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Full Length Research Paper

Biopotential of some *Trichoderma* spp. against cotton root rot pathogens and profiles of some of their metabolites

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The antagonistic activity of five selected isolates of *Trichoderma* spp. (*Trichoderma harzianum* CCM340, *Trichoderma koningii* CCM341, *Trichoderma longibranchiatum* NRRL11236, *Trichoderma viride* DSM63065 and *Trichoderma viride*) against *Fusarium oxysporum* f.sp. vasinfectum and *Rhizoctonia solani*, the causal agents of root rot of cotton plants grown in saline and non-saline soil, was studied *in vitro* and *in vivo*. Results indicated that *F. oxysporum* and *R. solani* were mostly inhibited by *T. viride* and least inhibited by T. longibranchiatum NRRL11236. Greenhouse test showed that for both pathogens, *T. koningii* CCM341 gave the highest disease control percentage (100%) in saline and non-saline soil and that *T. harzianum* CCM340, *T. longibrachiatum* NRRL11236 and *T. viride* DSM63065 gave the same disease control percentage (100%) in non-saline soil. In addition, the effect of *Trichoderma* isolates on several growth parameters of cotton plants was also studied. On the other hand, study on metabolites such as amino acids (AA), fatty acids (FA) and plant growth hormones (PGH) produced by these *Trichoderma* isolates showed that *T. viride* was the highest in AA production and the only isolate that produced lauric acid (FA). The highest amount of gibberellic acid, indol-3-acetic acid and abscisic acid was produced by *T. viride* DSM63065, *T. harzianum* CCM340 and *T. koningii* CCM341, respectively. Also, the pH and temperature effects on *Trichoderma* mycelial growth were also evaluated. However, the application of *Trichoderma* spp. to soil infested with *F. oxysporum* and *R. solani* provided a good way for protection of cotton plants. Also, *Trichoderma* metabolites such as amino acids, fatty acids and plant growth hormones may be developed into several commercial biological control products to prevent development of several pathogenic fungi and / or to induce plant growth response. Evaluation of pH and temperature effects on *Trichoderma* isolates mycelial growth showed that *Trichoderma* strains were found to be able to display activities under a wider range of pH values and that they are mesophili.

Key words: *Fusarium oxysporum, Rhizoctonia solani*, cotton growth parameters, biocontrol agent, salinity.

INTRODUCTION

Salinity is one of the major problems that face the farmers all over the world. Salt-affected soils occupy more than 7% of the earth's land surface, and represent a major limiting factor in crop production (Tian et al., 2004). More than 25% of irrigated land is saline in Egypt, Iran, Iraq, India, Pakistan and Syria (Choukr-Allah, 1996). Egypt is one of the countries that have been highly affected by salinity and a large area of its agricultural land is now rated as un-productive due to salinity. Increasing soil salinity is a very serious and growing problem in Egypt. Soil salinity inhibits plants growth by closing the stomata, which reduces the CO_2/O_2 ratio in the leaves and thus inhibits CO_2 fixation (Epstein, 1989). As a result, the rate of leaf elongation, enlargement and cells division is reduced (Allen et al., 1998).

Cotton is not only the most important fiber crop in the

world, but also the second best potential source for plant proteins after soybean and the fifth best oil-producing plant after soybean, palm-tree, colza and sunflower (Texier, 1993).

Cotton is infected by a number of pathogens inducing different diseases, among them the damping-off caused by *Rhizoctonia solani* and so the plant suffers heavy losses particularly during the early stage of crop growth (Nawar, 2008). Diseased seedlings showed damping-off in which the fungus attacked the basal part of the stem and eventually led to collapse of the plant on the soil surface. Many bare areas appeared in the diseased fields. *Fusarium oxysporum* and *Rhizoctonia solani* are reported to be active participants in the cotton seedling disease complex with varying degrees of virulence. Moreover, *F. oxysporum* f. sp. vasinfectum, causing Fusarium wilt of cotton disease (*Gossypium* spp. L.), occurs in most major cotton production regions of the world (Colyer, 2001).

Several studies have been made to characterize the fungal flora of soil adhering to the roots of numerous growing or cultivated plants in many parts of the world (Mazen and Shaban, 1983; Abdel Hafez et al., 1990, 1995; Nagaraja, 1991; Rajendra and Saxena, 1991; Abdelzaher et al., 2000; Maghazy et al., 2008).

The genus *Trichoderma* includes the most common saprophytic fungi in the rhizosphere and is found in almost any soil. The mycoparasite ability of *Trichoderma* spp. against economically important aerial and soil borne plant pathogens (Papavizas and Lumsden, 1980; Dubey et al., 2007), the ability to compete with pathogens and to induce resistance among others (Vinal et al., 2008) allows the development of different bio-control strategies. Several *Trichoderma* spp. reduce the incidence of soil borne plant pathogenic fungi under natural conditions (Calvet et al., 1990). Recently there have been numerous attempts to use *Trichoderma* spp. against soil borne pathogens such as *Sclerotinia, Fusarium, Pythium* and *Rhizoctonia* species (Dubey et al., 2007; Hassanein, 2010). *Trichoderma harzianum, Trichoderma viride, Trichoderma virens, Trichoderma hamatum, Trichoderma roseum* and *Trichoderma koningii* are the species that are most often used as biological controls for pathogens. *Trichoderma* produced in recent years, have been developed into several commercial biological control products to prevent development of several pathogenic fungi.

Different isolates of *Trichoderma* have various strategies for fungus antagonism as well as several indirect effects on plant health. Therefore, one of the most interesting aspects in biology is the study of the mechanisms employed by bio-control agents that affect disease control. Possible mechanisms of antagonism applied by *Trichoderma* spp. includes nutrient and niche competition, antibiosis by producing volatile components and non-volatile antibiotics (Harman and Hadar, 1983) that act as inhibitors against many soil-borne fungi, and last parasitism (Dennis and Webster, 1971). Also, synergism between different forms of action modes occurs as the natural condition for the biocontrol of fungal pathogens.

Trichoderma produces a plethora of secondary metabolites with biological activity (Ghisalberti and Sivasithamparam, 1991; Sivasithamparam and Ghisalberti, 1998; Vinal et al., 2008). The term "secondary metabolite" includes a heterogeneous group of different natural compounds possibly related to survival functions for the producing organism, such as competition against other micro- and macroorganisms, symbiosis, metal transport, differentiation, etc. (Denman and Fang, 2000). Includes in this group are antibiotics, which are natural products able to inhibit microbial growth. Ghisalberti et al. (1990) demonstrated that the bio-control efficacy of *T. harzianum* against *Gaeumannomyces graminis* is related to the production of pyrone-like antibiotics.

The production of secondary metabolites by *Trichoderma* spp. is strain dependent and includes antifungal substances belonging to a variety of classes of chemical compounds among which peptaiboles, which are linear oligopeptides of 12-22 amino acids rich in α-aminobuteric acid, N-acetylated at the N-terminus and containing an amino alcohol at the C-terminus (Le Doan et al., 1986; Rebuffat et al., 1989).

