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Effect of consortium of *Trichoderma harzianum* isolates on growth attributes and *Sclerotinia sclerotiorum* rot of brinjal

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Abstract : An experiment was conducted in the year 2008-09 and 2009-10 and evaluated the effect of individual and consortium of *Trichoderma harzianum* isolates BHU-51 and BHU-105 challenged with *Sclerotinia sclerotiorum* on growth attributes of plants and their biocontrol potential against *Sclerotinia sclerotiorum* rot of brinjal. The *Trichoderma* treated plants showed greater growth and vigor of the plants than untreated pathogen inoculated control. In the field trial shoot length, chlorophyll content and yield were recorded maximum in consortium treated seedlings and followed by individual *Trichoderma* treated seedlings whereas the lowest was recorded in untreated pathogen inoculated control. Mean Disease Rating (MDR) was recorded maximum in the control whereas the minimum was observed in the consortium treated seedlings. The maximum Percent Disease Reduction (PDR) was also recorded in the seedlings treated with consortium.

Key Words: *Trichoderma*, Brinjal, *Sclerotinia sclerotiorum*, Biocontrol

Introduction

Food problem and malnutrition are a serious challenge to both developed and developing countries of the world. Among the vegetables, brinjal (*Solanum melongena* L.) is the major crop that has achieves tremendous popularity over the last century. It is grown in practically every country of the world in fields and greenhouses. Production of brinjal is highly concentrated, with more than 90 per cent of output coming from five countries. China is the top producer (56.75% of world output) and India is second (27.55%); Egypt, Iran and Turkey round out the top producing nations. The productivity of brinjal in our country is 17.5 MT/ha while in Japan 32.0, Italy 28.2 and Turkey 30.2 MT/ha (FAO 2012). It is estimated that 10% of crops are lost due to plant diseases worldwide each year, which can lead to considerable financial losses for the farmers, but also to social concerns in underdeveloped countries where some areas are entirely dependent upon specific crops (Strange and Scott 2005). Many soil borne pathogens cause huge losses in the crops. However, the sclerotia forming soil borne pathogen (Pth) *Sclerotinia sclerotiorum*, is major threat to production of brinjal, because resistant varieties against this pathogen is rare or not available. *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most devastating and cosmopolitan plant pathogen. The fungus infects over 400 species of plants worldwide including important crops and numerous weeds. Control of these pathogens is difficult because of their ecological behaviour, it's extremely broad host range and the high survival rate of sclerotia under various environmental conditions. For this reason, efficient strategies to control these pathogens are urgently needed. Numerous studies have shown that biological control offers an environmentally friendly alternative to protect plants from soilborne pathogens (Harman 2011; Singh *et al*., 2011). *Trichoderma* species are worldwide in occurrence and are present in nearly all the soils and other diverse habitats. These versatile fungi acts upon pathogens by applying various mechanisms like mycoparasitism, nutrient competition by secreting antifungal metabolites.

The use of combination of two or more than two compatible microorganisms may provide improved disease control over the single biocontrol application. Use of multiple antagonistic microorganisms in combination may enhance the level and consistency of control by providing multiple mechanisms of action and it may also more stable to that rhizosphere community and effectiveness over a wide range of environmental conditions (Singh and Singh 2006; Srivastava *et al*., 2010). The aim of the present study was to evaluate the biocontrol activity of *Trichoderma* on *Sclerotinia*

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sclerotiorum, especially in combination, that may reduce the incidence of *Sclerotinia sclerotiorum* and increase the vigor index and yield of brinjal crop.

Materials and Methods

The present study was carried out in the glass house at Department of Mycology and Plant Pathology whereas the field experiments (2 m×2 m plots) were carried out at agricultural field of Banaras Hindu University, Varanasi during 2008-09 and 2009-10. In this study we used two *Trichoderma* isolates, *Trichoderma harzianum* BHU-51 (GenBank accession no. JN 618343.), *Trichoderma harzianum* BHU-105 (GenBank accession no. JN 618344), and their consortium (BHU-51+BHU-105). There were four treatments *viz.* T1-Control challenged with pathogen, T2-*Trichoderma* BHU-51+ BHU-105 treated and challenged with pathogen, T3- *Trichoderma* BHU-51 treated and challenged with pathogen and T4-*Trichoderma* BHU-105 treated and challenged with pathogen. In order to prepare pathogen inoculum of *S. sclerotiorum* sand maize meal media was used. Seeds of brinjal (*Solanum melongena* L.; variety- Sweekar-321) were procured from the market used in the study. Seedlings of the cultivars were raised in pots filled with sterilized soil (121 ^{0}C and 15 lbs pressure for 1 h for two consecutive days). They were transplanted according the treatments after 5 weeks of sowing.

