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Evaluation of antagonists isolated from rhizosphere of Mungbean against *Macrophomina phaseolina*

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ABSTRACT: *Efficacy of antagonists isolated from rhizosphere of Mungbean against Macrophomina phaseolina, Aspergillus niger, Trichoderma atroviride, T. harzianum, T. viride and Bacillus subtilis gave distinct antagonistic reactions, showing stunting of Macrophomina phaseolina colony and a clear zone of inhibition between colonies of antagonist and the pathogen was developed. Trichoderma harzianum was most effective among all antagonists in reducing dry root rot of mungbean followed by Trichoderma viride, Trichoderma atroviride, Bacillus subtilis and Aspergillus niger.*

Key words: *Rhizosphere , Macrophomina phaseolina , antagonist.*

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] is grown in almost all parts of the country in summer and *kharif* season in Northern and Southern India. In India, it is the third important pulse crop after chickpea and pigeonpea and occupies 34.4 lac hectare with 14 lac tonnes of production and productivity 407 kg/ha (Anonymous, 2013). The major mungbean growing states in India are Andhra Pradesh, Orissa, Maharashtra and Rajasthan. In Rajasthan, the total area of mungbean is 7.9 lac hectare with 2.34 lac tonnes of production and 302 kg/ha of productivity (Anonymous, 2013). The important mungbean growing districts in the state are Jaipur, Bhilwara, Bharatpur, Sri Ganganagar, Jodhpur, Kota and Udaipur. Mungbean is being infected by several fungal, bacterial and viral diseases. The major fungal diseases are dry root rot (*Macrophomina phaseolina* (Tassi) Goid.), web blight, *Rhizoctonia solani* Khun (*Thanatephorus cucumeris*), powdery mildew (*Erysiphe polygoni* DC), *Cercospora* leaf spot (*Cercospora canescens* Ellis and Martin) and anthracnose (*Colletotrichum dematium* and *C. lindemuthianum*). But, dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid. is considered as the most devastating disease in all the mungbean growing areas of country. The fungus *M. phaseolina* infects more than 500 plant species worldwide (Wyllie, 1993, Sinclair, 1982) and causes charcoal rot disease in several agronomically important crops includ-ing soybean, maize, sorghum and cotton. The disease is quite wide spread across the Rajasthan state due to congenial weather conditions and causes considerable yield losses (Philip *et al*., 1969, Grewal, 1988). The pathogen may infect almost all parts of plants i.e. root, stem, branches, petioles, leaves and pods. Seed infection due to *Macrophomina phaseolina* ranges from 2.2 to 15.7 per cent which may cause losses in grain yield to the extent of 10.8 per cent and protein content of 12.3 per cent (Kaushik *et al*., 1987). The pathogen being soil-borne and its propagules distributed randomly in soil is difficult to be controlled by fungicide. Moreover, the fungicides are effective only on the active metabolic stage of the propagules and not on resting structure. Soil application of fungicides is an expansive and deleterious to non target microflora. Biological control has become a critical component of plant disease management and it is a practical and safe approach in various crops (Patel and Anahosur, 2001). Bioprotectants provide unique opportunity for crop production, since they grow, proliferate, colonize and protect the newly-formed plant parts to which they were not initially applied (Harman, 1991). Microorganisms isolated during the course of studies

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were tested for antagonism to *Macrophomina phaseolina.* The microorganisms indicating such properties were utilized for possible biological control of mungbean dry root rot by addition of the cultures of antagonists to infested soil.

MATERIALS AND METHODS

Isolation and Purification

Dry root rot infected mungbean samples were collected from different field locations, *viz.* Bikaner, Sawaimadhopur, Churu, Hisar, Delhi, Sri Ganganagar, IARI-5143, Jalana (Maharastra)-5156, Junagarh-6486 and Narnaul. The root samples of diseased plants were used for isolation. The roots were thoroughly washed with tap water to remove soil. Small pieces of about 0.5 cm length were surface sterilized with 0.1 per cent mercuric chloride solution for 2 minutes. Three washings with sterilized distilled water given, placed on Potato Dextrose Agar (Peeled potato 200 g, dextrose 20 g and agar agar 20 g in 1000 ml distilled water) slant in a laminar flow and incubated at 28 ± 1°C temperature for growth for seven days (Table 1).

