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# Integrated management of Fusarium wilt of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceris* with microbial antagonist, botanical extract and fungicide

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**The present study was carried out to assess the efficacy of an integrated management strategy for Fusarium wilt of chickpea that combined the use of microbial antagonist, botanical extract and fungicide. Before setting the experiment in field micro plots, a series of *in vitro* and *in vivo* experiments were conducted to select a virulent isolate of *F. oxysporum* f. sp. *ciceris*, an effective antagonistic isolate of *Trichoderma harzianum*, a fungitoxic botanical extract and an appropriate fungicide. The isolate FS1 of *F. oxysporum* f. sp. *ciceris* appeared to be most virulent to chickpea cultivar BU-Chola-1 and selected as test pathogen. Among the 20 isolates screened, *T. harzianum* isolate T-75 showed the highest (75.89%) inhibition of the radial growth of *F. oxysporum* f. sp. *ciceris* in dual culture assay on PDA. Absolute inhibition (100.00%) of colony growth of *F. oxysporum* f. sp. *ciceris* was observed where fungicide Provax-200 at 100 ppm was used. *Azadirachta indica* leaf extract gave maximum inhibition (55.19%) of radial growth of *F. oxysporum* f. sp. *ciceris* at all concentrations. The integration of soil treatment with *T. harzianum* isolate T-75 and *Az. indica* leaf extract and seed treatment with Provax-200 appeared to be significantly superior in reducing Fusarium wilt and in improving seed yield of chickpea compared to any single or dual application of them in the field. The results of this study exhibit the importance of integrating selective microbial antagonist, botanical extract and fungicide to achieve appropriate management of Fusarium wilt and increase of seed yield in chickpea in Bangladesh.**

**Key words:** Integrated management, Fusarium wilt, *Fusarium oxysporum* f. sp. *ciceris,* chickpea (*Cicer arietinum* L.), antagonists, botanicals, fungicides.

## INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a vital source of plant-derived edible protein in many countries. Chickpea also has advantages in the management of soil fertility, particularly in dry lands and the semiarid tropics (Singh and Saxena, 1996). Indian subcontinent accounts for 90% of the total world chickpea production (Juan et al., 2000). Yet chickpea yields (0.88 tons/ha) in Bangladesh (BBS, 2011) have fallen below expectation. Low yield of chickpea attributed to its susceptibility to several fungal, bacterial, and viral diseases. Among the diseases affecting chickpea, vascular wilt caused by an important obligate biotroph *Fusarium oxysporum* f. sp. *ciceris*

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(Padwick) Matuo and K. Sato is considered one of the limiting factors for its low productivity. Although the disease is wide spread in the chickpea growing areas of the world, it is most prevalent in the Mediterranean Basin and the Indian subcontinent (Jalali and Chand, 1992). Fusarium wilt epidemics cause significant annual losses of chickpea yields which, account for 10 to 15% of the total yield and sometimes escalate to 100% under conditions favorable for disease (Navas-Cortés et al., 2000). *F. oxysporum* f. sp*. ciceris* infects chickpea at seedling as well as at flowering and pod forming stage (Grewal, 1969), with more incidence at flowering and podding stage if the crop is subjected to sudden temperature rise and water stress (Chaudhry et al., 2007). Following infection of host roots, the fungus enters the xylem tissues and spreads rapidly up through the vascular system, becoming systemic in the host tissues, and may directly infect the seed. Translocation of water and nutrients is severely prevented by blockage of vessels, resulting in stomatal closure, wilting and death of leaves, often followed by death of the whole plant (Cho and Muehlbauer, 2004). Early wilting causes more loss than late wilting, but seeds from late-wilted plants are lighter, rough and dull than those from healthy plants (Haware and Nene, 1980). *F. oxysporum* f. sp. *ciceris* can survive as mycelium and chlamydospores in seed and soil, and also on infected crop residues, roots and stem tissue buried in the soil for up to 6 years (Singh et al., 2007).

