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Biological control of tomato damping off caused by *Sclerotium rolfsii*

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Abstract
In vitro evaluation was conducted with twenty four isolates representing twenty four isolates representing two different species *Trichoderma harzianum* and *Trichoderma viride* and twelve different *Baccillus subtilis* and *Pseudomonas fluorescence*. These potential biocontrol agents were tested for their efficacy against phytopathogenic fungi *Sclerotium rolfsii* through dual culture technique. Therefore, these effective biocontrol agents can be used for greenhouse studies to confirm the feasibility of using in tomato damping off disease management. The combination of potential *Trichoderma harzianum* -1 and *Pseudomonas fluorescence* bacterial biocontrol agents (T_5 and T_6 treatments) also proved effective in increasing germination and to reduce pre and post emergence collar rot in the pots when inoculated with *Sclerotium rolfsii*. The treatments, T_6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1) and T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-3) recorded 52.08 and 49.17 percent germination respectively when inoculated with *Sclerotium rolfsii.* The lowest incidence (47.92 percent) of pre emergence damping off was recorded in T_6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1). Among the bio-control treatments the lowest (54.17 and 59.17) post emergence damping off incidence was recorded in T_6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1) at 30 and 50 DAS. The combination *Trichoderma harzianum* -1and *Pseudomonas fluorescence*-3 biocontrol agents ($T_5$and T_6) treatment also proved effective in increasing the shoot and root weight and fresh and dry weight of tomato plants when inoculated with *Sclerotium rolfsii*.

Keywords: *Sclerotium rolfsii, Trichoderma harzianum, Pseudomonas fluorescence*, tomato damping off.

1. Introduction
Tomato (*Lycopersicon esculentum* Mill.) is the second leading vegetable crop worldwide. Due to increasing demand, tomato has a great potential for increased commercialization. Tomato plants are susceptible, among other fungi, to *Sclerotium rolfsii*, a soil-borne pathogen that attacks over 500 different plant species (Wydra, 1996) [21]. *S. rolfsii* is a major problem in tomato crops in the warm, moist tropical regions of the world (Aycock, 1966) [4], causing damping-off I nursery seedlings as well as stem rot, wilting and blight in adult plants (Flores-Moctezuma *et al*., 2006) [13], with consistent loss of production (De Curtis *et al*., 2010) [10]. Synthetic chemical fungicides have long been used to reduce the incidence of plant diseases. However, they are costly, can have negative effects on the environment, and may induce pathogen resistance. Consequently, biological control, including the use of microorganisms or their antibiotics, offers an attractive alternative or supplement to pesticides for the management of plant diseases without the negative impact of chemical control (Wang *et al*., 1999) [20]. *T. harzianum* was found to be an effective biological control agent for protecting a number of crop plants from damage induced by *S. rolfsii* under both greenhouse and field conditions (Elad *et al*., 1980) [11]. *T. harzianum* is a common soil species and is used in biological control of a variety of plant-pathogenic fungi. The application of fungi as biological control agents, especially *T. harzianum*, parasitized *S. rolfsii* (Desai and Schlosser, 1999) [8]. *Pseudomonas* spp. are common soil bacteria easily cultured from most agricultural soils and plant rhizospheres. They have been studied in considerable detail because of their ability to promote plant growth, either by directly stimulating the plant or by suppressing pathogens (Rosas *et al*., 2009) [18]. Several research groups have tested the efficacy of antagonistic microbes for the control of *S. rolfsii* (Errakhi *et al*., 2007) [12]. which induces diseases difficult to control because of the production of sclerotia that represent resistant survival structures (Elad, 1995) [11].

Fluorescent *pseudomonads* have been reported as promising biological control agents against *S. rolfsii* in tomato.

