

Original Research Article

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Evaluation of Antagonistic Potential of Biocontrol Agents against *Macrophomina Phaseolina* (tassi) Goid. causing Stem and Root Rot of Sesame [*Sesamum Indicum* L.] under *In vitro* and *In vivo*

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ABSTRACT

Keywords

Sesame, Stem and root rot, *Macrophomina phaseolina*, Antagonists, Fungicides, Dual culture, Poisoned food technique, Management

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Effect of eight antagonists viz., *Trichoderma atroviride*, *T. harzianum*, *T. virens*, *T. fasciculatum*, *T. asperellum*, *T. viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were studied for their antagonism against *Macrophomina phaseolina* by dual culture method and six fungicides viz. Azoxystrobin 23 SC, Carbendazim (12%) + Mancozeb (63%) 75 WP, Carbendazim (25%) + Mancozeb (50%) 75 WS were evaluated at two different concentrations (500 ppm and 1000 ppm) by poisoned food technique against the same pathogen. Out of eight antagonists evaluated, *Trichoderma atroviride* showed strong antagonistic effect against the pathogen with highest growth inhibition (60.00%) and among the six fungicides, carbendazim (12%) + mancozeb (63%), carboxin (37.5%) + thiram (37.5%), carbendazim (25%) + mancozeb (50%) and carbendazim were proved to be effective with cent per cent growth inhibition of the pathogen at both the concentrations (500 and 1000 ppm) tested. The effective biocontrol agents and chemicals which were found promising under laboratory studies were further evaluated for the management of stem and root rot of sesame under pot conditions. The highest germination (100%), higher shoot (17.06 cm) and root (4.03 cm) length, vigour index (2032) and minimum plant mortality (8.22%) was achieved through treatment T₄ i.e. seed treatment with *T. harzianum* (2×10^8 cfu/g) AAU isolate @ 10 g/ kg seeds + soil application of *T. harzianum* (2×10^8 cfu/g) enriched FYM @ 100g/pot.

Introduction

Sesame (*Sesamum indicum* L.) is an ancient and traditional oilseed crop of India, cultivated in about 74 million hectare area and producing 0.82 million tons. The main reason for the low productivity of sesame is due to the attack of various diseases. Among the fungal diseases, stem and root rot also called

charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. is widely distributed and highly destructive right from the establishment phase of crop (Dinakaran and Mohammed, 2001), causing up to 50 per cent or more disease incidence in field resulting in heavy yield losses (Chattopadhyay *et al.*, 2002). Yield losses have been estimated up to 57 per cent when there is about 40 per cent

infection (Maiti *et al.*, 1988) and about 5-100% yield loss as estimated by Vyas (1981). Further loss in yield at the rate of 1.8 kg/ha due to 1 per cent increase in the incidence has been reported (Murugesan *et al.*, 1978). *Macrophomina phaseolina* (Tassi) Goid is one of the most destructive necrotrophic fungal pathogens that infect more than 500 plant species across 75 families. Under moisture stress condition, the fungus causes many diseases like seedling blight, collar rot, stem rot, charcoal rot and dry root rot. The most common symptoms of the disease are the sudden wilting of 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).

In this study, antagonistic effect of biocontrol agents and efficacy of various fungicides were evaluated under *in vitro* and *in vivo* against *Macrophomina phaseolina* causing stem and root rot of sesame and result are presented hereunder.

Materials and Methods

The eight antagonistic microorganisms (Table 1) employed for the *in vitro* antimicrobial assay were obtained from Department of Plant Pathology, BACA, AAU, Anand. The antagonistic fungal cultures were maintained on PDA culture media and bacterial cultures were maintained on nutrient agar media. The assay for antagonism was performed on PDA media on Petri plates by the dual culture method. The mycelial disc (5 mm diameter) of pathogen and fungal antagonists were placed on the same dish 6 cm from each other. To test for antagonistic bacteria, a 5 mm of mycelial disc of pathogen cultures was placed on the one side of a Petri plate containing PDA medium. A loopful of bacteria was then streaked 3 cm away from the disc of *Macrophomina* isolates on the same plate. Paired cultures were incubated at 28±1°C. The

plates inoculated only with test pathogens served as control. The experiment was conducted with three repetitions of each treatment.

