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# Field Efficacy of *Trichoderma harzianum* and *Rhizobium* against Wilt Complex of Chickpea

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**A B S T R A C T**

The experiment was conducted at farmer's fields of Village Nawgaon, Block-Bilha, Dist- Bilaspur (Chhattisgarh), India. The study was performed under assured irrigated condition of upland in paddy-chickpea-paddy cropping system. Studies focused on the efficacy of bio-control agents at natural field condition through evaluation and demonstration leads to maximum productivity of chickpea. The seed treated with *Trichoderma harzianum* + *Rhizobium* along with soil application of *T. harzianum* enriched FYM found most effective to reduce the incidence of wilt complex of chickpea. The same treatment combination was superior in case of root nodulation, fresh wet and dry wet of chick pea plants. Maximum yield obtained 8.44 q/ha in treatment (seed treatment of *T. harzianum* + *Rhizobium* along with soil application of *T. harzianum* enriched FYM) followed by Seed Treatment by *T. harzianum*+ *Rhizobium* (7.57q/ha). Integrated Disease Management (IDM) approach was carried out to combat chickpea wilt with a combination of bio agents and organic amendments. Soil application of bio agents enriched with FYM proved additive effect over the seed treatments.

## Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop in India has first ranks in production and consumption in the world. Although, chickpea is predominantly consumed as a pulse, dry chickpea is also used in preparing a variety of foods, processed foods, sweets and condiments and green fresh chickpeas are commonly consumed as a vegetable (Wallace *et al.*, 2016).

In India, productivity of chickpea is very low as compared to other countries due to various biotic and abiotic factors. More than fifty soil and seed borne pathogens have been reported on this crop which is causes significant crop losses. Among various soil borne diseases, wilt (*Fusarium oxysporum* f. sp. *ciceris*), dry root rot (*Rhizoctonia bataticola*), collar rot (*Sclerotium rolfsii*) are very destructive and appeared every year in Chhattisgarh (Nene *et*

*al.,* 1996; Ghosh *et al.,* 2013). The disease is challenged the cultivation of chickpea in Chhattisgarh in Paddy-Chickpea-Paddy cropping system due to uncomposed organic matter on the soil surface which help to persist the large number of fungi and bacteria.

The wilting pathogens are seed-borne in nature (Pande *et al.,* 2007) as well as soil borne (Jimenez-Fernandez *et al.,* 2011). The mycelium and chlamydospores can survive in seed and soil, and also infected crop residues, roots and stem tissue buried in the soil more than the six years in absent of host plant (Haware *et al.,* 1986). Pathogenic action appear as yellowing and drying of leaves from base upward, drooping of petioles and rachis, improper branching, withering of plants, browning of vascular bundles, finally wilting and necrosis of plants (Westerlund *et al.,* 1974; Leslie and Summerell, 2006) reported the initial symptom of the disease to be acropetal vein clearing of leaves. The chickpea wilt incited by soil and seed borne disease could be managed by the integration of various practices like using resistant varieties, seed treatment with chemicals, seed and soil application of bioagents and amendment of soils with oilseeds cakes (Nikam *et al.,* 2007). *T. harzianum* earlier proved as a potential bio-agent of soil borne plant pathogens (Kumar and Dubey 2001; Dubey, 2003). Unfortunately, most of the wild isolates of *Trichoderma harzianum* are highly sensitive to carbendazim, a commonly used fungicide as seed treatment in chickpea. Genetic modification of biocontrol agents through mutation by physical and chemical means has been used to produce biocontrol agents with greater tolerance to toxicants, enhanced antagonistic potential and improved survival in the agro-ecosystems (Mukherjee and Mukhopadhyay, 1993).

In present time, due to the use of harmful potential and conventional systemic and non-systemic fungicides to managing soil diseases are inadequate, uneconomical and cause ecological problem due to residues in soil and crops, many countries has big swings to use of eco-friendly protection methods. Due to the residual problem, export quality of many countries also reduces. In the present context, biological management of wilt complex with bioagents are safer, economical, effective, easily colonizes in the rhizosphere, the strong mechanism in soil pathogens with no residual effects on the arial plant parts (Dubey and Suresh, 2007; Panwar and Gaur, 2012). Keeping in the view, investigation were taken to minimize the incidence, study the efficacy of bio-control agents at natural field condition in small and large area through evaluation and demonstration leads to maximum productivity of chickpea.

