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Biological control of tomato damping off caused by *Rhizoctonia solani*

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Abstract
In vitro evaluation was conducted with 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 *Rhizoctonia solani* 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 lowest incidence (38.33 per cent) of pre emergence damping off was recorded in T_6 (seed treatment with *Pseudomonas fluorescence*-2 + soil application with *Trichoderma harzianum* -1). Among the bio-control treatments the lowest (43.75 and 48.33) 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*-2 bacterial 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 *Rhizoctonia solani*.

Keywords: *Rhizoctonia solani*, *Trichoderma harzianum*, *Pseudomonas fluorescence*, Tomato and damping off.

1. Introduction
Tomato (*Lycopersicon esculentum* L.) is considered one of the most important economic vegetable crops in India. However, there are many constraints that come in the way of tomato production. Often, it is affected by many diseases leading to substantial losses in yield however; it is susceptible to over 200 pathogens that cause severe destruction for this plant and consequent great reduction in the yield. *Rhizoctonia solani* is the main casual of the tomato damping off disease in tomato plants. Moreover, *Rhizoctonia solani* are highly destructive pathogens of both greenhouse and field grown tomatoes causing damping-off diseases (De Curtis *et al*., 2010) [4]. Various methods for controlling such diseases have been investigated including the use of resistant varieties (Brisa *et al*., 2007) [1], chemical control, plant volatile compounds (El-Mougy *et al*., 2007) [7] and biological control, (Dubey *et al*., 2007) [5]. Limited information is available on its sustainable management and is generally treated by chemical applications. Overuse of the chemical may result in environmental, human health and pest resistance problem. The increasing awareness of fungicide-related hazards has emphasized the need for adopting biological methods as an alternative disease control method, which is also ecofriendly (Khare *et al*., 2010) [13]. Biological control is an efficient and environmentally friendly way to prevent damping-off disease. Many microbial species such as *Trichoderma* spp (Hafez *et al*., 2013) [8], *Pseudomonas fluorescence* and *Bacillus subtilis* have been shown to effectively control plants pathogens (Sivasakthi *et al*., 2014) [22]. The development of formulations and delivery systems for biocontrol by using antagonistic microorganisms to suppress the incidence of diseases caused by soil borne pathogens is a great importance (Çigdemand Merih, 2005) [2]. Ideal formulation additives should improve the biocontrol efficacy of the antagonist but should not support the growth of the pathogen or cause any damage to the host plant (Wiyono *et al*., 2008) [23]. The objective of the present study was to evaluate some antagonistic fungal and bacterial agents against *Rhizoctonia solani in vitro* and *in vivo*. Preparation of different carrier formulations of antagonistic fungal and bacteria, its effect on damping off of cantaloupe in greenhouse conditions.

2. Material and Methods

2.1 Mass Multiplication of Pathogen

The test pathogen *R. solani* isolated form diseased plants and multiplied on sorghum grains. Sorghum grains were pre-soaked in 2 per cent 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 *Rhizoctonia solani* grown on PDA. Seven discs per flask were added and the flasks were incubated for three weeks at 28 ± 2 ^{0}C.

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 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*-2 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 (Jeyarajan *et al.*, (1994) [12]. 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.

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

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

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 *Rhizoctonia solani* under artificially inoculated conditions in glass house on tomato cv. Arka Vikas. The effect of different treatments on per cent seed germination, pre and post emergence damping off was studied and the results are presented here under.

3.1a: Per cent germination

The results of experiments are presented in Table 4.14 and Plate 17, showed that the germination of seeds of tomato in all the treatments significantly higher (50.0 to 68.33) as against 27.50 per cent in the inoculated control (Fig. 4.6).

Among the individual treatments T_7 (seed treatment with Carbendazim) recorded highest (68.33) germination per cent followed by T_6 (seed treatment with *Pseudomonas fluorescence*-2 + soil application with *Trichoderma harzianum* -1), T_5(seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2) with 61.67 and 60.83 percent respectively, which were statistically on par. While T_2 (soil application with *Trichoderma harzianum* -1) recorded least (50.0) per cent germination of tomato seeds.