The aim of the present investigation was to study: (i) The capability of five Trichoderma isolates belonging to four species (*T. harzianum* CCM340, *T. koningii* CCM341, *T. longibranchiatum* NRRL11236, *T. viride* DSM63065 and *T. viride*) to produce some metabolites such as amino acids, fatty acids and plant growth hormones. (ii) The effect of different temperatures and pH values on mycelia growth of the tested *Trichoderma* species. (iii) The *in vitro* biological potential of the selected *Trichoderma* spp. against *F. oxysporum* and *R. solani*. (iv) Greenhouse experiments to study the role of these *Trichoderma* spp. in controlling *F. oxysporum* and *R. solani* and their effects on some growth parameters of cotton plants in saline and non-saline soil.

MATERIALS AND METHODS

Trichoderma spp.

Trichoderma spp. used in this study (*T. harzianum* CCM340, *T. koningii* CCM341, *T. longibranchiatum* NRRL11236, *T. viride* DSM63065) were obtained from Egypt Microbial Culture Collection (EMCC) (Cairo MIRCEN). However, T. viride was isolated from the rhizosphere of cotton plants cultivated in saline soil from El-Fayoum and El-Beheira governorates in Egypt.

Production of amino acids, fatty acids and plant growth hormones by *Trichoderma* spp.

Amino acids, fatty acids and plant growth hormones analysis of *Trichoderma* spp. were carried out in the Research Park, Faculty of Agriculture, Cairo University, Cairo, Egypt.

Media

PDA (Potato dextrose agar) was used for cultivation of the *Trichoderma* spp. The same medium without agar was then used for extraction of the amino acids, fatty acids and plant growth hormones.

Amino acids

Hydrolysis of amino acids

Acid hydrolysis was carried out according to the method of Block et al. (1958). The dried grinded sample (100 mg) was hydrolyzed with 6N HCl (10 ml) in a sealed tube at 110°C in an oven for 24 h. The excess of HCl was then freed from 1 ml hydrolyzed under vacuum with occasionally addition of distilled water, then evaporated to dryness. The HCl free residue was dissolved in extract (2 ml) of diluting buffer (0.2 M, pH 2.2).

Preparation of diluting solution of buffer 0.2 M Na, pH 2.2

The buffer is used for the dilution of both samples and standards to required concentration. It will be sufficient if prepared according to the following recipe: citric acid 14.0 g/L, sodium chloride 11.5 g/L, thiodiglycol 5.0 g/L and sodium azide 0.1 g/L.

Fatty acids

Lipid extraction

The method of AOAC (2000) was conducted for lipid extraction from sample using chloroform methonal (2:1 v/v). The lipids in chloroform were dried over anhydrous sodium sulfate, then the solvent was removed by heating at 60°C under vacuum.

Separation of fatty acids

The lipid samples were saponified over – night with ethanoic KOH (20%) at room temperature. The fatty acids were freed from their potassium salts by acidification with hydrochloric acid (5N), followed by extraction with ether or (pt. ether 40 to 60°C). The ether extract was washed three times with distilled water then dried over anhydrous sodium sulfate, and filtered off (Vogel, 1975).

Preparation of diazomethane

Diazomethane was prepared from methylamine hydrochloride as reported by (Vogel, 1975) as follows: methylamine solution (100 ml) was placed in a stoppered 500 ml flask and concentrated hydrochloric acid (78 ml) and water was added to bring the total weight to 250 g. Urea (150 g) was introduced and the mixture was boiled gently under reflux for 200 min and vigorously for 15 min, the solution was cooled to room temperature, then sodium nitrite (55 g) was added at 0°C. A mixture of 300 g crushed ice and 50 g concentrated sulfuric acid was prepared in 1500 ml beaker surround by a bath of ice and salt. Cold methyl urea – nitrite solution was added slowly with mechanical stirring at such rate, that the temperature did not rise above 0°C. The crystalline nitrosomethyl urea was filtered at once then drained well and dried in vacuum desiccators. Aqueous potassium hydroxide solution (60 ml, 50 % w/w) and ether (200 ml) were placed in 500 ml round bottomed flask. The mixture was cooled to 5°C, and then nitrosomethyl urea (20.6 g) and ether (80 ml) were added. The ethereal layer was separated using separating funnel and dried over pellets of potassium hydroxide for 2 to 3 h.

Methylation of fatty acids with diazomethane

Fatty acids produced from lipid samples and standard fatty acids were dissolved in a little methanol and the ethereal solution of diazomethane was added in a small portion until gas evolution ceased. The mixture acquired a pale yellow color that indicated the addition of excess of diazomethane, the reaction mixture was left for 10 min and ether was evaporated under nitrogen stream at room temperature. Two drops of redistilled chloroform solution was added to dissolve the fatty acids methyl esters and 10 ml of this solution were injected into the gas chromatography.

Sources of standard fatty acids

A set of standard fatty acids of 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1 and 22:0 with a stated purity of 99% by GLC was purchased from Nu – check Prop. The purity of each fatty acid methyl ester was checked by GLC and gave one peak.

Identification and determination of fatty acids by gas liquid chromatography (GLC)

The method described by Farag et al. (1986) was applied for determination of fatty acids by GLC. The methyl esters of fatty acids obtained from oil of samples and standard materials were analyzed with a Pye Unicam Series 304 gas chromatography equipped with dual flam ionization detector and dual channel recorder. The separation of fatty acid methyl esters was conducted using a coiled glass column (1.5 m x 4 mm) packed with Diatomite (100 to 120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8°C per min to 190°C, then isothermally at 190°C for 25 min with nitrogen at 30 ml/min.

Fractionation of unsaponifiables

The unsaponifiables were also fractionated on a coiled glass column (2.8 m x 4 mm) packed with Diatomite (100 to120 mesh) and coated with 3% OV – 17. The oven temperature was programmed at 10°C/min from 70°C, then isothermally at 270°C for 25 min and nitrogen flow rate was 30 ml/min. Detector injector temperatures and hydrogen, air flow rates were generally 300°C, 280°C and 33 ml, 330 ml/min, respectively. Peak identification was performed by comparison the retention time (RT) of each compound with those of standard materials. The linear relationship between log retention times of the standard hydrocarbons and the number of carbon atoms of these compounds was used to characterize the unavailable authentic hydrocarbons. Peak area was measured by using a computing integrator (PU 4810, Philips).

Plant growth hormones

The method of extraction was essentially similar to that adopted by Shindy and Smith (1975) and described by Hashem (2000). The fungal mycelia were collected and ground in cold 80% methanol. The macerated mycelia were transferred to a flask with methanol and the volume was adjusted to 20 ml of methanol for each g fresh weight of sample. The mycelia were extracted for 2 h at 0°C and then were vacuum filtered through filter paper Whatman No. 12.

