Ethiop. J. Biol. Sci. **14(2): 129–145, 2015**
 ISSN: 1819-8678

EVALUATION OF ANTAGONISTIC ACTIVITIES OF *TRICHODERMA* ISOLATES AGAINST FUSARIUM WILT (*FUSARIUM OXYSPORUM*) OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) ISOLATES

Takele Wolebo[1], Tesfaye Alemu[1,*] and Fassil Assefa[1]

ABSTRACT: The study was initiated with the objective of controlling tomato wilt disease (*Fusarium oxysporum*) using *Trichoderma* isolates as biocontrol agents. *F. oxysporum* was isolated from diseased tomato plants grown in five selected kebeles of Dugda Bora and Adami Tulu Jido-Kombolcha woredas of the Central Rift Valley (CRV) region of Ethiopia. The pathogenicity of *F. oxysporum* was determined on three different tomato varieties namely Cochoro, Miya and Fetane that were grown in 20 cm plastic pots containing 3 kg of autoclaved soil under the greenhouse. The antagonistic effect of *Trichoderma* isolates against the test pathogen was tested both *in vitro* and *in vivo* conditions. From the three tomato varieties, Miya was more susceptible to *F. oxysporum* infection than both Cochoro and Fetane varieties. The antagonistic effects of *Trichoderma* isolates on the mycelial growth of the test pathogens, AUT9, AUT 8 and AUT10, showed 66%, 61% and 58% inhibition, respectively, on the mycelial growth of *F. oxysporum* isolate. All *Trichoderma* isolates achieved maximum mycelial growth at 25°C and minimum mycelial growth at 15°C. From the current comparative *in vivo* and *in vitro* (green house) studies it is evident that the most effective antagonist of the *Trichoderma* isolates to *F. oxysporum* was AUT9 and the most resistant tomato variety was Fetane.

Key words/phrases: Antagonism, Antibiosis, Pathogen, Pesticide, Resistance, Symptom.

INTRODUCTION

Lycopersicon esculentum Mill. is a horticultural plant that belongs to the Solanaceae family and it is grown worldwide for its edible fruit, tomato (Kocchar, 1981). In Ethiopia, it is grown on small-scale by farmers whereas processed types are mainly produced in large scale horticultural farms (MoARD, 2009). Farmers are more interested in tomato production than other vegetables for their multiple harvests because of its high profitability and its potential to improve household income and nutrition. With net income of about 11,000 to 14,000 Ethiopian Birr/ha, it is considered to be the most profitable vegetable (Lemma and Herath, 1994).

[1] Department of Microbial, Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. E-mail: tesfayealemu932@gmail.com; assefafassil2012@gmail.com
* Author to whom all correspondence should be addressed

Tomato production is generally affected by several abiotic and biotic factors, one of which *Fusarium* wilts caused by *Fusarium oxysporum* f. sp. *Lycopersici*. It is one of the most important diseases, and is highly destructive to tomatoes grown in greenhouse; in the field in many warm regions of the world, it is known to cause up to 50% yield loss (Larkin and Fravel, 1998; Borrero *et al*., 2004). In Ethiopia, tomato and other vegetables are widely cultivated in the Central Rift Valley (CRV) region of Ethiopia. Dagnatchew Yirgou and Stewart (1967) reported the various fungal and bacterial diseases and disorders that can affect tomatoes during the growing season. Among the fungal diseases, *Fusarium oxysporum* f. sp. *Lycopersici* is a highly destructive pathogen of both greenhouse and field grown tomatoes in warm vegetable production areas.

The disease caused by this fungus is characterized by wilted plants, yellowed leaves and minimal or absent crop yield. There may be a 30 to 40% yield loss (Kirankumar *et al*., 2008). De Cal *et al*. (1997) reported that *Fusarium* wilt of tomato is an intractable problem because management strategies such as cultivar resistance and chemical control are not always appropriate. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. A variety of biological control agents are available for use (Pal and Gardener, 2006).

Trichoderma are common soil fungi that have been known to control several plant pathogens that caused soil-borne pathogens in a wide range of crops (Koch, 1999). They are effective in suppressing different soil borne pathogens (Hoyos-Carvajal *et al*., 2009; Shanmugaiah *et al*., 2009) by improving their nutrient uptake (Yedidia *et al*., 2001); defense level against biotic, abiotic and physiological stresses in germinating seeds and seedlings (Mastouri *et al*., 2010; Hoitink *et al*., 2006). The antifungal abilities of these beneficial microbes have been known for a long time and there have been extensive efforts to use them for other plant diseases (Samuels, 1996). However, effective adoption requires a very good understanding of the complex interactions of the host, the biocontrol agents and the specific environment. Therefore, this study was initiated to isolate, identify and characterize the *Trichoderma* isolates under *in vitro* and *in vivo* conditions for use against *Fusarium* wilt from different areas (environmental settings).

MATERIALS AND METHODS

Isolation of *Trichoderma* isolates from the soil samples

Nineteen (19) soil samples were collected from the rhizosphere of tomato grown in the fields of the Central Rift Valley (CRV), East Shewa Zone, Oromiya Regional State, Ethiopia especially from Dugda Bora and Adami Tulu-Jido Kombolcha districts/woredas. Geographically, the study areas are located at 134 km and 160 km from Addis Ababa and between 8°01′–8°25′ N latitude and 38°32′–39°04′ E longitude and 7°37′–8°04′ N latitude and 38°32′–39°04′ E longitude, respectively (Fig. 1).