Isolation of antagonists from rhizosphere of Mungbean

Soil samples were collected from rhizosphere of healthy Mungbean plants. Suitable dilutions were made using serial dilution technique (dilution of 10^{-4} used for fungi, 10^{-5} used for bacteria). Taking appropriate volume from each dilution were plated on PDA (potato dextrose agar) and NA (nutrient agar) media for fungi and bacteria respectively and incubated for 2-3 days at 28 °C. Isolated colonies were transferred and purified on respective media slants and used for further studies.

Testing of antagonists to *Macrophomina phaseolina in vitro*

Trichoderma and other species isolated from infested soil were tested for their antagonism to *Macrophomina phaseolina* on Czapek's dox agar medium (Agar-agar 15.0 g, sodium nitrate 2.0 g, dipotassium hydrogen phosphate 1.0g, potassium chloride 0.50g, magnesium sulphate 0.50g, ferrous sulphate 0.01g, sucrose 30.0g, distilled water 1000 ml (Conn 1921) in Petri dishes.

Dual Culture Method

The antagonistic potential of each antagonist was studied. A 5mm diameter disc of antagonist was placed individually at one end of the Petri dish containing Czapek's dox agar medium and just opposite to that a 5 mm diameter disc of the pathogen was placed. Three replications were maintained for each antagonist. In control, the pathogen alone was inoculated. The Petri dishes were incubated at 28 ± 1°C for seven days in a BOD incubator and observations were recorded. Microorganisms which inhibited growth of *M. phaseolina* in above method were termed as antagonistic microorganisms.

Paper disc plate method

For bacterium *Bacillus subtilis* Paper Disc Plate Method (Loo *et al*., 1945) was followed. Circular disc (5 mm diameter) of whatman filter (No.42) were cut and after dipping in filterate of *Bacillus subtilis* were placed 2 cm inward from the periphery of Petri dishes at three equidistance places, having in the centre the inoculum of pathogen.The inoculated dishes were placed in incubator at 28?1°C for a week and observations were recorded. Each microorganism and the pathogen placed in Petri dishes were opposite to each other. Radial growth of *M. phaseolina* was recorded and inhibition per cent was calculated using formula:

$$\text{Per cent growth inhibition} = \frac{C - T}{C} \times 100$$

C = Radial growth of *M. phaseolina* in control (mm)
T = Radial growth of *M. phaseolina* in presence of antagonist (mm)

Antagonist in Green House conditions

The highly pathogenic isolate of *Macrophomina phaseolina* isolated from mungbean was multiplied on sand maize medium for 15 days. The antagonistic microorganism *Trichoderma* spp. *Bacillus* spp. and other spp. were also multiplied on sterilized sorghum grains side by side. The separate media for pathogen and antagonists were taken with the view that they will not utilize the medium of each other for growth and multiplication in pots. After multiplication of both organisms on their respective media, *Macrophomina phaseolina* and antagonist were added in equal quantity in pots filled with sterilized soil. Seeds of highly susceptible variety RMG-62 after surface sterilization with 0.1% mercuric chloride were sown in each pot and ten plants in each pot were maintained after germination. A control with only pathogen was maintained. The disease was recorded till there was 100 per cent mortality in control pots. Soil sample for isolation of antagonists and pathogen were also taken at two stages i.e. pre sowing in unsterilized soil and after 100 per cent mortality in

control in sterilized soil. Population of pathogen (*Macrophomina phaseolina*) and antagonists was calculated on the basis of per 'g' soil. Per cent root rot incidence and disease control in experiment were calculated by using the following formulae:

$$Root\ rot\ incidence\ (\%) = \frac{Number\ of\ diseased\ plants}{Total\ number\ of\ plants} \times 100$$

$$Disease\ control\ (\%) = \frac{Root\ rot\ incidence\ in\ inoculated\ control\ (\%) - Root\ rot\ incidence\ in\ treatment\ (\%)}{Root\ rot\ incidence\ in\ inoculated\ control\ (\%)} \times 100$$