The residue were returned to the flask with fresh volume of methanol and stirred for 30 min with magnetic stirrer and then filtered again. The procedure was repeated once more and the combined extracts were evaporated to the aqueous phase in a rotatory flask evaporated. The aqueous phase (10 to 30 ml) was adjusted to pH 8.6 with 1% (w/v) NaOH and partitioned three times with equal volumes of ethyl acetate. The combined ethyl acetate fraction was evaporated to dryness and held for further purification. The aqueous phase was adjusted to pH 2.8 with 1% HCl (v/v) and re-partitioned three times with equal volumes of ethyl acetate. The remaining aqueous phase was discarded and the combined acidic ethyl acetate phase was reduced to 5 ml (fraction I) to be used for gas chromatography (GC) determination of gibberellic acid (GA3), indol -3- acetic acid (IAA) and abscesic acid (ABA). The dried ethyl acetate fraction was dissolved in 80% methanol. The methanol was evaporated under vacuum leaving an aqueous phase which adjusted to pH 2.8 with1% HCl and partitioned three times with equal volumes (25 to 50 ml) of ethyl acetate. The ethyl acetate phase were combined (fraction II) reduced to 5 ml, stored at – 20°C until GC analysis for neutral auxins as indol acetonitrile (IAN). The remaining aqueous phase was adjusted to pH 5.5 with 1% NaOH and partitioned three times with 50 to 100 ml of water saturated n-butanol. All n-butanol phases were combined (fraction III), reduced to 5 ml volume, stored at – 20°C to be used for cytokinin analysis using High Performance Liquid Chromatography (HLPC).

To estimated the amount of GA3 (fraction I), IAA and ABA, the plant hormone fractions and standard ones were methylated according to Vogel (1975) to be ready for GC analysis. Flame ionization detector was used for identification and determination of acidic hormones using Helwett Packered Gas Chromatography (5890). The chromatography was fitted and equipped with HP-130 m x 0.32 mm x 0.25 µm capillary column coated with methyl silicone. The column oven temperature was programmed at 10°C/min from 200°C (5 min) to 260°C and kept finally to 10 min. Injector and detector temperature were 260 and 300°C, respectively. Gases flow rates were 30, 30, 300 cm/sec for N_2, H_2 and air, respectively and flow rate inside column was adjusted at 2 ml/min. Standards of GA3, IAA and ABA were used.

The retention times (RT) of peaks of authentic samples were used in identification and characterization of peaks of samples under investigation (Shindy and Smith, 1975). Peak identification was performed by comparing the relative retention time of each peak with those of GA3, IAA and ABA standards. Peak area was measured by triangulation and the relative properties of the individual compounds were therefore obtained at various retention times of samples.

Effect of temperature and pH

To evaluate the influence of pH and temperature on the *Trichoderma* mycelia growth, a 5 mm diameter mycelia block was cut from the 7-day-old margin of each *Trichoderma* species colonies by a cork borer (No.3). The cut block was then placed in PDA plates that were adjusted to pH 5, 7 and 8 with 0.1 N HCl and NaOH before autoclaving. Incubation followed at 20 ± 1, 25 ± 1 and 30 ± 1°C. The colony diameters of the *Trichoderma* spp. were measured in three replicates each day after inoculation.

Isolation of fungal pathogens

Cotton plants showing disease symptoms (wilting or yellowing) were obtained from saline soils from El-Beheira and El-Fayoum governorates fields and non-saline soils from Shebeen El-Kanater in Kaliubia governorate in Egypt. The roots and stem bases of the infected plants were rinsed in tap water and the necrotic portion were excised and cut into 2 mm pieces. The surface was sterilized with 2.5% sodium hypochlorite (NaClO) for one min and rinsed in 4 successive changes of sterile distilled water. These pieces were then planted on PDA medium supplemented with antibiotic to eliminate bacterial growth and incubated for 7 days at 28°C under 12 h of photoperiod.

The growing fungal colonies were transferred to new PDA plates and pathogens were identified according to their cultural and morphological features using references of Gilman (1957) and Booth (1977) by The Microbiology Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

Dual culture technique

The antagonistic activity of *Trichoderma* spp. against *F. oxysporum* and R. solani was evaluated by dual culture technique as described by Morton and Strouble (1955). A 5 mm diameter mycelia disc from the 7-day-old margin of the Trichoderma spp. culture was placed on a plate where each pathogen (*F. oxysporum* and *R. solani*) was then placed on the opposite of the plate at equal distance from the periphery. The experimental design used was a completely randomized with three Petri dishes for each isolate. In the control plates (without *Trichoderma* spp.), a sterile agar disc was placed at opposite side of the pathogens inoculated plates. Inoculated plates were incubated at 25 ± 1°C for 7 days. Two, four and six days after the incubation period, radial growth of pathogen isolates was measured. PDA plates were used in triplicates and inhibition percentage of average radial growth was calculated in relation to the controls growth as follows:

$$L = [(C – T)/C] \times 100$$

Where L is inhibition of radial mycelia growth; C is radial growth measurement of the pathogen in control; T is radial growth of the pathogen in the presence of *Trichoderma* isolates (Edington et al., 1971).

Preparation of pathogens inocula

To prepare the inoculum of each pathogen, maize grain-based inoculum was prepared by adding 25 g of grains with distilled water (100 ml) into 500 ml conical flasks and kept overnight. The flasks were then autoclaved at 121°C for 30 min on 3 consecutive days (Wong et al., 1984). Under aseptic condition, the sterilized maize grains were inoculated with five agar discs from the actively growing margin of pathogens colony. The flasks were incubated at 28 ± 2°C for 2 weeks. The full grown flasks were then used for the study.

Preparation of *Trichoderma* spp. inocula

Trichoderma spp. inocula were prepared by placing 25 g of moist wheat bran into 500 ml conical flask and autoclaved at 121°C for 30 min on three successive periods as described by (Roiger and Jeffers, 1991). Under aseptic condition, the wheat bran was inoculated with five agar plugs from the actively growing margins of *Trichoderma* colonies. The flasks were incubated at 28 ± 2°C for 2 weeks and occasionally shaken to improve uniformity of microorganism growth.

Greenhouse experiments

Pathogenicity test

Pathogenicity test was carried out under greenhouse conditions. Sterilization of soil was carried out by autoclaving at 121°C for one hour. Pathogens inocula for pathogenicity test and for bioassay

were obtained by growing each pathogen on PDA medium and multiplied on sterilized maize grains as previously mentioned. Three pots each containing 3 kg sterilized soil, were prepared and three treatments were carried out. The first and the second pots containing sterilized soil infested with each pathogen and the third one served as a control. Ten cotton seeds (var. Giza 88) were surface sterilized by immersion in 70% ethanol for 1 min, then in 1.0% sodium hypochlorite for 30 s, washed for several times with sterile water and finally sown in each pot. Three replicates were prepared for each treatment. Pots were irrigated with equal amounts of sterile water. The growing cotton seedlings were observed daily for the severity of pre- and post-emergence damping-off, root rot and foliar symptoms for 15 and 60 days after sowing respectively (Leath et al., 1989).

Soil infestation

Wheat bran colonized with *Trichoderma* spp. (1% wt of colonized wheat bran based inoculums / wt of sterilized soil) was thoroughly dispersed through two sets of pots. Each pot was then filled with 3 kg of soil. The first set containing sterilized saline soil and the second containing sterilized non-saline soil. One week before adding the pathogen and sowing, the pots were watered twice a week. Then maize grains that got infested with pathogens were thoroughly dispersed through sterilized saline and non-saline soil (1% wt of colonized maize seeds / wt of sterilized soil). The positive control (+ve control) consisted of saline and non-saline soil that was treated with each pathogen. Negative control (-ve control) had therefore the sterile non-treated soil. Immediately after adding the pathogens, surface sterilized cotton seeds were coated with *Trichoderma* spp. by immersing the seeds for 3 h in their spore suspension.

