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Evaluation of antagonistic activity and plant growth promotion by paste formulation of *Trichoderma harzianum*

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Abstract
The present study was proposed to test the efficacy of a formulation of *T. harzianum* suitable for application through seed treatment. Paste formulation containing starch 10 percent + copper sulphate 20 ppm at pH 4 was tested *in vitro* for their effect on the mycelial growth of 9 important pathogens namely *Rhizoctonia solani, Macrophomina phaseolina, Pythium aphanidermatum, Colletotrichum lindemuthianum, Phytophthora infestans, Botrydiplodia theobromae, Mycosphaerella musicola, Fusarium oxysporum* f.sp. *lycopersici* and *Sclerotium rolfsii* was studied which showed that the paste formulation inhibited the mycelial growth of all the 9 pathogens tested at all five concentration. The higher concentration of paste formulation had more inhibitory effect than lower concentration against all the pathogen. The paste formulaton of *T. harzianum* has significantly increased seed germination, shoot length, root length, seedling mean, dry matter production, vigour index in blackgram, chilli, cotton, sunflower, and tomato. Seed treatment @ 5 g/Kg of seed has recorded significantly higher colonization of seed surface in black gram, chilli, cotton and tomato and in the case of sunflower, seed treatment @ 3g/Kg of seed has recorded the highest colonization.

Keywords: Colonization study, germination assay, paste formulation, *Trichoderma harzianum*, Seed treatment

Introduction
Plant diseases need to be controlled to maintain the quality and abundance of food, feed and fiber produced by growers around the world. Different approaches may be used to prevent, mitigate or control plant diseases. Beyond good agricultural practices, growers often rely heavily on chemical fertilizers and pesticides. Such inputs to agriculture have contributed significantly to the spectacular improvements in crop productivity and quality over the past 100 years. Today, there are strict regulations on chemical pesticide use and there is political pressure to remove the most hazardous chemicals from the market. Additionally, the spread of plant diseases in nature may preclude successful application of chemicals, because of the scale to which such might have to be applied. Even though the expanded use of fungicides remains the prime means of control, they are now being replaced by eco-friendly measures that are more specific towards the target pathogen. (Parthasarathy *et al.*, 2016) [18]. A variety of biological controls are available for use, but further development and effective adoption will require a greater understanding of the complex interactions among plants, people and the environment (Mcspadden and Fravel, 2002) [15]. Biological control of diseases has been practiced as an alternative to control of plant disease using fungicides. *Trichoderma* spp. is the most widely exploited antagonistic fungi for the management of plant diseases world wide. Though biological control of plant diseases using *Trichoderma* spp. has been impressive and consistent under *in vitro* conditions, numbers of reports indicate wide variations in the disease control effeciency under field conditions. The genus *Trichoderma* are worldwide in occurrence and easily isolated from soil, decaying wood and other forms of plant organic matter. Dennis and Webster (1971) [6] described the antagonistic properties of *Trichoderma* in terms of antibiotic production and hyphal interactions in the control of *Sclerotium rolfsii.* Several *Trichoderma* species were formulated in a commercial production for the protection and growth enhancement of a number of crops in several countries such as the United States (Mcspadden and Fravel, 2002) [15]. One of the most interesting aspects of the science of biological control is the study of the mechanisms employed by biocontrol agents to effect disease control. Past research indicates that the mechanisms are many and varied, even within the genus *Trichoderma*.

