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Evaluation of Seed Bio-priming against Chilli (*Capsicum frutescence* L.) Diseases *in vivo*

Rinkal Chauhan and PR Patel

Abstract
An experiment was to know the efficacy of various seed bio-priming against chilli diseases *in vivo*. Chilli (*Capsicum frutescence* L.) damping-off, anthracnose and fusarium wilt can be common mainly caused by *Pythium* sp., *Colletotrichum capsici* and *Fusarium solani.* Seed bio-priming of chilli seeds *in vivo* revealed maximum per cent seed germination 59.4% in *P. fluorescens* applied at imbibition @10gm/kg and these treatment was also found significantly superior over the rest and also reduce per cent disease incidence damping off, anthracnose and fusarium wilt of chilli by 21.6%, 9.0% and 18.9%, respectively. Seed bio-priming with *P. fluorescens* applied at imbibition @10gm/kg was also found promoting plant growth activity significantly higher after 10, 20 and 30 days of seed sowing in seedling height, shoot length and root length.

Keywords: Seed, bio-priming, Chilli, *Capsicum frutescence*, disease, *in vivo*

Introduction
Chilli (*Capsicum frutescens* L.) is most widely cultivated vegetable crop in the world. It is a solanaceous fruit vegetable mainly cultivated for its vegetable green fruits and for dry chilli as the spice of commerce. It is a rich source of Vitamin C, A and B. In India, it is an important cash crop, which is grown for the both domestic and export market.

Chilli is affected by number of seed borne diseases *viz.*, damping off (*Pythium* sp.), anthracnose or fruit rot (*Colletotrichum capsici*), fusarium wilt (*Fusarium* sp.), phytophthora fruit rot (*Phytophthora sp*.) and stem rot (*Sclerotium rolfsii*) which cause Sudden Death Syndrome (SDS) is the most important and serious that eventually leads to quick and rapid death of plants.

Seed priming is a pre-sowing seed treatment which leads to a physiological state that enables seed to germinate more efficiently. The majority seed treatments are based on seed imbibition allowing the seed to go through the first reversible stage of germination but do not allow radical protrusion through seed coat. Seeds keeping their desiccation tolerance are then dehydrated and can be stored until final sowing. During subsequent germination, primed seeds exhibit a faster and more synchronized germination and young seedlings are often more vigorous and resistant to abiotic stresses than seedlings obtained from unprimed seeds.

Material and Methods
Chilli cv. GVC 111 seeds were subjected to the following bio-agents by two methods, biopriming applied at imbibition and after imbibition. (Jogani and John, 2014).

Seed bio-priming applied at imbibition
Seed bio-priming is treating seed with bio-agents and incubating under warm and moist condition until just prior to radical emergence. One gram of chilli seeds of variety GVC 111 were bio-primed with inoculum produced by using talcum powder as carrier. Ten gram of talc formulation of the bio-agents along with 0.1 g gum arabic used as adhesive and mixed in 25 ml of water. Pre-treated chilli seeds (surface sterilized with 0.1 % $HgCl_2$ for 2-3 min followed by three washing with sterile water) soaked in the slurry at room temperature for 24 hours and then sowing in open field condition.

Seed bio-priming after imbibition
Seeds of chilli were imbibed in aerated water (50 g seed per 500 ml water) and then dried at room temperature. Formulation of the bio-agent in talc along with 0.1 g gum arabic was dusted on seeds (0.01 g per gram of seed) after seeds were imbibed and dried. Then transferred on moist filter paper in Petri-plate for sowing in open field condition.

As per the methodology given, 1 g of chilli seeds were treated and sown in the nursery plot or open field conditions under each treatment along with control. The seeds were sown in lines 10 cm apart, and 50 seeds in each line of one meter. The beds were regularly watered daily with rose can.
The observation on different parameters was carried out as follows

1. Per cent germination of seeds
The observations on germinated and ungerminated seeds were counted from each of the treatments. Emergence of seedling from the seed was considered as successful germination. Three replications were maintained for each of the treatments. The germination was expressed as percentage of the ratio of number of normal seedlings to the sum of the normal, abnormal and ungerminated seeds, *i.e.,* total sown seeds. (Khare and Bhale, 2000) [4].
The germination percent was calculated by following formula:

$$\text{Germination Percentage (\%)} = \frac{\text{Total no. of germinated seeds}}{\text{Total no. of seeds sown}} \text{ X } 100$$

