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Down-Regulation of Damping-off and Root Rot Diseases in Lentil Using kinetin and *Trichoderma*.

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ABSTRACT

Damping–off and root rot diseases caused by several soil-borne pathogenic fungi are the most responsible for high reduction in lentil yield. The aim of the present study is to evaluate the efficiency of kinetin and *Trichoderma* for controlling these diseases and lentil productivity compared with Rizolex-T50 fungicide. *Rhizoctonia solani* and *Fusarium oxysporum* are the common causal pathogens which isolated from infected lentil collected from different fields of Dakahlia Governorate, Egypt. In greenhouse: both tested fungi were pathogenic and caused pre- and post-emergence damping-off. Lentil cultivar Giza 9 was more susceptible for infected with both pathogenic fungi. *F. oxysporum* was most aggressive in post-emergence damping-off. On the other side, Rizolex-T50 recorded the highest reduction in emergence damping-off in both lentil cultivars, *Trichoderma harzianum* come next. Anatomically, *R. solani* caused complete disruption of the epidermal and cortical cells in both cultivars of lentil roots, Giza 9 was very affective. In field: Lentil; Giza 4 cultivar was more tolerant for infected with damping-off and root rot diseases under natural infection. The application of both *Trichoderma* species and two concentrate of kinetin led to significant reduction in damping-off and dead plants. *T. harzianum* was more effective. Giza 4 was the best cultivar in photosynthetic pigments as compared with Giza 9. Except Rizolex-T50, all treatments increased significantly photosynthetic pigments. Kinetin at 100 ppm was more effective. Meanwhile, *T. harzianum* gave the highest values of total phenols content. Kinetin at level 100 ppm gave the highest average of growth parameters (plant height and branches number plant^{-1})and yield components (pods number plant^{-1}, 1000 seed weight and seed yield (kg/fed)). Giza 4 cultivar recorded the highest yield, while Giza 9 was the best in seed quality. The highest seed protein% occurred under the application of *T. viride*. Meanwhile, kinetin at 100 ppm gave the high phosphorus percentage in the seed of lentil. Therefore, application of kinetin at 100 ppm as well as *T. harzianum* as seed soaking could be recommended for controlling soil-borne diseases in addition to improving growth and productivity of lentil.

Keywords: Lentil, damping-off, root rot, kinetin, *Trichoderma*.

INTRODUCTION

Lentil (*Lens esculinta* L.) is one of the most important legumes for human diets in Egypt. It is cultivated for non-endospermic seeds, which rich in protein content ranging from 22-35 % (Rahman *et al*., 2012). In addition, lentil can be considered as friendly crop to the environment because its supply of the soil by nitrogen fixation.

Several biotic stresses adversely affect in lentil yields. Soil-borne diseases are a major limitation to improve production efficiency and crop quality of lentil. In Egypt, damping-off, root rot and vascular wilt are the most diseases responsible for high reduction in lentil yield, sometimes total loss in the yield (Morsy, 2005 and El-Shennawy *et al*., 2010). Infection of fungal diseases let to decrease in productivity due to the damage which occur in roots, leaves, stems and pods as well as discoloration of seeds (Taylor et al., 2007). Damping-off occur either before plant emergence (pre-emergence damping-off) or in the young seedlings (post-emergence damping-off). Damping-off symptoms are yellow seedlings while no secondary roots or a brown/black tap roots, and plant death. Damping-off and root rot diseases caused by several fungi as *Rhizoctonia solani*, *Fusarium* spp., *Macrophomina phaseolina* and *Sclerotinia sclerotiorum* (Morsy, 2005, El-Shennawy *et al*., 2010 and El-Hersh *et al*., 2011). Moreover, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lentis* is one of the most important diseases and is a major factor that limits the successful cultivation of lentil worldwide (Saxena, 1993).

The control of soil-borne diseases depends mainly on fungicidal treatment with difficult the control of these pathogens due to their persistency in the soil and wide host range (Rauf, 2000). The use of fungicides cause hazards to human health and increase environmental pollution. Hence, there is an urgent to apply alternative safe efficient methods against these diseases.