In order to make the most effective use of biocontrol agents for the control of plant diseases, we must understand how the agents work and what their limitations are. We can then develop effective means of culturing, storing, formulating, applying and utilizing biocontrol agents so that we harness their best effort for disease control. However, *Trichoderma* formulations as conidia or chlamydospore have restricted gradients of dispersion and reproduction in soils. The germination of conidia or chlamydospore is vulnerable to soil fungistasis (Xu *et al*., 2004) [22]. The introduced *Trichoderma* spores can lyse before germination and growth of germinating spores can not occur due to non-availability of continuous nutrient supply. This greatly limits the use of *Trichoderma* spores in controlling soil-borne diseases (Papavizas, 1985; Hoitink and Boehm, 1999) [17, 10]. *Trichoderma* is a genus of asexually reproducing fungi that is present in all types of soils. *Trichoderma* species have been recognized as antagonists of soil-borne and foliage pathogens and as efficient decomposers of cellulosic waste materials. Moreover, they have the ability to increase plant growth and induce plant resistance. Along with mycoparasitism, antibiotics and competition, induced resistance is one of the most important mechanisms of *Trichoderma* action against fungal plant pathogens. Strategies to enhance biocontrol ability of *Trichoderma* include use of composts (Cumagun, 2012) [5]. The fungus is a valuable source for the commercial production of enzymes and helpful in recycling cellulosic waste materials while producing useful byproducts (Samuels, 1996). *Trichoderma* received the most attention as fungal antagonists not only of soil-borne pathogens (Amin *et al*., 2010) but also of foliage pathogens such as *Botrytis cinerea* (Elad, 1994) [7]. This is because of the ability of some of its species to produce enzymes which inhibit other fungi. *Trichoderma* can function at the same time both as microbial antagonists and plant symbionts (Lima *et al*., 1997 and Mohamed *et al*., 2010) [14, 16].

Materials and Methods

All the laboratory experiments were conducted at the Department of Plant Pathology Tamil Nadu Agricultural University (TNAU), Coimbatore. The culture of *Trichoderma harzianum* was obtained from the National Bureau of Agriculturally important Insects (ICAR), Hebbal, and Bengaluru. The *T. harzianum* was further sub cultured on Potato Dextrose Agar (PDA). The cultures were maintained under refrigerated condition (4˚C) and used periodically for preparing different formulations. The seeds of Tomato cv. PKM 1 and Chilli cv. PKM 1 were obtained from the Department of Vegetable Crops, Horticultural College and Research Institute, seeds of Blackgram cv. Co 6 was obtained from Department of Pulses, seeds of Sunflower cv. CO 4 was obtained from Department of Oilseeds (TNAU) and Cotton cv. RCH II seeds were obtained from retail shop Lawley road, Coimbatore and used throughout the experiment.

Effect of *T. harzianum* paste formulation on against various plant pathogens

Among different levels of starch, copper sulphate and pH tested, the paste formulation containing 10 percent starch, copper sulphate at 20 ppm and pH 4 was found optimum in sustaining the viability of *T. harzianum* propagules. Hence this combination was used for further studies. The efficacy of paste formulation of *T. harzianum* against important plant pathogens namely *R. solani, M. phaseolina, P. aphanidermatum, C. lindemuthianum, P. infestans, B. theobromae, M. musicola, F. oxysporum* f.sp. *lycopersici* and *S. rolfsii* was tested by dual culture technique using PDA medium (Dennis and Webster, 1971) [6]. The mycelial discs of pathogen (5mm diameter) were placed at one end of the petriplate (90 mm). Diffferent concentrations of developed paste formulation *viz*., 0.5, 1, 2, 3 and 5 gram in 100 ml sterile water were prepared. Sterile filter paper discs (5mm diameter) were dipped in different concentration of the formulations for 2 minutes and placed on the opposite end of the petriplate. Paper discs dipped in sterile water for 2 minutes served as control. The observations were taken when the pathogen in control plate, covered the entire petriplate (90mm). Percent inhibition of mycelial growth of different pathogens was recorded.

Germination test

The germination test was conducted by following the procedure outlined in ISTA (1999) rules using paper (Between papers) medium. Five replicates of 125 seeds (25 seeds /replication) treated with different concentration (0.5, 1, 2, 3 and 5g /Kg of seeds) of paste formulation each was germinated in a germination room maintained at 25±2°C temperature and 90 percent RH. At the end of 7 days for blackgram, 14 days for chillies and cotton, 12 days for sunflower, 13 days for tomato of sowing, the number of normal seedlings in each replication was counted and the germination was calculated and expressed in percentage.

Germination percentage = No of seed germination / Total number of seed ×100

Root length

Ten normal seedlings taken at random from standard germination test were used for root length measurement. Root length was measured from the collar region to the tip of the primary root. The mean value was calculated and expressed in cm.

Shoot length

The seedlings used for root length measurement were again measured for shoot length from the collar region to the growing tip of the shoot. The mean value was calculated and expressed in cm.