2. Material and Methods

2.1 Mass Multiplication of Pathogen

The test pathogen *Sclerotium rolfsii* isolated form diseased plants and multiplied on sorghum grains. Sorghum grains were pre-soaked in 2 percent sucrose solution overnight, drained and boiled in fresh water for 30 minutes and drained again. This was transferred into 1000 ml flasks @ 400 g and autoclaved for 15 lb psi for 20 minutes. The flasks were allowed to cool at room temperature and inoculated with 5 mm discs of 3 to 4 day old culture of grown *Sclerotium rolfsii* on PDA. Seven discs per flask were added and the flasks were incubated for three weeks at 28 ± 2 ^{0}C. (Pande *et al*., 1994) [17].

2.2 Sterilization of soil

Soil was sterilized with formaldehyde for 3 days, after three days inoculum of respective pathogens multiplied on sorghum grains was mixed at the rate of 20g/kg of soil in upper 10 cm layer of pot soil. Pots were sprinkled with water and incubated for two days after covering with polythene bags. Poly bags (18×18) were filled with sterilized soil (formaldehyde sterilized) containing red and black soil and FYM in the ratio 1:1:1 were used for this study. In all three replications for each treatment were tried. The details of each treatment are as follows the table-

Table 1: Details of the treatment

Treatments	Description
T_1	Seed treatment (10g kg^{-1} seed) with potential fungal bio-agent
T_2	Soil application (20g kg^{-1} soil) with potential fungal bio-agent at the time of planting
T_3	Seed treatment (10g kg^{-1} seed) with potential bacterial bio-agent
T_4	Soil application (20g kg^{-1}soil) with potential bacterial bio-agent at the time of planting
T_5	T_1 (Seed treatment with potential fungal bio-agent) + T_4 (Soil application with potential bacterial bio-agent at the time of planting)
T_6	T_3 (Seed treatment with potential bacterial bio-agent) + T_2 (Soil application with potential fungal bio-agent at the time of planting)
T_7	Seed treatment with standard fungicide - 0.1 g kg^{-1} seed.
T_8	Inoculated control

Plants were grown for a period of 50 days *i.e.* till the period of harvest and the data on pre-emergence and post emergence damping off (%) at 10, 30, 50 DAS was recorded. Seeds of tomato cv. Arka vikas used in experiments and seed treatment with talc based potential fungal and bacterial antagonist *T. harzianum* -1 and *P. fluorescence*-3 were used and treated @ 10 g kg^{-1} of the seed using gum (5 ml kg^{-1}) as sticker. The treated seeds were spread over a clean paper and dried in a cool and shady place. The seeds were sown immediately after drying. The talc based formulations of fungal and bacterial bioagents were applied to soil @ 20 g kg^{-1} soil. The seeds of tomato cv. Arka Vikas were treated with Carbendazim @ 1 g kg^{-1} seed using gum (5 ml/kg) as sticker and the treated seeds were used for sowing. The various Treatments and their combinations from T_1 to T_8 with three replications were imposed on tomato seedling to record. The various Treatments and their combinations from T_1 to T_8 with three replications were imposed on tomato seedling to record.

1. $$\text{Percent germination (PG)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

2. $$\text{Percent disease incidence (PDI)} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

The growth parameters like shoot length, root length, total seedling length, fresh and dry weights were record.

3. Results and Discussion

3.1 Bio-control of damping off disease on tomato cv. Arka Vikas

The effect of potential bio-control agents alone or in combination was studied for their ability to enhance seed germination and to reduce the damping off incidence caused by *Pythium debaryanum*, *Rhizoctonia solani* and *Sclerotium rolfsii* under artificially inoculated conditions in glass house on tomato cv. Arka Vikas. The effect of different treatments on percent seed germination, pre and post emergence damping off was studied and the results are presented here under.

3.1a Percent germination

The results of experiments are presented in the Table-2, Fig.1 and Plate-18, showed that the germination of seeds of tomato in all the treatments significantly higher (62.92 to 38.75) as against 29.17 percent in the inoculated control.

