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BIOCONTROL POTENTIAL OF MIXTURE OF *TRICHODERMA* ISOLATES ON DAMPING-OFF AND COLLAR ROT OF TOMATO

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ABSTRACT

Sclerotium rolfsii is a notorious phytopathogen causing serious damage to a wide variety of hosts. Management of this pathogen requires large inputs of chemical pesticides to treat the affected soil which leads to environmental pollution. Two *Trichoderma* isolates BHU51 and BHU105 were tested singly and in combination for their biocontrol potential against *S. rolfsii* under glass house and field conditions. All *Trichoderma* treatments significantly reduced the damping-off severity in tomato which was observed up to 30 days after sowing (DAS). In consortium of *Trichoderma* (BHU51+BHU105) incidence of damping-off was recorded only 22% while in control it was found 54.67%. Highest shoot length (15.16 cm), root length (3.56 cm) and their respective fresh weight (887.53 mg and 47.33 mg) and dry weight (54.27 mg and 4.45 mg) also found highest in the mixture of *Trichoderama* treatment followed by single *Trichoderma* treatments while the lowest was recorded in the control in glass house conditions. Highest vigor index (1301.67) was recorded in the mixture of *Trichoderma* treatment while the lowest (465.67) was found in control. The minimum mean disease rating (1.71 and 1.96), maximum percent disease reduction (42.85 and 44.32) and highest shoot length, chlorophyll content and yield was recorded in the consortium of *Trichoderma* in field experiments in both the years. The results indicated that a consortium of compatible *Trichoderma* isolates can lead to greater protection and ultimately greater yield over single *Trichoderma* isolate.

INTRODUCTION

Tomato is one of the economically important vegetable crops grown worldwide. It is susceptible to more than 200 plant pathogens that cause severe destruction (Hafez *et al.*, 2013). *Sclerotium rolfsii* is one of the important pathogen of tomato crop. *S. rolfsii* is a notorious phytopathogen causing serious damage to a wide variety of hosts. The major diseases caused by it are southern blight, stem and collar rot, damping-off, etc. It produces a large number of melanin rich sclerotia which increases its biological and chemical tolerance towards degradation thus increasing its persistence in soil for many years (Chet, 1975, Punja, 1985). Management of this pathogen with synthetic fungicides is not very effective as it requires large inputs of pesticides to treat the affected soil which leads to environmental pollution and development of fungicide resistance in the pathogen. Therefore an effective and environment friendly method for plant protection is biological control of fungal plant pathogens. In recent decade, numerous microbial antagonists are widely used that are capable of suppressing various soil-borne diseases. Management of *S. rolfsii* using antagonistic microorganisms has been reported in many crops including tomato (Elad *et al.*, 1980, Singh *et al.*, 2014), brinjal (Singh and Singh, 2014), sugar beet (Upadhyay and Mukhopadhyay, 1986) and tomato and pepper (Mao *et al.*, 1998). The application of formulation of native isolates of *Trichoderma* spp. would more closely mimic the rhizospheric micro environment and thus enhance the efficacy of disease control. *Trichoderma* species are present in nearly all the soils and control phytopathogens through various mechanisms including mycoparasitism, antibiosis and competition (Harman *et al.*, 2012; Singh *et al.*, 2011). In last many years single species of a biocontrol agent were used for the management of the plant pathogens but in different environmental conditions sometimes performance were inconsistent. In order to achieve consistent performance, consortium of strains with divergent quality is desired. Many studies on combination of biological control agents with different qualities for plant disease management have been done which included mixture of fungi, mixtures of fungi and bacteria, mixtures of bacteria that improved their biocontrol efficiency (Singh and Singh, 2014, Singh *et al.*, 2013, Abeysinghe, 2009). It increases the efficiency of biological control by providing multiple mechanism of action; maintain consistency over wide range of environmental conditions (Srivastava *et al.*, 2010). *Trichoderma* is one of the potent biocontrol agents for the management of soilborne diseases. The use of mixture of compatible *Trichoderma* isolates may show increased growth promotion activities and biocontrol potential as compared to single isolates. The use of *Trichoderma* may help in reducing the excess use of hazardous chemical pesticides and can improve economy of farmers. Keeping above points in mind the main objectives of the present study was to investigate the potential of mixture of two compatible *Trichoderma* isolates over single isolate on plant growth promotion activities, yield and management of *S. rolfsii* rot of tomato in glass house as well as field conditions.

