The Bioscan 8(3): 801-804, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

TRICHODERMA HARZIANUM AND *PSEUDOMONAS* SP. MEDIATED MANAGEMENT OF *SCLEROTIUM ROLFSII* ROT IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

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KEYWORDS
Trichoderma, *Pseudomonas*, *Sclerotium rolfsii*, Tomato

Received on :
04.03.2013

Accepted on :
25.05.2013

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ABSTRACT
Trichoderma spp. and *Pseudomonas* spp. are known for their biocontrol potential and growth promotion activity. Combination of these two microbes was used to assess the synergistic effect of compatible isolates for plant growth promotion and management of *S. rolfsii*. *Trichoderma harzianum* and *Pseudomonas* and their combination were applied as seed and seedlings treatment in tomato. Consortium treatment showed the greater plant growth promotion activity in comparison to solo treatment. Consortium treatment showed highest chlorophyll content (1.05mgg^{-1} fresh weight of leaves) while in the pathogen inoculated control lowest amount was recorded (0.8mgg^{-1} fresh weight of leaves). The lowest mean disease rating (MDR) 1.96 and maximum percent disease reduction (PDR), 53.23% recorded in consortium treatment. The yield was also significantly higher in bioagents treated treatments, T3 (37.41 tons h^{-1}), T4 (36.60 tons h^{-1}), T5 (43.84 tons h^{-1}) and untreated unchallenged treatment (45.29 tons h^{-1}) than untreated challenged control (24.07 tons h^{-1}). From these results it can be concluded that the application of consortium of compatible bioagents will enhance the plant growth and biological control of phytopathogens in contrast to treatment with single bioagent.

INTRODUCTION

Tomato (*Lycopersicon esculentum)* is an important vegetable crop grown throughout the world. It is rich in lycopene which is an active antioxidant present in vegetarian diet. India is the second largest producer of tomato with a record yield of 16826000 MT (FAOSTAT, 2011). There are many fungal diseases of tomato which not only decrease the production but also deteriorate the quality of the produce. A large number of environmentally hazardous chemical pesticides are applied for management of these diseases. *Sclerotium rolfsii* Sacc., is one of the most devastating soil borne sclerotia forming phytopathogen which causes wilting, blight, basal stem rot and fruit rot in tomato (Aycock, 1966; Punja, 1988; Tindall, 1983. Southern blight disease caused by this pathogen is a major problem in tomato (Aycock, 1966). Since the sclerotia are the main resting bodies and highly persistent in the soil making it difficult to manage by the use of synthetic pesticides (Papavizas and Lewis, 1989). Biocontrol agents (BCAs) are the most eligible candidates for managing such sclerotia forming phytopathogens. *Trichoderma* spp. is one of the best alternatives for the management of this pathogen (Singh and Singh, 2012; Kumar *et al*., 2012). *T. harzianum* employs various mechanisms like mycoparasitism, antibiosis and competition to colonize *S. rolfsii* hyphae and sclerotia, ultimately causing host death (Djonovic *et al.*, 2006; Harman, 2006; Singh *et al.,* 2011; Harman *et al*., 2004). *Trichoderma* spp. and *Pseudomonas* spp. have been reported to secrete an array of chemically diverse anti-microbial secondary metabolites and hydrolytic enzymes such as proteases, cellulases, chitinases, lipasees etc., which help it in host recognition and pathogen control (Srivastava *et al*. 2010; Harman, 2011 and Harman *et al*., 2012; Hermosa *et al*. 2012, Shanmugaiah *et al*. 2009). Key feature of using compatible strains of plant growth promoting and biocontrol microorganisms such as *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., etc., in a consortium is to maximize plant growth and biological control of phytopathogens has been globally demonstrated (Singh and Singh, 2012; Yobo et al., 2009; Srivastava *et al*. 2010).

The main aim of this study was to assess the cumulative effect of a consortium of *Pseudomonas* sp. and *Trichoderma harzianum*, in compararison to their individualistic effect on tomato in glasshouse. These treatments were challenged with *S. rolfsii* to determine the biocontrol potential of the consortium. Growth parameters were also assessed to verify the synergistic effect of the consortium.

