



Combination of soil application and seed treatment formulations of *Trichoderma* species for integrated management of wet root rot caused by *Rhizoctonia solani* in chickpea (*Cicer arietinum*)

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ABSTRACT

The efficacy of seed dressing and soil application formulations developed from the isolates of *Trichoderma viride* (IARI P1; MTCC 5369), *T. virens* (IARI P3; MTCC 5370) and *T. harzianum* (IARI P4; MTCC 5371) was evaluated individually and in combinations under pot and field experiments during the winter seasons of 2005–06, 2006–07 and 2007–08 for the management of wet root rot caused by *Rhizoctonia solani* Kühn and improvement in the yield of chickpea. Under pot experiments, *T. virens* based seed dressing formulation, viz. Pusa 5SD and soil application formulations, viz. Pusa Biopellet 16G (PBP 16G) and Pusa Biogranule 6 (PBG 6) were found to be superior to other formulations in reducing wet root rot incidence and increasing the seed germination, shoot and root lengths in chickpea. Under field experiments, a combination of soil application of PBP 16G (*T. virens*) and seed treatment with either a combination of Pusa 5SD (*T. virens*) and carboxin or Pusa 5SD (*T. virens*) alone was superior to any of these formulations individually in increasing the seed germination, shoot and root lengths and grain yield and reducing the wet root rot incidence in chickpea. Seed treatment was more effective than soil application for all the parameters. Both soil application and seed treatment formulations enhanced the growth of the plants indicating growth promoting ability of the isolates used for development of the formulations. The efficacy of the formulations evaluated for the first time against wet root rot of chickpea.

Key words: Bio-formulations, Disease management, Seed treatment, Soil application

Chickpea (*Cicer arietinum* L.) is an important pulse crop widely cultivated in India as well as other tropical, sub-tropical and temperate regions of the world. India is recognized globally as major player in pulse production, contributing 25% of the global production. Chickpea occupies an area of 8.21 mha and its production is 7.35 million tonnes with an average productivity of 895 kg/ha (Agricultural Statistics at a Glance 2010). Among various factors attributing to low productivity of chickpea, diseases are important. It is estimated that yield loss due to insects and diseases ranges from 5–10% in temperate regions and 50–100% in tropical region (Van Emden *et al.* 1988). Chickpea is prone to many diseases including wet root rot caused by *Rhizoctonia solani* Kühn, which is considered as one of the factors for low productivity of chickpea. The disease is widespread in the chickpea growing areas of the world and in India it is prevalent in all chickpea-growing states and causes an annual loss

around 8–10% (Vishwa Dhar and Chaudhary 2001). *R. solani*, the anamorph of *Thanatephorus cucumeris* (Frank) Donk, is a widespread soil-borne pathogen. It affects many important agricultural and horticultural crops worldwide, causing several diseases (Gonzalez Garcia *et al.* 2006). Control of *R. solani* is difficult because of wide host range and its ability to survive through sclerotia under adverse environmental conditions. In practice, control of diseases caused by *R. solani* relies mainly on fungicides (Kataria and Gisi 1996). However, increasing concern about the health and environmental hazards associated with the use of agrochemicals has resulted in the search for viable alternatives. Hence, biological control is being considered as a substitute or a supplement to reduce the use of chemical pesticides (Compant *et al.* 2005).

In recent years, eco-friendly organic farming technologies for plant protection have been gaining importance. The genus *Trichoderma* is highly effective against several phytopathogenic fungi including *R. solani* causing seed and soil - borne diseases of several economically important crops (Howell 2003). The potential of *Trichoderma* species in managing diseases caused by *R. solani* has been demonstrated

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in soybean (Raguchander *et al.* 1998), mungbean (Dubey and Patel 2001, Singh and Chand 2006), potato (Ishtiaq and Raziq 2006), faba bean (El-Mougy and Abdel-Kader 2008), tomato (Montealegre *et al.* 2010) and bean (Abd-El-Khair *et al.* 2010).

In the present study, performance of seed dressing and soil application formulations developed from *Trichoderma* species was evaluated under pot and field experiments in different modes of application to develop an eco-friendly and sustainable integrated management strategy for wet root rot of chickpea. The isolates of *Trichoderma* species used for development of formulations were found effective against *R. solani* (Dubey 2000, Dubey 2003), *R. bataticola* (Dubey 2002; Dubey *et al.* 2009), *Fusarium solani* f. sp. *pisi* (Kumar and Dubey 2001) and *F. oxysporum* f. sp. *ciceris* (Dubey *et al.* 2007). The main objective of the study was to integrate various formulations and methods of their application for effective management of the disease and higher grain yield of chickpea.

