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Evaluation of Bio-agents against Different Isolates of *Macrophomina phaseolina* (Tassi) Goid Causing Root Rot in Castor

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ABSTRACT

Keywords

Macrophomina phaseolina isolates, Castor root rot, Efficacy of bio-agents

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The antagonistic efficacy of five different bio-agents *viz.*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma longibrachiatum*, *Pseudomonas fluorescens* and *Bacillus subtilis* was tested against 25 isolates of *Macrophomina phaseolina* causing castor root rot by dual culture techniques under *in vitro* conditions. Variation in respect of growth inhibition of *M. phaseolina* was observed among the bio-agents as well as isolates. Among five bio-agents, significantly maximum mean growth inhibition (53.19 %) was recorded by *T. viride* followed by *T. harzianum* (49.81 %) while minimum growth inhibition (26.79 %) was recorded by *P. fluorescens* followed by *B. subtilis* (28.66 %). Among different 25 isolates of castor root rot pathogen, significantly maximum mean growth inhibition (35.31 %) was recorded of Mp-25 followed by Mp-7 (32.83 %) which was at par with Mp-10 (32.03 %), Mp-11 (31.98 %) and Mp-12 (31.98 %). Maximum growth inhibition of most of the isolates of *M. phaseolina* was observed by *T. viride* except Mp-4, 8, 16, 21 and 24 in which maximum growth inhibited by *T. harzianum*. Interaction effect of different bio-agents and isolates also showed significant variation in respect of per cent growth inhibition.

Introduction

The fungus *Macrophomina phaseolina* (Tassi) Goid. a soil inhabiting an important root pathogen in many crops. The disease appears at different growth stage of castor crop and hence, it is named as spike blight, stem blight, twig blight, collar rot and root rot (Moses and Reddy, 1987). Biological control is a potential non-chemical means for plant disease control by reducing the harmful effects of a pathogen through the use of other living entities. Since the *M. phaseolina* is a soil borne fungus and possess greater problem in managing the disease. Soil borne diseases are difficult to control. Seed treatment with fungicides does not protect the crop for long periods. Soil drenching with fungicides are not economical and they may establish imbalances in the microbial community unfavorable for activities of beneficial organisms (Jeyarajan *et al.*, 1991). It is now widely recognized that biological control of plant pathogens using antagonistic fungi and bacteria is a distinct possibility for the future and can be successfully utilized especially within the framework of integrated disease management system (Muthamilan and Jeyarajan, 1996). Use of antagonistic organisms against *Macrophomina* root rot has been well documented in several crops (Mukhopadhyay, 1987; Raguchander *et al.*, 1995).

Materials and Methods

Collection and purification of fungal isolates

The castor plants with typical root rot symptoms were collected from different locations of castor growing areas of Gujarat state (Table 1) and then they were subjected to tissue isolation separately on potato dextrose agar (PDA) medium in Petri plates. Purified culture disc (5 mm) of each of the isolates grown on PDA was transferred to PDA slant separately and incubated for four days at 30 ± 2^{o}C until the surface of PDA slant was covered with a dense sclerotial layer of the fungal culture. The culture tubes were labeled and stored at 4 ± 1^{o}C temperature in refrigerator for further investigation.

Collection of bio-agents

Two fungal bio-agents *viz., Trichoderma harzianum*, *T. viride* and two bacterial bio-agents *viz*., *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sadarkrushinagar (Guj.), while one fungal bio-agent of *T. longibrachiatum* was obtained from Navsari Agricultural University, Navsari (Guj.).

Bio assay study

The five known bio-agents were evaluated for their effectiveness against 25 isolates of castor root rot pathogen (*M. phaseolina*) by dual culture technique. The test bio-agents and pathogen isolates were grown separately on solidified PDA in the sterilized Petri plates aseptically. Mycelial disc (5 mm) from four days old actively growing culture of bio-agents and test pathogen isolates were separately cut aseptically from the periphery of the colony with the help of sterilized cork borer and placed on solidified PDA in the sterilized Petri plates aseptically approximately 60 mm away from each other. Test pathogen and bio-agents were subjected for growth and comparison. All inoculated Petri plates were incubated at 30 ± 2^{o}C temperature in BOD incubator. Observations on radial growth in each of the Petri plates were measured periodically and final observations were recorded when control plate was fully covered with the growth of test pathogen. The Per cent growth inhibition (PGI) was calculated by the following equation as adopted by Bliss (1934).

$$PGI = \frac{C-T}{C} \times 100$$

Where,

PGI = Per cent growth inhibition
C = Colony diameter (mm) in control
T = Colony diameter (mm) in treatment

Results and Discussion

Variation in respect of growth inhibition of *M. phaseolina* was observed among the bio-agents as well as isolates.

