



Synergistic effect of antagonistic microflora and farmyard manure (FYM) to reduce wilt disease in chickpea caused by *Fusarium oxysporum* f sp *ciceri*

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ABSTRACT

Wilt disease of chickpea caused by *Fusarium oxysporum* f sp *ciceri* (Pudwick) Synd. and Hans. is serious problems across the country. The experiment was conducted at the farm College of Agriculture, Rajasthan Agricultural University, Bikaner in for two crop seasons 2007-08 and 2008-09 to manage the wilt disease of chickpea. Chickpea variety RSG-44 was used for this experiment. For the purpose, two antagonist, i.e. *Trichoderma harzianum* and *Pseudomonas fluorescens* were applied as seed treatment (alone @ 8 g/ kg seeds; in combination of both bioagents @ 4 g + 4 g/ kg seeds) and soil application (alone @ 10 kg/ ha; in combination of both bioagents @ 5 kg + 5 kg/ ha) along with FYM @ 5, 10, and 15 tonnes/ ha in the field. Minimum wilt disease incidence 23.65% and 25.35% was found in the treatment T₁₂: *T. harzianum*+*P. fluorescens* seed treatment (4+4) kg/seed) and soil application (5+5) kg/ha along with FYM @ 15 tonnes/ h followed by T₁₁:*T. harzianum* +*P. fluorescens* seed treatment (4+4) kg/seed) and soil application (5+5) kg/ha along with FYM @ 10 tonnes/ ha (27.67 and 28.61%) and T8: *P. fluorescens* ST (8 g/ kg seed + SA (10 kg/ h + FYM 15 tonnes/ha (28.51 and 29.48%) in 2007-08 and 2008-09 under field conditions respectively. The plant growth, i.e. root and shoot lengths, dry weight and seed yield were found higher when *T. harzianum* and *P. fluorescens* were used along with higher dose of FYM, i.e. 15 tonnes/ha. A significant variation was recorded among the treatments. The organic carbon content of the soil was increased by increasing the dose of FYM from 5 to 15 tonnes/h irrespective of bioagents. The *Fusarium* population was suppressed by the two bioagents used alone or in combinations. The population of *T. harzianum* and *P. fluorescens* was higher in rhizosphere soil when FYM was applied at higher dose (15 tonnes/ha). Our result findings indicate that microbial bioagents and FYM have synergistic effect on reducing the wilt incidence in chickpea and promote the plant growth significantly.

Key words: Antagonist, Chickpea, *Cicer arietinum*, *Fusarium oxysporum* f. sp. *ciceri*

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown in tropical, subtropical and temperate regions of the world. It is world's third most important pulse crop after beans and peas with India accounting for approximately 65% of area and 64% of production of the world (FAO 1993, FAO 2008). The overall productivity of chickpea in India is comparatively low due to various biotic and abiotic stresses. It is also realized that lack of diffusion of appropriate production and protection technologies is a limitation of chickpea production in the country (Anonymous 2003). Nearly, 50 different types of pathogens have been reported in this crop from different parts of the world. Among the fungal diseases, chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Snyder and Hans is one of the important limiting factors of chickpea production in India. The disease causes substantial yield losses which

may reach even 100 per cent under favourable weather conditions (Nene 1980, Jalali and Chand 1992).

Management of *Fusarium* wilt of chickpea is difficult to achieve as the fungus is soil-borne, survive through resistant structure, i.e. chlamydospores in soil and the crop remains susceptible all throughout the growth stages (Kaiser *et al.* 1994, Haware *et al.* 1996). Fungicidal seed treatment has been recommended for managing this disease in almost all the chickpea growing regions of the world. However, there is a growing concern about health and environmental hazards and persistence of toxic residues in nature. Moreover, continuous use of fungicides leads to development of resistant or tolerant strains of the pathogen towards fungicides. Hence, biological management of root diseases of various field crops including chickpea using microbial antagonists such as *Trichoderma* spp. *Pseudomonas fluorescens*, *Bacillus* spp. etc. has drawn the attention of growers and researchers throughout the world (Mukhopadhyay *et al.* 1992, Hervas *et al.* 1997, Saikia *et al.* 2003, Khan and Gangopadhyay 2008, Jayalakshmi *et al.* 2009, Merkuze and Getachew, 2012, Animisha *et al.* 2012, Subhani *et al.* 2013). FYM is rich source of organic matter

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and nutrients to support the growth of microbes including antagonistic bacteria and fungi in soil. There is urgent need to know the influence of FYM on efficacy of bioagents to reduce the incidence of soil borne plant diseases as well as plant growth and also fate of inoculated bioagents in the field.