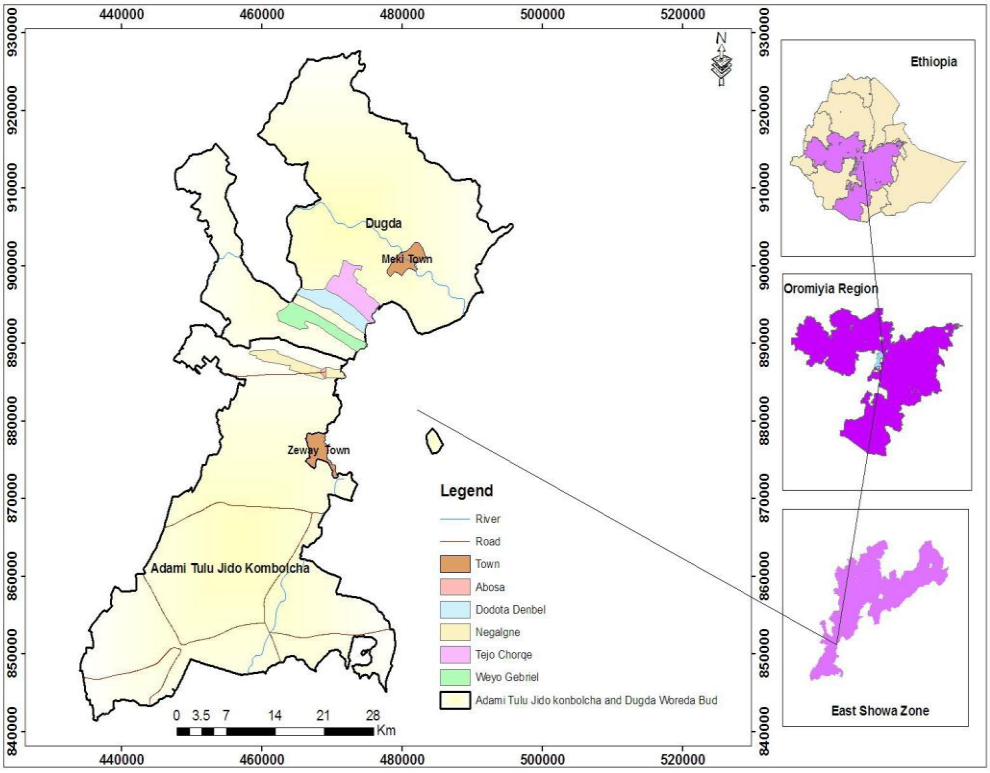


Fig.1. Map of the study areas.

They were homogeneously mixed and carefully sorted to remove stones and other unwanted soil debris using 2.0 mm sieve. Members of the genus *Trichoderma* and *Trichoderma* species were isolated from the soil samples according to Kader *et al*. (1999). Soil suspensions were prepared by adding 1.0 g homogeneously mixed soil to 9 ml sterile distilled water and mixed thoroughly for 15 minutes on rotary shaker. Immediately, each suspension was serially diluted. From appropriate dilution, 0.1 ml was spread plated on

potato dextrose agar (PDA) with an antibiotic Neomycin sulfate at a concentration of 0.1 g/l and incubated at 25°C for 7 days. Based on the appearance, *Trichoderma* colonies were repeatedly transferred to purify the cultures on PDA. The cultural characteristics and microscopic structures of the *Trichoderma* isolates were characterized using standard methods (Rifai, 1969; Chowdhry, 1996). All the eleven *Trichoderma* isolates were maintained on PDA slants at 4°C for further studies.

Isolation of *Fusarium oxysporum* isolates

Fusarium oxysporum was isolated from diseased tomato plants that showed typical symptoms of *Fusarium* wilt according to Katan *et al*. (1991). The isolates were tested for their virulence on the host, and the most virulent isolate *F*. *oxysporum* isolate RV121 was preserved as the test organism for further studies.

The effect of culture media on the mycelia growth of *Trichoderma* isolates

The effect of different culture media on the mycelial growth of *Trichoderma* isolates for the production of maximum conidial yield was investigated by growing them on three different culture media namely, Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Czapeck Dox Agar (CDA). An agar disc of 5 mm diameter was cut from 7 days old culture of *Trichoderma* isolates and inoculated into the freshly prepared culture media. Each medium was prepared in triplicate and incubated at 25°C. After 7 days of incubation, the diameter of the mycelium growth was measured and recorded (Aneja, 2005; Negash Hailu and Tesfaye Alemu, 2010).

The effect of pH on the mycelial growth of *Trichoderma* isolates

The optimal pH for mycelial growth and the production of maximum conidial yield were determined by growing them on various pH values. The Potato Dextrose Broth (PDB) (200 g potato, 20 g dextrose per litre of distilled water) was prepared and adjusted to different pH levels (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) in order to obtain the optimum and suitable pH value for the growth of the *Trichoderma* isolates. These pH ranges were adjusted by using 1N HCl and 1N NaOH. The media was autoclaved and a disc of 5 mm diameter inoculum was taken from the margin of 7 days old culture grown on PDA and inoculated into the 250 ml flasks containing 100 ml PDB and incubated at 25°C for 15 days. After 15 days of growth in the potato dextrose broth (PDB), mycelial dry weight was measured by using electronic sensitive balance (Zuriash Mamo and Tesfaye

Alemu, 2012).

The effect of temperature on the mycelial growth of *Trichoderma* isolates

The majority of filamentous fungi are mesophilic, growing at temperatures within the range of 10–35°C, of which most grow with temperatures between 15 and 30°C (Tesfaye Alemu and Kapoor, 2004). To evaluate the effect of temperature on the mycelial growth of *Trichoderma* isolates, three *Trichoderma* isolates were grown on Potato Dextrose Agar (PDA) for determining their comparative growth rate. In each of Petri plate 25 ml of molten PDA medium was aseptically poured. The mycelial disc of each *Trichoderma* isolate, 5 mm in diameter was taken from the edge of 7 days old culture which grown on PDA by using sterile cork borer and placed at the centre of 90 ml Petri plate containing freshly prepared PDA. The plates were incubated at five different temperature ranges (15°C, 20°C, 25°C, 30°C and 35°C). Mycelial diameter of each *Trichoderma* isolate in a plate was measured in millimetre at right angle by using plastic ruler. Three replicate plates were used for each *Trichoderma* isolates.

