



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2017; 5(5): 447-452

© 2017 IJCS

Received: 03-07-2017

Accepted: 04-08-2017

M Rajendraprasad

Department of Plant Pathology,
College of Agriculture, PJTSAU
Hyderabad, Andhra Pradesh,
India

B Vidyasagar

Department of Plant Pathology,
College of Agriculture, PJTSAU
Hyderabad, Andhra Pradesh,
India

G Uma Devi

Department of Plant Pathology,
College of Agriculture, PJTSAU
Hyderabad, Andhra Pradesh,
India

SR Koteswar Rao

Department of Entomology,
College of Agriculture, PJTSAU
Hyderabad, Andhra Pradesh,
India

Correspondence**M Rajendraprasad**

Department of Plant Pathology,
College of Agriculture, PJTSAU
Hyderabad, Andhra Pradesh,
India

Biological control of tomato damping off caused by *Pythium debaryanum*

M Rajendraprasad, B Vidyasagar, G Uma Devi and SR Koteswar Rao

Abstract

In vitro evaluation was conducted with twenty four isolates representing two different species *Trichoderma harzianum* and *Trichoderma viride* and twelve different *Bacillus subtilis* and *Pseudomonas fluorescence*. These potential biocontrol agents were tested for their efficacy against phytopathogenic fungi 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* spp and *Pseudomonas fluorescence* bacterial biocontrol agents (T₅ and T₇ treatments) also proved effective in increasing germination and to reduce pre and post emergence damping off in the pots when inoculated with *Pythium debaryanum*. The lowest incidence (45.00 percent) of pre emergence damping off was recorded in T₅ (seed treatment with *Trichoderma harzianum* - 7 + soil application with *Pseudomonas fluorescence*-3). Among the bio-control treatments the lowest (47.92 and 52.08) post emergence damping off incidence was recorded in T₅ (seed treatment with *Trichoderma harzianum* -7 + soil application with *Pseudomonas fluorescence*-3) at 30 and 50 DAS. The combination *Trichoderma* spp and *Pseudomonas fluorescence* bacterial biocontrol agents (T₆ and T₇) treatment also proved effective in increasing the shoot and root weight and fresh and dry weight of tomato plants when inoculated with *Pythium debaryanum*.

Keywords: *Pythium debaryanum*, *Trichoderma harzianum*, *Pseudomonas fluorescence*, Tomato damping off.

1. Introduction

Tomato (*Lycopersicon esculentum*. Mill) is one of the important vegetable crop in India and world. It is considered as 'Protective food' because of its nutritive value and year round production throughout the country. Soil borne fungal pathogens such as *Pythium* spp., *Rhizoctonia solani* and *Sclerotium rolfsii* infects the tomato crop causing damping off disease and is becoming a potential threat to its cultivation. *Pythium debaryanum*, a member of class Oomycetes, is one of the important soil-borne plant pathogen and it causes great loss in agriculture production. This fungus like organism is an unspecialized parasite that has a wide host range. Young tissues and plants are infected and affected much more severely by this pathogen. It causes damping off disease in several plants including tomato; it affects the plant both in pre and post emergence stage in nursery beds. Even though this pathogen can be controlled by some fungicides, nowadays researchers are more interested on biological control agents and their antifungal metabolites due to the notification of resistance development in the pathogen. Biological control is an alternative approach to the chemical fungicides and it may be a safe, effective and ecofriendly method for plant disease management. Damping-off of tomato caused by *Pythium debaryanum* was reduced by the application of talc based formulation of *Trichoderma viride* and *Pseudomonas fluorescence* in nursery beds before sowing. Besides reducing the pre - and post-emergence damping off these antagonists' increased the root length, shoot length and biomass production of tomato seedlings. These antagonists significantly reduced the population of *P. debaryanum* in soil. Damping-off is an important disease of tomato, causing significant losses in nurseries where young susceptible transplants are produced. A pot culture experiment was conducted in the greenhouse to study the efficacy of effective fungal or bacterial bio-control agents against test pathogen.

2. Material and Methods

2.1 Mass Multiplication of Pathogen

The test pathogen *Pythium debaryanum* isolated from 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 *Pythium debaryanum* grown on PDA or carrot agar. Seven discs per flask were added and the flasks were incubated for three weeks at 28 ± 2 °C.

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(or) 0.3 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* -7 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 (Jeyarajan *et al.*, (1994). The seeds of tomato cv. Arka Vikas were treated with Thiram @ 3 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 T₁ to T₈ with three replications were imposed on tomato seedling to record.