MATERIALS AND METHODS

Experiment was conducted at Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, in controlled condition under polyhouse to see the effect of single *Pseudomonas* sp. and *Trichoderma harzianum* and effect of their interaction on

the yield, growth and development of tomato and management of collar rot caused by *S. rolfsii*. Experiment was laid out as randomized block design having five treatments: T1: Control without bioagents and without pathogen, T2: Treatment challenged with *S. rolfsii* pathogen only, T3: *Pseudomonas* sp. treated and challenged with *S.rolfsii* T4: *Trichoderma harzianum* treated and challenged with *S.rolfsii*, T5: *Trichoderma harzianum* + *Pseudomonas*, consortium treated and challenged with *S.rolfsii*, having three replication each. Net plot size was 2.0 m × 2.0 m. Seed of tomato cultivar Navodya was obtained from market used in this study.

Isolation of pathogen and biocontrol agents (BCAs)

Sclerotium rolfsii, a causal agent of collar rot was isolated from infected collar region of tomato plant from the vegetable form of the Banaras Hindu University, Varanasi, India. *Trichoderma harzianum* and *Pseudomonas* sp. was isolated from the rhizosphere soil and characterized. All the microbes were grown and maintained at 4^0C. *In vitro* compatibility of the *Trichoderma* isolate and *Pseudomonas* sp. was assessed on solid media.

T. harzianum and *Pseudomonas* spp. and their consortium formulations (4g) containing approximately 10^8 spores/g were mixed separately with 1% (w/v) sterile carboxymethyl cellulose (CMC) sticker suspensions (20 mL) in 100 mL glass beakers to form a slurry. Sterile distilled water was used for the CMC sticker suspensions. Seeds were then added to each slurry suspension, mixed and allowed to soak for 30 min. The treated seeds were placed in sterile 90 mm Petri dishes and air-dried on a laminar ûow bench overnight at room temperature. Similarly consortium of compatible Control seeds were treated with mixtures of CMC suspension and talcum powder only in the same manner as mentioned with the treatments.

Evaluation of plant increased growth response

Observations were taken consecutively two years 2008 and 2009. Plant growth promotion parameters such as shoot length, root length, number of branch per plant, leaves per plants, fresh shoot and root weight, dry shoot and root weight and total chlorophyll content were recorded at the blooming stage of the plant. Disease incidence was observed in the term of MDR and PDR in bioagents treated treatments, consequently the yield was also notified in terms of tons per hectare and percent increase over control.

Seeds were grown in a greenhouse condition for 4 weeks in pot under sterilized soil condition. A field trial was established in August and was run until December. The seedlings were treated with *Pseudomonas* sp. and *T. harzianum* and by their consortium according to treatments and transplanted in the respective plots of polyhouse of Banaras Hindu University, Varanasi. The various measurements of plant growth responses were made in polyhouse. Plant heights were measured from soil surface to apical buds. 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 weight. After 72hr, plant dry weights were determined.

Assessment of growth promotion and damping-off in glasshouse condition

Seedling trials were carried out in pots kept under glasshouse conditions. Treated dry tomato seeds were shown into trays filled with sterilized soil. Hundred seeds were planted per pot and were evaluated for shoot length, root length, their fresh and dry weight and percent damping off of seedlings. Pots were filled with sterilized soil and mixed with the pathogen inoculums @5g /kg of soil, except in the uninoculated control. The cumulative damping off of seedlings and shoot length, root length, their fresh and dry weight were recorded 30 days after sowing.

Assessment of plants

Inoculums of *S. rolfsii* inoculated @ 50g m^{-2} plot size (inoculums grown on sand maize media) was inoculated in the plot which was pre selected for transplanting of seedlings before 10 days of transplanting. Seedlings, transplanted were observed weekly for the symptoms of blight, wilting or damping off and for the signs of *S. rolfsii*. The incidence of damping off in seedlings was expressed as a percentage of the total number of plants. In the grown populations, disease severity was estimated by scoring individual plants on a 0-5 visual scale described by Latunde-Dada (1993) of increasing severity: 0 = no symptom; 1 - slight infection, mycelial mat on soil surface only; 2 - moderate infection, wilting and mycelial mat start covering on stem base; 3- wilting and mycelial mat on stem base with few sclerotia; 4 - severe infection, advanced wilting, sclerotia abundant at stem base; 5 = plant dead.

