SYMPTOMATOLOGY, PATHOGENICITY AND EFFECT OF ANTAGONISTS ON GROWTH OF *Macrophomina phaseolina* INCITING ROOT ROT IN MUNGBEAN UNDER *IN VITRO* CONDITIONS

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ABSTRACT

The infected plants showed typical symptoms of root rot exhibited easily uprooting, rotting root hairs, blackish tap roots, crossed brownish cracking, less vegetative growth, shrunken leaves and roots, instant plant death, wilting, etc. The pure culture was isolated from naturally infected plants and inoculated in healthy plants. Then plant showed similar symptoms to the naturally infected plants afterward pure culture was obtained through re-isolation. Isolated and re-isolated were similar confirmed through Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi and was identified as Macrophomina phaseolina with their identity No.10718.18. Mycelial growth of fungal bioagents viz., Trichoderma asperellum, T. harzianum, T. viride and T. virens appeared on streak of bacterial bioagent i.e. Pseudomonas fluorescens without any antagonistic effect on each other considered as a compatible bioagents. Among the bioagents studied, T. asperellum was found most potential bioagent for suppressing mycelium growth (18.13 mm) and per cent growth inhibition (79.85 %) against M. phaseolina.

KEY WORDS: Antagonists, Mungbean, Pathogenicity, Symptomatology

INTRODUCTION

Mungbean irritated from many diseases caused by biotic stresses *viz.*, fungi, bacteria, viruses, nematodes and other abiotic stresses. Diseases caused by fungi which are responsible for drastically reduction in yield of mungbean are dry root rot [*Macrophomina phaseolina* (Tassi) Goid.], web blight [*Rhizoctonia solani* Kuhn = *Thanatophorus cucumeris*], powdery mildew [*Erysiphe polygoni* DC], cercospora leaf spot [*Cercospora canescens* Ellis and Martin] and anthracnose [*Colletotrichum dematium* and *C. lindemuthianum*) (Grewal, 1988). Among them, *Macrophomina phaseolina* (Tassi) Goid a causal organism of dry root rot is one of the leading important disease and it is known as the most dangerous pathogen which infects wide range of hosts (Sandhu and Singh, 1998). This fungus attack on different plant parts such as root, stem and pod of host plants and microsclerotia appears on plant parts. Yield loss due to the seed infection in mungbean is 10.8 per cent (Kaushik *et al*., 1981). Soil and seed borne nature of this pathogen is a paramount problems for functional effective management.

Looking to the importance of this disease, symptomatology, pathogenicity and effect of antagonists on growth of *Macrophomina phaseolina* inciting root rot under *in vitro* conditions was studied.

MATERIALS AND METHODS

The diseased samples were collected from Pathology farm near library. The infected samples carried in sample bags and brought in the laboratory for confirmation through microscopic observation of diseased tissues. Infected portion of plants were first washed with tap water and then infected tissue adjoining with healthy tissues were cut into small pieces of 5 to 7 mm and surface sterilized by dipping in 0.1 % sodium hypochlorite solution for one minute followed by three washing with sterilized distilled water. These sterilized pieces were placed on Potato Dextrose Agar (PDA) medium poured in Petri plates under aseptic condition. These plates were incubated for good growth of pathogen at 27 ± 1^0C in BOD incubator. Mycelial growth obtained after 48 hrs of incubation was sub-cultured and further purified by hyphal tip method. The isolate thus obtained was used for pathogenicity. After proving pathogenicity the pathogen was reisolated from infected tissues and maintained by periodical transfer on PDA slants under refrigerator throughout the investigation.

Symptomatology and pathogenicity

Typical characteristics of dry root rot were identified in plants through visual investigation and microscopic observation and confirmed the pathogen. The typical symptoms of dry root rot on mungbean plants were observed in field conditions. An infected plant parts were placed between blotting papers after drying used for current investigation. The pathogenicity was confirmed with various inoculation methods. The symptoms of dry root rot were compared with naturally infected plants.

Seed inoculation

Vigorous and healthy seed of cultivar GM-4 was gently mixed with 7 days old pure culture of *M. phaseolina*. Pot filled with sterilized soil was used for sowing of *M. phaseolina* inoculated seeds. The uninoculated seeds were sown as a control. Glasshouse was used for proving pathogenicity and regularly irrigated as per requirements. Observations on seed germination and seedling mortality were recorded.