MATERIALS AND METHODS

The pot experiments were conducted in a wire net house during winter crop seasons of 2005–06 (temperature 8.0–24.7°C, relative humidity 42–89% and rain fall 3.2mm during experimentation) and 2006–07 (temperature 8.9–23.6°C, relative humidity 45–88% and rain fall 58.5 mm during experimentation) at Indian Agricultural Research Institute (IARI), New Delhi in completely randomized design (CRD) to evaluate the performance of different soil application and seed dressing formulations of *T. viride* Pers. ex Gray (IARI P-1: MTCC No. 5369), *T. virens* (Miller, Giddens & Foster) Arx (IARI P-3: MTCC No. 5370) and *T. harzianum* Rifai (IARI P-4 MTCC No. 5371). The isolates of *Trichoderma* species were isolated from suppressive soil and maintained on potato dextrose agar (PDA) slants in Division of Plant Pathology, IARI, New Delhi. The culture of *R. solani* was isolated from chickpea roots and maintained on PDA medium. Five seed dressing formulations, viz. Pusa 2SD, Pusa 5SD, Pusa 8SD, Pusa 17SD and Pusa 26SD developed from the three species of *Trichoderma* (Table 1) were evaluated individually in three replications in one set of experiment. The seed dressing formulations were prepared by mixing their ingredients. Before adding carboxymethyl cellulose, the fine mixture (200 mesh) of substrates was dried for 12 hr at 60°C in a hot air oven. A fine powder of the substrates was made in a grinder. The mixture of substrates was inoculated with the inoculum of *Trichoderma* species grown on sorghum (*Sorghum vulgare* Pers.) grains (Dubey *et al.* 2009).

Six granular formulations, viz Pusa Biogranule (PBG) 1, PBG2, PBG3, PBG4, PBG5 and PBG6 and 12 pellets/beads formulations, viz. PBP2G, PBP3G, PBP4G, PBP5G, PBP8G, PBP9G, PBP10G, PBP11G, PBP14G, PBP15G; PBP16G and PBP17G (Table 1) were evaluated as soil

application in three replications in the second set of experiment. The formulations were prepared according to the procedures described earlier by Dubey *et al.* (2009). Pusa Biopellets (PBP) were prepared by mixing their respective ingredients in a blender and the mixture was autoclaved. Fifteen-day old conidia of *Trichoderma* (10^8 conidia/ml) were added. The product was pipetted into solution of calcium gluconate. Pusa Biogranules (PBG) were prepared by mixing their respective ingredients (sterilized at 72°C for three days) in a grinder with 15-day old inoculum of *Trichoderma* grown on potato dextrose broth (50 ml).

Surface sterilized plastic pots (15cm diameter) were filled with sterilized (formalin 1.0%) soil (2 kg/pot) and inoculated with 15 days old inoculum of *R. solani* grown on sorghum grains at 7.5 g/kg soil 5 days before sowing (Dubey *et al.* 2009). The crop was sown on 8 November, 2005 and 15 November, 2006. Each pot was sown with 10 seeds of susceptible chickpea cv. BG 1103 treated with various formulations separately at 4 g/kg seed (10^8 cfu/g) as dry seed dressing. Granules (10^8 cfu/g) and pellets (10^8 cfu/pellet) were applied in pot soil at the time of sowing at 1 g/pot (20 pellets).

The field experiments were conducted during the winter seasons of 2006–07 (temperature 9.8–24.2°C, relative humidity 48–87% and rain fall 112.9 mm) and 2007–08 (temperature 8.7–24.7°C, relative humidity 51–85% and rain fall 1.8 mm) in a split-plot factorial design. The soil treatments constituted the main plots and the seed treatments the sub plots. Each sub plot experimental unit was 6.0 m² with five rows. Forty seeds per row were sown 10 cm apart at 30 cm row spacing. Twenty-four treatment combinations (Table 5) consisting of two different factors as soil application and seed treatment were evaluated. Two soil application formulations, PBG 6 (10^8 cfu/g) and PBP 16G (10^8 cfu/pellet) of *T. virens*, were evaluated as main treatment factors. Seed dressing formulations, Pusa 5SD of *T. viride*, Pusa 5SD of *T. harzianum* and Pusa 5SD of *T. virens* individually and in combination with carboxin (vitavaxTM) were evaluated as seed treatments. Untreated soil and seeds were served as control. All treatments were replicated three times in an experiment. Randomization of factors was made at each level. The experiment was conducted in a field infested with *R. solani* by the incorporation of a 15-day old inoculum grown on sorghum grains, as mentioned earlier, at the rate of 13 g sub/plot. Susceptible chickpea cv. BG 1103 was sown on 14 November 2006 and 23 November 2007 at 5 cm depth in furrows. N 25 kg/ha and P₂O₅ 50 kg/ha were applied. The granules and pellets were applied at 0.5 g/m² (2.25 g/sub plot) at the time of sowing in furrows. The seeds were treated with carboxin (vitavaxTM) at 2g/kg seed while Pusa 5SD was used at 4 g/kg of seed (10^8 cfu/g) separately and for integrated treatment with half doses of carboxin (1g/kg seed), followed by the bio-formulation.