Bioassay of the isolates on their antagonism to the test pathogens

Preparation of inocula of *Trichoderma* isolates

Trichoderma isolates were prepared as proposed by Smith *et al*. (1990). The conidial inoculum for each *Trichoderma* isolate was prepared by adding sterile distilled water to 7 days old cultures under aseptic conditions and centrifuged at 5000 rpm for 10 minutes. The conidial spore suspension was adjusted to 1.5 x 10^6 conidia/ml by using heamocytometer and aseptically transferred into 250 ml flasks containing 100 ml potato dextrose broth (PDB). The flasks were incubated for 15 days on a rotary shaker at 121 revolutions per minute (rpm). The young germinated mycelia were harvested by filtration using sterilized cloth and aseptically washed with sterile distilled water.

In vitro assay of *Trichoderma* isolates against mycelial growth of *F. oxysporum* isolates

The antagonism test was made using the dual culture technique (Tesfaye Alemu and Kapoor, 2004). Two agar discs 5 mm in diameter were cut from 7 days old PDA grown culture of the *Trichoderma* isolates and the test pathogen, *F. oxysporum* isolate RV121. The test pathogen was first placed on the centre of the PDA plates, and after 3 days the antagonist *Trichoderma* isolates were separately inoculated 3 cm apart from each other and

incubated at 25°C for about 7 days (Negash Hailu and Tesfaye Alemu, 2010). The radial growth of pathogen in the control and radial mycelial growth of pathogen in dual culture with antagonist and the width of the zone of inhibition were measured as the smallest distance between the colonies in the dual culture plate (Tesfaye Alemu and Kapoor, 2004).

$$I = \frac{(R1-R2) \times 100}{R1}$$

Where I = Percent inhibition in mycelial growth; R1 = Radial growth of *Fusarium oxysporum* in the control; R2 = Radial growth of *Fusarium oxysporum* isolate in dual culture

Assay of culture filtrates of *Trichoderma* isolates on the mycelial growth of *F. oxysporum* isolates

Secondary metabolites were extracted from the *Trichoderma* isolates as described by Vinale *et al.* (2006). The effect of the culture filtrates of the *Trichoderma* isolates on the test organism was evaluated according to the method of Agarry *et al*. (2005). Accordingly, isolates were grown two hundred fifty (250) ml flasks containing 100 ml of PDB on potato dextrose broth (PDB). The experiment was done in triplicates and the plates were incubated at 25°C for 7 days. The culture was centrifuged at 9000 rpm for 30 minutes. Five (5) ml of filtrate of the culture the isolates was added in sterilized and cooled PDA (45°C) and prepared in plates. Then 5 mm disc taken from the periphery of a 7 days old culture of *F. oxysporum* isolate was placed at the centre of the Petri plates, and incubated for 7 days at 25°C. The radial growth of the pathogen was measured and compared to the control growth where the filtrate was replaced with equal amount of sterile distilled water (Tesfaye Alemu and Kapoor, 2004).

***In vivo* evaluation on the antagonistic activity of *Trichoderma* isolates against the test pathogen**

The antagonistic activities of *Trichoderma* isolates on the test pathogen was undertaken in pot culture under greenhouse conditions according to Bell *et al*. (1982). Six seeds from three tomato varieties (Cochoro, Miya and Fetane), obtained from Melkassa Agricultural Research Centre (MARC), a branch of the Ethiopian Institute of Agricultural Research (EIAR) were surface-sterilized using 2% sodium hypochlorite (NaOCl) and 70% ethanol. They were sown in 20 cm diameter plastic pots filled with 3 kg of autoclaved soil. After seedling emergence, about 20 ml of spore suspension of the test pathogen at a concentration of 1.5×10^6 conidia/ml was

inoculated into each seedling by drenching/flooding method from 7 days culture of the isolates. The control seedlings were inoculated with equivalent amount of sterile distilled water. The experiment included the following treatments: 1) seedlings without pathogen infestation (control); 2) Soil treated with the test pathogen only, 3) The test pathogen + *Trichoderma* isolates. The experiment was arranged using a complete randomized block design (CRBD) and three replicates for each experiment. Percentage of disease incidence (% DI) was recorded (Ishikawa *et al*., 2005; Tesfaye Alemu and Kapoor, 2007).

$$\text{Percent Disease Incidence} = \frac{\text{Number of diseased plants} \times 100}{\text{Total number of plants}}$$

Data analysis

All the experiments were performed in triplicates. Statistical analysis of mycelial growth of *Trichoderma* isolates on different culture media, temperature, and pH and mean comparisons of isolates, mycelial growth inhibition and disease incidence based on different parameters were analyzed by least significant difference (L.S.D.) test at probability of 0.05 to identify significant effect of the treatments. ANOVA analysis was done with the SPSS statistics analysis software (SPSS Institute Inc., Cary, NC 2006).

RESULTS

Cultural and microscopic characterization of *Trichoderma* isolates

The *Trichoderma* isolates were characterized by the presence of fast growing colonies producing white, green, or yellow cushions on potato dextrose agar medium, and sporulating filaments with side branched conidiophores with spherical to ovoid green coloured spores (Fig. 2A).