Table 2: Effect of bio-control agents on management of damping off caused by *Rhizoctonia solani* 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 (10 g kg^{-1} seed) with *Trichoderma harzianum*-1	54.17 (47.38)	45.83 (42.57)	50.42 (45.22)	57.50 (49.32)
T_2	Soil application (20 g kg^{-1} soil) with *Trichoderma harzianum*-1	50.00 (44.98)	50.00 (44.98)	56.67 (48.81)	65.00 (53.71)
T_3	Seed treatment (10 g kg^{-1} seed) with *Pseudomonas fluorescence*-2	56.67 (48.81)	43.33 (41.145)	49.17 (44.50)	55.42 (48.09)
T_4	Soil application (20 g kg^{-1}soil) with *Pseudomonas fluorescence*-2	50.42 (45.22)	49.58 (44.74)	54.17 (47.35)	60.00 (50.75)
T_5	$T_1 + T_4$	60.83 (51.25)	39.17 (38.71)	44.17 (41.62)	47.50 (43.54)
T_6	$T_3 + T_2$	61.67	38.33	43.75	48.33

		(51.75)	(38.20)	(41.37)	(44.02)
T_7	Seed treatment with Carbendazim 0.1 g kg^{-1} seed	68.33 (55.83)	31.67 (34.13)	41.67 (40.16)	45.83 (42.58)
T_8	Inoculated control	27.50 (31.51)	72.50 (58.44)	81.25 (64.32)	86.67 (68.58)
	CD	5.763	5.763	4.268	3.871
	SE(d)	2.722	2.722	2.016	1.828
	SE(m)	1.925	1.925	1.425	1.293

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

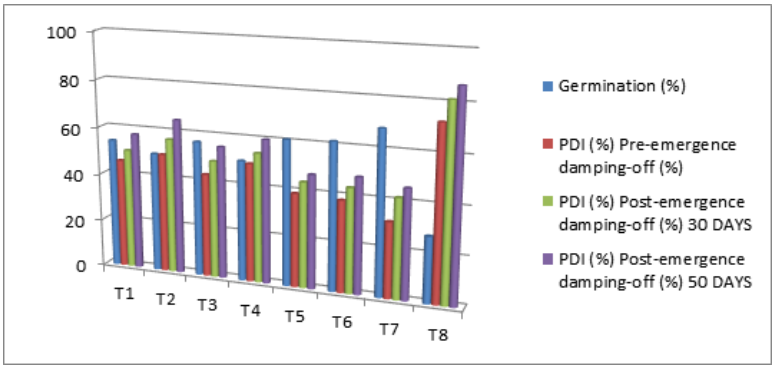

Fig 1: Effect of bio-control agents and their combinations on management of germination (%), pre-emergence damping- off (%), post-emergence damping-off (%) at -30 and 50 days- damping off disease caused by *Rhizoctonia solani.*
T_1 - Seed treatment with *Trichoderma harzianum*-1, T_2- Soil application with *Trichoderma harzianum*-1 at the time of planting, T_3- Seed treatment with *Pseudomonas fluorescence* -2, T_4- Soil application with *Pseudomonas fluorescence* -2 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.

3.1b: Pre and post emergence damping off incidence

It is obvious from Table 4.14, Plate.17 and Fig.4.7 that, all the treatments significantly reduced the disease incidence over control. The pre-emergence damping off incidence ranged between 31.67 to 50.0 per cent in various treatments. The minimum pre emergence damping off was recorded in the treatment T_7 (seed treatment with Carbendazim) compared to control (72.50).

Among the bio-control treatments the lowest (38.33) per cent pre-emergence damping off incidence was recorded in T_6 (seed treatment with *Pseudomonas fluorescence*-2+ soil application with *Trichoderma harzianum* -1) followed by T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2), T_3 (seed treatment with *Pseudomonas fluorescence*-2), T_1 (seed treatment with *Trichoderma harzianum* -1), T_4 (soil application with *Pseudomonas fluorescence*-2) and T_2 (soil application with *Trichoderma harzianum* -1) with 39.17, 43.33, 45.83, 49.58 and 50.0 respectively. The treatments T_5 and T_6, T_5 and T_7, T_3 and T_5, T_2 and T_4, T_1 and T_2 were statisticallypar.

