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Influence of biocontrol agents on sesame root rot

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Macrophomina phaseolina (Tassi) Goid, a soil borne plant pathogen has a wide host range causing root rot, seedling blight, collar rot, stem rot of various crops. Here attempts had been made to control root rot of sesame caused by *M. phaseolina* by suppressive organisms following *in vitro* and *in vivo* studies. Five isolates of *Bacillus subtilis* namely BS-12, BS-14, BS-17, BK-1, BM-1, *Pseudomonas fluorescens* and *Trichoderma harzianum* were taken as antagonists in the present studies.

In vitro studies revealed that, in case of dual culture technique, highest mycelial inhibition was recorded in BS-17 (up to 65.56 %).

Under *in vivo* studies *M. phaseolina* was grown in sand maize meal medium and inoculated in the pots @ 200 g/pot. The symptom of root rot disease appeared within 10 days of sowing and continued up to 30 days. *In vivo* studies revealed that all the antagonists reduced the root rot of sesame significantly, when applied as soil drenching or in combination of seed soaking and soil drenching. Combination of seed soaking and soil drenching gave better results than the soil drenching alone. Here among the antagonists maximum disease control was observed by the *T. harzianum* which gave up to 66.17 % of sesame root rot disease control followed by isolate BS-12 (up to 65.27 %) and BS.17 (up to 65.25 %).

Key words : Sesame, *Macrophomina phaseolina, P. fluorescens, T. harzianum, Bacillus* sp.

INTRODUCTION

Sesame is an important oil seed crop, extensively cultivated in the states of Gujarat, Uttar Pradesh, West Bengal, Orissa, Rajasthan, Andhra Pradesh etc. The root rot of sesame is caused by *Macrophomina phaseolina* (Tassi) Goid. Sclerotia of *M. phaseolina* are black, round to oblong in shape and measure 50-300 μ in diameter. The disease is soil borne as well as seed borne. The primary source of inoculum is mainly soil in the form of sclerotia and seed in the form of mycelia. Various antagonistic bacteria and fungi have great potentialities as biocontrol agent. In our present investigation 5 isolates of *Bacillus* sp. namely BS-12, BS-14, BS-17, BM-1, BK-1 and *Trichoderma* sp. and *Pseudomonas fluorescens* have been used as biocontrol agents against root rot of sesame caused by *Macrophomina phaseolina.*

MATERIALS AND METHODS

The experiment was conducted in the sick pots and in the laboratory as *in vivo* and *in vitro* study. A susceptible sesame variety was taken as test plant. Isolates of *Bacillus* sp. were taken from the Bacteriology Laboratory, Department of Plant Pathology, B.C.K.V.. *Pseudomonas fluorescens* was isolated from the rhizosphere of jute and *Trichoderma harzianum* was taken from Department of Plant Pathology, B.C.K.V..

Macrophomina phaseolina, the test pathogen, was isolated from the naturally infected jute seedlings. Isolates of *Bacillus* sp. were grown in Nutrient Agar (NA) medium and in Nutrient Broth. *P. fluorescens* was grown in King's B Medium where as *M. phaseolina* and *T. harzianum* were grown in Potato Dextrose Agar medium for maintenance and study. *In vitro* study was carried out by Dual Culture Technique. Under *in vivo* study, mass culture of the fungus was necessary for conducting the pot experiment for assaying the antagonistic activity of the organisms towards the disease. For this purpose, *M. phaseolina* was grown in Petriplate. Sand maize meal (sand 70 g, crushed maize meal 30 g and re-

quired amount of water) medium was prepared in polypropylene packets and inoculation was done by *M. phaseolina.* Total operation was done carefully in laminar airflow cabinet so that contamination would not occur. All the inoculated medium were incubated at 30^{0}C±1^{0}C for 15 days for mass culture.

Garden soil and farmyard manure in 5 : 1 ratio were mixed and filled in earthen pots of 30 cm diameter. Fifteen days old culture of *M.phaseolina* grown on sand maize meal medium was thoroughly mixed with the soil @ 200 g/pot. The disease symptoms were generally observed after 10 days of sowing and continued up to 30 days.

