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Full Length Research Paper

Application of *Trichoderma harziunum* T22 as a biofertilizer supporting maize growth

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This study was conducted to investigate the effect of *Trichoderma harzianum* strain (T22) on the growth and development of maize (*Zea mays*) plants. *T. harzianum* was applied to the grains in two different treatments. The first one was performed by inoculating the soil with the air dried growth of the tested fungus in three different levels (2, 4 and 6 g kg^{-1}) before sowing. The second treatment was conducted by treating the seeds with different concentrations of metabolic solution of *T. harzianum* (100, 200 and 300 µl) before sowing time. We compared these two types of treatments to determine which one has more benefit for improving the growth of maize plants. Results obtained revealed that the application of *T. harzianum* caused increases in all measured parameters which include growth parameters, chlorophyll content, starch content, nucleic acids content, total protein content and phytohormones content of maize plants, when applied to the soil or the seeds but the magnitude of these increases was much more pronounced in case of plants developed from seeds treated with various concentrations of metabolic solution of *T. harzianum*. The sodium dodecyl sulphate-polyacryalmide gel electrophresis (SDS-PAGE) electrophoretic pattern of proteins extracted from maize leaves revealed the appearance of newly protein bands. It was concluded that the uses of *T. harzianum* improves the growth and development of maize plants.

Key words: *Trichoderma*, maize, growth-promoting effect, resistance response, inoculum, phytohormones, protiens (SDS-PAGE).

INTRODUCTION

Trichoderma harzianum is a fungal genus found in many regions of the world. These fungi are ubiquitous in a wide variety of environments, showing up in soil, forests, wood, and paper, among other locations (Harman et al., 2004a). These fungi grow as symbiotic relationships with plants and they promote abundant root growth so that they have plenty of roots to grow. *Trichoderma* species have been widely studied for their capacity to enhance plant growth, produce antibiotics, parasitize other fungi and compete with deleterious plant microorganisms so that they are used as biofertilisers and biological agent (Adams et al., 2007; Bais et al., 2006). Recently, several attempts have been undertaken to apply *Trichoderma* spp. as bio stimulants of seedling establishment, enhancement of plant growth and elicit plant defense (Shanmugaiah et al., 2009; Celar and Valic, 2005). Until recently, these traits were considered to be the basis for an indication of how *Trichoderma* exert beneficial effects on plant growth and development. However, it is becoming increasingly clear that certain strains also have substantial direct influence on plant development and crop productivity (Harman, 2006).

T. harzianum may be used as alternative to the chemicals to suppress the wilt pathogen and raise the yield of tomato (Mushtaq and Upadhyay, 2011). Chlorophyll content increased when seed was coated with *T. harzianum* T969 (Rasool et al., 2011). Also some species of *Trichoderma* have the ability to promote plant growth, as a result of different mechanisms, such as solubilization of phosphates, micronutrient and minerals, such as Fe, Mn and Mg that have important role in plant growth as well as indirectly with the control of the major and minor root infesting pathogens in rhizosphere, and improve nutrient uptake (Hoyos-Carvajal et al., 2009), as well as improve plant defense level against biotic and/or abiotic stress (Mastouri et al., 2010).

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This study aims to find if *Trichoderma* sp. had additional and promoting effects on vegetative growth through the analysis of some indicating factors, such as germination rate, root elongation, plant height, fresh weight of the plants and some physiological and biochemical changes on maize plants.

MATERIALS AND METHODS

Plant material

Grains of maize cultivar, *Zea mays* L. used in this investigation was obtained from the Agricultural Research Center (ARC), El-Dokkey, Giza, Egypt.

Fungal preparation

T. harzianum strain (T22) isolates selected for this study were obtained from NRRL (Agricultural Research Service Culture Collection) United States Department of Agriculture (USDA), New Orleans, Louisiana 70179. Cell suspensions of *T. harzianum* strain 22 were prepared by culturing the fungus in Czapek broth medium at 25°C for 7 days. The resulting culture was filtered through cheesecloth to separate mycelial fragments, washed by centrifugation (10,000 rpm for 15 min).

The *Trichoderma* was applied to the seeds in two different methods:

(1) In the first one the *Trichoderma* mycelia was air dried for 72 h and inoculated in loam based garden soil in concentrations of 2, 4 and 6 g of the powdered culture / kg of the soil before sowing time.
(2) In the second method, maize seeds were treated with the metabolic solution (as the culture supernatant of the fungus) in concentrations of 100, 200 and 300 µl for one hour before sowing.