Among the individuals treatments T_7 (seed treatment to Carbendazim) recorded highest (62.92) germination percent followed by T_6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum*-1), T_5 (seed treatment with *Trichoderma harzianum* -1+ soil application with *Pseudomonas fluorescence*-3) with 52.08 and 49.17 percent respectively, which were statistically on par. While T_4 (soil application with *Pseudomonas fluorescence*-3) recorded least 38.75 percent germination of tomato seeds.

The other treatments T_1 (seed treatment with *Trichoderma harzianum* -1), T_2 (soil application with *Trichoderma harzianum* -1) and T_3 (seed treatment with *Pseudomonas fluorescence*-3) recorded the percent germination of 42.08, 39.17 and 45.00 respectively.

Table 2: Effect of bio-control agents on management of damping off caused by *Sclerotium rolfsii* on Tomato cv. Arka Vikas

Treatments	Description	Germination (%)	PDI (%)		
			Pre-emergence damping off (%)	Post-emergence damping off (%)	
				30 DAYS	50 DAYS
T_1	Seed treatment (20 g kg^{-1}soil) with *Trichoderma harzianum* -1	42.08 (40.42)	57.92 (49.53)	64.17 (53.21)	68.33 (55.73)
T_2	Soil application (20 g kg^{-1} soil) with *Trichoderma harzianum* -1	39.17 (38.70)	60.83 (51.25)	65.83 (54.24)	71.67 (57.92)
T_3	Seed treatment (10 g kg^{-1} seed) with *Pseudomonas fluorescens*-3	45.00 (42.11)	55.00 (47.85)	59.58 (50.50)	64.58 (53.46)
T_4	Soil application (20 g kg^{-1}soil) with *Pseudomonas fluorescens*-3	38.75 (38.47)	61.25 (51.49)	66.25 (54.48)	71.25 (57.57)
T_5	T_1 + T_4	49.17 (44.50)	50.83 (45.46)	56.25 (48.57)	61.25 (51.48)
T_6	`T_3 + T_2	52.08 (46.17)	47.92 (43.78)	54.17 (47.37)	59.17 (50.26)
T_7	Seed treatment with Carbendazim 0.1g kg^{-1} seed	62.92 (52.48)	37.08 (37.47)	45.42 (42.35)	53.75 (47.13)
T_8	Inoculated control	29.17 (32.65)	70.83 (57.31)	78.33 (62.24)	83.33 (65.92)
	CD SE(d) SE(m)	3.373 1.593 1.126	3.373 1.593 1.126	2.973 1.404 0.993	3.745 1.769 1.251

Statistical Design: CRD;
* Mean of three replications
Figures in parentheses are angular transformed value

3.1b Pre and post emergence damping off incidence

It is obvious from Table-2 and Fig.1 that, all the treatments significantly reduced the disease incidence over control. The pre-emergence damping off incidence ranged between 37.08 to 61.25 percent in various treatments. The low (37.08) pre emergence damping off was recorded in the treatment T_7 (seed treatment with Carbendazim) compared to control, where 70.83 percent of disease incidence recorded.

Among the bio-control treatments the lowest incidence (47.92) percent was recorded in T_6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1) followed by T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-3), T_2 (soil application with *Trichoderma harzianum* -1), T_4 (soil application with *Pseudomonas fluorescence*-3), T_1 (seed treatment with *Trichoderma harzianum* -1), and T_3 (seed treatment with *Pseudomonas fluorescence*-3) with 50.83, 60.83, 61.25, 57.92 and 55.0 respectively. The treatments T_6 and T_5, T_2 and T_4, T_1 and T_3 were statistically on par.