Statistical analysis

Each treatment in all experiments was in triplicate and data analysed statistically with the help of Completely Randomized Design (CRD). The per cent value was transformed into angles corresponding to that as follows (Snedecor and William, 1967). Angles = Arcsin "Percentage

RESULTS AND DISCUSSION

Testing of microorganisms against *Macrophomina phaseolina* isolate *in vitro*:

Seventeen fungi *viz. Trichoderma harzianum, T. viride, T. atroviride, Alternaria alternata, Aspergillus niger, A. clavatus, A. flavus, A. japonicus, A. nidulans, Curvularia lunata, Chaetomium globosum, Cochliobolus lunatus, Drechslera halodes, Fusarium oxysporum, Penicillium aurantiogriseum, P. javanicum* and *Rhizopus stolonifer* and one bacterium *Bacillus subtilis* isolated from rhizosphere soil of mungbean were tested for their antagonistic activity against Bikaner isolate of *Macrophomina phaseolina* on Czapek's dox agar medium *in vitro* (Table 2).The antagonistic reaction of isolated fungi and bacteria against pathogen was classified as mild, intermediate and antagonist. All the microorganisms showed reaction to *Macrophomina phaseolina.* Two microbes *viz. Penicillium aurantiogriseum* and *Penicillium javanicum* which showed reactions up to 30 per cent inhibition of growth were termed as mild antagonist. Eleven microbes which formed inhibition zone between 31 to 50 per cents *viz. A. alternate, A. clavatus, A. flavus, A. japonicus, A. nidulans, Curvularia lunata, Chaetomium globosum, Cochliobolus lunatus, Drechslera halodes, Fusarium oxysporum* and *Rhizopus stolonifer* were termed as intermediate antagonist. Four fungi and one bacterium *viz. Trichoderma viride, T. harzianum, T. atroviride, Aspergillus niger* and *Bacillus subtilis* which developed a zone of inhibition more than 50 per cent were termed as antagonist . Mutual antagonism and mutual intermingling of the pathogen and antagonist in few cases were also observed.

Antagonism in Green House condition

Four fungi and one bacterium *viz. Trichoderma harzianum, Trichoderma viride, Trichoderma atroviride, Aspergillus niger* and *Bacillus subtilis* in susceptible RMG-62 variety of mungbean were able to reduce the dry root rot incidence in sterilized soil filled in earthen pots. Highly virulent Bikaner isolate was taken in this experiment. An apparent correlation between the decrease in disease incidence of dry root rot of mungbean and the magnitude of population/g soil of *Macrophomina phaseolina* and antagonists have been illustrated (Table 3). The mean population of *Macrophomina phaseolina* and antagonists was optimum in naturally infested unsterilized soil of mungbean (pre-sowing). Population of each antagonist assayed revealed its increase over *Macrophomina phaseolina.* The mean population of *Macrophomina phaseolina* observed with treatment of *Trichoderma harzianum, Trichoderma viride, Trichoderma atroviride, Aspergillus niger* and *Bacillus subtilis* was 2636.67, 3268.17, 3920.78 5365.10 and 4390.42 respectively, in sterilized soil. In control *Macrophomina phaseolina* population was 7038.26. A significant decrease in incidence of disease by antagonists was found as compared to control. *Trichoderma harzianum, Trichoderma viride, Trichoderma atroviride, Bacillus subtilis* and *Aspergillus niger* minimized dry root rot incidence (%) in mungbean upto 76.67, 70.00, 66.67 53.33 and 43.34 respectively as compared to control where 100 per cent mortality was observed. *Trichoderma harzianum* was most effective among the antagonists trial in minimizing the disease followed by *Trichoderma viride, Trichoderma atroviride, Bacillus subtilis* and *Aspergillus niger*. The microorganisms isolated from initial infested soil and rhizosphere of resistant MSJ-118 and susceptible RMG-62 varieties of mungbean were tested for their antagonistic reactions against the pathogen *Macrophomina phaseolina.* All the microorganisms showed reaction to *Macrophomina phaseolina.* In present investigations, majority of the microorganisms were intermediate with pathogen and formed inhibition zone. The antagonistic reaction of isolated fungi and bacteria against pathogen was classified as mild, intermediate and antagonist. Two microbes *viz. Penicillium aurantiogriseum* and *Penicillium javanicum* were mild in reaction. The microbes *viz. Alternaria alternata, A. clavatus, A. flavus, A. japonicus, A. nidulans, Curvularia*

