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Bioefficacy of various strains of *Trichoderma and Pseudomonas* spp. against damping-off of cauliflower

AJAY KUMAR CHOUDHARY[1], NARINDER SINGH[2], DALJEET SINGH[3] and SHIVETA RAINA[4]

Punjab Agricultural University, Ludhiana, Punjab 141 004

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ABSTRACT

Trichoderma and *Pseudomonas* strains were tested against damping-off of cauliflower (*Pythium aphanidermatum and Rhizoctonia solani*). Dual culture antagonism assays were carried out on potato dextrose agar and King's B medium to determine the effect of *Trichoderma* and *Pseudomonas* strains respectively, on mycelial growth of the pathogens. Antagonistic effect on *P. aphanidermatum* and *R. solani* was 53.1 and 44.6% respectively by *T. harzianum* (Th5) where as *P. fluorescens* (Pf3) showed maximum inhibition against *P. aphanidermatum* and *R. solani* of 60 and 70% respectively. Finally, the selected potent strains of *Trichoderma* spp (Th5 and Tv1) and *P. fluorescens* Pf3, were evaluated on cauliflower seedling development and as a potential antagonist for controlling cauliflower damping-off caused by *P. aphanidermatum* and *R.solani*, under field conditions. *T. harzianum* (Th5) resulted significantly reduction in damping off disease, promoted the plant growth and enhanced the yield.

Key words: Cauliflower, Damping-off*, P. aphanidermatum, P. fluorescens, Trichoderma* spp.

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is one of the major vegetable belonging to family Brassicaceae (Faruk 2016). Cauliflower and other *Brassica* plants such as broccoli and cabbage are cold season crops and they grow poorly in hot weather (Krish *et al.* 2007). Cauliflower is attacked by several diseases, mostly caused by fungi and bacteria leading to severe crop losses. Damping-off caused by *Pythium aphanidermatum* and *Rhizoctonia* spp. in vegetable crops is economically very important due to its wide host range and worldwide occurrence (Sharma and Sain 2005).

Pythium spp. are ubiquitous soil borne pathogens which cause damping–off and root rot diseases in many plant species such as cauliflower, cabbage, broccoli, carrot, cucumber, melon and turf grass (Abdelzaher 2004). *Pythium* spp. could be controlled by steaming the soil which is feasible on a small scale. Although, fungicides could supply a good suppression of *Pythium* diseases, they have hazardous effects on the environment. Currently, increasing attention has been paid to the biological control through the use of antagonistic microorganisms such as fungi and bacteria as an alternative to fungicides (Gravel *et al.* 2005; Rosenzweig *et al.* 2001) The damping off incited by *R. solani* Kuhn is a major constraint in the production of cauliflower seedlings. *R. solani* is essentially soil-borne pathogen which caused heavy losses under favorable conditions (Seema and Devaki 2010). The management of this disease is difficult owing to long saprophytic survival ability of the pathogen in soil (Dey 2005). Reduction or elimination of soil borne inoculum is the only effective solution to overcome the problem and this may be achieved through use of effective fungal or bacterial antagonists.

Biological and cultural control measures are two alternatives feasible options to the synthetic pesticides in an integrated diseases management program (Harman *et al.* 2004). Control of the plant diseases by chemicals can be spectacular but the increasing use of potentially hazardous pesticides and fungicides in agriculture has been the growing concern. Efficacy and environmental concerns, as well as pathogen resistance to some pesticides, have encouraged research into biological control (Stephens *et al.* 1993).

Soil has enormous untapped potential antagonistic microbes, which are helpful in reducing pathogen inoculums through different mode of actions such as competition for nutrients and space, antibiosis, mycoparasitism, production of siderophores and lytic enzymes (Dixit *et al*. 2015) Seed treatment with biocontrol agents is one of the most suitable methods for biocontrol of soil borne pathogens in the spermosphere and rhizosphere (Harman 1991). Many biological control agents such as *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas* spp. suppress diseases caused by *Pythium* spp and *R. solani* (Moller *et al*.2003, Carisse *et al*.2003). *Trichoderma* spp. are well documented as effective biological control agents of plant diseases caused

[1]Ph D Student (e mail: choudhary.ajay11@gmail.com),[2]Senior Plant Pathologist (e mail:narindermutti@pau.edu), [3]Senior Plant Pathologist (e mail: pau_daljeet2@pau.edu), [4]Research Fellow (e mail: rainashiveta@ymail.com), Department of Plant Pathology.

by soil borne fungi (Abdel- Kader 1997, Okot *et al*. 2011)

The present study was carried out to evaluate bioefficacy of *T. harzianum, T. viride* and *P. fluorescens* against damping-off disease of cauliflower.