2. Per cent disease incidence
The per cent incidence of damping off, anthracnose and fusarium wilt by each of differentiated pathogens from field experiment was calculated. Infected seedlings representing typical disease symptoms were collected in fresh polyethylene bags and brought to laboratory for further studies. The percent infection was calculated by following formula.

$$\text{Per cent infection (\%)} = \frac{\text{No. of infected seeds}}{\text{Total Number of seeds}} \text{ X } 100$$

3. Seedling height (cm) at 10, 20 & 30 days after sowing.
4. Root & Shoot length (cm) at 10, 20 & 30 days after sowing.

Results and Discussion
The experiment was laid out in randomized block design with three replications. Methodology was adopted as per mention in materials and methods. *In vivo*, study was conducted to check out the efficacy of seeds bio-priming with five antagonistic bio-agents *viz., T. viride, T. harzianum, P. fluorescens, B. subtilis* and *P. lilacinus* against damping off, anthracnose and wilt diseases. The observation were also recorded on percent germination and seedling height, shoot length and root length at 10, 20 and 30 days after seed sowing.

1. Percentage germination of seeds
Per cent seed germination was significantly observed higher in the seed biopriming with *P. fluorescens* applied at imbibition (59.4%) followed by *T. harzianum* applied at imbibition (55.3%) and lowest seed germination was observed in control (45.6%). (Table 1) (Fig. 1)

Table 1: Effect of seed bio-priming on chilli seed germination *in vivo*

S. No.	Treatment	Per cent germination
T_1	*Trichoderma viride* applied at imbibition	54.8
T_2	*T. viride* applied after imbibition	50.9
T_3	*Trichoderma harzianum* applied at imbibition	55.3
T_4	*T. harzianum* applied after imbibition	51.1
T_5	*Pseudomonas fluorescens* applied at imbibition	59.4
T_6	*P. fluorescens* applied after imbibition	52.5
T_7	*Bacillus subtilis* applied at imbibition	51.5
T_8	*B. subtilis* applied after imbibition	50.7
T_9	*Paecilomyces lilacinus* applied at imbibition	49.8
T_{10}	*P. lilacinus* applied after imbibition	48.1
T_{11}	Hydro priming (Water only)	49.9
T_{12}	Control (Without any treatment)	45.6
S.Em ±		1.02
CD at 5%		2.99
CV %		3.42

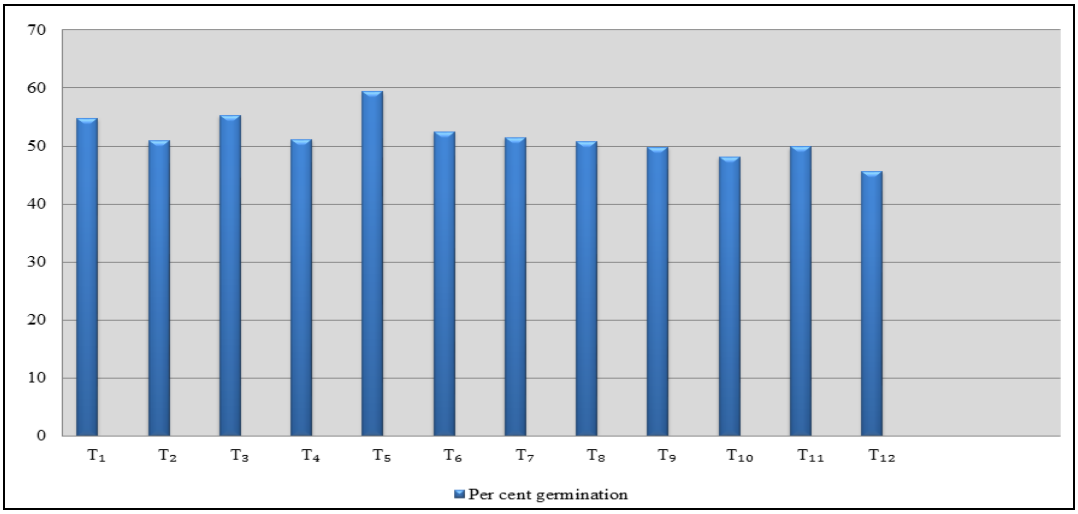

Fig 1: Effect of seed bio-priming on chilli seed germination *in vivo*

2. Per cent disease incidence

Per cent disease incidence of all the three diseases *viz.*, damping off, anthracnose and wilt were significantly observed minimum in the seed bio-priming with *P. fluorescens* applied at imbibition after 30 days of sowing.