Soil inoculation

Sterilized soil in pot was inoculated with *M. phaseolina* through pathogen multiplied sorghum grains and mixed inoculated sorghum seeds in 4-5 cm outer layer of soil. Polythene cover was used to avoid contamination. Healthy and un-inoculated seeds of mungbean were sown in pots. Un-inoculated soil and seed treatment was maintained as a check. Seedling mortality was recorded after 10 days of sowing upto 60 days of crops.

Seed and soil inoculation

Combination of seed and soil inoculation method was followed as mentioned above.

Bioagents

The potential fungal and bacterial biocontrol agents maintained at the Department of Plant Pathology, B. A. College of Agriculture, AAU, Anand were used for current investigation. These bioagents (*T.asperellum, T. harzianum T. virens T. viride P. fluorescens* tested *in vitro* against *M. phaseolina* by dual culture methods (Plate 1)

Observations recorded

Pathogen inhibition was measured at an interval of 24 hours until the control plate was covered with pathogen mycelium. Inhibition of pathogen was recorded as per formula given by Vincent (1927).

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

Where,

C= Mean diameter of mycelial colony in control plates (mm)

T= Mean diameter of mycelial colony in treated plates (mm)

RESULTS AND DISCUSSION

Symptoms

The infected plants showing characteristic symptoms of root rot were collected from field. The infected plants exhibited discoloration of tap roots, longitudinal cracking of the stems, stunting, wilting, *etc*., (Plate 2).

Isolation and Purification

The infected plant samples brought to the laboratory and were subjected for isolation by tissue isolation technique to obtain the culture of pathogen causing root rot. The cultures, thus, obtained were further purified by hyphal tip method. The pure cultures of the pathogen were maintained by periodical transfer on PDA slants throughout the investigations (Plate 3). Thilagavathi *et al.* (2007) isolated *M. phaseolina* from green gram plants showing typical root rot symptoms and pure cultures of the pathogen were obtained by the single hyphal tip method.

Morphology and Identification of the Pathogen

Fungi morphologically diversified in characters *viz*., visual appearance and growth patterns, mycelium, sclerotia, *etc*,. were used as primary identification. Hyphal pattern and sclerotia were observed through microscopic visualization by preparing slide from pure culture of pathogen.

Mycelium

The fungal growth begins on autoclaved nutrient medium with whitish appearance and mycelium slowly scattered in entire space of the Petri plates within four to six days. Plenty number of white colored mycelium present on aerial region, dichotomy appearance observed on basal region of the Petri plates. The mycelium colored was white in earlier growth, but after sometime sudden changes observed like mycelium became black with plenty of black sclerotia (Plate 4). The hyphae construct an angle of 90 degrees with septa was observed through microscopic visualization. The hyphae measured as 2.1 to 8.1 µm in diameter.

Sclerotium

The pure culture was maintained in incubator after one fortnight plenty number sclerotia observed in pure culture of pathogen. Slide was prepared from 17 days old pure culture to study the sclerotial features through a ocular micrometer. Plenty of the sclerotia were observed in microscopic visualization and wide range of diameter was recorded between 69.25 to 189.75 µm with 129 µm mean diameter (Plate 4).

Identification and pathogenicity

The A1 and A2 pure culture were isolated from mungbean plants and confirmed through identification number 10,718.18 at Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi. The A1 pure culture was isolated from naturally infected plants and inoculated in healthy plants and inoculated plant produced similar symptoms to the naturally infected plants afterward A2 pure culture was obtained through re-isolation. A1 (isolated) and A2 (re-isolated) were similar and confirmed through Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi. Different inoculation techniques were used in proving pathogenicity among them combination of soil and seed inoculation method was found effective compared to other (Table 1 and Plate 5). Typical symptoms observed in combined application of soil and seed inoculation method after ten

days of sowing. However, untreated plants did not reveal any disease symptom.

Maximum disease incidence was recorded in combination of soil and seed inoculation method as compared to other treatments. Diseased plants reveals symptoms like easily uprooting, rotting of root hairs, blackening tap roots, crossed brownish cracking, less vegetative growth, shrunken leaves and roots, instant plant death and wilting, *etc*.

Testing of antagonism of the bioagents to the pathogen

Efficiency of four fungal bioagents *viz*. *T. asperellum*, *T. harzianum*, *T. virens*, *T. viride* and one bacterial bioagent *i.e. P. fluorescens* were studied for their antagonism against *M. phaseolina* by dual culture method.