Table 1 Bio-formulations developed and used in the present study

Formulation	Ingredient
Pusa 2SD	Talc powder - 47.5% + sabudana powder (<i>Manihot esculenta</i> Crantz) -47.5% + carboxymethyl cellulose (CMC) -5%
Pusa 5SD	Peat powder - 47.5% + sabudana powder -47.5% + CMC - 5%
Pusa 8SD	Multani soil - 47.5% + sabudana powder -47.5% + CMC-5%
Pusa 17SD	Karanj cake (<i>Pongamia glabra</i> Vent) powder - 47.5% + sabudana powder -47.5% + CMC-5%
Pusa 26SD	Pulse bran powder- 47.5% + sabudana powder -47.5% + CMC-5%
PBP 2G	Sodium alginate + pioneer + water (1:10:100 w/w/v) for <i>T. viride</i>
PBP 3G	Sodium alginate + pioneer + rice + water (1:5:5:100 w/w/w/v) for <i>T. viride</i>
PBP 4G	Sodium alginate + pioneer + sabudana + water (1:5:5:100 w/w/w/v) for <i>T. viride</i>
PBP 5G	Sodium alginate + pioneer+ wheat bran + water (1:5:5:100 w/w/w/v) for <i>T. viride</i>
PBP 8G	Sodium alginate + pioneer + water (1:10:100 w/w/v) for <i>T. harzianum</i>
PBP 9G	Sodium alginate + pioneer + rice + water (1:5:5:100 w/w/w/v) for <i>T. harzianum</i>
PBP 10G	Sodium alginate + pioneer + sabudana + water (1:5:5:100 w/w/w/v) for <i>T. harzianum</i>
PBP 11G	Sodium alginate + pioneer+ wheat bran + water (1:5:5:100 w/w/w/v) for <i>T. harzianum</i>
PBP 14G	Sodium alginate + pioneer + water (1:10:100 w/w/v) for <i>T. virens</i>
PBP 15G	Sodium alginate + pioneer + rice + water (1:5:5:100 w/w/w/v) for <i>T. virens</i>
PBP 16G	Sodium alginate + pioneer + sabudana + water (1:5:5:100 w/w/w/v) for <i>T. virens</i>
PBP 17G	Sodium alginate + pioneer+ wheat bran + water (1:5:5:100 w/w/w/v) for <i>T. virens</i>
PBG 1	Wheat bran (400g), kaolin powder (100g) and acacia (<i>Acacia nilotica</i> L.) gum powder (50g) for <i>T. viride</i>
PBG 2	Wheat bran (400g), kaolin powder (100g) and acacia gum powder (50g) for <i>T. harzianum</i>
PBG 3	Wheat bran (400g), kaolin powder (100g) and acacia gum powder (50g) for <i>T. virens</i>
PBG 4	Pulse bran (400g), kaolin powder (100g) and acacia gum powder (50g) for <i>T. viride</i>
PBG 5	Pulse bran (400g), kaolin powder (100g) and acacia gum powder (50g) for <i>T. harzianum</i>
PBG 6	Pulse bran (400g), kaolin powder (100g) and acacia gum powder (50g) for <i>T. virens</i>

SD, Seed dressing; Pioneer™, aluminium silicate; PBP, Pusa biopellet; PBG, Pusa biogranule

Table 2 Effect of different seed dressing formulations of *Trichoderma* species on seed germination, shoot and root lengths and wet root rot incidence of chickpea under pot soil infested with *R. solani*