Trichoderma Isolates (BHU-51and BHU-105) formulations containing approximately 10^8 spores /g were mixed separately with 1% (w/v) sterile carboxymethyl cellulose (CMC) to form slurry. Seeds were then added to each slurry suspension, mixed and allowed to soak for 30 min. The *Trichoderma* treated seeds air-dried on a laminar flow bench. For combined inoculation of compatible *Trichoderma*, isolates (BHU-51+ BHU-105) formulations were prepared separately as described above and mixed in equal amount (1:1) before seed treatment. Control seeds were treated with mixtures of CMC suspension and talcum powder only. Thirty five days old seedlings of brinjal seedlings were treated by dipping them into the *Trichoderma* suspension prepared as above and left for half an hour in one liter beaker and used for transplanting.

Surface disinfected seeds were first inoculated with mycelial suspension of pathogens followed by various talc preparations of *Trichoderma* isolates separately and tested for their plant growth promotion activity using standard roll towel method (ISTA 1993). The vigor index was calculated as described by 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, induced and increase the emergence of seedling, increase plant height, fresh and dry weight was tested. Two *Trichoderma* isolates (BHU-51 and BHU-105) and their mixture (BHU-51+ BHU-105) were used. Trials were carried out in pots kept under glasshouse conditions. Pots were filled with 1.5 kg of sterilized soil and mixed with the pathogen inoculums @ 5g /Kg of soil. Treated dry brinjal seeds were planted into 15 cm diameter pots according to the treatments. Hundred seeds were planted per pot and were evaluated for germination and damping off of seedlings. The seedling emergence was counted at regular interval. The germination and cumulative damping off of seedlings were recorded 30 days after sowing. The incidence of damping off in seedlings was expressed as a percentage of the total number of plants. The number of seedlings which had survived their shoot length, root length and fresh and dry weight of shoot and root of surviving plants were recorded from each replicate. Concerning fresh and dry weights, plants were washed under running tap water to remove soil from roots; plants were then dried at 80 °C in drying oven after recording fresh weights. After 72 h, plant dry weights were determined. For the field study, *S. sclerotiorum* inoculums grown on sand maize media was inoculated in the field, which were pre selected for transplanting of seedlings, before 7 days of transplanting. Field grown brinjal were observed at regular interval for the symptoms of rot and wilting, caused by *S. sclerotiorum*. For the *Sclerotinia sclerotiorum* the severity index of plants grown in the field was calculated as described by (Grau *et al.*, 1982). Mean Disease Rating (MDR) and Per cent disease reduction (PDR) was calculated by the formula given by Pal *et al.* (2001). The shoot length was measured at 60 days after transplanting. The yield was recorded at regular intervals and expressed in kg /plot. Chlorophyll analysis was done by harvesting fresh leaves at 60 days after transplanting (DAT). The leaves were crushed in 80% acetone. The amount of chlorophyll content was determined by taking absorbance at 645 and 663nm (Arnon, 1949) and was expressed as mg chlorophyll per gram fresh weight. Experiments were designed in Randomized Block Design (RBD), Values from different experiments shown in tables were mean ± standard deviation (SD) of at least three determinations and analyzed by analysis of variance (ANOVA).The treatment means were compared with level of significance $p = 0.05$ (Gomez and Gomez, 1984).

Results and Discussion

All the *Trichoderma* treated plants invariably showed the significantly different observations as compared with untreated pathogen inoculated control. The maximum incidence of damping-off of seedlings were observed 37.33 % in the *S. sclerotiorum* inoculated control while the minimum was recorded 13.67% in the consortium treated seeds against *S. sclerotiorum*. Germination per centage of seeds, shoot length, root length and their fresh and dry weight was also recorded maximum (93.33%, 11.00 cm, 5.0 cm, 459.03 mg, 23.61 mg, 38.25 mg and 3.23 mg) in the seeds treated with consortium of *Trichoderma* isolates (BHU-51+BHU-105) and followed by individual *Trichoderma* BHU-51 (93.00%, 10.67 cm, 4.17 cm, 417.90 mg, 21.35 mg, 34.55 mg and 2.95 mg) and BHU-105 (92.00%, 10.67 cm, 4.50 cm, 445.96 mg, 19.93 mg, 33.07 mg and 2.86 mg) treated seeds whereas the minimum (85.67%, 8.33 cm, 3.17 cm, 290.95 mg, 15.95 mg, 19.27 mg and 1.85 mg) respectively, was recorded in *S. sclerotiorum* inoculated control (Table-1 and 2). The vigor index was also recorded significantly greater in the *Trichoderma* BHU-51+BHU-105 (1282.21), BHU-51 (1197.01) and BHU-105 (1209.69) treated seeds compared with *S. sclerotiorum* inoculated control (519.66) (Table 2).