MATERIALS AND METHODS

Trichoderma harzianum, Trichoderma viride and *Pseudomonas fluorescens* were isolated from cauliflower rhizospheric soils of Punjab. Sample were brought to laboratory and stored at 4°C until used. Five–fold serial dilution of each soil samples were prepared in sterilized distilled water and 0.5 ml diluted sample was poured on the surface of (TSM) (Elad *et al.* 1982), Plates were incubated at 28 ± 2°C for 96 h. Morphologically different colonies appearing on the plates were purified on the Potato Dextrose Agar (PDA). The purified isolates were preserved at 4°C and were used during the course of study.

Isolation of *Pseudomonas fluorescens* was carried out on King's B medium (King et al. 1954). One gram of rhizospheric soil sample was suspended in 9 ml of sterile distilled water. Samples were serially diluted and 0.1 ml of sample was spread on King's B medium plates. The colonies exhibiting fluorescence were picked up and streaked on to the slants for maintenance, purified on King's B medium plates, identified and also designated as Pf1, Pf2, Pf3 and Pf4 which stands for *P. fluorescens* isolates. These were used for further studies.

Damping off pathogens of cauliflower plant, i.e *Pythium aphanidermatum* and *Rhizoctonia solani* used for present investigation were obtained from diseased plants of cauliflower. The culture of *P. aphanidermatum* was isolated and maintained on corn meal agar at 25±1°C with regular subculturing at an interval of 15 days. *Rhizoctonia solani* was isolated from the infected plants and maintained on Potato Dextrose Agar (PDA) at 25±1°C with regular sub culturing at an interval of 30 days. Their virulence was tested by growing cauliflower in the glass house in soil mixed with the culture of their isolates (1.5% w/w basis). Most virulent isolates were selected for further investigation.

In vitro antagonistic efficacy of *T. harzinium* and *T. viride* against *P. aphanidermatum* and *R. solani* was studied by dual cultural technique of Kucuk and Kivanc (2003). Petridishes (90 mm) containing 20 ml of sterile PDA were inoculated with a 5mm diameter plug of 7 day old pure culture of antagonistic fungi and pathogens. Mycelial disc of each fungus was placed on opposite poles of PDA plates and incubated at 25°C

To evaluate the antagonistic activity of *Pseudomonas fluorescens* isolates against *P. aphanidermatum* and *R. solani*, 5 mm diameter mycelium disc of test pathogens was placed on side of plate and bacterial isolates were streaked at 3 cm distance from the disc on King, B agar medium (Maurya *et al*. 2014). The zone of inhibition was observed after 7 days of incubation at 25 °C.

$$\% \text{ Inhibition} = (R - r)/R \times 100$$

Where r is the radius of the fungal colony in presence of the bacterial colony and R is the maximum radius of the fungal colony in absence of the bacterial colony.

Based on *in vitro* studies *Trichoderma harzianum* (Th5), *Trichoderma viride* (Tv1) and *Pseudomonas fluorescens* (Pf3) were selected for further field studies. The field experiments were conducted at Plant Pathology Farm, PAU, Ludhiana; to study the effectiveness of the talc based formulations of *T. harzianum* (Th5), *T. viride* (Tv1) and *P. fluorescens* (Pf3) against the pathogens *P. aphanidermatum* and *R. solani* causing damping-off of cauliflower. The experiments were conducted in randomized block design (RBD) with three treatments and three replications. The antagonistic were applied on cauliflower variety Pusa chetki as seed dressing (15g/kg of seed), soil (1 kg of bioformulation in 25 kg of FYM/acre primed for 24 hr) and seed + soil application and were sown in lines. For comparsion, seed treatment with Captan (Heterocyclic nitrogen compound) (0.2%) was taken as standard chemical control against damping off of cauliflower.The % seed germination, disease incidence, inhibition of disease development and parameters of plant growth promotion were recorded.