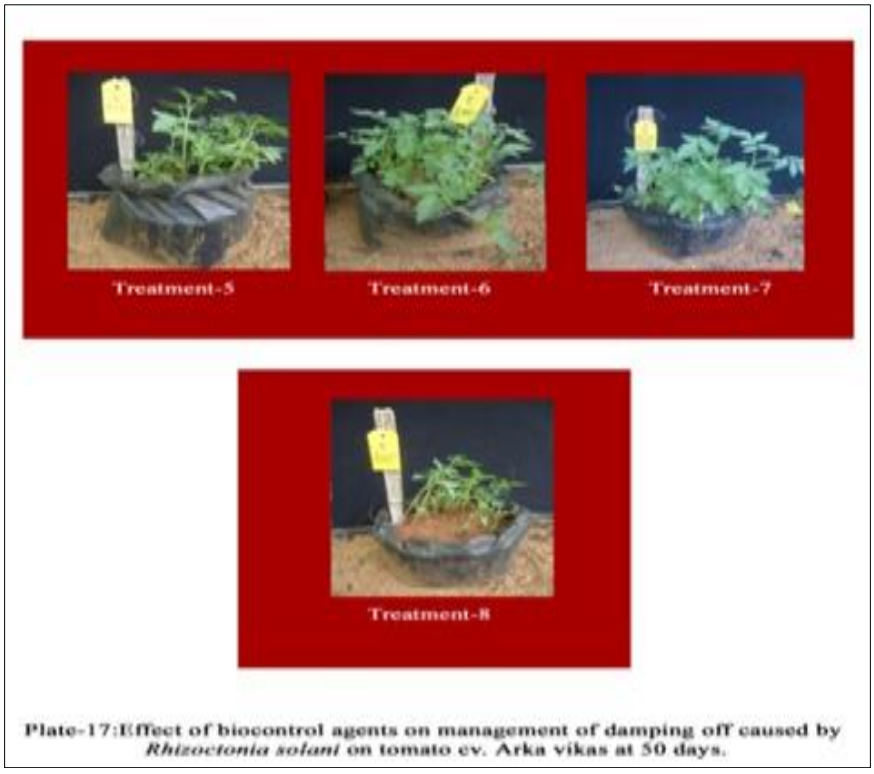

Plate-17:Effect of biocontrol agents on management of damping off caused by *Rhizoctonia solani* on tomato cv. Arka vikas at 50 days.

Post emergence damping- off incidence at 30 DAS ranged from 41.67 to 56.67 per cent when compared to 81.25 per cent in control. The lowest (41.67) post emergence damping off incidence recorded in T_7 treatment (seed treatment with Carbendazim) when compared to inoculated control. Among the bio-control treatments the lowest (43.75) post emergence damping off incidence was recorded in T_6 (seed treatment with *Pseudomonas fluorescence*-2 + soil application with *Trichoderma harzianum* -1), followed by T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2), T_3 (seed treatment with *Pseudomonas fluorescence*-2), T_1 (seed treatment with *Trichoderma harzianum* -1), T_4 (soil application with *Pseudomonas fluorescence*-2) and T_2 (soil application with *Trichoderma harzianum* -1) with 43.75, 44.17, 49.17, 50.42, 54.17 and 56.67 percent respectively.

After 50 DAS sowing, the post emergence damping off ranged from 47.50 to 65.0. The least (47.50) disease incidence was recorded in the treatment T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2) followed by T_7 (seed treatment with Carbendazim), T_6 (seed treatment with *Pseudomonas fluorescence*-2 + soil application with *Trichoderma harzianum* -1), T_3 (seed treatment with *Pseudomonas fluorescence*-2), T_1 (seed treatment with *Trichoderma harzianum* -1), T_4 (soil application with *Pseudomonas fluorescence*-2), T_2 (soil application with *Trichoderma harzianum* -1) with 45.83, 48.33, 55.42, 57.50, 60.0 and 65.0 per cent respectively. However the treatments T_5 and T_6, T_6 and T_7, T_5 and T_7, T_1 with T_3 were statically on par.

In the present study, the combination of seed treatment with *Pseudomonas fluorescence*-2+ soil application of *Trichoderma harzianum* -1 (T_6) was found to be effective in promoting seed germination and in reducing pre and post emergence damping off incidence. Similar observation on the efficacy of *Trichoderma* and bacterial biocontrol agents on the *R. solani* were also made by Lo *et al.* (1996) [16], Dubey (2000) [3] Jayalaksmi *et al.* (2003) [10] Rudresh *et al* (2005) [20] and Kumar *et al* (2008) [15].

