Welfare, Government of India*. 2020

Anonymous, Production of Crops. FAOSTAT, Food and Agriculture Organization of the United Nations. 2020

El-Fikki., El-Deep A. A., Mohmmad F. G., and Khalifa M.M.A. Controlling sesame charcoal rot by *Macrophomina phaseolina* under field conditions by using resistant cultivars, seed and soil. *Egypt Journal of Phytopathology*. (2004;**32**(1-2) 103-118.

Javed M. S., Wahid A., and Idress M. Fungi associated with sesame seeds and their frequency. *Pakistan Journal of Phytopathology*. (1995);**7**(2) 174-176.

Khare, M. N., and Jharia H. K., (2002). Biostressors and their management in sesame. Integrated crop management of sesame and niger. PC Unit, AICRP Sesame and Niger, JNKVV, Jabalpur.2002;82-94

Kumar S., Aeron A., Pandey P., and Maheshwari D. K. Ecofriendly management of charcoal rot and wilt diseases in sesame (Sesamu*m indicum* L.) In: D. K. Maheshwari (ed.) *Bacteria in Agrobiology*: *Crop Ecosystems*. 2011; pp. 387-405

Kumar, S. and Goel, P. D., A great ancient oilseed sesamum. *Farmers and Parliament.*, **12** (1994) 6-7.

Maiti, S., Hedge, M. R. and Chattopadhyay, S. B., (1988). Handbook of Annual Oilseed Crop. Oxford and IBH Publ. Co. Pvt. Ltd., New Delhi.1988

Rangaswami, G. (1972). *Diseases of crop plants in India*. Prentice Hall of India Pvt. Ltd., New Delhi, 1972; 520p

Saharan G. S., Mehta N. and Sangwan M.S. Diseases of oilseed crops. Indus Publishing Co New Delhi. .2005; 643p

Shyu, Y. S. and Hwang, L. S. Antioxidative activity of crude extract of lignin glycosides from unroasted Burma black sesame meal. *Food Research International*. 2002;**35**: 357-365.

Verma M. L., Prasad D. and Puri S. N., (2002). Fungal and bacterial diseases of sesame and their management. Crop pest and diseases management: Challenges for the mellenium. Jyoti Publishers and Distributors. Dehradun, India. 2002; 161-192

Vincent, J. M. Distortion of fungal hyphae in presence of certain inhibitors. *Nature*. 1927; **15**:850

Vyas, S. C. Diseases in *Sesamum* in India and their control. *Pesticides*. 1981; **15**:9-10.

Worasatit N., Suddhiyam P. and Kampai S. Study of disinfecting method against *Macrophomina sp.* infected on sesame seed. Ubon Ratchathai Field Crops Research Center, Muang, Ubon Ratchathai, Thailand. 2007

Zhang X., Rong C. Y., Liu S.I. and Feng X. Y. Evaluation of sesame germplasm resistant to *Macrophomina phaseolina* and *Fusarium oxysporum*. *Chinese Journal of Oil Seed Crop Sciences*. 2001; **23**(4):23-27.