RESEARCH ARTICLE

MANAGEMENT OF CHICKPEA WILT (*Fusarium oxysporum F.SP. CICERIS*) USING *Trichoderma* SPP.

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ABSTRACT

Thirty-eight *Trichoderma* isolates were collected from fields grown with chickpea in six districts of Northwestern Ethiopia in 2007 and 2008 and purified. Some isolates were identified to be *Trichoderma harzianum*, *T. koningii* and *T. pseudokoningii*. In *in vitro* tests the effect of *Trichoderma* isolates on colony growth of *Fusarium oxysporum* f.sp. *ciceris* and effect of seed treatment with *Trichoderma* isolates against chickpea wilt in glass house were studied at Amhara Region Agricultural Institute. In glasshouse experiment the treatments were arranged in randomized complete block design in three replications. Data on colony diameter *in vitro* tests and seedling emergence, wilt incidence, fresh and dry shoot weight in the glass house experiment were collected and were analyzed using the SAS system for windows V8 in ANOVA. In the *in vitro* tests *Trichoderma* isolates showed differences in their colony growth and antagonistic potential. Sixteen isolates showed competition potential, seventeen mycoparasitic and five lysis effects on *F. oxysporum* f.sp. *ciceris*. In glasshouse experiment, five *Trichoderma* isolates, two of which had shown mycroparasitic effect, two competition effect and one lysis effect were tested as seed treatment on two chickpea varieties namely *Adet* local and Shasho against fusarium wilt as compared with untreated control. Significant differences were observed among the treatments in reducing wilt incidence on *Adet* local and Shasho varieties. However, Shasho showed low levels of disease incidence compared to the *Adet* local. Lower incidence was recorded on *Adet* local with *Trichoderma* isolate Tr6 (mycoparasitic ability) and for Shasho with *Trichodrema* isolate Tr5 (competition ability), respectively. Significant differences were recorded in fresh and dry weight of shoots in the *Trichoderma* treated *Adet* local over the control. *Trichoderma* isolates improved the plant growth also. Highest fresh and dry shoot weight of Adet local was recorded with the isolate Tr3 (competition ability) followed by Tr6 (mycoparasitic). Significant differences were also recorded in fresh and dry shoot weight in the *Trichoderma* treated Shasho variety over the control. However the highest record was for Tr6 (mycoparasitic) followed by Tr7(mycoparasitic). The result showed that the potential of *Trichoderma* in reducing wilt incidence, delaying disease onset. Our study revealed that biological control agents such as *Trichoderma* can be a useful component of integrated chickpea fusarium wilt management and further study is also important under field conditions.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the important food legumes of Ethiopia and is grown mainly in the central, north, northwest, south and eastern highlands (Bejiga *et al*., 1995; Merkuz, 2012a). The crop is widely attacked by soil-borne diseases resulting in severe yield losses. Among the soil-borne diseases affecting chickpea, fusarium wilt caused by *Fusarium oxysporum* f.sp. *ciceris* is the major one, wherever the crop is grown in Ethiopia (Mengistu and Negussie, 1994; Negussie, 1995; Merkuz *et al*., 2011a). The disease can be managed using resistant cultivars, adjusting of sowing dates, fungicidal seed treatment biofumigation and biocontrol agents (De *et al*., 1996; Navas-Cortes *et al*., 1998; Meki, *et al*., 2009; Merkuz *et al*., 2011b). However, the use of fungicides is not usually

effective as it is used mainly for the seed borne inoculum and the effect is short lived. Highly resistant varieties are neither available nor can be effective against different races of the pathogen prevalent in the country (Meki, *et al*., 2009; Merkuz *et al*., 2011c; Merkuz, 2012b). Biological control using microbes is becoming a critically needed component of plant disease management, particularly in reducing root diseases (Nautiyal, 2000; Meki *et al*., 2009). Use of biocontrol agents is much safer and is presumed to be less polluting to the environment than the chemical pesticides (Sumeet and Mukerji, 2000). However, this method has been given little attention in Ethiopia. Biocontrol agents control plant pathogens through different mechanisms of action such as competition, antibiosis, mycoparasitism, lysis, production of siderophores and so on (Sumeet and Mukerji, 2000). During competition, biocontrol agents compete for nutrient and space with pathogens and are rapid colonizer and proliferate at a rate