All the data were in triplicates and were represented as mean±standard deviation. The variations in the results obtained with respect to seed germination, damping off incidence and plant growth parameters were statistically analyzed using one way Analysis of Variance (ANOVA) by using the data analysis SPSS software version 22. Treatment means separated by Duncan's multiple range test (DMRT) were determined by the magnitude of F value (P=0.05)

RESULTS AND DISCUSSION

Isolation and identification of Trichoderma spp.

Antagonists were identified using both morphological and growth parameters of the isolates. Six isolates corresponded to the following species: *T. harzianum* (five isolates) and *T. viride* (one isolate). *Trichoderma harzianum* Th2, Th3, Th4 and Th5 were rapid in growth whereas Th1 and Tv1 were slow and medium in growth respectively. The colony color varied from light green to green in Th1, Th2, Th3, Th4 and Th5. The light yellow to yellow pigment was present in one isolates of Tv1.The growth of mycelium was aerial in Th2 and Th5 isolates but in case of Th1, Th3 and Th4 it was non-aerial.

Isolation and identification of Pseudomonas fluorescens

Four *P. fluorescens* isolateswere evaluated for their cultural, morphological and biochemical characteristics as described in Bergey's manual of determinative bacteriology (Holt *et al.* 1994). All *P. fluorescens* strains were gram negative. Isolates Pf1 and Pf4 had bluish green colour colony, Pf2 had greenish yellow while Pf3 had fluorescent green coloured colony. All strains were methyl red negative, Voges-Proskauer positive and were able to reduce nitrate. Similar results are also reported by Meera and Balabaskar (2012) they isolated thirty five (Pf1…….Pf35) isolates of *Pseudomonas fluorescens* from the rhizosphere of rice fields

and among these, seven (Pf4, Pf6, Pf8, Pf11, Pf13, Pf15 and Pf20) isolates showed bright fluorescens under UV light.

In vitro testing of T. harzianum, T. viride and P. fluorescens isolates against damping off pathogens

Five isolates of *T. harzianum* were found promising against *P. aphanidermatum*, as they reduced the pathogen growth by 36.1 to 53.1% within seven days of post inoculation (Table 1). Th5 showed maximum inhibition of 53.1% followed Th1 (48.5%). *In vitro* analysis also revealed that the *T. harzianum* Th5 and Th3 significantly inhibited the growth of *R. solani.* The results presented in Table 1 show that Th5 inhibited the growth of *R. solani* by 44.6%. Singh *et al*. (2012) also conducted *in vitro* experiments to evaluate the effects of four isolates of *Trichoderma* species viz. *T. harzianum* (Th), *T. viride*, *T. asperellum* and *T. longibrachiatum* against *P. aphanidermatum*. Out of four isolates *T. harzianum* (Th) recorded maximum growth inhibition (60.3%) against *P. aphanidermatum* and produced more amounts of volatile and non-volatile metabolites. The inhibition of pathogen may be also attributed to the production of secondary metabolites by antagonists such as glioviridin, viridin and gliotoxin. (Shabir and Rubina 2010, Kamlesh and Gurjar 2002; Muhammad and Amusa 2003).

The results by the dual culture technique also indicated that the four isolates of *P. fluorescens* inhibited growth of tested fungi significantly. In case of *P. aphanidermatum*, a maximum inhibition of 60.0% was recorded by *P. fluorescens* Pf3 and minimum of 28.3% was recorded with isolate Pf2. In case of *Rhizoctonia solani*, the maximum inhibition of 70% was exhibited by Pf3 and minimum, 37.1% was recorded with isolate Pf1 (Table 2). Similar results were also reported by many other investigators (Andersen *et al*. 2003; Carisse *et al.* 2003; Leclère *et al.*2005). They reported the inhibitory effects of antagonistic fungi and bacteria such as *Trichoderma* spp. and *P. fluorescen*s on growth reduction of *P. ultimum* and *Rhizoctonia solani* under *in vitro* conditions. The inhibition in the growth of the pathogen could be attributed to antibiosis, hyperparasitism (We *et al.* 1986) or production of chitinase and *β*-1, 3 glucanase enzymes