Experimental condition

Maize grains were surface sterilized by immersing in 70% ethanol for 2 min and then in 0.2% sodium hypochlorite (NaoCl) for 3 min. They were washed for several times with sterile distilled water then divided into three groups as follows:

(1) The 1st group was left without any treatments and served as the control plants (5 pots).
(2) The 2nd group represented the seeds grown in soil inoculated with the air dried mycelia of *T. harzianum* (5 pots/ level).
(3) The 3rd group represents the treated seeds soaked in 100, 200 and 300 µl of the metabolic solution (as the culture supernatant of *T. harzianum*) for one hour before sowing (5 pots/ concentration).

Fifteen seeds were placed into 30 cm diameter clay pots, each filled with about 4 kg of loam based garden soil. The seeds were sown at 2 to 3 cm depth in each pot and when seedlings growth became (~7 old days), the seedling density was reduced to 12 seedlings /pot. The pots were watered every 48 h with equal amount of water. When the developed plants reached 40 days old, 5 plants were carefully uprooted from the soil of each treatment; samples were collected for the following analyses:

Growth parameters

Plant height, number of leaves, leaf area, fresh weight and dry matter of shoots and roots were determined for each treatment.

Photosynthetic pigments

Chlorophyll a, b, and carotenoids were extracted from the leaves and estimated by the method of Metzner et al. (1965).

Starch content

Starch content was assessed by colorimeter using iodine solution in potassium iodide (Magel, 1991).

Total RNA and DNA

Total RNA and DNA were estimated by the method of Schmidt and Thannhauser (1945).

Total protein

Total protein was done in plant extracts by the method of Lowry et al. (1951).

Endogenous phytohormones

The method of extraction was essentially similar to that adopted by Shindy and Smith (1975). To estimate the amounts of acidic hormones [indole acetic acid (IAA), gibberellic acid (GA_3) and abscisic acid (ABA)], the plant hormone fractions and standard ones were methylated according to Vogel (1975) to be ready for GC analysis.

SDS-PAGE protein

SDS-PAGE protein was determined according to the method of Laemmli (1970).

Tissue processing for electron microscope studies

Maize leave samples (2 mm^3) were fixed by immersion in 3% (v/v) glutaraldehyde in 0.1 m sodium cacodylate buffer (pH 7.2) for 2 h at room temperature and post-fixed with 1% (w/v) osmium tetroxide in the same buffer for 1 h at 4°C. Samples were dehydrated in a graded ethanol series and embedded in Epon 812 (JBEM Chemicals Pointe-Claire, Quebec, Canada). Thin sections (0.7 mm), cut from the Epon-embedded material using glass knives, were mounted on glass slides and stained with 1% (v/v) aqueous toluidine blue prior to examination with an Axioscope microscope (Zeiss, Jena, Germany). For each treatment, an average of five samples from three different leaves were investigated and examined under the electron microscope.

RESULTS

Growth parameters

Our results highlight the important role of *T. harzianum* on plant growth promotion. The positive effects of *T. harzianum* in both applications methods (either as soil inoculated with *T. harzianum* or treating the seeds

Table 1. Effect of different experimental treatments of *Trichoderma harzianum* on growth parameters of maize plant (*Zea mays*).

Treatments	Conc.	Plant height (cm)	Root length (cm)	No. of leaves/ plant	Area of leaves (cm^2)	Fresh weight of shoots (g)	Dry weight of shoots (g)	Fresh weight of roots (g)	Dry weight of roots (g)
Control		23.00± 0.82	33.67 ± 1.74	7.00 ± 0.73	566 ± 1.31	21.74 ± 2.11	2.52 ± 0.70	4.23 ± 0.53	1.42 ± 0.44
Plants grown in soil inoculated with air dried mycelia (g /kg soil	2	21.67** ± 1.15	31.33** ± 0.58	6.33** ± 0.86	504** ± 0.92	19.63** ± 1.93	2.25** ± 0.65	3.77** ± 0.36	1.29** ± 0.31
	4	22.51** ± 1.31	35.33** ± 0.86	6.67** ±0.93	604** ± 1.53	20.92** ±1.65	2.41** ± 0.58	4.12** ± 0.44	1.38** ± 0.23
	6	24.67** ± 1.02	41.00** ± 0.77	7.00 ± 1.00	650** ± 0.95	22.20** ± 1.42	3.07** ± 0.94	4.57 ** ± 0.32	1.50** ± 0.56
L.S.D. at:	5%	0.16	0.51	0.04	7.65	0.14	0.04	0.04	0.01
	1%	0.22	0.73	0.06	10.99	0.20	0.06	0.06	0.02
Plants developed from seeds treated with culture supernatant	100 µl	26.67** ± 0.92	50.67** ± 1.52	7.69** ± 0.58	997** ± 1.16	28.12** ± 1.80	8.70** ±0.75	8.70 ** ± 0.51	1.70** ± 0.40
	200 µl	26.00** ± 1.12	49.67** ± 1.15	7.33** ± 0.87	972** ± 1.35	26.60** ± 1.65	7.38** ± 0.69	7.38 ** ± 0.47	1.66** ± 0.51
	300 µl	26.00** ± 1.00	42.33** ± 1.62	7.00 ± 0.67	726** ± 0.98	25.83** ± 1.51	6.49** ± 0.80	4.99 ** ± 0.37	1.51** ± 0.36
L.S.D. at:	5%	0.20	0.97	0.04	25.54	0.34	0.33	0.26	0.02
	1%	0.29	1.40	0.06	36.73	0.49	0.47	0.37	0.02

