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Additive Effect of Soil Application with *Trichoderma* Enriched FYM Along with Seed Treatment and Drenching with *Trichoderma* Formulation for Management of Wet Root Rot caused by *Rhizoctonia solani* in Chickpea

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Efficacy of seed dressing, soil application and drenching with *Trichoderma harzianum* Th3, *T. viride*, Carbendazim, Dithane M-45 and Carbendazim+Mancozeb against wet root rot of chickpea caused by *Rhizoctonia solani* was evaluated under field experiment during rabi seasons of 2012-13 and 2013-14. The treatments were also evaluated for improvement in plant growth parameters and yield of chickpea cv Pratap Chana-1. Soil application with *Trichoderma* enriched farm yard manure (FYM) and Neem cake in different plots was done one week before sowing and seed dressing was done with antagonists and fungicides individually at the time of sowing. At 50 days after sowing drenching with antagonists and fungicides was done. Soil application, seed dressing and drenching with *T.harzianum* Th3 was found to be most effective in improving germination, shoot length, root length and thereby increasing vigour index of plants. Seed dressing, soil application and drenching with formulation of *T. harzianum* Th3, reduced wet root rot disease incidence most effectively than only seed dressing along with drenching. Study showed that soil application with *Trichoderma* enriched FYM gives an additive effect to seed dressing and drenching in reducing disease incidence.

Key words: *Trichoderma*, root rot, chickpea, seed treatment, soil application, drenching

Chickpea (*Cicer arietinum*) is a widely cultivated cool-season grain legume in India and accounts for approximately 65% of area and 64% of production of the world's chickpea production. India is producing 18.09 million tons of pulses from an area of 26.28 million hectare, which is 33% of world area and 22% of world's production of pulses (FAO, 2008; Agricultural Statistics at a Glance, 2011). Among other pulses chickpea grown on maximum area of 8.17 million ha cover 31% area of total pulse production in India. Chickpea accounts for production of 7.48 million tones which accounts for 41.3% production of grain legumes with an average productivity of 915 kg/ha in India (Agricultural Statistics at a Glance, 2011). Chickpea is mainly grown in soils with residual moisture in the post rainfall season as a sole crop or as a crop mixed with wheat, mustard and sorghum. Rajasthan State is an important chickpea growing region in India with 0.88 million ha cultivated area thus ranking fourth in area and fifth in production (Agricultural Statistics at a Glance 2011). However, about 2-3 million tons of pulses are imported annually to meet the domestic consumption requirement.

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*.

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1988). Bagri et al., 2004 observed that Chickpea suffers from seed borne fungal diseases viz, black root rot, dry root rot, wet root rot, seed rotting, root rot, stem rot, crown rot, foot rot, sclerotinia wilt and gray mould. Amongst these disease dry root rot caused by *Macrophomina phaseolina* and wet root rot, caused by *Rhizoctonia solani*, reported to cause severe losses right from seedling to maturity of the crop (Khan 2007). This results in dismal national productivity of 915 Kg/ha. The productivity of chickpea is far less than national average in Rajasthan (460 kg/ha). Thus there is an urgent need to increase production and productivity of pulses especially chickpea in Rajasthan and country as a whole by developing more specific and cost effective interventions for management of root rot of chickpea.

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). *R. solani* is a soil borne pathogen hence use of chemical fungicide to control the pathogen in inadequate and uneconomical. Antagonists could provide better remedy to minimize the incidence of root rot and other soil borne diseases (Panwar and Gaur, 2012). 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 in soybean (Raguchander et al. 1998), mungbean (Dubey and Patel 2001), 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). There are several reports on the application of biocontrol agents to the soil and other growing media either before or at the time of planting for control of wide range of fungal pathogens. Such applications are ideally suited for green house and nursery. *Trichoderma* is capable of colonizing farm yard manure (FYM) and therefore application of colonized FYM to the soil is more appropriate and beneficial. This is the most effective method of application of *Trichoderma* particularly for the management of soil borne diseases (Ramanujam *et al.*, 2010). Application of bioagents in combination with FYM enhanced the plant growth parameters significantly that is dry weight, root length, and grain yield (Rehman, 2013). Biological management of *R. solani* by addition of *Trichoderma* formulations by enriching it in FYM for soil treatment before sowing is a potential mechanism as find out during this study.

