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Growth Promotion and *Fusarium* Wilt Disease Management Ecofriendly in Chickpea by *Trichoderma asperellum*

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Abstract

Chickpea, *Cicer arietinum* L., is one of the best legumes for human consumption and widely grown in India, and other parts of the world. *Fusarium* wilt caused by *Fusarium oxysporum ciceri*, is the most important disease of chickpea throughout the world and particularly in the India. The effect of 10^8 spore/ml of *T.asperellum* gave maximum (96.00 a) percentage of seed germination. In minipot trial, there were 25% increased of percentage of germination of seeds and shoot length but 27% increase of root length and 45% increase of vigour index in compare to control. In microplot trial, 75.25 % and 67.15 % crop protection respectively in two consecutive years were achieved by applying *T asperellum*. Similarly pulse yields and plant dry weight (25.9 & 30.12) were higher in compare with control and than both *F.oxysporum ciceri* and *F.o.c.+ T. asperellum* treated plots. Microplot field trials *T.asperellum* treated plots gave higher number of pods per plant (47.25 ; 44.50) than control, *F.oxysporum ciceri* treated, and *F.oxysporum ciceri + T.asperellum* treated plots (8.25 & 12.25). *T. asperellum* treated plants yielded maximum functional nodules. Therefore, *T.asperellum* is very active growth promotion including enhancing functional root nodules and fusarial wilt management in chickpea crop. This biocontrol agent (BCA) and Plant growth promotion (PGP) agent in a dose of 10^8spore /ml by seed dressing may be alternative to chemical fertilizers and fungicides in chickpea cultivation and other crops.

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Keywords

Biocontrol, growth promotion, *T.asperellum*, Chickpea, root nodule.

Introduction

Soil fertility or status of NPK (Nitrogen, Phosphate and Potassium) including micronutrient has the profound role in plant growth. Lack of soil nutrients such as NPK and plant disease continue to threaten crop production in modern agriculture and play a direct role in net loss of crop productivity in agriculture. With the advent of chemical fertilizers and pesticides /fungicides, it was thought that a permanent and reliable solution of soil fertility and crop pathogens have been achieved but it has been proved that chemical fertilizers and pesticide application are not safe to the environment as the toxicants cause environmental pollution and has harmful effects on human beings (PAN, 2007). Chemical fertilizers may have harmful effects on the soil and its life, especially when they are very concentrated and water soluble (Smith *et al*., 2008). Ammonium sulphate is a very strong biocide, hindering nitrogen fixation and killing nematodes and earthworms. Superphosphate has a

negative effect on free-living nitrogen-fixing bacteria **(**Primavesi and Primavesi, 1990). Nitrate levels above 10 mg/L(10 ppm) in groundwater can cause 'blue baby syndrome' (acquired methemoglobinemia), leading to hypoxia (which can lead to coma and death if not treated) (Ward *et al*., 2005; Powler, 2006).Unfortunately to gain a target crop production with chemical fertilizers and pesticides, over 100 species of non target organisms are adversely affected (Alabouvette and Couteadier, 1992). Despite realization of adverse effects of chemical fertilizers and pesticides on plants, animals and environment, they are being applied indiscriminately (Eckert and Ogawa,1988; PAN, 2007) Current problems include the continued development of fungicide resistance among pathogens **(**Spotts and Cervantes, 1986; Holmes and Eckert, 1999; Kanetis *et al*., 2008; Smilanick, 2011) Recently, US Dept of Health and Human Services (2008) reported that the chemicals benomyl, carbamates and mancozeb are carcinogenic. The triazoles fungicides are causing reproductive defect of male (Goetz *et al*., 2007) and female mice (Rockett *et al*., 2006). All humans now carry a body burden of persistent pesticides, many of which are linked to chronic health effects (Schafer *et al*., 2004; PAN, 2007). Some pesticides are even carcinogenic and causing some human cancer such as colorectal cancer (Lee *et al*., 2007), breast cancer (Abdalla *et al*., 2003), leukemia and non-Hodgkin's lymphoma in childhood (Meinert *et al*., 2000). However, the potential impact on environment as well as health largely limits their application (Eckert *et al*., 1994). Hence, to reduce the use or dose of chemicals, one possibility is to utilize the disease suppressing activity and plant growth promoting capacity of certain microorganisms in agrifields which should be highly ecofriendly. Such microorganisms are commonly referred to as biological control (biocontrol) agents (BCA) and plant growth promoters (PGP).and their commercial formulations are as biopesticides and biofertilizer.