Keeping these points in view, the present investigation was undertaken to study the effect of antagonistic microbes and FYM on incidence of wilt disease, plant growth and yield of chickpea crops and also population dynamics of antagonists under field conditions.

MATERIALS AND METHODS

A field experiment was conducted on management of chickpea wilt using bioagents and farmyard manure (FYM) for two crop season, i.e. 2007-08 and 2008-09 using chickpea variety RSG-44 at Division of Plant Pathology, College of Agriculture, Rajasthan Agricultural University, Bikaner. Talc based formulations of two bioagents, viz. *Trichoderma harzianum* (6.3×10^9 cfu/ml), and *P. fluorescens* (7.2×10^9 cfu/ml) prepared in laboratory were used alone and in combination (*T. harzianum* + *P. fluorescens*). Seeds of chickpea var. RSG-44 were treated with @ 8g/kg seeds individual bioagent and (4g + 4g) kg/seeds in combination of bioagents. Similarly, for soil application, the bioagents were applied @ 10 kg/ha individual bioagent and (5 kg + 5 kg)/ha in combination of bioagents. Farmyard manure (FYM) was applied in the field @ 5, 10 and 15 t/ha before sowing of seeds.

The effect of FYM alone as well as in combination with bioagents was tested in the present trial. Total sixteen treatments including control such as T₁: *T. harzianum* seed treatment (ST) 8 g/ kg seeds + soil application (SA) 10 kg/ha, T₂: *T. harzianum* ST 8 g/ kg seed + SA 10 kg/ha + FYM 5 tonnes/ha, T₃: *T. harzianum* ST 8 g/kg seed + SA 10 kg/ha + FYM 10 tonnes/ha, T₄: *T. harzianum* ST 8 g/kg seed + SA 10 kg/ha + FYM 15 tonnes/ha, T₅: *P. fluorescens* ST 8 g/kg seed + SA 10 kg/ha, T₆: *P. fluorescens* ST 8 g/kg seed + SA 10 kg/ha + FYM 5 tonnes/ha, T₇: *P. fluorescens* ST 8 g/kg seed + SA 10 kg/ha + FYM 10 tonnes/ha, T₈: *P. fluorescens* ST 8 g/kg seed + SA 10 kg/ha + FYM 15 tonnes/ha, T₉: *T. harzianum* + *P. fluorescens* ST (4+4) g/kg seed + SA (5+5) kg/ha, T₁₀: *T. harzianum* + *P. fluorescens* ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 5 t/ha, T₁₁: *T. harzianum* + *P. fluorescens* ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 10 t/ha, T₁₂: *T. harzianum* + *P. fluorescens* ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 15 tonnes/ha, T₁₃: FYM 5 t/ha, T₁₄: FYM 10 t/ha, T₁₅: FYM 15 t/ha and T₁₆: Control (without bioagent and FYM application) were tested following Randomized Block Design having plot size 4×3 m² with three replications. The trial was conducted under artificial soil infestation conditions. For this purpose, sand maize meal inocula of *F. oxysporum* f. sp. *ciceri* was applied at 50 g per plot (4×3 m²) and mixed thoroughly on top surface soil using a hand rack. Standard agronomic practices recommended for cultivation of chickpea crop in this region was followed. In case of control, the untreated seeds were

sown in unamended plots. Observations on wilt incidence were recorded periodically and calculated percent disease incidence and percent disease control as

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants germinated}} \times 100$$

$$\text{Disease control (\%)} = \frac{\text{Diseased incidence in inoculated control (\%)} - \text{Diseased incidence in treatment (\%)}}{\text{Disease incidence in inoculated control (\%)}} \times 100$$

The grain yield was also recorded at after harvest the crop. The dry weight of chickpea plants as well as shoot and root lengths of the plants were recorded after harvesting of the crop and calculated dry weight.