Growth substances (bio-regulators) also called plant growth regulators are organic compounds other than nutrients which modify plant physiological processes. Some authors stated that the role of plant regulators in plant diseases is not clearly identified. Because the increasing evidence of both pathogens and host have the capacity to synthesize various plant growth regulators(Singh *et al*., 1997 and El-Masri *et al*., 2002).However, many authors studied the effect of growth substances on reduction of fungal disease infection in many plants. Khalil, (2002) and Metwally *et al*., (2006) reported that soaking peanut seeds with growth substances significantly reduced root rot disease. Moreover, Abd El-Hai *et al*., (2012) found that using growth substances decreased pre and post-emergence damping-off in soybean. Kinetin is a member of cytokinins group. The recent work has revealed that plant-originated cytokinins augment plant immunity together with salicylic acid signaling (Choi et al., 2010). Cytokinins enhance chlorophyll synthesis, or prevent or delay its degradation, which enhance plant growth followed by increase the plant defense mechanisms (Robert-Seilaniantz *et al*., 2007). Kinetin induces osmosis in seeds and might be limiting factor under stress condition (Sakr, 1996). Moreover, it increases protein synthesis during germination. While, Perty *et al*., (2009) stated that the molecular mechanisms of cytokinin action in disease resistance to a wide spectrum of pathogens and the reason for the inverse effects of cytokinins on plant responses against biotrophic pathogens and viral infection have remained elusive.

Trichoderma species are used for disease control and yield increases. *Trichoderma* induced systemic or localized resistance. In addition to, their substantial influence on plant growth and development (Harman, 2006, Reino *et al*., 2008 and Saber *et al*., 2009). *Trichoderma* produces lytic enzymes and antifungal antibiotics (Almassi *et al*., 1991 and Haran *et al*., 1996). The biocontrol mechanisms of *Trichoderma* strains includes both directly mechanisms as a mycoparasitism and indirectly mechanisms by competing for nutrients and space, modifying environmental conditions, promoting plant growth and plant defense as well as antibiosis (Shakeri and Foster, 2007 and Gajera *et al*., 2013).

The present study was undertaken to evaluate the efficiency of kinetin and *Trichoderma* for controlling damping-off and root rot diseases and increasing lentil productivity as compared with the fungicide Rizolex-T50. Also, the changes of the anatomical structure of roots in lentil plants which occurred by the pathogenic fungi were studied.

MATERIALS AND METHODS

Seeds and tested chemicals

Lentil seeds cvs. Giza 4 and Giza 9 were obtained from Legume Crops Res. Dept., Field Crop Res., Instit., Agric. Res. Cent., Giza, Egypt. Whereas kinetin (6-Furfuryl amino purine) and Rizolex- T50 (Tolclofos-methyl) were obtained from Al-Gomhoria Chemical Company, Egypt.

Isolation of *Trichoderma* spp.

Two fungi of *Trichoderma* species i.e. *T. harzianum* and *T. viride* were isolated from phyllosphere of healthy lentil plants collected from Dakahlia Governorate using selective medium of Elad *et al*., (1991). Fungal cultures of *Trichoderma* were purified by hyphal tip technique and identified on the basis of cultural and microscopic morphological characters (Barnett and Hunter, 1972 and Bissett, 1991).

Pathogens

Rhizoctonia solani and *Fusarium oxysporum* were isolated from naturally infected lentil plants cultivated in Dakahlia Governorate, showing damping-off and root rot symptoms. Both isolated fungi were identified on the basis of cultural and microscopic morphological characters according to Barnett and Hunter (1972) and Booth (1985).

Fungal inocula preparation

Inocula of *R. solani* and *F. oxysporum* were prepared using sorghum: coarse sand: water (2: 1: 2 v/v) media. The media were mixed, bottled and autoclaved for 2 hours at 1.5 air pressure. The sterilized media were inoculated using agar discs obtained from the periphery of 5-day old colony of both isolated fungi. The inoculated media were incubated at 25 ºC for 15 days, then used for soil infestation in greenhouse experiment for studying the pathogenicity test and changes of the anatomical structure of lentil roots.

Greenhouse experiment

a- Pathogenicity test

Pathogenicity test of both isolated fungi was carried out under greenhouse conditions. The response of two lentil cultivars for tested treatments under soil infestation with causal pathogens was testing in pot experiment. Inoculum of each isolate was added to the autoclaved clayey soil at a rate of 0.3% (w/w) then putted in sterilized pots (25 cm in diameter), filled up with 3.5 kg soil. Infested pots watered twice at 3 days intervals before sowing to enhance growth and distribution of fungal inoculum. Seeds of both lentil cultivars were soaked in kinetin (50 and 100 ppm) and spore suspension of both *Trichoderma* sp. (10^7 conidia^{-1}) for 8 hours and sown in pots at 15 seeds/pot. At the same time, untreated seeds were sown as check treatment and also seed coating with Rizolex-T50 (3 g/kg seeds) were sown as a fungicide treatment. Three replicates were used in each particular treatment. The percentage of pre and post-emergence damping-off were recorded at 15 days and 30 days after sowing, respectively.

b- Anatomical structure of lentil roots

Specimens from roots of lentil were taken after 35 days from sowing for studying the anatomical structure of lentil roots in infected region. Specimens were killed and fixed in Formalin Alcohol Acetic acid mixture (F: A: A) at a rate of (1: 18: 1 v/v). Then washed and dehydrated in alcohol series and embedded in paraffin wax (52-54 ºC melting point). Sections at 15 µ thick were prepared by a rotary microtome, stained in crystal violet and erythrosine, cleared in xyol and mounted in Canada balsam (Gerlash, 1977). Sections were examined microscopically for determining the anatomical changes which occurred by the pathogenic fungi compared with healthy plants.