The soil biology includes the rhizosphere concept (soil immediately surrounding plant roots) which was first introduced by Hiltner (1904) where microbe populations are stimulated by root activities (Mc. Cully, 2005). *Trichoderma* sps are active rhizosphere colonisers (Tronsmo and Harman, 1992) and act as BCA and PGP. The use of PGP/BCA offers an attractive way to replace chemical fertilizer, pesticides, and supplements; most of the isolates result in a significant increase in plant height, root length, and dry matter production of shoot and root of plants. Microorganisms such as *Bacillus, Rhizobium and Pseudomonas* amongst bacteria, and *Aspergillus* sp, *Penicillium* sp *Trichoderma* sp amongst fungi offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants (Sharma, 2003; Chen *et al*., 2006). Current developments in our understanding of the functional diversity, rhizosphere colonizing ability, mode of actions and judicious application are likely to facilitate their use as reliable components in the management of sustainable agricultural systems (Zaidi *et al*., 2009). It has also been established that biocontrol agents enhance growth by producing growth stimulating factors (Windham *et al*., 1986, Ponmurugan and Baby, 2006b).

Trichoderma has a superior capacity to mobilize and take up soil nutrients compared to other organisms (Chet *et al*., 1997). Enhanced growth response of several plants, such as tomato(Ozbay *et al*., 2004; Vinale *et al*., 2008), bean (Inber *et al*., 1994), cucumber (Kleifield and Chet, 1992) pepper, lettuce (Vinale *et al*., 2008) were recorded by application of *Trichoderma.* The application of *Trichoderma* increased both root and shoot growth of plant. There are numerous soil microflora involved in the synthesis of auxins in pure culture and soil. Some of the P-solubilizing bacteria and fungi act as plant growth promoters due to their ability to produce IAA (Souchie *et al*., 2007). Biological Control of pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods and also found that many isolates of *Trichoderma* spp. by producing non-volatile metabolites, volatile metabolites, enzymes which were active against a range of pathogenic fungi (Chet *et al*., 2009; Barakat *et al*., 2006; Karthikeyan *et al*., 2006; Eziashi *et al*., 2007; Mukhopadhyay, 2009). *T. harzianum* colonizes *S. rolfsii* hyphae, disrupts mycelial growth and kills this pathogen. *Trichoderma* are used as biopesticide, biofertilizer or fertility promoter (Harman *et al*., 2004; Harmen, 2006; Vinale *et al*., 2008). Chickpea, *Cicer arietinum* L., is one of the best legumes for human consumption and widely grown in India, Northern Sudan and other parts of the world. It is the world's fourth most important pulse crop after soybeans (*Glycine max* L.), beans (*Phaseolus vulgaris* L.) and peas (*Psium sativum* L.) (FAO, 2012). In India, chickpea is ranked first in terms of production and consumption in the world. About 65% of global area with 68% of global production of chickpea is contributed by India (Amarender and Devraj, 2010). *Fusarium* wilt caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceri* (Padwick) Erwin. Matuo and Sato, is the most important soil-borne disease of chickpea throughout the world and

particularly in the Indian Subcontinent, the Mediterranean Basin and California (Nene and Reddy, 1987; Dubey *et al*., 2007). The International strategies include minimum use of chemicals for checking the pathogen, pollution, encouragement of beneficial biological agents to reduce pathogen inoculums (Bendre and Barhate, 1998; Harman and Kubicek, 1998). The main objectives of this work are to apply one strain of *Trichoderma asperellum* in chickpea field for growth promotion and *Fusarium* wilt management ecofriendly without applying chemical fertilizers and fungicides for clean environment.

Material and Methods

Isolation and purification of fungal antagonist

Fungus was isolated from agrisoil; by dilution plate technique in PDA and Rose Bengal Agar medium supplemented with antibiotic at 28±2^{O}C temperature. Purification was done by single hyphal tip method (Dhingra and Sinclair, 1986).

Phenotypical Identification of fungal antagonist

The fungal isolate was phenotypically identified with cultural characteristics like culture growth rate, colony texture, margin, color, exude, reverse plate characters etc. and microscopical (morphological) characterstics i.e. spore length and breadth, spore type, spore septum, hyphal type, septum formation. The collected data comparing with fungal published key (Domsch *et al*., 1980) revealed the identity of the fungus.