Among the different five antagonists, significantly maximum mean growth inhibition (53.19 %) was recorded by *T. viride* followed by *T. harzianum* (49.81 %) and *T.*

longibrachiatum (41.25 %). Minimum mean growth inhibition (26.79 %) was recorded by *P. fluorescens* followed by *B. subtilis* (28.66 %).

Among the different 25 isolates, significantly maximum mean growth inhibition (35.31 %) was recorded of Mp-25 followed by Mp-7 (32.83 %) which was at par with Mp-10 (32.03 %), Mp-11 (31.98 %) and Mp-12 (31.98 %). Significantly, minimum mean growth inhibition (23.15 %) was recorded in Mp-23 and was at par with Mp-8 (22.69 %).

Maximum growth inhibition of most of the isolates of *M. phaseolina* was observed by *T. viride* except Mp-4, 8, 16, 21 and 24 in which maximum growth inhibited by *T. harzianum*. Significantly *T. harzianum* inhibited maximum growth (58.61 %) of Mp-21 and was statistically at par with *T. harzianum* inhibited Mp-12, *T. viride* inhibited Mp-2, 3, 5, 7, 10, 13, 15, 17, 21, 22, 25 and *T. longibrachiatum* inhibited Mp-25 (55.31-58.44 % inhibition). Interaction effect of different bio-agents and isolates also showed significant variation in respect of per cent growth inhibition (Table 2).

Table.1 List of isolates of *M. phaseolina* obtained from different locations of castor growing areas of Gujarat

Sr. No.	Isolates	Location		
		Village	Taluka	District
1	Mp-1	Sardarkrushinagar	Dantiwada	Banaskantha
2	Mp-2	Silasana	Dhanera	Banaskantha
3	Mp-3	Gathaman	Palanpur	Banaskantha
4	Mp-4	Pepalu	Lakhani	Banaskantha
5	Mp-5	Bhakhar	Dantiwada	Banaskantha
6	Mp-6	Jaska	Vadnagar	Mahesana
7	Mp-7	Vadali	Vadali	Sabarkantha
8	Mp-8	Thalvada	Vadnagar	Mahesana
9	Mp-9	Karnasar	Tharad	Banaskantha
10	Mp-10	Gangundra	Dantiwada	Banaskantha
11	Mp-11	Laxmipura	Unjha	Mahesana
12	Mp-12	Santalpur	Vanthali	Junagadh
13	Mp-13	Bagadu	Junagadh	Junagadh
14	Mp-14	Araniyala	Mendarda	Junagadh
15	Mp-15	Dervan	Keshod	Junagadh
16	Mp-16	Lushala	Vanthali	Junagadh
17	Mp-17	Barawala	Mendarda	Junagadh
18	Mp-18	Mendarda	Mendarda	Junagadh
19	Mp-19	Khumbhadi	Vanthali	Junagadh
20	Mp-20	Khorasha	Vanthali	Junagadh
21	Mp-21	Sogadi	Jamjodhpur	Jamnagar
22	Mp-22	Khadpipali	Mendarda	Junagadh
23	Mp-23	Dhutarpar	Jamjodhpur	Jamnagar
24	Mp-24	Jamnagar	Jamnagar	Jamnagar
25	Mp-25	Supedi	Dhoraji	Rajkot

Table.2 Growth inhibition of twenty-five isolates of *M. phaseolina* by different bio-agents *in vitro*