MATERIALS AND METHODS

Seeds of chickpea cv Pratap Chana-1, a widely cultivated but susceptible to root rot, obtained from farm store of Agricultural Research Station (ARS), Banswara. The disease response of this cv Pratap Chana-1was regularly observed and surveyed at farmers' fields and was confirmed by screening experiment carried out in root rot sick plot at ARS farm during rabi season of 2011-12 (Data not presented). Thus the pathogen was not artificially inoculated and the entire experiment was conducted on natural infection conditions. The powdered bioformulation of *T. harzianum* Th3 isolate was obtained from Biological control unit of Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi. Another formulation of *T.viride* was obtained from State IPM laboratory, Banswara.

This field experiment was conducted in root rot sick plot at Agricultural Research Station, MPUAT, Banswara, Rajasthan, India, during Rabi season of 2012-13 and 2013-14 for two consecutive years to develop economical and cost effective management practice for wet root rot in chickpea. This trial was laid out in Randomized Block Design (RBD) with three replications by maintaining each plot size of 6m x 10m. There were eight treatments including T1- Seed treatment with *T. harzianum* TH3 @ 6g/kg seeds +Soil treatment with *Trichoderma* enriched FYM (10kg formulation in

200 kg FYM)+ Drenching with *T.harzianum* Th3@ 6g/lit water at 50 DAS; T2- Seed treatment with *T. viride* @ 6g/kg seeds +Soil treatment with *Trichoderma* enriched FYM (10kg formulation in 200 kg FYM) + Drenching with *T.viride* @ 6g/lit water at 50 DAS; T3- Seed treatment with *T.harzianum* Th3 + Drenching with *T.harzianum* Th3@ 6g/lit water at 50DAS; T4- Seed treatment with *T.viride* @ 6g/kg seeds + Drenching with *T. viride* @ 6g/lit water at 50DAS; T5- Seed treatment with Carbendazim 50 WP (0.2%) + Soil treatment with Neem cake@ 500kg/ha+ Drenching with carbendazim (0.2%) at 50 DAS; T6- Seed treatment with Dithane M-45 75% WP (0.3%)+ Soil treatment with Neem cake@ 500kg/ha + Drenching with Dithane M-45(0.3%) at 50 DAS; T7- Seed treatment with Carbendazim+ mancozeb (Saaf ™ 0.2%)+Soil treatment with Neem cake @500kg/ha +Drenching with carbendazim 12%+Mancozeb 63%WP (Saaf ™ 0.2%) at 50DAS; T8- Control. The organic amendment, Neem cake @ 500 Kg ha^{-1} was incorporated in to the soil one week before sowing. For soil application, formulation of *T. harzianum* Th3 and *T. viride* were individually mixed with thoroughly decomposed farm yard manure (FYM) in 1:20 ratio. Optimum moisture was maintained and dumped in a pit for 20 days. The covered pit was opened on 20th day, *Trichoderma* mycelium was proliferated throughout the FYM, and it was mixed well and spread evenly in the field plots one week before sowing. The treated and untreated seeds were sown in sick plot (having 65-70 sclerotia g^{-1} soil) on 13 December 2012 and 25 November 2013. Drenching treatment was done at 50 days after sowing. The plants were raised with recommended agronomical practices. Observations on initial plant stand (Germination%) were recorded 20 days after sowing and plant mortality due to wet root rot was recorded thrice at 25 days interval. At the time of count, moribund plants were removed from the plots, cause of mortality was confirmed and plants killed due to *R. solani* were recorded. Per cent disease incidence (PDI) was calculated and data were subjected to angular transformation before statistical analysis. Seedling vigour of 40 randomly selected 90 days old chickpea plants form each plot were measured for shoot length and root length at the time of harvest. Experiment was repeated twice with three replicates. Germination percentage, shoot length and root length were recorded and vigour index was calculated with the formula Vigour Index=Germination% x (Mean Shoot length + Mean root length). At maturity, crop was harvested and grain yield was weighed in Kg/ha.

The data obtained in the field experiments were analyzed using analysis of variance for RBD (Gomez and Gomez, 1984). Critical difference values were calculated at 5 per cent probability level were compared using Fisher's least significance difference test.