The organic content of soil was determined following the method described by Singh *et al.* (2005). One gram of soil sample was taken into 500 ml dry conical flask. Ten milliliter of 1N K₂Cr₂O₇ and 20 ml of conc. H₂SO₄ were added to it. They were swirled a little and kept on an asbestos sheet for 30 minutes. Slowly 200 ml of distilled water and 10 ml of orthophosphoric acid were added. After that 1ml of diphenylamine indicator was added. Ferrous ammonium sulphate solution (0.5 N) was taken in 50 ml burette. The content was titrated until green colour starts appearing. The organic content was calculated using following formula:

$$\text{Organic carbon (\%)} \text{ in soil} = \frac{10(B-S) \times 0.003 \times 100}{B \times \text{Wt. of sample (g)}}$$

whereas B, Titrate values of blank (ml) and S, titrate values of sample (ml).

As described above, soil samples were collected from rhizosphere of chickpea plant after 90 days of inoculation, mixed thoroughly and air dried in shade for 48 hr. Ten gram soil was added to 90 ml of sterile distilled water in 250 ml of Erlenmeyer flask and shaken gently for 5 min. Serial dilutions were prepared from the stock soil suspension up to 10⁵. A 0.2 ml suspension of desired dilution was added on to the surface of *Fusarium* selective medium, for *F. oxysporum* f. sp. *ciceri* (Papavizas 1967), *Trichoderma* selective medium (Elad and Chet 1983) for *T. harzianum* and *Pseudomonas agar fluorescens* (PAF) media for *P. fluorescens* in Petri dishes and spread uniformly and incubated at 26± 2°C for 2-7 days. The colonies developed on in the Petri dishes were counted.

The data of per cent disease incidence and organic carbon content in all the treatments were transformed to their Arcsin values (Fisher and Yates 1963). The statistical analysis of the data of all the laboratory and green house experiments were done following Completely Randomized Design. The data of field experiments were analyzed following Randomized Block Design (Cochran and Cox 1957).

RESULTS AND DISCUSSION

Disease control efficacy of *Trichoderma harzianum*

Table 1 Synergistic effect of FYM and bioagents on wilt incidence of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* under field condition

| Treatment | Disease incidence (%) | | Disease control (%) |
|--|-----------------------|---------------|---------------------|
| | 2007- 08 | 2008-09 | |
| <i>T. harzianum</i> Seed Treatment (ST) 8 g/kg seed + Soil Application (SA) 10 kg/ha | 37.54 (37.78)* | 39.84 (39.14) | 52.90 |
| <i>T. harzianum</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 5 tonnes/ha | 35.98 (29.97) | 37.20 (30.54) | 55.45 |
| <i>T. harzianum</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 10 tonnes/ha | 33.68 (27.75) | 35.63 (28.82) | 57.80 |
| <i>T. harzianum</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 15 tonnes/ha | 30.29 (25.32) | 31.84 (26.01) | 62.18 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha | 36.21 (36.99) | 37.80 (37.80) | 54.94 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 5 tonnes/ha | 34.37 (28.91) | 36.84 (30.03) | 56.65 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 10 tonnes/ha | 31.19 (27.01) | 31.85 (27.31) | 61.62 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 15 tonnes/ha | 28.51 (23.97) | 29.48 (24.40) | 64.70 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha | 35.52 (31.64) | 38.08 (32.90) | 55.20 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 5 tonnes/ha | 31.34 (27.51) | 33.26 (28.42) | 60.68 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 10 tonnes/ha | 27.67 (24.86) | 28.61 (25.30) | 65.74 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 15 tonnes/ha | 23.65 (21.68) | 25.35 (22.49) | 70.17 |
| FYM 5 tonnes/ha | 63.73 (52.98) | 65.72 (54.17) | 21.20 |
| FYM 10 tonnes/ha | 60.32 (51.40) | 61.16 (51.46) | 26.05 |
| FYM 15 tonnes/ha | 57.14 (49.12) | 61.25 (51.51) | 27.93 |
| Control (Without bioagent and FYM application) | 78.93 (62.85) | 85.34 (67.60) | |
| SEm ± | (1.94) | (1.32) | |
| CD (P=0.05) | (5.60) | (3.83) | |