Table 1 *In vitro* screening of *Trichoderma harzianum*, *Trichoderma viride* against *P. aphanidermatum* and *Rhizoctonia solani* causing damping off of cauliflower

Isolates	Radial growth of *P. aphanidermatum* (cm) (Mean ± SD) [1]	Growth inhibition of *P. aphanidermatum* (%)	Radial growth of *R. solani* (cm) (Mean ± SD) [1]	Growth inhibition of *R. solani* (%)
Th1	2.42±0.07^{cd}	48.5	4.65±0.30^{b}	34.5
Th2	2.79±0.26b	40.6	4.53±0.26b^{c}	36.1
Th3	3.00±0.20^{b}	36.1	4.00±0.20^{d}	43.6
Th4	2.58±0.10^{c}	45.1	4.20±0.20c^{d}	40.8
Th5	2.20±0.05^{d}	53.1	3.93±0.15^{d}	44.6
Tv1	3.02±0.50^{b}	35.9	4.60±0.25b^{c}	35.2
Control	4.70±0.15^{a}		7.10±0.10^{a}	

[1]Mean ± SD (Standard deviation); values labeled with different letters are significantly different from the control level by Duncan test at 95% confidence.Th1, Th2, Th3, Th4, Th5= *Trichoderma harzianum* isolates, Tv1= *Trichoderma viride*

Table 2 *In vitro* screening of different isolates of *Pseudomonas fluorescens* against *P. aphanidermatum and Rhizoctonia solani* damping off of cauliflower

Isolates	*P. aphanidermatum*			*R. solani*		
	Radial Growth (Mean ± SD)[1]	Zone of Inhibition (Mean ± SD)[1]	Inhibition %	Radial growth (Mean ± SD)[1]	Zone of inhibition (Mean ± SD)[1]	Inhibition (%)
PF1	3.1±0.32^{b}	0.37±0.21^{c}	48.3	4.0±0.3^{a}	1.0±0.44^{b}	42.8
PF2	4.3±0.35^{a}	0.40±0.20^{c}	28.3	4.4±1.4^{a}	0.9±0.26^{b}	37.1
PF3	2.4±0.31^{c}	1.80±0.26^{a}	60.0	2.1±0.15^{c}	1.9±0.10^{a}	70.0
PF4	3.4±0.45^{b}	0.97±0.25^{b}	43.3	3.2±0.2^{b}	1.1±0.15^{b}	54.2
Control	6.00			7.00		

[A]Mean ± SD (Standard deviation); values labeled with different letters are significantly different from the control level by Duncan test at 95% confidence. PF1, PF2, PF3, PF4 = *Pseudomonas fluorescens* isolates.

which degrade the cell wall leading to lyses of mycelium of the pathogen (Ahmed and Baker 1987).

Effect of different isolates of selected antagonists in cauliflower

Seed germination, inhibition of disease were maximum and disease incidence was less in seed, soil and seed + soil combination application talc based formulations of different bioagents, i.e *T. harzianum* (Th5*), T. viride* (Tv1) and *P. fluorescens* (Pf3) as compared to the control. Data presented in the Table 3 reveals that the maximum percentage of seed germination 90.3% was recorded in case of seed treatment *T. harzianum* (Th5), whereas minimum percentage was recorded in *P. fluorescens* (Pf3) (77.9%) as compared to control (44.2%). Minimum percentage of disease incidence was recorded in case of seed treatment with *T. harzianum* (Th5 6.9%) and the maximum percentage was recorded in *P. fluorescens* (Pf3) (21.4%) as compared to the control (53.7%). Enhancement of seed germination due to *Trichoderma* species was also reported by Mukhtar (2008). It was noticed by Tjamos *et al.* (1992) that *T. harzianum* controls *R. solani* by competing for both rhizosphere colonization and nutrients. Disease incidence of cauliflower, water melon and cotton was reported to be reduced considerably by the application of *T. harzianum* (Sivan and Chet 1986)

Maxmimum percentage inhibition of disease was recorded in case of seed treatment with *T. harzianum* (Th5) 82.5. whereas minimum percentage was recorded in *P. fluorescens* (Pf3) (60.3%) Similarly maximum percentage of seed germination in case of soil treatment with *T. harzianum* (Th5) was 93.4 and the minimum percentage was recorded in *P. fluorescens* (Pf3) (78.5%) as compared to the control (46.1%). Minimum percentage of disease incidence recorded in case of soil treatment was with *T. harzianum* (Th5) 6.9% and the maximum percentage was recorded in *P. fluorescens* (Pf3) (21.4%) as compared to the control (53.7%).