Means ± SD (*n* = *3*) of measurements on each three plants,**highly significant change.

with metabolic solution of T. harzianum prior to sowing) on growth promotion and vigor were observed as early as shown in Table 1 and Figures 1,2 and 3. All growth parameters of maize plant which includes: plant height, root length, number of leaves/ plant, area of leaves, fresh and dry weights of shoots and roots were increased as compared to the control plants. Also, results showed that as Trichoderma concentrations increase in soil, all growth parameters were increased with the exception of the lowest concentration (2 g/kg) which caused slight decreases in the measured growth parameters.

Photosynthetic pigments

Results showed significantly higher values in the content of plant photosynthetic pigments in both applications of *T. harzianum* (Table 2). The magnitude of such increases were much more pronounced in plants grown from the seeds treated with the metabolic solution of *T. harzianum* especially by using the lower concentration (100 µl). On the other hand, application of *T. harzianum* to the soil in concentrations of 2 and 4 g/kg caused decreases in this content in comparison to control but the same content was gradually increased as the concentration of *Trichoderma* was increased in the soil from 2 to 6 g/kg. Results show also that the content of chlorophyll (b) was decreased by inoculation of the soil with all concentrations of *T. harzianum*.

Starch content

Results indicate that *T. harzianum* highly significantly increased the starch content in roots and shoots of maize plants by using both applications of *T. harzianum* as compared to control (Table 3). It is obvious from the result that the response of shoots to application of *Trichoderma* is higher than roots in comparison to untreated plants, moreover, the content of starch reached the highest values in the shoots of plants produced from the seeds treated with *T. harzianum* prior to sowing. On the other hand, the same content recorded the lowest values as a result of inoculation of the soil with 2 and 4 g/kg.

Total protein content

Data represented in Table 3 showed the effect of *T. harzianum* applications on total protein content of roots and shoots of maize plants, the total

Figure 1. Control plants (A); Plants grown in soil inoculated with *T. harzianum* (2 g/kg soil) (B); Plants grown in soil inoculated with *T. harzianum* (4 g/kg soil) (C); Plants grown in soil inoculated with *T. harzianum* (6 g/kg soil) (D).

Figure 2. Control plants (A); Plants developed from seeds treated with 100 µl of the metabolic solution of *T. harzianum* (E); Plants developed from seeds treated with 200 µl of the metabolic solution of *T. harzianum* (F); Plants developed from seeds treated with 300 µl of the metabolic solution of *T. harzianum* (G).

Figure 3. Enhanced shoot development of plants developed from seeds treated with 100, 200 and 300 µL of the metabloic solution of T. *harzianum*

Table 2. Effect of different experimental treatments of *Trichoderma harzianum* on the contents of photosynthetic pigments (mg/g FW) of the leaves of maize plants (*Zea mays*).

Treatments	Conc.	Chl a	Chl b	Chl a+b	Car	Total pigments
Control		3.25 ± 0.09	3.57 ± 0.21	6.82 ± 0.16	2.85 ± 0.05	9.67 ± 0.27
Plants grown in soil inoculated with *T. harzianum* (g /kg soil)	2	1.97** ± 0.08	2.31** ± 0.09	4.28** ± 0.13	2.36**±0.07	6.64** ± 0.21
	4	3.10** ± 0.19	2.73 ** ± 0.05	5.82** ± 0.12	3.56 **± 0.05	9.38* ± 0.34
	6	3.37** ± 0.16	2.98** ± 0.10	6.35** ± 0.15	4.45**± 0.12	10.80** ± 0.43
L.S.D. at:	5%	0.08	0.07	0.14	0.11	0.22
	1%	0.11	0.09	0.20	0.16	0.31
Plants developed from seeds treated with metabolic solution of *T. harzianum*	100 µl	6.17** ± 0.07	4.30** ± 0.21	10.47** ± 0.16	6.23** ± 0.35	16.70** ± 0.59
	200 µl	5.13** ± 0.05	4.50** ± 0.01	9.63** ± 0.08	5.31** ± 0.16	14.94** ± 0.42
	300 µl	4.62** ± 0.11	4.14** ± 0.10	8.76** ± 0.09	2.97 ± 0.13	11.73** ± 0.23
L.S.D. at:	5%	0.15	0.05	0.19	0.24	0.41
	1%	0.22	0.07	0.28	0.35	0.59