Before applying the spore suspension, cotton seeds were treated with 0.2% carboxymethyl cellulose (CMC) as an adhesive agent. Each group of seeds (ten / pot) was placed into pots containing the required soil treatments. The seeds were sown to a depth of 3 cm in each pot and when emergence was complete, the seedlings density were then thinned to five seedlings per pot. Each treatment was replicated three times. Each set of pots were watered with equal amount of water.

The following treatments were carried out for each pathogen (*F. oxysporum* and *R. solani*) in each set of pots (saline and non-saline):

1. Seeds + pathogen (+ve control).
2. Seeds + pathogen + *T. harzianum* CCM340.
3. Seeds + pathogen + *T. koningii* CCM341.
4. Seeds + pathogen + *T. longibranchiatum* NRRL11236.
5. Seeds + pathogen + *T. viride* DSM63065.
6. Seeds + pathogen + *T. viride*.
7. Seeds (non-treated) + non-infested soil (- ve control).

Sixty days after seedling emergence, the growing cotton plants were examined to determine the percentage of disease incidence and disease control.

Disease assessment

The following formulas were used in calculation:

$$\text{Percentage of pre-emergence damping-off} = \frac{\text{No.of ungerminated seeds/pot}}{\text{No.of sown seeds/pot}} \times 100$$

$$\text{Percentage of post-emergence damping-off} = \frac{\text{No.of died seedlings/pot}}{\text{No.of sown seeds/pot}} \times 100$$

$$\text{Percentage of disease incidence} = \frac{\text{No.of infected plants}}{\text{Total No.of plants}} \times 100$$

$$\text{Percentage of disease control} = 100 - \text{disease incidence}$$

Effect of *Trichoderma* spp. in improving cotton growth parameters in saline and non-saline soil

Cotton plants were harvested 60 days after seedling emergence. The whole plants were carefully removed and washed in order to remove any soil particles from shoot and root systems. Growth parameters measures include: shoot height (cm), root length (cm) and fresh as well as dry weights of shoot and root (gm).

RESULTS

Production of amino acids, fatty acids and plant growth hormones by *Trichoderma* species

Trichoderma spp. was cultured on PDA. The PDA medium (without agar) was used for extraction of the fungal amino acids (AA), fatty acids (FA) and plant growth hormones (PGH).

Amino acid profiles

The AA profiles of the investigated *Trichoderma* spp. were screened (Table 1). The results show that most of the AA included aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine (14 AA) were detected in all the investigated *Trichoderma* species. Although proline was detected only in *T. koningii* CCM341, *T. viride* DSM63065 and *T. viride*, so each of them contains even 15 AA. Interestingly, *T. viride* showed the highest quantity of all AA detected in comparison with the other *Trichoderma* spp.

Fatty acid profiles

The fatty acid profiles of the investigated *Trichoderma* spp. were determined (Table 2). The results show that most of the fatty acids (FA) such as myristic, palmitic, stearic, oleic and linoleic were detected in all of the investigated *Trichoderma* species. However, linolenic was detected in all *Trichoderma* spp. except *T. harzianum* CCM340; arachidic was detected in *T. harzianum* CCM340, *T. longibranchiatum* NRRL11236 and *T. viride* DSM63065; palmitoleic was detected in *T. viride* DSM63065 and *T. viride* while lauric was detected in *T. viride* only (Table 2). The highest number of FA (8) was detected in T. viride DSM63065 *and T. viride*. On the other hand, *T. longibranchiatum* NRRL11236 produced seven FA while *T. harzianum* CCM340 and *T. koningii* CCM341 each produced only six FA.

Table 1. The amino acid profiles of *Trichoderma* spp. grown on PDA. Data expressed as the percentage of amino acid present.

Amino acid (g/100 g)	*T. harzianum* CCM340	*T. koningii* CCM341	*T. longibranchiatum* NRRL11236	*T. viride* DSM63065	*T. viride*
Aspartic acid	5.50	6.27	5.00	5.23	39.00
Threonine	1.18	1.30	0.99	2.77	5.71
Serine	1.02	1.08	1.13	2.56	7.83
Glutamic acid	2.23	5.38	3.00	4.18	19.02
Proline	-	0.14	-	0.04	0.32
Glycine	3.67	3.86	3.64	5.45	21.22
Alanine	3.28	3.00	2.52	4.19	16.59
Valine	3.07	2.64	2.50	2.60	18.10
Isoleucine	1.07	0.77	0.60	3.29	5.42
Leucine	2.74	3.23	1.83	4.01	13.44
Tyrosine	3.35	3.35	3.74	1.37	23.49
Phenylalanine	1.26	1.79	1.01	1.33	4.58
Histidine	2.14	2.05	1.89	1.53	8.68
Lysine	1.77	3.36	3.33	1.70	16.99
Arginine	0.68	0.74	0.93	0.73	2.96

Table 2. The fatty acid profiles of *Trichoderma* spp. grown on PDA. Data expressed as the percentage of fatty acid present.

Fatty acid	Number of fatty acid	Area [relative (%)]				
		T. harzianum CCM340	*T. koningii* CCM341	*T. longibranchiatum* NRRL11236	*T. viride* DSM63065	*T. viride*
Lauric	$C_{12:0}$	-	-	-	-	1.32
Myristic	$C_{14:0}$	47.07	25.53	70.30	73.20	5.62
Palmitic	$C_{16:0}$	14.22	25.92	8.26	6.00	27.44
Palmitoleic	$C_{16:1}$	-	-	-	0.69	1.57
Stearic	$C_{18:0}$	1.40	1.70	0.85	0.66	2.52
Oleic	$C_{18:1}$ (ω_9)	6.47	16.47	1.93	2.98	9.49
Linoleic	$C_{18:2}$ (ω_6)	16.44	21.60	7.95	10.75	1.74
Linolenic	$C_{18:3}$ (ω_3)	-	3.70	1.39	4.50	2.82
Arachidic	$C_{20:1}$	0.80	-	0.61	0.73	-

Plant growth hormones profiles

The PGH profiles of *Trichoderma* spp. were determined. PGH such as gibberellic acid (GA3), indol-3-acetic acid (IAA) and abscisic acid (ABA) were detected in all *Trichoderma* species. Results show that the highest amount of GA3, IAA and ABA could be found in *T. viride* DSM63065, *T. harzianum* CCM340 and *T. koningii* CCM341 with values of 176.20, 69.18 and 45.40 mg, respectively (Figure 1).

Effect of temperature and pH

Trichoderma spp. mycelia growth was statistically different in respect of the selected temperature and pH. The highest mycelia growth of *T. harzianum* CCM340 and *T. longibranchiatum* NRRL11236 was supported by a temperature of 25°C. The mycelia growth of *T. koningii* CCM341 and *T. viride* was highest at 30°C (Figure 2). Furthermore, the highest mycelia growth of *T. longibranchiatum* NRRL11236 and T. viride was at pH 5, that of *T. harzianum* CCM340 and T. koningii CCM341 at pH 7 and of *T. viride* at pH 8 (Figure 3). The experiment was designed by completely randomized design (Factorial). The colony diameters of the *Trichoderma* isolates were measured in three replicates each day after inoculation. The means were statistically analyzed by analysis of variance (ANOVA) and Least Significant Difference (LSD) test at 5% significant level with SAS software (SAS, 1985) InstituteInc., Cary, NC, USA).