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

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

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

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* 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 presented in Table (2), Plate 1 and Figure-1 showed that the percent germination of tomato seeds

in all the treatments (39.58 to 57.08) was significantly higher as against 26.25 percent in the inoculated control.

Among the individuals treatments, T₇ (seed treatment with thiram) recorded highest (57.08) percent germination followed by T₅ (seed treatment with *Trichoderma harzianum* -7 + soil application with *Pseudomonas fluorescence*-3), T₆ (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -7) with 55.08 and 51.25 percent respectively, which were statistically on par. However treatment T₂ (soil application with *Trichoderma harzianum* -7) recorded least (39.58 percent) germination of tomato seeds.

3.1b Pre and post emergence damping off incidence

It is obvious from Table-2 and Plate. 1 that, all the treatments significantly reduced the disease incidence over control. The pre-emergence damping off incidence ranged between 42.92 to 60.42 percent in various treatments. The minimum pre emergence damping off incidence was recorded in the treatment T₇ (seed treatment with thiram) when compared to control (73.75 percent).

Among the bio-control treatments, the lowest damping off incidence (45.00) was recorded in T₅ (seed treatment with *Trichoderma harzianum* -7 + soil application with *Pseudomonas fluorescence*-3) followed by T₆ (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -7), T₃ (seed treatment with *Pseudomonas fluorescence*-3), T₁ (seed treatment with *Trichoderma harzianum* -7), T₄ (soil application with *Pseudomonas fluorescence*-3) and T₂ (soil application with *Trichoderma harzianum* -7) with 48.75, 51.25, 52.92, 55.0 and 60.42 respectively. The treatments T₅ and T₆, T₅ and T₇, T₃ and T₄ and T₁ and T₃ were statistically on par.

The post emergence damping-off incidence ranged from 47.08 to 65.83 percent at 30 DAS in various treatments. The lowest (47.08) post emergence of damping off incidence was recorded in T₇ treatment (seed treatment with thiram) when compared to inoculated control (84.58). Among the bio-control treatments the lowest (47.92) incidence was recorded in T₅ (seed treatment with *Trichoderma harzianum* -7 + soil application with *Pseudomonas fluorescence*-3) followed by T₆ (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -7), T₃ (seed

treatment with *Pseudomonas fluorescence-3*), T₁ (seed treatment with *Trichoderma harzianum -7*), T₄ (soil application with *Pseudomonas fluorescence-3*) and T₂ (soil application with *Trichoderma harzianum -7*) with 53.33, 55.42, 58.33, 61.25 and 68.83 percent respectively.

The post emergence damping off after 50 DAS sowing was ranged from 52.08 to 70.0 when compared to control (87.10). The least (52.08) was recorded in the treatment T₅ (seed treatment with *Trichoderma harzianum -7* + soil application with *Pseudomonas fluorescence-3*) followed by T₇ (seed treatment with thiram), T₆ (seed treatment with *Pseudomonas fluorescence-3* + soil application with *Trichoderma harzianum -7*), T₃ (seed treatment with *Pseudomonas fluorescence-3*), T₁ (seed treatment with *Trichoderma harzianum -7*), T₄ (soil application with *Pseudomonas fluorescence-3*) and T₂ (soil application with *Trichoderma harzianum -7*) with 54.17, 57.50, 60.0, 61.67, 66.25 and 70.0 percent respectively. However the treatments T₅ and T₇, T₃ and T₆, T₃ and T₁ were statistically on par.

In the present study, all the treatments enhanced seed germination and suppressed pre and post emergence damping off incidence. Among all the treatments, T₅ (seed treatment with *Trichoderma harzianum -7* + soil application with *Pseudomonas fluorescence-3*) recorded maximum percent germination with minimum percent of damping off incidence.

The seed treatment with thiram (T₇) supported maximum germination and reduced the incidence of damping off. The effectiveness of Thiram and other chemicals in controlling tomato damping off was reported by Krishnamoorthy and Baskeran (1990). Among the bio-control treatments, T₅ (seed treatment with *Trichoderma harzianum -7* + soil application with *Pseudomonas fluorescence-3*) was found to be best in increasing the germination of the seeds and reducing the damping off incidence compared to individual treatments. The effectiveness of *Trichoderma* and several bacterial biocontrol agents in reduction of tomato damping off incidence, was reported by Wolfhechel and Funck Jensen (1992), Mehata *et al.* (1995) [8], Dinakaran and Ramakrishnan (1996) [2], Karthikeyan *et al.* (1999) [5].