lunata, Chaetomium globosum, Cochliobolus lunatus, Drechslera halodes, Fusarium oxysporum and *Rhizopus stolonifer* were intermediate in reaction while a few developed a distinct zone of inhibition between the colonies of antagonist and pathogen. Microorganisms which developed well marked zone of inhibition on Czapek's dox agar plates comprised four fungi and a bacteria *viz. Trichoderma viride, T. harzianum, T. atroviride, Aspergillus niger* and *Bacillus subtilis* were found antagonist. Mishra and his associates (2011) found *Trichoderma viride* antagonistic to *Macrophomina phaseolina* incitant of dry root disease in mungbean. *Trichoderma viride* inhibited growth of pathogen upto seventy per cent. Veena *et al.* (2014) from S.V. Agricultural College, Tirupati observed that *Trichoderma* isolate-7 inhibited the growth of *Rhizoctonia bataticola* upto 83.33 per cent. Similarly, Tandel *et al.* (2014) found eight antagonists against *Macrophomina phaseolina* causing blight in greengram as observed in present in present investigation.The antagonistic microorganisms present in rhizosphere can play an important role for the disease resistance

Table 1
Pathogenicity of ten isolates of *M. phaseolina* on RMG-62 variety of mungbean in sterilized and unsterilized soil

Isolate	*Sterilized*	*Unsterilized*	*Average*
Bikaner	100 (90)	83.33 (66.14)	91.67 (78.07)
Churu	80 (63.43)	66.67 (54.78)	73.34 (59.11)
Delhi	56.67 (48.85)	53.33 (46.92)	55 (47.89)
Hisar	73.33 (59)	63.33 (52.78)	68.33 (55.89)
IARI-5143	60 (50.77)	56.67 (48.85)	58.34 (49.81)
Jalana-5156	50 (45)	43.33 (41.15)	46.67 (43.08)
Junagarh-6486	63.33 (52.78)	53.33 (46.92)	58.33 (49.85)
Narnaul	33.33 (35.22)	26.67 (30.79)	30 (33.01)
Sawaimadhopur	60 (50.85)	46.67 (43.08)	53.34. (46.97)
Sri Ganganagar	70 (57)	66.67 (54.78)	68.34 (55.89)
Average	64.67 (55.29)	56 (48.62)	60.33 (51.95)
	S. Em.±	CD (P=0.05)	CD (P=0.01)
Isolates	1.76	5.00	6.66
Soil types	0.79	2.23	2.98
Isolates x Soil types	2.49	7.07	9.42
CV (%)	8.29		

Table 2
Effect of microorganisms on the growth of *Macrophomina phaseolina* on Czapek's dox agar medium