Dry matter production

The ten seedlings used for growth measurement were air dried for 8h and then in a hot air oven maintained at 85±2°C for 16 hours; cooled in silica gel desiccators for 30min. The dry weight of seedlings was recorded using electrically operated top pan balance. The mean dry weight of the seedlings was determined and recorded as mg 10 seedlings $^{-1}$.

Vigour index

Vigour index values were computed using the following formulae and the mean values were expressed in whole number (Abdul-Baki and Anderson, 1973).

Vigour index I = Germination x Seedling length (cm).

Vigour index II= Germination x Dry matter production (g).

Effect of *T. harzianum* paste formulation on colonization of seed surface

An experiment was conducted to study the effect of five different concentrations of paste formulation *viz*., 0.5, 1, 2, 3 and 5 percent on colonization of seed surface in five crops namely black gram, chilli, cotton, sunflower and tomato. For each crop, 125 seeds were taken and soaked in 5 different

concentrations of the paste formulation in a beaker for 10 minutes. Seeds soaked in sterile water served as control. After 10 minutes of soaking, the seeds were shade dried on a sterile blotter paper for 10 minutes. The seeds were placed on germination papers @ 25 seeds/paper. Totally 125 seeds were used for each crop. The seeds were incubated in laboratory condition for 72 hours. After 72 hrs, the seeds were examined for colonization by *T. harzianum* and percent of seeds colonized by *T. harzianum* was worked out and expressed in percentage.

Statistical analysis

The data obtained were statistically analyzed (Gomez and Gomez, 1984) [9] and the treatment means were compared by Duncan' package used for analysis was IRRISTAT version 92 developed by the International Rice Research Institute, Biometrics Unit, The Philippines.

Result

Effect of *T. harzianum* paste formulation on the radial growth of plant pathogens

Based on the study conducted using different combinations of starch, copper sulphate and pH, paste formulation containing starch 10 percent + copper sulphate 20 ppm at pH 4 was found superior in maintaining the viability of *T. harzianum* propagules even after 120 days of storage. Hence this formulation was used for further experiments. This paste formulation was tested *in vitro* for their effect on the mycelial growth of 9 important pathogens namely *R. solani*, *M. phaseolina*, *P. aphanidermatum*, *C. lindemuthianum*, *P. infestans*, *B. theobromae*, *M. musicola*, *F. oxysporum* f.sp. *lycopersici* and *S. rolfsii* was studied. The paste formulation at all five concentration tested significantly inhibited the mycelial growth of all the 9 pathogens tested. The higher concentration of paste formulation had more inhibitory effect than lower concentration against all the pathogens. In the case of *R. solani*, the percent inhibition ranged from 58.16 to 74.07 percent. Like wise, in *P. aphanidermatum* the percent inhibition ranged from 53.09 to 92.03 percent. In *S. rolfsii* the percent inhibition ranged from 50.00 to 53.71 percent. In the case of *M. musicola* the percent inhibition ranged from 62.96 to 76.66 percent. In *F. oxysporum* f.sp. *lycopersici* the percent inhibition ranged from 54.63 to 65.18 percent. In *C. lindemuthianum* the percent inhibition ranged from 70.07 to 82.22 percent. In *M. phaseolina* the percent inhibition ranged from 45.55 to 55.55 percent. In *B. theobromae* the percent inhibition ranged from 44.44 to 55.18 percent. In *P. infestans* the percent inhibition ranged from 42.22 to 62.55 percent (Table 1).

Table 1a: Effect of *T. harzianum* paste formulation on the radial growth of various plant pathogens

Treatments	*R. solani*		*P. aphanidermatum*		*S. rolfsii*		*M. musicola*		*F. oxysporum* f.sp. *lycopersici*	
	Mycelial growth of the pathogen (mm)	Percentage of inhibition over control	Mycelial growth of the pathogen (mm)	Percentage of inhibition over control	Mycelial growth of the pathogen (mm)	Percentage of inhibition over control	Mycelial growth of the pathogen (mm)	Percentage of inhibition over control	Mycelial growth of the pathogen (mm)	Percentage of inhibition over control
T_1 (0.5% of paste formulation)	37.667^{c}	58.16	43.000^{e}	53.09	45.000^{b}	50.00	33.333^{c}	62.96	36.333^{b}	54.63
T_2 (1% of paste formulation)	29.000^{b}	52.23	31.667^{d}	64.81	43.667^{ab}	51.48	32.333^{c}	64.07	33.333^{ab}	56.66
T_3 (2% of paste formulation)	27.667^{b}	69.25	26.333^{c}	70.74	42.000^{a}	53.33	30.000^{bc}	66.67	32.667^{ab}	63.70
T_4 (3% of paste formulation)	27.667 ^{b}	69.25	17.000^{b}	81.11	42.00^{a}	53.33	25.667^{ab}	71.48	31.667^{ab}	64.81
T5 (5% of paste formulation)	23.333^{a}	74.07	7.167 ^{a}	92.03	41.66^{a}	53.71	21.000 ^{a}	76.66	31.333^{a}	65.18
Control	90.00^{d}	-	90.00^{f}	-	90.00^{c}	-	90.00^{d}	-	90.00^{c}	-