The disease is primarily managed by resistance breeding programs. But high pathogenic variability and mutability limit the sustainability and effectiveness of any naturally selected resistance against the pathogen (Nimalkar et al., 2006). Management of Fusarium wilt with fungicides is uneconomical and difficult to achieve because of the soil and seed-borne nature of the pathogen (Ahmad et al., 2010). Moreover, the application of fungicides causes groundwater pollution, loss of non-target beneficial flora and evolving fungicidal resistance variants of the pathogen. The recontamination of the pathogen in the fungicide-treated soil often flourishes faster due to the absence of competitive microflora leading to higher incidence of disease in susceptible host (Jamil et al., 2010). As such in the present context, biological management of wilt with bioagents offers a great promise. *Trichoderma harzianum* is one efficient biocontrol agent that is successfully used to suppress Fusarium wilt (Khan et al., 2004; Dubey et al., 2007). Similarly, amending soil with plant extracts significantly reduces Fusarium wilt in the field (Chand and Singh, 2005). However, biological suppression of plant disease is often subjected to ecological limitations and is not sufficient alone to escape the pathogen under field conditions. Instead, biological control when used in combina-tion with other management strategies offer potential for suppression of disease under field conditions. Therefore, management of Fusarium wilt of chickpea should be based on strategies that combine the use of additive or synergistic combinations of biotic, cultural, and chemical control measures (Landa et al., 2004). The objective of the present research was to conduct a comprehensive study to find out the best individual treatment among various control measures for Fusarium wilt and identify the benefits of integrating the best one of them. In this study, we conducted a series of *in vitro* experiments comprising antagonists, organic amendments and fungicides to assess their efficacy in inhibition of *F. oxysporum* f. sp. *ciceris* and finally, a field experiment to develop integrated management strategy for Fusarium wilt of chickpea by combining best treatments in the field.

## MATERIALS AND METHODS

### Microorganisms and plant materials

All the isolates (T -1, T -2, T -3, T -8, T -9, T -10, T -11, T -12, T -16, T -18, T -20, T -25, T -36, T -52, T -68, T -70, T -71, T -72, T -75, and T -77/2) of *T. harzianum* and *F. oxysporum* f. sp. *ciceris* (FS1, FS2, FS3 and FS5) were collected from the stock cultures of the Plant Pathology Laboratory of Banghabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. Isolate FS1, FS2, FS3 and FS5 of *F. oxysporum* f. sp. *ciceris* were originally isolated from infected plants of chickpea variety BARI-2, BARI-3, BARI-5 and BU Chola-1, respectively. Chickpea variety "BU Chola-1" was used as host plant throughout study.

### Preparation of inoculum of *F. oxysporum* f. sp. *ciceris*

Wheat grains were soaked in water overnight and excess water was drained off. About 100 g water soaked grains were taken into 500 ml Erlenmeyer flask, sealed by cotton plug and were sterilized for 30 min at 121°C under 1.02 atm pressure in an autoclave. Sterilized wheat grains were inoculated with 10 mycelial discs (5 mm) obtained from the actively growing margin of 4-day-old PDA cultures of *F. oxysporum* f. sp. *ciceris*. Flasks were incubated at 25 ± 2°C for 10 to 12 days. They were shaken by hand after two to three days interval for even growth. Inocula of all isolates were prepared separately. The completely colonized wheat grain was air dried on brown paper for two days at laboratory (23 to 25°C) temperature and stored at 4°C until use.

### Pathogenicity test of *F. oxysporum* f. sp. *ciceris* isolates on BU Chola-1 plants

All isolates were subjected to the preliminary pathogenicity test on chickpea cultivar BU Chola-1. Earthen pots (15 cm) were filled with sterilized soil at 1 kg/pot. Wheat grain inoculum of each isolate of *F. oxysporum* f. sp. *ciceris* was thoroughly mixed with the soil at the rate of 20 g/kg soil. Control pots were prepared using sterilized soil only. Fifteen (15) seeds of BU Chola-1 were sown in each pot and grown in the net house. The seed emergence was recorded 21 days after sowing. Observations on number of plants wilted in each pot were recorded at 30, 45 and 60 days after sowing. The plants that showed dropping petioles, rachis and leaflets without any external rotting in the roots, but dark brown discoloration of internal xylem was considered as wilted (Nene et al., 1991). The causal agent of wilt incidence was confirmed after re-isolation of the pathogen from the infected root and stems of chickpea plants. The percent wilt incidence was calculated on the basis of initial plant count and total number of wilted plants in each pot.