Dual culture technique

The study of the Trichoderma spp. and the pathogens in

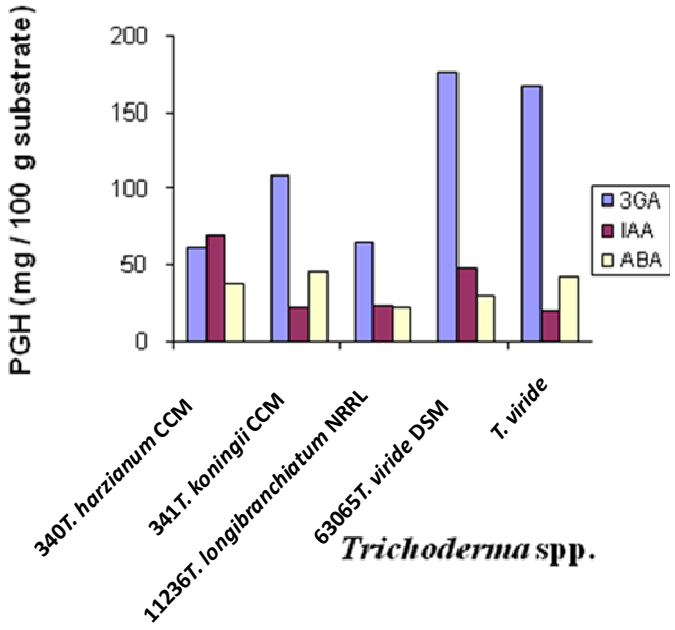

Figure 1. Plant growth hormones (PGH) profiles of *Trichoderma* spp. grown on PDA.

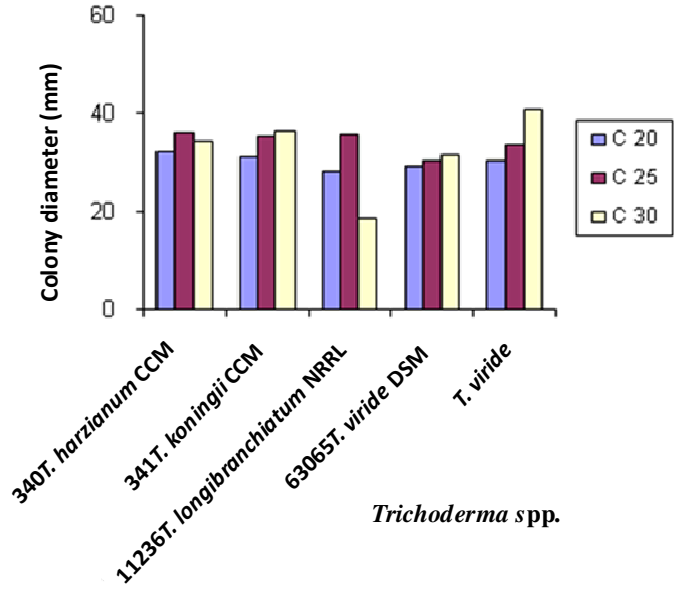

Figure 2. Effect of temperature on the mycelia growth of *Trichoderma* spp.

dual culture showed that all of the Trichoderma spp. had a marked significant inhibitory effect on the growth of the pathogens compared with their control (Figure 4). By 48 h after interaction between mycelia of *Trichoderma* spp. and the pathogens mycelia, a clear zone of interaction was formed in all *Trichoderma*-pathogen combination. Maximum pathogen growth inhibition occurred in the interaction of *T. viride* with *R. solani* (89.36% radial

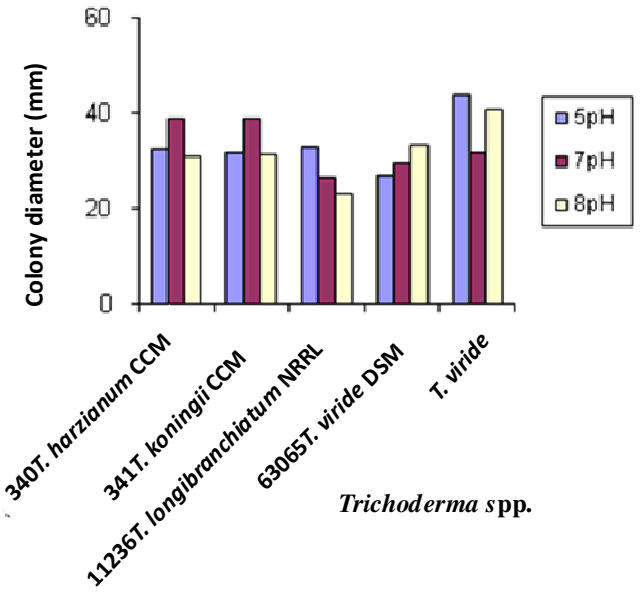

Figure 3. Effect of pH on the mycelia growth of *Trichoderma* spp.

growth reduction). *F. oxysporum* and *R. solani* were most inhibited by *T. viride* and gave radial growth reduction of 81.39 and 89.36 % respectively while both pathogens were least inhibited by *T. longibranchiatum* NRRL11236 (Figure 4).

Greenhouse experiments

Pathogenicity test

Concerning *F. oxysporum*, cotton plants were more vulnerable to get attacked by this pathogen in saline soil (47.22% disease incidence) than in non-saline soil (16.66% disease incidence) (Table 3). On the other hand, cotton plants were more vulnerable to get attacked by *R. solani* in non-saline soil (72.22% disease incidence) than in saline soil (33.33% disease incidence) (Table 4). Generally it can be said that saline soil is more favorable for pathogenicity of *F. oxysporum* in cotton plants while non-saline soil is more favorable for pathogenicity of *R. solani*.

Effect of *Trichoderma* spp. on damping-off, disease incidence and disease control of cotton plants in saline and non-saline soil

Tables 3 and 4 show the effect of *Trichoderma* spp. on pre- and post-emergence damping-off, disease incidence and disease control of cotton plants infested with *F. oxysporum* and *R. solani* in pre- and post-emergence damping-off of *T. viride* was 20% while in non-saline soil it was 33.33%. For *R. solani*, in saline soil, the percentages of pre- and post-emergence damping-off

