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MANAGEMENT OF ROOT ROT OF URDBEAN (*PHASEOLUS MUNGO*) WITH *TRICHODERMA SPP.* IN RAINFED AREAS OF JAMMU AND KATHUA DISTRICTS

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ABSTRACT
All the fungal bio control agents viz. Three isolates of *Trichoderma viride*, *T. harzianum* and *T. virens* were found effective against root rot disease of urdbean under *in vitro* and *in vivo* condition. Among them, *Trichoderma viride* isolate 2 was observed as most effective, which inhibited the radial growth of *Macrophomina phaseolina* with the maximum inhibition percentage (19.30, 39.50, 56.00 and 72.10) % after 2, 4, 6 and 8 days over control *in vitro* condition. Under *in vivo* condition at Samba district *T. viride* isolate 2 was highly effective in the management of the root rot disease was (0.40, 17.60 and 25.00) % and similarly at Kathua district (0.42, 17.80 and 24.00) % after 40, 60 and 80 days over control. The pooled data of both the years showed that the maximum root length (9.72cm), shoot length (27.85cm), root weight (4.78g) and shoot weight (11.84g) at Samba district and at kathua district root length (9.92cm), shoot length (27.90cm), root weight (4.90g) and shoot weight (11.90g) was also recorded with the same treatment. However it was observed that *T. viride* isolate 2 was most effective against root rot of urdbean and showed maximum root length, shoot length, root weight and shoot weight

INTRODUCTION

Pulses are known to be among the most vulnerable crops to the attacks of pests and diseases causing huge production losses. Urdbean is an important short duration grain legume crops with wide adaptability, low input requirement and have the ability to improve soil fertility by fixing atmospheric nitrogen and is grown on about 3.24 million hectares with annual production of 1.52

million tons. The crop is thought to be of Indian origin as evidenced by occurrence at archaeological sites in the Indian Subcontinent (NCIPM). It is also known as black gram (*Vigna mungo*). Archaeological studies have shown that it was cultivated in the country as far back as 2200 B.C. Based on seed color and other characteristics urdbean has been grouped under two main types viz. var. mungo with large black seed and early maturity and var. *viridis* with small greenish seed and late maturity. It is an important pulse crop and serves as a major source of dietary protein for majority of people. It is also cultivated in three different season, viz., kharif, rabi and summer. Although the crop is grown in all the season but maximum area is occupied under kharif season. The major urdbean growing states of the country are Maharashtra, Andhra Pradesh, Madhya Pradesh, Uttar Pradesh, Tamil Nadu, Karnataka and Rajasthan. The nutritive value of urdbean lies in its high and easily digestible protein and contains approximately 25-28% protein, 1.0-1.5% oil, 3.5% - 4.5% fiber, 4.5-5.5% ash and 62-65% carbohydrates on dry weight basis. There may be some variability in protein percentage due to environment and genotype effect and that may range from 19-29%(NCPIM Field guide 2011).

Many soil borne fungi play a major role in causing several diseases, among them root rot is the major problem of urdbean in rainfed areas**.** *M. phaseolina* (Tassi) Goidanich causes root rot of more than 500 crops (Kishore K. Babu *et al.,* 2007). *Rhizoctonia bataticola* (Taub.) Butler (= *Sclerotium batatiicola* Taub.) (Pycnidial stage: *Macrophomina phaseolina*) is a diverse omnipresent soil-borne fungal pathogen, in-fecting more than 500 plant species. Macrophomina (Tassi) Goid, a soil inhabiting fungus is an important root rot/stem canker, stalk rot or charcoal rot of over 400 plant species including pigeon pea (Lokesha, N. M. and Benagi, V. I. 2007, Maharishi, 1986). The pathogen causes different types of diseases *viz.,* seedling blight, root rot, charcoal rot, wilt, stalk rot, stem blight, fruit rot, seedling decay and leaf blight in crop plants (Dhingra and Sinclair 1978). *R. bataticola* causes up to 60% yield loss. Different isolates of *R. bataticola,* obtained from different plant spe-cies, and plant parts of the same host showed variability (Prameela and Singh 1998; Meena *et al.,* 2006). 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 with fungicides. Seed treatment with fungicides, soil drenching with fungicides does not protect the crop for long periods and also not economically favourables, they may establish imbalances in the microbial community unfavorable for activities of beneficial organism (Jeyarajan *et al.,*1991). In addition, continuous use of the same fungicides for the same pathogen results in the development of resistant strains of the pathogen, besides polluting the environment (Muthukrishnan, 1989). It is now widely recognized that biological control of plant pathogens using antagonistic fungi is a distinct possibility for the future and can be successfully utilized especially within the frame work of integrated disease management system (Muthamilan and Jeyarajan, 1996). Use of antagonistric organiams against Macrophomina root rot has been well documented in several crops (Mukhopadhyay, 1987; Raghuchander *et al*., 1995). In this regard, the efficacy of three isolates of three biocontrol agent (viz. *Trichoderma viride, T. harzianum* and *T. virens)* against *Macrophomina phaseolina* both in dual cultura techniques and in vivo condition was investigated.