*Figures in parentheses are angular transformed values

and *Pseudomonas fluorescens* used as seed treatment (ST) and soil application (SA) along with FYM was evaluated against wilt of chickpea under field conditions during crop season 2007-08 and 2008-09. The wilt incidence was significantly suppressed by treating chickpea seeds with bioagent and soil application along with FYM application in field before sowing the seeds. Minimum wilt disease incidence 23.65 and 25.35 was found in treatment T₁₂: *T. harzianum*+*P. fluorescens* were used in combination as seed treatment (4 + 4) g/kg seed and soil application (5+5) kg/ha along with FYM 15 t/ha followed by followed by T₁₁: *T. harzianum*+*P. fluorescens* seed treatment (4+4) kg/seed and soil application (5+5) kg/ha along with FYM @10 t/ h (27.67 and 28.61%) and T₈: *P. fluorescens* ST (8 g/ kg seed) + SA (10 kg/ h + FYM 15 t/h) (28.51 and 29.48%) in 2007-08 and 2008-09 under field conditions respectively (Table 1). The seed treatment with bioagents alone or combination was also found significantly effective to reduce wilt incidence without application FYM in the field. However, the level of disease control was higher when the two bioagents were used in combination as compared to individual bioagents. The level of FYM application was significantly showed variation in disease reduction by increasing the level of dose of FYM decreases the disease incidence. The efficacy of microbial antagonist, viz. *T. harzianum*, *T. viride*, *P. fluorescens* and *B. subtilis* plus *P. fluorescens* in chickpea wilt has been reported (Agrawal *et al.* 2002, Merkuiz and Getachew 2012, Animisha *et al.*

2012, Subhani *et al.* 2013). The present study clearly revealed the disease control efficacy of the bioagents was significantly enhanced in FYM amended plots. The combined use of biocontrol agents, i.e. *T. harzianum* and *T. viride* and FYM significantly checked the cumin wilt under greenhouse and field conditions (Gangopadhyay and Ram Gopal 2009 and Chawala and Gangopadhyay 2009). The combined seed treatment of *T. viride* was more effective as compared to individual bioagent treatment in reducing *Fusarium* wilt of pigeon pea (Sethuraman and Vidhyasekaran 1994).

The root and shoot lengths of chickpea plants was significantly increased in response to bioagent treatments either used alone or in combination with FYM. Both the root and shoot lengths was higher in *T. harzianum*+*P. fluorescens* treated seeds as compared to the individual bioagents treatments. Maximum root length (17.13 cm and 15.53 cm) shoot length (50.53 cm and 48.43 cm) was recorded in treatment T₁₂: *T. harzianum* + *P. fluorescens* were used in combination as seed treatment (4 + 4) g/kg seed and soil application (5+5) kg/ha along with FYM 15 tonnes/ha followed by followed by T₁₁ and T₈ in 2007-08 and 2008-09 (Table 2). The data also showed that some other combination treatments like *T. harzianum* used alone as SA and ST along with higher dose of FYM, i.e. 10 and 15 tonnes/ha also enhanced root and shoot lengths. The results showed that maximum dry weight of chickpea plants (45.50 g/plant and 44.86 g/plant) was found in treatment T₁₂ (*T. harzianum* + *P. fluorescens* ST (4+4) g/kg seed + SA

Table 2 Synergistic effect of FYM and bioagents on plant growth and seed yield of chickpea plant under field condition