RESULTS AND DISCUSSION

The treatments significantly ($P<0.05$) reduced the wet root rot incidence and increased the seed germination, shoot length and root length in comparison to those of control. Though no treatment gave 100% germination but the seed and soil application of *T. harzianum* and *T. viride* provided 94-96% germination while treatments with chemicals cause little lower germination of 91-93% during both the years of experimental field trials. Significantly high percentage of germination (95% in 2012-13 and 96% in 2013-14) of chickpea seeds when treated with *T. harzianum* Th3 formulation along with soil application of *T. harzianum* enriched FYM was recorded as compared to the control. The treatment with *T. viride*+FYM formulation also resulted significant increase in germination (96% in 2012-13 and 94% in 2013-14) of chickpea seeds (Table 1). These results indicated a certain role of *Trichoderma* formulations in promoting seed germination of chickpea seeds. Results are in conformity with the findings of work done by Hassan *et al*., 2013 which suggests that there was no significant variation in germination on treatment with two different strains of *Trichoderma* spp. However insignificantly low germination (91-93%) was recorded when chickpea seeds were treated with fungicides. The present results are supported by the observations that *Trichoderma* species produces growth factors which increase the rate of seed germination (Benitez *et al.*, 1998).Earlier studies also observed enhancing seed germination with treatment of *Trichoderma* spp. in several host pathogen systems (Kumar and Dubey , 2001).

Shoot length and root length were significantly differentiated among treatments of *T.*

Table1. Effect of seed treatment, soil application and drenching with *Trichoderma* formulation and various fungicides on plant growth promoting parameters of chickpea plants under condition of soil infestation with *R.solani*

Treatment	Seed germination (%)		Shoot length(cm)		Root length(cm)	
	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
ST with *T. harzianum* TH3 @ 6g/kg seeds +SA with *Trichoderma* enriched FYM (10 formulation in 200 kg FYM)+ Drenching with TH3 at 50 DAS	*95^{ab}	96^{a}	43.0^{a}	45.2^{a}	11.3^{b}	12.4^{a}
ST with *T. viride* @ 6g/kg seeds +SA with *Trichoderma* enriched FYM (10 formulation in 200 kg FYM) + Drenching with *T.viride* at 50 DAS	96^{a}	94^{ab}	41.0^{b}	44.0^{ab}	10.5^{bc}	8.6^{c}
ST with *T. harzianum* TH3 + Drenching with TH3 at 50DAS	92^{c}	91^{c}	38.5^{c}	41.3^{c}	9.3^{d}	11.2^{b}
ST with *T. viride* + Drenching with *T.viride* at 50DAS	93^{d}	94^{ab}	40.0^{cd}	39.6^{cd}	10.1^{bc}	12.4^{a}
ST with Carbendazim (0.2%)+ SA with Neem cake@ 500kg/ha+ Drenching with carbendazim at 50 DAS	91^{c}	93^{b}	38.0^{c}	39.8^{cd}	10.5^{bc}	9.1^{c}
ST with Dithane M-45(0.3%)+ SA with Neem cake@ 500kg/ha + Drenching with Dithane M-45(0.3%) at 50 DAS	93^{d}	93^{b}	38.5^{cd}	40.0^{d}	12.5^{a}	11.5^{b}
ST with Carbendazim+ mancozeb (Saaf™0.2%)+SA with Neem cake @500kg/ha +Drenching with carbendazim+Mancozeb (Saaf™0.2%) at 50DAS	91^{c}	92^{d}	36.0^{e}	37.4^{d}	11.0^{b}	12.3^{a}
Control	73^{e}	85^{e}	30.5^{f}	32.4^{e}	7.4^{e}	10.2^{d}
SEm	0.42	0.33	0.34	0.61	0.24	0.41
CV	8.3	9.4	7.8	10.4	8.1	8.3
CD(0.05)	1.3	1.1	1.1	1.8	0.75	1.2

ST- Seed treatment; SA-Soil application;
*Figures were angular transformed before analysis
Values within column with different letters are significantly different at 5% level by using Fisher's least significance difference test

Table 2. Effect of seed treatment, soil application and drenching with *Trichoderma* formulation and various fungicides on per cent disease incidence and yield of chickpea under condition of soil infestation with *R.solani*