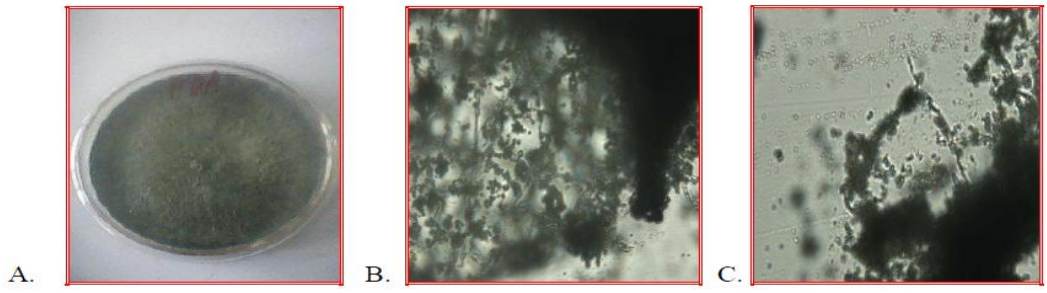


Fig. 2. A. Morphology of *Trichoderma* isolate on PDA medium, B. Microscopic observation of *Trichoderma* isolate AUT9 spores and C. Microscopic observation of *Trichoderma* isolate AUT10 spores.

Growth characteristics of the *Trichoderma* isolates on different media

All the *Trichoderma* isolates showed the largest and smallest and minimum growth diameter on Malt Extract agar (MEA) and Czapeck Dox's Agar, respectively (Table 1). The data also indicated that *Trichoderma* isolate AUT8 and isolate AUT10 showed the maximum and minimum mycelial growth on all types of culture media, respectively. In general, the highest mycelial growth was observed from *Trichoderma* isolates AUT8, AUT9 and AUT10 corresponding to 60.89, 58.44 and 57.11 mm on malt extract agar (MEA), respectively, after 7 days of incubation (Table 1).

Table 1. The effect of different culture media on the mycelial growth of *Trichoderma* isolates after 7 days of incubation at 25°C.

Culture media	***Trichoderma* isolates (Mycelial diameter in mm) (Mean ± SD)**		
	AUT8	**AUT9**	**AUT10**
PDA	57.67 ± 28.40^{b}	56.33 ± 28.50^{b}	52.89 ± 28.00^{b}
MEA	60.89 ± 28.70^{a}	58.44 ± 28.60^{a}	57.11 ± 28.70^{a}
CDA	39.33 ± 25.24^{c}	36.67 ± 24.64^{c}	34.33 ± 24.17^{c}

[a] Each value is an average of three replicates ± standard deviation. Data followed by the same letter are not significantly different (P≤0.05), according to Duncan's multiple range test

The effect of temperature on the mycelial growth of *Trichoderma* isolates

All isolates are best at 25°C, whereas slow mycelial growth was recorded at a temperature of 15°C (Table 2). The maximum mycelial growth of 66.11, 61.44 and 59.68 mm was displayed at 25°C by *Trichoderma* isolates AUT8, AUT9 and AUT10, respectively.

Table 2. The effect of temperature on the mycelial growth of *Trichoderma* isolates on PDA medium after 5 days of incubation.

Temperature (°C)	***Trichoderma* isolates (Mycelial diameter in mm) (Mean ± SD)**		
	AUT8	**AUT9**	**AUT10**
15°C	36.44 ± 24.77^{e}	34.44 ± 23.19^{e}	31.78 ± 20.87^{e}
20°C	45.56 ± 22.40^{d}	43.44 ± 21.60^{c}	40.56 ± 22.40^{c}
25°C	66.11 ± 28.00^{a}	61.44 ± 26.40^{a}	59.68 ± 27.50^{a}
30°C	62.00 ± 29.00^{b}	55.00 ± 32.20^{b}	50.78 ± 33.90^{b}
35°C	51.56 ± 22.16^{c}	48.56 ± 21.50^{bc}	46.22 ± 19.22^{bc}

[a] Each value is an average of three replicates ± standard deviation. Data followed by the same letter are not significantly different (P≤0.05), according to Duncan's multiple range test

The effect of pH on the mycelial growth of *Trichoderma* isolates

The effect of pH on the mycelial growth of *Trichoderma* isolates is shown in Fig. 3. The best mycelial growth was recorded at pH 5.5 and pH 3.5 from the isolate AUT8 with a mycelial biomass of 1228.80 mg and 1133.20 mg, respectively. The least mycelial growth of 552 mg was measured from isolate AUT8 at a pH 7.0. In general, good mycelial growth for

Trichoderma isolate AUT8 was recorded at pH between 5.5–6.5. With regard to *Trichoderma* isolate AUT9, the best mycelial growth was detected at pH 6.5 with mycelial biomass of 722.70 mg. Most isolates did not show good growth as pH decreased to 3.

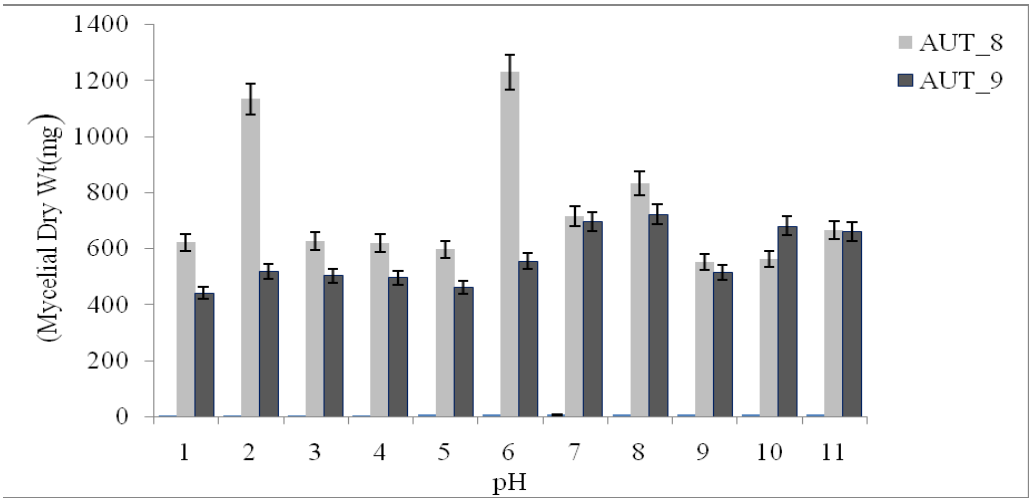


Fig. 3. The effect of pH on the mycelial growth of *Trichoderma* isolates grown on potato dextrose broth (PDB).