Field experiments

The field experiments were carried out in naturally infested soil of Tag El-Ezz Agricultural Research Station Farm, Dakahlia Governorate, Egypt during 2012/2013 and 2013/2014 winter seasons. Two cultivars of lentil seeds (Giza 4 and Giza 9) were treated with the same previous treatments in the greenhouse experiment and untreated control were used. Treated and untreated seeds were sown in 20th of November in the first season and 16th of November in the second one. The experimental layout was split plot design with three replicates. The main plot were occupied by cultivars while sub-plots were occupied by treatments. The area of each sub-plot was 3.5 x 3 m (10.5 m^2) consisting of 5 rows. Two seeds/hill with 5 cm apart between hills were planted(700 seeds/sub-plot). Within plant growth, the same treatments were used as a foliar spraying two times with 15 days interval beginning from 45 days after sowing. Other agricultural practices were carried out as usual. The percentage of pre- and post-emergence damping-off and also dead plants (resulted from root rot and/or wilt) were determined after 15, 30 and 70 days from sowing, respectively.

Physiological activities

Samples were taken at 70 days from planting to determine the following physiological activities of lentil plants.

a- photosynthetic pigments

The blades of the third leaf from plant tip (terminal leaflet) were taken to determine photosynthetic pigments (chlorophyll a, b and carotenoids) which extracted with methanol after adding traces of sodium carbonate (Robinson and Britz, 2000) and determined according to Mackinney (1941).

b- Total phenolic compounds

The total phenolic compounds were determined in fresh shoot using Folin-Ciocalteau reagent according to Malik and Singh (1980).

Growth and yield characters

Samples were taken at harvesting to estimate plant height (cm), number of branches, pods number/plant, weight of 1000 seeds (g) and seed yield (kg fed^{-1})

Seed quality

After harvesting, lentil seeds were dried at 70 ºC, grounded and analyzed for phosphorus percentage according to Chapman and Pratt (1965) and total nitrogen by semi-micro Kjeldahl (Pregl, 1945). The percentage of protein was calculated by multiplying N% by 6.25.

Statistical analysis

The obtained data were analyzed with the statistical analysis software CoStatV6.4. Means were compared using Least Significant Difference (LSD) at p ≤ 0.05 according to Gomez and Gomez (1984).

RESULTS

Isolation of root rot fungi and pathogenicity test

Rhizoctonia solani and *F. oxysporum*, the causal pathogens of lentil root rot were isolated from naturally infected plants. Both pathogens after their identification were investigated for pathogenicity, the percentage of damping-off (pre-and post) in lentil cultivars (Giza 4 and Giza 9) under greenhouse condition are presented in Table 1. The two tested fungi were pathogenic and caused pre- and post-emergence damping-off. The highest percentage of pre-emergence damping-off occurred under infected soil with *R. solani*. While, *F. oxysporum* was the most aggressive in post-emergence damping-off. Giza 9 was the most susceptible cultivar for infected with both pathogenic fungi. The results also show that the fungicide Rizolex-T50 recorded the highest reduction in pre- and post-emergence damping-off in both lentil cultivars.

Concerning the effects of kinetin and *Trichoderma*, data in the same table show that *T. harzianum* came next to fungicide followed by *T. viride* then kinetin at 100 ppm in reducing lentil damping-off and root rot diseases.

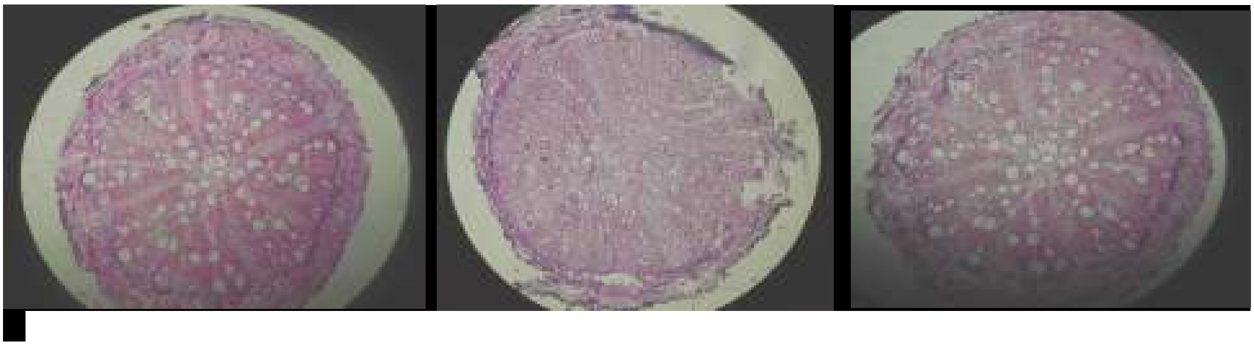
Table 1. Effect of kinetin and Trichoderma on damping-off of lentil plant under greenhouse conditions