Post emergence damping- off incidence at 30 DAS ranged from 45.42 to 66.25 percent among the various treatments. The lowest (45.42) percent post emergence of damping off incidence was recorded in T_7 treatment (seed treatment with Carbendazim) when compared to inoculated control (78.33). Among the bio-control treatments the lowest (54.17) post emergence incidence was recorded in T_6 (seed treatment with *Pseudomonas fluorescence*-3 + Soil application with *Trichoderma harzianum* -1), followed by T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-3), T_3 (seed treatment with *Pseudomonas fluorescence*-3), T_1 (seed treatment with *Trichoderma harzianum* -1), T_2 (soil application with *Trichoderma harzianum* -1), T_4 (soil application with *Pseudomonas fluorescence*-3) at the time of planting with 56.25, 59.58, 64.17, 65.83 and 66.25 percent respectively.

After 50 DAS sowing, the post emergence damping off ranged from 53.75 to 71.67 among the various treatments. The least (53.75) damping off incidence was recorded in the treatment T_7 treatment (seed treatment with Carbendazim), followed by T_6 (seed treatment with *Pseudomonas fluorescence*-3+ soil application with *Trichoderma harzianum* -1), T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-3), T_3 (seed treatment with *Pseudomonas fluorescence*-3), T_1 (seed treatment with *Trichoderma harzianum* -1), T_2 (Soil application with) *Trichoderma harzianum* -1, T_4 (soil application with *Pseudomonas fluorescence*-3) with, 59.17, 61.25, 64.58, 68.33, 71.67 and 71.25percent respectively. However the treatments T_6 and T_5, T_4 and T_2, were statistically on par.

In the present study, it was found that the combination of seed treatment with *Pseudomonas fluorescens*-3 + soil application of *Trichoderma harzianum* -1 (T_6) was effective in promoting seed germination and in reducing pre and post emergence damping off incidence. Efficacy of these biocontrol agents against *S. rolfsii* was also studied by Asghari and Mayee (1991) [3], Biswas and Sen (2000) [5], Das *et al.* (2000) [6], Vanitha and Suresh (2002) [19] Ansari (2005) [2].

Ansari (2005) [2] observed a high (51.11%) seed germination of soybean, when seeds treated with *Pseudomonas fluorescence* followed by *Trichoderma* and thiram + Carbendazim (2:1). Both *Pseudomonas* and *Trichoderma* was found effective in controlling collar rot disease. The results clearly indicated that the synergistic effect of the fungal and bacterial bio control agents when compared to individual biocontrol treatments.