Mean Disease Rating (MDR) and Per cent disease reduction (PDR) were calculated by the formula given by Pal *et al*. (2001). Severity of disease was assessed as percent mortality caused by *S.rolfsii* in untreated challenged and treated, challenged treatments were recorded. The percent reduction in disease was calculated in respect to challenged control. The yield was also recorded in respect to challenged control. Experiments were designed in Randomized Block Design (RBD), Values from different experiments shown in tables were mean of data recorded in two years experiment with ± 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

Effect of bioagents on increased growth response on seedlings

Consortium of *Pseudomonas* sp. and *T. harzianum* displayed a significant increase in shoot length in comparison to control. Although T1 measured highest in shoot length, root length, fresh and dry weight but in challenged conditions the treatment T5 showed significantly improved shoot length (14.19 cm), root length (5.62 cm) followed by single *Pseudomonas* sp. (T3) and *T. harzianum* (T4) treatments. While the lowest for the same was recorded in the control (T2). Damping off caused caused by *S. rolfsii* was significantly suppressed in T3, T4 and T5 treatments in comparison to challenged untreated control (T2). The maximum seedling rot was recorded in T2 (74.67%) while in treatment T3, 14.33%; 19.67% in T4 and 11.33% in T5 was recorded. Our results are in agreement with Srivastava *et al*. (2010) as they also demonstrated that by the use of similar consortium *Fusarium* wilt in tomato was significantly controlled.

Table 1: Efficacy of *Pseudomonas* sp., *Trichoderma harzianum* and their consortium on growth attributes and incidence of damping-off of tomato caused by *Sclerotium rolfsii,* 30 DAS.

Treatments	Shoot length (cm)	Fresh wt. of shoot (mg)	Dry wt. of shoot (mg)	Root length (cm)	Fresh root weight (mg)	Dry root weight (mg)	Seedling damp off (%)
T1	14.94 ± 0.12	860.87 ± 31.38	52.60 ± 1.92	5.89 ± 0.39	42.67 ± 1.76	4.28 ± 0.30	-
T2	8.42 ± 0.42	490.77 ± 28.73	34.28 ± 4.18	3.33 ± 0.58	26.55 ± 1.65	2.12 ± 0.13	74.67 ± 4.16
T3	11.83 ± 0.44	776.53 ± 36.87	47.54 ± 1.86	5.20 ± 0.98	36.60 ± 2.91	3.20 ± 0.08	14.33 ± 2.08
T4	11.36 ± 0.56	690.13 ± 22.68	48.28 ± 2.05	5.39 ± 0.84	37.33 ± 1.91	3.37 ± 0.48	19.67 ± 2.08
T5	14.19 ± 0.64	829.87 ± 10.60	51.63 ± 0.86	5.62 ± 0.71	39.57 ± 2.44	3.92 ± 0.29	11.33 ± 2.08
CD at 5%	0.88	47.36	4.70	1.51	3.26	0.58	5.10

All the values in the table are mean of the three replicates with ± standard deviation.

Table 2: Effect of *Pseudomonas* sp., *Trichoderma harzianum* and their consortium on growth attributes of tomato crop against *Sclerotium rolfsii* under controlled polyhouse conditions.

Treatments	Shoot length (cm)	No. of leaves plant^{-1}	No. of branch plant^{-1}	Fresh weight of shoot (g)	Dry weight of shoot (g)	Chlorophyll content mg g^{-1} fw
T1	69.67 ± 4.04	27.67 ± 2.52	6.00 ± 1.00	89.00 ± 3.61	13.03 ± 0.49	1.05 ± 0.05
T2	40.67 ± 2.08	15.33 ± 1.53	3.33 ± 0.58	48.33 ± 1.53	5.20 ± 0.82	0.81 ± 0.06
T3	59.67 ± 4.16	22.67 ± 2.08	5.00 ± 1.00	72.67 ± 3.51	9.40 ± 0.46	1.04 ± 0.05
T4	58.00 ± 3.00	23.00 ± 2.00	4.33 ± 0.58	69.33 ± 2.52	9.00 ± 0.46	0.98 ± 0.03
T5	70.00 ± 4.00	26.33 ± 1.53	5.67 ± 0.58	85.00 ± 2.00	11.83 ± 0.35	1.05 ± 0.08
CD at 5%	6.94	3.99	1.61	5.20	1.08	0.11

All the values in the table are mean of the three replicates with ± standard deviation.