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out numbering the pathogen (Chincholkar *et al*, 2000; Sumeet and Mukerji, 2000). Antibiosis is an imoportant mechanism used by biocontrol agents to suppress diseases by produce volatile and non-volatile antibiotics which disrupt the cell contents of pathogenic microorganisms before coming in contact with the biocontrol agent (Dennis and Webster, 1971; Yong *et al*., 1985). Lysis is by secretion of cell wall degrading enzymes, such as chitinases and β-1, 3-glucanases as suppression of plant diseases (Wu *et al*., 1986). According to Loper (1988), siderophore producing microbes act as biocontrol agents by limiting the amount of iron available to potential plant pathogens. Mycoparasitism occurs when one fungus exists in intimate association with another, from which it derives some or all of its nutrients while conferring no benefits in return (Lewis *et al*., 1991). The mechanism of mycoparasitism involves different kinds of interactions like coiling of hyphae around the pathogen, penetration and production of haustoria (Nigam *et al*., 1997). *Trichoderma* species have been found to possess great potential in plant disease management (Calvet *et al*., 1990; De *et al*., 1996). Different *Trichoderma* species have been extensively tested as biocontrol agents against wide range of plant pathogens and several of them have been found potent against many soil-borne plant pathogenic fungi (Calvet *et al*., 1990; De *et al*., 1996; Reddy *et al*., 2000; Meki *et al*., 2009). Seed treatment is a strategy to control soil-borne diseases, which is less laborious, cheap, and preferred by farmers (Merkuz *et al*., 2011a). Antagonistic microorganisms applied to seeds prior to planting colonize the rhizosphere of seedlings and thus are present at or near the pathogen's infection court where they act by competition, antibiosis, mycoparasitism, lysis and so on in the rhizosphere substrate (Harman, 1991).

Seed pelleting with *T. harzianum* using 5×10^9 c.f.u./ml reduced the infection by *M. phaseolina* in bean and chickpea and reduced the root rot incidence by 90% in urd-bean (Jeyarajan *et al*., 1994). The efficacy of seed treatment method can be improved through solid matrix priming (Harman, 1991). Solid matrix priming is a process in which moistened seeds are mixed with an organic carrier and the moisture content of the mixture is brought to a level just below that required for seed sprouting. Slurry of *Trichoderma* is first added to seeds followed by addition of lignite and water. The primed seeds are incubated for four days at 80-100% RH. During this period, *Trichoderma* colonizes seed and sporulation is evident. In *Pythium ultimum* infested soil, the stand was increased to 70-80% as against 10% in the control. In tomato seed, the population of *Trichoderma* increased by ten-fold due to solid matrix priming (Harman, 1991).In Ethiopia, *Trichoderma viride* has been found to reduce the radial growth of *F. solani* and mortality of faba bean due to root rot caused by this pathogen (Tesfaye, 1999). Temesgen (2002) found that *T. hamatum* and *T. koningii* were highly antagonistic against *F. solani* affecting faba bean. *T. harzianum* and unidentified *Trichoderma* isolate T23 significantly reduced severity of fusarium wilt caused by race 3 and delayed disease onset (Meki *et al*., 2009). However, *Trichoderma* spp. prevalent in Northwestern Ethiopia, which is one of the major chickpea producer areas, has not been explored for management of chickpea wilt. Therefore, this study was undertaken with the objective of evaluating the potential of *Trichoderma* isolates from Northwestern Ethiopia for controlling chickpea wilt.

MATERIALS AND METHODS

Collection of soil samples, isolation, purification and identification of *Trichoderma* isolates

A total of 101 soil samples were collected randomly from different major chickpea growing districts in Northwestern Ethiopia as indicated in Table 1 (Merkuz, 2011). From each field, soil samples were collected from relatively healthy-looking chickpea plant from about 10 cm depth. Samples were collected in plastic bags, dried in the laboratory and grounded with mortar and pestle and then kept in the refrigerator at 4 °C until needed. From each sample, 10 g of soil was added to 90 ml of distilled sterilized water and vigorously shaken using a shaker for 20-30 minutes. From this, five fold serial dilutions were made by pipeting 10 ml into additional dilution water. From the final dilution (10^{-5}), aliquots of 1 ml each were spread on 9 cm diameter plates, containing 20 ml of PDA and to reduce bacterial contaminants antibiotic (50 mg/l streptomycin) were added and incubated at 25 °C for 7-10 days. *Trichoderma* colonies which developed on PDA, were identified based on visual and microscopic observations and according Rifai (1969), Samuel *et al*. (2002) and some isolates identified at CAB-International Plant Clinic and reference cultures maintained at Debre Zeit Agricultural Research Centre. Isolates were kept in test tubes containing autoclaved sand, wheat bran and chickpea bran mixed with molasses (10g sand, 20 g wheat bran + 20 g chickpea bran + 5 ml molasses) at 4^0C (Elad *et al*., 1980).