### *In vitro* screening of *T. harzianum* isolates against *F. oxysporum* f. sp. *ciceris*

A total of 20 isolates of *T. harzianum* were tested for antagonistic activity against *F. oxysporum* f. sp. *ciceris* to select the most effective antagonist as biocontrol agent of the disease. Mycelial discs (5 mm) were cut from the edge of a 4-day-old colony of *T. harzianum* and *F. oxysporum* f. sp. *ciceris* and were placed simultaneously on the edge of the each PDA plate at opposite direction. Four replicate plates were used for each isolate. The plates that received only discs of *F. oxysporum* f. sp. *ciceris* served as control. The plates were incubated in the laboratory at room temperature (25±2°C). Inhibition percentage of *F. oxysporum* f. sp. *ciceris* was calculated based on the growth of the pathogen on PDA plates after 5 to 7 days of incubation following the formula as suggested by Sundar et al. (1995):

$$\% \text{ Inhibition} = \frac{X - Y}{X} \times 100$$

Where, X is the mycelial growth of the pathogen (*F. oxysporum* f. sp. *ciceris*) in the absence of antagonist and Y is the mycelial growth of the pathogen (*F. oxysporum* f. sp. *ciceris*) in the presence of the antagonist.

### *In vitro* evaluation of selected plant extracts on the radial growth of *F. oxysporum* f. sp. *ciceris*

An *in vitro* test was conducted to determine the effect of aqueous extract of three cost-effective and commonly available botanicals such as *Zingiber officinale* (Family-Zingiberaceae) rhizome, *Allium cepa* (Family-Amaryllidaceous) bulbs and freshly harvested *Azadirachta indica* (Family-Meliaceae) leaves on colony growth of *F. oxysporum* f. sp. *ciceris* isolate FS1. The *Z. officinale* rhizomes and *Al. cepa* bulbs were collected from local supplier and were commonly known as “Bangladeshi Ginger” and “Bangladeshi Onion”, respectively. All the selected botanicals were cleaned with tap water to remove the dust particle and crushed in a blender with sterile distilled water (1:1, w/v). The macerated extract was filtered through three-folded cheese cloth to remove fibrous and suspended material; this extracts were used as crude aqueous extract for experimental works. Individual extract was added to the PDA medium during preparation at 10.0, 20.0 and 40% concentration. PDA was autoclaved and approximately 15 ml of medium was poured into each 9.0 cm Petri dish. After solidification, each plate was inoculated with a 5 mm mycelial disc of 4-day-old colony of *F. oxysporum* f. sp. *ciceris*. The plates without plant extract served as control. The inoculated plates were incubated at 25±2°C. The radial growth was recorded after 5 to 7 days of incubation when the fungus covered the plates completely in control. The percent inhibition (PI) of the fungus over control was calculated by using the formula of Sundar et al. (1995).

### *In vitro* evaluation of selected fungicides against *F. oxysporum* f. sp. *ciceris*

Three fungicides namely Bavistin 50% WP (Carbendazim, Methyl-benzimidazol-2-yl carbamate) (BASF Bangladesh Limited, Dhaka, Bangladesh), Ridomil 250 EC (Metalaxyl, N-(2,6-Dimethylphenyl)-N-(Methoxyacetyl)-alanine methyl ester) (Syngenta Bangladesh Limited, Dhaka, Bangladesh), and Provax-200 (Carboxin, 5,6-dihydro-2-methyl-1,4-oxathin-3-carboxanilide) (Hossain Enterprise CC Limited, Dhaka, Bangladesh) were tested against colony growth of *F. oxyporum* f.sp. *ceceris* isolate FS1. Fungicides were used at 100, 250 and 500 ppm concentration in autoclaved PDA medium by poisoned food techniques (Dey et al., 1991). 5 mm diameter agar disc of test fungi was cut from 4-day old culture and placed in the center of Petri plates containing different concentration of fungicides. The plates without fungicides served as control. The inoculated plates were incubated at (25±2°C). The radial growth was recorded after 5 to 7 days of incubation when the fungus covered the plates completely in control. The percent inhibition of the fungus over control was calculated by using the formula of Sundar et al. (1995).