## Materials and Methods

### Bioagents as seed treatments

To evaluate the efficacy of bio-agent and bio-fertilizer for effective management of wilt complex of chickpea, the experiment was conducted at farmers' fields of Village Nawgaon, Block- Bilha, Dist- Bilaspur (C.G.), India situated at Latitude 22°12'07.5"N and Longitude 82°07'59.6"E during rabi season of 2013-14, as need based study of the district Bilaspur. The study was performed under assured irrigated condition of upland in paddy-chickpea-paddy cropping system. The wilt resistance variety JG-74 was taken for experiments with a seed rate of 75 kg/hac and follow recommended standard agronomic practice. The experiments were conducted in Randomized block design and size of field under each treatment was 0.50 acres with four treatments and five replications as shown in table 1. All bioagents used in the present investigation were procured from SBCL, Thakur Chhedilal Barrister College of Agriculture and Research

Station, Bilaspur, India. Chickpea seeds were coated with the talc based formulation of antagonistic microorganisms, whereas combination of these two microorganism seeds were treated first by *Trichoderma* then apply jaggary solution (1%) followed by rhizobium culture (Godhani *et al.,* 2010). Untreated farmers practices were kept to serve as control.

## Soil application by Bio-agents

For the soil treatment *Trichoderma* inoculums is prepared with FYM in advance, mix 6.25 kg of *Trichoderma harzianum* (Talc based formulation) in 250 kg of well-rotted farm yard manures (FYM) and cover it for 07 days with gunny bags, sprinkle the heap with water intermittently. Turn the mixture in every 3-4 days interval. *Trichoderma* mycelium was proliferated throughout the FYM and it was mixed well, sieved and then used for soil treatment per hac (Ranasingh *et al.,* 2006; Nikam *et al.,* 2007; Godhani *et al.,* 2010; Jambhulkar *et al.,* 2015). Treated the seeds by *T. harzianum, Rhizobium* and both of them as described earlier @ 10g/kg of seed. Treated seeds and value added FYM is sown in the field with the seed cum fertilizer drill.

## Observation recorded and calculation

Wilt incidence was recorded after 30, 60 DAS (Days after sowing) and at harvesting time four (1×1 m) quadrates were randomly selected in each field and infected plants were counted in each quadrate.

Based on infected and total number of plants, disease incidence was calculated. Twenty-five plants from each field were uprooted to determine dry-matter production. Two-month-old plants were randomly uprooted from each field (10 plants/field) to count the root nodules. Pink and healthy nodules were counted as functional nodules, dark-brown and degenerated ones as nonfunctional nodules and other parameter fresh wet, yield were also recorded. The pathogens were identified based on the symptoms of plants and pathogen associated by serial dilution techniques on the basis of colony and conidial characteristics (Jalali and Chand, 1992).

Wilt incidence were expressed according to the following formula:

$$Wilt\ incidence\ (\%) = \frac{Total\ No.of\ wilted\ \ plant}{Total\ No.of\ \ plants\ observed} \times 100$$

The benefit cost ratio was also calculated based on the market rate and calculated as follows

$$BCR = \frac{Gross\,\mathrm{Re}turn}{Total\,\mathrm{cos}t}$$

## Statistical analysis

The calculated data was worked out from the observed data. Later the data subjected to simple RCBD (ANOVAs) during statistical analysis Panse and Sukhatme (1985).

## Results and Discussion

## Incidence of wilt complex

Result presented in table 2. indicated that the seed treated with *T. harzianum* + *Rhizobium* along with soil application of *T. harzianum* enriched FYM found most effective to reduce the incidence of wilt complex of chickpea (7.25%) and maximum reduction of mortality (74.80%) followed by combination of seed treatment by *Trichoderma harzianum*+ *Rhizobium* (8.21 and 71.50%) and *T. harzianum alone (*8.82 and 69.34 %), Rhizobium alone (12.26 and 57.38 %) incidence and reduction in mortality respectively, compared to the untreated

control 28.77 % mortality. All treatments significantly reduced the wilt complex in comparison of untreated control (Figure 1). Similarly, Jambhulkar *et al.,* (2015) reported bio-protectants applied to seeds and soil application with *T. harzianum* enriched FYM the incidence is only 5.8% compared to control 31.0% against *Rhizoctonia solani*.

The 68.62 per cent reduction in mortality against wilt of chickpea in integrated approaches as seed treatments with *Trichoderma* + PSB+ Rhizobium and soil application *Trichoderma* was reported by Singh *et al.,* (2016). Performance of *T. harzianum* against *Fusarium oxysporum f.sp. ciceri* was better (41.63%) than *Trichoderma viride* (Kala *et al.,* 2016). Dubey *et al.,* (2012) suggested that soil application + seed treatment with *Trichoderma* formulation enhanced the growth of plants and reduced the wet root rot of chickpea. Mahmood *et al.,* (2013) tested six biological agents under in vitro, glasshouse and field condition and found that 78.78, 63.95 and 60.79% reduction over control when treated the seed with *P. fluorescens, T. harzianum and Rhizobium* respectively. Prasad *et al.,* (2002) who had reported soil application of *T. viride* and *T. harzianum* one week before sowing as more effective in reducing wilt and wet root rot of chickpea.