Isolation and identification of the target pathogen

Fusarium oxysporum f.sp. *ciceris* cultures isolated from wilted chickpea plant showing browning of vascular tissue isolates were identified microscopically by their morphological characteristics such as abundance of micro and fewer macro conidia, white to creamy-white color on PDA medium and production of chlamydospores on sand-maize meal after 10 days of incubation. Microconidia were cylindrical, slightly curved and were produced on short, unbranched monophialides (Haware and Nene, 1982; Haq and Jamil, 1995). An isolate from Dembia district was finally selected due to higher frequency of isolation of *F. oxysporum* f.sp. *ciceris* in the sampled chickpea growing fields of the district. Isolate of the pathogen was maintained in culture tubes containing 10g of fine autoclaved sand (Haware and Nene, 1982) and used as stock culture of the target organism through out the study. The pathogenecity of this isolate was tested by using the susceptible variety, JG-62. Surface disinfected seeds were sown in plastic pots containing sterilized soil. On top of each seed, 1 mm disc of *Fusarium* isolate from its pure culture was placed and covered with fine sterilized soil. Plants were watered as required and observed weekly for symptom development.

In vitro evaluation of *Trichoderma* isolates for antagonistic potential to *Fusarium oxysporum* f.sp. *ciceris*

Pure cultures of the isolated *Trichoderma* spp were screened individually for their antagonistic activity against *F. oxysporum* by dual culture technique (Chet *et al*., 1979b). *Trichoderma* isolates showed competition, mycoparasitic and lysis effects on the pathogen, which were further confirmed as follows:-

Each *Trichoderma* isolate was tested for competition ability with colony growth of *F. oxysporum*. *Trichoderma* isolate and *F. oxysporum* were inoculated in the same culture plate 4-cm apart on petri dishes (9 cm diameter) containing PDA. Six mm diameter mycelial plugs of the test isolate and that of the pathogen was placed on PDA plates and each test was replicates in three plates inoculated only with *F. oxysporum* served as control. After incubation for 5 days at 25°C, colony diameter of both antagonist and pathogen was measured (as the average of two cross diameters).

Antagonists selected for their mycoparasitic and lytic mechanisms were tested specifically for their mycoparasitic and lytic activity. Conidial suspension of *F. oxysporum* was prepared by washing one-to two-week old PDA cultures. Spores were suspended by gently scraping the colony surface with sterile loop. The suspension was transferred to a sterile test tube and spores were counted using a haemocytometer and the concentration of spores was adjusted to about 10^{-5} conidia/ml *F. oxysporum* spore suspension and spread in to PDA culture plates. When sufficient growth had occurred (medium covered by the mycelium), 6mm diameter hole was made at the center of the cultures of *F. oxysporum* using a cork borer. Then the space left open was replaced with 6mm diameter piece of *Trichoderma* culture. The test plates were then incubated at 25^{0}C. Each test was replicated three times. The growth of the *Trichoderma* isolate inoculated at the center was inspected at 24 hr interval for the next 5 days and the width of the overlapping mycoparsitic and pathogen mycelium lysed around the colony of the antagonist was measured (Bell *et al*., 1982; Chet *et al*., 1993a). Microscopic examination also confirmed the lytic activity that was underway.

Soil sterilization, inoculum preparation and inoculation

Soils for the experiments were collected form field at Adet Agricultural Research Center experimental station used for growing chickpea. Samples were taken from a depth of 10cm and autoclaved for 90 minutes. The pathogen isolate was multiplied in petridish (9 cm diameter) containing 20 ml of PDA by transferring a small amount of infested sand from the test tube. From 7-day old culture, two discs (1 cm diameter each) were removed with sterile cork borer and transferred to 100g of sand: maize meal (9:1, autoclaved twice at 121°C for 20min.) in each of the 370-ml glass jars and incubated for 14 days at 25°C with 12h light and 12h dark at approximately $36\mu E m^{-2}s^{-1}$ (Jimenez-Gasco *et al*., 2001). The inoculum raised on 100g sand; maize mixture in each glass jar was mixed thoroughly with 2 kg sterilized vertisol soil and mixed in 20 cm diameter plastic pots. The pots were disinfected with 2.5% sodium hypochlorite solution for 5 min., rinsed in distilled water and air-dried. The isolate were allowed to become established in the infested soil for one week before planting of chickpea and was watering sterilized water as necessary.