Formulation	Seed germination (%)	Shoot length (cm)	Root length (cm)	Wet root rot incidence (%)
Pusa 2SD (<i>T. harzianum</i>)	85.0 ^{cde}	47.0 ^{cd}	12.2 ^{bcd}	17.6 ^{cd}
Pusa 5SD (<i>T. harzianum</i>)	90.4 ^{bc}	51.6 ^b	12.4 ^{bc}	11.6 ^{ab}
Pusa 8SD (<i>T. harzianum</i>)	67.9 ^{fg}	43.9 ^{ef}	11.5 ^{defg}	36.9 ^g
Pusa 17SD (<i>T. harzianum</i>)	77.5 ^{def}	46.1 ^d	11.4 ^{efg}	26.6 ^{ef}
Pusa 26SD (<i>T. harzianum</i>)	72.1 ^{fg}	42.7 ^{gh}	12.8 ^{ab}	35.0 ^{fg}
Pusa 2SD (<i>T. viride</i>)	87.1 ^{bcd}	41.1 ^h	10.9 ^g	16.8 ^{cd}
Pusa 5SD (<i>T. viride</i>)	92.5 ^{bc}	46.0 ^d	11.8 ^{cdef}	10.4 ^{ab}
Pusa 8SD (<i>T. viride</i>)	75.4 ^{def}	41.1 ^h	11.5 ^{defg}	29.6 ^{efg}
Pusa 17SD (<i>T. viride</i>)	83.0 ^{cde}	43.2 ^{fg}	11.3 ^{efg}	16.3 ^{bcd}
Pusa 26SD (<i>T. viride</i>)	75.9 ^{ef}	41.4 ^{gh}	11.2 ^{fg}	30.4 ^{efg}
Pusa 2SD (<i>T. virens</i>)	94.2 ^{ab}	51.3 ^b	12.3 ^{bcd}	8.5 ^a
Pusa 5SD (<i>T. virens</i>)	100.0 ^a	55.8 ^a	13.4 ^a	6.3 ^a
Pusa 8SD (<i>T. virens</i>)	79.2 ^{def}	48.5 ^c	11.3 ^{efg}	24.1 ^{de}
Pusa 17SD (<i>T. virens</i>)	86.7 ^{cde}	47.1 ^{cd}	11.8 ^{cdef}	15.5 ^{bc}
Pusa 26SD (<i>T. virens</i>)	79.2 ^{def}	45.4 ^{de}	11.8 ^{cdefg}	23.4 ^{cde}
Control (untreated seed)	56.3 ^g	35.8 ⁱ	8.3 ^h	55.2 ^h

SD, Seed dressing.

Values within a column represent the mean of two years (2005–06 and 2006–07).

Values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test.

The seed germination was recorded 15 days after sowing. Wet root rot was recorded on the basis of the number of

diseased plants out of the total germinated seeds. Shoot and root lengths of the plants were recorded at flowering stage of

the crop on the basis of randomly selected 10 plants/plot in field experiments, and five plants/pot in pot experiments. In the field experiment, at maturity the crop was harvested and grain yield was weighed in kg/plot.

The data of pot experiments were analyzed as per the procedure of CRD. The data of field experiments were statistically analyzed as per the procedure of split-plot design for the test of significance (Gomez and Gomez 1984). The transformed angular values of the data recorded in percentage were used for analysis. The data were subjected to the analysis of variance (ANOVA) for individual year and mixed model (pooled for both the years) analysis by using SAS Software (SAS Institute, version 9.1, Cary, NC). The statistical significance was assessed at $P < 0.05$, and Fisher's least significance difference test was used to separate means.

RESULTS AND DISCUSSION

Effect of seed dressing formulations in pot experiments

The seed treatment formulations significantly ($P < 0.05$) increased the seed germination, shoot and root lengths and decreased the wet root rot incidence in comparison to those of control (Table 2). The effect of year was non-significant for all the parameters recorded. The seeds treated with Pusa 5SD (*T. virens*) provided 100% seed germination, 55.8 cm shoot and 13.4 cm root lengths and 6.3% wet root rot incidence. The seed germination and wet root rot incidence recorded in Pusa 5SD (*T. virens*) were statistically at par with those of Pusa 2SD (*T. virens*), Pusa 5SD (*T. viride*) and Pusa 5SD (*T. harzianum*). Root length of Pusa 5SD (*T. virens*) was statistically on par with that of Pusa 26 SD (*T. harzianum*), whereas shoot length of Pusa 5SD (*T. virens*) was significantly superior to others.