MATERIALS AND METHODS

Investigation was carried out under both *in vitro* and *in vivo* condition in the kharif season year 2009 and 2010 with objectives to find out the efficacy of the different biological control agents for the eco-friendly management of root rot disease of urdbean with the improvement of yield attributing character i.e., the root length, shoot length, root weight, shoot weight of the urdbean plant were conducted in the Advanced Centre for Rainfed Agriculture, Dhiansar, Bari-Brahmana SKUAST-Jammu and KVK-Kathua.

Field survey of Urdbean growing areas of farmer's fields of seven villages of Samba and Kathua districts were conducted for the assessment of losses caused by root rot diseases during the ongoing kharif season (2009). The disease incidence in different locations in Kathua district was –Barwal (11.66%), Samba district- Patyari (11.52%), Rayean (12.00%), and Painthi (10.60%). No disease was however, recorded in Govindsar, Saktachak (Kathua) and Kharha Madana (Samba district) as shown in the table 1. The disease samples have been collected and are under process of isolation of the pathogen(s) responsible for causing root rot disease in Urdbean. Same locations were chosen for field survey of Urdbean growing areas of farmer's fields. Seven villages of Samba and Kathua districts were conducted for the assessment of losses caused by root rot disease during the ongoing kharif season (2010). The disease incidence in different locations in Kathua district was – Barwal (10.00%), Samba district - Patyari (14.20%), Rayean (14.00%), and Painthi (12.50%). No disease was however, recorded in Govindsar, Saktachak (Kathua) and but in Kharha Madana (Samba district) 12.15% disease was recorded. The root rot disease causing pathogen *Macrophomina spp*. (*Rhizoctonia spp*. were isolated from the diseased samples.

Isolation of *R. bataticola* isolates

Pulse crops (red gram, green gram, cowpea, soybean, blackgram) showing typical root rot symptoms were col-lected from the infected farmer's field. The infected portion collected from roots, shoots and seeds were surface sterilized with 0.1% mer-curic chloride for 30 sec, washed subsequently in three changes of sterile distilled water, and placed on Potato dextrose agar (PDA) medium. It was purified by the single hyphal tip method. Pure culture of the different isolates of *R. bataticola* was maintained on PDA slants for further studies.

Mass multiplication of *R. bataticola* inoculum

The isolates of the fungus were multiplied in sand maize medium (Riker and Riker 1936). Sand and ground maize seeds were mixed in a ratio of 19:1 moistened to 50 % moisture content, filled in polypropylene bags, and autoclaved at 20 psi for two hours. Four actively grow-ing mycelial discs (5 mm) of the pathogen isolates were inoculated into each polypropylene bag under aseptic condition. The polypropylene bags were then incubated at room temperature (28 ± 2ºC) for 15 days, and the inocu-lum thus obtained was used for the experiments.

Morphological studies

Macrophomina phaseolina of urdbean were grown in PDA and the growth pattern was studied at a room temperature of 28 ± 2ºC. The colony color, growth of colony, and colony characters were measured 3 DAI (Days after inoculation). Under *in vitro* condition three resident isolates of bio-control agents viz. *T. viride*, *T. harzianum*, *T. virens* were isolated from the soils of two districts viz. Samba and Kathua of Jammu division. Isolate 1 and 2 were isolated from the Kathua and isolate 3 was isolated from the Samba district. The infected plant samples of root rot infection were collected with foreceps and washed with water and the portions were cut in such a way that it contained both the diseased and healthy looking. The infected samples were wiped with cotton swap dipped in 70% ethanol followed by light flaming. The samples were washed in three changes of sterile water and blot dry on clean, sterile paper towel to remove sterilant. Asceptically transferred the samples and put on nutrient medium usually in 3-5 portions/plate, well separated/ nutrient agar (PDA supplemented with streptomycin) plates. Incubate the inoculated plates, in an inverted position, at 25ºc for 3-5 days and observe for the development of colonies.