Jayalaksmi *et al.* (2003) [10] reported that seed treatment with *Pseudomonas fluorescence* and other bacterial strains was more effective in reducing seed mortality and increased seed germination of rice. Similarly, Montealegre *et al.* (2014) [18] observed a high per cent germination of tomato when seeds treated with native isolates of *Trichoderma* spp. Mujeebur Rahman *et al.* (2014) [19] also reported the effectiveness of *Trichoderma* strains in the control of *Fusarium* and *Rhizoctonia solani* on chick pea.

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 greenhouse 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 pathogen check. Maximum shoot length of 37.36 cm was recorded in T_7 (seed treatment with Carbendazim) followed by T_6 (seed treatment with *Pseudomonas fluorescence*-2+ soil application with *Trichoderma harzianum* -1), T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2), T_3 (seed treatment with *Pseudomonas fluorescence*-2), T_1 (seed treatment with *Trichoderma harzianum* -1) and T_2 (soil application with *Trichoderma harzianum* -1) with 36.66, 32.80 22.93, 22.00, 20.33 cm, while T_4 treatment (soil application with *Pseudomonas fluorescence*-2), recorded minimum shoot length of 19.66 cm. The treatments T_1 and T_3 on par with each other with regard to shoot length.

3.2b: Root length

It is obvious from the Table-3 and Fig-2 that treatment T_7 (seed treatment with Carbendazim) recorded a significantly highest root of 7.96cm followed by T_6 (seed treatment with *Pseudomonas fluorescence*-2 + soil application with *Trichoderma harzianum* -1), T_5 (seed treatment with *Trichoderma harzianum* -1+ soil application with *Pseudomonas fluorescence*-2) with 7.12, 6.80cm respectively. The treatment T_5 and T_6 was on par with each other. Among all the treatments, T_4 (soil application with *Pseudomonas fluorescence*-2) recorded minimum root length of 4.50 cm.

The other treatments T_3 (seed treatment with *Pseudomonas fluorescence*-2), T_1 (seed treatment with *Trichoderma harzianum* -1) and T_2 (soil application with *Trichoderma harzianum* -1) recorded root length of 6.33, 6.16 and 5.43 cm respectively.

Table 3: Effect of biological control treatments of *Rhizoctonia solani* on growth parameters of Tomato cv. Arka vikas At 50 days.

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	22.00	6.16	28.16	3.36	0.33
T_2	Soil application (20 g kg^{-1} soil) with *Trichoderma harzianum*-1 at the time of planting	20.33	5.43	25.86	3.26	0.30
T_3	Seed treatment (10 g kg^{-1} seed) with *Pseudomonas fluorescence*-2	22.93	6.33	29.13	3.50	0.35
T_4	Soil application (20 g kg^{-1}soil) with *Pseudomonas fluorescence*-2 at the time of planting	19.66	4.50	24.36	3.20	0.27
T_5	$T_1 + T_4$	32.80	6.80	39.20	4.10	0.41
T_6	$T_3 + T_2$	36.66	7.12	43.79	4.43	0.43
T_7	Seed treatment with Carbendazim 0.1g kg^{-1} seed	37.36	7.96	44.32	4.63	0.46
T_8	Inoculated control	13.66	3.10	16.76	2.18	0.18
	CD SE(d) SE(m)	1.82 0.74 0.52	0.32 0.15 0.10	1.52 0.72 0.51	0.36 0.17 0.12	0.05 0.02 0.01