Values listed are expressed in units of mg/g fresh weight; Means ± SD (*n=3*) of measurements on each three plants, **highly significant change, *significant change.

protein content was highly significantly increased by inoculation of the soil with 6 g/kg, whereas using the concentrations of 2 and 4 g/kg decreased this content as compared to the control plants. It is clear that the content of total protein in both roots and shoots were higher in plants grown from seeds treated with the metabolic solution of *T. harzianum* prior to sowing than that of plants grown in soil inoculated with *T. harzianum.* The maximum increase in such content was recorded by using 100 µl of the metabolic solution.

Nucleic acids content

The effects of both applications with *T. harzianum* on nucleic acids content of roots and shoots were

Table 3. Effect of different experimental treatments of *Trichoderma harzianum* on starch and total protein contents of maize plant (*Zea mays*).

Treatments	Conc.	Starch content (mg/g DW)		Total protein (mg/g FW)	
		Roots	Shoots	Roots	Shoots
Control		204.05 ± 6.01	220.14 ± 7.21	11.34 ± 0.86	21.48 ± 0.25
Plants grown in soil inoculated with *T. harzianum* (g /kg soil)	2	206.70** ± 8.08	8.92 ** ± 0.51	19.84* ± 0.74	21.48 ± 0.25
	4	218.14 ** ± 6.30	9.36 ** ± 0.50	20.59** ± 0.66	19.84* ± 0.74
	6	232.44 ** ± 5.96	14.64 ** ± 0.24	22.55** ± 0.48	20.59** ± 0.66
L.S.D. at:	5%	4.68	1.31	0.32	0.14
	1%	6.73	1.88	0.46	0.21
Plants developed from seeds treated with metabolic solution of *T. harzianum*	100 µl				
	200 µl	228.14** ± 5.70	262.13** ± 7.23	18.28** ± 0.37	26.37** ± 0.73
	300 µl	225.12** ± 4.22	254.25** ± 8.10	17.62** ± 0.69	26.21** ± 0.74
L.S.D. at:	5%	1.38	2.50	0.42	0.30
	1%	1.98	3.59	0.60	0.43

Means ± SD (*n* = *3*) of measurements on each three plants, **highly significant change.

Table 4. Effect of of different experimental treatments of *Trichoderma harzianum* on RNA and DNA contents of roots and shoots of maize plant (*Zea mays*).

Treatments	Conc.	RNA		DNA	
		Roots	Shoots	Roots	Shoots
Control		7.04 ± 0.30	8.71 ± 0.14	0.31 ± 0.30	0.35 ± 0.08
Plants grown in soil inoculated with *T. harzianum* (g /kg soil)	2	6.12** ± 0.19	7.62** ± 0.23	0.25** ± 0.21	0.30** ± 0.07
	4	6.94* ± 0.17	8.18** ± 0.28	0.29** ± 0.33	0.32** ± 0.03
	6	7.74** ± 0.41	8.99** ± 0.54	0.37** ± 0.62	0.39** ± 0.04
L.S.D. at:	5%	0.08	0.07	0.01	0.00
	1%	0.12	0.11	0.01	0.01
Plants developed from seeds treated with metabolic solution of *T. harzianum*	100 µl	9.09** ± 0.55	10.08** ± 0.34	0.41** ± 0.46	0.42** ± 0.05
	200 µl	8.88** ± 0.47	9.83** ± 0.51	0.37** ± 0.50	0.40** ± 0.06
	300 µl	7.98** ± 0.24	9.27** ± 0.65	0.35** ± 0.44	0.38** ± 0.04
L.S.D. at:	5%	0.12	0.08	0.01	0.00
	1%	0.17	0.11	0.01	0.01

Values listed are expressed in units of mg/g dry weight, means ± SD (*n*=*3*) of measurements on each three plants, **highly significant change.

represented in Table 4. The results demonstrate that the contents of nucleic acids were highly significantly increased as the concentration of *T. harzianum* was increased in the soil. Moreover, treatments of the seeds with various concentrations of metabolic solution of the used fungus prior to sowing caused increases in the contents of RNA and DNA of the roots and shoots of the produced plants particularly by using the lowest concentration (100 µl).

Endogenous phytohormone

Results shown in Table 5 revealed that, the contents of GA_3 and IAA were significantly decreased as a result of inoculation of the soil with 2 and 4 g of *T. harzianum.*

Table 5. Effect of of different experimental treatments of *Trichoderma harzianum* on contents of endogenous GA_3, IAA and ABA of maize plant (*Zea mays*).