The reduction or suppression of damping off incidence by bio-control agents maybe due to production of hydrolytic enzymes secreted by bio-control agents which help in entry of antagonists to pathogens through penetration with direct lysis and degradation of cell walls as reported by Chet and Baker (1981) [1]. Similarly Elad *et al.* (1987) [3] also reported the ability of *Trichoderma* isolates to control *P. aphanidermatum*, *S. rolfsii* in soil was achieved through hydrolytic enzyme production. It is evident from the results that the application of antagonists in combination showed synergistic effect on *P. aphanidermatum*.



Plate 1: effect of biocontrol agents on management of damping off caused by *Pythium debaranum* on tomato cv Arka Vikas at 50 days.

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 Table-(3) and Figure-(2).

3.2a Shoot length

There was significant increase in shoot length in various treatments compared to pathogen check. Maximum shoot length of 35.0 cm was recorded in T₇ (seed treatment with thiram) followed by T₆ (seed treatment with *Pseudomonas*

fluorescence-3 + soil application with *Trichoderma harzianum -7*), T₅ (seed treatment with *Trichoderma harzianum -7* + soil application with *Pseudomonas fluorescence-3*), T₃ (seed treatment with *Pseudomonas fluorescence-3*), T₁ (seed treatment with *Trichoderma harzianum -7*) and T₂ (soil application with *Trichoderma harzianum -7*) with 33.0, 29.0, 25.0, 24.0 and 21.0 cm respectively, while T₄ treatment (soil application with *Pseudomonas fluorescence-3*), recorded minimum shoot length of 20.0 cm. The treatments T₁ and T₃ were on par with each other with regard to shoot length.

3.2b Root length

It is obvious from the Table -3 and Figure-2, that treatment T₇ (seed treatment with thiram) recorded a significantly highest root length 7.20 cm followed by T₆ (seed treatment with *Pseudomonas fluorescence*-3 + Soil application with *Trichoderma harzianum* -7), T₅ (Seed treatment with *Trichoderma harzianum* -7 + Soil application with *Pseudomonas fluorescence*-3) with 6.96, 6.43 cm respectively. The treatments T₆ and T₇ were on par with each other. Among all the treatments, T₄ (soil application with *Pseudomonas fluorescence*-3) recorded minimum root length of 2.92 cm. The other treatments T₃, T₁ and T₂ recorded root length of 3.76, 3.50 and 3.23 cm respectively.

3.2c Total length

The total length also increased significantly over control. The treatment T₇ (seed treatment with thiram) recorded with 42.20 cm followed by T₆ (seed treatment with *Pseudomonas fluorescence*-3 + Soil application with *Trichoderma harzianum* -7), T₅ (seed treatment with *Trichoderma harzianum* -7 + soil application with *Pseudomonas fluorescence*-3), T₃ (Seed treatment with *Pseudomonas fluorescence*-3), T₁ (seed treatment with *Trichoderma harzianum* -7) and T₂ (soil application with *Trichoderma harzianum* -7), recorded 39.96, 35.43, 28.76, 27.50 and 24.43 cm respectively when compared to control (16.20 cm). While the treatment T₄ (soil application of *Pseudomonas fluorescence*-3) recorded the minimum total length of 22.92 cm.

3.2d Fresh weight

The fresh weight of tomato seedlings increased in all the treatments compared to control (Fig.4.13). The treatment T₇ (seed treatment with thiram) recorded 4.06 g followed by T₆ (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -7), T₅ (seed treatment with *Trichoderma harzianum* -7 + soil application with *Pseudomonas fluorescence*-3), T₃ (Seed treatment with *Pseudomonas fluorescence*-3), T₁ (seed treatment with *Trichoderma harzianum* -7) and T₂ (soil application with *Trichoderma harzianum* -7), recorded 3.80, 3.70, 3.53, 2.60

and 2.52 g respectively, when compared to control (1.66 g). While the treatment T₄ (soil application of *Pseudomonas fluorescence*-3) recorded minimum fresh weight of 2.40 g.