The consortium (*T. viride* + *T. harzianum* + *T. hamatum*) found very effective for control of chickpea wilt due to synergistic effect. The results of present investigations are in conformity with the results of Amalraj *et al.,* (2012); Shabir-U-Rehman *et al.,* (2013); Rani and Mane (2014).

## Root nodulation, fresh wet, dry wet

Number of effective root nodulation per plant was found highest when chickpea seed is treated with combination of *T. harzianum* + *Rhizobium* and soil application of *T. harzianum* enriched FYM 38.23 nodules per plant followed by Rhizobium alone 36.80, *T. harzianum* + *Rhizobium* 35.90 and *T. harzianum* alone 34.40 nodules per plant. All treatments significantly increases the root nodulation compared to the untreated control 29.95. Similarly fresh wet of chickpea plant showed highest in combination of seed and soil treatments, 46.27 followed by *T. harzianum* + *Rhizobium* 45.93, *Rhizobium* alone 45.09 and *T. harzianum* 41.73g/plant. These treatments showed significantly differences compared to the control 37.37g. All culture treated treatment showed non-significant differences among each other except with *T. harzianum* alone. The dry weights of chickpea plants range between 18.30 to 31.28g. The highest dry weight recorded with seed and soil was treated by bio-agents and bio-fertilizers (31.28g) followed by combination of seed treatment (27.25g), *Rhizobium* alone (22.38g) and *T. harzianum* alone (21.91g) compared to untreated control (18.30g) (Figure 2). Individual treatment showed non-significantly differences among each other whereas combination of seed and soil treatments showed critical differences. Our findings are in conformity with the Maya *et al.,* (2012) recorded 35.20 root nodulation and 34.02g dry wet treated the chickpea seed with rhizobium culture. Rajsekhar *et al.,* (2016) reported 41.30 nodulation and 2.67g dry wet in treated with *T. harzianum*. The results of present investigations are in conformity with the findings of Shabir-U-Rehman *et al.,* (2013), Khan *et al.,* (2005), Amalraj *et al.,* (2012). *T. harzianum* earlier proved as a potential bio-agent of soil borne plant pathogens (Dubey, 1998, 2000; Kumar and Dubey, 2001; Dubey, 2003) was found effective against *Fusarium oxysporum* f. sp. ciceris, may be used alone or in combination with carboxin as a seed treatment for the management of the disease (Dubey *et al.,* 2007).