Molecular Identification of fungal antagonist by PCR method

Genomic DNA extraction

Genomic DNA of fungus was extracted by modified CTAB method (Chutima *et al*., 2010). and purified by fungal DNA Purification Kit (HiMedia Laboratories Pvt. Ltd.). The concentration of DNA was checked by electrophoresis in 1% agarose gel stained with ethidium bromide under UV light.

PCR of ITS1-5.8S- ITS-2 of r DNA

Genomic DNA from fungus was extracted and the internal transcribed spacer (ITS1-5.8S-ITS2) regions were amplified by PCR technology using with DNA amplification reagent kit manual (GeNei) along with fungus specific forward primer ITS-1 F (CTTGGTCATTTAGAGGAA-GTAA) (Gardes and Bruns,1993) and the reverse primer ITS-4 (TCCTCCGCTTATTGATA-TGC) (White *et al*., 1990). PCR was carried out using the following protocol, modified from Gardes and Bruns (1993); initial denaturation at 95˚C for 85s, followed by 37 cycles of denaturation at 95˚C for 30s, annealing at 55˚C for 50s, extension at 72˚C for 10 min. The reaction was held at 4˚C. Amplicon was electrophoresed in a 1% Agarose gel stained with ethidium bromide and visualized under UV. Concentration of the amplicon was checked in UV-Vis Spectrophotometer, the ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA. A ratio of ~1.8 is generally accepted as "pure" for DNA. The amplicon was purified using Gene JET gel Extraction Kit (Thermo Scientific).

Gene Sequencing

Sequencing of PCR product was carried out in Sci Genome Labs Pvt Ltd, Kerala, India and the sequence obtained was submitted to NCBI Gene Bank. The sequence analysis was carried out using bio-informatics tool BLAST of NCBI.

Gram plant protection and plant seedling growth promoting experiments

Preparation of spore suspension of *T.asperellum* antagonists

Trichoderma asperellum was subcultured in slants of PDA for ten days. After ten days, cotton plugs of culture tubes were unplugged and 10 ml of sterilized distilled water was filled inside. Then cotton plug again fitted. The culture tubes were jerked for 15 minutes for dislodging the spores in water. Then water with spores was taken out in beaker. The number of spore/ml was measured by haemocytometer. The spore suspension was made as per requisition by adding sterile water.

Preparation of inoculum of *F. oxysporum ciceri* for field trial

Pure culture of the wilt fungus *F. oxysporum ciceri* was cultured in laboratory. The fungus was mass cultured on broken maize seeds. The seeds were soaked overnight in a 5% sucrose and 30 mg /L chloramphenicol solution. The soaked seeds were transferred to 500 ml conical flasks and autoclaved twice at 15 kg cm^{2}, 121^{0}C, for 20 minutes. Thereafter, the flasks were inoculated with pure

culture of *F. oxysporum ciceri* and incubated at 28 ± 2^0C for 10 days in an incubator. For soil inoculation, fungus colonised seeds (500 g) were ground in a mixer-grinder and asuspended in 10 ml tap water. The suspension (10:1) was spread uniformly on a microplot of 3 x 2 m to achieve an inoculums level of 1.5 g colonised seeds kg $^{-1}$ soil. Soil inoculation was done two days before seed was sown. Pots were arranged in randomized design. After sowing the treated or untreated seeds, minipots were observed every day for pre- emergence and post-emergence wilts of seedlings. The number of pre-emergence and post-emergence wilted seedlings or non wilted seedlings was recorded. The data were statistically analysed.

Effect of *T. asperellum* on seed germination, root length & shoot length of chickpea

i) Petri plate culture: A suspension of spore (10^8/ml) fungal was made in sterile distilled water. 10 gms of fresh & disease free seeds of chickpea c.v. B-110 were dipped in 100ml suspension of spore of antagonist for overnight, then they were placed in the wetted blotting papers placed in petri dishes. In each petri dish 20 seeds were taken. Five sets were prepared. The sets were placed in room temperature. Control or untreated sets were also arranged. Seedling raised from seeds treated with *T.asperellum* recorded and vigour index values based on germination and seedling length were also calculated statistically by the following formula (Abdul-Baki and Anderson, 1973)

Vigour index = [Mean Root length + Mean Shoot length] x Percentage of Seed germination

ii) In minipot culture: Twenty minipot (plastic tea cup) were filled with fertile soil. A suspension of spore (10^8/ ml) fungal antagonist was made in sterile distilled water. Ten gms of fresh & disease free chickpea seeds were dipped in 100ml suspension of spore of antagonists for overnight, then they were sown in minipot. In each pot one seed was sown. One control set (10 minipot each with one seedling) where seeds were soaked in sterile water over night was arranged. After 15 days the treated & non-treated seedling of chickpea were uprooted and the soils adherence on roots were washed. The water of washed seedling was blotted by blotting paper. The root length, & shoot length were measured by mm scale. The number of root branches of both treated and control (untreated) was recorded. The data collected were statistically (t –test) analysed.