| Treatment | Root length (cm) | | Shoot length (cm) | | Dry weight (g/ plant) | | Seed yield (q/ha) | |
|--|------------------|---------|-------------------|---------|-----------------------|---------|-------------------|---------|
| | 2007-08 | 2008-09 | 2007-08 | 2008-09 | 2007-08 | 2008-09 | 2007-08 | 2008-09 |
| <i>T. harzianum</i> Seed Treatment (ST) 8 g/kg seed + Soil Application (SA) 10 kg/ha | 14.32 | 12.52 | 37.80 | 36.00 | 33.24 | 31.78 | 10.42 | 9.89 |
| <i>T. harzianum</i> ST 8 g/kg seed + SA 10 kg/ha+ FYM 5 tonnes/ha | 15.53 | 13.23 | 39.33 | 37.03 | 36.34 | 33.72 | 11.33 | 10.88 |
| <i>T. harzianum</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 10 tonnes ha ⁻¹ | 15.60 | 14.00 | 44.65 | 42.85 | 38.53 | 37.71 | 12.12 | 11.48 |
| <i>T. harzianum</i> ST 8 g/kg seed + SA 10 kg/ha+ FYM 15 tonnes/ha | 16.27 | 14.17 | 46.73 | 44.63 | 40.36 | 39.79 | 13.50 | 13.08 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha | 14.40 | 12.30 | 38.27 | 36.17 | 30.21 | 28.64 | 10.34 | 10.40 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 5 tonnes/ha | 15.80 | 14.00 | 41.80 | 39.50 | 33.77 | 32.28 | 11.64 | 11.11 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 10 tonnes/ha | 16.09 | 13.79 | 44.13 | 42.53 | 36.56 | 34.29 | 12.34 | 11.92 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 15 tonnes/ha | 16.33 | 14.73 | 47.42 | 45.62 | 39.21 | 38.38 | 13.65 | 13.12 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha | 14.40 | 12.30 | 38.40 | 36.10 | 35.53 | 33.97 | 11.47 | 10.94 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 5 tonnes/ha | 15.80 | 13.70 | 41.13 | 39.53 | 37.64 | 34.93 | 12.67 | 12.14 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 10 tonnes/ha | 17.07 | 14.77 | 47.24 | 46.17 | 39.41 | 38.57 | 13.56 | 13.11 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 15 tonnes/ha | 17.13 | 15.53 | 50.53 | 48.43 | 45.50 | 44.86 | 14.13 | 13.49 |
| FYM 5 tonnes/ha | 10.07 | 7.97 | 39.53 | 37.23 | 27.34 | 25.92 | 5.05 | 4.63 |
| FYM 10 tonnes/ha | 11.27 | 9.17 | 40.27 | 38.67 | 29.63 | 28.33 | 6.66 | 6.13 |
| FYM 15 tonnes/ha | 13.47 | 11.67 | 41.67 | 39.87 | 32.40 | 30.39 | 7.28 | 6.75 |
| Control (Without bioagent and FYM application) | 8.70 | 6.40 | 21.10 | 19.00 | 10.56 | 10.34 | 4.54 | 3.47 |
| SEm ± | 0.56 | 0.68 | 2.15 | 1.36 | 0.83 | 1.57 | 0.42 | 0.56 |
| CD (P=0.05) | 1.61 | 1.97 | 6.22 | 3.92 | 2.39 | 4.55 | 1.21 | 1.62 |

(5+5) kg/ha + FYM 15 tonnes/ha) by T_4 and T_{11} in 2007-08 and 2008-09 respectively (Table 2). The chickpea grain yield was significantly enhanced when bioagents treated seeds were sown in FYM amended soils. The yield was highest (14.13 q/ha and 13.49 q/ha) in treatment T_{12} followed by T_8 (13.65 q/ha and 13.12 q/ha) and T_{11} (13.56 q/ha and 13.11 q/ha) in 2007-08 and 2008-09 respectively. In fact the grain yield was statistically at par when *T. harzianum* or *P. fluorescens* were used alone or in combinations along with FYM at 15 tonnes/ha. The results also showed that root and shoot lengths and dry weight of chickpea plants was significantly increased, even when FYM was applied alone at all the three doses in the field. Although, application of FYM in the field enhanced the positive effect of bioagents significantly. These treatments are responsible for increasing the shoot and root lengths, dry weight of plants and grain yield (Dileep Kumar 1999, Merkuze and Getachew 2012). Significant enhancement in different plant growth parameters, viz. root and shoot lengths and dry weight of pea seedlings was recorded in treatments with different strains of *P. fluorescens* either alone or in combination

(Negi *et al.* 2005).

Estimation of soil organic carbon

The organic carbon content of the soil samples collected from treated plots were analyzed after harvest of chickpea crop and Table 3 reveals that organic carbon content was highest in treatment T_{12} (0.128% and 0.122%) followed by T_{11} and T_8 . The organic carbon content of the soil was increased by increasing the dose of FYM irrespective of bioagent used alone or in combination.