Seed treatment experiment

Five *Trichoderma* isolates, two of which having mycoparasitic effect viz., Tr6 (*Trichoderma harzianum*) and Tr7 (*Trichoderma pseudokoningii*); two having competition ability viz., Tr3 (*Trichoderma koningii*) and Tr5 (unidentified *Trichoderma species*); and one having lysing ability namely Tr34 (unidentified *Trichoderma sp.*) were

evaluated as seed treatment against *F. oxysporum* f.sp. *ciceris* in pot experiment. The *Trichoderma* isolates were grown in Erlenmeyer flasks each containing 200 ml of potato dextrose broth for 1 week at 25^{0}C and thoroughly mixed with the medium using magnetic stirrer and after filtration, the spore suspension was adjusted to 5×10^{8} cfu/ml with a haemocytometer and supplemented with 0.01% Tween 80 as spreader (Calvet *et al*. 1990). Two chickpea varieties, *Adet* local (susceptible), Shasho (moderately susceptible) were used. Chickpea seeds were surface sterilised by immersing in 2.5% Sodium hypochlorite solution for 2–3 min followed by washing three times with sterilised distilled water and coated with *Trichoderma* spore suspensions and incubated in Solid Matrix Priming (SMP) (Harman 1991). Ten grams of seeds were immersed in 5 ml *Trichoderma* spore suspension for 30 min and transferred to moistened charcoal in a tray and incubated at 25^{0}C for 2 days. Five treated seeds were sown in each pot containing *F. oxysporum* f.sp. *ciceris* infested soil. Untreated seeds, coated only with water + Tween 80, served as a control. Each treatment was replicated three times in RCBD. Highly susceptible variety, JG-62 was also planted for evaluation of the disease pressure in the pots.

Data collection and Analysis

Data on colony diameter of *Trichodrema* isolates and pathogen in dual culture was measured daily and descriptive analysis was used (Gomez and Gomez, 1984). In the pots, disease incidence was recorded at weekly interval after sowing and percentages of wilted plants calculated based on the stand count. Fresh and dry weights of shoots were recorded at early stage of flowering. Data were subjected to analysis of variance (ANOVA) using the SAS system for windows V8 and means were compared using student- Newman-Keuls multiple-range test (SAS, 2001).

RESULTS

In vitro test of Trichoderma spp. against chickpea wilt pathogen

All the isolates of *Trichoderma* tested for antagonistic activity against *F. oxysporum* f.sp. *ciceris* showed different degree of antagonism to the pathogen. *In vitro* test indicated that *Trichoderma* isolates had competition, mycoparasitic and lysis effect on the pathogen *F. oxysporum* f.sp. *ciceris* (Table 2). Sixteen isolates coded as Tr2, Tr3, Tr5, Tr8, Tr10, Tr17, Tr18, Tr22, Tr28, Tr30, Tr31, Tr32 Tr35, Tr36, Tr37 and Tr38 were having competition effect and showed differences in their linear growth rate in five days after incubation. The linear colony growth among isolates ranged from 4.87 to 5.96 cm in five days incubation. Isolate Tr3 had the fastest growth rate (5.96 cm) followed by isolates Tr5 (5.78 cm), Tr37 (5.57 cm) and Tr8 (5.36) in linear colony growth. Isolates Tr2 (4.85 cm), Tr31 (4.87cm) and Tr32 (4.87 cm) were the slowest in their colony growth. However all those isolates showed competition effect on the growth of the pathogen in the dual culture over the control. Seventeen isolates coded as Tr1, Tr4, Tr6, Tr7, Tr9, Tr11, Tr12, Tr13, Tr14, Tr15, Tr16, Tr20, Tr21, Tr23, Tr25, Tr27 and Tr29 had mycoparasitic effect on the pathogen, in which they over grew the pathogen with different magnitude levels and some showed difference in their linear growth rate in five days after incubation in over grow