Effect of *T.asperellum* on chickpea protection from wilt disease

Hundred minipot (plastic tea cup) were filled with fertile soil. A suspension of spore (10^8spores/ ml) *T. asperellum* was made in sterile distilled water. 100gms of fresh & disease free chickpea seeds were dipped in 100ml suspension of spore of the antagonist for overnight, then they were sown in minipot. In each minipot 5ml of spore suspension (10^8spores/ ml) of *F. oxysporum ciceri* and 5ml of spore suspension (10^8spores/ ml) of fungal antagonist. One seed was sown in each minipot. Similarly a suspension of spores (10^8spores/ ml) of *F. oxysporum ciceri* was made in sterile distilled water. 100gms of fresh & disease free chickpea seeds were dipped in 100ml suspension of spores for overnight, then they were sown in minipot and then only 5ml of spore suspension of *F. oxysporum ciceri* was mixed in these miniport .One control set (10 minipot each with one seedling) where seeds were soaked in sterile water over night was arranged. For each treatment 10 minipots were taken. All treated or control (untreated set) was run. The number of germinated and non-germinated seeds was recorded and percent of seed germination was calculated. The length of shoot and root of germinated seeds were measured by mm scale or mm graph paper by 24 hr. intervals up to 15 days.

Effect of *T.asperellum* on protection of chickpea from wilt and growth promotion in mini plot field trial

A field of 25 x 15 m was prepared in which 30 microplots (3 x 2 m) were demarcated by 25 cm wide, raised margins. Each treatment was applied on three micro- plots, distributed in a completely randomized block design in the field. A suspension of spore (10^8spores/ ml) *T.asperellum* was made in sterile distilled water. 100gms of fresh & disease free chickpea seeds were dipped in 100ml suspension of spore of *T. asperellum* for overnight, then they were sown in three rows (57 seeds /row) in the micro -plots, irrespective of whether the wilt fungus was added into the soil. The field was irrigated a week after sowing. At maturity, four months after sowing, twenty –five plants from each microplot were uprooted to determine dry matter production and grain yield. Two month old plants were randomly uprooted from each microplot (10 plants / micro-plot) to count the root nodules. Pink and healthy nodules were counted as functional nodules, dark-brown and degenerated ones as non-functional nodules. Wilt incidence (%) and wilt severity were recorded on two and a half month old plants. Wilt severity (%) was

scored on a 0-5 scale: 0=no wilt; 1= 1-20% : 2= 21-40% ;3= 1-60% ;4= 61-80% ; 5= 81-100%

The rhizosphere population of the wilt fungus and the bio-agents was estimated monthly from December to April using the dilution plate method. The *Fusarium oxysporum f. sp. ciceri* was identified on the basis of colony and conidial characteristics (Jalali and Chand, 1992). Wilt incidence and severity were expressed according to the following formulae:

$$\text{Wilt incidence (\%)} = \frac{\text{No. of wilted plants in a microplot}}{\text{Total No. of plants in a microplot}} \text{X } 100$$

$$\text{Wilt severity (\%)} = \frac{\text{Wilted branches in a plant}}{\text{Total No. of branches in a plant}} \text{X100}$$

The experiment was conducted for two successive growing seasons (20012-2013 & 2013-2014) under identical conditions. Observations from the twenty-five plants of each microplot were averaged and considered one replicate.

Since three microplots were used for each treatment, there were therefore three replicates. Data on plant growth and yields were recorded. Wilt incidence was angularly transformed before analysis. Least significance difference (LSD) was calculated at P =0.05 for all variables to compare individual treatments.