RESULTS AND DISCUSSION

Antagonistic study in vitro

The dual culture study revealed that all the antagonists i.e. five isolates of *Bacillus subtilis, Pseudomonas fluorescens* and *Trichoderma harzianum* inhibited the pathogen's growth significantly. The inhibition ranged from 42.22 to 65.56 %. The maximum inhibition i.e. 65.56 % was shown by isolate BS-17 followed by isolate BM-1 (58.89 %), BK-1 (54.44 %) and *Pseudomonas fluorescens* (54.44 %). Other isolates like BS-14 (up to 52.22 %), BS-12 (up to 47.78 %) and *Trichoderma harzianum* (up to 44.44 %) were also seemed to be promising antagonists. Here bacterial antagonists were more effective in inhibition of mycelial growth of *M. phaseolina.*

Table 1 : Inhibition of mycelial growth of *M. phaseolina* by antagonists (Dual culture technique) *in vitro* (1st set)

Antagonist	Colony diameter (mm)		Inhibition	Inhibition of
	Pathogen	Antagonists	Zone(mm)	pathogen (%)
BM-1	37 (1.568)	49 (1.690)	4 (0.602)	58.98
BS-14	45 (1.653)	42 (1.623)	3 (0.477)	50.0
BS-12	49 (1.690)	39 (1.591)	2 (0.301)	45.56
P. fluorescene	44 (1.643)	37 (1.568)	9 (0.954)	51.11
BK-1	41 (1.612)	44 (1.643)	5 (0.698)	54.44
BS-17	31 (1.491)	53 (1.724)	6 (0.778)	65.56
Trichoderma harzianum	52 (1.716)	35 (1.544)	3 (0.477)	42.22
Inoculated Control	90 (1.954)	—	—	—
S. Em±	0.005	—	—	—
C D at 5 %	0.016	—	—	—
C D at 1 %	0.02	—	—	—

* Figure in parenthesis are log transformed values

Table 2 : Inhibition of mycelial growth of *M. phaseolina* by antagonists (Dual culture technique) *in vitro* (2st set)

Antagonist	Colony diameter (mm)		Inhibition	Inhibition of
	Pathogen	Antagonists	Zone(mm)	pathogen (%)
BM-1	38 (1.579)	49 (1.69)	3 (0.477)	57.78
BS-14	43 (1.633)	43 (1.633)	4 (0.602)	52.22
BS-12	47 (1.672)	40 (1.602)	3 (0.477)	47.78
P. fluorescene	41 (1.612)	42 (1.623)	7 (0.845)	54.44
BK-1	43 (1.633)	42 (1.623)	5 (0.698)	52.22
BS-17	33 (1.518)	53 (1.724)	4 (0.602)	63.33
Trichoderma harzianum	50 (1.698)	36 (1.556)	4 (0.602)	44.44
Inoculated Control	90 (1.954)	—	—	—
S. Em±	0.021	—	—	—
C D at 5 %	0.063	—	—	—
C D at 1 %	0.086	—	—	—

* Figures in parenthesis are log transformed values

Study of antagonism in vivo

Effect of seed soaking and soil drenching of antagonists on per cent mortality of root rot of sesame

Five isolates of *Bacillus subtilis* and *T. harzianum* were grown for 72 h in PD broth medium and *P. fluorescens* was grown in King's B broth medium. The population of the baterial suspension was estimated by using Mc. Farland Scale to be 10 x 10^8 cells/ml. Before sowing, the sesame seeds were soaked separately in each suspension respectively for one and half. Then it was dried under shade and broadcasted in the sick pots. The suspensions were poured to the sick pots respectively according to the treatments. Three replications were maintained for each treatment including inoculated and uninoculated control. Per cent mortality was recorded from 10 days after sowing up to 30 DAS. Results were presented in Table 3 and Table 4.

The treatments showed remarkable variations. The maximum root rot mortality were recorded in the treatment of BS-14 (39.83 %), BM-1 (39.26 %). In *P. fluroescens* it was 35.48 % and in *T. harzianum* it was 35.36 %. The maximum per cent of disease control was observed in *T. harzianum* (66.17 %), followed by BS-12 (65.27 %) and BS-17 (65.25 %). Per cent disease control showed by other antagonist were also encouraging.