Values are means of 3 replications.

In a column, means followed by common letters are not significantly different at the 5% level by DMRT.

Table 1b: Effect of *T. harzianum* paste formulation on the radial growth of important plant pathogens

Treatments	*C. lindemuthianum*		*M. phaseolina*		*B. theobromae*		*P. infestans*	
	Mycelial growth of the pathogen (mm)	Percentage of inhibition over control	Mycelial growth of the pathogen (mm)	Percentage of inhibition over control	Mycelial growth ofthe pathogen (mm)	Percentage of inhibition over control	Mycelial growth of the pathogen (mm)	Percentage of inhibition over control
T_1 (0.5% of paste formulation)	23.333^{b}	70.07	49.0^{c}	45.55	50.000^{c}	44.44	52.0^{d}	42.22
T_2 (1% of paste formulation)	21.66^{ab}	75.92	46.7^{bc}	48.67	48.667^{c}	45.92	43.0^{c}	52.22
T_3 (2% of paste formulation)	21.333^{ab}	76.29	45.0^{bc}	50.00	43.333^{b}	51.85	42.0^{bc}	53.33
T_4 (3% of paste formulation)	21.000^{ab}	76.67	42.3^{ab}	53.00	43.333^{b}	51.85	40.7^{b}	54.77
T5 (5% of paste formulation)	16.000^{a}	82.22	40.0^{a}	55.55	40.333^{a}	55.18	33.7^{a}	62.55
Control	90.00^{c}	-	90.00^{d}	-	90.00^{d}	-	90.00^{e}	-

Effect of different concentration of *T. harzianum* paste formulation on Blackgram, cv. CO 6

The effect of different concentration of *T. harzianum* paste formulation on the germination and seedling vigour of Blackgram cv. CO 6 was studied. Among the treatments, T_5 – (5g/Kg of seed) has recorded significantly higher seed germination (96.8%), shoot length (17.6 cm), root length (15 cm), seedlings mean (32.44 cm), dry matter production (366 mg), vigour index I (3140) and vigour index II (35.26). All other treatments have recorded significantly higher germination percentage, shoot length, root length, seedlings mean, dry matter production, vigour index I and vigour index II when compared to control (Table 2).

Table 2: Effect of *T. harzianum* paste formulation on the germination and seedling vigour of Blackgram cv. CO 6

S. No	Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Seedlings mean (cm)	Dry matter production (mg)	Vigour index I	Vigour index II
1	T_1 - (0.5g/kg of seed)	84.0^{bc}	13.4^{d}	13.0^{bc}	26.20^{c}	222^{c}	2205^{cd}	19.38^{d}
2	T_2 - (1g/kg of seed)	84.8^{bc}	15.0^{bcd}	13.8^{ab}	28.79^{b}	232^{c}	2480^{bc}	23.50^{c}
3	T_3 - (2g/kg of seed)	86.4^{bc}	15.8^{abc}	14.0^{ab}	29.87^{b}	282^{b}	2528^{bc}	23.91^{c}
4	T_4 - (3g/kg of seed)	92.8^{ab}	16.6^{ab}	14.4^{ab}	30.54^{b}	328^{a}	2841^{ab}	30.58 ^{b}
5	T_5 - (5g/kg of seed)	96.8^{a}	17.6^{a}	15.0^{a}	32.44^{a}	366^{a}	3140^{a}	35.26^{a}
6	Control	81.6^{c}	14.0^{cd}	11.6^{c}	25.77^{c}	218^{c}	2103^{d}	18.13^{d}

Values are means of 5 replications.
In a column, means followed by common letters are not significantly different at the 5% level by DMRT.