Treatments	2012-13		2013-14		Pooled	Yield (kg/ha)	
	PDI	%ROC	PDI	%ROC	mean PDI	2012-13	2013-14
ST with *T. harzianum* TH3 @ 6g/kg seeds +SA with *Trichoderma* enriched FYM (10 formulation in 200 kg FYM)+ Drenching with TH3 at 50 DAS	5.0^{a}	81.6	6.6^{a}	80.9	5.8^{a}	1481.0^{a}	1520.6^{a}
ST with *T. viride* @ 6g/kg seeds +SA with *Trichoderma* enriched FYM (10 formulation in 200 kg FYM) + Drenching with *T.viride* at 50 DAS	6.9^{bc}	74.6	9.5^{bc}	72.5	8.2^{b}	1323.4^{ab}	1264.0^{bc}
ST with *T.harzianum* TH3 + Drenching with TH3 at 50 DAS	7.7^{d}	71.7	10.5^{bc}	69.6	9.1^{bc}	1281.2^{ab}	1271.1^{b}
ST with *T.viride* + Drenching with *T. viride* at 50 DAS	8.9^{d}	67.3	11.2^{d}	67.6	10.1^{bcd}	1134.5^{c}	1252.2^{bc}
ST with Carbendazim (0.2%)+ SA with Neem cake@ 500kg/ha+ Drenching with carbendazim at 50 DAS	9.2^{d}	66.2	11.7^{d}	66.2	10.4^{cd}	1110.3^{c}	1030.7^{cd}
ST with Dithane M-45(0.3%)+ SA with Neem cake@ 500kg/ha + Drenching with Dithane M-45(0.3%) at 50 DAS	5.9^{a}	78.3	8.9^{b}	74.3	7.4^{a}	1231.7^{ab}	1123.1^{bcd}
ST with Carbendazim+ Mancozeb (Saaf™0.2%)+SA with Neem cake @500kg/ha +Drenching with Carbendazim+Mancozeb (Saaf™0.2%) at 50DAS	6.5^{bc}	76.1	9.7^{bc}	71.9	8.1^{bc}	1040.0^{c}	970.8^{d}
Control	27.2^{e}	-	34.6^{e}	-	31.0^{e}	830.1^{d}	708.4^{e}
SEm	0.46		0.81		0.73	29.4	26.3
CV	8.3		10.1		8.6	12.4	11.5
CD(0.05)	1.5		2.2		2.1	93.1	84.3

ROC-Reduction over control
PDI-Per cent Disease incidence; ST- Seed treatment; SA-Soil application;
*Figures were angular transformed before analysis
Values within column with different letters are significantly different at 5% level by using Fisher's least significance difference test

harzianum Th3 and *T.viride*, when used alone or along with FYM. Also carbendazim, Dithane M-45 and Carbendazim+mencozeb (Saaf) when used in combination with Neem cake showed varied level of growth promotion (Table 1). Shoot length was in the range of 30.5 to 45.2 cm. The maximum shoot length (45.2 cm in 2013-14) was recorded when chickpea seeds treated with formulation of *T.harzianum* Th3 sown in seedbed treated with *T.harzianum* Th3 enriched FYM and drenched with suspension of *T. harzianum* Th3 formulation while the lowest shoot length (30.5 cm) was reported for untreated control. Table 1 reveals significant differences in chickpea root length among different treatments and soil applications, it was ranged from 7.4-12.5 cm. Maximum root length (12.5 cm) was recorded when seeds were treated with Dithane M-45 sown in seedbed applied with Neem cake and later drenched with solution of Dithane M-45(0.3%). Root length of seeds treated with *T. harzianum* Th3 sown in seedbed applied with *T. harzianum* Th3 enriched FYM (12.4cm) was significantly at par with the best treatment of Dithane M-45. These observations clearly inferred that highest vigour index (5158.5 in 2012-13 and 5529.6 in 2013-14) values appeared in treatment having maximum seed germination, shoot length and root length. Thus seeds treated with *T.harzianum* Th3 sown in seedbed applied with *T. harzianum* enriched FYM, were found to be most vigorous with highest vigour index (Fig1.). Srivastava (2004) reported that root colonization by *Trichoderma* strains frequently enhances root growth and development. The strains of *Trichoderma* increased root development in several crops, under both green house and field conditions (Harman *et al.*, 2004).