pathogen and the linear colony over grow among isolates ranged from 0.86 to 1.21cm. Isolate Tr6 had the fastest over grow rate (1.21 cm) followed by isolates Tr7 (1.13 cm), Tr15 (1.09 cm) and Tr16 (0.99cm) in linear colony over grow. Isolates Tr1, Tr11, Tr13, Tr14, and Tr25 were (0.86 cm) and the slowest in their colony over grow. However all isolates showed mycoparasitic effect on the pathogen in the test. It was also observed five *Trichoderma* isolates Tr19, Tr24, Tr26, Tr33, and Tr34 showed lysis effect on the pathogen after over growing. The linear colony lysis ranged from 0.57 to 0.97cm and the highest lysis for isolates Tr34 (0.97cm) followed by Tr33 (0.76cm) and the lowest by Tr24 (0.59), Tr19 (0.58cm) and Tr26 (0.57) were recorded in five days incubation. Microscopic examination also confirmed the lytic activity that was underway. The challenged mycelia of the pathogen showed a disintegrated appearance when examined microscopically. Lysis of fungus mycelium by antagonistic microorganisms is one form of antagonism and had been reported in soilborne diseases (Chet *et al.*, 1979b). The pathogen could not be able to grow again when transferred on to fresh media after 15 to 20 days of attack by antagonists having mycoparasitic and lysis mechanisms. In pathogenecity test of the pathogen using susceptible variety of JG-62 was observed completely wilted after three weeks.

0.05) differences were observed among treatments in reducing wilt incidence when compared to the control (Table 2). However, *Trichoderma* species tested gave limited effect in reducing disease. Significant ($P < 0.05$) differences were observed among all the treatments with *Adet* local and Shasho chickpea varieties. However, Shasho showed low levels of disease compared to the Adet local (Table 2).

Effect of *Trichoderma* seed treatment on plant above ground mass

Significant ($P < 0.05$) differences were recorded in fresh and dry weight of shoots in the *Trichoderma* treated *Adet* local seed over the control (Table 3). However the highest recorded was for the isolate Tr3 followed by Tr6 in fresh and dry weight (Table 3). Significant ($P < .05$) differences were also recorded in fresh and dry shoot weight in the treated Shasho seed over the control (Table 4). However the highest was recorded for Tr6 followed by Tr7 (Table 4). In *Adet* local treated treatment wilting was delayed by one week after sowing for onset of disease (Fig. 1). Delay of the onset of disease was observed by two weeks on treated variety Shasho (Fig. 2). However disease occurrence was observed in

Table 1. Number of *Trichoderma* isolates collected from soils of different chickpea growing districts of Northwestern Ethiopia

District	No. of isolates	Isolate code[1]
Dembia	6	Tr3, Tr7, Tr11, Tr14, Tr16; Tr25
Gondarzuria	9	Tr2; Tr5, Tr6, Tr13, Tr23, Tr26, Tr28; Tr31; Tr33
Libo-kemkem	6	Tr1; Tr4, Tr9; Tr15; Tr17; Tr21
Fogera	5	Tr8, Tr12, Tr18; Tr20; Tr22
Enemay	8	Tr10; Tr19; Tr24; Tr27;Tr29;Tr30;Tr32; Tr 38
Dejen	4	Tr34, Tr35, Tr36, Tr37
Total	38	

[1]= the letter Tr represents *Trichoderma*

Table 2. Effects of *Trichoderma* seed treatment on mean percent chickpea wilt incidence

Seed treatment	Variety**	
	Adet local	Shasho
Tr6(*Trichoderma harzianum*)	31.7c	22.7c
Tr3 (*T. koningii*)	35.0c	26.7bc
Tr34 (*T. spp.*)	35.8bc	31.3b
Tr5 (*T. spp*)	38.3bc	22.0c
Tr7(*T. pseudokoningii*)	39.2bc	28.0bc
Control (water)	48.3a	36.0a
P-value	0.0002	‹.0001

** Values in each column with the same letter are not significantly difference at 5% probability
*Water treated=control, Tr6 &Tr7 =Mycoparasitic effect, Tr3 & Tr5=Competition effect, Tr34=Lysis effect

Table 3. Effects of *Trichoderma* seed treatment on fresh and dry shoot weight of *Adet* local variety

Seed treatment	Fresh weight (g)	Dry wt weight(g)
Tr3 (*T. koningii*)	4.24a	2.44a
Tr6 (*T. harzaniumi*)	4.23a	2.37b
Tr7(*T. pseudokoningi*)	4.14ab	2.35bc
Tr5 (*T. spp.*)	4.04b	2.33c
Tr34 (*T. spp.*)	4.03b	2.32c
Control (water)	4.00b	2.30c
P-value	0.0013	‹.0001

Values in each column with the same letter are not significantly difference at 5% probability
Water treated=control, Tr6 &Tr7 =Mycoparasitic effect, Tr5 & Tr3=Competition effect, TrL34 =Lysis effect.