MATERIALS AND METHODS

Sclerotia forming soilborne plant pathogen *S. rolfsii* was isolated from the infected tomato plants from vegetable field of Banaras Hindu University, Varanasi. The culture of pathogens were maintained on PDA and stored at 4°C for further studies. Pathogens inoculum was prepared in sand maize meal media. Two *Trichoderma* isolates (BHU51 and BHU105) were used individually and their mixture (BHU51+ BHU105) in this study. Seeds and seedlings were treated according to the methods described by Yobo *et al.* (2009), briefly the seeds were treated with slurry of individual and combinations of *Trichoderma* isolates and were allowed to soak for 30 min. The treated seeds were placed in sterile 90 mm Petri plates and air dried in laminar flow bench overnight at room temperature and then used for glass house study. Thirty days old seedlings of tomato (*Lycopersicum esculentum*; variety- Navodya) grown in sterilized soil were used for field study. Seedlings were treated by dipping their roots in the *Trichoderma* suspension for 30 min and then transplanted into experimental plots (2m×2m).

For the vigor index measurement, surface disinfected seeds were first inoculated with mycelial suspension of the pathogens followed by various talc preparations of *Trichoderma* isolates separately (Singh and Singh, 2012). The formulation of the *Trichoderma* isolates were prepared and seed treatment was done after pathogen treatment, and tested for their plant growth promotion activity using standard roll towel method (ISTA, 1993). The germination percentage of seeds was recorded and the vigor index was calculated as described by Abdul-Baki and Anderson (1973), using the formula:

Vigor index = Percent germination × seedling length (shoot length + root length).

The ability to reduce damping-off of seedlings and increase the emergence of seedlings, plant height, fresh and dry weight was tested. Experiments were carried out in pots pre-inoculated with pathogen @ 5g /kg of sterilized soil kept under glasshouse conditions (Singh and Singh, 2012). *Trichoderma* treated hundred seeds were planted per pot and were evaluated for germination and damping off of seedlings. The incidence of damping off in seedlings was expressed as a percentage of the total number of plants. For the field study, inoculums of *S. rolfsii* was inoculated with methods described by Singh *et al.* (2013) with some modifications, briefly 100 g/ m^2 (inoculums grown on sand maize media) was inoculated in the field, pre-selected for transplanting the seedlings, before 7 days of transplanting. Field grown tomato plants were observed at regular intervals for the symptoms of wilting, collar rot or damping-off caused by *S. rolfsii*. In the field grown populations, disease severity was estimated by scoring individual plants randomly. Disease severity of *S. rolfsii* was calculated by scoring individual plants on a 0-5 visual scale as described by Latunde-Dada (1993). Mean disease rating (MDR) and per cent disease reduction (PDR) were calculated by the formula given by Pal *et al.* (2001). Shoot length was measured at 60 days after transplanting. The yield was recorded at regular intervals and expressed in kg/plot. Chlorophyll content analysis was done by harvesting fresh leaves at 60 days after transplanting (DAT). The amount of chlorophyll content was determined by the method described by Arnon (1949) and was expressed as mg chlorophyll per gram fresh weight.

All the data were analyzed by analysis of variance (ANOVA). Results of the experiments mean ± standard deviation (SD) of at least three replications. The treatment means were compared with level of significance $p = 0.05$ (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The effect of *Trichoderma* isolates on germination of seeds and growth attributes of seedlings and damping-off caused by *S. rolfsii* are presented in Table 1 (a, b). The germination percentage was recorded higher in the *Trichoderma* treated seeds in comparison to pathogen inoculated control. The

Table 1 (a, b): Efficacy of *Trichoderma* isolates (BHU51 and BHU105) on growth attributes and incidence of damping-off of tomato caused by *Sclerotium rolfsii* in glass house condition

(a)

Treatments	Germination rate (%)	Incidence of damping-off (%)	Shoot length (cm)	Shoot fresh weight (mg)	Shoot dry weight (mg)
Control (*S. rolfsii*)	83.33±3.21	54.67±5.13	8.32±0.59	474.10±7.76	37.62±1.13
BHU51+BHU105+ *S. rolfsii*	92.00±2.65	22.00±2.00	15.16±0.45	887.53±9.81	54.27±1.68
BHU51 + *S. rolfsii*	92.33± 3.51	27.00±2.00	11.76±0.21	586.97±11.25	50.94±1.08
BHU105 + *S. rolfsii*	92.67± 1.53	28.33±2.08	11.96±0.32	836.53±13.2	53.27±3.64
LSD (P= 0.05)	5.28	3.89	0.96	17.56	4.94