3.2e Dry weight

The dry weight of tomato seedlings increased significantly in all the treatments compared to control. The treatments T₇ (seed treatment with thiram) recorded 0.43g followed by T₆ (seed treatment with *Pseudomonas fluorescence*-3 + Soil application with *Trichoderma harzianum* -7), T₅ (seed treatment with *Trichoderma harzianum* -7 + Soil application with *Pseudomonas fluorescence*-3), T₃ (seed treatment with *Pseudomonas fluorescence*-3), T₁ (Seed treatment with *Trichoderma harzianum* -7) and T₂ (Soil application with *Trichoderma harzianum* -7), recorded 0.40, 0.39, 0.35, 0.26 and 0.23 g respectively when compared to control (0.17). While the treatment T₄ (soil application of *Pseudomonas fluorescence*-3) recorded minimum dry weight of 0.21 g.

In the present study, among the bio-control treatments, T₆ (seed treatment with *Pseudomonas fluorescence*-3 + Soil application with *Trichoderma harzianum* -7) treatment was found to be effective to enhance the shoot length, root length and fresh and dry weight over other treatments.

The results are in agreement with the results of Manoranjitham *et al.* (1999) who reported that combined seed treatment with talc based formulations *T. viride* + *P. fluorescence* recorded maximum shoot length (4.45 cm), root length (13.50 cm), dry matter production (6.77 mg) against *P. aphanidermatum*.

Combined seed treatment with *T. viride* and *B. subtilis* resulted in increased fresh and dry weight of shoots, roots and nodules of broad bean apart from controlling infection by *F. solani* (Yehia *et al.* 1982)^[10]. While the results of treatments T₆ (combined seed treatment of ThM₁ + *P. fluorescence*) is in agreement with Jensen *et al.* (2002)^[11] who reported that *B. subtilis* in combination with *T. harzianum* when given as a seed treatment, resulted in increased biomass of dry apart from decreasing the severity of *F. solani* f.sp *phaseoli* infection.

Table 2: Effect of bio-control agents on management of damping off caused by *Pythium debaryanum* 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 <i>Trichoderma harzianum</i> -7.	47.08 (43.30)	52.92 (46.65)	58.33 (49.78)	61.67 (51.74)
T ₂	Soil application (20 g kg ⁻¹ soil) with <i>Trichoderma harzianum</i> -7.	39.58 (38.96)	60.42 (51.00)	65.83 (54.22)	70.00 (56.77)
T ₃	Seed treatment (10 g kg ⁻¹ seed) with <i>Pseudomonas fluorescence</i> -3.	48.75 (44.26)	51.25 (45.69)	55.42 (48.09)	60.00 (50.75)
T ₄	Soil application (20 g kg ⁻¹ soil) with <i>Pseudomonas fluorescence</i> -3.	45.00 (42.11)	55.00 (47.83)	61.25 (51.48)	66.25 (54.46)
T ₅	T ₁ + T ₄	55.00 (47.85)	45.00 (42.11)	47.92 (43.78)	52.08 (46.17)
T ₆	T ₃ + T ₂	51.25 (45.69)	48.75 (44.26)	53.33 (46.89)	57.50 (49.29)
T ₇	Seed treatment with Thiram 0.3 g kg ⁻¹ seed	57.08 (49.07)	42.92 (40.89)	47.08 (43.30)	54.17 (49.29)
T ₈	Inoculated control	26.25 (30.77)	73.75 (59.19)	84.58 (66.92)	87.50 (69.33)
	CD	3.986	3.986	3.051	3.268
	SE(d)	1.882	1.882	1.441	1.544
	SE(m)	1.331	1.331	1.019	1.092

Statistical Design: CRD;

* Mean of three replications

Figures in parentheses are angular transformed value

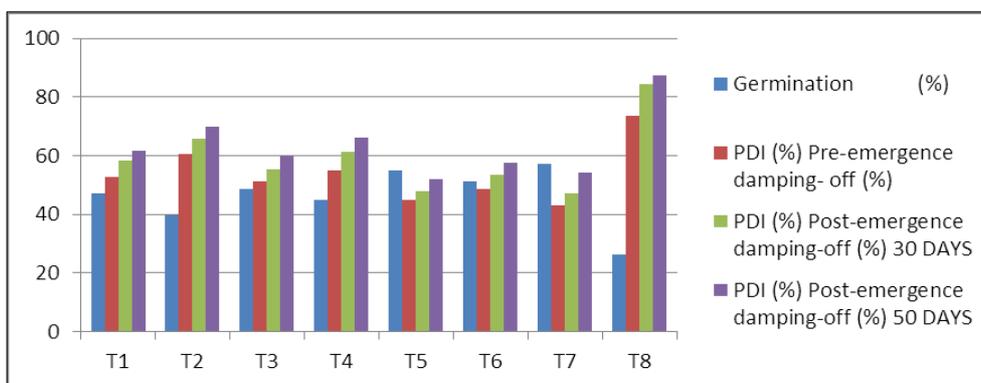
Table 3: Effect of bio control agents of *Pythium debaryanum* on growth parameters of tomato cv. Arka vikas at 50 days crop