In vitro assay of *Trichoderma* isolates against *Fusarium oxysporum* isolate

The antagonistic effect of *Trichoderma* isolates against the test pathogen, *Fusarium oxysporum* isolate is shown in Table 1 and Fig 4. Accordingly, *Trichoderma* isolate AUT9 showed the highest percentage inhibition of the growth of the test pathogen with inhibition percentage of 50.83% compared to isolates AUT10 and AUT8 with percent inhibition of 41.99% and 28.24%, respectively (Table 3).

Table 3. *In vitro* evaluation of *Trichoderma* isolates on the mycelial growth *Fusarium oxysporum* isolates in the dual culture on PDA medium after 7 days of incubation at 25℃.

***Trichoderma* isolates**	**Mycelial growth of *F. oxysporum* isolate (mm)**	**Percent inhibition of mycelial growth of *F. oxysporum* isolate**
AUT8	20.30	28.24
AUT9	13.91	50.83
AUT10	16.41	41.99
Control	28.29	0.00

Each value is an average of three replicates

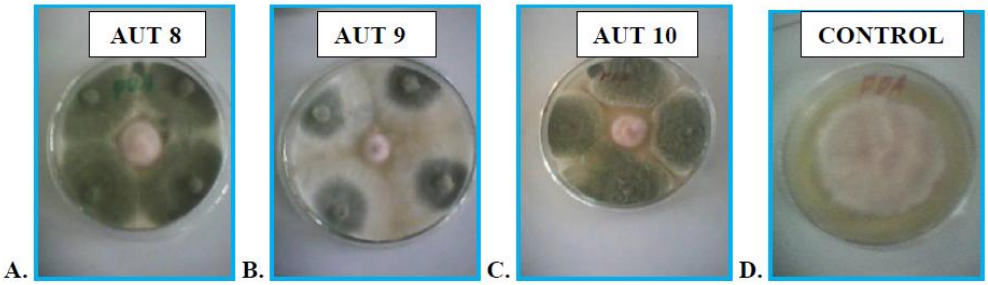


Fig. 4. The antagonistic effect of *Trichoderma* isolates on the mycelial growth of *Fusarium oxysporum* isolate in the dual culture technique A. *Trichoderma* isolate (AUT8) B. *Trichoderma* isolate (AUT9) C. *Trichoderma* isolate (AUT10) and D. Control.

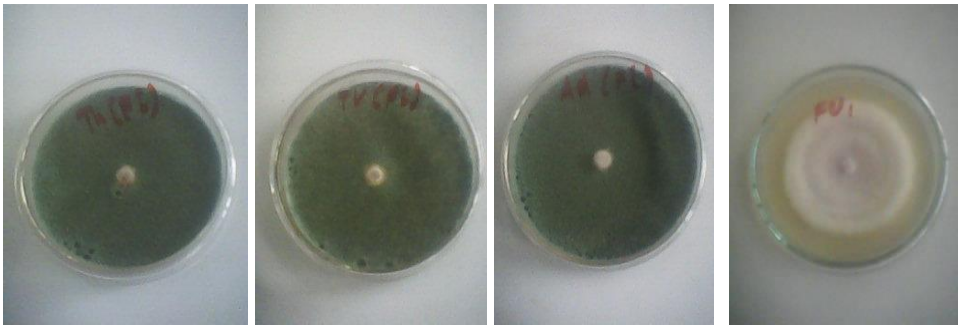
Assay of culture filtrates from *Trichoderma* on growth of the test pathogen

From the antagonistic *Trichoderma* isolates, culture filtrate of isolate AUT9 showed the maximum growth inhibition of the test pathogen with percent inhibition of 66% followed by 58% inhibition by isolate AUT10 (Table 4).

Table 4. The effect of culture filtrates of *Trichoderma* isolates on the mycelial growth of *Fusarium oxysporum* isolate after 4 days of incubation on PDA.

Isolates	**Mycelial growth of pathogen in the culture filtrates of *Trichoderma* isolates (diameter in mm)**	**Average % inhibition of mycelial growth of the test pathogen**
AUT8	10.44	61
AUT9	9.10	66
AUT10	11.24	58
Control	26.78	0.00

Each value is an average of three replicates



A B C D

Fig. 5. The effect of culture filtrate of *Trichoderma* isolates on the mycelial growth of *Fusarium oxysporum* isolate. A. *Trichoderma* isolate (AUT8); B. *Trichoderma* isolate (AUT9); C. *Trichoderma* isolate (AUT10) and D. *Fusarium oxysporum* isolate.

In vivo evaluation of antagonistic activity of *Trichoderma* isolates against the test pathogen under greenhouse condition

Wilting symptoms were observed on all the three tomato varieties after 60 days of sowing, but Miya variety was more susceptible to *Fusarium* wilt compared to the Cochoro and Fetane varieties (Fig. 6). The symptoms were yellowing of lower leaves and discolouration of stem. The re-isolated strains from the diseased plants were similar to the original test pathogen *F. oxysporum*. All the tomato varieties showed significant (p=0.05) differences between the treatments and negative controls. Among the tomato varieties, Miya variety showed the shortest shoot length, lowest shoot and root fresh weights, and lowest shoot and root dry weights in all the treatments.