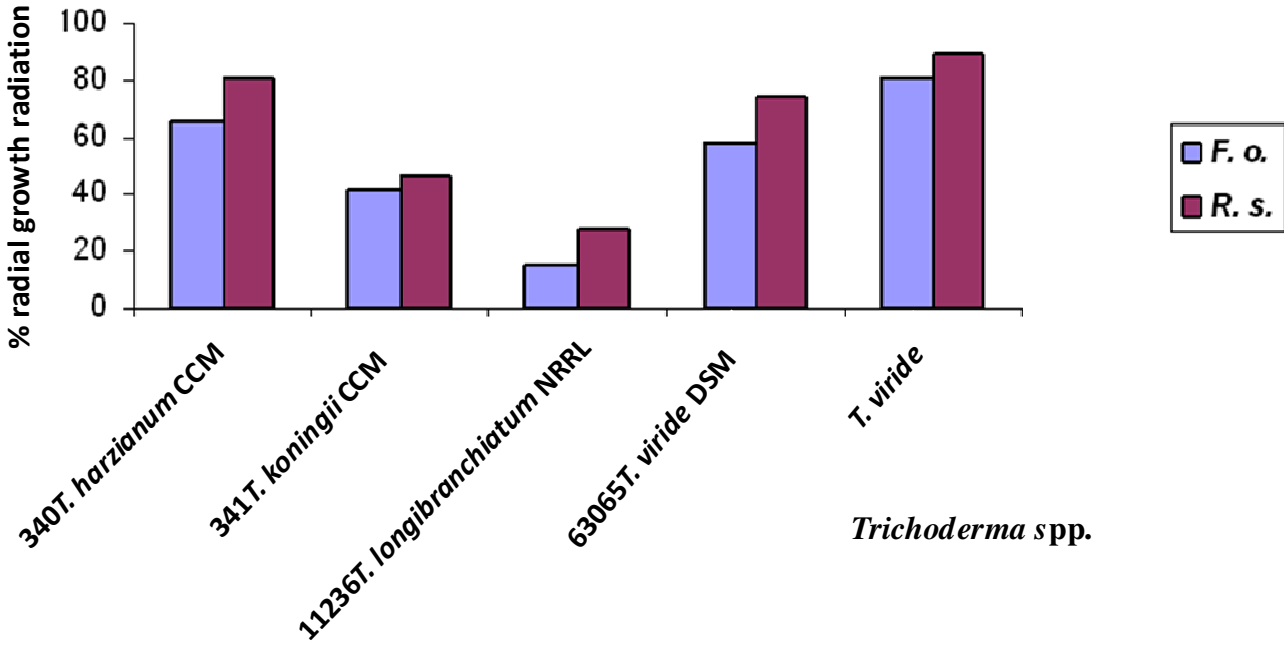

Figure 4. *In vitro* growth inhibition of *F. oxysporum* and *R. solani* by *Trichoderma* spp. using dual culture.

Table 3. Effect of *Trichoderma* spp. on damping-off, disease incidence and disease control of cotton plants infested with *F. oxysporum* in saline and non-saline soil.

Treatment	Saline soil				Non-saline soil			
	Damping-off (%)		DI (%)	DC (%)	Damping-off (%)		DI (%)	DC (%)
	Pre-emergence	Post-emergence			Pre-emergence	Post-emergence		
Seeds + *F.o.* (+ve control)	36.66^{c}	40^{c}	47.22^{a}	-	53.33^{b}	46.66^{b}	16.66^{b}	-
Seeds + *F.o.*+ *T. harzianum* CCM340	43.33^{d}	36.66^{d}	16.66^{d}	83.33^{b}	36.66^{d}	40^{c}	0^{c}	100^{a}
Seeds + *F.o.*+ *T. koningii* CCM341	23.33^{e}	23.33^{e}	0^{e}	100^{a}	40^{c}	40^{c}	0^{c}	100^{a}
Seeds + *F.o.*+ *T. longibrachiatum* NRRL11236	46.66^{b}	46.66^{b}	16.66^{d}	83.33^{b}	56.66^{a}	56.66^{a}	0^{c}	100^{a}
Seeds + *F.o.*+ *T. viride* DSM63065	46.66^{a}	50^{a}	41.66^{b}	58.33^{d}	56.66^{ab}	56.66^{a}	0^{c}	100^{a}
Seeds + F.o.+ *T. viride*	20^{f}	20^{f}	25^{c}	75^{c}	33.33^{d}	33.33^{d}	33.33^{a}	66.66^{b}
Seeds (non-treated) + non- infested soil (-ve control)	13.33^{g}	13.33^{g}	16.66^{d}	-	20^{e}	20^{e}	0^{c}	-
F value	574.1	343.3	237.5	361.5	625.2	281.4	112	132.2
L.S.D.	1.01	0.66	1.5	1.32	2.5	1.57	0.11	5.1

DI = disease incidence, DC = Disease control (Data were statistically analyzed by analysis of variance (ANOVA), statistical analysis cycle (SAS) followed by Duncan's multiple range test ($P \leq 0.05$) (Duncan, 1955). Means having the same letters vertically are not significant at $p \leq 0.05$ according to Duncan's multiple range tests).

Table 4. Effect of *Trichoderma* spp. on damping-off, disease incidence and disease control of cotton plants infested with *R. solani* in saline and non-saline soil.

Treatment	Saline soil				Non-saline soil			
	Damping-off (%)		DI (%)	DC (%)	Damping-off (%)		DI (%)	DC (%)
	Pre-emergence	Post-emergence			Pre-emergence	Post-emergence		
Seeds + *R.s.* (+ve control)	43.33[a]	43.33[a]	33.33[b]	-	53.33[a]	46.66[a]	72.22[a]	-
Seeds + *R.s.+ T. harzianum* CCM340	36.66[b]	40[b]	16.66[e]	83.33[b]	36.66[c]	36.66[c]	0[c]	100[a]
Seeds + *R.s.+ T. koningii* CCM341	23.33[d]	20[e]	0[f]	100[a]	43.33[b]	43.33[b]	0[c]	100[a]
Seeds +*R.s.+T. longibrachiatum* NRRL11236	36.66[b]	36.66[c]	30.55[c]	69.44 [d]	36.66[c]	36.66[c]	0[c]	100[a]
Seeds + *R.s.+ T. viride* DSM63065	33.33[c]	33.33[d]	50[a]	50[e]	36.66[c]	36.66[c]	0[c]	100[a]
Seeds+*R.s.+T. viride*	13.33[e]	16.66[f]	25[d]	75[c]	16.66[e]	13.33[e]	66.66[b]	33.33 [b]
Seeds (non-treated) + non- infested soil (-ve control)	13.33[e]	13.33[g]	16.66[e]	-	20[d]	20[d]	0[c]	-
F value	403.9	358.4	675.3	429.6	289.8	378.2	789.6	849.2
L.S.D.	1.05	0.32	0.31	1.07	0.38	0.34	0.18	0.34

DI = disease incidence, DC = Disease control (Data were statistically analyzed by analysis of variance (ANOVA), statistical analysis cycle (SAS) followed by Duncan's multiple range test (P≤0.05) (Duncan, 1955). Means having the same letters vertically are not significant at p≤0.05 according to Duncan's multiple range tests).

were 13.33 and 16.66% respectively, while in non-saline soil the percentages were 16.66 and 13.33% respectively (Table 4). Results also indicate that for both pathogens, *T. koningii* CCM341 gave the highest disease control percentage (100%) in saline and non-saline soil and that *T. harzianum* CCM340, *T. longibrachiatum* NRRL11236 and *T. viride* DSM63065 gave the same disease control percentage (100%) in non-saline soil (Tables 3 and 4). According to the analysis of variance it yielded significant "F" value in pre-emergence and post-emergence with saline soil and non-saline soil at the different treatments.

Effect of *Trichoderma* spp. on some growth parameters of cotton plants in saline and non-saline soil

The biological activity of the tested *Trichoderma* spp. was assessed for their effect upon the growth and development of cotton plants grown in saline and non-saline soils. Tables 5 and 6 show increase in the growth parameters including shoot height, root length, fresh weight and dry weight of shoots, and roots of plants treated separately with different *Trichoderma* spp. against F. oxysporum and *R. solani* compared with the non-treated plants (+ve control).