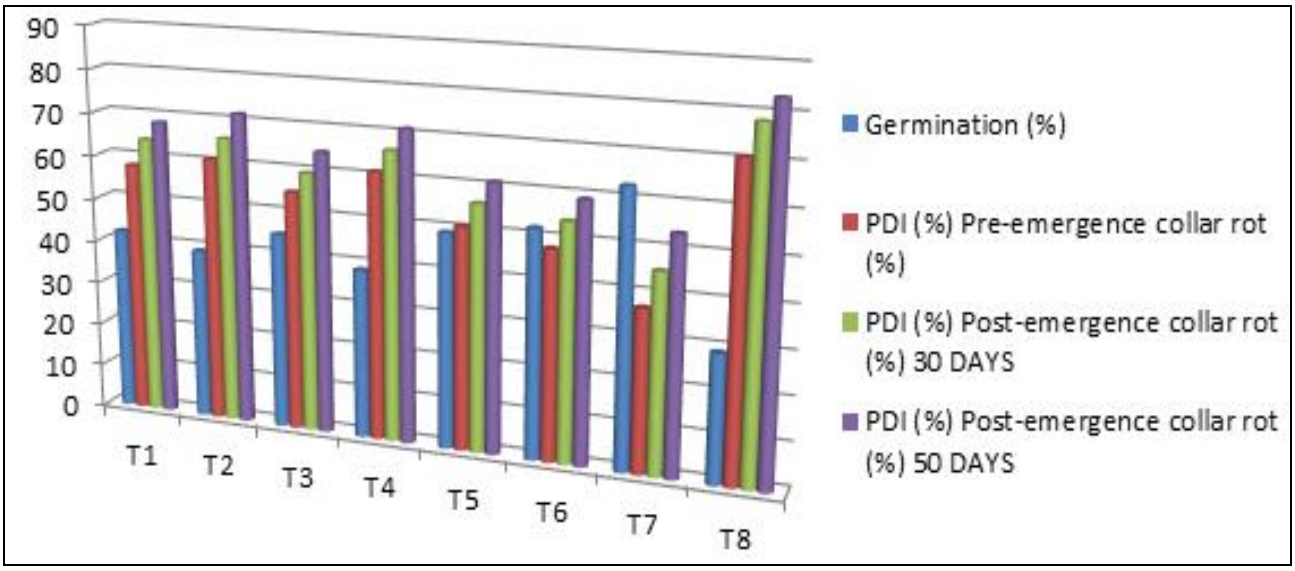

Fig 1: Effect of bio-control agents and their combinations on management of germination (%), pre-emergence damping- off (%), post-emergence damping-off (%) at -30 and 50 days and damping off disease caused by *Sclerotium rolfsii.*

T_1 - Seed treatment with *Trichoderma harzianum*-1, T_2- Soil application with *Trichoderma harzianum*-1 at the time of planting, T_3- Seed treatment with *Pseudomonas fluorescence* -3, T_4- Soil application with *Pseudomonas fluorescence* -3 at the time of planting, T_5-T_1 + T_4 , T_6-T_3 +T_2, T_7- Seed treatment with Carbendazim, T_8 - Inoculated control.

3.2 Effect of biocontrol agents on growth parameters of tomato cv. Arka vikas

The influence of biocontrol agents alone and in combination on the growth parameters such as shoot length, root length, total length and fresh and dry weight were studied in pots under green house conditions and the results are presented here under in pathogen wise.

3.2a Shoot length

There was significant increase in shoot length in various treatments compared control (Table-3) and (Fig.2). Maximum shoot length of 23.46 cm was recorded in T_7 (seed treatment with Carbendazim) treatment followed by T_5 (seed treatment with *Pseudomonas fluorescence*-3 + Soil application with *Trichoderma harzianum* -1), T_6 (seed treatment with *Pseudomonas fluorescence*-3 + Soil application with *Trichoderma harzianum* -1), T_1 (seed treatment with *Trichoderma harzianum* -1), T_3 (seed treatment with *Pseudomonas fluorescence*-3) and T_2 (soil application with *Trichoderma harzianum* -1) with 21.16, 20.0,18.46, 17.66 and 15.66 cm respectively. While T_4 treatment (soil application with *Pseudomonas fluorescence*-3) recorded minimum shoot length of 15.43 cm. The treatments T_1 and T_3 on par with each other with regard to shoot length.

Table 3: Effect of bio-control agents of *Sclerotium rolfsii* on growth parameters of tomato cv. Arka vikas at 50 days crop.

Treatments	Description	Shoot length (cm)	Root length (cm)	Total length	Fresh weight (g)	Dry weight (g)
T_1	Seed treatment (10 g kg^{-1} seed) with *Trichoderma harzianum* -1	18.46	3.20	21.66	1.93	0.21
T_2	Soil application (20 g kg^{-1} soil) with *Trichoderma harzianum* -1 at the time of planting	15.66	3.10	19.76	1.60	0.16
T_3	Seed treatment (10 g kg^{-1} seed) with *Pseudomonas fluorescens*-3	17.66	4.13	19.79	1.83	0.19
T_4	Soil application (20 g kg^{-1}soil) with *Pseudomonas fluorescens*-3 at the time of planting	15.43	3.0	18.43	1.61	0.17
T_5	T_1 + T_4	21.16	6.46	27.57	2.84	0.27
T_6	T_3 + T_2	20.00	7.26	27.26	2.73	0.25
T_7	Seed treatment with Carbendazim 0.1 g kg^{-1}seed	23.46	8.83	32.29	3.43	0.35
T_8	Inoculated control	12.03	2.03	14.6	1.10	0.12
	CD	1.21	0.839	1.13	0.35	0.060
	SE(d)	0.57	0.431	0.53	0.16	0.028
	SE(m)	0.40	0. 282	0.38	0.11	0.020