Effect of soil application formulations in pot experiments

Except seed germination recorded in PBG 1, all the soil application formulations significantly ($P < 0.05$) increased the seed germination, shoot and root lengths and decreased the wet root rot incidence in comparison to those of control (Table 3). Except root length, the effect of year was non significant for all the parameters recorded. PBP 16G (*T. viride*) provided 100% seed germination, 54.2 cm shoot and 14.3 cm root lengths and 10.0% wet root rot incidence. The seed germination recorded in PBG 5, PBG 6, PBP 4G and PBP 15G were non-significant to each other and ranked second after PBP 16G. The shoot and root lengths and the wet root rot incidence recorded in PBP 15G and PBP 4G were statistically on par with those of PBP 16G.

Integrated management in field experiments

Except root length, the effect of soil application on the seed germination, shoot length, wet root rot incidence and grain yield was significant ($P < 0.05$). The effect of the year and the interaction effect of the year with soil application and

Table 3 Effect of different soil application formulations of *Trichoderma* species on seed germination, shoot and root lengths and wet root rot incidence of chickpea under pot soil infested with *R. solani*

Soil application	Seed germination (%)	Shoot length (cm)	Root length (cm)	Wet root rot incidence (%)
<i>Granules</i>				
PBG 2 (<i>T. harzianum</i>)	78.3 ^{ghi}	38.6 ^j	10.9 ^{gh}	33.5 ^h
PBG 5 (<i>T. harzianum</i>)	95.0 ^b	45.4 ^{fgh}	12.7 ^{cd}	22.0 ^{cdef}
PBG 1 (<i>T. viride</i>)	74.6 ^{ij}	44.4 ^{ghi}	10.9 ^{gh}	43.7 ⁱ
PBG 4 (<i>T. viride</i>)	80.0 ^{fghi}	43.4 ^{hi}	11.3 ^{efg}	33.1 ^{gh}
PBG 3 (<i>T. virens</i>)	85.9 ^{cdef}	47.8 ^{cde}	11.3 ^{efg}	24.1 ^{defg}
PBG 6 (<i>T. virens</i>)	95.0 ^b	48.5 ^{cd}	12.5 ^d	16.0 ^{bc}
<i>Pellets/Beads</i>				
PBP 8G (<i>T. harzianum</i>)	74.6 ^{hi}	42.3 ⁱ	9.9 ⁱ	35.2 ^{hi}
PBP 9G (<i>T. harzianum</i>)	80.4 ^{fghi}	45.8 ^{efg}	11.8 ^e	30.0 ^{fgh}
PBP 10G (<i>T. harzianum</i>)	87.5 ^{cde}	47.8 ^{cde}	12.6 ^{cd}	19.2 ^{cd}
PBP 11G (<i>T. harzianum</i>)	88.4 ^{bc}	39.2 ^j	10.8 ^{gh}	31.9 ^{gh}
PBP 2G (<i>T. viride</i>)	74.6 ^{hi}	44.4 ^{ghi}	10.5 ^{hi}	32.2 ^{gh}
PBP 3G (<i>T. viride</i>)	84.2 ^{defg}	44.9 ^{fgh}	11.0 ^{fgh}	22.4 ^{cdef}
PBP 4G (<i>T. viride</i>)	94.6 ^b	52.0 ^{ab}	13.7 ^{ab}	12.2 ^{ab}
PBP 5G (<i>T. viride</i>)	86.7 ^{bcd}	38.8 ^j	11.7 ^e	28.3 ^{efgh}
PBP 14G (<i>T. virens</i>)	83.8 ^{efgh}	47.0 ^{def}	12.8 ^{cd}	20.3 ^{cde}
PBP 15G (<i>T. virens</i>)	92.9 ^b	50.1 ^{bc}	13.2 ^{bc}	12.6 ^{ab}
PBP 16G (<i>T. virens</i>)	100.0 ^a	54.2 ^a	14.3 ^a	10.0 ^a
PBP 17G (<i>T. virens</i>)	85.0 ^{efg}	44.0 ^{ghi}	11.6 ^{ef}	18.1 ^{bcd}
Control (Untreated soil)	63.8 ⁱ	30.9 ^k	7.8 ^j	56.1 ^j

PBG, Pusa Biogranule, PBP, Pusa Biopellet, G, Prepared in calcium gluconate. The values within a column represent the mean of two years (2005–06 and 2006–07).