Table 3 : Effect of seed soaking and soil drenching of antagonists on per cent mortality of root rot of sesame (1st set)

Antagonist	Replication			Mean
	R_1	R_2	R_3	
BM-1	36.36 (37.05)	39.13 (38.70)	34.28 (35.79)	37.18
BS-14	40.38 (39.41)	42.22 (40.51)	37.5 (37.76)	39.22
BS-12	34.88 (36.15)	32.60 (34.82)	30.95 (33.77)	34.73
P. fluorescene	31.57 (34.14)	35.89 (36.75)	31.70 (34.27)	35.05
BK-1	33.33 (35.24)	36.73 (37.29)	34.78 (36.09)	36.20
BS-17	34.04 (35.67)	31.25 (33.96)	32.35 (34.63)	34.75
Trichoderma harzianum	31.11 (33.90)	31.57 (34.14)	30.43 (33.46)	33.83
Inoculated Control	81.39 (64.38)	79.06 (62.73)	76.19 (60.73)	62.61
Uninoculated Control	5.12 (12.92)	6.81 (15.12)	4.87 (12.66)	13.56
S. Em±				0.591
C D at 5 %				1.77
C D at 1 %				2.44

* Angular transformed values are in parenthesis

Table 4 : Effect of seed soaking and soil drenching of antagonists on per cent mortality of root rot of sesame (2nd set)

Antagonist	Replication			Mean
	R_1	R_2	R_3	
BM-1	38.63 (38.41)	40.00 (39.23)	41.66 (40.16)	39.26
BS-14	43.75 (41.38)	44.89 (42.02)	34.78 (36.09)	39.83
BS-12	35.89 (36.75)	35.29 (36.39)	32.60 (34.82)	35.98
P. fluorescene	34.14 (35.73)	35.71 (36.69)	31.37 (34.02)	35.48
BK-1	34.28 (35.79)	34.04 (35.67)	35.71 (36.69)	36.05
BS-17	36.58 (37.17)	31.81 (34.33)	32.72 (34.88)	35.46
Trichoderma harzianum	32.65 (34.82)	32.55 (34.76)	35.41 (36.51)	35.36
Inoculated Control	63.04 (52.54)	73.33 (58.89)	56.36 (48.62)	53.35
Uninoculated Control	2.5 (9.10)	4.65 (12.38)	4.76 (12.52)	11.33
S. Em±				1.311
C D at 5 %				3.93
C D at 1 %				5.41

* Angular transformed values are in parenthesis

Table 5 : Effect of soil application of antagonists before seed sowing (Pre-sowing) on per cent mortality of root rot of sesame (1st set)

Antagonist	Replication			Mean
	R_1	R_2	R_3	
BM-1	42.10 (40.45)	46.15 (42.76)	41.17 (39.87)	41.02
BS-14	47.91 (43.80)	51.31 (45.74)	48.83 (44.31)	44.61
BS-12	45.45 (42.36)	46.34 (42.88)	40.00 (39.23)	41.49
P. fluorescene	46.15 (42.76)	43.18 (41.03)	48.88 (44.31)	42.70
BK-1	44.82 (42.02)	51.02 (45.57)	46.93 (43.22)	43.60
BS-17	41.02 (39.82)	43.90 (41.50)	45.23 (42.25)	41.19
Trichoderma harzianum	41.17 (39.87)	45.94 (42.65)	43.58 (41.27)	41.26
Inoculated Control	59.09 (50.18)	68.29 (55.67)	76.31 (60.87)	55.57
Uninoculated Control	5.12 (13.05)	6.52 (14.77)	2.50 (9.10)	12.30
S. Em±				1.364
C D at 5 %				4.09
C D at 1 %				5.63

* Angular transformed values are in parenthesis

Table 6 : Effect of soil application of antagonists before seed sowing (Pre-sowing) on per cent mortality of root rot of sesame (2nd set)