Damping-off

Per cent disease incidence of damping-off was significantly reduced in all the treatments over control. It was significantly lower in seed biopriming with *P. fluorescens* applied at imbibition (21.6%) followed by *T. harzianum* applied at imbibition (22.4%) and maximum per cent disease incidence was observed in control (35.9%). (Table 2) (Fig. 2)

Table 2: Effect of seed bio-priming on chilli diseases after 30 days of seed sowing *in vivo*

S. No.	Treatment	Damping off PDI (%)	Anthracnose PDI (%)	Fusarium wilt PDI (%)
T_1	*Trichoderma viride* applied at imbibition	23.8* (16.4)**	14.3* (6.2)**	21.0* (12.9)**
T_2	*T. viride* applied after imbibition	28.9 (23.4)	20.3 (12.1)	28.2 (22.4)
T_3	*Trichoderma harzianum* applied at imbibition	22.4 (14.6)	13.7 (5.7)	19.6 (11.3)
T_4	*T. harzianum* applied after imbibition	27.9 (22.0)	20.1 (11.9)	27.1 (20.9)
T_5	*Pseudomonas fluorescens* applied at imbibition	21.6 (13.6)	9.0 (2.6)	18.9 (10.6)
T_6	*P. fluorescens* applied after imbibition	27.5 (21.4)	19.5 (11.2)	26.5 (19.9)
T_7	*Bacillus subtilis* applied at imbibition	23.5 (16.2)	15.4 (7.2)	24.4 (17.2)
T_8	*B. subtilis* applied after imbibition	31.7 (27.7)	20.7 (12.7)	28.4 (22.7)
T_9	*Paecilomyces lilacinus* applied at imbibition	26.0 (19.6)	18.0 (9.6)	25.2 (18.2)
T_{10}	*P. lilacinus* applied after imbibition	32.0 (28.2)	20.9 (12.9)	29.4 (24.2)
T_{11}	Hydro priming (Water only)	33.2 (30.1)	21.25 (13.4)	28.3 (22.7)
T_{12}	Control (Without any treatment)	35.9 (34.6)	25.3 (18.4)	32.7 (29.4)
S.Em ± CD at 5% CV %		1.15 3.39 7.13	1.08 3.20 10.3	0.92 2.71 6.16