Values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test.

seed treatment were non-significant. Soil application of PBP 16G (*T. virens*) provided the highest seed germination (66.6%), shoot (60.3 cm) and root (13.3 cm) lengths and grain yield (2200.5 kg/ha) and the lowest wet root rot incidence (24.2%) and except the wet root rot incidence, all parameters recorded in this treatment were statistically similar with those of PBG 6 (*T. virens*) (Table 4).

Except shoot and root lengths recorded in carboxin treated seeds, all seed treatments significantly ($P < 0.05$) increased the seed germination, shoot and root lengths, grain yield and decreased the wet root rot incidence (Table 5). The seeds treated with a combination of Pusa 5SD (*T. virens*) and carboxin provided the highest seed germination, shoot and root lengths, grain yield and the lowest wet root rot incidence and except wet root rot incidence, all other variables recorded in this treatment was statistically at par with those of Pusa 5SD (*T. virens*) treated seeds. Antagonists applied to seeds before planting colonies the rhizosphere of seedlings and

Table 4 Effect of different soil application formulations of *Trichoderma virens* on seed germination, shoot and root lengths, wet root rot incidence and grain yield of chickpea under field infested with *R. solani*.

Soil application	Seed germination (%)	Shoot length (cm)	Root length (cm)	Wet root rot incidence (%)	Grain yield (kg/ha)
PBG 6 (<i>T. virens</i>)	64.3 ^{ab}	58.7 ^a	12.9 ^{ab}	28.6 ^b	2100.1 ^a
PBP 16G (<i>T. virens</i>)	66.6 ^a	60.3 ^a	13.3 ^a	24.2 ^a	2200.5 ^a
Control (no soil application)	61.3 ^b	55.7 ^b	12.2 ^b	38.6 ^c	1966.8 ^b

Values within a column represent the average of two years (2006–07 and 2007–08)

Values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test

Table 5 Effect of different seed treatments on germination, shoot and root lengths, wet root rot incidence and grain yield of chickpea under field infested with *R. solani*

Seed treatment	Seed germination (%)	Shoot length (cm)	Root length (cm)	Wet root rot incidence (%)	Grain yield (kg/ha)
Pusa 5SD (<i>T. viride</i>)	65.5 ^{cd}	59.3 ^{cd}	13.1 ^{ab}	26.4 ^d	2138.1 ^{bc}
Pusa 5SD (<i>T. viride</i>) + carboxin	67.6 ^{bc}	60.5 ^{bc}	13.3 ^{ab}	23.2 ^c	2178.8 ^b
Pusa 5SD (<i>T. harzianum</i>)	61.9 ^e	57.1 ^d	12.5 ^{bc}	34.5 ^f	2018.4 ^{de}
Pusa 5SD (<i>T. harzianum</i>) + carboxin	63.3 ^{de}	58.1 ^{cd}	12.7 ^{bc}	31.9 ^e	2058.0 ^{cd}
Pusa 5SD (<i>T. virens</i>)	68.7 ^{ab}	62.8 ^{ab}	13.8 ^a	16.7 ^b	2308.7 ^a
Pusa 5SD (<i>T. virens</i>) + carboxin	70.1 ^a	63.3 ^a	14.0 ^a	14.4 ^a	2345.6 ^a
Carboxin	63.3 ^{cd}	53.6 ^e	11.8 ^{cd}	43.8 ^g	1920.3 ^e
Control (untreated seed)	51.9 ^f	51.2 ^e	11.3 ^d	52.7 ^h	1744.3 ^f

Values within a column represent the average of two years (2006–07 and 2007–08)

Values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test

thus are present at or near the pathogen's infection court, where they act by producing antifungal or antibiotic compounds, through hyperparasitism, or by competitively colonising spermosphere and rhizosphere substrates (Taylor and Harman 1990). Seed treatment is an attractive delivery system of fungal bioprotectants (Wright *et al.* 2003). Bioprotectants applied to seeds may not only protect seeds (Sivan and Chet 1986) but also may colonise and protect roots and may increase plant growth. It is evident that the antagonistic bioagent can affect the plant's resistance to a pathogen either by inducing the basal level of defense reactions immediately after treatment or by enhancing a capacity for rapid and effective activation of cellular defence responses (Conrath *et al.* 2002). In the present study, seed treatments enhanced the seed germination, shoot and root lengths and reduced wet root rot incidence. Pusa 5SD based on *T. virens* was found superior to other formulations for reducing the wet root rot incidence and increasing the seed germination, plant growth and grain yield of chickpea. The strain of *Trichoderma* species used in the present study showed growth-enhancing ability. *Trichoderma* spp. are effective biocontrol agents for a number of soil-borne plant pathogens, and some are also known for their ability to enhance plant growth. It was suggested that *Trichoderma* also affects induced systemic resistance mechanism in plants. Analysis of signal molecules involved in defense mechanism and application of specific inhibitors indicated the

involvement of jasmonic acid and ethylene in the protective effect conferred by *Trichoderma* spp (Shoresh *et al.* 2005).