Further, Six fungicides belonging to different chemical groups at two different concentrations (Table 2) were tested for their efficacy *in vitro* against *M. phaseolina* using "Poisoned food technique". The required quantities of each test fungicides were incorporated in a conical flask containing 100 ml melted PDA medium so as to get required concentration in parts per million (ppm). The flask containing poisoned medium was well shaken to facilitate uniform mixture of fungicides and 20 ml was poured in each sterilized Petri plates. On solidification of the medium, the plates were inoculated in the centre by placing 5 mm diameter mycelial disc cut aseptically with the help of cork borer from seven days old pure culture of *M. phaseolina*. Three repetitions were kept for each concentration of respective fungicide. The inoculated plates were incubated at 28±1°C. The growth of test fungus on non-poisoned PDA served as a control. Observation on the radial growth (mm) was recorded from 24 h of incubation at 28±1°C till the complete growth of test pathogen in control plates. The per cent growth inhibition (PGI) over control was calculated by using formula given by Vincent (1947).

$$PGI = \frac{DC - DT}{DC} \times 100$$

Where,

PGI = Per cent growth inhibition

DC = Mean diameter of mycelial colony in control treatment (mm)

DT = Mean diameter of mycelial colony in treated set (mm)

The effective biocontrol agents and chemicals which were found promising under laboratory

studies were further evaluated for the management of stem and root rot of sesame under pot conditions.

The sterilized soil was weighed and filled in surface sterilized earthen pots (30 cm diameter). *Macrophomina phaseolina* culture multiplied on sorghum grains for 20 days (containing mycelium and sclerotial bodies) by using modified method of Kataria and Grover (1976) in a proportion of 1:10 by thoroughly mixing it in the upper 4-5 cm layer of soil and allowed to stabilize for one week. After a week of application of *M. phaseolina* in soil, seeds were sown in these pots @10

seeds/pot. The treatment combinations used as a seed treatment and soil application as given in Table 3.

The seedling vigour index was calculated using the formula as given by Abdul Baki and Anderson (1973).

Vigour index = (Mean root length + Mean shoot length) x Per cent germination

The per cent seedling mortality was calculated by using the following formula given by Pandey *et al.*, (1989):

$$\text{Per cent mortality} = \frac{\text{Number of seeds or seedlings rotted}}{\text{Total no. of seeds sown}} \times 100$$

Results and Discussion

The result presented in Table 1 revealed that most of the antagonists tested against *M. phaseolina* under *in vitro* were effective in checking the growth of the pathogen. Out of eight antagonists tested, least growth of the pathogen was recorded in *T. atroviride* (36.00 mm) which was at par with *T. harzianum* (38.00 mm), *T. vires* (40.00 mm), *T. fasciculatum* (40.00 mm) and *T. asperellum* (41.00 mm). *Bacillus subtilis* (86.00 mm) and *P. fluorescens* (90.00 mm) were less effective having maximum growth of the pathogen. With respect to growth inhibition, *T. atroviride* (60%), *T. harzianum* (57.78%), *T. vires* (55.56%), *T. fasciculatum* (55.56%) and *T. asperellum* (54.44%) were significantly inhibited the pathogen. Whereas, *B. subtilis* (4.44%) were least effective, while *P. fluorescens* showed completely ineffective where colony growth was as same as control (90.00 mm).

It is evident from these studies that among all the antagonists evaluated by dual culture method, *T. atroviride*, *T. harzianum*, *T. vires*,

T. fasciculatum and *T. asperellum* consistently showed strong antagonistic property against *M. phaseolina* compared to the other antagonists tested, hence considered as potential antagonists.

The results of the present findings are more or less similar with the results obtained by Kumar *et al.*, (2013) and Karthikeyan *et al.*, (2015).

The results presented in Table 2 indicated that under *in vitro* all fungicides significantly reduced the growth of *M. phaseolina* as compared to control but all the fungicides and their concentrations significantly differed within themselves. The result revealed that mycelial growth of the pathogen completely (100%) inhibited by most of the fungicide tested namely carbendazim (12%) + mancozeb (63%), carboxin (37.5%) + thiram (37.5%), carbendazim (25%) + mancozeb (50%) and carbendazim. The next better in order of merit were azoxystrobin at 1000 ppm (61.00%), tebuconazole at 1000 ppm (60.11%), azoxystrobin at 500 ppm (50%) and tebuconazole at 500 ppm (12.96%) inhibited the growth of *M. phaseolina*.

Table.1 Antagonism of different bioagents against *M. phaseolina* under dual culture technique

Tr. No.	Antagonists	Colony diameter of pathogen (mm)*	Growth inhibition over control (%)
T ₁	<i>Trichoderma atroviride</i>	36.00	60.00
T ₂	<i>Trichoderma harzianum</i>	38.00	57.78
T ₃	<i>Trichoderma virens</i>	40.00	55.56
T ₄	<i>Trichoderma fasciculatum</i>	40.00	55.56
T ₅	<i>Trichoderma asperellum</i>	41.00	54.44
T ₆	<i>Trichoderma viride</i>	41.33	54.08
T ₇	<i>Pseudomonas fluorescens</i>	90.00	0.00
T ₈	<i>Bacillus subtilis</i>	86.00	4.44
T ₉	Control (Test pathogen only)	90.00	-
	S.Em.±	1.69	-
	CD at 5%	5.02	-
	CV (%)	5.25	-

*Mean of three repetitions

Table.2 Evaluation of different fungicides against *M. phaseolina* by poisoned food technique