However, Fetane variety showed the highest shoot length, shoot and root fresh weights, shoot and root dry weights in all the treatments. All tomato varieties showed the highest shoot length, shoot and root fresh weights when treated with *Trichoderma* isolate AUT9 (Table 5). The disease incidence was slightly lower on varieties treated with isolate AUT9 compared with those treated with isolate AUT8. Accordingly, Miya, Cochoro and Fetane varieties treated with AUT9 showed disease incidence of 19.44, 16.67 and 11.11%, compared with the disease incidence of 27.78, 22.22 and 21.22%, treated with *Trichoderma* isolate AUT8, respectively. The untreated varieties (control groups) showed the highest disease incidence of 83.33, 77.78 and 72.22%, with their respective varieties, respectively (Table 6). In general, Miya variety was more susceptible to *Fusarium* wilt than Cochoro and Fetane varieties.

Table 5. The effect of *Trichoderma* isolates on tomato plant shoot length, shoot and root fresh weights and dry weights inoculated with *Fusarium oxysporum* isolates under greenhouse condition.

Tomato varieties	Treatments	Plant fresh weight (g) Mean ± SD		Plant dry weight (g) Mean ± SD		Shoot length (cm)
		Shoot	Root	Shoot	Root	Mean ± SD
1. Cochoro	AUT8 + FoI	61.83 ± 18.81^{c}	15.18 ± 3.79^{c}	11.67 ± 2.53^{c}	1.70 ± 0.79^{c}	39.00 ± 8.66^{d}
	AUT9 + FoI	136.33 ± 30.14^{b}	35.67 ± 10.01^{a}	20.33 ± 5.50^{b}	4.93 ± 0.90^{a}	52.33 ± 3.51^{b}
	AUT10 + FoI	69.18 ± 30.72^{c}	16.18 ± 6.66^{c}	12.40 ± 6.73^{c}	2.33 ± 1.17^{b}	41.33 ± 2.50^{c}
	Positive control (Seed only)	175.00 ± 44.93^{a}	32.33 ± 6.79^{b}	30.17 ± 3.75^{a}	5.50 ± 1.32^{a}	57.67 ± 6.51^{a}
	Negative control	41.67 ± 29.88^{d}	10.50 ± 4.92^{d}	9.67 ± 3.21^{c}	1.33 ± 0.50^{d}	36.67 ± 6.43^{d}
2. Miya	AUT8 + FoI	40.33 ± 7.02^{c}	11.67 ± 1.53^{c}	7.00 ± 2.29^{d}	1.97 ± 0.40^{d}	29.00 ± 4.36^{a}
	AUT9 + FoI	77.50 ± 44.33^{b}	24.33 ± 22.23^{b}	17.33 ± 13.32^{b}	4.50 ± 1.32^{b}	43.67 ± 14.43^{a}
	AUT10 + FoI	59.83 ± 47.79^{c}	22.68 ± 18.56^{b}	10.43 ± 7.54c	2.87 ± 2.15^{c}	41.33 ± 13.65^{a}
	Positive control (Seed only)	161.00 ± 74.98^{a}	49.33 ± 15.22^{a}	31.67 ± 20.60^{a}	6.90 ± 2.65^{a}	58.33 ± 7.64^{a}
	Negative control	37.33 ± 21.13^{d}	8.00 ± 1.80^{d}	6.50 ± 1.00^{d}	1.17 ± 0.35^{e}	28.67 ± 5.03^{a}
3. Fetane	AUT8 + FoI	70.00 ± 50.57^{b}	21.00 ± 7.94^{b}	12.10 ± 5.37^{c}	2.10 ± 0.36^{c}	48.33 ± 9.71^{b}
	AUT9 + FoI	119.83 ± 58.87^{a}	47.00 ± 19.94^{a}	14.67 ± 6.43^{b}	6.43 ± 3.39^{b}	56.33 ± 3.51^{a}
	AUT10 + FoI	115.67 ± 67.45^{a}	24.67 ± 4.16^{b}	13.17 ± 8.91^{b}	3.90 ± 0.78^{b}	54.40 ± 7.21^{a}
	Positive control (Seed only)	133.33 ± 32.32^{a}	47.50 ± 22.79^{a}	24.00 ± 14.93^{a}	7.40 ± 6.60^{a}	59.00 ± 4.58^{a}
	Negative control	64.00 ± 32.75^{b}	13.33 ± 5.51^{c}	10.33 ± 4.51^{d}	1.70 ± 0.61^{d}	44.00 ± 2.00^{b}

[a] Each value is an average of three replicates. Data followed by the same letters are not significantly different (P≤0.05) according to Duncan's multiple range test.

Table 6. Percentage of disease incidence on three tomato varieties inoculated with *Fusarium oxysporum* isolate under greenhouse condition.

Tomato varieties	Treatments	Disease incidence (%)
1. Cochoro	AUT8 + FoI	22.22
	AUT9 + FoI	16.67
	AUT10 + FoI	18.32
	Positive control (Seed only)	5.56
	Negative control (FoI only)	77.78
2. Miya	AUT8 + FoI	27.78
	AUT9 + FoI	19.44
	AUT10 + FoI	25.00
	Positive control (Seed only)	8.33
	Negative control (FoI only)	83.33
3. Fetane	AUT8 + FoI	21.22
	AUT9 + FoI	11.11
	AUT10 + FoI	16.67
	Positive control (Seed only)	2.78
	Negative control (FoI only)	72.22

Note: FoI means *Fusarium oxysporum* isolate

Fig. 6. The effect of different varieties to *Fusarium oxysporum* isolate Cochoro variety (A-E), Miya Variety (E-J) and Fetane Variety (K-O).

DISCUSSION

The isolates from diseased plants showed variations in their pathogenicity. *F. oxysporum* isolate RV121 was the most pathogenic of all the isolates. It was a tour collection, preserved and used as a test pathogen for this study. It was also clearly evident from this study that different *Trichoderma* isolates responded differently to various temperatures ranges. Mean mycelial growth for all *Trichoderma* isolates was maximum at 25°C. It goes well with the findings of Lilly and Barnett (1951) report that states temperature to affect almost every function of the fungal plant pathogens and that of Papavizas

(1985) who reported that different species of *Trichoderma* have their own ecological preferences.