**Fig.1** Efficacy of bioagents against wilt complex of chickpea

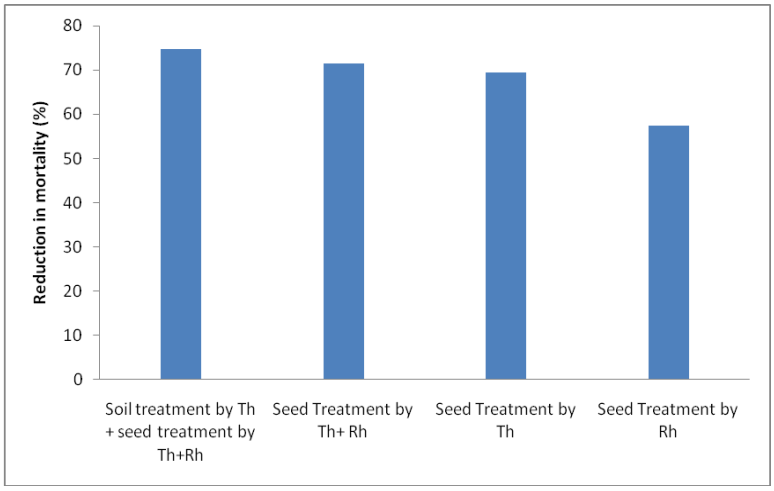


**Fig.2** Effect of bioagents on nodulation fresh wet, dry wet and yield of chickpea

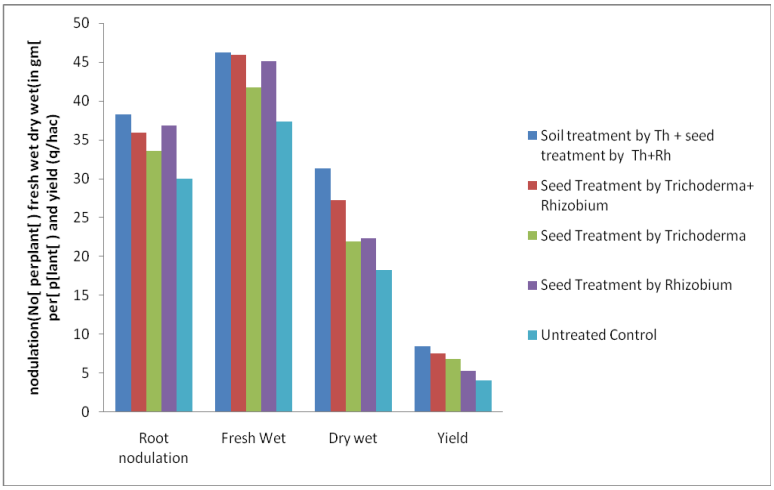


**Table.1** Treatments details used in experiment

| Sr. No. | Treatments | Dose /kg of seed |
|---|---|---|
| 1 | Seed treatment with *Trichoderma harzianum* 10% WP (CFUs $10^{10}$/g) | 10g |
| 2 | Seed treatment with *Rhizobium species*10% WP (CFUs $10^{10}$/g) | 10g |
| 3 | Seed treatment by *Trichoderma harzianum* and *Rhizobium species* | 10g |
| 4 | Soil treatment with *Trichoderma harzianum* + seed treatment with *T. harzianum* followed by *Rhizobium* 10% WP (CFUs $10^{10}$/g) | 250kg/hac of value added FYM and @10g/kg of seed |
| 5 | Untreated Seed (FP) | - |

FP= Farmer's Practice

**Table.2** Efficacy of bio-agent and bio-fertilizer against wilt complex of chickpea

| Treatments | Treatments Detail | Disease Incidence (%) | Root nodulation | Fresh Wet | Dry wet | Yield (q/ha) | B:C |
|---|---|---|---|---|---|---|---|
| $T_1$ | Seed Treatment by *Trichoderma harzianum* | 8.82 (17.18) | 34.40 | 41.73 | 21.91 | 6.82 | 1:2.37 |
| $T_2$ | Seed Treatment by *Rhizobium* | 12.26 (20.33) | 36.8 | 45.08 | 22.38 | 5.32 | 1:1.85 |
| $T_3$ | Seed Treatment by *T. harzianum*+ *Rhizobium* | 8.21 (16.51) | 35.9 | 45.93 | 27.25 | 7.57 | 1:2.58 |
| $T_4$ | Soil application with *T. harzianum* + seed treatment with *T. harzianum* followed by *Rhizobium* 10% WP (CFUs $10^{10}$/g) | 7.25 (15.52) | 38.23 | 46.27 | 31.28 | 8.44 | 1:2.95 |
| $T_5$ | Untreated Control | 28.77 (32.41) | 29.95 | 37.37 | 18.308 | 4.06 | 1:1.45 |
| | SEM+ | 1.82 | 1.89 | 1.71 | 1.25 | 0.41 | |
| | CD at 5% | 4.12 | 4.28 | 3.88 | 2.84 | 0.93 | |
| | CV % | 11.92 | 7.86 | 5.71 | 7.90 | 9.87 | |

## Yield and benefit cost ratio

Grain yields ranged from 4.06 to 8.44 q/ha, a similar trend was also recorded as found in earlier parameters. All treatments showed significantly differences in grain yields. Maximum yield obtained 8.44 q/ha in treatment $T_4$ followed by $T_3$ (7.57q), $T_1$ (6.82 q) $T_2$ 5.32 q/ha with least at untreated control 4.06 q/ha. Similarly highest benefit cost ratio was recorded in treatment $T_4$ (1:2.95) followed by $T_3$ (1:2.50), $T_1$ (1:2.37) and least with untreated control (1:1.45) (Table 2). Our findings are inconformity of Jambhulkar *et al.,* 2015 obtained yield of 14.18 q/ha treated the seed by *T. harzianum* + soil application of enriched *T. harzianum* FYM. Increases the grain yield of chickpea 52.16% and highest benefit cost ratio 3.61 (Singh *et al.,* 2016). Our findings are in agreement with the earlier findings of Ainmisha and Zacharia (2011), Amalraj *et al.,* (2012), Shabir-U-Rehman *et al.,* (2013) and Maya *et al.,* (2012). The tetra-inoculants (*R. leguminosarum* + *A. chroococcum* + *P. aeruginosa* + *T. harzianum*) should be used as effective biofertilizer for chickpea (*Cicer arietinum* L.) production under sustainable agriculture.

In conclusion, Chickpea seed sown after treated with *Trichoderma harzianum* + *Rhizobium@10g/kg* along with soil application of *T. harzianum* enriched FYM 250 kg/ hac to reduce the incidence of wilt complex of chickpea.

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