Growth inhibition of M. phaseolina

All the antagonists significantly helped in inhibiting the mycelial growth of *M. phaseolina* over control. Significantly minimum mycelial growth (18.13 mm) and maximum growth inhibition (79.85%) was observed in treatment T_1 *i.e. T. asperellum* followed by treatment T_4 *i.e. T. viride* (21.37 mm & 76.25%) after 8 days of incubation. Treatment T_5 *i.e. P. fluorescens* recorded minimum mycelial growth inhibition (38.10 mm, 57.67 %) (Table 2 & Plate 6). Thilagavathi *et al.* (2007) the antagonistic effect of *Trichoderma viride* (strains Tv1 and Tv13), *Pseudomonas fluorescens* (Pf1 and Pf15) and *Bacillus subtilis* (Bs16) individually and in combination against *M. phaseolina* causing root rot in green gram. Among all, *T. viride* (strains Tv1 and Tv13) showed maximum growth inhibition of the pathogen.

CONCLUSION

The infected plant showing typical characteristic symptoms ofroot rot were collected from field. The infected plants exhibited discoloration of tap roots, longitudinal cracking of the stems, stunting, wilting, *etc*. The A1 pure culture was isolated from naturally infected plants and inoculated in healthy plants and inoculated plant produced similar symptoms to the naturally infected plants afterward A2 pure culture was obtained through re-isolation. A1 (isolated) and A2 (re-isolated) were similar and confirmed through Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi.Fungal bioagents *viz.,T. asperellum T. harzianum, T. viride* and *T. virens* mycelial growth appeared onstreak of bacterial bioagents *i.e.P. fluorescens*without any antagonistic effect on each other so considered as a compatible bioagents. All the bioagents were effective for supressing the mycelium growth and maximize the growth inhibition of *M. phaseolina* compared to control. Four fungal bioagents and one bacterial bioagent, fungal bioagents were efficient compared to bacterial bioagent in relation to mycelium growth and maximum per cent inhibition. Especially, T_1 *i.e. T. asperellum,* recorded least mycelial growth 18.13 mm and maximum growth inhibition of 79.85 per cent.

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agents improves the management of dry root rot (*Macrophomina phaseolina*) in greengram. *Phytopathologia Mediterranea*, **46**(2): 157-167.

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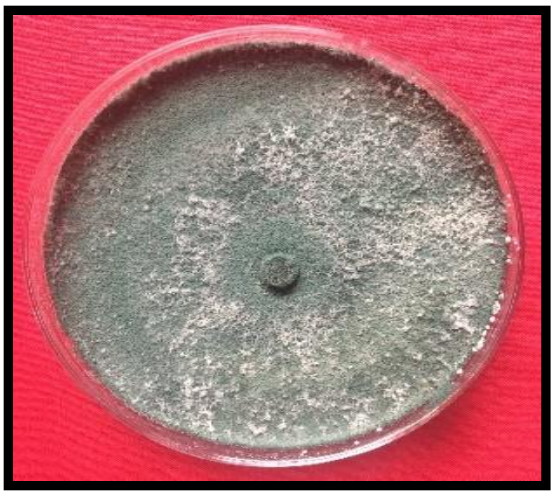
Table 1: Inoculation techniques for proving pathogenicity

Method of Inoculation	Number of Plants Inoculated	Number of Plants Infected	Disease Incidence (%)
Seed inoculation	10	4	40
Soil inoculation	10	6	60
Seed & soil inoculation	10	9	90
Control	10	0	0

Table 2: *In vitro* antagonism of bioagents with pathogen

Tr. No.	Bio-agents	*M. phaseolina*	
		Mycelial Growth (mm)	Growth Inhibition (%)
T_1	*T. asperellum*	18.13	79.85
T_2	*T. harzianum*	29.33	67.41
T_3	*T. virens*	32.88	63.47
T_4	*T. viride*	21.37	76.25
T_5	*P. fluorescens*	38.10	57.67
T_6	Control	90.00	0.00
S. Em. ±		0.28	--
C.D. at 5 %		0.85	--
C.V. %		1.25	--



T. asperellum

T. virens

P. fluorescens

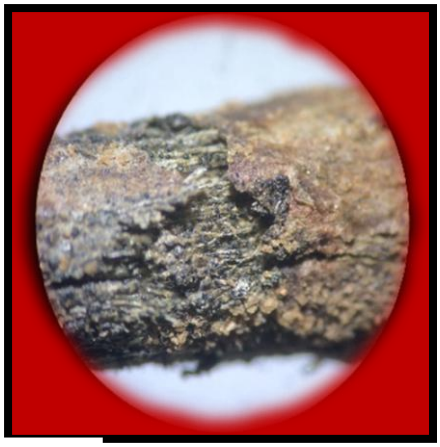
T. viride

T. harzianum

Plate 1: Bioagents viz., T. asperellum, T. viride, T. harzianum, T. virens and P. fluorescens