*Figures outside paranthesis are arc sine transformed values

** Figure indicate original values

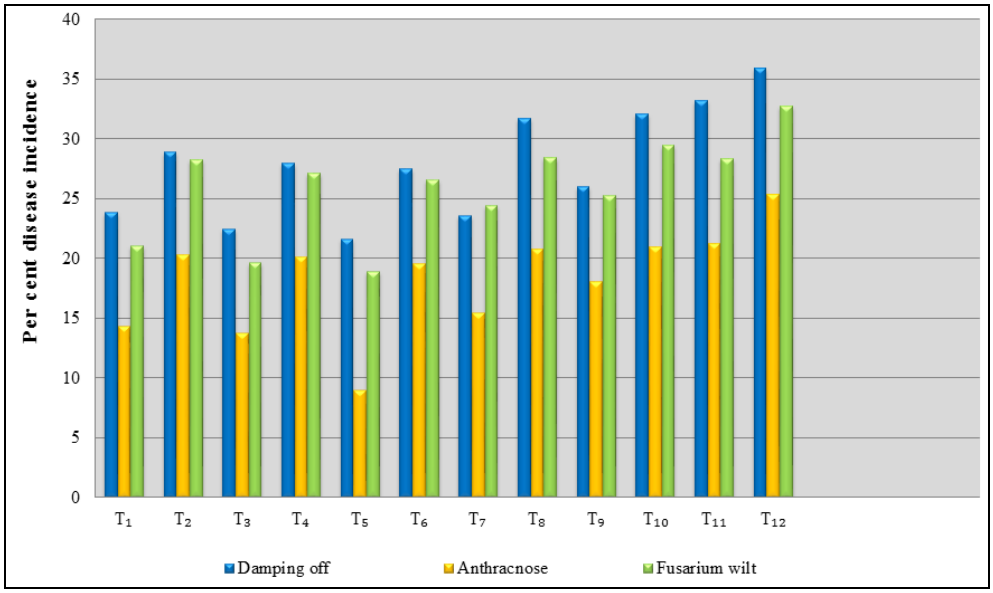

Fig 2: Effect of seed biopriming on chilli diseases after 30 days of seed sowing *in vivo*

Anthracnose

Per cent disease incidence of anthracnose was significantly reduced in all the treatments over control. It was significantly lower in seed biopriming with *P. fluorescens* applied at imbibition (9.0%) followed by *T. harzianum* applied at imbibition (13.7) and maximum per cent disease incidence was observed in control (23.3%). (Table 2) (Fig. 2)

Fusarium Wilt

Per cent disease incidence of fusarium wilt was significantly reduced in all the treatments over control. It was significantly lower in seed biopriming with *P. fluorescens* applied at imbibition (18.9%) followed by *T. harzianum* applied at imbibition (19.6) and maximum per cent disease incidence was observed in control (32.7%). (Table 2) (Fig. 2)

The control of damping-off, anthracnose and fusarium wilt diseases by bio-primed seeds of chilli was related to reduction of incidence of seed colonization by the pathogens due to reduced exudation of nutrients from the primed seeds upon imbibition of water. Moreover, the direct antagonistic ability of *P. fluorescens* and *T. harzianum* against soil borne pathogens. Trichoderma reduce growth infection caused by pathogen by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interaction and enzyme secretion. Combination between seed priming and bio control agents has improved the rate of germination and uniformly of emergence of chilli crop and reduced damping-off, anthracnose and fusarium wilt disease incidence.

It is very clear from this study that seed bio-priming with *P. fluorescens* applied at imbibition significantly reduced damping off, anthracnose and wilt diseases.

3. Seedling Height

The data of effect of seed bio-priming on seedlings height are depicted in table 3 and fig. 3. The seedling height was significantly longer in all the treatments. Among the treatments, significantly longer seedling height was recorded after 10, 20 and 30 days of seeds sowing after biopriming with *P. fluorescens* applied at imbibition was recorded 10.1cm, 13.4cm and 21.2cm seedling height, respectively and followed by *T. harzianum* applied at imbibition recorded 9.3cm, 12.8cm and 20.3cm, respectively. Lowest seedling height was observed in control 6.8cm, 10.7cm and 12.3cm, after 10, 20 and 30 days of sowing, respectively.

Table 3: Effect of seed bio-priming on chilli seedling height after 10, 20 and 30 days of sowing of seeds *in vivo*

S. No.	Treatment	Seedling height after sowing (cm)			
		10 days	20 days	30 days	Mean
T_1	*Trichoderma viride* applied at imbibition	9.1	12.2	19.7	13.6
T_2	*T. viride* applied after imbibition	7.9	11.9	15.8	11.8
T_3	*Trichoderma harzianum* applied at imbibition	9.3	12.8	20.3	14.1
T_4	*T. harzianum* applied after imbibition	8.9	11.6	16.1	12.2
T_5	*Pseudomonas fluorescens* applied at imbibition	10.1	13.4	21.2	14.9
T_6	*P. fluorescens* applied after imbibition	8.5	11.8	16.4	12.2
T_7	*Bacillus subtilis* applied at imbibition	8.6	11.6	18.0	12.7
T_8	*B. subtilis* applied after imbibition	7.9	11.2	14.4	11.1
T_9	*Paecilomyces lilacinus* applied at imbibition	7.9	11.4	17.3	12.2
T_{10}	*P. lilacinus* applied after imbibition	7.7	11.0	13.7	10.8
T_{11}	Hydro priming (Water only)	7.4	10.9	13.0	10.4
T_{12}	Control (Without any treatment)	6.8	10.7	12.3	9.9
S.Em ±		0.35	0.39	0.97	
CD at 5%		1.03	1.15	2.83	
CV %		7.30	5.81	10.14	