### Integration of *T. harzianum* T-75, *Az. indica* leaf extract and Provax-200 for management of Fusarium wilt disease and improvement of yield of chickpea in the field

A field experiment under artificially inoculated condition was conducted to determine the integrated effect of *T. harzianum* isolate T-75, *Az. indica* leaf extract and Provax-200 on Fusarium wilt of chickpea during the November 2009 to March 2010 in research field of BSMRAU. This experiment included the following eight treatments: $T_1$, Untreated seeds + inoculum of *F. oxysporum* f. sp. *ciceris* (control); $T_2$, Untreated seeds + inoculum of *T. harzianum* T-75 + inoculum of *F. oxysporum* f. sp. *Ciceris;* $T_3$, Untreated seeds + *Az. indica* leaf extract + inoculum of *F. oxysporum* f. sp. *Ciceris;* $T_4$, Provax-200-treated seeds + inoculum of *F. oxysporum* f. sp. *Ciceris;* $T_5$, Untreated seeds + inoculum of *T. harzianum* T-75 + *Az. indica* leaf extract + inoculum of *F. oxysporum* f. sp. *Ciceris;* $T_6$, Provax-200-treated seeds + inoculum of *T. harzianum* T-75 + inoculum of *F. oxysporum* f. sp. *Ciceris;* $T_7$, Provax-200-treated seeds + *Az. indica* leaf extract + inoculum of *F. oxysporum* f. sp. *Ciceris;* $T_8$, Provax-200 treated-seeds + inoculum of *T. harzianum* T-75 + *Az. indica* leaf extract + inoculum of *F. oxysporum* f. sp. *Ciceris.*

Inocula of *T. harzianum* T-75 were prepared on sterilized wheat grain following the same procedure as described for *F. oxysporum* f. sp. *ciceris*. Seed treatment with Provax-200 was done by thoroughly mixing 100 g seed, 0.2 g fungicide and a small amount of distilled water in a conical flask. Individual plot size was 1.5 × 1 m and plot to plot distance was 0.5 m. Each plot was prepared by a good tillage. Inoculum of *F. oxysporum* f. sp. *ciceris* isolate FS1 was mixed with soil of relevant plots at the rate of 90 g/m$^2$ soil and moistened to about 50% water holding capacity. Inoculum of *T. harzianum* T-3 at 50 g/m$^2$ was mixed thoroughly in the soil of selected treatment plots. Freshly prepared *Az. indica* leaf extract (10%, w/v) was mixed in the soil of treatment plots at the rate of 2 L/m$^2$. All treatments to the soil were done seven days before seed sowing. Seeds of BU Chola-1 were sown at the seed rate of 7.5 g/microplot. The seed emergence was recorded 21 days after sowing. Observations on number of plants wilted in each microplot were recorded at 30, 45, and 60 days after sowing. The causal agent of wilt incidence was confirmed after re-isolation of the pathogen from the infected root and stems of chickpea plants. The percent wilt incidence was calculated on the basis of initial plant count and total number of wilted plants in each microplot. At maturity, seed weight and grain yield were recorded from each microplot.

### Experimental design and statistical analysis

*In vitro* and pot culture studies were done using completely randomized design and each treatment had four replications. The field experiment was conducted in Randomized Complete Block Design with eight treatment combinations replicated four times. Data were analyzed statistically using the MSTAT-C computer program (Michigan State University, Michigan, USA). The mean values were compared by Fisher LSD (*P = 0.05*).

**Table 1.** Pathogenicity test of *F. oxysporum* f. sp. *ciceris* against BU Chola-1 variety of chickpea.

| Isolate of *F. oxysporum* f. sp. *ciceris* | % Wilted plant (mortality) |
|---|---|
| FS1 | 70.30[a]* |
| FS2 | 40.00[c] |
| FS3 | 60.00[b] |
| FS5 | 57.28[b] |
| Control | 0.00 |

*Mean values within a column having a common letter do not differ significantly (*P=0.05*).