Crossed brownish cracking

Blackish collar region



PYCNIDIA ON STEM

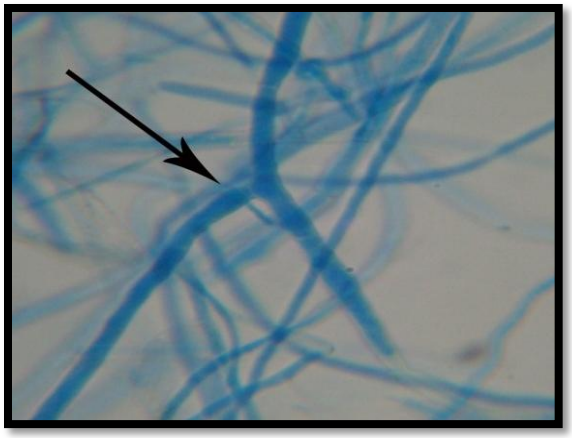
Infected root

Bark shredding

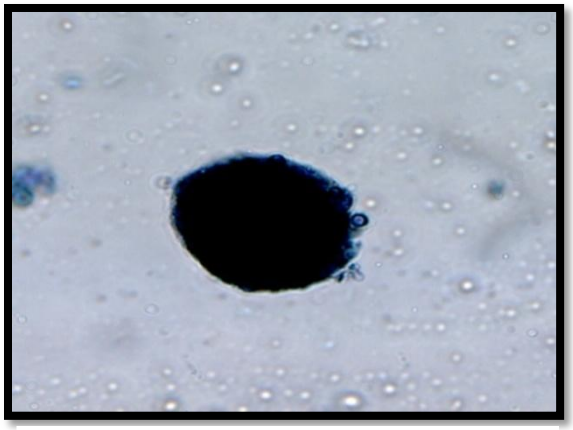
PLANT DEATH AND WILTING

Plate 2: Typical symptoms of dry root rot on different parts of mungbean caused by M. phaseolina

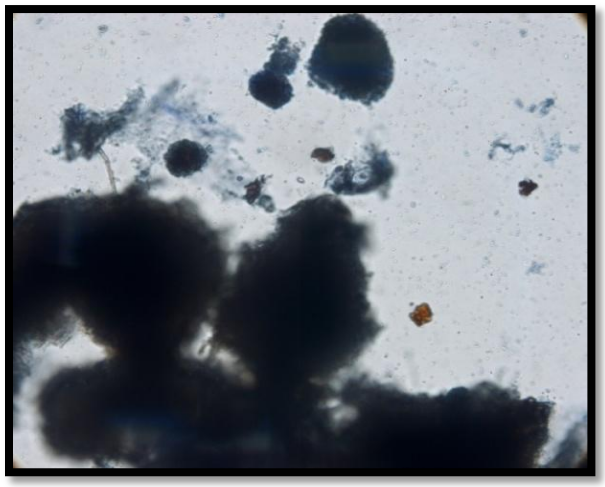
Plate 3: Pure culture of M. phaseolina isolated from infected root of mungbean



Right angled mycelium



Close-up view of sclerotia



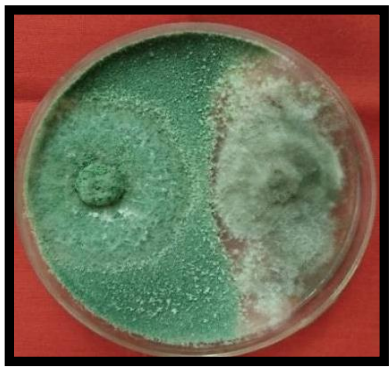
Mass of sclerotia

Plate 4: Photomicrograph of morphological characters of M. phaseolina

(A) Seed inoculation (B) Soil inoculation (C) Seed and soil inoculation (D) Control

Plate 5: Pathogenicity test of M. phaseolina in mungbean with different inoculation techniques

T. asperellum

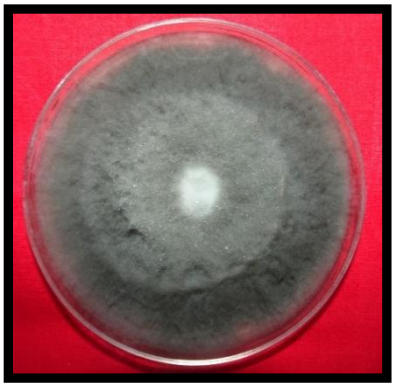


T. harzianum

T. virens

T. viride

P. fluorescens



Control

Plate 6: In vitro antagonism of bioagents with pathogen

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