The antagonistic properties of all the three isolates of *Trichoderma* were evaluated following the dual culture techniques as per the mycological techniques. Per cent growth inhibition was calculated by Vincent's [9] formula, which is as follows:

Whereas,

$$\text{Percent inhibation}: \frac{C - T}{C} X100$$

C = Diameter of the colony in check (coverage of both diagonals)

T = Diameter of colony in treatment (average of both diagonals)

Growth of mycelia of *Trichoderma spp*. alone and coated around hyphae of the soil-borne fungi has reported by many workers (Ch *et al*., 1987; Weindling 1932). The efficacy of bio

agents was also evaluated *in vivo* conditions (Mukhopadhyay 1987). All the ten treatments including control were replicated thrice. The experiments were conducted in the plot of 1x1m having 10 plants each. Planting distance was maintained as recommended. Per cent root rot of incidence was calculated by following formula (Kumari *et al.,* 2012)

$$\%\ \text{Root rot incidenc e} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} X100$$

Seeds were treated with bioagents spore suspension having spore load of 1 x 10^6 spores/ml and distilled water in case of control for 10 minutes before sowing. All the bio-agents used in the experiment were mass multiplied on the PDA and incubated at 25 + 2ºc for two weeks for preparation of spore suspension. The mycelium mat with spore were harvested from flasks, blended and filtered through 3-layered muslin cloth. Final spore load of each bio-agent was diluted to 1x10^9 spores/ml using distilled water. Both the pathogenic fungi were mass multiplied on barnyard millet (1:1w/w) medium and incorporated in the seed treatment 4gm per kg of seed. The experiments regarding yield attributing characters was conducted in field. The observation regarding disease incidence was recorded after 40, 60 and 80 DAS and the root length, shoot length, root weight and shoot weight of urdbean plant was recorded at 80 DAS.

Statistical analysis

The data were analyzed subjected to analysis of variance (ANOVA) and

RESULTS AND DISCUSSION

In the present study *Trichoderma viride, T. harzianum, T. virens* were tested *in vitro* and *in vivo* condition. Under *in vitro* condition it is clear from the results that the radial growth of *Macrophomina phaseolina* was significantly reduced in *Trichoderma viride* isolate 2 followed by *T. viride* isolate 1 after 2, 4, 6 and 8 days after inoculation during both the years of investigation (Table 2). *T. viride* isolate 2 was the most effective in which maximum growth inhibition (19.30, 39.50, 56.00 and 72.10) % of *M. phaseolina* was recorded. It was followed by *T. viride* isolate 1 and *T. viride* isolate 3. After 4th day of inoculation all the fungal biocontrol agents started growing nature rapid sporulation or secretion of cell wall lytic enzymes in dual culture. These findings are in confirmity of earlier findings of Deshmukh and Raut (1992) reported *T. harzianum* and *T. viride* were effective inhibiting the mycelial growth of *M. phaseolina* and reducing the disease incidence in pot experiment. Hussain *et al*.(1990) observed that *T. spp.* were effective in controlling the infection of *M. phaseolina* in mungbean. Manczinger *et al*. (2002) reported that *Trichoderma harzianum*, *T. viride* and *T. polysporum* have a strong antagonistic against soil borne pathogens.

The results of the experiment under *in vivo* showed that all the bio control agents were effective in controlling root rot of urdbean during both the years of experimentation (table 3 and 4) at Samba and Kathua districts of Jammu division. *T. viride* isolate 2 registered the least root rot incidence (0.40, 17.60 and 25.00)% at Samba district as compared with control (10.00, 40.20 and 60.30)% and at Kathua it was recorded (0.42, 17.80 and 24.00) % as compared with control (11.00, 41.50 and 61.20)% after 40, 60 and 80 days during both the years during both the years respectively. This was closely followed by *T. viride* isolate 1.The result was in concordance with various workers Elad *et al*.(1979); Elad *et al*. (1980); Elad *et al*. (1986); Kehri and Chandra (1991); Jeyarajan *et al*.

Table 1: Field survey of Urdbean growing areas of farmer's fields (Disease Incidence %)

S. No.	Locations	2009	2010
1.	Barwal	11.66	10.00
2.	Govindsar	No disease	No disease
3.	Saktachak	No disease	No disease
4.	Patyari	11.52	14.20
5.	Rayean	12.00	14.00
6.	Kharha Madana	No disease	12.15
7.	Painthi	10.60	12.50

Table 2: Antagonistic efficacy of bio-control agents against root rot of urdbean *in vitro*

Treatments	2 days inhibition percentage over control	4 days inhibition percentage over control	6 days inhibition percentage over control	8 days inhibition percentage over control
T_1 (*T. viride* isolate 1)	18.4	38.9	54.9	67.8
T_2 (*T. viride* isolate 2)	19.3	39.5	56	72.1
T_3 (*T. viride* isolate 3)	18.1	38.2	54.1	66.3
T_4 (*T. harzianum* isolate 1)	17.6	34.8	52.1	64.5
T_5 (*T. harzianum* isolate 2)	16.4	32.3	50.8	62.3
T_6 (*T. harzianum* isolate 3)	17.1	34.2	52.2	63.3
T_7 (*T. virens* isolate 1)	15.7	29.5	48.2	60.1
T_8 (*T. virens* isolate 2)	13.1	26.7	45.3	61.5
T_9 (*T. virens* isolate 3)	15.4	27.2	47.4	58.5
SE(m) ±	0.098	0.095	0.12	0.077
$CD_{0.05}$	0.292	0.282	0.357	0.23