Results and Discussion

Phenotypical Identification of Fungi

Phenotypically the fungus was identified as *Trichederma asperellum.*

Molecular identification by ITS 1-5.8S-ITS 2 of 18S r DNA

BLAST analysis was done with help of NCBI nucleotide sequence analysis; data analysis of nucleotide sequence (632 bp) of isolated fungi was showed 100% homology with published sequence of *Trichoderma asperellum.* The sequence was submitted in NCBI Bankit and published with the Gene Bank accession number KM604669.1

Effect of *T. asperellum* on seed germination of Chickpea in Petri dishes

The effect of concentration of spore *Trichoderma asperellum* on seed germination of chickpea indicated that 10^8 spore/ml of *Trichoderma asperellum* gave maximum (96.00 a) percentage of seed germination followed by 10^7, 10^6, 10^5 spores/ml (Table 1). Seedling raised from seeds treated with *T.asperellum* recorded significantly higher vigour index (2158.30) values based on germination and seedling length which were more than control (1454.17) (Table 4). The above result agreed with the finding of Krishanmoorthy (1987) and he reported increase germination & seedling vigour of tomato and chilli respectively by *Trichoderma*. Moreover, some workers (Benitez *et al*., 1998; Kumar and Dubey, 2001) also reported beneficial effect of *Trichoderma*s on seed germination.

Effect of *Trichoderma asperellum* on growth promotion of Chickpea in mini pot trial

In mature disease free seeds (10) of chickpea were dipped in spore suspension (10^8spore /ml) for 24 hrs and sown in minipots in randomized block design. 10 seeds were dipped in sterile distilled water and sown in 10 minipots and it was treated as control. After 15 days shoot length, root length and branches of roots of 10 treated plants were 17.9 cm, 4.7 cm and 10.9 cm respectively while in control they were 13.3 cm, 7.4 cm and 3.8cm respectively (Table 2). After data were statistically analysed (t-test), it was interesting to note that *T.asperellum* increases significant length of shoot and number of root branch of chickpea. But effect of *T.asperellum* on the length of main roots was not statistically significant at 5% and 1% level. On the other hand, the calculated t value of root length (1.746) is smaller than tabulated value (2.262) at 0.05 levels and tabulated value (3.250) at 0.01 levels at 9 df. So Null hypothesis is accepted. Therefore, it can be calculated that main root length of chickpea treated with *T.asperellum* is not significantly different from main root length of chickpea (control untreated).

Calculated t value of shoot length (6.73) and root branches (5.428) are greater than tabulated t value (2.262) at P = 0.05 level and also than tabulated t value (3.250) at P = 0.01 level at 9 df. Thus the null hypothesis is rejected. It can be concluded that Shoot length and

root branch of Chickpea of *T. asperellum* treated plants are statistically significantly different from control (untreated).There was 25% increased of percentage of germination of seeds and shoot length but 27% increase of root length and 45% increase of vigour index of *T. asperellum* treated Chickpea in compare to control (Table 2).There was a reduction in the morphological characters of the Sorghum in control but *Trichoderma* treatments, there was 40% increased shoot, root length, fresh weight dry weight and vigour index of the plant, when compared to control. The reason for the increase in growth parameters and vigour index may be certain plant growth hormones and secondary metabolites produced by *P. fluorescens* and *T. harzianum* which are known to have increased growth rate as reported by Lynch and Hobbie (1991) and Kimura *et al*. (1992).

The study of protection of chickpea from wilt disease by applying *T. asperellum* in minipot trial

Spore suspension of *T. asperellum* was prepared and diluted according to requirement; seeds of chickpea were dipped over night before sowing. In pot trial the study of protection of wilt disease by *T. asperellum* was conducted. The results (Figure 2) revealed that minipots treated with *F. oxysporum ciceri* caused 95% wilt of Chickpea but when both *F. oxysporum ciceri* and *T. asperellum* were treated in the pots, *T. asperellum* checked 70% of wilt of Chickpea. It suggested that without using any fungicides, Chickpea can be protected from wilt by seed treatment with *T. asperellum* (10^5/spore/ml). As it was minipot trials, field trial may proved its actual potentiality.