F. oxysporum

Results in Table 5 indicate that in saline soil, the treatment with *T. koningii* CCM341 gave the highest values of shoot height, fresh weight of shoot and root as well as dry weight of root. The highest root length though could be achieved with the treatment of *T. harzianum* CCM340. Also dry weight of shoot showed highest results with *T. viride* compared with other treatments. On the other hand, *T. viride* showed lowest shoot height and root length values, just like *T. longibrachiatum* NRRL11236 gave the lowest values of fresh weights of shoot and root and dry weight of root. In non- saline soil, *T. koningii* CCM341 gave the highest values of all growth parameters tested while *T. viride* gave the lowest values of all parameters except fresh and dry weights of root which is detected in the treatment with *T. longibrachiatum* NRRL11236 (Table 5).

R. solani

Results in Table 6 revealed that in saline soil, *T. harzianum* CCM340, *T. koningii* CCM341 and *T. longibrachiatum* NRRL11236 gave the highest values of dry weight of shoot, fresh weight of root and shoot height respectively while *T. viride* DSM63065 and *T. harzianum* CCM340 were the superiors in increasing root length and fresh weight of shoot values respectively. Again *T. viride* gave the lowest values of all growth parameters tested. In non-saline soil, *T. koningii*

Table 5. Effect of *Trichoderma* spp. on some growth parameters of cotton plants infested with *F. oxysporum* in saline and non-saline soil.

Treatment	Saline soil						Non-saline soil					
	SH	RL	FWS	FWR	DWS	DWR	SH	RL	FWS	FWR	DWS	DWR
Seeds + *F.o.* (+ve control)	-0.558	-0.368	0.168	-0.171	-0.119	-0.167	-0.274	-0.287	0.108	0.455	0.234	-0.571
Seeds + *F.o.+ T. harzianum* CCM340	0.297	0.203	0.237	0.771	-0.025	0.357	1.219	0.976	0.704	0.188	0.620	0.364
Seeds + *F.o.+ T. koningii* CCM341	0.491	0.125	1.304	9.458	-0.234	1.643	1.705	1.198	1.560	1.313	1.810	1.636
Seeds + *F.o.+ T. longibrachiatum* NRRL11236	0.197	0.180	-0.062	0.125	-0.112	0.143	1.071	0.942	0.640	0.083	0.692	0.182
Seeds + *F.o.+ T. viride* DSM63065	0.120	0.065	0.163	-0.188	-0.396	-0.214	1.235	0.976	0.704	0.188	0.620	0.364
Seeds + *F.o.+ T. viride*	-0.095	0.006	0.769	0.867	0.690	0.250	-0.055	0.150	-0.479	-0.396	0.278	0.455
Seeds (non-treated) + non- infested soil (-ve control)	25.1	14.2	6.65	0.41	1.76	0.12	25.2	8.23	3.81	0.33	0.64	0.07

SH = Shoot height (cm); FWS = Fresh weight of shoot (g); DWS = Dry weight of shoot (g); RL = Root length (cm); FWR = Fresh weight of root (g); DWR = Dry weight of root (g). (SPSS statistical software package (V. 17.0, Echosoft Corp., USA, 2008) and Z score (also known as normal deviate score, standard score or critical ratio score) were used for data analysis. It is defined as how many units of control, the parameter lays away from the control. It may be up or down from the control target value).

Table 6. Effect of *Trichoderma* spp. on some growth parameters of cotton plants infested with *R. solani* in saline and non-saline soil.

Treatment	Saline soil						Non-saline soil					
	SH	RL	FWS	FWR	DWS	DWR	SH	RL	FWS	FWR	DWS	DWR
Seed s + *R.s.* (+ve control)	-0.458	-0.587	-0.817	-0.805	-0.886	-0.833	0.284	0.084	-0.711	-0.727	-0.875	0.001
Seeds + *R.s.+ T. harzianum* CCM340	3.090	0.826	9.574	8.625	12.550	8.500	-0.008	0.055	0.281	-0.018	0.300	-0.071
Seeds + *R.s.+ T. koningii* CCM341	1.912	0.677	7.279	27.125	3.050	10.500	0.415	0.211	0.462	0.456	0.567	0.786
Seeds + *R.s.+ T. longibrachiatum* NRRL11236	3.125	1.082	6.959	11.250	11.550	13.000	0.384	0.502	0.610	0.456	0.867	0.571
Seeds + *R.s.+ T. viride* DSM63065	2.993	2.635	8.836	8.357	10.550	9.500	0.125	0.001	0.184	-0.175	0.375	-0.286
Seeds + *R.s.+ T. viride*	0.297	0.289	0.524	1.360	1.156	1.333	-0.036	0.166	0.021	0.982	0.483	0.786
Seeds (non-treated) + non- infested soil (-ve control)	25.1	14.2	6.65	0.41	1.76	0.12	25.2	8.23	3.81	0.33	0.64	0.07

SH = Shoot height (cm); FWS = Fresh weight of shoot (g); DWS = Dry weight of shoot (g); RL = Root length (cm) DWR = Dry weight of root (g)); FWR = Fresh weight of root (g). (SPSS statistical software package (V. 17.0, Echosoft Corp., USA, 2008) and Z score (also known as normal deviate score, standard score or critical ratio score) were used for data analysis. It is defined as how many units of control, the parameter lays away from the control. It may be up or down from the control target value).

CCM341 gave the highest values of shoot height and dry weight of root while *T. viride* gave the highest values of fresh and dry weights of root. Also, *T. longibrachiatum* NRRL11236 gave the highest values of root length and fresh and dry weights of shoot. On the other hand, *T. viride* DSM63065 gave the lowest values of root length while *T. harzianum* CCM340 gave the lowest values of shoot height, fresh and dry weights of root and dry weight of shoot (Table 6).

Generally with *F. oxysporum*, *T. koningii* DSM63065 showed the most efficiency by increasing most growth parameters tested while *T. viride* was the least effective one in both saline and non-saline soil. Also, with *R. solani*, *T. viride* had the lowest effect in increasing all growth parameters in saline soil. However *T. longibrachiatum* NRRL11236 was here the most effective one and *T. harzianum* CCM340 showed the least effectiveness in non-saline soil.

DISCUSSION

Trichoderma is a genus of asexual fungi found in the soils of all climatic zones. *Trichoderma* is a secondary opportunistic invader, a fast growing

fungus, a strong spore producer, a source of cell wall degrading enzymes, and an important antibiotic producer (Vinal et al., 2008). Numerous strains of this genus are rhizosphere competent and are able to degrade hydrocarbons, chlorophenolic compounds, polysaccharides and xenobiotic pesticides used in agriculture (Harman and Kubicek, 1998; Harman et al., 2004). *Trichoderma* species are well known as bio-control agents of several crop diseases (Tsuen Lo and Yih Lin, 2002). The main bio-control mechanisms that *Trichoderma* utilizes in direct confrontation with fungal pathogens are mycoparasitism (Papavizas, 1985; Harman and Kubicek, 1998; Howell, 2003) and antibiosis (Howell, 1998; Sivasithamparam and Ghisalberti, 1998).