Population dynamics of *Fusarium oxysporum* f. sp. *ciceri* and bioagents

Population of *Fusarium oxysporum* f. sp. *ciceri* in rhizosphere soil was enumerated at 90 days after sowing (DAS) of chickpea seeds using selective media. The pathogen propagules in soil were significantly least in all the treatments as compared to control. The population of *F. oxysporum* f. sp. *ciceri* in soil was relatively low (17.33×10^5 cfu/g and 17.92×10^5 cfu/g) in *T. harzianum* plus *P. fluorescens* along with FYM 15 tonnes/ha treatment (T_{12})

Table 3 Effect of FYM and bioagents on organic carbon and population of *Fusarium oxysporum* f.sp. *ciceri*, *Trichoderma harzianum* and *Pseudomonas fluorescens* in rhizospheric soil of chickpea under field condition

| Treatment | Organic carbon (%) | | Population of <i>F. o. f. sp. ciceri</i> | | Population of <i>T. harzianum</i> | | Population of <i>P. fluorescens</i> | |
|--|--------------------|---------------|--|---------|-----------------------------------|---------|-------------------------------------|---------|
| | 2007-08 | 2008-09 | 2007-08 | 2008-09 | 2007-08 | 2008-09 | 2007-08 | 2008-09 |
| <i>T. harzianum</i> seed treatment (ST) 8 g/kg seed + Soil Application (SA) 10 kg/ha | 0.067 (1.48)* | 0.051 (1.29) | 24.50 | 25.75 | 8.90 | 8.51 | 7.10 | 6.59 |
| <i>T. harzianum</i> ST 8 g/kg seed + SA 10 kg/ha+ FYM 5 tonnes/ha | 0.070 (1.52) | 0.064 (1.45) | 23.34 | 24.37 | 9.50 | 8.82 | 7.90 | 7.63 |
| <i>T. harzianum</i> ST 8 g/kg seed + SA 10 kg/ha+ FYM 10 tonnes/ha | 0.090 (1.72) | 0.083 (1.65) | 21.42 | 22.96 | 10.70 | 10.47 | 8.50 | 8.21 |
| <i>T. harzianum</i> ST 8 g/kg seed + SA 10 kg/ha+ FYM 15 tonnes/ha | 0.107 (1.87) | 0.098 (1.79) | 19.88 | 20.30 | 11.20 | 11.04 | 8.96 | 8.31 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha | 0.069 (1.51) | 0.054 (1.32) | 24.31 | 25.14 | 4.51 | 4.28 | 10.10 | 9.66 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 5 tonnes/ha | 0.078 (1.60) | 0.072 (1.54) | 22.16 | 22.91 | 4.90 | 4.68 | 11.10 | 10.30 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 10 tonnes/ha | 0.085 (1.67) | 0.078 (1.60) | 20.91 | 22.12 | 5.20 | 4.88 | 13.50 | 13.21 |
| <i>P. fluorescens</i> ST 8 g/kg seed + SA 10 kg/ha + FYM 15 tonnes/ha | 0.119 (1.98) | 0.110 (1.90) | 19.21 | 20.19 | 6.30 | 6.17 | 14.80 | 14.30 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha | 0.066 (1.47) | 0.057 (1.37) | 22.12 | 23.09 | 9.20 | 8.80 | 11.50 | 10.67 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 5 tonnes/ha | 0.091 (1.73) | 0.082 (1.64) | 20.98 | 22.49 | 11.60 | 10.76 | 12.40 | 11.98 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 10 tonnes/ha | 0.105 (1.86) | 0.101 (1.82) | 19.22 | 19.63 | 12.00 | 11.75 | 13.00 | 12.56 |
| <i>T. harzianum</i> + <i>P. fluorescens</i> ST (4+4) g/kg seed + SA (5+5) kg/ha + FYM 15 tonnes/ha | 0.128 (2.04) | 0.122 (2.00) | 17.33 | 17.92 | 12.50 | 12.33 | 14.20 | 13.18 |
| FYM 5 tonnes/ha | 0.063 (1.44) | 0.050 (1.28) | 41.00 | 43.95 | 4.40 | 4.17 | 6.40 | 6.12 |
| FYM 10 tonnes/ha | 0.083 (1.65) | 0.076 (1.58) | 37.00 | 39.26 | 5.00 | 4.78 | 7.10 | 6.59 |
| FYM 15 tonnes/ha | 0.095 (1.77) | 0.086 (1.68) | 35.60 | 36.81 | 5.80 | 5.44 | 8.00 | 7.83 |
| Control (Without bioagent and FYM application) | 0.049 (1.27) | 0.048 (1.26) | 47.35 | 50.76 | 3.50 | 3.43 | 5.00 | 4.83 |
| SEm ± | (0.042) | (0.037) | 0.60 | 1.45 | 0.21 | 0.31 | 0.33 | 0.40 |
| CD (P=0.05) | (0.12) | (0.11) | 1.73 | 4.20 | 0.61 | 0.89 | 0.96 | 1.14 |