3.2c: Total length

The total length also increased significantly over control (Fig.2). The treatment T_7 (seed treatment with Carbendazim) recorded 44.32cm followed by T_6 (seed treatment with *Pseudomonas fluorescence*-2 + soil application with *Trichoderma harzianum* -1), T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2), T_3 (seed treatment with *Pseudomonas fluorescence*-2), T_1 (seed treatment with *Trichoderma harzianum* -1) and T_2 (soil application with *Trichoderma harzianum* -1), followed by recorded 43.79, 39.20, 29.13, 28.16 and 25.86 cm respectively when compared to control (16.76 cm). While the treatment T_4 (soil application with *Pseudomonas fluorescence*-2) recorded minimum total length of 24.36 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 4.63 g followed by T_6 (seed treatment with *Pseudomonas fluorescence*-2+ soil application with *Trichoderma harzianum* -1), T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2), T_3 (seed treatment with *Pseudomonas fluorescence*-2), T_1 (seed treatment with *Trichoderma harzianum* -1) and T_2 (soil application with *Trichoderma harzianum* -1) recorded 4.43, 4.10, 3.50, 3.36 and 3.26 respectively, when compared to control (2.18 g). While the treatment T_4 (soil application with *Pseudomonas fluorescence*-2) recorded minimum fresh weight of 3.20 g.

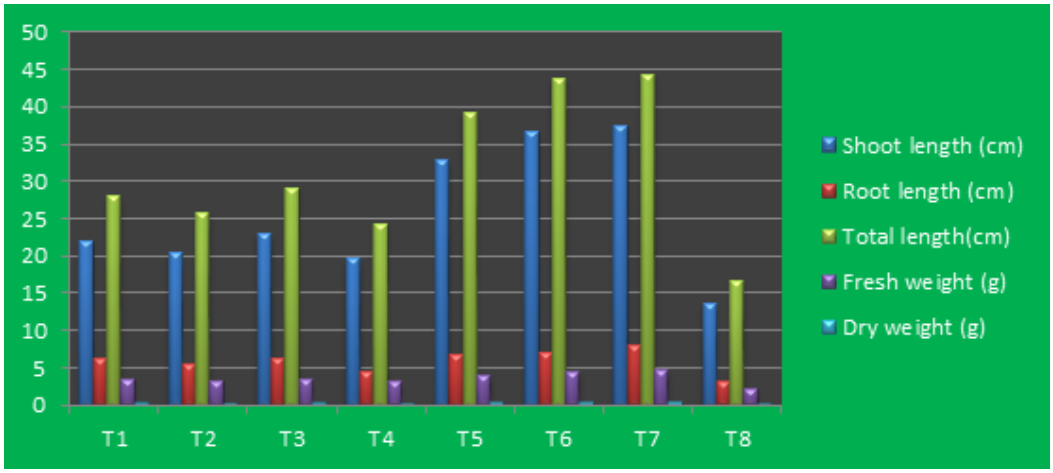

Fig 2: Effect of biological control treatments on growth parameters of Root length (cm), Shoot length (cm), Total length (cm), Fresh weight (g) and Dry weight (g) of tomato cv. Arka vikas (*Rhizoctonia solani).*
T_1 - Seed treatment with *Trichoderma harzianum*-1, T_2- Soil application with *Trichoderma harzianum*-1 at the time of planting, T_3- Seed treatment with *Pseudomonas fluorescence* -2, T_4- Soil application with *Pseudomonas fluorescence* -2 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.

3.2e: 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 0.46 g followed by T_6 (seed treatment with *Pseudomonas fluorescence*-2 + soil application with *Trichoderma harzianum* -1), T_5(seed treatment with *Trichoderma harzianum* -1+ soil application with *Pseudomonas fluorescence*-2), T_3 (seed treatment with *Pseudomonas fluorescence*-2), T_1 (seed treatment with *Trichoderma harzianum* -1) and T_2 (soil application with *Trichoderma harzianum* -1), recorded 0.43, 0.41, 0.35, 0.33 and 0.30 g respectively, when compared to control (0.18 g).While the treatment T_4 (soil application with *Pseudomonas fluorescence*-2) recorded minimum dry weight 0.27 g.

In the present study the seed treatment with Carbendazim (T_7) and seed treatment with *Pseudomonas fluorescence*-2 and soil application of *Trichoderma harzianum*-1enhanced germination per cent, shoot and root length and fresh and dry weight. Increase in growth parameters was reported by Yehai *et al.* (1982) [24], Krishnamoorthy and Bhaskaran (1990) [14], Dinakaran and Ramakrishnan (1996) [6], Manoranjitham *et al.* (2000) [17].