Maximum percentage inhibition of the disease was recorded in case of soil treatment with *T. harzianum* (Th5) (87.2%) and the minimum percentage was recorded in *P. fluorescens* (Pf3) (60.2%). In case of seed+soil treatment with *T. harzianum* (Th5) the maximum percentage of seed germination (93.8%) and the minimum was recorded in *P. fluorescens* (Pf3) (73.1%) as compared to the germination in control (43.8%). Minimum percentage of disease incidence

Table 3 Effect of different isolates of selected antagonists applied as seed, soil and seed + soil treatments on germination incidence and inhibition of damping off in cauliflower

Treatment	Seed treatment			Soil treatment			Seed +soil treatment		
	Seed germination (%)[1]	Disease incidence (%) [1]	Inhibition of disease (%)[1]	Seed germination (%)[1]	Disease incidence (%)[1]	Inhibition of disease (%)[1]	Seed germination (%)[1]	Disease incidence (%)[1]	Inhibition of disease (%)[1]
Trichoderma harzianum (Th5)	90.3 ± 0.17b	6.9 ± 083de	82.5 ± 2.02b	93.4 ± 0.93a	6.9 ± 0.83d	87.2 ± 1.28 a	93.8 ± 0.87 a	6.1 ± 0.32 e	89.0 ± 0.37 a
Trichoderma viride(Tv1)	82.3 ± 0.66f	17.9 ± 2.12bc	68.3 ± 2.51e	82.0 ± 2.21bc	17.9 ± 2.12 bc	66.6 ± 2.46 d	77.6 ± 0.30 d	22.3 ± 0.62 c	60.2 ± 0.36 b
Pseudomonas fluorescens (Pf3)	77.9 ± 0.35g	21.4 ± 2.42 b	60.3 ± 5.53f	78.5 ± 246c	21.4 ± 2.42 b	60.2c ± 2.74	73.1 ± 0.10 e	26.9 ± 1.83 b	52.0 ± 2.34 d
Trichoderma harzianum + *Trichoderma viride* (Th5+Tv1)	85.8 ± 0.22c	9.0 ± 3.06d	74.6 ± 1.72c	90.7 ± 3.27a	9.0 ± 3.06 d	83.2 ± 5.15 ab	86.7 ± 0.60 b	13.3 ± 0.35 d	76.3 ± 0.19 b
Trichoderma harzianum + *Pseudomonas fluorescens* (Th5+Pf3)	85.1 ± 0.24d	9.7 ± 1.11d	73.4 ± 1.65cd	90.2 ± 0.90a	9.7 ± 1.11 d	81.8 ± 1.87 b	84.6 ± 1.06 c	15.3 ± 0.50 d	72.7 ± 1.40 c
Trichoderma viride + *Pseudomonas fluorescens* (Tv1+Pf3)	83.0 ± 0.22e	15.1 ± 2.72c	69.5 ± 3.17de	84.2 ± 2.69b	15.7 ± 2.72 c	70.7 ± 3.74 c	78.7 ± 1.09 d	21.2 ± 2.36 c	62.1 ± 3.47 e
Captan (Heterocyclic nitrogen compound)	94.5 ± 0.29a	4.1 ± 0.76e	90.1 ± 2.20a						
Control (No treatment)	44.2 ± 0.64h	53.7 ± 2.37a		46.1 ± 2.36d	53.7 ± 2.37 a		43.8 ± 1.19 g	56.7 ± 1.07 a	

[1]Mean ± SD (Standard deviation); values labeled with different letters are significantly different from the control level by Duncan test at 95% confidence

Table 4 Effect of different isolates of selected antagonists applied as seed, soil and seed + soil treatments on shoot and root length of cauliflower