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 <i>Trichoderma harzianum</i> -7	24	3.50	27.50	2.60	0.26
T ₂	Soil application (20 g kg ⁻¹ soil) with <i>Trichoderma harzianum</i> -7 at the time of planting	21	3.23	24.23	2.52	0.23
T ₃	Seed treatment (10 g kg ⁻¹ seed) with <i>Pseudomonas fluorescence</i> -3	25	3.76	28.76	3.53	0.35
T ₄	Soil application (20 g kg ⁻¹ soil) with <i>Pseudomonas fluorescence</i> -3 at the time of planting	20	2.92	22.92	2.40	0.21
T ₅	T ₁ + T ₄	29	6.43	35.43	3.70	0.39
T ₆	T ₃ + T ₂	33	6.96	39.96	3.80	0.40
T ₇	Seed treatment with Thiram 0.3 g kg ⁻¹ seed	35	7.20	42.20	4.06	0.43
T ₈	Inoculated control	15	1.20	16.20	1.66	0.17
	CD	1.50	0.25	1.44	0.459	0.053
	SE(d)	0.84	0.12	0.68	0.217	0.025
	SE(m)	0.45	0.9	0.48	0.153	0.018

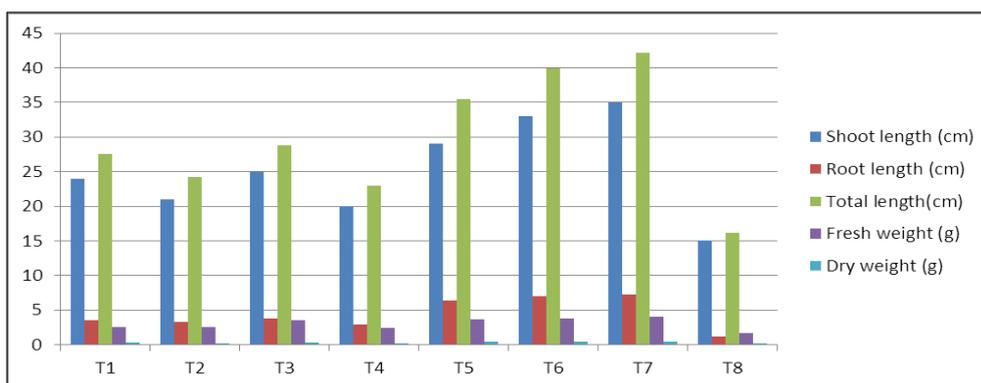
Statistical Design: CRD;

* 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 and damping off disease caused by *Pythium debaryanum*.

T₁ - Seed treatment with *Trichoderma harzianum*-7, T₂- Soil application with *Trichoderma harzianum*-7 at the time of planting, T₃- Seed treatment with *Pseudomonas fluorescence* -3, T₄- Soil application with *Pseudomonas fluorescence* -3 at the time of planting, T₅-T₁ + T₄, T₆- T₃ +T₂, T₇- Seed treatment with thiram, T₈ - Inoculated control.

**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 (*Pythium debaryanum*).

T₁ - Seed treatment with *Trichoderma harzianum*-7, T₂- Soil application with *Trichoderma harzianum*-7 at the time of planting, T₃- Seed treatment with *Pseudomonas fluorescence* -3, T₄- Soil application with *Pseudomonas fluorescence* -3 at the time of planting, T₅-T₁ + T₄, T₆- T₃ +T₂, T₇- Seed treatment with thiram, T₈- Inoculated control.