Statistical Design: CRD ;

* Mean of three replications

Figures in parentheses are angular transformed value

3.2b Root length

It is obvious from Table-3 and Fig.2 that treatment T_7 (seed treatment with Carbendazim) recorded a significantly highest root length of 8.83 cm followed by T_6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1), T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-3) with 7.26, 6.46 cm respectively. The treatments T_5 and T_6 were on par with each other. Among all the treatments, T_4 (soil application with *Pseudomonas fluorescence*-3) recorded minimum root length of 3.0 cm. The other treatments T3 (seed treatment with *Pseudomonas fluorescence*-3), T_1 (seed treatment with *Trichoderma harzianum* -1) and T_2 (soil application with *Trichoderma harzianum* -1) recorded root length of 4.13, 3.20 and 3.10 cm respectively.

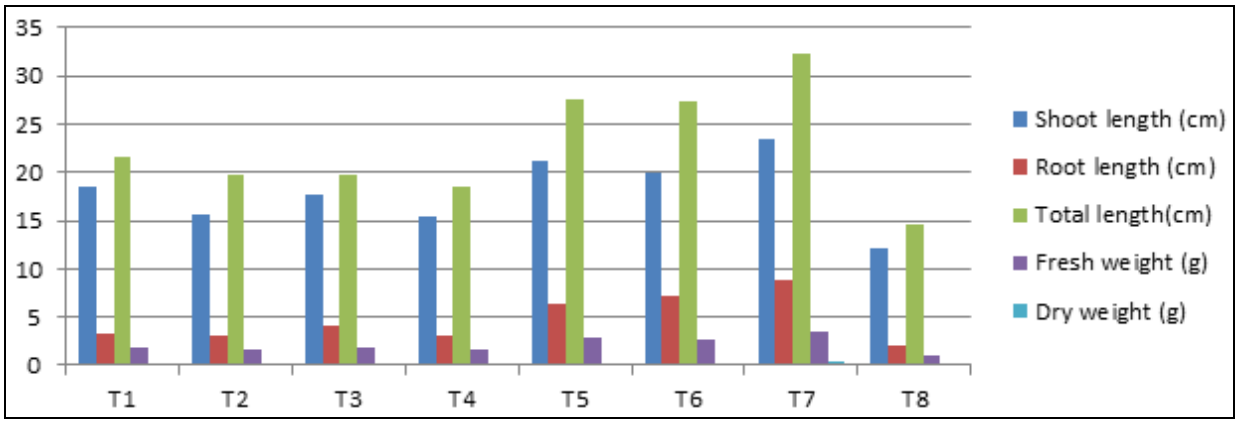

Fig 2: Effect of biological control treatments on growth parameters of Root length (cm), shoot length (cm), Total length (cm), Fresh weight (g) and Dry weight (g) of tomato cv. Arka vikas (*Sclerotium rolfsii).*

T_1 - Seed treatment with *Trichoderma harzianum*-1, T_2- Soil application with *Trichoderma harzianum*-1 at the time of planting, T_3- Seed treatment with *Pseudomonas fluorescence* -3, T_4- Soil application with *Pseudomonas fluorescence* -3 at the time of planting, T_5-T_1 + T_4 , T_6-T_3 +T_2, T_7- Seed treatment with Carbendazim, T_8 - Inoculated control.