Among the combined seed treatment, the results obtained in treatment T_{10} where *P. fluorescens* was applied to soil are in agreement with Hoflich (1994) [9] who reported that *P. fluorescence* increased root length, lateral root development, shoot and root dry matter and seed yield of winter wheat against soil borne root pathogens *i.e. Fusarium solani*, *F. oxysporum*. Application of *P. aeruginosa* to soil resulted in growth promotion of chilli seedlings in addition to suppressing the root infection by *F. solani*, *R. solani* (Siddiqui *et al*. 2001) [21].

The results obtained in the treatment T_7 are in agreement with Jayaraj and Radha Krishanan (2003) [11] who also reported that seed treatment with Carbendazim followed by soil application of Carbendazim resistant mutants of *T. harzianum* resulted in increased plant biomass apart from reducing the infection by *R. solani.*

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 damping off caused by *R. solani* under artificially inoculated conditions in glass house on tomato cv. Arka Vikas. Seed and soil application of *Trichoderma harzianum* -1 and *Pseudomonas fluorescence*-2 for *R. solani* and combination of both the bioagents was tested against the pathogens. Fungicidal seed treatment with Carbendazim for was *R. solani* also included as one of the treatment in the present experiment.

The per cent germination of tomato seeds in all the treatments was significantly higher when compared with the inoculated control in the presence of *R. solani*. The treatment, T_7 *i.e.*, seed treatment with Carbendazim for *R. solani* (68.33)

recorded highest per cent 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 three pathogens *R. solani.* seed treatment with *Pseudomonas fluorescence*-2 + soil application with *Trichoderma harzianum* -1(T_6) followed by Seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2 (T_5) recorded 61.67 and 60.83 per cent germination respectively when inoculated with *R. solani.* While T_2 (soil application with *Trichoderma harzianum* -1) recorded least (50.00 per cent) 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 *R. solani.* The pre emergence damping off caused by *R. solani* (31.67) was minimum when treated with Carbendazim. Among the bio-control treatments, seed treatment with *Trichoderma harzianum* -1 and *Pseudomonas fluorescence*-2 recorded low pre emergence damping off caused by *Rhizoctonia solani.*

But post emergence damping- off incidence caused by *Rhizoctonia solani* at 30 DAS ranged from 41.67 to 56.67 per cent when compared to 81.25 per cent in control. The lowest (41.67) incidence recorded in T_7 treatment (seed treatment with Carbendazim) followed by T_6 (seed treatment with *Pseudomonas fluorescence*-2 + soil application with *Trichoderma harzianum* -1) (43.75). After 50 DAS sowing, the post emergence damping off ranged from 47.50 to 65.0. The least (47.50) disease incidence was recorded in the treatment T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2) followed by T_7 (seed treatment with Carbendazim) with 45.83.

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 *Rhizoctonia solani*.

Maximum shoot and root length was recorded in T_7 (seed treatment with Carbendazim), while T_4 treatment (soil application with *Pseudomonas fluorescence*-2), recorded minimum shoot length of 19.66 cm.

The combination of fungal and bacterial treatments T_6 (seed treatment with *Pseudomonas fluorescens*-2 + soil application with *Trichoderma harzianum* -1) recorded shoot and root length of 36.6 and 7.12 cm respectively while T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2) recorded 32.80 and 6.80 respectively.

The total length also increased significantly over control in the treatment T_7 (seed treatment with Carbendazim), T_6 (seed treatment with *Pseudomonas fluorescence*-2 + soil application with *Trichoderma harzianum* -1) and T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2) with 44.32, 43.79 and 39.20 cm respectively. While the treatment T_4 (soil application with *Pseudomonas fluorescence*-2) recorded minimum total length of 24.36 cm in *Rhizoctonia solani* infected soil.

The fresh and dry weight (4.63 g and 0.46 g) of seedlings increased in the treatment T_7 (seed treatment with Carbendazim), followed by T_6 (seed treatment with *Pseudomonas fluorescence*-2+ soil application with *Trichoderma harzianum* -1) and T_5 (seed treatment with *Trichoderma harzianum* -1 + soil application with *Pseudomonas fluorescence*-2) with 4.43 and 0.43 g and 4.10 and 0.41 g respectively. While the treatment T_4 (soil application with *Pseudomonas fluorescence*-2) recorded minimum fresh and dry weight of 3.20 g and 0.27 g in the presence of *Rhizoctonia solani.*

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