Effects of Trichoderma seed treatment on incidence of fusarium wilt of chickpea

It was observed that JG-62 susceptible check was completely wilted in the 3rd and 4th assessments and hundred percent seedling emergences during the experiment. Significant ($P <$

susceptible check JG-62 and control in both varieties with in the first week after sowing (Fig. 1 and 2).

DISCUSSION

Fusarium oxysporum f.sp. *ciceris* and *Trichoderma* spp. share the same ecological niche and it is common to find the two

Table 4. Effects of *Trichoderma* seed treatment on fresh and dry shoot weight of variety Shasho

Seed treatment	Fresh weight (g)	Dry weight(g)
Tr6 (*T. harzaniumi*)	5.91a	4.00a
Tr7(*T. pseudokoningi*)	5.82ab	3.94ab
Tr3 (*T. koningii*)	5.73bc	3.90abc
Tr5 (*T. spp.*)	5.70c	3.84bc
Tr34 (*T. spp.*)	5.60c	3.80c
Control (water)	4.20d	2.51d
P-value	‹.0001	‹.0001

Values in each column with the same letter are not significantly difference at 5% probability
Water treated=control, Tr6 &Tr7 =Mycoparasitic effect, Tr3 & Tr5=Competition effect, Tr34 = Lysis effect.
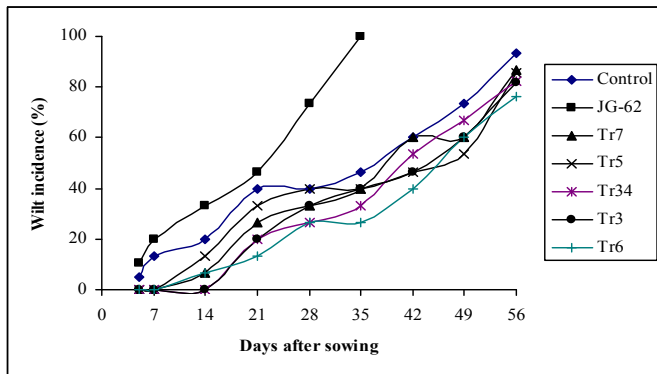
Fig. 1. Disease progress curve of fusarium wilt on Adet local chickpea treated with *Trichoderma* isolates
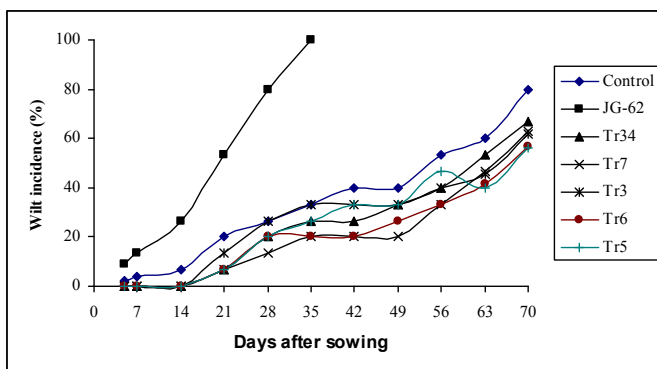
Fig. 2. Disease progress curve of fusarium wilt on variety Shasho chickpea treated with Trichoderma isolates

organisms in the rhizosphere of the same plant. For a better understanding of such phenomenon, detailed studies of their distribution and relationship have proved relevant in generating important biocontrol systems. Analysis of 101 soil samples from the rhizosphere of plants in the chickpea growing areas yielded 38 pure *Trichoderma* isolates (Table 1). Claude *et al.* (1993) expressed that microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front-line for root against attack by pathogens. Moreover, indigenous biocontrol agents are better adapted to survive in the natural environment, where control is intended and hence are suitable for inundate augmentation (Merkuz *et al.*, 2011a; Prior and Street, 1997). Singh *et al.*, (1995) reported that *Trichoderma* species occur in nearly all soils all over the world. The *Trichoderma* isolates from northwest Ethiopia showed diversity. From the isolates, *T. harzianum, T. koningi* and *T. pseudokoningii* were identified. Though bioagents are commonly found in the rhizosphere of chickpea plants the population level in soil is required for effective disease reduction (Sumeet and Mukerji, 2000). Low population of antagonist or its decline in soil over a long period often resulted in failure of suppression of pathogens. This shows that a certain level of *Trichoderma*