Treatment	Shoot length/plant (cm) [1]			Root length/plant (cm) [1]			Total length/plant (cm) [1]		
	Seed treatment	Soil treatment	Seed + soil treatment	Seed treatment	Soil treatment	Seed + soil treatment	Seed treatment	Soil treatment	Seed + soil treatment
Trichoderma harzianum (Th5)	10.67 ± 0.15^{a}	10.93 ± 0.37^{a}	11.50 ± 0.20^{a}	3.77 ± 0.15^{a}	4.00 ± 0.26^{a}	4.47 ± 0.15^{a}	14.44 ± 0.15^{a}	14.43 ± 0.32^{a}	15.97 ± 0.20^{a}
Trichoderma viride (Tv1)	9.33 ± 0.15^{cd}	9.57 ± 0.20^{c}	9.87 ± 0.15^{c}	2.43 ± 0.15^{c}	2.30 ± 0.20^{c}	2.13 ± 0.35^{de}	11.86 ± 0.25^{e}	11.87 ± 0.11^{d}	12.00 ± 0.45^{e}
Pseudomonas fluorescens (Pf3)	9.90 ± 0.52^{abc}	9.57 ± 0.25^{c}	9.33 ± 0.20^{c}	2.50 ± 0.20^{c}	2.47 ± 0.20^{c}	2.53 ± 0.15^{c}	12.00 ± 0.20^{de}	12.04 ± 0.30^{d}	11.86 ± 0.11^{e}
Trichoderma harzianum + *Trichoderma viride* (Th5+Tv1)	10.53 ± 0.37^{ab}	10.87 ± 0.37^{a}	11.30 ± 0.20^{b}	3.03 ± 0.20^{b}	3.30 ± 0.17^{b}	3.67 ± 0.15^{b}	13.70 ± 0.36^{b}	14.17 ± 0.17^{b}	14.97 ± 0.32^{b}
Trichoderma harzianum + *Pseudomonas fluorescens* (Th5+Pf3)	10.16 ± 0.25^{ab}	10.33 ± 0.37^{b}	10.27 ± 0.25^{b}	3.67 ± 0.15^{a}	3.53 ± 0.05^{b}	3.83 ± 0.15^{b}	13.74 ± 0.23^{b}	13.86 ± 0.15^{b}	14.10 ± 0.26^{c}
Trichoderma viride + *Pseudomonas fluorescens* (Tv1+Pf3)	9.80 ± 0.60^{bc}	10.43 ± 0.20^{b}	10.30 ± 0.20^{c}	2.47 ± 0.15^{c}	2.47 ± 0.15^{c}	2.37 ± 0.15^{cd}	12.70 ± 0.20^{c}	12.90 ± 0.10^{c}	12.67 ± 0.20^{d}
Captan (Heterocyclic nitrogen compound)	8.76 ± 0.60^{d}			3.20 ± 0.10^{b}			12.33 ± 0.15^{cd}		
Control (No treatment)	7.97 ± 0.28^{e}	7.77 ± 0.15^{d}	8.07 ± 0.15^{d}	1.97 ± 0.15^{d}	1.83 ± 0.15^{d}	1.83 ± 0.23^{e}	10.00 ± 0.10^{f}	9.60 ± 0.28^{e}	9.90 ± 0.30^{f}

[1]Mean ± SD (Standard deviation); values labeled with different letters are significantly different from the control level by Duncan test at (95%) confidence

(6.1%) was recorded in case of seed + soil treatment with *T. harzianum* (Th5) and the maximum percentage was recorded in *P. fluorescens* (Pf3) (22.3%) as compared to the control (56.7%). Maxmimum percentage inhibition of disease (89%) was recorded in case of seed+soil treatment with *T. harzianum* (Th5) and the minimum percentage was recorded in *P. fluorescens* (Pf3) (52%).

Effect of different isolates of selected antagonists on growth parameters and yield of cauliflower