(b)

Treatments	Root length (cm)	Root fresh weight (mg)	Root dry weight (mg)	Vigor index
Control (*S. rolfsii*)	2.00±0.00	26.55±1.65	2.21±0.21	465.67±71.25
BHU51+BHU105+ *S. rolfsii*	3.56±0.21	47.33±0.71	4.45±0.18	1301.67±40.28
BHU51 + *S. rolfsii*	3.20±0.09	31.07±0.96	3.04±0.11	1144.78±42.18
BHU105 + *S. rolfsii*	2.39±0.19	32.57±0.25	3.17±0.13	1182.17±36.24
LSD (P= 0.05)	0.25	1.65	0.36	98.93

All the values are average of three replications and the values represent ± SD

Table 2: Effect of treatment with different *Trichoderma* isolates on shoot length, chlorophyll content, disease incidence and yield of tomato against *S. rolfsii* under field conditions

Treatments	Shoot length (cm)60 DAT	Chlorophyll content, (mg g^{-1}fw) 60DAT	Mean disease rating	Disease reduction (%)	Yield (kg/plot)
2008-09					
Control (*S. rolfsii*)	55.67±3.51	0.785±0.056	3.19±0.15	-	6.17±0.50
BHU51+BHU105+ *S. rolfsii*	76.67±3.06	1.045±0.013	1.71±0.15	42.85±2.76	11.40±1.59
BHU51 + *S. rolfsii*	70.33±2.52	0.994±0.017	1.99±0.06	37.76±2.09	9.46±0.53
BHU105 + *S. rolfsii*	70.33±3.21	0.999±0.017	2.02±0.09	37.15±2.23	8.83±0.67
LSD (P= 0.05)	6.83	0.05	0.18	3.06	1.94
2009-10					
Control (*S. rolfsii*)	52.67±4.16	0.742±0.032	3.83±0.20	-	5.99±0..48
BHU51+BHU105+ *S. rolfsii*	76.00±2.00	1.034±0.010	1.96±0.13	44.32±0.23	12.97±0.79
BHU51 + *S. rolfsii*	70.00±2.00	0.994±0.025	2.36±0.15	38.05±2.72	10.73±0.65
BHU105 + *S. rolfsii*	68.67±1.53	0.951±0.021	2.30±0.15	38.94±1.75	9.91±0.52
LSD (P= 0.05)	5.17	0.04	0.36	2.75	1.42

All the values are average of three replications and the values represent ± SD

incidence of damping-off was found maximum in the pathogen inoculated control (54.67%) and lowest in the plants treated with the consortium of *Trichoderma* isolates BHU51+BHU105 (22.00%) while the individual application of *Trichoderma* isolate namely BHU51 and BHU105 treated seeds, the incidence of damping-off was 27.00% and 28.33% respectively. Results revealed that all the *Trichoderma* treatments significantly reduced the damping-off. Similar results were also reported by Abd-El- Khair *et al.* (2010) and Singh *et al.* (2014) that confirms our findings. They reported that *Trichoderma* spp. reduced the damping-off caused by *Fusarium solani* and *Rhizoctonia solani* in bean and tomato crops. After 30 days of showing tomato seeds, it was observed that the shoot length was higher in the *Trichoderma* treated plants when compared with *S. rolfsii* inoculated control. The highest shoot length and root length was recorded in the plants treated with consortium of *Trichoderma* isolate BHU51+BHU105, 15.16 cm and 3.56 cm respectively which was significantly different from all other treatments and followed by application of individual *Trichoderma* isolate BHU51 treated treatment, 11.76 cm and 3.20 cm and *Trichoderma* isolate BHU105, 11.96 cm and 2.39 cm and the least was recorded in control 8.32 cm and 2.00 cm respectively. The maximum fresh and dry shoot weight (887.53 mg and 54.27 mg) and root weight (47.33 mg and 4.45 mg) was recorded in consortium and followed by single *Trichoderma* treated treatments while the lowest was found in control (Table 1 a, b). Singh *et al.* (2014) also find the similar results by using single and mixture of *Trichoderma* against *R. solani* that were in favor of our findings. They find that use of consortium of *Trichoderma* significantly increase the plant growth parameters including shoot, root length and their fresh and dry weight and nutrient uptake as well. Singh and Singh (2014) also reported that the use of mixture of *Trichoderma*, increase the level of defence releted enzymes in the plant that protect the plant from the infection caused by *R. solani*. The vigor index was done according to ISTA paper roll towel method and it was found that the pathogen challenged and consortium treated plants showed maximum vigor index (1301.67) and minimum was in the *S. rolfsii* challenged plants (465.67) (Table-1 b). Shanmugaiah *et al.* (2009) reported that use of biocontrol agents like *Trichoderma* and *Pseudomonas* sp. as seed treatment of cotton, increased shoot length, root length, their fresh and dry weight and greater vigor index was observed. He also reported that biocontrol agents significantly reduced the incidence of soil borne pathogens like *Rhizoctonia solani* and *Macrphomina phaseolina* which endorse our findings. Kumar *et al.* (2012) also reported that *Trichoderma* spp. have strong biocontrol potential against sclerotia forming soil borne plant pathogens such as *R. solani* and *S. rolfsii*. Srinivasan and Mathivanan (2009) used microbial consortia of two *Bacillus* spp., *Pseudomonas aeruginosa* and *Streptomyces fradiae* under field conditions, and he recorded significantly disease reduction, increase seed germination, plant height and yields that are confirm our results.