*Figures in parentheses are angular transformed values

as compared to the rest of other treatments. The *Fusarium* population was also effectively suppressed by the *T. harzianum* and *P. fluorescens* used alone along with FYM 15 tonnes/ha. However, the population level of the pathogen was statistically at par *T. harzianum* and *P. fluorescens* treatments at FYM 15 tonnes/ha enumerated 90 DAS. The magnitude of reduction of pathogen population was higher at higher doses of FYM.

The two test bioagents in chickpea rhizosphere soil were enumerated in respective selective media at 90 DAS. The population of *T. harzianum* (12.50×10^5 cfu/g and 12.33×10^5 cfu/g) and *P. fluorescens* (14.20×10^5 cfu/g and 13.18×10^5 cfu/g) was higher in rhizosphere soil treated with T₁₂. The results clearly revealed that by increasing in

level of FYM, the population of antagonists *T. harzianum* and *P. fluorescens* in rhizosphere soil was also increased. The recovery of *P. fluorescens* from soil was less in *T. harzianum* treatments or only FYM was used. The survival of bioagent was relatively higher at 60 DAS in comparison to 90 DAS. Bareja and Lodha (2002) recorded that the population of *T. harzianum* in compost prepared from residues of *Prosopis juliflora* and *Calotropis procera*. Zaidi and Singh (2004) used chickpea manure, press mud, FYM and vermicompost for multiplication of *T. harzianum* and *P. fluorescens* alone and together. All the four substrate supported multiplication of *T. harzianum* and *P. fluorescens*.

The higher disease control recorded in present studies may be due to suppression of *F. oxysporum* f. sp. *ciceri*

population in rhizosphere soil *vis-a-vis* enhanced population of antagonist in soil. Our results indicate that application of bioagents and FYM have synergistic effect on reduction of wilt disease incidence caused by *F. oxysporum* f. sp. *ciceri*, enhancement of plant growth adding organic content in the soil. The population of antagonists is enhanced by application of FYM, which directly enhance the organic content in soil. It is suggested that combined treatment of *T. harzianum* and *P. fluorescens* seed treatment as well soil application along with application farmyard manure (15 q/ha) may be applied under farmer's field conditions to manage *Fusarium* wilt and improve plant growth of chickpea effectively.