Sr.No.	Isolates	Growth inhibition (%)					Mean
		Bio-agents					
		Th	*Tv*	*Tl*	*Pf*	*Bs*	
1	Mp-1	44.98(49.97)	47.56(54.46)	42.40(45.48)	30.52(25.79)	33.75(30.87)	32.20(28.40)
2	Mp-2	44.74(49.55)	48.64(56.34)	39.51(40.48)	32.67(29.14)	33.39(30.29)	33.16(29.92)
3	Mp-3	44.74(49.74)	48.74(56.51)	44.98(47.57)	33.02(29.70)	32.67(29.14)	34.01(31.29)
4	Mp-4	47.62(54.57)	46.21(52.11)	40.21(41.68)	32.07(28.19)	28.86(23.30)	32.50(28.87)
5	Mp-5	45.40(50.70)	49.45(57.74)	37.93(37.79)	31.85(27.85)	37.24(36.62)	32.50(28.87)
6	Mp-6	40.94(42.94)	44.10(48.43)	43.31(47.05)	21.45(13.37)	32.26(28.49)	30.34(25.52)
7	Mp-7	46.49(52.60)	49.86(58.44)	39.76(40.91)	38.03(37.95)	35.59(33.87)	34.96(32.83)
8	Mp-8	41.51(43.92)	40.31(41.85)	32.83(29.39)	23.23(15.56)	32.82 (29.3)	28.45(22.69)
9	Mp-9	44.25(48.69)	47.15(53.75)	41.36(43.66)	36.02(34.58)	37.06 36.32)	34.31(31.77)
10	Mp-10	45.40(50.70)	48.73(56.49)	38.02(37.94)	35.96(34.48)	38.95 39.52)	34.47(32.03)
11	Mp-11	44.18(48.57)	47.71(54.72)	46.41(52.46)	36.04(34.62)	32.60(29.03)	34.44(31.98)
12	Mp-12	48.05(55.31)	46.65(52.88)	40.32(41.87)	34.88(32.70)	36.73(35.77)	34.44(31.98)
13	Mp-13	44.90(49.83)	48.83(56.66)	38.65(39.01)	32.43(28.76)	33.50(30.46)	33.05(29.74)
14	Mp-14	43.87(48.03)	47.64(54.60)	46.28(52.23)	26.12(29.38)	26.13(19.40)	31.67(27.57)
15	Mp-15	43.98(48.22)	47.98(55.19)	36.53(35.43)	34.42(31.95)	34.06(31.38)	32.83(29.39)
16	Mp-16	46.74(53.04)	45.57(50.99)	37.29(36.7)	21.64(13.60)	24.34(16.99)	29.26(23.89)
17	Mp-17	45.70(51.22)	48.92(56.82)	39.25(40.03)	32.12(28.27)	35.93(34.43)	33.65(30.70)
18	Mp-18	42.89(46.32)	45.57(50.99)	36.42(35.25)	32.11(28.25)	27.46(21.26)	30.74(26.13)
19	Mp-19	45.87(51.52)	46.89(53.30)	33.36(30.24)	29.89(24.83)	28.65(22.99)	30.78(26.19)
20	Mp-20	42.56(45.75)	44.36(48.88)	45.07(50.12)	21.30(13.20)	28.45(22.69)	30.29(25.44)
21	Mp-21	49.96(58.61)	48.64(56.34)	41.48(43.87)	34.42(31.95)	28.61(22.93)	33.85(31.03)
22	Mp-22	46.57(52.74)	48.17(55.52)	39.57(40.58)	31.08(26.65)	33.19(29.97)	33.09(29.81)
23	Mp-23	40.11(41.51)	41.54(43.98)	33.10(29.82)	28.70(23.06)	29.12(23.68)	28.76(23.15)
24	Mp-24	44.01(48.27)	42.60(45.82)	36.86(35.98)	33.87(31.06)	28.70(23.06)	31.01(26.54)
25	Mp-25	46.85(53.23)	48.90 (56.79)	48.21(55.59)	35.46(33.66)	39.36(40.22)	36.46(35.31)
	Mean	44.89(49.81)	46.83(53.19)	39.96(41.25)	31.17(26.79)	32.37(28.66)	

	Bio-agents	Isolates	Bio-agents × Isolates
S. Em.±	0.15	0.30	0.74
C.D. at 5 %	0.42	0.85	2.07

Figures in parentheses are re-transformed values of arcsine transformation;
Th =Trichoderma harzianum; Tv = T. viride; Tl= T. longibrachiatum;
Pf = Pseudomonas fluorescens; Bs = Bacillus subtilis

The fungal bio-agents *T. viride* and *T. harzianum* exhibited strong inhibition of the growth of *M. phaseolina* caused root rot in different crops (Suriachandraselvan *et al.*, 2004; Kartikeyan *et al.*, 2006; Chaudhary *et al.*, 2010; Kumar *et al.*, 2013; Karthikeyan *et al.*, 2015). The bacterial bio-agents *P. fluorescens* and *B. subtilis* were also found effective against *M. phaseolina* caused root rot in different crops (Ahmad and Srivastva, 2000; Lokesha and Benagi, 2007; Afouda *et al.*, 2012; Ashwini *et al.*, 2014; Malleshwari, 2014; Savaliya *et al.,* 2016). The results of present study are in agreement with the earlier research workers.

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