The seedlings of brinjal treated with consortium of the *Trichoderma* isolates *viz.,* BHU-51+BHU-105 showed the highest shoot length (73.42 cm) followed by individual *Trichoderma* BHU-51 (64.17 cm) and BHU-105 (69.5 cm) treated seedlings against *S. sclerotiorum.* Minimum shoot length was observed in the untreated, *S. sclerotiorum* inoculated control plants (49.78 cm) in the year 2008-09 after 60 days of transplanting (DAT). Chlorophyll content and yields were also found maximum in the consortium treated plants (1.017 mg/g FW and 9.643 kg/plot) which were significantly higher than the untreated, *S. sclerotiorum* inoculated control plots (0.783 mg/g FW and 5.302 kg/plot) respectively. The mean disease rating was observed in *S. sclerotiorum* inoculated, untreated control 3.03, while in the consortium treated and pathogen inoculated treatment it was only 1.82 in the first field trial (2008-09). The maximum disease reduction was recorded in consortium treated plants against *S. sclerotiorum* (39.37%), followed by individual *Trichoderma* BHU-51 (34.04%) and BHU-105 (37.38%) treated plants (Table 3). A similar trend was also observed in the second field trial (2009-10) for all these parameters (Table 4).

The use of *Trichoderma* spp. as biocontrol agent and its influence on seed germination and seedling vigor has been well documented. *Trichoderma* spp. also increases growth of shoot and root and productivity as well (Harman *et al*., 2004). The seeds of brinjal treated with consortium of *Trichoderma harzianum* (BHU-51+BHU-105) isolates and individually BHU-51 and BHU-105 significantly increased (p=0.05%) their germination per centage, shoot length, root length and their fresh and dry weight comparison to untreated pathogen inoculated control. In the field trial growth response of seedlings treated with *Trichoderma harzianum* also recorded-higher than control. These results indicated that the use of *Trichoderma* as consortium of compatible isolates enhanced the growth and vigor of the plants compared with *Trichoderma* alone. Srivastava *et al*. (2010) and Singh and Singh (2006) also reported that the use of

Table 1. Efficacy of *Trichoderma* isolates on growth attributes and incidence of damping-off of brinjal caused by *Sclerotinia sclerotiorum*

Treatments	Germination rate (%)	Incidence of Damping-off (%)	Shoot Length (cm)	Shoot Fresh Weight (mg)	Shoot Dry Weight (mg)
Control with pathogen (Pth)	85.67±3.51	37.33±3.21	8.33±0.58	290.95±11.15	19.27±0.74
BHU -051 + BHU-105+ Pth	93.33±2.08	13.67±1.53	11.00±0.50	459.03±9.32	38.25±0.98
BHU- 051 + Pth	93.00±3.00	17.33±1.53	10.67±0.58	417.90±12.35	34.55±0.91
BHU-105 + Pth	92.00±3.00	19.67±1.53	10.67±0.58	445.96±6.24	33.07±1.04
LSD (P= 0.05)	5.46	4.09	1.21	20.74	1.77

All the values are average of three replications with ± standard deviation

Table 2. Efficacy of *Trichoderma* isolates on growth attributes and incidence of damping-off of brinjal caused by *Sclerotinia sclerotiorum*

Treatments	Root Length (cm)	Root Fresh Weight (mg)	Root Dry Weight (mg)	Vigour index
Control with pathogen (Pth)	3.17±0.29	15.95±0.44	1.85±0.21	519.66±68.19
BHU -051 + BHU-105+ Pth	5.00±0.50	23.61±1.08	3.23±0.13	1282.21±13.51
BHU- 051 + Pth	4.17±0.76	21.35±1.26	2.95±0.10	1197.01±39.41
BHU-105 + Pth	4.50±0.50	19.93±1.24	2.86±0.30	1209.69±89.48
LSD (P= 0.05)	0.88	2.17	0.38	111.89

All the values are average of three replications with ± standard deviation

Table 3. Effect of treatment with different *Trichoderma* isolates on shoot length, chlorophyll content, disease incidence and yield of brinjal against *Sclerotinia sclerotiorum* under field conditions during 2008-09