In the current study the highest growth diameter on the solid media was obtained by *Trichoderma* isolate (AUT8) at pH 5.5. Similarly, the highest mycelial growth of *Trichoderma* isolate (AUT9) was observed at pH 6.5. Similarly, Jackson *et al*. (1991) obtained optimum biomass production of *Trichoderma* isolates at pH ranges between 4.6 and 6.8. Also, Seyis and Aksoz (2005) observed that activity of *Trichoderma harzianum* was maximum around pH 5. It has been demonstrated that *Trichoderma* strains are active under a wide range of pH (Kredics *et al*., 2003). In dual culture the maximum mycelial growth inhibition was obtained by *Trichoderma* isolate (AUT9). However, Negash Hailu and Tesfaye Alemu (2010) observed the highest mycelial growth inhibition of 70.9% by *T. viride* on another test pathogen *F. xylarioides* isolates (Fx.22).

Similarly, the culture filtrate of *Trichoderma* isolate AUT9 gave the highest mycelial growth inhibition of 58% on the test pathogen. This is similar to the study of the culture filtrate of *Trichoderma viride* by Negash Hailu and Tesfaye Alemu (2010). The authors showed mycelial growth inhibition of 63.6% of the test pathogen *Fusarium xylarioides* compared to the control. Similarly, the antagonistic activity of different *Trichoderma* isolates against different phytopathogenic fungi such as *Rhizoctonia solani*, and *Sclerotium rolfsii* was reported by Deshmukh and Raut (1992). The presence of inhibitory activity in culture filtrates on the test pathogen had been shown from *Trichoderma harzianum* in dual cultures with *F. oxysporum* f. sp. *lycopersici* indicating secretion of diffusible non-volatile inhibitory substance by the antagonist, *Trichoderma* (Grodona *et al*., 1997; Behzad *et al*., 2008).

It was observed that Miya and Fetane varieties were the most susceptible resistant varieties to *Fusarium oxysporum* wilt, respectively. All *Trichoderma* isolates increased shoot length, shoot and root fresh weights of the tomato plants compared to pathogen inoculated (negative control). *Trichoderma* isolate AUT9 showed the maximum shoot length, shoot and root fresh weights on three tomato varieties. Similarly, Ozbay and Newman (2004) indicated that *Trichoderma harzianum* strains significantly increased the height, shoot and root dry weight in tomato seedlings transplanted into pots under greenhouse conditions. Similarly, Altomare *et al*. (1999) reported that *Trichoderma* species increase growth of the plant by providing them with solubilized phosphates and micronutrients in the soil. From this study it

is possible to conclude that *Trichoderma* isolates AUT9 was the most effective to control *Fusarium oxysporum* both *in vitro* and *in vivo* conditions. The performance and growth of tomato plants increased by treating them with this isolate. It was also observed that Fetane variety was the most resistant to *Fusarium* wilt of tomato (*Fusarium oxysporum*) under greenhouse conditions.

ACKNOWLEDGEMENTS

The authors greatly acknowledge the Department of Microbial, Cellular and Molecular Biology and Institute of Biotechnology, College of Natural and Computational Sciences of the Addis Ababa University for funding and providing the laboratory facilities and supply.

REFERENCES

Agarry, O.O., Akinyosoye, F.A. and Adetuyi, F.C. (2005). Antagonistic properties of microogranisms associated with cassava (*Manihot esculenta* Crantz) products. *Afr. J. Biotechnol*. **4**: 627–632.

Altomare, C., Norvell, W.A., Bjorkman, T. and Harman, G.E. (1999). Solubilization of phosphates and micronutrients by plant growth promoting and biocontrol fungus *Trichoderma harzianum* strain 1295-22. *Appl. Environ. Microbiol*. **65**: 2926–2933.

Aneja, K.R. (2005). **Experiments in Microbiology, Plant Pathology and Biotechnology**. 4th edn., New Age International Pvt. Ltd., India.

Behzad, H., Mousa, T., Mohammad, R.M. and Mahdi, D. (2008). Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. *Afr. J. Biotechnol*. **7**: 967–972.

Bell, D.K., Well, H.D. and Markham, C.R. (1982). *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology* **72**: 379–382.

Borrero, C., Trillas, M.I., Ordales, J., Tello, J.C. and Aviles, M. (2004). Predictive factors for the suppression of Fusarium wilt of tomato in plant growth media. *Phytopathology* **94**: 1094–1101.

Chowdhry, P.N. (1996). Identification of *Trichoderma* species. Division of Plant Pathology, Indian Agricultural Research Institute.

Dagnatchew Yirgou and Stewart, R.B. (1967). Index of plant diseases in Ethiopia. Experimental, station bulletin no. 30. College of Agriculture, Haile Sillassie University, Debrezeit.

De Cal, A., Pascual, S., and Melgarejo, P. (1997). A rapid laboratory method for assessing the biological control potential of *Penicillium oxalicum* against Fusarium wilts of tomato. *Plant Pathol*. **46**: 699–707.

Deshmukh, P.P. and Raut, J.G. (1992). Antagonism by *Trichoderma* species on five plant pathogenic fungi. *New Agric.* **3**(2): 127–130.

Grodona, I., Hermosa, M.R., Tejada, M., Gomis, M.D., Mateos, P.F., Bridge, P., Monte, E. and García-Acha, I. (1997). Physiological and biochemical characterization of *Trichoderma harzianum*, a biological control agent against soil borne fungal plant pathogens. *Appl. Environ. Microbiol*. **63**: 3189–3198.

Hoitink, H.A.J., Madden, L.V. and Dorrance, A.E. (2006). Systemic resistance induced by

Trichoderma spp.: Interactions between the host, the pathogen, the biocontrol agent, and soil organic matter quality. *Phytopathology* **96**: 186–189.

Hoyos-Carvajal, L., Orduz, S. and Bissett, J. (2009). Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*. *Biol. Control* **51**(3): 409–416.