Antagonist	Replication			Mean
	R_1	R_2	R_3	
BM-1	43.13 (41.03)	43.18 (41.03)	46.66 (43.05)	41.70
BS-14	48.78 (42.26)	44.73 (41.96)	51.06 (45.57)	43.93
BS-12	47.05 (43.28)	40.00 (39.23)	42.10 (40.45)	40.98
P. fluorescene	42.50 (40.69)	48.48 (44.08)	45.65 (42.48)	42.41
BK-1	45.94 (42.65)	46.34 (42.88)	44.68 (41.90)	42.47
BS-17	45.00 (42.13)	44.18 (41.61)	42.85 (40.86)	41.53
Trichoderma harzianum	44.11 (41.61)	45.23 (42.25)	42.50 (40.69)	41.51
Inoculated Control	79.41 (63.01)	72.72 (58.50)	60.97 (51.30)	57.60
Uninoculated Control	2.32 (8.72)	2.63 (9.28)	4.34 (11.94)	9.98
S. Em±				1.422
C D at 5 %				4.26
C D at 1 %				5.87

* Angular transformed values are in parenthesis

Effect of soil application of antagonists before seed sowing (pre-sowing) on per cent mortality of root rot of sesame

The antagonists were grown in their respective broth medium. The antagonists suspension of about 200 ml was then separately added to each sick pot one day before sowing of sesame seeds. The population of bacterial suspension was estimated to be 12×10^8 cells/ml. The seeds were broadcasted and three replications were also maintained for each treatment along with control. Per cent mortality of root rot were counted from 10 days after sowing up to 30 days and the results were shown in Table 5 and Table 6.

Different treatments showed the variable results. The highest per cent disease control among the bacterial antagonists was recorded in isolate BS-12 (59.08 %), followed by BM-1 (58.98 %), BS-17 (58.81 %), BK-1 (57.53 %) and BS-14 (56.07 %). *P. fluorescens* and *T. harzianum* were also established as promising antagonists against *M. phaseolina* and recorded up to 57.59 % and 58.74 % disease control respectively.

Seed treatment with bacilli antagonists had presented some encouraging information regarding disease control, caused by mostly soil borne pathogens. Soyabean plants infected with root infecting fungi, *Rhizoctonia solani, Macrophomina phaseolina* and *Fusarium* sp. was significantly reduced by the seed treatment with *B. subtilis*. (Farzana *et al.*, 1991). Biological control of *M. phaseolina* by *Bacillus subtilis* on chickpea was studied by Shaid *et al* (2000). This has been confirmed with the present work. Inhibition of *M. phaseolina* by *P. fluorescens* was already known and indicated by several workers. Inoculation of bean seed with *Pseudomonas* sp. showed inhibitory effect against *M. phaseolina* causing root rot (Sanchez *et al.*, 1994). In another work, biocontrol of *Rhizoctonia solani* in cowpea by the treatment of *P. fluorescens* was also reported. This has been confirmed with our present works.

According to Jayashree *et al.* (2000), *Pseudomonas fluorescens* effectively inhibited the mycelial growth of *M. phaseolina* causing root rot of sesame. When *P. fluorescens* applied as seed treatment followed by soil application, this could very effectively reduce the root rot of sesame caused by *M. phaseolina.* In this present work, *P. fluorescens* showed up to 54.44 % inhibition of pathogen under *in vitro*. The per cent disease control by *P. fluorescens* in soil application method was recorded up to 57.59 % whereas up to 64.95 % disease control was also recorded in plants treated with *P. fluorescens* as seed soaking followed by drenching.

The number of research works had been done world wide to control *M. phaseolina* by means of several species of *Trichoderma* as biocontrol agent. Biswas *et al.* (2000) showed the significant inhibitory effect of *Trichoderma* against *M. phaseolina* causing stem rot of jute. The application of *T. harzianum* to sesame as soil application reduced the root rot of sesame caused by *M. phaseolina.* This has been confirmed with the present work. Under *in vitro* condition, suppression of the mycelial growth of *M. phaseolina* by *T. harzianum* was recorded upto 44.44 %. But under *in vivo* condition *T. harzianum* when applied on soil, reduced the root rot upto 58.74 %, whereas it become up to 66.17 % when *T. harzianum* was applied as seed treatment followed by soil application.

A challenge to the pathogen by the antagonist has been expressed. The possibility of using a *Bacillus* inoculum for lowering the pressure of root rot of sesame caused by *Macrophomina phasoelina* (Tassi) Goid is clearly expressed from the present work. This has opened up an area of possible use of *Bacillus* sp.. *Pseudomonas fluorescens* not only as plant growth promoting rhizobacteria but also as suppressor in the soil against the plant pathogen to lower the disease pressure.

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