## RESULTS AND DISCUSSION

### Pathogenicity of *F. oxysporum* f. sp. *ciceris* isolates

The pathogenicity test of the four selected isolates of *F. oxysporum* f. sp. *ciceris* namely FS1, FS2, FS3 and FS5 was done in chickpea plants in the pot culture experiment. All the isolates of *F. oxysporum* f. sp. *ciceris* were found pathogenic to chickpea and produced typical Fusarium wilt symptoms, while control pots without inoculum of *F. oxysporum* f. sp. *ciceris* did not show any wilt incidence. The wilt incidence caused by four isolates varied from 57.28 to 70.03% (Table 1). Isolate FS2 showed the least wilt incidence, while isolate FS1 showed the highest and was selected as a test pathogen for the rest of the experiment. The present study suggests a considerable variation in virulence among the tested isolates of *F. oxysporum* f. sp. *ciceris.* Fusaric acid is thought to be involved in wilt symptom development and a positive correlation exists between the production of the compound and the fungus virulence in the host (Türkkan and Dolar, 2010). Variation in the virulence within isolates of *F. oxysporum* f. sp. *ciceris* isolates has previously been demonstrated and led to the designation of pathogenic races (Bayraktar and Dolar, 2012). Until now, eight races of *F. oxysporum* f. sp. *ciceris* have been identified by their reactions with differential chickpea lines (Gurjar et al., 2009). This high pathogenic variability among different races complicates control measures to the pathogen and causes the heavy losses.

### *In vitro* antagonism of *T. harzianum* isolates against *F. oxysporum* f. sp. *ciceris*

Twenty (20) isolates of *T. harzianum* were tested against *F. oxysporum* f. sp. *ciceris* isolate FS1 on PDA by dual culture technique. All the isolates of *T. harzianum* caused significant reduction in the mycelial growth of the pathogen *in vitro* compared to the control (Table 2). Isolate T-75 showed the highest (75.89%) reduction of the radial growth of *F. oxysporum* f. sp. *ciceris* followed by T-3 (70.33%), T-12 (70.33%), T-20 (69.22%) and T-25 (68.11%). The lowest radial growth inhibition of *F. oxysporum* f. sp. *ciceris* was observed by the isolate T-9 (55.56%). These results indicate that most of the *T. harzianum* isolates tested in the present study were potential antagonists against *F. oxysporum* f. sp. *ciceris*. The antagonistic effect of *T. harzianum* isolates against *F. oxysporum* f. sp. *ciceris* has already been reported by some investigators (Poddar et al., 2004; Dubey and Suresh, 2006). Mechanisms for inhibition of pathogenic fungi by *T. harzianum* include antibiosis, lysis, competition and mycoparasitism (Cook and Baker, 1983). The presence of inhibition zones in dual cultures between *F. oxysporum* f. sp. *ciceris* and *T. harzianum* suggested secretion of diffusible non-volatile antibiotic substances by the *T. harzianum* isolates (Dubey and Suresh, 2006). The most effective isolate (T-75) from this study was selected for being utilized in the field evaluation against Fusarium wilt disease.

### *In vitro* evaluation of plant extracts on radial growth of *F. oxysporum* f. sp. *ciceris*

*In vitro* evaluation of plant extract revealed that all the plant extracts tested had considerable inhibitory effect on the radial growth of *F. oxysporum* f. sp. *ciceris* isolate FS1 (Table 3). The results also indicated the relatively higher fungitoxicity of *Az. indica* extract to control mycelial growth of the *F. oxysporium* f. sp. *ciceris*, showing more than 50% inhibition at lowest concentrations (10%). Extracts of *Z. officinale* and *Az. cepa* had nearly equal effective behavior against the fungus, showing 45 to 48% inhibition of the radial growth at different concentrations. Aqueous extracts of *Az. indica*, *Az. cepa* and *Z. officinale* were previously found significantly pronounced in inhibiting the mycelial growth of different fungi (Benkeblia, 2004; Hassanein et al., 2008; Banso et al., 2009). Singh et al. (1980) reported that growth of four soil borne pathogens including *F. oxysporum* f. sp. *ciceris* was effectively inhibited by aqueous extracts of leaf, trunk bark, fruit pulp and oil of *Az. indica*. Mukhtar (2007) also reported that aqueous leaf extract of *Az. indica* is highly effective in reducing the mycelial growth of *F. oxysporum* f. sp. *ciceris*. The fungal growth inhibition by aqueous extract of *Az. indica* is associated with alteration or disruption of a variety of cellular components such as deformation of the mycelium, vacuolation of the mycelial cytoplasm and herniation of the cytoplasmic contents