Ishikawa, R., Shirouzu, K., Heitefuss, H., Nakashita, H.Y., Lee, T., Motoyama, I., Yamaguchi, T. and Arie, T. (2005). Foliar spray of validamycin A or validoxylamine A controls tomato Fusarium wilt. *Phytopathology* **95**: 1209–1216.

Jackson, A.M., Whipps, J.M. and Lynch, J.M. (1991). Effects of temperature, pH and water potential on growth of four fungi with disease biocontrol potential. *World J. Microbiol. Biotechnol*. **7**: 494–501.

Kader, A.J., Omar, O. and Feng, L.S. (1999). ASENA Review of Biodiversity and Environmental Conservation (ARBEC). http://www.arbec.com.my/pdf/art12 sepoct99.pdf.

Katan, T., Zamir, D., Sarfati, M., and Katan, J. (1991). Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f. sp. *radicis lycopersici. Phytopathology* **81**: 255–262.

Kirankumar, R., Jagadeesh, K.S., Krishnaraj, P.U. and Patil, M.S. (2008). Enhanced growth promotion of tomato and nutrient uptake by plant growth promoting rhizobacterial isolates in presence of tobacco mosaic virus pathogen. *Karnataka J. Agric. Sci.* **21**(2): 309–311.

Kocchar, S.D. (1981). **Tropical Crops. A Text book on Economics Botany**. Macmillan Publishers, London and Basingstoke.

Koch, E. (1999). Evaluation of commercial products for microbial control of soil-borne plant disease. *Crop Prot*. **18**: 119–125.

Kredics, L., Antal, Z., Manczinger, L. and Szekeres, A. (2003). Influence of environmental parameters on *Trichoderma* strains with biocontrol potential. *Food Technol. Biotech*. **41**(1): 37–42.

Larkin, R.P. and Fravel, D.R. (1998). Efficacy of various fungal and bacterial biocontrol organisms for the control of Fusarium wilt of tomatoes. *Plant Dis*. **82**: 1022–1028.

Lemma, D and Herath, E. (1994). Varietal development on vegetables. In: **Proceeding of the Second National Horticultural Workshop**, pp. 1–3 (Herath, E. and Lemma, D., eds.). Addis Ababa.

Lilly, V.G. and Barnett, H.L. (1951). **Physiology of Fungi**. McGraw Hill Book Company Inc., New York.

Mastouri, F., Bjorkman, K. and Harman, G.H. (2010). Seed treated with *Trichoderma harzianum* alleviates biotic, abiotic and physiological stresses in germinating seeds and seedlings. *Phytopathology* **100**: 1213–1221.

MoARD (2009). Course for training of trainers on improved horticultural crop technologies. Rural Capacity Building Project, MoARD, Addis Ababa.

Negash Hailu and Tesfaye Alemu (2010). *In vitro* evaluation of antagonistic potential activity and assay of culture filtrates of *Trichoderma harzianum* and *T. viride* against coffee wilt disease (*Fusarium xylarioides*) isolates. *Ethiop. J. Biol. Sci*. **9**(1): 67–77.

Ozbay, N. and Newman, S.E. (2004). Effect of *Trichoderma harzianum* strains to colonize tomato root and improve transplant growth. *Pak. J. Biol. Sci*. **7**: 253–257.

Pal, K.K. and Gardener, B.M. (2006). **Biological Control of Plant Pathogens**. Ohio State University, OARDC, Wooster, Ohio.

Papavizas, G.C. (1985). *Trichoderma* and *Gliocladium*: Biology, ecology and potential for

biocontrol. *Annu. Rev. Phytopathol.* **23**: 23–54.

Rifai, M.A. (1969). A revision of the genus *Trichoderma*. *Mycol. Pap.* **116**: 1–56.

Samuels, G.J. (1996). *Trichoderma*: A review of biology and systematics of the genus. *Mycol. Res*. **100**: 923–935.

Seyis, I. and Aksoz, N. (2005). Investigation of factors affecting xylanase activity from *Trichoderma harzianum* 1073 D3. *Braz. Arch. Biol. Technol*. **48**(2): 187–193.

Shanmugaiah, V., Balasubramanian, N., Gomathinayagam, S., Monoharan, P.T., and Rajendran, A. (2009). Effect of single application of *Trichoderma viride* and *Pseudomonas fluorescens* on growth promotion in cotton plants. *Afr. J. Agric. Res*. **4**: 1220–1225.

Smith, V.L., Wilcox, W.F. and Harman, G.E. (1990). Potential for biological control of *Phytophthora* root and crown rots of apple by *Trichoderma* and *Gliocladium* species. *Phytopathology* **80**: 880–885.

Tesfaye Alemu and Kapoor, I.J. (2004). *In vitro* evaluation of *Trichoderma* and *Gliocladium* spp against Botrytis corm rot (*Botrytis gladiolorum*) of Gladiolus. *Pest Manage. J. Ethiop.* **8**: 97–103.

Tesfaye Alemu and Kapoor, I.J. (2007). *In vivo* evaluation of *Trichoderma* species against Botrytis corm rot/ blight of Gladiolus. *Ethiop. J. Biol. Sci.* **6**(2): 165–171.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Barbetti, M.J., Li, H., Woo, S.L. and Lorito, M. (2006). Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. *Lett. Appl. Microbiol*. **43**: 143–148.

Yedidia, I., Srivastva, A.K., Kapuluik, Y. and Chet, I. (2001). Effect of *Trichoderma harzianum* on microelement concentration and increased growth of cucumber plants. *Plant Soil*. **235**(2): 235–242.

Zuriash Mamo and Tesfaye Alemu (2012). Evaluation and optimization of agro-industrial wastes for conidial production of *Trichoderma* isolates under solid state fermentation. *J. Appl. Biosci*. **54**: 3870–3879.