The effects of *T. harzianum, T. viride* and *P. fluorescens* on different growth characters of cauliflower seedlings showed significant variation. Data given in Table 4 shows that the maximum shoot length in seed treatment was recorded in *T. harzianum* Th5 (10.6 cm) followed by Th5+Tv1 (10.5cm), maximum root length was recorded in Th5 (3.7cm) followed by Th5+Pf3 (3.6cm) and maximum total length was recorded in Th5 (14.4 cm) followed by Th5+Tv1 (13.7cm). In soil treatment the maximum shoot length was recorded in *T. harzianum* Th5 (10.9 cm) followed by Th5+Tv1 (10.8cm), maximum root length was recorded in Th5 (4.0 cm) followed by Th5+Pf3 (3.5cm) and maximum total length was recorded in Th5 (14.4 cm) followed by Th5+Tv1 (14.1cm). Same was in case of seed + soil treatment where the maximum shoot length were recorded in *T. harzianum* (Th5) (11.5 cm) followed by Th5+Tv1 (11.3cm), maximum root length was recorded in Th5 (4.4 cm) followed by Th5+Pf3 (3.8 cm) and maximum total length was recorded in Th5 (15.9 cm) followed by Th5+Tv1 (14.9cm). Manoranjitham *et al.* (2000) reported that soil application of *T. viride* and *P. fluorescens* effectively controlled the pre-emergence and post-emergence damping off of tomato and increased the shoot length, root length and dry matter production of tomato seedlings. Similarly Joshi *et al* (2010) demonstrated that plant growth promotion measured as root and shoot lengths is significantly higher in treatment than in control.

The highest yield was obtained in seed treatment of Th5 (38 q/acre) followed by Th5+Tv1 (37.56 q/acre) as compared to control where it was 16.44 q/acre, similarly highest yield was obtained in soil treatment with *T. harzianum* (Th5) (38.8 q/acre) followed by Th5+Tv1 (37.44 q/acre) as compare to control where it was 16.8 qtls/acre. Among seed+soil treatments highest yield was obtained in seed+soil treatment with *T. harzianum* Th5 (41.5 q/acre) followed by Th5+Tv1 (37.56 q/acre) as compared to control where it was 20.0 qtls/acre and in standard chemical treatment yield was 34.4 q/acre (Table 5). Rini and Sulochana 2006 also

Table 5 Effect of different isolates of selected antagonists applied as seed, soil and seed + soil treatments on yield of cauliflower

Treatment	Yield(q/acre)[1]		
	Seed treatment	Soil treatment	Seed + soil treatment
Trichoderma harzianum (Th5)	38.00 ± 0.20 a	38.89 ± 0.15 a	41.56 ± 1.10 a
Trichoderma viride Tv1	31.56 ± 0.25bc	34.11 ± 0.52ab	32.67 ± 0.36 ac
Pseudomonas fluorescens Pf3	31.11 ± 0.15 c	33.53 ± 0.60 b	28.44 ± 0.21 c
Trichoderma harzianum + *Trichoderma viride* Th5+Tv1	37.56 ± 0.20 a	37.44 ± 0.11ab	37.56 ± 0.20ab
Trichoderma harzianum + *Pseudomonas fluorescens* Th5+Pf3	36.22 ± 0.40ab	35.11 ± 0.28ab	35.56 ± 1.04ab
Trichoderma viride + *Pseudomonas fluorescens* (Tv1+Pf1)	34.89 ± 0.55abc	34.35 ± 0.76ab	35.22 ± 0.25abc
Captan(Heterocyclic nitrogen compound)	34.44 ± 0.76abc		
Control (No treatment)	16.44 ± 0.25 d	16.89 ± 0.20 c	20.00 ± 0.20 d

[1]Mean ± SD (Standard deviation); values labeled with different letters are significantly different from the control level by Duncan test at 95.0% confidence.

evaluated isolates of *Trichoderma* (*T. harzianum* TR20 and *T. pseudokoningii* TR17) and *Pseudomonas fluorescens* (P28 and P51) alone and in combination and reported promoted plant growth and yield in chilli.

Jayaraj *et al.* (2006) also reported that the seed treatment with *T. harzianum* formulations reduced the incidence of damping-off disease of tomato up to 74% and enhanced plant biomass under greenhouse and field conditions. Cuevas (2005) also have reported the presence of the fungus in the soil in sufficient population which resulted in more mineral nutrients especially P and Zn available for the plant use which increased crop growth and yield.

From the above findings it can be concluded that the Th5 isolate of *T. harzianum* showed *in vitro* inhibition of two fungal phytopathogens (*P. aphanidermatum and R. solani*) and enhanced the growth of the cauliflower root system and showed promising results in controlling cauliflower damping-off caused by *P. aphinidermatum* and *R. solani* under field conditions.

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