Except seed germination, shoot and root lengths recorded in the interaction of PBG 6 × untreated seeds and seed germination in PBG 16G × untreated seeds, shoot and root lengths in no soil application × carboxin treated seeds, the effect of interactions of soil application and seed treatment on seed germination, shoot and root lengths, wet root rot incidence and grain yield was significantly ($P < 0.05$) superior to those of the control. The interaction of soil application of PBP 16G and seed treatment with Pusa 5SD (*T. virens*) + carboxin provided the highest seed germination (72.2%), shoot (66.2 cm) and root (14.5 cm) lengths, grain yield 2509.6 kg/ha and the lowest wet root rot incidence (9.1%) in chickpea (Table 6). The next effective interaction was the soil application with PBP 16G and seed treatment with Pusa 5SD (*T. virens*). Except wet root rot incidence, all the variables recorded in these two treatment interactions were statistically similar. The interaction of soil application of PBG 6 and seed treatment with Pusa 5SD (*T. virens*) + carboxin was also found effective.

Soil application of alginate pellet (10^5 cfu/g) formulation of *T. harzianum* mutant significantly reduced the infection of *R. solani* in tomato (Montealegre *et al.* 2010). Biocontrol agents generally do not perform well enough under field conditions to compete with chemical fungicides (Brunner *et al.* 2005), but the formulations evaluation in the present

Table 6 Effect of interaction of soil application and seed treatment on germination, shoot and root lengths, wet root rot incidence and grain yield of chickpea under field infested with *R. solani*.

Soil application × seed treatment	Seed germination (%)	Shoot length (cm)	Root length (cm)	Wet root rot incidence (%)	Grain yield (kg/ha)
PBG 6 × Pusa 5SD (<i>T. viride</i>)	66.5 ^{bcde}	59.4 ^{defg}	13.2 ^{cdefgh}	23.4 ^{fg}	2144.5 ^{defg}
PBG 6 × Pusa 5SD (<i>T. viride</i>)+carboxin	67.6 ^{bcd}	60.9 ^{cde}	13.4 ^{bcdef}	22.0 ^{ef}	2169.7 ^{def}
PBG 6 × Pusa 5SD (<i>T. harzianum</i>)	62.2 ^{ghij}	57.8 ^{fgh}	12.7 ^{ghijkl}	33.9 ^{jk}	2041.2 ^{ghij}
PBG 6 × Pusa 5SD (<i>T. harzianum</i>)+carboxin	63.7 ^{fgh}	58.4 ^{efg}	12.8 ^{efghijk}	30.6 ^{hi}	2079.6 ^{gghi}
PBG 6 × Pusa 5SD (<i>T. virens</i>)	67.9 ^{bc}	63.1 ^{bc}	13.8 ^{abcde}	16.2 ^d	2285.5 ^{bc}
PBG 6 × Pusa 5SD (<i>T. virens</i>)+carboxin	69.3 ^b	64.3 ^{ab}	14.0 ^{abc}	13.9 ^c	2324.1 ^b
PBG 6 × carboxin	64.9 ^{bcd}	54.5 ^{ij}	12.0 ^{ijklmn}	41.0 ^l	1939.2 ^{ijkl}
PBG 6 × untreated seed	52.2 ^k	51.6 ^{kl}	11.5 ^{mno}	47.5 ⁿ	1816.7 ^m
PBP 16G × Pusa 5SD (<i>T. viride</i>)	67.1 ^{bcde}	61.6 ^{cd}	13.7 ^{abcde}	19.9 ^l	2251.9 ^{bcd}
PBP 16G × Pusa 5SD (<i>T. viride</i>)+carboxin	70.3 ^b	62.9 ^{bc}	13.9 ^{abcd}	15.5 ^{cd}	2322.3 ^b
PBP 16G × Pusa 5SD (<i>T. harzianum</i>)	64.2 ^{efg}	58.6 ^{efg}	12.9 ^{efghij}	28.2 ^h	2106.0 ^{efgh}
PBP 16G × Pusa 5SD (<i>T. harzianum</i>)+carboxin	66.8 ^{bcde}	60.1 ^{def}	13.0 ^{defghi}	25.5 ^g	2124.1 ^{efgh}
PBP 16G × Pusa 5SD (<i>T. virens</i>)	73.0 ^a	64.8 ^{ab}	14.3 ^{ab}	11.1 ^b	2469.6 ^a
PBP 16G × Pusa 5SD (<i>T. virens</i>)+carboxin	74.2 ^a	66.2 ^a	14.5 ^a	9.1 ^a	2509.6 ^a
PBP 16G × carboxin	65.5 ^{cdef}	55.3 ^{hij}	12.3 ^{hijklm}	39.3 ^l	1970.4 ^{ijk}
PBP 16G × untreated seed	51.2 ^k	52.8 ^{jk}	11.7 ^{lmn}	44.8 ^m	1849.6 ^{lm}
No soil application × Pusa 5SD (<i>T. viride</i>)	63.0 ^{ghi}	56.8 ^{ghi}	12.4 ^{ghijklm}	35.7 ^k	2017.8 ^{hijk}
No soil application × Pusa 5SD (<i>T. viride</i>)+carboxin	64.8 ^{defg}	57.7 ^{fgh}	12.6 ^{efghijkl}	32.0 ^{ij}	2044.4 ^{ghij}
No soil application × Pusa 5SD (<i>T. harzianum</i>)	59.1 ^j	55.0 ^{ij}	11.9 ^{klmn}	41.4 ^l	1908.2 ^{klm}
No soil application × Pusa 5SD (<i>T. harzianum</i>)+carboxin	59.6 ^{ij}	55.7 ^{hi}	12.2 ^{ijklm}	39.5 ^l	1970.4 ^{ijk}
No soil application × Pusa 5SD (<i>T. virens</i>)	65.2 ^{cdefg}	60.5 ^{cde}	13.3 ^{cdefg}	22.9 ^f	217.6 ^{cdef}
No soil application × Pusa 5SD (<i>T. virens</i>)+carboxin	66.7 ^{bcde}	59.3 ^{defg}	13.4 ^{bcdef}	20.3 ^e	2203.0 ^{cde}
No soil application × carboxin	59.6 ^{hij}	51.1 ^{kl}	11.2 ^{no}	51.0 ^o	1851.2 ^{lm}
No soil application × untreated seed	52.3 ^k	49.3 ^l	10.6 ^o	65.6 ^p	1566.7 ⁿ