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REFERENCES

- Agrawal S C, Sharma S and Prasad K V V. 2002. Efficacy of biological components in wilt management of chickpea. *Indian Journal of Pulses Research* **15** (2): 177– 8.
- Animisha, Zacharia S, Jaiswal K K and Pandey P. 2012. Integrated management of chickpea wilt incited by *Fusarium oxysporum* f. sp. *ciceri*. *International Journal of Agricultural Research* **7** (5): 284–90.
- Anonymous. 2003. Carvan, ICARDA, Ethiopia, Issue No. 18/19.
- Bareja M and Lodha S. 2002. Enhancing the population of *Trichoderma harzianum* utilizing farm wastes. *Journal of Mycology and Plant Pathology* **32** (3) : 395–6.
- Chawla N and Gangopadhyay S. 2009. Integration of organic amendment and bioagents in suppressing cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini*. *Indian Phytopathology*. **62** (2) : 209–16.
- Cochran W G and Cox G M. 1957. *Experimental Designs*, Second Edition. John Wiley & Sons, Inc., New York.
- Dileep Kumar B S. 1999. Fusarial wilt suppression and crop improvement through two rhizobacterial strains in chickpea growing in soils infested with *Fusarium oxysporum* f. sp. *ciceri*. *Biol-fertil. Soils Berlin, Germany* **29** (1) : 87–91.
- Elad Y and Chet I. 1983. Improved selective media for the isolation of *Trichoderma* or *Fusarium* spp. *Phytoparasitica* **11**: 55–8.
- FAO. 2008. Tapes about statistics of food crops. Food and Agriculture Organization, Rome, Italy.
- FAO. 1993. *FAO yearbook production 1992*, vol. 46. FAO, Rome, Italy, pp 105–15.
- Fisher R A and Yates F. 1963. *Statistical Tables for Biological, Agricultural and Medical Research*. Oliver and Boyd, Edinburgh, London, 146 pp.
- Gangopadhyay S and Ram Gopal. 2010. Evaluation of *Trichoderma* spp. along with farmyard manure for management of *Fusarium* wilt of cumin (*Cuminum cyminum* L.) *Journal of Spices Aromatic Crops* **19** (1&2) : 57-60.
- Haware M P, Nene Y L, and Natarajan M. 1996. Survival of *Fusarium oxysporum* f. sp. *ciceri* in soil in the absence of chickpea. *Phytopathology Mediterranea* **35**: 9–12.
- Hervas A, Landa B and Jimenez-Diaz R M. 1997. Influence of chickpea genotype and *Bacillus* sp. on protection from *Fusarium* wilt by seed treatment with nonpathogenic *Fusarium oxysporum*. *European Journal of Plant Pathology* **103**: 631–42.
- Jalali B L and Chand H. 1992. Chickpea wilt. (In) *Plant Disease of International Importance*, Vol I, pp 429–44. *Diseases of Cereals and Pulses*. Singh U S, Mukhopadhyay A N, Kumar J and Chaube H S (Eds) Prentice Hall, Englewood Cliffs, NJ.
- Jayalakshmi S K, Raju S, Usha Rani S, Benagi VI and Sreeramulu K. 2009. *Trichoderma harzianum* L₁ as a potential source for lytic enzymes and elicitor of defense responses in chickpea (*Cicer arietinum* L.) against wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri*. *Australian Journal Crop Science* **3**(1): 44–2.
- Kaiser W J, Alcalá-Jiménez A R, Hervás-Vargas A, Trapero-Casas J L, Jiménez-Díaz R M. 1994. Screening of wild *Cicer* species for resistance to races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris*. *Plant Disease* **78**: 962–7.
- Khan M A and Gangopadhyay S. 2008. Efficacy of *Pseudomonas fluorescens* in controlling root rot of chickpea caused by *Macrophomina phaseolina*. *Indian Journal of Mycology and Plant Pathology* **38**(3): 580–7.
- Merkuz A and Getachew A. 2012. Management of chickpea wilt (*Fusarium oxysporum* f.sp. *ciceris*) using *Trichoderma* spp. *International Journal of Current Research*, **4** (5): 128–34.
- Mukhopadhyay A N, Shrestha S M and Mukherjee P K. 1992. Biological seed treatment for control of soil-borne plant pathogens. *FAO Plant Protection Bulletin* **40**: 100.
- Negi Y K, Garg S K, and Kumar J. 2005. Cold-tolerant fluorescent *Pseudomonas* isolates from Garhwal Himalayas as potential plant growth promoting and biocontrol agents in pea. *Current Science* **89** (12) : 2 151–6.
- Nene Y L. 1980. Diseases of chickpea. (In) *Proceedings of the International Workshop on Chickpea Improvement*, 28 February-2 March 1979, pp 171–8.
- Papavizas G C. 1967. Evaluation of various media and antimicrobial agents for isolation of *Fusarium* from soil. *Phytopathology* **57** : 848–52.
- Saikia R, Singh T, Kumar R, Srivastava J, Srivastava A K, Singh K and Arora D K. 2003. Role of salicylic acid in systemic resistance induced by *Pseudomonas fluorescens* against *Fusarium oxysporum* f.sp. *ciceri* in chickpea. *Microbiological Research* **158**(3): 203–13.
- Sethuraman R and Vidhyasekaran P. 1994. Effective control of pigeonpea wilt by *Pseudomonas fluorescens*. *Journal of Phytopathological Society*. **47** (3) : 289–90.
- Subhani M N, Sahi S T, Ali L, Hussain S, Iqbal J, Hussain N. 2013. Management of chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris* through antagonistic microorganisms. *CJPP*. **1**(1):1–6.
- Zaidi N W and Singh U S. 2004. Multiplication of *Trichoderma harzianum* on cowdung. *Indian Phytopathology* **57** : 189–92.