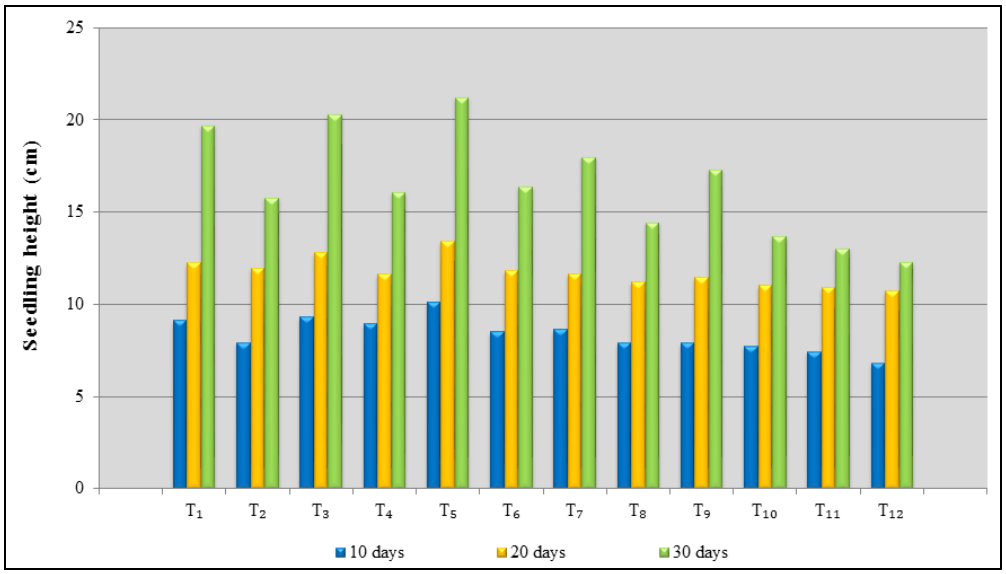

Fig 3: Effect of seed bio-priming on chilli seedling height after 10, 20 and 30 days of sowing of seeds *in vivo*

4. Shoot length

The shoot length was significantly longer in all the treatments as compared to the control. Among these, significantly longer shoot was recorded after 10, 20 and 30 days of seeds sowing treated with biopriming with *P. fluorescens* applied at imbibition was found 8.5cm, 9.0cm and 13.2cm, respectively and followed by *T. harzianum* applied at imbibition recorded with 8.2cm, 8.6cm and 12.9cm shoot length, respectively. Minimum shoot length was observed in control 6.2cm, 6.2cm and 7.9cm, after 10, 20 and 30 days of sowing, respectively. (Table 4) (Fig. 4)

Table 4: Effect of seed bio-priming on chilli shoot length after 10, 20 and 30 days of sowing of seeds *in vivo*

S. No.	Treatment	Shoot length after sowing (cm)			
		10 days	20 days	30 days	Mean
T_1	*Trichoderma viride* applied at imbibition	7.9	8.3	12.2	9.4
T_2	*T. viride* applied after imbibition	7.3	7.2	9.5	8.0
T_3	*Trichoderma harzianum* applied at imbibition	8.2	8.6	12.9	9.9
T_4	*T. harzianum* applied after imbibition	7.4	7.3	9.9	8.2
T_5	*Pseudomonas fluorescens* applied at imbibition	8.5	9.0	13.2	10.2
T_6	*P. fluorescens* applied after imbibition	7.7	7.6	10.2	8.5
T_7	*Bacillus subtilis* applied at imbibition	7.7	7.8	11.5	9.0
T_8	*B. subtilis* applied after imbibition	7.5	7.3	9.3	8.0
T_9	*Paecilomyces lilacinus* applied at imbibition	7.4	7.9	10.6	8.6
T_{10}	*P. lilacinus* applied after imbibition	7.2	7.1	9.0	7.7
T_{11}	Hydro priming (Water only)	6.8	6.7	8.7	7.4
T_{12}	Control (Without any treatment)	6.2	6.2	7.9	6.7
S.Em ±		0.24	0.25	0.40	
CD at 5%		0.70	0.74	1.17	
CV %		5.56	5.73	6.64	