Treatments	Conc.	Levels of endogenous hormones		
		GA_3	IAA	ABA
Control		16.20 ± 0.34	25.94 ± 0.41	7.24 ± 0.67
Plants grown in soil inoculated with *T. harzianum* (g /kg soil)	2	12.67** ± 0.25	18.55** ± 0.56	12.63** ± 0.59
	4	14.28** ± 0.61	26.89* ± 0.72	9.16 ** ± 0.46
	6	22.62** ± 0.59	35.46** ± 0.33	6.80* ± 0.52
L.S.D. at:	5%	0.54	0.86	0.33
	1%	0.78	1.23	0.47
Plants developed from seeds treated with metabolic solution of *T. harzianum*	100 µl	31.58** ± 0.46	48.16** ± 0.76	3.59** ± 0.63
	200 µl	29.27** ± 0.37	40.80** ± 0.62	4.84** ± 0.71
	300 µl	23.18** ± 0.55	36.29** ± 0.54	5.29** ± 0.36
L.S.D. at:	5%	0.85	1.15	0.19
	1%	1.23	1.65	0.27

Values listed are expressed in units of mg/100 g fresh weight, means ± SD (*n=3*) of measurements on each three plants, **highly significant change, *significant change.

However, the same treatments increased the contents of ABA than that of untreated control plants. Application of 6 g of *T. harzianum* caused an increase in the contents of GA_3 and IAA accompanied by reduction in the content of ABA. Table 5 reveals also that treating the seeds with different concentrations of metabolic solution of *T. harzianum* prior to sowing significantly increased the contents of GA_3 and IAA, while activities of ABA were markedly decreased. Treatment with 100 µl was found to be more effective than other concentrations.

Anatomical changes

It is obvious from our results (Figures 4 to 11) that the treatments with *T. harzianum* caused smaller and irregular lignification in vessel elements of the xylem in the transverse sections of the leaves of maize plants as compared to the control. This reduction in the amount of liginin was much more pronounced by using 100 µl of the culture supernatant of *T. harzianum* since the walls of xylem vessel elements were thinner than those in the control; this result confirm that such treatment caused the highest morphological growth.

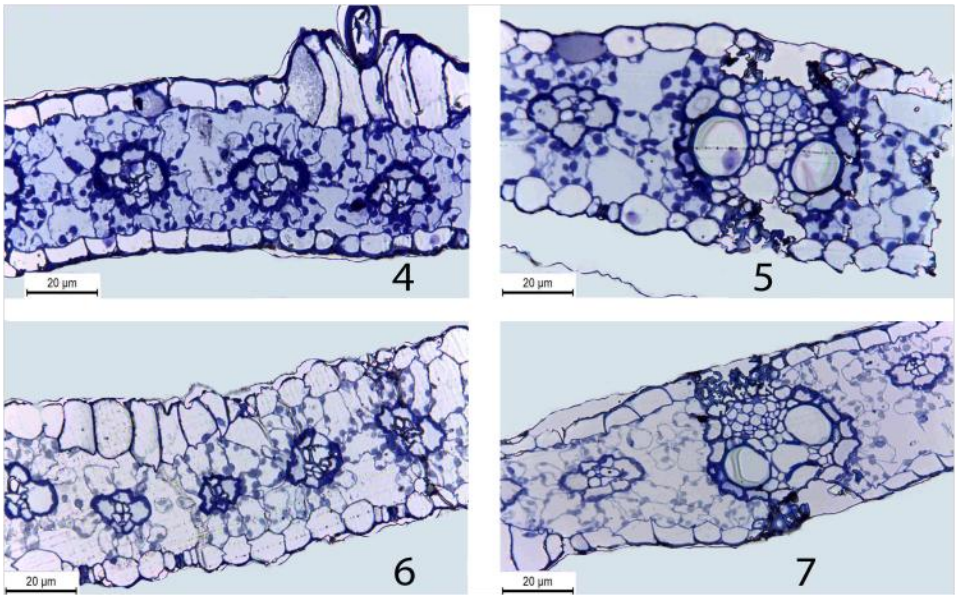
Protein electrophoretic pattern

The changes in the protein electrophoretic pattern in maize leaves exposed to both applications of *T. harzianum* exhibit an increase in the number of protein bands as compared to the control, Table 6 and Figure 12. The maximum increases in the total number of protein bands was observed in plants developed from seeds treated with 300 ul of metabolic solution prior to sowing (11 bands). On the other hand, the number of protein bands decreased to 8 bands in plants grown in soil inoculated with 4 g/kg as compared to the control (9 bands). Newly protein bands appeared in leaves of plants grown in soil inoculated with different concentrations of *T. harzianum* having the molecular weights of 29.62, 17.98 and 16.40 kDa. Simultaneously, the electropherograms of leaves of plants produced from the seeds treated with metabolic solution of *T. harzianum* showed the induction of 3 specific newly protein bands having the molecular weights of 29.62, 22.40 and 17.98 kDa respectively. Furthermore, protein bands with molecular weights of 119.9, 85.87, 72.93, 61.71, 44.71, 41.87 and 38.29 kDa were present in all treatments as well as in the untreated control. The results also revealed that both applications of *T. harzianum* improved the electrophoretic banding pattern of proteins, as well as the banding intensity of leaves as compared with the banding profile seen in the control plants.