The study of protection of chickpea from wilt disease and Plant growth promotion (Number of pods/plant, Yield/plant, Plant dry weight and functional nodules)in two years (2012-13 & 2013-14) mini plot field trials by applying *T .asperellum*

The study of protection of chickpea from wilt disease by applying *T. asperellum* in microplot field trial was conducted for two years (2012-13, 2013- 14). It revealed that 75.25 % and 67.15 % crop protection respectively in two consecutive years were achieved by applying *T. asperellum* in seed treatment (Fig 3). There was a significant decline in the yield of chickpea infected by *F. oxysporum ciceri* (Jalali and Chand, 1992). Moreover for plant growth promotion (Number of pods/plant, Yield/plant, Plant dry weight and functional nodules) two years (2012-13 & 2013-14) microplot field trials (Table 4) showed that in both year *T. asperellum* treated plots gave higher number of pods per plant (47.25 ; 44.50) than control, *F. oxysporum ciceri* treated, and *F.oxysporum ciceri* + *T. asperellum* treated plots (8.25 & 12.25). Similarly pulse yields and plant dry weight (25.9 and 30.12) were higher in compare with control, and then both *F. oxysporum ciceri* and *F.o.c.* + *T. asperellum* treated plots. The two month- old plants were randomly uprooted from each microplot (10 plants/ microplot) to count functional and non-functional nodule. It revealed that *T. asperellum* treated plants yielded maximum functional nodules. The number of functional nodules increased by 33% with *T. harzianum* (Khan *et al*., 2004). That there is a significant decline in the yield of chickpea infected with *F. oxysporum* f. sp. *ciceri* has already been reported in India (Jalali and Chand, 1992). Infection with *F. oxysporum* f. sp. *ciceri* suppressed the rhizobial nodules on the roots of chickpea plants. The bioagents significantly increased the number of functional nodules. Rhizobacteria (*Pseudomonas* spp.) and mycoparasites (*Trichoderma* spp.) are known to synergise *Bradyrhizobium* spp. (Khan *et al.,* 2004). .Effectiveness of *T. viride*, *T. harzianium* and *T.* (*Gliocladium*) *virens* against chickpea wilt complex has also been reported by Sonawane and Pawar (2001), Tewari and Mukhopadhyay (2001) and Gupta *et al.* (2003).Application of *Trichoderma* as BCA can bring substantial changes in plant metabolism to promote plant growth, increase nutrient availability, improve crop production and enhance disease resistance (Harman *et al*., 2004).

In chickpea, *T. harzianum,* enhanced the top length, pod numbers, total dry biomass and yield than control (Rai and Singh, 2004). Biological control of *F. oxysporum ciceri* by non pathogenic *Fusarium* was recorded by Kaur *et al*., (2003) Similarly, the results of this field trial were corroborated with the findings of former workers (Khan *et al.,* 2004). The pool data of results indicated that *T. asperellum* treated plants gave 21.12% better yield of number of pod, 40% better pulse yield, 35.51% of better yield of functional nodules in compare to *F.oxysporum ciceri* treated and 11.33% of increase of number of Pod, 18.43% of pulse yield, 15.83 of plant dry weight and 17.58% of functional nodules in compare of control (untreated). Certain isolates of *Trichoderma*, from the rhizosphere of some cultivated crops, displayed the activity of plant growth promotion as well as disease suppression (Hyakumachi, 1994; Chang *et al*., 1986).

The mean values of top length, total dry biomass of rice showed significant increase when treated with *T. harzianum* in comparison to control (Rai and Singh,

2004). Andrabi *et al*. (2010) conducted experiments for controlling wilt disease of chickpea by various strategies and recorded that seed coating with *T. asperellum* resulted in minimum disease incidence (9.24%). The primary mechanisms of disease suppression by BCA are production of antimicrobial secondary metabolites such as siderophore (Neilands, 1981), antibiotics (Keel *et al*., 1992), volatile substances (eg HCN, NH_3) (Wei *et al*., 1991; Ghosh *et al*., 2014), plant hormones (Sharaf and Farrage, 2004), and other mechanisms. *Trichoderma* utilizes mycoparasitism to mitigate phytopathogenesis (Harman and Kubicek, 1998; Pan and Ghosh, 1997) and antibiosis (Sivasithamparam and Ghisalberti, 1998). The efficacy of *Trichoderma* as BCA is believed to involve antibiotic production(eg. gliotoxin, viridin), ethyl acetate (Claydown *et al.,* 1987) and some cell wall degrading enzymes (Larito *et al.,* 1976; Bello *et al.,* 1997,Vinale *et al*., 2009; Howell, 2003).

Trichoderma species produces growth factors which increase the rate of seed germination (Benitez *et al*., 1998; Kumar and Dubey, 2001). Some studies reported that the reduction in disease incidence and increase in seed germination lead to higher yield in *Trichoderma* treated seeds and soil (Podder *et al*., 2004). 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 or field conditions (Harman *et al*., 2004).