3.2c Total length

The total length also increased significantly over control (Table-3, Fig.2). The treatment T_7 (seed treatment with Carbendazim) recorded with 32.29 cm followed by T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-3), T_6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1), T_1 (seed treatment with *Trichoderma harzianum* -1), T_3 (seed treatment with *Pseudomonas fluorescence*-3) and T_2 (soil application with *Trichoderma harzianum* -1) recorded 27.57, 27.26, 21.66, 19.79 and 19.76 respectively when compared to control (14.06 cm). While the treatment T_4 (soil application with *Pseudomonas fluorescens*-3) recorded minimum total length of 18.43 cm.

3.2d Fresh weight

The fresh weight of tomato seedlings increased in all the treatments compared to control (Fig.2). The treatment T_7 (seed treatment with Carbendazim) recorded 3.43 g followed by T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-3), T_6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1), T_1 (seed treatment with *Trichoderma harzianum* -1), T_3 (seed treatment with *Pseudomonas fluorescence*-3), T_4 (soil application with *Pseudomonas fluorescence*-3) and recorded 2.84, 2.73, 1.93, 1.83, 1.61 g respectively, when compared to control (1.10g). While the treatment T_2 (soil application with *Trichoderma harzianum* -1) recorded minimum fresh weight of 1.60 g**.**

3.2e Dry Weight

The dry weight of tomato seedlings increased significantly in all the treatments compared to control (Fig.2). The treatments T_7 (seed treatment with Carbendazim) recorded 0.35g followed by T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-3), T_6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1), T_1 (seed treatment with *Trichoderma harzianum* -1), T_3 (seed treatment with *Pseudomonas fluorescence*-3) and T_4 (soil application with *Pseudomonas fluorescence*-3) recorded 0.27, 0.25, 0.21, 0.19 and 0.17 g respectively when compared to control (0.12 g). While the treatment T_2 (soil application with *Trichoderma harzianum* -1) recorded minimum dry weight of 0.16 g.

In the present investigation, the treatment T_7 (seed treatment with Carbendazim) was enhanced the shoot and root length and fresh and dry weight of plants. Among bio-control treatments, combination of *Trichoderma harzianum* -1 and *Pseudomonas fluorescence*-3 *i.e*. T_5 and T_6 were proved superior in increasing the shoot and root length and fresh and dry weight. Results are in agreement with Krishnamoorthy and Bhaskaran (1990) [15], Dinakaran and Ramakrishnan (1996) [9].

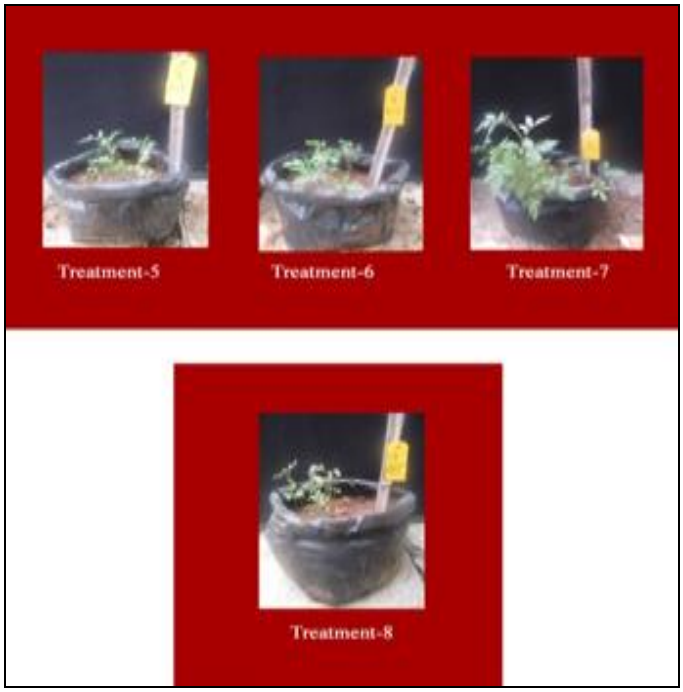

Fig 1: Effect of biocontrol agents on management of damping off caused by *Sclerotium rolfsii* on tomato cv arka vikas at 50 days