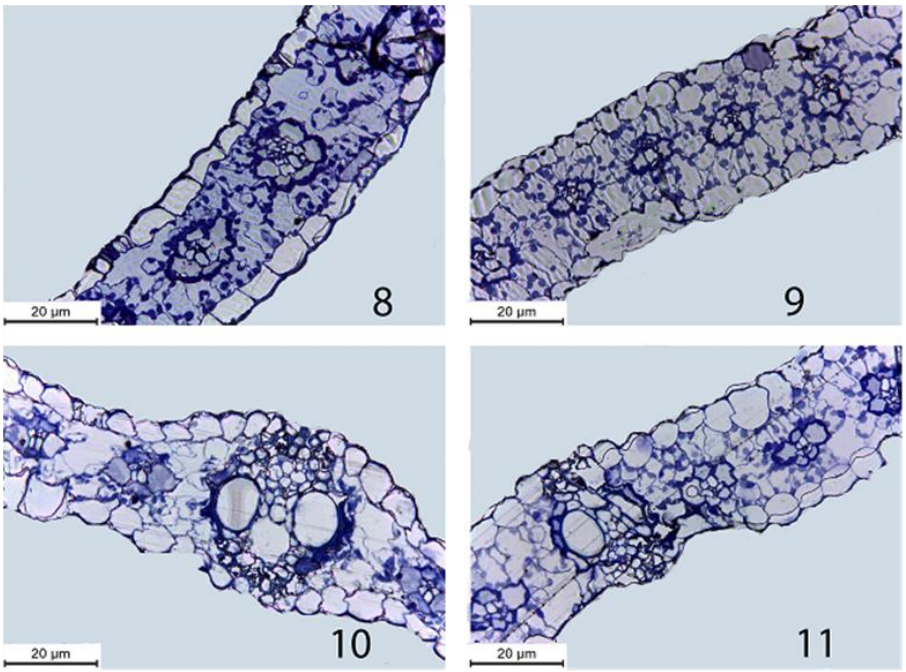
DISCUSSION

Fungi in the genus *Trichoderma* and rhizobacteria in the genera *Pseudomonas*, *Bacillus*, *Streptomyces*, *Enterobacter* and others have evolved multiple mechanisms that result in the improvements in plant resistance to disease and plant growth and productivity (Shoresh et al., 2010).

Our results reveal that the *Trichoderma* treatments highly significantly increased the growth of maize plants as compared to the control, this may be attributed to that *Trichoderma*-treated plants were able to enhance nutrient



Figures 4 to 7. Transverse sections of *Zea mays* leaves. (4) Portion of a transverse leave section of the control plant showing the arrangement of vascular bundles and other leave tissues. (5) Portion of a transverse leave section of plants grown in soil inoculated with *T. harzianum* (2 g /kg soil). (6) Portion of a transverse leave section of plants grown in soil inoculated with *T. harzianum* (4 g /kg soil). (7) Portion of a transverse leave section of plants grown in soil inoculated with *T. harzianum* (6 g /kg soil).



Figures 8 to 11. Transverse sections of *Zea mays* leaves. (8) Portion of a transverse leave section of the control plant showing the arrangement of vascular bundles and other leave tissues. (9) Portion of a transverse leave section of plants developed from seeds treated with 100 µl of the metabolic solution of *T. harzianum* before sowing. (10) Portion of a transverse leave section of plants developed from seeds treated with 200 µl of the metabolic solution of *T. harzianum* before sowing. (11) Portion of a transverse leave section of plants developed from seeds treated with 300 µl of the metabolic solution of *T. harzianum* before sowing.

Table 6. Electrophoretic pattern and protein bands intensity (%) of the protein in leaves of maize plants (*Zea mays*) subjected to different experimental treatments of *T. harzianum*.