**Table 2.** Screening of *T. harzianum* isolates against the radial growth of *F. oxysporum* f. sp. *ciceris* in dual culture technique.

| *T. harzianum* isolate | % Inhibition of radial growth of *F. oxysporum* f. sp. *ciceris* |
|---|---|
| T-1 | 64.78$^{de}$* |
| T-2 | 61.44$^{f}$ |
| T-3 | 70.33$^{b}$ |
| T-8 | 56.67$^{ghi}$ |
| T-9 | 55.56$^{i}$ |
| T-10 | 64.11$^{de}$ |
| T-11 | 64.11$^{de}$ |
| T-12 | 70.33$^{b}$ |
| T-16 | 58.56$^{g}$ |
| T-18 | 65.89$^{cd}$ |
| T-20 | 69.22$^{b}$ |
| T-25 | 68.11$^{bc}$ |
| T-52 | 58.56$^{g}$ |
| T-36 | 63.33$^{ef}$ |
| T-68 | 66.30$^{cd}$ |
| T-70 | 55.89$^{hi}$ |
| T-71 | 58.11$^{gh}$ |
| T-72 | 65.22$^{de}$ |
| T-75 | 75.89$^{a}$ |
| T-77/2 | 65.22$^{de}$ |
| Control | 0.00 |

*Mean values within a column having a common letter do not differ significantly (*P=0.05*).

**Table 3.** Effect of different botanical extracts on suppression of the radial growth of *F. oxysporum* f. sp. *ciceris.*

| Botanical extract | Concentration (%, w/v) | % Inhibition of radial growth of *F. oxysporum* f. sp. *ciceris* |
|---|---|---|
| | 10 | 51.48$^{c}$* |
| *Azadirachta indica* | 20 | 53.70$^{b}$ |
| | 40 | 55.19$^{a}$ |
| | 10 | 45.18$^{f}$ |
| *Zingiber officinale* | 20 | 46.67d$^{e}$ |
| | 40 | 47.44$^{d}$ |
| | 10 | 45.93$^{ef}$ |
| *Allium cepa* | 20 | 46.30d$^{e}$ |
| | 40 | 48.52$^{cd}$ |
| | 10 | 0.00 |
| Control | 20 | 0.00 |
| | 40 | 0.00 |

*Mean values within a column having a common letter do not differ significantly (*P = 0.05*).

(Abyaneh et al., 2005). Hence, *Az. indica* leaf extract was selected as a key component in the integrated management study of Fusarium wilt of chickpea in the field.

### *In vitro* evaluation of selected fungicides against *F. oxysporum* f. sp. *ciceris*

Our results showed that Bavistin 50 WP, Provax-200 and Ridomil 250 EC significantly inhibited the radial growth of the *F. oxysporum* f. sp. *ciceris* isolate FS1 at all selected concentrations compared to the control (Table 4).

However, Provax-200 was found as the most effective fungicides, completely (100%) inhibiting the radial growth of the fungus even at lowest concentration. Bavistin 50 WP showed a 72.93, 80.73 and 84.80% inhibition of radial growth of *F. oxysporum* f. sp. ciceris at 100, 250

**Table 4.** Effect of fungicidal treatments on the radial growth of *F. oxysporum* f. sp. *ciceris.*

| Fungicide | Concentration (ppm) | % Inhibition of radial growth of *F. oxysporum* f. sp. *ciceris* |
|---|---|---|
| | 100 | 72.93[d]* |
| Bavistin 50 WP | 250 | 80.73[c] |
| | 500 | 84.80[b] |
| | 100 | 68.90[e] |
| Ridomil 250 EC | 250 | 70.00[e] |
| | 500 | 72.57[d] |
| | 100 | 100.00[a] |
| Provax-200 | 250 | 100.00[a] |
| | 500 | 100.00[a] |
| | 100 | 0.00 |
| Control | 250 | 0.00 |
| | 500 | 0.00 |