Effect of different concentration of *T. harzianum* paste formulation of Chilli cv. PKM 1

The effect of different concentration of *T. harzianum* paste formulation on the germination and seedling vigour of Chilli cv. PKM 1 was studied. Among the treatments, T_5 – (5g/Kg of seed) has recorded significantly higher seed germination (90%), shoot length (4.81 cm), root length (11.5 cm), seedlings mean (16.32 cm), dry matter production (40 mg), vigour index I (1481) and vigour index II (3.60) (Table 3).

Table 3: Effect of *T. harzianum* paste formulation on the germination and seedling vigour of Chilli cv. PKM 1

S. No	Treatment	(%)	Shoot length (cm)	Root length (cm)	Seedlings mean (cm)	Dry matter production (mg)	Vigour index I	Vigour index II
1	T_1 - (0.5g/kg of seed)	76.00^{c}	4.40 ^{bc}	10.390^{cd}	14.798^{cd}	20^{a}	1123^{d}	1.140^{d}
2	T_2 - (1g/kg of seed)	78.80^{bc}	4.48^{abc}	10.470^{cd}	14.950^{cd}	20^{a}	1179^{cd}	1.840^{c}
3	T_3 - (2g/kg of seed)	79.20^{bc}	4.57^{abc}	10.784^{bc}	15.330^{bc}	20^{a}	1214^{c}	1.840^{c}
4	T_4 - (3g/kg of seed)	86.00^{ab}	4.70 ^{ab}	11.254^{ab}	15.964^{ab}	20^{a}	1371^{b}	2.260^{b}
5	T_5 - (5g/kg of seed)	90.80^{a}	4.81 ^{a}	11.510^{a}	16.320^{a}	40^{a}	1481^{a}	3.600^{a}
6	Control	75.20^{c}	4.32 ^{c}	10.130^{d}	14.460^{d}	10^{a}	1086^{d}	0.960^{d}

Values are means of 5 replications.
In a column, means followed by common letters are not significantly different at the 5% level by DMRT.

Effect of different concentration of *T. harzianum* paste formulation on Cotton cv. RCH II

The effect of different concentration of *T. harzianum* paste formulation on the germination and seedling vigour of Cotton cv. RCH II was studied. Among the treatments, T_5 – (5g /Kg of seed) has recorded significantly higher seed germination (88%), shoot length (10.54 cm), root length (20.80 cm), seedlings mean (31.35 cm), dry matter production (338 mg), vigour index I (2757) and vigour index II (29.63) (Table 4).

Table 4: Effect of *T. harzianum* paste formulation on the germination and seedling vigour of Cotton cv. RCH II

S. No	Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Seedlings mean (cm)	Dry matter production (mg)	Vigour index I	Vigour index II
1	T_1 - (0.5g/kg of seed)	70.40^{c}	8.98^{c}	16.65^{d}	25.64 ^{d}	300^{bc}	1806^{cd}	21.08^{c}
2	T_2 - (1g/kg of seed)	71.20^{c}	9.57^{bc}	17.93^{c}	27.50 ^{c}	310^{bc}	1962^{c}	21.93^{c}
3	T_3 - (2g/kg of seed)	78.40^{b}	9.62^{b}	18.84^{bc}	28.46 ^{bc}	310^{bc}	2232^{b}	24.36^{b}
4	T_4 - (3g/kg of seed)	80.80^{b}	9.99^{ab}	19.62^{b}	29.61 ^{b}	316^{b}	2392^{b}	25.49^{b}
5	T_5 - (5g/kg of seed)	88.00^{a}	10.54^{a}	20.80^{a}	31.35 ^{a}	338^{a}	2757^{a}	29.63^{a}
6	Control	69.60^{c}	8.96^{c}	16.49^{d}	25.46 ^{d}	292^{c}	1771^{d}	20.33^{c}

Values are means of 5 replications.
In a column, means followed by common letters are not significantly different at the 5% level by DMRT.