The production of secondary metabolites by *Trichoderma* spp. is strain dependent and includes antifungal substances belonging to a variety of classes of chemical compounds. They were classified by Ghisalberti and Sivasithamparam (1991) into three categories: (1) volatile antibiotics: most of the isocyanide drevatives; (2) water-soluble compounds: heptelidic acid or koningic acid; (3) peptaiboles, which are linear oligopeptides of 12-22 amino acids rich in α-aminαobuteric acid, N-acetylated at the N-terminus and containing an amino alcohol at the C-terminus (Le Doan et al., 1986; Rebuffat et al., 1989). Also, studies of Hanson and Howell (2004) indicated that phytoalxin synthesis in cotton is elicited by a protein produced by *T. virens*. In addition, the mode of action of the amino acids by *Trichoderma* spp. may involve phytoalexins accumulation that may act as inhibitors to plant pathogens (Cohen and Coffey, 1986). Also, the direct production of growth stimulating factors (plants hormones or growth-factors) by *Trichoderma* spp. that increased plant growth response was documented (Windham et al., 1986; Kleifield and Chet, 1992; Wang et al., 2000). In this study, we characterized and evaluated different amino acids, fatty acids and plant growth hormones such as gibberellic acid, indol-3-acetic acid and abscisic acid of all investigated *Trichoderma* spp. that may be one of the important factors for biological control of cotton plant.

It has been shown that temperature and pH are two key parameters to manipulate growth, sporulation and the saprophytic ability as well as the production of volatile and non-volatile metabolites involved in nutrition, competition, mycoparasitism and extracellular enzymes that disintegrate cell wall of fungi. Therefore, it is important to gain as much as possible information about the effect of temperature and pH on the mycelia growth. In this work, Trichoderma spp. showed different optima of temperatures and pH values for their mycelia growth. These results were in accordance with Kredics et al. (2003) and Hajieghrari et al. (2008) who showed that the optimum temperature for growth differs among the *Trichoderma* spp. although most *Trichoderma* strains are mesophilic and are active under a wide range of pH.

It is also widely known that environmental parameters such as abiotic (soil type, soil temperature, soil pH, water potential etc.) and biotic (plant species and variety, microbial activity of the soil) factors as well as other factors such as method and timing of application may have an influence on the biological control efficacy of Trichoderma and other isolates (Barakat, 2008; Hajieghrari et al., 2008; Hassanein et al., 2010).

In this study, the results of dual culture revealed that all *Trichoderma* spp. tested substantially reduced the colony growth of *F. oxysporum* and *R. solani* with various degrees. These results were in accordance with that of Ozbay and Newman (2004). The presence of an inhibition zone without the hyphae contact suggests the secretion of non-volatile inhibitory substances by the Trichoderma isolates. It is important to mention that *Trichoderma* spp. are known to produce a number of antibiotics such as Trichodermin, Trichodermol, Harzianum A and Harzianolide (Dennis and Webster, 1971; Kucuk and Kivanc, 2004) as well as some cell wall degrading enzymes such as chitinases, glucaneses that break down polysaccharides, chitins and B-glucanase, thereby destroying cell wall integrity (Elad, 2000). These may also play a major role in mycoparasitism because of changes in cell wall integrity prior than penetration.

Microbial antagonism has been used with some success for controlling damping-off and root rot diseases of plants. Howell (2002) and Vinal et al. (2008) indicated that the biological control of pre-emergence damping-off by *Trichoderma virens* could be related to its ability to degrade seed-emitted compounds that stimulate pathogen propagule germination. On the other hand, the induction of plant defense responses by some strains of *Trichoderma* plays a pivotal role in successful damping-off control of *R. solani* on cotton (Howell et al., 2000; Vinal et al., 2008).

In the present investigation, most tested *Trichoderma* spp. were efficient in the reduction of pre- and post-emergence damping-off caused by *F. oxysporum* in saline and non- saline soil. Also, *T. koningii* CCM341 was superior in the reduction of root rot disease incidence in both types of soil.

The study results showed that salinity may affect the capability of *Trichoderma* spp. to control *F. oxysporum* and *R. solani* root rot disease of cotton plant since most of the tested *Trichoderma* spp. gave the highest percentage of disease control in non-saline soil with both pathogens. Generally, disease control percentage is higher in non-saline soil than in saline one. This finding was in harmony with that of Mohamed and Haggag (2006) who reported that salinity is one of the most widespread constraints to soil fertility. Also, it has been proven that salt stress had significant detrimental effects on growth, sporulation and antagonistic activity of the wild type isolates of *Trichoderma* spp. against *F. oxysporum* (Kredics et al., 2001).

Generally, salinity may have an effect on *Trichoderma* spp. in controlling *F. oxysporum* and *R. solani* root rot in cotton plants. It may as well have an effect on the efficiency of these two pathogens to cause the disease,

since most of the tested *Trichoderma* spp. gave highest disease control percentage (100%) in non-saline soil with both pathogens.

In the present study, *Trichoderma* spp. showed various degrees of increased plant growth responses. The results suggested that various unknown factors might interact to mediate responses. The factors might result in rhizosphere affinity or survival ability of these species in different crops (Lo et al., 1997). Enhanced root and shoot growth as well as plant vigor has been observed following application of *Trichoderma* spp. to many crops. For example, Lo et al. (1997) reported that T. harzianm increased plant vigor bentagrass. Bjorkman et al. (1994) also reported that the fungus increased both root and shoot growth of corn. Also, Ozbay and Newman (2004) showed that *Trichoderma* spp. have evolved numerous mechanisms that are involved in attacking other fungi and so enhance plant and root growth. The enhanced growth response of several plants following application of *Trichoderma* spp. has also been well documented (Kleifield and Chet, 1992). Also, Tsuen Lo and Yih Lin (2002) reported that some selected isolates of *Trichoderma* spp. increased the root length and lateral root numbers of cucumber seedlings.

The increased growth response induced by *Trichoderma* spp. is not yet fully understood. However, several possible mechanisms have been suggested to explain this phenomenon of increased plant growth. These factors include (1) control of deleterious root microorganisms, that were not causing obvious diseases, (2) direct production of growth stimulating factors (plants hormones or growth-factors), (3) increased nutrients uptake through enhanced root growth or promoted availability of necessary nutrients, (4) reduction of the concentration of substances in soil that are inhibitory to plant growth (Windham et al., 1986; Kleifield and Chet, 1992; Wang et al., 2000). The increased growth response induced by *Trichoderma* has been reported to not only be the result of only one but of several factors (Kleifield and Chet, 1992). Harman (2000, 2001) reported that T. harzianum could improve nitrogen use efficiency in maize and also could solubilize a number of poorly soluble nutrients such as manganese (Mn4+) and copper (Cu^{2+}).

In conclusion, the application of *Trichoderma* spp. or their metabolites for crop protection such as the host defense inducers and antibiotics can be produced cheaply in large quantities on an industrial scale, easily prepared from the fungal biomass, dried and formulated for spray or drench applications. Consequently, more detail studies in the various strains of Trichoderma are still needed in order to provide a better understanding of the mechanisms of controlling damping-off and root rot and also promoting plant growth responses of cotton plants.

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