Table 3: Effect of *Pseudomonas* sp., *Trichoderma harzianum* and their consortium on root development, incidence of disease and yield of tomato crop against *Sclerotium rolfsii* under controlled polyhouse conditions.

Treatments	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Mean disease rating	Percent disease reduction	Production (tons ha^{-1})
T1	30.67 ± 2.08	25.33 ± 2.52	7.03 ± 0.42	-	-	45.29 ± 2.41
T2	17.33 ± 1.53	13.33 ± 1.53	2.87 ± 0.31	3.71 ± 0.28	-	24.07 ± 3.56
T3	25.33 ± 2.52	20.00 ± 1.73	5.83 ± 0.25	1.96 ± 0.09	47.01 ± 6.20	37.41 ± 2.80
T4	25.67 ± 2.52	19.67 ± 2.52	5.43 ± 0.40	2.08 ± 0.21	43.90 ± 3.77	36.60 ± 2.14
T5	29.00 ± 2.00	23.00 ± 2.00	6.73 ± 0.60	1.74 ± 0.13	53.23 ± 0.80	43.84 ± 2.88
CD at 5%	3.46	4.33	0.87	0.29	6.08	5.57

All the values in the table are mean of the three replicates with ± standard deviation.

The data from the Table 1 and Table 2 indicated that the seeds treated with consortium and individual of these bioagents, showed the significantly higher shoot length, root length, fresh and dry weight, number of branch, leaves than the challenged control. Besides these growth parameters, bioagents also improved chlorophyll content in contrast to control.

The data from the present investigation (Table 2) showed that percent shoot length was increased by 46.72, 42.61 and 72.12% over challenged control in the treatments T3, T4, & T5 respectively while in pathogen challenged treated control it was recorded to be 40.67cm and in untreated unchallenged control it was found 69.67cm. The highest fresh and dry weight of shoots was recorded in T5, 85.00g and 11.83g respectively, which was significantly higher than challenged control (48.33g and 5.20g respectively). The number of leaves, branch length, root length, root fresh and dry weight was also recorded to be significantly higher than challenged control (Table 2).

Evaluation of antagonistic potential

Five treatments as mentioned in table 3 were tested for their antagonistic potential in controlled environment. The tested isolates reduced mortality of tomato plants caused by *S. rolfsii* at variable rates; mean of percent disease reduction ranged from 43.90 to 53.23, highest being 53.23% in the consortium of *Pseudomonas* spp. and *Trichoderma harzianum*. Significant reduction in the incidence of disease with lowest MDR (1.96) was recorded in plants treated with consortium whereas maximum MDR was recorded in the pathogen inoculated control (3.71). Production of the tomato was also found significantly higher than the pathogen inoculated control. Highest production was recorded in the treatment T1 while in T5 it was recorded to be the highest followed by T3 and T4 treatments (Table 3).

It has been reported that *Trichoderma* spp. and *Pseudomonas* spp. are potential biocontrol agents for the management of various soilborne phytopathogens by its ability to secrete antimicrobial secondary metabolites that acts upon the pathogen. *Trichoderma* mycoparasitism is suggested to play an important role in the management of sclerotia forming phytopathogens by colonizing them (Kumar *et al*., 2012, Singh and Singh, 2012) while secretion of antimicrobial metabolites by *Pseudomonas* spp. improves their biocontrol potential (Asha *et al*., 2011). Bell *et al*. (1982), Pan and Bhagat (2008) reported that *Trichoderma* spp. significantly inhibited *S. rolfsii* that supports our result. Duffy *et al*. (2004) also reported the beneficial effect of *Pseudomonas* spp. on growth of plants and reduction in disease severity in various horticultural crops. Radjacommare *et al*. (2004), El-Katatny *et al*. (2000) and Yedidia *et al*. (2000) reported that during interaction of biocontrol agents and plant-pathogens, a array of defense related proteins viz. β-1, 3 glucanase, chitinase, peroxidase, polyphenol oxidase etc., are induced in plants resulting in reduction of disease incidence. From the present study it may be concluded that the seeds and seedlings treatment with consortium of these bio-agents (*Trichoderma* spp., *Pseudomonas* spp.) results in plant growth promotion, yield and simultaneously reduce

the disease severity in contrast to application of individual bioagent.

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