use mycoparasitism as one of the mechanisms of control. Nigam (1997) pointed out mycoparasitism occurs when intimate association exists between the pathogen and the biocontrol agent and involves coiling of hyphae around the pathogen, penetration, production of haustoria and lysis of hyphae. It was also reported that *T. harzianum* showed mycoparasitism by coiling around and lysis on hyphae of *F. oxysporum* f.sp. *ciceris* and *T. harzianum* is also known to produce certain volatile and non-volatile compounds (Dennis and Webster, 1971; Singh *et al.* 1997). It is also observed through time the mycoparasitic isolates gradually over grow whole of the pathogen. Wells (1988) also indicated that time to completely control the pathogen growth is proportional to the virulence potential of the isolates. In this test isolate Tr34 and Tr33 showed good lysis effect on the pathogen. According to Papavizas and Lumsden (1980) the mechanism involved in the control of pathogens by *Trichoderma* species are antibiosis, lysis, competition and mycoparastism. In the pot experiment, *Trichoderma* isolates showed reducing wilt incidence compared with water treated control. Treatments with Tr3 and Tr6 on *Adet* local and Tr6 and Tr5 on Shasho showed reducing wilt incidence by 28-35% and 37-39% respectively better over the control. Treated *Adet* local delayed disease onset by one week over control. Delaying disease onset will give the plant a chance to escape and give better yield because early wilting causes more yield loss than late wilting (Navas-cortes *et al.*, 2000). Treated Shasho was well managed against wilt incidence and delayed disease onset than treated *Adet* local. The result indicated that *Trichoderma* species could be more effective when integrated with moderately susceptible or resistant varieties. This is in agreement with the finding of De *et al.* (1996), who reported that coating moderately susceptible chickpea cultivars with *B. subtillis, G. virens, T. harzianum* and *T. viride* significantly controlled *Fusarium* wilt by 30-46%. The efficacy of biocontrol agents as seed treatment can be affected by different factors, such as seed size and chemical nutrients released from seeds. As Shasho seeds have larger size, they can accommodate more spores of the bioagent than small-sized seeds. Furthermore, after treatment with *Trichoderma* spore suspension, seeds were incubated in SMP process. According to Harman (1991), in this SMP process *Trichoderma* grow on the surface of seeds and increase in numbers utilizing seed exudates that normally leach from seeds in the pre-emergence process. Thus, differences in the amount and type of the exudates can cause variation in growth and reproduction of the bioagent on the surface of the seeds, which in turn may bring variation on the efficacy of the bioagent. The experiment showed that treated *Adet* local and Shasho showed significant differences among the treatments in plant growth over the control. *Trichoderma* isolates have been found to be capable of controlling chickpea wilt under rainfed condition on sandy loam soils (Inam-ul-Haq *et al.*,

2009, Merkuz, 2012a). In Ethiopia, chickpea is mostly grown under rainfed conditions and hence can be managed by developing *Trichoderma* based mycofungicides. Boureghda and Bouznad (2009) also reported high degree of chickpea wilt control by *T. harzianum, T. atroviride* and *T. longibrachiatum*. Temesgen (2002) indicated *T. hamatum* and *T. koningii* controlled soil borne infection of 30 day-old seedlings by *R. solani* and *Fusarium* spp. and increased the grain yield of faba bean. Observation in this work implied that mycofungicides belonging to the genus *Trichoderma* occur naturally in Northwestern Ethiopia. *Trichoderma* isolates have been found to be capable of controlling chickpea wilt under rain fed conditions on sandy loam soils (Inan-ul-Haq *et al.*, 2009, Merkuz, 2012a). In Ethiopia, chickpea is mostly grown under rain fed conditions and hence can be managed by developing *Trichoderma* based mycofungicides. The present study showed significant wilt incidence reduction and delay of disease onset by *Trichoderma* seed treatment over control. Further studies are required to verify this result under field conditions together with appropriate carrier to the biocontrol agent. The integration of biocontrol agents, with resistant cultivars will increase the effectiveness of chickpea fusarium wilt management under field conditions.

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