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 *Pythium debaryanum* under artificially inoculated conditions in glass house on tomato cv. Arka Vikas. Seed and soil application of

Trichoderma harzianum -7 and *Pseudomonas fluorescence*-3 for *Pythium debaryanum* and combination of both the bioagents was tested against the pathogens. Fungicidal seed treatment with Thiram for *Pythium* was 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 *Pythium debaryanum*. The treatment, T₇ i.e., seed treatment with thiram for *Pythium* (57.08) 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 three pathogens *Pythium debaryanum*. Seed treatment with *Trichoderma harzianum* -7 + soil application with *Pseudomonas fluorescence*-3 (T₅) followed by seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -7(T₆) recorded 55.08 and 51.25 percent germination respectively when inoculated with *Pythium debaryanum*. While T₂ (soil application with *Trichoderma harzianum* -7) recorded least (39.58 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 *Pythium debaryanum*. The pre emergence damping off caused by *Pythium debaryanum* (42.92) was minimum when treated with thiram. Among the bio-control treatments, seed treatment with *Trichoderma harzianum* -7 and *Pseudomonas fluorescence*-3 recorded low pre emergence damping off caused by *Pythium debaryanum*.

The data on post emergence damping off was recorded at 30 and 50 DAS. Seed treatment with thiram (T₇) showed lowest incidence of (47.08, 52.08) followed by T₅ (seed treatment with *Trichoderma harzianum* -7 + soil application with *Pseudomonas fluorescence*-3) (47.92 and 52.08) incidence *Pythium debaryanum* when compared to inoculated control (84.58, 70.0) at 30 and 50 DAS.

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 *Pythium debaryanum*.

Maximum shoot and root length was recorded in T₇ (seed treatment with thiram) in *Pythium debaryanum* (35.0cm, 7.2cm) inoculated pots. The combination of fungal and bacterial treatment T₆ (seed treatment with *Pseudomonas fluorescence*-3+ soil application with *Trichoderma harzianum* -7) recorded high shoot and root length of 33.0, 6.96 cm respectively in *Pythium* inoculated plants while T₅ (seed treatment with *Trichoderma harzianum* -7 + soil application with *Pseudomonas fluorescence*-3) recorded 29.0 and 6.43 cm respectively.

The total length also increased significantly over control in the treatment T₇ (seed treatment with thiram 23.46 cm 8.83 cm) recorded with 42.20 cm and the low (22.92) in T₄ (soil application of *Pseudomonas fluorescence*-3). The combination of fungal and bacterial treatment T₆ and T₅ recorded 39.96, 35.43 cm respectively.

The fresh and dry weight of tomato seedlings also increased in the treatment T₇ (seed treatment with thiram) with 4.06 g and 0.43 g respectively. Among the biocontrol treatments T₆ (seed treatment with *Pseudomonas fluorescence*-3 + soil application with *Trichoderma harzianum* -7) recorded fresh and dry weight of 3.80 and 0.40 g while T₅ recorded 3.70 and 0.39 g respectively.

5. References

1. Chet I, Baker R. *Trichoderma hamatum*, its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. Microb Ecol. 1981; 7:29-38.

2. Dinakaran D, Ramakrishnan D. Studies on the control of tomato damping off with *Trichoderma viride*. Plant Disease Research. 1996; 11(2):148-149.
3. Elad Y, Chet I Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Canda Journal Microbiology. 1987; 28:719-725.
4. Jayarajan R, Ramakrishnan G. Efficacy of *Trichoderma* formulation against root rot disease of grain legumes. Petria giornale di patologia delle piante. 1994, 1:137.
1. Jensen CS, Percich JA, Graham PH. Integrated management strategies of bean root rot with *Bacillus subtilis* and *Rhizobium* in Minnesota. Field Crops Res. 2002; 74:107-115.
5. Karthikeyan A. Effect of organic amendments, antagonistic *Trichoderma viride* and fungicides on seed and collar rot of groundnut. Plant Disease Research. 1999; 11:72-74.
6. Krishnamoorthy AS, Bhaskaran R. Biological control of damping off disease of tomato caused by *Pythium indicum*. Journal of Biological control. 1990; 4(1):52-54.
7. Manorajitham SK, Prakasam V, Rajappan K. Effect of antagonists on *Pythium aphanidermatum* and the growth of chilli seedlings. Biological control. 1999; 13:101-106.
8. Mehata RD, Patel K, Roy KK, Mehta MH. Biological control of soil borne fungal pathogens with *Trichoderma harzianum*. Journal of Mycology and Plant pathology. 1995; 25(1):128.
9. Wolfhechel H, Jensen D. Use of *Trichoderma harzianum* and root rot of cucumber caused by *Pythium ultimum*. Journal of Plant Pathology. 1992; 136:221-230.
10. Yehia AH, El-Hassan SA, El-Bahadli AH. Biological seed treatment to control of *Fusarium* rot of broad bean. Egyptian journal of phytopathology. 1982; 14:59-66.