Tr. No.	Fungicides	Colony diameter of pathogen* (mm)		Growth inhibition over control (%)	
		Concentration (ppm)			
		500	1000	500	1000
T ₁	Azoxystrobin23 SC	45.00	34.56	50.00	61.00
T ₂	Carbendazim (12%) + Mancozeb (63%)75 WP	0	0	100.00	100.00
T ₃	Tebuconazole2 DS	78.33	35.90	12.96	60.11
T ₄	Carboxin (37.5%) + Thiram (37.5%) 75 WP	0	0	100.00	100.00
T ₅	Carbendazim (25%) +Mancozeb (50%)75 WS	0	0	100.00	100.00
T ₆	Carbendazim50 WP	0	0	100.00	100.00
T ₇	Control	90.00	90.00	-	-
	S.Em.±	0.76	0.48	-	-
	CD at 5%	2.32	1.46	-	-
	CV (%)	4.35	3.64	-	-

*Mean of three repetitions

Table.3 Management of stem and root rot of sesame in pot under glass house condition

Tr. No.	Treatments	Germination (%)	Shoot length (cm) 15 DAG	Root length (cm) 15 DAG	Seedling vigour index	Plant mortality due to <i>M. phaseolina</i> (%)
T ₁	Seed treatment with <i>Trichoderma atroviride</i> (2×10^8 cfu/g) AAU isolate @ 10 g/ kg seeds	96.67	10.80	2.46	1282	30.00* (25.00)**
T ₂	T ₁ + soil application of <i>T. atroviride</i> (2×10^8 cfu/g) enriched FYM @ 100 g/ pot	96.67	14.26	3.26	1769	26.66 (20.13)
T ₃	Seed treatment with <i>T. harzianum</i> (2×10^8 cfu/g) AAU isolate @ 10 g/ kg seeds	86.67	13.86	3.06	1467	76.66 (94.68)
T ₄	T ₃ + soil application of <i>T. harzianum</i> (2×10^8 cfu/g) enriched FYM @ 100 g/ pot	100.00	17.06	4.03	2032	16.66 (8.22)
T ₅	Seed treatment with carbendazim + mancozeb 75WP @ 3 g/ kg seeds	93.33	15.63	3.26	1764	33.33 (30.19)
T ₆	Seed treatment with carboxin + thiram 75 WP @ 3 g/ kg seeds	90.00	17.03	3.46	1845	23.33 (15.68)
T ₇	Seed treatment with carbendazim 50 WP @ 3 g/ kg seeds	83.33	16.20	3.66	1655	60.00 (75.00)
T ₈	Control (Untreated check)	70.00	8.63	1.83	732	83.33 (98.65)
	S.Em.±	5.03	0.13	0.09		2.04
	CD at 5%	15.10	0.39	0.27		6.12
	CV (%)	11.59	1.61	5.00		8.57

Note: *Figures those outside the parentheses are arcsine transformed values,

**Figures in the parentheses are original values

It is evident from the results that the growth inhibition of *M. phaseolina* increased while increasing in the concentration of the fungicides. Carbendazim (12%) + mancozeb (63%), carboxin (37.5%) + thiram (37.5%), carbendazim (25%) + mancozeb (50%) and carbendazim were proved to be most effective at all the tested concentration.

The result similar to the present investigations was also achieved by Magar et al., (2011), Chaity et al., (2012) and Shahid and Khan

(2016).

The data presented in Table 3, revealed the various combinations of bioagents and fungicides were used in the management of stem and root rot of sesame in pot under glasshouse condition. The effects of all the treatments were found significantly superior over control in managing the stem and root rot of sesame. The highest germination (100%), higher shoot (17.06 cm) and root (4.03 cm) length, vigour index (2032) and minimum plant mortality (8.22%) was

achieved through treatment T₄ i.e. seed treatment with *T. harzianum* (2×10⁸cfu/g) AAU isolate @ 10 g/ kg seeds + soil application of *T. harzianum* (2×10⁸cfu/g)enriched FYM @ 100g/pot.

The next better in order to minimize the plant mortality was treatment T₆ (15.68%) i.e. seed treatment with carboxin + thiram 75 WP @ 3 g/ kg seeds, followed by T₂(20.13%) i.e. seed treatment with *T. atroviride*(2×10⁸cfu/g) AAU isolate @10 g/ kg seeds + soil application of *T. atroviride*(2×10⁸cfu/g) enriched FYM @100g/pot

The result of the present finding showed that seed treatment with *T. harzianum* (2×10⁸cfu/g) AAU isolate @ 10 g/ kg seeds + soil application of *T. harzianum* enriched FYM @ 100g/pot was excellent in order to minimize the plant mortality.

The present results are in contradiction with the results of Savaliya *et al.*, (2016) who reported that *T. viride* and *T. harzianum* were least effective in managing sesame root rot under *in vivo*.

The present result are in agreement with the findings of Pandey *et al.*, (2017) who reported that *T. viride* and *T. harzianum* significantly checked the incidence of root rot of chickpea.

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