Treatments	Shoot Length (cm)	Chlorophyll content, (mg g^{-1} Fresh weight)	Mean Disease Rating	Disease Reduction (%)	Yield (kg/plot)
Control with pathogen (Pth)	49.78±2.67	0.783±0.06	3.03±0.14	-	5.302±0.71
BHU -051 + BHU-105+ Pth	73.42±4.89	1.017±0.04	1.82±0.09	39.37±1.77	9.643±0.92
BHU- 051 + Pth	64.17±4.13	0.962±0.05	2.10±0.03	34.04±1.53	8.362±0.96
BHU-105 + Pth	69.5±6.76	0.977±0.04	1.94±0.15	37.38±1.56	7.742±1.34
LSD (P= 0.05)	10.42	0.09	0.20	3.17	2.13

All the values are average of three replications and the values represent ± standard deviation

Table 4. Effect of treatment with different *Trichoderma* isolates on shoot length, chlorophyll content, disease incidence and yield of brinjal against *Sclerotinia sclerotiorum* under field conditions during 2009-10

Treatments	Shoot Length (cm)	Chlorophyll content, (mg g^{-1} Fresh weight)	Mean Disease Rating	Disease Reduction (%)	Yield (kg/plot)
Control with pathogen (Pth)	46.08±5.54	0.809±0.06	3.24±0.18	-	4.985±0.51
BHU -051 + BHU-105 + Pth	72.25±5.65	1.008±0.03	1.62±0.12	44.96±1.63	10.099±0.90
BHU- 051 + Pth	64.58±4.20	0.984±0.06	1.96±0.17	38.62±2.78	8.705±0.10
BHU-105 + Pth	69.58±5.45	0.949±0.03	1.80±0.09	41.43±3.50	9.015±0.65
LSD (P= 0.05)	11.66	0.11	0.32	5.08	1.57

All the values are average of three replications and the values represent ± standard deviation

combination of bioagents are more effective than single isolates treatments and also reported that the biopriming of seeds with *Trichoderma* and fluorescent *Pseudomonas* increase seed germination and reduce the incidence of disease. Our findings of seed germination, growth and development and vigor of the plants also similar with the findings of other authors this may be due to the ability of *Trichoderma* isolates to survive and colonize in the root and rhizosphere (Harman *et al*., 2004). Researchers reported that rhizosphere competent isolate produces number of metabolites in the rhizosphere which are responsible for *Trichoderma* colonization, plant growth promotion and reduction in damping-off. The fungi *Trichoderma* spp. has the abilities to increase photosynthetic efficiency. The vigor index and yield was further validate the growth and yield response of *Trichoderma* especially in the consortium which was far better than the singly *Trichoderma* treated seedlings.

Application of compatible *Trichoderma* isolates both singly or in combination effectively suppressed the disease severity and increased percent disease reduction. Similar results were also recorded by Pal *et al.* (2001) by using plant growth promoting rhizobacteria against *Macrophomina phaseolina, Fusarium moniliforme* and *Fusarium graminearum*. It also has been reported that *Trichoderma* penetrate the root cortex which increase the lignifications and induced the resistance level in treated plants against the pathogen attack and also increases the hydrolytic enzymes and stimulates the plant defense mechanism (Harman 2011, Singh *et al*., 2011).

The application of *Trchoderma*, especially in the consortium form, are more useful than the individual because of two or more than two compatible isolates of same or different species with same or different quality work synergistically and give the high impact of their use. Reduction in disease by using *Trichoderma* isolates reduce the chemical pesticide loads on the crop which will be in the favor of farmers (cost: benefit ratio) and consumers as well.

सारांश

वर्ष 2008–09 और वर्ष 2009–10 में *ट्राइकोडर्मा हारजिएनम* के एकल (बी.एच.यू.–51 और बी.एच.यू.–105) और उनके मिश्रण (बी. एच.यू.–51 + बी.एच.यू.–105) के प्रभाव का मूल्यांकन बैगन के पौधों की वृद्धि और *स्क्लेरोटिनिया स्क्लेरोटियोरम* के खिलाफ जैव–नियंत्रण क्षमता का परीक्षण किया गया। ट्राइकोडर्मा से उपचारित पौधों में अनुपचारित पौधों की अपेक्षा अधिक वृद्धि पाई गयी। क्षेत्र परीक्षण में एकल ट्राइकोडर्मा उपचारित बेहन के सापेक्ष ट्राइकोडर्मा मिश्रण से उपचारित पौधों में तने की लंबाई और क्लोरोफिल अधिक पाया गया, जबकि सबसे कम तने की लंबाई और क्लोरोफिल अनुपचारित पौधों में दर्ज किया गया। औसत रोग रेटिंग अनुपचारित पौधों में अधिकतम दर्ज की गयी, जबकि न्यूनतम औसत रोग रेटिंग ट्राइकोडर्मा मिश्रण से उपचारित पौधों में पाया गया। अधिकतम प्रतिशत रोग कटौती ट्राइकोडर्मा मिश्रण शोधित पौधों में दर्ज की गयी।

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