Each value is mean of three replicates

Table 3: Effect of fungal bio-control agents on disease and yield attributing characteristics of urdbean plant (pooled data 2009-10, 2010-2011) at Samba districts

Treatments	Root length (cm)	Shoot length (cm)	Root weight (g)	Shoot weight (g)	Disease Incidence 40	60	90
T_1 (Control)	2.33	4.33	0.4	1.85	10	40.2	60.3
T_2(*T. viride*1)	8.87	25.97	4.15	10.97	0.45	19.2	27
T_3 (*T. viride2*)	9.72	27.85	4.78	11.84	0.4	17.6	25
T_4 (*T. viride* 3)	8.37	25.5	3.8	10.61	0.5	20.3	28
T_5 (*T. harzianum* 1)	8.2	20.45	3.35	9.93	0.6	25	31
T_6 (*T. harzianum* 2)	6.95	15.95	2.59	8.45	0.7	26	33.6
T_7 (*T. harzianum* 3)	7.92	19.1	2.83	9.54	0.68	25.3	32.4
T_8(*T. virens* 1)	6.25	15.18	2	7.78	0.75	27	34.3
T_9 (*T. virens* 2)	4.27	12.06	1.8	6.2	0.85	29.1	38.9
T_{10} (*T. virens* 3)	5.3	13.23	1.95	6.85	0.79	27.8	35.6
SE(m) ±	0.011	0.013	0.024	0.014	0.091	0.052	0.054
$CD_{0.05}$	0.034	0.04	0.071	0.043	0.272	0.157	0.162

Each value is mean of three replicates

Table 4: Effect of fungal bio-control agents on disease and yield attributing characteristics of urdbean plant (pooled data2009-10, 2010-2011) at Kathua districts

Treatments	Root length(cm)	Shoot length (cm)	Root weight (g)	Shoot weight (g)	Disease Incidence 40	60	90
T_1 (Control)	2.35	4.5	0.45	1.9	11	41.5	61.2
T_2(*T. viride*1)	8.8	26	4.3	11	0.48	19.5	25.5
T_3 (*T. viride2*)	9.92	27.9	4.9	11.9	0.42	17.8	24
T_4 (*T. viride* 3)	8.47	25.8	3.9	10.7	0.53	20.5	26.8
T_5 (*T. harzianum* 1)	8.32	20.95	3.5	9.95	0.58	25.3	28
T_6 (*T. harzianum* 2)	6.9	15.8	2.6	8.5	0.69	26.5	32.2
T_7 (*T. harzianum* 3)	7.98	18.7	3	9.8	0.62	25.8	30.4
T_8(*T. virens* 1)	6.35	14.4	2.2	7.8	0.75	27.2	33
T_9 (*T. virens* 2)	4.87	12.1	2	6.2	0.84	29.5	38
T_{10} (*T. virens* 3)	5.8	13.8	1.8	6.9	0.79	27.8	36
SE(m) ±	0.031	0.067	0.048	0.041	0.036	0.395	0.456
$CD_{0.05}$	0.093	0.202	0.145	0.122	0.107	1.182	1.364

Each value is mean of three replicates

(1991); Mustafa *et al*. (2009); Kousalya and Jeyarajan (1990); Ramakrishnan *et al*. (1994).

The data regarding the effect of fungal biocontrol agents on the growth of urdbean plant indicated that the *T. viride* isolate 2 recorded the maximum root length(9.72cm), shoot length(27.85cm), root weight (4.78g) and shoot weight (11.84g) respectively over control during both the years of experimentation(table 3) at Samba district. Similarly trial conducted at Kathua district recorded maximum root length (9.92cm), shoot length (11.90cm), root weight (4.90g) and shoot weight (11.90g) was with *T. viride* isolate 2 (Table 4). Gaffar and Mallik (1991) also applied successfully *Trichoderma* as biocontrol agent against *Macrophomina phaseolina* in urdbean. Kehri and Chandra (1991) applied *T. viride* a seed coating agents *M. phaseolina* causing dry root in mungbean. Hussain *et al* .(1990) observed that the Trichoderma harzianum and Gliocladium virens were effective in controlling the infection of M. phaseolina in mungbean. Similarly Manczinger *et al*. (2002) reported that Trichoderma spp. have a strong antagonistic effect against soil borne fungi.

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