*Mean values within a column having a common letter do not differ significantly (*P=0.05*)

and 500 ppm, respectively. An inhibition of 68.90, 70.00 and 72.57%, radial colony growth of *F. oxysporum* f. sp. *ciceris* was observed with Ridomil 250 EC at 100, 250 and 500 ppm, respectively. Sugha et al. (1995) observed that carboxin fungicide (Thiram) alone and in combination was highly effective in inhibiting *in vitro* mycelial growth of *F. oxysporum* f. sp. *ciceris* and in reducing wilt incidence both under glass house and field conditions. Gupta et al. (1997) screened six fungicides against *F. oxysporium* f. sp. *ciceris* and reported carbendazim at 100 mg/ml as most effective in inhibiting the growth of fungus *in vitro*. However, carbendazim such as Bavistin 50 WP checked the growth of *T. harzianum* completely at all the concentrations, while Provax-200 allowed the normal growth of fungus even at 500 ppm (Akhter et al. 2013). Dubey et al. (2007) reported that the efficacy of *Trichoderma* species was enhanced in combination with carboxin.

This is because carboxin fungicides have high specificity to members of Basidiomycetes, a few Deuteromycetes and the Phycomycetes, but limited activity towards other fungi (Edgington and Barron, 1967). Consequently, inclusion of Provax-200 in the application schedule of integrated study with *T. harzianum* is more compatible than that of Bavistin 50 WP. The carboxin fungicide Provax-200 inhibits succinate dehydrogenase complex (syn. Complex) and interrupts electron transport in the mitochondrial respiratory chain of target fungi, so the fungi cannot produce vital energy to form ATP (Mathre, 1971).

## Integrated management of Fusarium wilt and seed yield improvement of chickpea in the field

Integration of *T. harzianum* T-75, *Az. indica* leaf extract and Provax-200 was evaluated in the field in controlling Fusarium wilt and in increasing seed yield of chickpea. Our results show that all the treatments with *T. harzianum* T-75, *Az. indica* leaf extract and Provax-200 were significantly superior to the control and varied from each other (Table 5). The highest percentage (22.61%) of wilted plants was observed in control plots ($T_1$, control), where untreated chickpea seeds were sown in field soil inoculated with *F. oxysporum* f. sp. *ciceris* isolate FS1. The lowest percentage of wilted plant (10.11%) was observed in the treatment $T_8$, where integrated control measures with *T. harzianum* T-75*, Az. indica* extract and Provax-200 were incorporated. The next lowest percent wilt incidence (13%) was observed in $T_5$ and $T_7$ treatment, where *Az. indica* extract was combined with *T. harzianum* T-75 and Provax-200, respectively. Similarly, significant variation among the treatments was observed for 100 seed weight and seed yield. The lowest 100 seed weight (14.00 g) and seed yield (1.05 t/ha) was observed in $T_1$, which was followed by $T_4$. Treatment $T_2$, $T_3$, $T_5$, $T_6$ and $T_7$ showed statistically similar effect on 100 seed weight and seed yield, while the highest 100 seed weight (20.20 g) and seed yield (2.25 t/ha) was observed in T8. These results indicate that the integrated effect Provax-200, *T. harzianum* T-75 and *Az. indica* extract is significantly superior over any single or combined effect of them in reducing Fusarium wilt and improving yield of chickpea. Our results obtained on the integrated management of chickpea wilt are in conformity with the findings of Sultana and Gaffar, (2010) and Nikam et al. (2007) who reported that the soil borne diseases of crops incited by species of *Fusarium* are cost-effective to be managed through integration of microbial antagonist, fungi toxicants or organic amendment.