Effect of different concentration of *T. harzianum* paste formulation on Sunflower cv. CO 4

The effect of different concentration of *T. harzianum* paste formulation on the germination and seedling vigour of Sunflower cv. CO 4 was studied. Among the treatments, T_5 – (5g/Kg of seed) has recorded significantly higher seed germination (89.60%), shoot length (16.69 cm), root length (16.60 cm), seedlings mean (33.70 cm), dry matter production (342 mg), vigour index I (2982) and vigour index II (30.56) (Table 5).

Table 5: Effect of *T. harzianum* paste formulation on the germination and seedling vigour of Sunflower cv. CO 4

S. No	Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Seedlings mean (cm)	Dry matter production (mg)	Vigour index I	Vigour index II
1	T_1 - (0.5g/kg of seed)	69.60^{bc}	15.90^{a}	10.80^{c}	26.66^{c}	244^{bc}	1858^{c}	16.83^{cd}
2	T_2 - (1g/kg of seed)	73.60^{b}	16.11^{a}	14.60^{b}	30.71^{b}	258^{bc}	2256^{b}	18.90^{cd}
3	T_3 - (2g/kg of seed)	74.40^{b}	16.18^{a}	15.00^{ab}	31.13^{ab}	270^{bc}	2317^{b}	20.17^{bc}
4	T_4 - (3g/kg of seed)	84.00^{a}	16.68^{a}	16.20^{ab}	32.60^{ab}	286^{b}	2739^{a}	24.30^{b}
5	T_5 - (5g/kg of seed)	89.60^{a}	16.69^{a}	16.60^{a}	33.27^{a}	342^{a}	2982^{a}	30.56^{a}
6	Control	61.60^{c}	15.76^{a}	10.40^{c}	26.32^{c}	236^{c}	1621^{c}	14.63^{d}

Values are means of 5 replications.
In a column, means followed by common letters are not significantly different at the 5% level by DMRT.

Effect of *T. harzianum* paste formulation on the germination and seedling vigour of Tomato cv. PKM 1

The effect of different concentration of *T. harzianum* paste formulation on the germination and seedling vigour of Tomato cv. PKM 1 was studied and the results are furnished in Table 6. Among the treatments, T_5 – (5 g/ Kg of seed) has recorded significantly higher seed germination (96%), shoot length (6.39 cm), root length (11.47 cm), Seedlings mean (17.87 cm), dry matter production (26 mg), vigour index I (1751) and vigour index II (2.47) (Table 6).

Tables 6: Effect of *T. harzianum* paste formulation on the germination and seedling vigour of Tomato cv. PKM 1

S. No	Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Seedlings mean (cm)	Dry matter production (mg)	Vigour index I	Vigour index II
1	T_1 - (0.5g/kg of seed)	84.00^{c}	4.49^{d}	10.38^{b}	14.87^{de}	16^{bc}	1247^{cd}	1.27^{c}
2	T_2 - (1g/kg of seed)	86.40^{bc}	5.40^{c}	10.56^{ab}	15.96^{cd}	22^{ab}	13780^{c}	1.91^{b}
3	T_3 - (2g/kg of seed)	92.80^{ab}	5.62^{bc}	10.93^{ab}	16.55^{bc}	22^{ab}	1551^{b}	2.24^{ab}
4	T_4 - (3g/kg of seed)	93.60^{a}	6.15^{ab}	11.17^{ab}	17.33^{ab}	24^{a}	1608^{ab}	2.35^{ab}
5	T_5 - (5g/kg of seed)	96.00^{a}	6.39^{a}	11.47^{a}	17.87^{a}	26^{a}	1715^{a}	2.47^{a}
6	Control	79.20^{c}	4.20^{d}	10.32^{b}	14.52^{e}	12^{c}	1149^{d}	1.12^{c}

Values are means of 5 replications.
In a column, means followed by common letters are not significantly different at the 5% level by DMRT.

Effect of *T. harzianum* paste formulation on colonization of seed surface in different crops

The effect of different concentration of *T. harzianum* paste formulation on colonization of seed surface in different crops was studied and resuslts are furnished in Table 7. All the treatments have significantly colonized the seed surface in all the five crops tested. Among the treatments, seed treatment @ 5 g/ Kg of seed has recorded significantly higher colonization of seed surface in black gram, chilli, cotton and tomato with 92, 92.4, 97.2 and 80 percent colonization of seeds respectively. However, in the case of sunflower, seed treatment @ 3g / Kg of seed has recorded the highest colonization of 98 percent (Table 7).