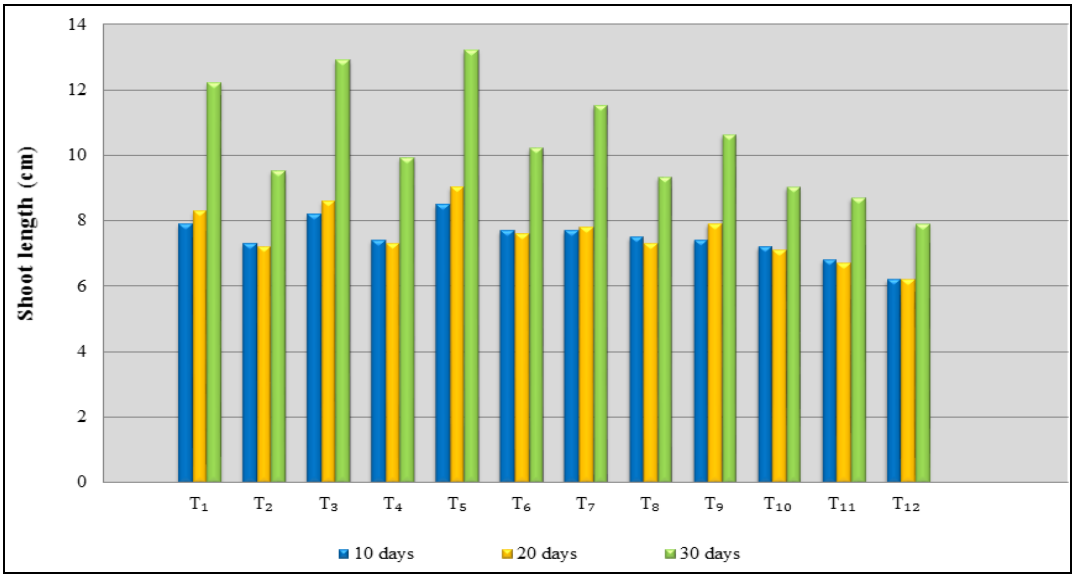

Fig 4: Effect of seed bio-priming on chilli shoot length after 10, 20 and 30 days of sowing of seeds *in vivo*

5. Root Length

The root length was significantly longer in all the treatments showed in table 5 and fig. 5. Among these, significantly longer root was recorded after 10, 20 and 30 days of seeds sowing treated with biopriming with *P. fluorescens* applied at imbibition was recorded 4.6cm, 5.8cm and 8.2cm root length, respectively and followed by *T. harzianum* applied at imbibition was recorded 4.3cm, 5.0cm and 7.6cm, respectively. Minimum root length was observed in control 2.7cm, 3.4cm and 5.2cm, after 10, 20 and 30 days of sowing, respectively.

Table 5: Effect of seed bio-priming on chilli root length after 10, 20 and 30 days of sowing of seeds *in vivo*

S. No.	Treatment	Root length after sowing (cm)			
		10 days	20 days	30 days	Mean
T_1	*Trichoderma viride* applied at imbibition	3.9	4.8	7.3	5.3
T_2	*T. viride* applied after imbibition	3.4	4.4	6.1	4.6
T_3	*Trichoderma harzianum* applied at imbibition	4.3	5.0	7.6	5.6
T_4	*T. harzianum* applied after imbibition	3.5	4.5	6.3	4.7
T_5	*Pseudomonas fluorescens* applied at imbibition	4.6	5.8	8.2	6.2
T_6	*P. fluorescens* applied after imbibition	3.6	4.5	6.5	4.8
T_7	*Bacillus subtilis* applied at imbibition	3.7	4.6	6.7	5.0
T_8	*B. subtilis* applied after imbibition	3.3	4.4	5.9	4.5
T_9	*Paecilomyces lilacinus* applied at imbibition	3.4	4.2	6.5	4.7
T_{10}	*P. lilacinus* applied after imbibition	3.3	4.2	5.8	4.4
T_{11}	Hydro priming (Water only)	3.0	4.1	5.8	4.3
T_{12}	Control (Without any treatment)	2.7	3.4	5.2	3.7
S.Em ±		0.25	0.32	0.46	
CD at 5%		0.74	0.94	1.35	
CV %		12.28	12.41	12.24	