4. Conclusion

A pot culture experiment was conducted to study the effect of potential fungal and bacterial bio-control agents alone or in combination for their ability to enhance seed germination and to reduce disease incidence of collar rot caused by *Sclerotium rolfsii* under artificially inoculated conditions in glass house on tomato cv. Arka Vikas. Seed and soil application of *Trichoderma harzianum* -1 and *Pseudomonas fluorescence*-3 for *Sclerotium rolfsii* and combination of both the bioagents was tested against the pathogens. Fungicidal seed treatment with Carbendazim for was *Sclerotium rolfsii* also included as

one of the treatment in the present experiment.

The percent germination of tomato seeds in all the treatments was significantly higher when compared with the inoculated control in the presence of *Sclerotium rolfsii.* The treatment, T_7 *i.e.*, seed treatment with Carbendazim for *Sclerotium rolfsii* (62.92) recorded highest percent germination in tomato. However, seed treatment and soil application of both fungal and bacterial bioagents were also found equally good in enhancing the germination of tomato when inoculated with the pathogen *Sclerotium rolfsii.* seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1(T_6) followed by Seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-3 (T_5) recorded 52.08 and 49.17 percent germination respectively when inoculated with *Sclerotium rolfsii.* While T_2 (soil application with *Trichoderma harzianum* -1) recorded least (39.17 percent) germination of tomato seeds.

It is observed that seed treatment with *Trichoderma* and soil application of bacterial isolate was effective for increasing the germination percentage when inoculated with *Sclerotium rolfsii.* The pre emergence collar rot caused by (37.08) was minimum when treated with Carbendazim. Among the bio-control treatments, seed treatment with *Trichoderma harzianum* -1 and *Pseudomonas fluorescence*-3 recorded low pre emergence collar rot caused by *Sclerotium rolfsii.*

But post emergence collar rot incidence caused by *Sclerotium rolfsii* at 30 DAS ranged from 45.42 to 66.25 percent when compared to 78.33 percent in control. The lowest (45.42) incidence recorded in T_7 treatment (seed treatment with Carbendazim) followed by T_6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1) (54.17). After 50 DAS sowing, the post emergence collar rot ranged from 53.72 to 71.67. The least (71.67) disease incidence was recorded in the treatment T_2 (soil application with *Trichoderma harzianum* -1) followed by T_7 (seed treatment with Carbendazim) with 53.75.

The influence of bio-control agents alone and in combination on the growth parameters such as shoot length, root length, total length, fresh and dry weight were also studied when inoculated with *Sclerotium rolfsii*.

The maximum shoot and root length of was recorded in T_7 (seed treatment with Carbendazim) treatment in the pots inoculated with *Sclerotium rolfsii* followed by T_5 and T_6 (20.0 and 6.46 cm). While T_4 treatment (soil application with *Pseudomonas fluorescence*-3) recorded minimum shoot and root length of 15.43 and 3.10 cm respectively.

The total length also increased significantly in the treatment T_7 (seed treatment with carbendazim) recorded with 32.29 cm followed by T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-3) T_6 (seed treatment with *Pseudomonas fluorescence*-3 + Soil application with *Trichoderma harzianum* -1) with 27.57 and 27.26 cm respectively.

The fresh and dry weight of tomato seedlings increased in the treatment T_7 (seed treatment with Carbendazim) with 3.43 g and 0.35 g. While the treatment T_2 (soil application with *Trichoderma harzianum* -1) recorded minimum fresh weight of 1.60 g and 0.16 g respectively.

The combination of fungal and bacterial treatments T_5 (seed treatment with *Trichoderma harzianum* -1+ soil application with *Pseudomonas fluorescence*-3) recorded a high fresh and dry weight of 2.84 g and 0.27 g while another treatment T_6 (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -1) recorded 2.73 and 0.25 g respectively.

5. References

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