Row No.	Molecular weight (kDa)	Plants grown in soil inoculated with *T. harzianum* (g /kg soil)				Plants developed from seeds treated with metabolic solution of *T. harzianum*		
		Protein intensity (%)				Protein intensity (%)		
		Control	2	4	6	100 µl	200 µl	300 µl
1	119.9	5.02	6.52	4.51	6.18	6.69	6.62	6.13
2	85.87	5.25	8.77	7.36	6.50	6.05	5.10	6.00
3	72.93	3.50	2.48	2.60	2.55	2.94	3.48	2.94
4	61.71	5.51	5.52	6.13	14.60	4.89	5.61	4.44
5	44.71	2.49	4.04	3.66	3.80	3.66	2.92	4.25
6	41.87	2.62	2.28	2.65	3.25	3.49	3.66	3.92
7	38.29	3.94	3.95	5.67	5.25	4.13	3.93	4.64
8	31.62	3.67	--	5.17	4.77	4.91	4.47	--
9	29.62	--	4.37	--	--	--	--	4.96
10	22.4	--	--	--	--	--	2.85	4.46
11	21.05	5.59	3.10	--	4.14	3.01	--	3.36
12	17.98	--	--	--	7.22	4.05	3.57	4.45
13	16.4	--	7.41	--	--	--	--	--
Total number of bands		9	10	8	10	10	10	11

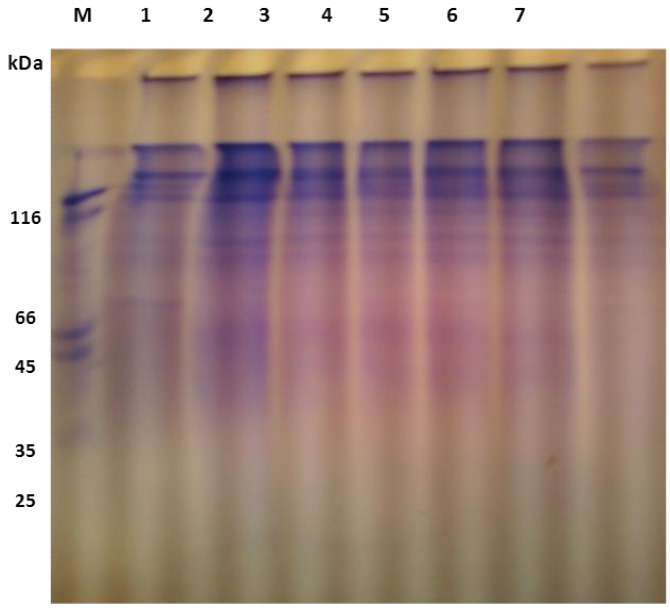


Figure 12. Electrophoretic banding profiles of protein extracted from the leaves of maize plants (*Zea mays*) as influenced by different experimental treatments of *Trichoderma harzianum*.

M: Marker protein.
Lane (1): Control
Lane (2): Plants grown in soil inoculated with *T. harzianum* (2 g /kg soil)
Lane (3): Plants grown in soil inoculated with *T. harzianum* (4 g /kg soil)
Lane (4): Plants grown in soil inoculated with *T. harzianum* (6 g /kg soil)
Lane (5): Plants developed from seeds treated with 100 µl of the metabolic solution of *T. harzianum*
Lane (6): Plants developed from seeds treated with 200 µl of the metabolic solution of *T. harzianum*
Lane (7): Plants developed from seeds treated with 300 µl of the metabolic solution of *T. harzianum.*

uptake, resulting in increasing root and shoot growth, and improving plant vigor to grow more rapidly and to enhance plant greenness, which might result in higher photosynthetic rates (Harman, 2006). Our results show that the increased growth response of plants caused by application of *Trichoderma* metabolites was more effective rather than other treatment, these results indicate that the method of *Trichoderma* introduction is also effective in the success of *Trichoderma* in seedling growth improvement (Harman et al., 2004). Improved plant growth might be due to increased solubility of insoluble plant nutrients by *Trichoderma* species (Kaya et al., 2009). Hexon et al. 2009 showed that *Trichoderma spp*. produced indole-3-acetic acid (IAA) that promoted lateral root formation in *Arabidopsis thaliana.* The root system is important for plant fitness because it provides anchorage, contributes to water use efficiency and facilitates the acquisition of mineral nutrients from the soil (Lopez et al., 2005a). Increased root size resulted into increased shoot size which translates into increased shoot biomass production indicating a beneficial effect of inoculation on plant growth and development. The enhanced plant growth by *Trichoderma* (*T. harzianum*) might be due to production of secondary metabolites which may act as an auxin like compound; these materials may lead to the development of the root system and an exploration of a large volume of soil (Vinale et al., 2008a, b). Development of the root system with production of some organic acids in the rhizosphere such as: gluconic, citric and/ or fumaric acids by *Trichoderma* which decrease soil pH, lead to increase solubility of the insoluble compound and an availability of micronutrient.

In this study, the results revealed that the *Trichoderma* treatments highly significantly increased photosynthetic pigments of maize plants as compared to control. Hexon et al. (2009) showed that *Trichoderma* spp. in *Arabidopsis thaliana* increased root size which resulted into increase in shoot size which translates into increase in the shoot biomass; these resulted in the increase of photosynthetic pigments. *Trichoderma*-T22 has been shown in maize to increase plant greenness (Mastouri, 2010; Shoresh et al., 2010).