Table.1 Effect of concentration of spore of T .asperellum on seed germination of Chickpea

Concentration of spore of *T. asperellum*	Percentage of seed germination
10^4	77.25 c (61.55)
10^5	80.75 c (64.01)
10^6	90.00 b (71.56)
10^7	95.50 a (77.75)
10^8	96.00 a (78.46)
Control	70.25 d (56.98)

Note: Mean values followed by a different letter indicated significant different (P=0.05), according to Duncan's multiple range test. (Data in the parentheses are angular trnsformed value of percentage)

Table.2 Effect of T.asperellum on growth promotion of Chickpea in mini pot trial

Plant No.	Shoot length(cm)		Root length(cm)		Root branch(cm)	
	Treated	Control	Treated	Control	Treated	Control
1.	18	15	02	11	04	03
2.	20	12	03	04	16	04
3.	19	14	07	09	08	03
4.	18	11	05	04	12	Nil
5.	16	13	05	10	12	04
6.	20	13	07	04	16	04
7.	18	13	05	05	12	05
8.	16	15	05	06	08	04
9.	17	12	02	05	09	05
10	17	15	06	16	12	06
Mean	17.9	13.3	4.7	7.4	10.9	3.8

Table.3 Calculated t- value, tabulated t- value of shoot, root length and root branches

	Shoot length	Root length	Root branches
Calculated t value	6.73	1.746	5.428
Tabulated t value (P=0.05)	2.262	2.262	2.262
Tabulated t value(P=0.01)	3.250	3.250	3.250

Table.4 Effect of *T. asperellum* on shoot length, root length, vigour index of Chickpea

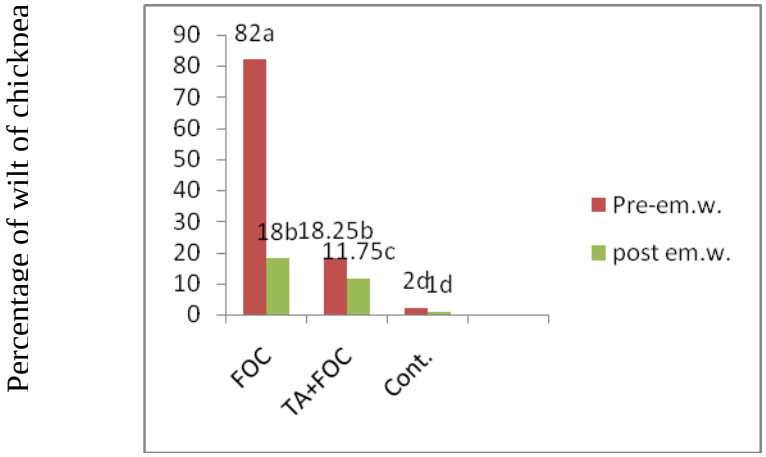
Sl no.	Treatment	Percentage of seed germination (%)	Shoot length (cm)	Root length (cm)	Vigour Index (VI)
1.	*T. asperellum*	95.50	17.9	4.7	2158.30
2.	Control	70.25	13.3	7.4	1454.17

Table.5 Effect of seed treatment on dry matter, yield, nodulation, wilt incidence and severity of chickpea plants inoculated with *Fusarium oxysporum* f. sp. ciceri

Treatment	No. of pods per plant			Yield plant (per g)			Plant dry weight (g)			Nodules/root system					
										Functional			Non-functional		
	2012-13	2013-14	Pool	2012-13	2013-14	pool	2012-13	2013-14	Pool	2012-13	2013-14	Pool	2012-13	2013-14	Pool
Control	39.10	42.25	81.35	7.22	9.50	16.72	21.9	25.25	47.15	35.00	14.20	49.20	39.00	12.20	51.20
F.oxysporum ciceri	32.00	40.37	72.37	5.05	7.25	12.30	17.5	19.50	37.00	26.0	12.50	38.50	32.00	10.20	42.20
T. asperellum	44.50	47.25	91.75	8.25	12.25	20.50	25.9	30.12	56.02	46.7	13.00	59.70	50.25	14.00	64.25
F.oxysporum ciceri + T. asperellum	35.00	42.25	77.25	6.50	8.15	14.65	23.2	26.50	49.70	32.05	13.8	46.05	36.00	14.20	50.20
SEm± CD (P≤0.05)	1.893 ± 2.341	0.987± 1.987		0.0123± 1.895	0.123 ± 1.564		1.987± 3.123	2.023 ± 3.786		2.675± 3.563	1.023 ± 2.060		1.993± 2.451	2.785± 4.312	