Values within a column represent the average of two years (2006–07 and 2007–08).

Values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test

study were found superior to fungicide carboxin used as seed treatment. *Trichoderma* species alone and in combination with ridomil inhibited the growth of *R. solani* causing canker in potato (Ishtiaq and Raziq 2006). Significant increase in the plant growth and grain yield clearly indicated the growth-promoting properties of *Trichoderma* strains used for the development of Pusa 5SD and PBP 16G.

The antagonistic activity of the genus *Trichoderma* against *R. solani* has been widely demonstrated (Dubey 2003,) Almeida *et al.* 2007, Hajieghrari *et al.* 2008. *Trichoderma* spp are effective biocontrol agents for a number of soil borne pathogens and are also known for their ability to enhance plant growth (Yedidia *et al.* 2003). Long-term activity of bio-priming seed treatment for biological control of faba bean root rot pathogens including *R. solani* was determined under greenhouse conditions (El-Mougy and Abdel-Kader 2008). In green house experiments, the species of *Trichoderma*, significantly reduced the disease and improve the growth and yield parameters. The levels of the chitinase, peroxidase and polyphenol oxidase activities highly increased in treated bean plants as compared to untreated plants (Abd-

El-Khair *et al.* 2010). Soil amendment with *T. harzianum* significantly increased the height and weight of plants and reduced the *R. solani* infection (Malik *et al.* 2005). In the present study, soil application of *Trichoderma* formulation also increased plant growth and grain yield and reduced root rot incidence but, seed treatment was more effective in comparison to soil application for reducing the disease and increasing the grain yield of chickpea. Application of *T. harzianum* as seed treatment significantly reduced the incidence of damping-off diseases in leguminous crops, ie faba bean, lentil, and chickpea, when planted in a soil naturally infested with *Fusarium* spp and *R. solani* (Abou-Zeid *et al.* 2003). In the present study *T. harzianum* and *T. viride* based soil application and seed-dressing formulations were also found effective for all the variables, but their performance was inferior than *T. virens* based Pusa 5SD and PBP 16G.

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