Sr.no	*Microorganisms*	*Microorganism inhibiting growth of Macrophomina phaseolina (%)*
1	*Alternaria alternata*	48.47(44.12)
2	*Aspergillus clavatus*	39.63(39.02)
3	*Aspergillus flavus*	41.44(40.07)
4	*Aspergillus japonicas*	40.70(39.64)
5	*Aspergillus nidulans*	38.62(38.42)
6	*Aspergillus niger*	56.96(49.00)
7	*Chaetomium globosum*	41.74(40.24)
8	*Cochliobolus lunatus*	36.35(37.08)
9	*Curvularia lunata*	42.23(40.53)
10	*Drechslera halodes*	39.60(38.99)
11	*Fusarium oxysporum*	40.55(39.55)
12	*Penicillium aurantiogriseum*	28.41(32.20)
13	*Penicillium javanicum*	27.52(31.64)
14	*Rhizopus stolonifer*	38.33(38.25)
15	*Trichoderma atroviride*	60.48 (51.05)
16	*Trichoderma harzianum*	66.35 (54.55)
17	*Trichoderma viride*	65.86 (54.25)
18	*Bacillus subtilis*	53.24 (46.86)
S. Em.		0.64
CD 5%		1.83
CD 1%		2.45
CV (%)		2.63
General mean		41.97

Table 3
Effect of antagonistic microorganisms to minimize dry root rot of mungbean in Green House conditions

Antagonist	*Mean population/g soil (pre-sowing)*		*Mean population/g soil (after 100% mortality in control)*			
	M. phaseolina	*Antagonist*	*M. phaseolina*	*Antagonist*	*Disease incidence (%)*	*Disease control(%)*
Control (Soil infected with *M. phaseolina* alone)	1872.69	-	7038.26	-	100.00	-
Aspergillus niger (Soil infected with *M. phaseolina* + *Aspergillus niger*)	1340.46	3042.69	5365.10	13474.18	56.66	43.34
T. atroviride (Soil infected with *M. phaseolina* + *T. atroviride*)	1404.43	3640.63	3920.78	12672.50	33.33	66.67
T. harzianum (Soil infected with *M. phaseolina* + *T. harzinum*)	1787.82	476.18	2636.67	3784.32	23.33	76.67
T. viride (Soil infected with *M. phaseolina* + *T. viride*)	1589.08	516.52	3268.17	3523.80	30.00	70.00
Bacillus subtilis (Soil infected with *M. phaseolina* + *Bacillus subtilis*)	1596.07	4213.17	4390.42	1433.33	46.67	53.33
S. Em. 2.96	CD 5% 9.13		CD 1% 12.80	CV (%) 11.94	General mean	46.47

or susceptibility of a variety. In present investigations, the microorganisms *viz. Aspergillus niger, Trichoderma atroviride, T. harzianum, T. viride,* and *Bacillus subtilis* were more in rhizosphere of resistant MSJ-118 variety than of susceptible RMG-62 variety. These antagonists found in present studies were responsible in reducing the population of *Macrophomina phaseolina* with the plant growth in rhizosphere of MSJ-118, thereby, creating conditions in rhizosphere for host resistance. But the population of antagonistic microorganisms in susceptible RMG-62 variety was reduced in the present investigations hence the population of pathogen was higher throughout plant growth.Although resistant varieties can solve the problem to minimize the soil borne diseases to a greater extent but they do not maintain their resistance for long due to constant variations in pathogen in nature, the changing rate of microorganisms in soil and other variable soil factors. Antibiotics secreted by antagonistic organisms can be utilized for inhibiting the development of the pathogen in soil, thereby, reducing the intensity of soil borne disease incited by plant pathogenic organisms, ultimately leading to biological management.In present studies, a direct correlation was found between the population of pathogen and per cent mortality of plants which was inversely proportionate to the population of antagonists. *Trichoderma harzianum* was most effective among the antagonists in checking the disease followed by *Trichoderma viride, Trichoderma atroviride, Bacillus subtilis* and *Aspergillus niger*. Similar findings have been observed by Malik and Dawar (2003), Indra and Subbiah (2003), Chandel (2007), Sarkar and Bhattacharyya (2008) and Jaiman and coworkers (2009) tested biocontrol agents inclusive *Trichoderma harzianum, T. viride, Bacillus* spp. against *Macrophomina phaseolina* in different crops and found the reduction of disease incidence with a increase of grain yield of tested crops.

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