The results obtained in this study revealed that starch content increased in both shoots and roots of maize plants treated with *T. harzianum* strain T22. Increased photosynthesis should have resulted in increased starch accumulation in seedlings (Michal and Gary, 2008). Shoots of *Trichoderma*-treated plants had higher starch contents. All of these data are consistent with the concept that in the presence of *T. harzianum* strain T22, energy metabolism via glycolysis and the tricarboxylic acid cycle is up-regulated. *Trichoderma* interaction with plant roots results in controlled activation of carbohydrate metabolic processes as well as enhancement of photosynthesis (Duncan et al., 2006). *T. harzianum* strain T22 on roots up-regulated maize proteins, the largest number of up-regulated proteins were involved in carbohydrate metabolism with substantial increases in proteins involved in photosynthesis, as well as disease and stress resistance (Alfano et al., 2007).

The results obtained demonstrated that proteins content of shoots and roots of maize plants treated with *T. harzianum* T22 were increased, these results may attributed to *Trichoderma* spp. increase uptake of nitrates and other ions (Harman, 2000). *Trichoderma* spp. activity increase biological nitrogen fixation in soil and nitrogen uptake by plant (Dordas and Sioulas, 2008). *T. harzianum* could produce nitrogen oxide (NO) which is that coding for enzyme involved in L-arginine which is important for protein biosynthesis arginine biosynthesis (Gong et al., 2007). *T. harzianum* inoculums in soybean grown gave higher percentage of crude protein (Egberongbe et al., 2010). In addition, numerous proteins induced in response to *Trichoderma* were those involved in stress and defense responses (Michal and Gary, 2008). Many studies have demonstrated that application of *Trichoderma* increased grain protein content in Chickpea (Alfano et al., 2007).

Inoculation of soil with *T. harzianum* T22 increase nucleic acids content in shoots and roots of maize plants as well as in plants developed from seeds treated with the metabolic solution of *T. harzianum* T22. In tomato plants inoculated with *Trichoderma hamatum*, the expression of stress, cell wall and RNA metabolism related genes were upregulated demonstrating similarities of plant responses to *T. Arabidopsis thaliana harzianum* (Alfano et al., 2007). *T. harzianum* which alterate the genetic systems were up-regulated proteins were the transcription factors and nuclear proteins RNA polymerase (Guyomarc et al., 2006).

Auxins are important plant regulators involved in many growth and behavioral processes, including those activated by *Trichoderma* spp. (Contreras et al., 2009). Hexon et al. (2009) showed that *Trichoderma* spp. produced indole-3-acetic acid (IAA) that promotes lateral root formation. The enhanced plant growth by *Trichoderma* (*T. harzianum* strain T22, T39 and A6) might be due to the production of secondary metabolites which may act as an auxin like compound (Vinale et al., 2008a, b).

Seed germination of cucumber increased by application of *Trichoderma,* which may be due to hormonal secretion like gibberellins, auxins (Akhtar et al., 2007). *Trichoderma virens* is able to produce the indolic compounds indole-3-acetic acid (IAA), and indole-3-ethanol, which may play roles in mediating plant growth promotion by this fungus. Also plant growth mechanisms of plant growth promoting fungi can be achieved by the production of plant growth regulators like auxins, gibberellins, and cytokinin (Hexon et al., 2009).

The decrease in the lignification which occurred in the vessels elements of the xylem confirm that *T. harzianum* is a very effective cellulolytic organism. These observations are in agreements with the finding of Gupta

et al. (2004) who reported *that T. viride* can degrade different side chains of lignin and bring about faster decomposition. Amouri and Gargouri (2006) reported that *T. harzianum* secrete higher amounts of cellulases which is the most efficient enzyme system for the complete hydrolysis of cellulosic substrates into its monomeric glucose.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was employed to assess the relative amounts and banding patterns of protein samples. The results in our study showed differences in responses of *Z. mays* plants to *T. harzianum* strain T22 treatments. In addition, in maize leaves ten proteins were identified compared to control. Proteins that are produced by *Trichoderma* spp. within plant cells may be involvement in defense-related proteins in plants interacting with *Trichoderma* (Harman and Shoresh, 2007).

Conclusion

Trichoderma isolates on plant growth and development have important economical implications such as shortening the plant growth period and time, as well as improving plant vigor to overcome biotic and/or abiotic stresses, resulting in increase plant productivity and yields. In addition, the reduction in lignification which was induced by *T. harzianum* strain T22 have a beneficial effect in enhancing fresh state of *Z. maize* stalks.

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