Table 7: Effect of *T. harzianum* paste formulation on colonization of seed surface in different crops

Treatment	Percentage of seeds colonized by *T. harzianum*				
	Blackgram	Chilli	Cotton	Sunflower	Tomato
T_1 - (0.5g/kg of seed)	76.00^{c}	56.40^{c}	89.60^{bc}	76.00^{d}	54.40^{b}
T_2 - (1g/kg of seed)	79.00^{bc}	63.60^{bc}	90.00^{b}	86.00^{c}	59.20^{b}
T_3 - (2g/kg of seed)	88.80^{ab}	70.40^{b}	91.20^{b}	92.00^{b}	76.00^{a}
T_4 - (3g/kg of seed)	91.20^{a}	85.60^{a}	92.40^{b}	98.00^{a}	78.00^{a}
T_5 - (5g/kg of seed)	92.00^{a}	92.40^{a}	97.20^{a}	96.00^{a}	80.00^{a}
Control	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{e}	0.00^{c}

Values are means of 5 replications.
In a column, means followed by common letters are not significantly different at the 5% level by DMRT.

Discussion

In the dual culture experiment, *T. harzianum* paste formulation had a significant inhibitory effect on the growth of selected phytopathogens compared to their respecive control. The inhibitory effect may be due to direct mycoparasitism or due to antibiosis or both. Since *Trichoderma* is a fast growing fungus, it reached the pathogen within 3-4 days and overgrew them in 8 – 10 days. The presence of an inhibition zone in dual culture without hyphal contact in treatments advocates the secretion of some diffusible non-volatile antibiotics by *T. harzianum*. Several reports are available on the *in vitro* inhibitory nature of *Trichoderma* spp. against several plant pathogens. Ashwani *et al*. (2011) reported that in the dual culture experiment, *T. viride* had a significant inhibitory effect on the growth of selected phytopathogens compared to their respecive control. Growth inhibition was higher in *F. oxysporum* (67.96%) followed by *R. solani* (48.67%), *Curvularia lunata* (44.32%), *Alternaria solani* (62.55%) and *Alternaria zinniae* (53.71%). The inhibition in radial growth of two interacting organisms in dual culture has been attributed to secretion of extracellular hydrolytic enzymes (Schirmbock *et al*., 1994) and by the production of antibiotics (Howell, 1998) or as well as some cell walls degrading enzyms such as chitinases, glucanases that break down polysaccharides, chitins and β – glucanases there by destroying cell wall intergrity (Elad, 2000). These may play a key role in mycoparasitism because of changes in cell wall intergrity prior to actual physical contact.

The fungus *T. harzianum* which was applied to pathogen free soil, induced, an increase in emergence of seedlings, plant

height, leaf area and dry weight. *Trichoderma* induced growth response has been reported for various plant species including bean, cucumber, periwinkle and petunia (Kleifeld and Chet, 1992) [13]. The capability of the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22 (T-22) to solubilize invitro some insoluble or sparingly soluble minerals via three possible mechanisms: acidification of the medium, production of chelating metabolites, and redox activity was studied. T-22 was able to solubilize MnO_2, metallic zinc, and rock phosphate in a liquid sucrose-yeast extract medium, as determined by inductively coupled plasma emission spectroscopy. Acidification was not the major mechanism of solubilization since the pH of cultures never fell below 5.0 and in cultures containing MnO_2 the pH rose from 6.8 to 7.4. This activity may explain, at least partially, the ability of T-22 to increase plant growth. Solubilization of metal oxides by *Trichoderma* involves both chelation and reduction. Both of these mechanisms also play a role in biocontrol of plant pathogens, and they may be part of a multiple-component action exerted by T-22 to achieve effective biocontrol under a variety of environmental conditions (Altomare *et al*., 1999) [1]. In the present study, the plant growth promoting activity of the paste formulation of *T. harzianum* was assessed on the basis of germination test and seedling vigour as determined by standard roll towel method (ISTA, 1993). In this study all the treated cultivars with 5g/kg of *T. harzianum* was shown best plant growth promotion activity. But, in case of sunflower plant growth promoting activity was observed very well even in 3g/kg of *T. harzainum*.

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