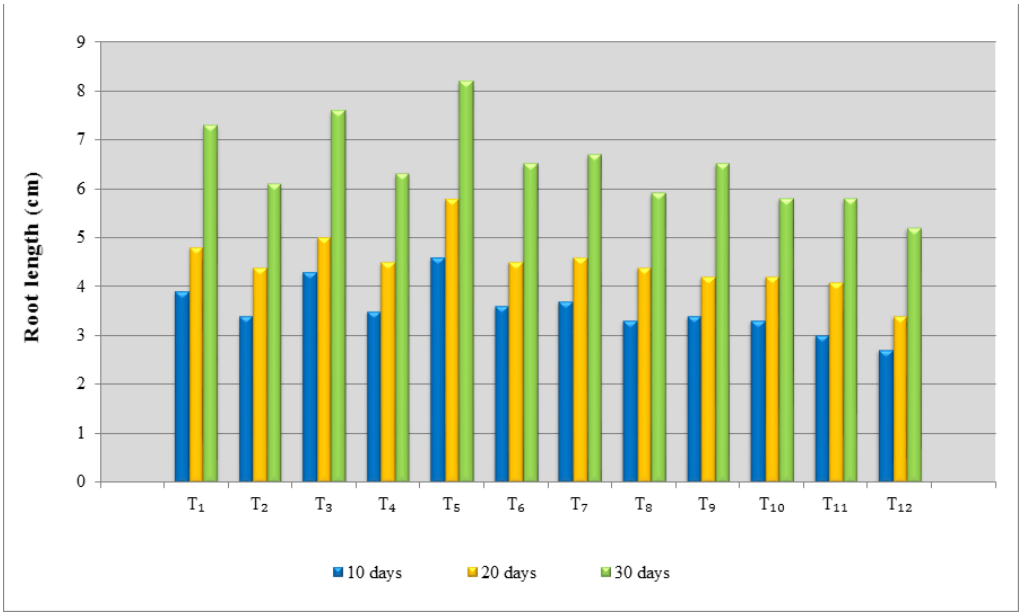

Fig 5: Effect of seed bio-priming on chilli root length after 10, 20 and 30 days of sowing of seeds *in vivo*

Bio-priming in which specific biological control agents are incorporated into the seed priming process, can be very effective in suppressing many disease caused by seed and soil borne pathogens. Moreover, bio-priming has great promise for enhancing the efficacy, shelf life and consistent performance of biological control agents. Biopriming of chilli seeds with *T. viride* and *T. harzianum* and bacterial antagonist *P. fluorescens* improved the seed germination. *T. harzianum* was found to be significantly effective for the enhancement of growth parameters such as root length, shoot length, root weight and shoot weight and for the suppression of root infecting fungi. Seed bio-priming can improve the physiological responses of plants and tolerance of seeds under the stress conditions.

From this experiment, it is clear that seed bio-priming with *P. fluorescens* applied at imbibition enhance the seedling height, shoot length and root length, these may be due to *P. fluorescens* induced the activities of polyphenol oxidase (PPO) is a copper containing enzyme that oxidizes phenolics to highly toxic quinines and is involved in the terminal oxidation of diseased plant tissue. It has been recognized for its role in disease resistance. The enhancement in chilli seedling growth could be attributed to suppression of deleterious micro-organisms and pathogens, the production of endogenous plant growth regulators such as gibberellins, cytokinins, and/or indole-acetic acid, an increased availability of minerals and other ions, and/or enhanced water uptake.

Our results are in harmony with earlier worker Verma and Dohroo (2005) [6], El-Mohamedy *et al.* (2006) [1] reported bio-primed of cowpea seeds caused a highly significant reduction in damping off and root rot and Khurrana Zaif *et al.* (2007) [5] reported hydro priming were positively affected on seed emerging rate, El-Mohamedy *et al.* (2008) [2].

In the present study, *P. fluorescens* was found potential bio agents for to bio-primed of chilli seeds and were found highly effective in inhibiting the seed and soil borne pathogen and increased the plant growth. Seed bio-priming with *P. fluorescens* @10 gm/kg seeds applied at imbibition found potential bio-agents to increased seed germination and reduced per cent disease incidence of damping-off, anthracnose and fusarium wilt and also found to increased seedling height, shoot length and root length.

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