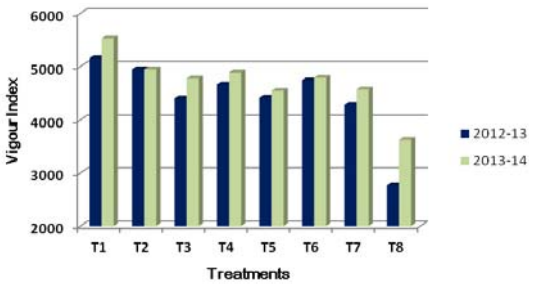

Fig. 1. Effect of seed treatment, soil application and drenching of various treatments on vigour index of chickpea plants

This was resulted in improved plant vigour in *Trichoderma* formulation treated plots. Seed treatment with *T. harzianum* TH3 @ 6g kg^{-1} seeds and soil application of *Trichoderma* enriched FYM (10kg formulation/200 kg FYM) along with drenching of *Trichoderma* formulation at 50 Days after sowing (DAS) was found significantly at par with that of seed treatment with Dithane M-45 (0.3%) and soil application with Neem cake @ 500 kg/ha followed by drenching with Dithane M-45 (0.3%) solution at 50 DAS. Both these treatments were found superior with rest of the treatments in reducing wet root rot incidence in chickpea but only treatment with *Trichoderma* and not the Dithane -45, helped to enhance plant growth promoting parameters. Antagonists applied to seeds before planting colonies the rhizosphere of seedlings and 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. Bioprotectants applied to seeds may not only protect seeds but also may colonise and protect roots and may increase plant growth. In this study it is reported that soil application with *Trichoderma* enriched FYM act as an additive effect in colonizing the *Trichoderma* in the rhizosphere and thereby reduce incidence of *Rhizoctonia solani*. Pooled data showed that only seed treatment with *T. harzianum* Th3 followed by its drenching at 50 DAS caused 9.1% disease incidence but when this treatment supplemented with soil application with *Trichoderma* enriched FYM, there was not only reduction in disease incidence to only 5.8% but also increased germination and other plant growth parameters. This showed that soil application of *Trichoderma* formulation created an additive effect to seed treatment by rapidly colonizing spermosphere and rhizosphere in reducing disease incidence and increasing plant growth parameters. Rehman *et al*., 2013 successfully evaluated *T.harzianum* and *T.viride* along with FYM and carbendazim as seed, seedbed treatment, drenching against root rot in chilli caused by *R. solani* and found significant reduction in disease incidence over control improvement in plant growth parameters. Our

results are also supported by the experimental results of Dubey *et al.,* 2012 which suggests that both soil application and seed treatment with *Trichoderma* formulation enhanced the growth of the plants and reduced wet root rot of chickpea.

The strain of *Trichoderma* species used in the present study also showed varied antagonism and growth-enhancing ability. *T.harzianum* TH3 strain of *Trichoderma* was obtained from Biological Control laboratory, Division of Plant Pathology, IARI, New Delhi and *T. viride* formulation was obtained from state IPM laboratory, Banswara. Among both these strains, *T.harzianum* TH3 was found superior in reducing disease incidence by ~81% as compared to application of *T. viride* formulation which can reduce the disease incidence to the maximum extent of 74.6% (Table 2). *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.

Soil application of Neem cake also gave promising results in reducing disease incidence of wet root rot. Reports suggest that *Trichoderma* strains when applied with fungicide or Neem cake, cause reduced colonization (Ainmisha and Zacharia 2011), thus in the present study soil application was done with Neem cake along with individual seed treatment by carbendazim (0.2%), Dithane M-45 (0.3%) and Carbendazim+ Mancozeb (Saaf™ 0.2%). Pooled data clearly showed that seed treatment with Dithane M-45 (0.3%) and soil application with Neem cake @ 500 kg/ha followed by drenching with solution of Dithane M-45 (0.3%) has reduced disease incidence to 7.4% which was at par with best treatment of *T.harziamum* TH3 application. Treatments having Dithane M-45 and Carbendazim+ mencozeb (Saaf™) along with soil application with Neem cake not only improved germination but also enhanced root length but application carbendazim (0.2%) with Neem cake less effective in enhancing root length (Table 1). Similar findings were reported by Ainmisha and Zacharia 2011, Saralamma and Reddy 2005. When effect of these treatments on yield of chickpea was recorded, highest yield of 1481.0 kg/ha and 1520.6 kg/ha respectively during 2012-13 and 2013-14 experiments was obtained on seed treatment with *T.harzianum* Th3 @ 6g/kg seeds and soil application of Th3 enriched FYM @ 10 kg *T.harzianum* Th3 formulation in 200 kg FYM along with drenching by *T.harzianum* Th3 formulation suspension at 50 days after sowing. Seed treatment, soil application and drenching with *T. viride* also produce significant yield of 1323.4 kg/ha and 1264 kg/ha respectively during 2012-13 and 2013-14 which was at par with the treatment of Dithane M-45 as seed treatment and drenching and soil application by Neem cake during both the years (Table 2).

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