The results presented in table 2, clearly indicated that in tomato field trials the shoot length, disease reduction, yield and chlorophyll content was recorded maximum in the consortium treated plants and also the mean disease rating was found minimum in the consortium treated plants during both the years of field trials. The maximum shoot length in 2008-09 was 76.67 cm in the *Trichoderma* consortium treated plants (BHU51+BHU105), followed by individual *Trichoderma* treated plants i.e. BHU51 and BHU105 (70.33 cm and 70.33 cm) respectively which was also significantly higher than the untreated pathogen inoculated control (55.67 cm) against *S. rolfsii*. Similar trend was also observed in the second year of field trial (2009-10). Chlorophyll content was also recorded maximum (1.045 mg g^{-1}fw and 1.034 mg g^{-1}fw) in the consortium (BHU51+BHU105) treated plants and followed by single *Trichoderma* BHU51 (0.994 and 0.994 mg g^{-1}fw) and BHU105 (0.999 and 0.951 mg g^{-1}fw) treated plants, while the minimum (0.785 and 0.742 mg g^{-1}fw) was found in control in both the years respectively. The MDR was recorded significantly higher in control (3.19 and 3.83) than the *Trichoderma* treated plants during 2008-09 and 2009-10 crop seasons respectively. The lowest MDR was recorded in the consortium treatment (1.71 and 1.96) respectively in both the trials against *S. rolfsii*. The maximum percent disease reduction (PDR) 42.85 and 44.32 were recorded in *Trichoderma* consortium (BHU51 + BHU105) treated treatment in both years respectively, followed by single *Trichoderma* treated treatments. The yield was also recorded maximum (11.40 kg/plot and 12.97 kg/plot) in the consortium treated treatments and followed by single *Trichoderma* BHU51 (9.46 kg/plot and 10.73 kg/plot) and BHU105 (8.83 kg/plot and 9.91 kg/plot) treated plants, which were significantly higher than the untreated *S. rolfsii* inoculated control (6.17 kg/plot and 5.99 kg/plot) in both the years respectively (Table 2). From the data in Table 1 and 2 it is clear that the use of *Trichoderma* as consortium of compatible isolates enhanced the growth attributes and yield and reduced the incidence of *S. rolfsii* compared to *Trichoderma* alone. Srivastava *et al.* (2010), Singh *et al.* (2014), Singh and Singh (2012) and Srinivasan and Mathivanan (2009) also reported that the uses of combination of bioagents are more effective than single isolates. Similar results were also recorded by Pal *et al.* (2001) by using plant growth promoting rhizobacteria against *Macrophomina phaseolina, Fusarium moniliforme* and *Fusarium graminearum*.

From the present study it can be concluded that the application of consortium of two compatible isolates of *Trichoderma* spp. significantly increased the plant growth parameters and reduced the incidence of *S. rolfsii*, a sclerotia farming soil borne plant pathogen and eventually increased the yield under field conditions. Hence it can be recommended that the use of mixture of two compatible *Trichoderma* isolates as one of the crop protection strategies for the management of sclerotial plant pathogens will be better than the single *Trichoderma* isolates.

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