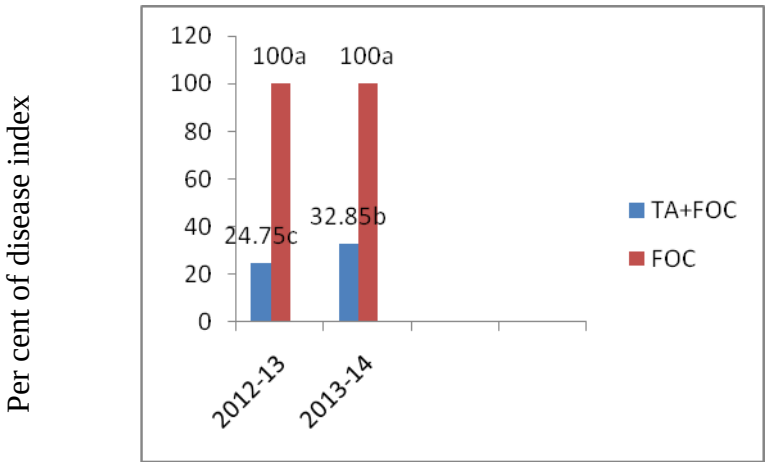
Fig.1 Effect of *Trichoderma asperellum* on growth promotion of Chickpea in mini pot trial (A) & measurement of the plants (B)

Fig.2 Percentage of pre- emergence and post emergence of wilt of chickpea with *F. oxysporum ciceri*, F.*oxysporum ciceri*+ *T. asperellum* and control(untreated)



Note: Mean values followed by a different letter indicated significant different (P=0.05), according to Duncan's multiple range test. (TA= *Trichoderma asperellum;* FOC=*Fusarium oxysporum ciceri*. ;Cont.=Control)

Fig.3 Biocontrol of wilt of chickpea by *T. asperellum* in mini plot trial



Note: Mean values followed by a different letter indicated significant different (P=0.05),according to Duncan's multiple range test. (TA= *Trichoderma asperellum;* FOC=*Fusarium oxysporum ciceri*.)

The growth promotion by *Trichoderma* may be due to production of secondary metabolites which may act as auxin like compounds (Vinale *et al*., 2009). Secondary metabolite such as harzianolide, antraquinoues, T39 butinolide isolated from *Trichoderma* were responsible for increase growth of wheat (Vinale *et al*., 2008) while harzianic acid increased the growth of canola (Vinale *et al*., 2009). Altomare *et al*. (1999) recorded that *T. harzianum* could improve nitrogen use efficacy and could solubilize a number of poorly nutrients such as Mn^{4+} , Fe^{3+} and Cu^{2+} ,etc enhancing more plant growth and development. The capacity of phosphate solubilization by *Trichoderma* sp and iron chelating siderophores production are other factors for plant growth promotion (Altomare *et al*., 1999; Benitez *et al*., 2004).

Conclusion

In conclusion, *Trichoderma* sp have capacity of growth promotion and crop protection from Fusarium wilt disease in agifields. This study revealed that 75.25 % and 67.15 % crop protection respectively in two consecutive years were achieved by applying *T. asperellum* in seed treatment. Mini plot field trials showed that in both year *T. asperellum* treated plots gave higher number of pods per plant (47.25;44.50) than control, *F.oxysporum ciceri* treated, and *F.oxysporum ciceri* + *T. asperellum* treated

plots (8.25 & 12.25). Similarly pulse yields and plant dry weight (25.9 & 30.12) were higher in compare with control, and than both *F.oxysporum ciceri* and *F.o.c.* + *T. asperellum* treated plots.

It revealed that *T. asperellum* treated plants yielded maximum functional nodules for N-nitrogen fixation and increases root branches allowing roots for more nutrient uptake. Other mechanism for growth promotion by our treated *T. asperellum* may be P-solubilization, siderophore production and other secondary metabolite production as reported by other workers. The efficacy of *Trichoderma* as BCA is believed to involve antibiotic production and secretion of hydrolytic enzymes and mycoparasitism. Our selected dose of spores (10^8spores/ml) yields maximum seed germination of Chickpea and this dose of *T. asperellum* by seed dressing technique gave appreciable crop protection and crop growth promotion .The present finding may suggest that application of *Trichoderma asperellum* in crop field may reduce or alternative to the application of chemical fertilizer and chemical fungicides to clean environment.

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