Different mechanisms have been suggested as being responsible for their combined or single effect on yield improvement and fungal inhibition. Treatment of *T. harzianum* resulted in greater growth, increased transpiration and reduced wilting index of *F. oxysporum* f. sp. *ciceris*-infected plants (Siddiqui and Singh, 2004). *T. harzianum* caused a drastic decrease in the rhizosphere population of *F. oxysporum* f. sp. *ciceris* and increased the number

**Table 5.** Effect of integrated use of *T. harzianum, Az. indica* extract and Provax-200 on Fusarium wilt and seed yield of chickpea.

| Treatment | Wilted plants (%) | 100 seed weight (g) | Yield/Plot (g) | Yield (t/ha) |
|---|---|---|---|---|
| $T_1$ | 22.61[a]* | 14.00[c] | 156.0[e] | 1.05[e] |
| $T_2$ | 16.67[bc] | 16.50[b] | 212.3[bcd] | 1.45[bcd] |
| $T_3$ | 15.47[cd] | 16.50[b] | 243.0[bc] | 1.62[bc] |
| $T_4$ | 18.45[b] | 14.27[c] | 180.0[de] | 1.20[de] |
| $T_5$ | 13.09[d] | 17.83[b] | 281.8[b] | 1.88[b] |
| $T_6$ | 15.48[cd] | 17.50[b] | 253.8[bc] | 1.69[b] |
| $T_7$ | 13.54[d] | 16.48[b] | 250.0[bc] | 1.67[b] |
| $T_8$ | 10.11[e] | 20.20[a] | 337.3[a] | 2.25[a] |

*Mean values within a column having a common letter do not differ significantly (*P=0.05*). $T_1$, Untreated seeds + inoculum of *F. oxysporum* f. sp. *ciceris* (control); $T_2$, untreated seeds + inoculum of *T. harzianum* T-75 + inoculum of *F. oxysporum* f. sp. *ciceris;* $T_3$, untreated seeds + *Az. indica* leaf extract + inoculum of *F. oxysporum* f. sp. *ciceris;* $T_4$, Provax-200-treated seeds + inoculum of *F. oxysporum* f. sp. *ciceris;* $T_5$, untreated seeds + inoculum of *T. harzianum* T-75 + *Az. indica* leaf extract + inoculum of *F. oxysporum* f. sp. *ciceris;* $T_6$, Provax-200-treated seeds + inoculum of *T. harzianum* T-75 + inoculum of *F. oxysporum* f. sp. *ciceris;* $T_7$, Provax-200-treated seeds + *Az. indica* leaf extract + inoculum of *F. oxysporum* f. sp. *ciceris;* $T_8$, Provax-200 treated-seeds + inoculum of *T. harzianum* T-75 + *Az. indica* leaf extract + inoculum of *F. oxysporum* f. sp. *ciceris.*

of functional nodules in the chickpea roots (Khan et al., 2004). Moreover, the induction of plant basal resistance and the attenuation of the hormonal disruption caused by the pathogen are both mechanisms by which *T. harzianum* can control Fusarium wilt (Martínez-Medina et al., 2010). Fresh *Az. indica* leaves and their aqueous extracts were assayed as significantly distinctive in reducing Fusarium wilt incidence in *Cicer arietinum* (Chand and Singh, 2005; Mukhtar, 2007). The bioactivity of *Az. indica* extracts was attributed by various compounds such as nimbin, nimbidin and salannin and the most important antifungal compound was azadirachtin (Lale and Abdulrahman, 1999). The *Az. indica* leaf extract may also produce volatile and nonvolatile substances during their decomposition in the soil and cause both volatile and nonvolatile fungistatic effect against soilborne pathogenic fungi (Dubey et al., 2009). The seed-treated fungicide Provax-200 WP is a perfect match for controlling fungi in Bangladesh soil, for achieving excellent seed germination and for protecting plants from fungal attacks during the seedling stage (Hossain and Teixeira Da Silva, 2012).

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is a major constraint to chickpea production in Bangladesh. There was no substantial host plant resistance to Fusarium wilt in the current chickpea cultivars. The present study concluded that the use of fungicide, microbial antagonist and plant extract could be three key measures for a rational integrated management of Fusarium wilt of chickpea in sustainable cropping systems in Bangladesh. In that approach, a fungicide possibly eliminates the seed and soil borne inoculum and a biocontrol agent with soil amendment (plant extract) takes care of the soil borne inoculum and increases crop productivity by improving nutrients status and soil tilth (Chattopadhyay and Sen, 1996, Basue and Das, 2003). By utilizing this strategy, chickpea would maintain their critical role in Bangladesh as a major source of protein and as a contributing factor in agriculture sustainability through improvement of soil fertility.

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