Treatment		*Rhizoctonia solani* Pre-emergence damping-off		*Rhizoctonia solani* Post-emergence damping-off		*Fusarium oxysporum* Pre-emergence damping-off		*Fusarium oxysporum* Post-emergence damping-off	
Variety									
Giza 4		13.72	b	9.61	b	11.44	b	15.89	B
Giza 9		17.11	a	12.11	a	14.17	a	19.94	A
Treatments									
Control		39.50	a	25.33	a	19.83	a	31.67	A
Kinetin 50		18.50	b	14.50	b	15.17	b	20.67	B
Kinetin 100		13.17	c	10.00	c	13.33	c	17.67	C
Trichoderma harzianum		7.33	e	5.17	e	10.00	e	13.00	E
Trichoderma viride		9.50	d	7.17	d	11.67	d	15.17	D
Fungicide		4.50	f	3.00	f	6.83	f	9.33	F
Interaction									
Giza 4	Control	36.67	b	22.33	b	17.67	b	26.00	B
	Kinetin 50	16.67	d	12.33	d	12.67	d	19.00	D
	Kinetin 100	11.67	e	9.33	e	11.67	de	16.33	De
	T.harzianum	6.00	h	4.67	hi	9.33	f	11.67	Fg
	T.viride	8.00	gh	6.67	fg	11.00	e	14.00	Ef
	Fungicide	3.33	i	2.33	j	6.33	g	8.33	H
Giza 9	Control	42.33	a	28.33	a	22.00	a	37.33	A
	Kinetin 50	20.33	c	16.67	c	17.67	b	22.33	C
	Kinetin 100	14.67	d	10.67	e	15.00	c	19.00	D
	T.harzianum	8.67	fg	5.67	gh	10.67	e	14.33	Ef
	T.viride	11.00	ef	7.67	f	12.33	d	16.33	De
	Fungicide	5.67	hi	3.67	ij	7.33	g	10.33	Gh

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

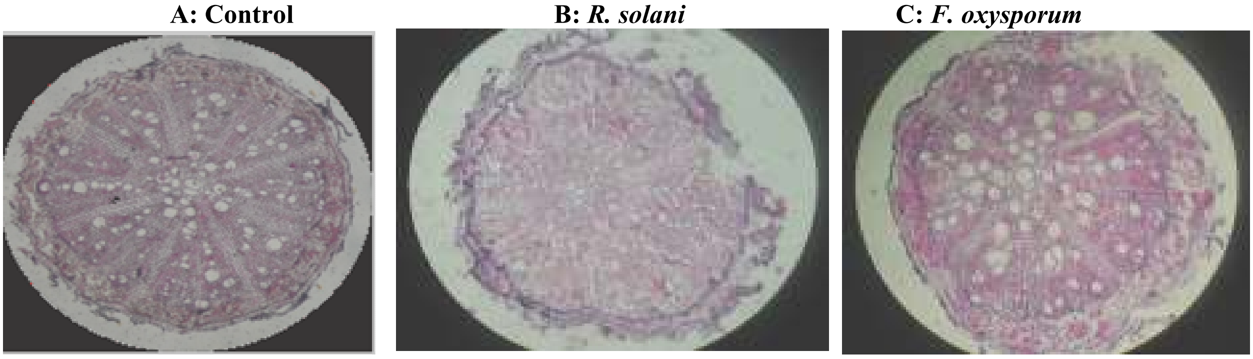
Root structure

Root cross sections of healthy and infected lentil plants by *R. solani* and *F. oxysporum* are illustrated in Figure 1 and 2. The structure of root from normal lentil plants showed well-formed structures of epidermis consists of closely packed elongated cells with thin walls, cortex may be homogenous and simple in structure and vascular cylinder that consists of xylem which forms discrete strands, alternating with the phloem strands. Clear differences of root structure were found among root sections obtained from normal healthy plants and infected plants with either *R. solani* or *F. oxysporum*. The anatomical structures observed in the cross sections of infected root showed remarkable differences occurred mainly in epidermis, cortex and littleness in vascular cylinder. *R. solani* led to complete destruction of the epidermis and cortex in both cultivars of lentil. The infected roots showed also severe plasmolysis in cortex, hydrolysis of cell components and degradation of primary cell wall. Giza 9 cultivar was very affective by both pathogens. Also, *R. solani* led to injurious effects on root structure more than *F. oxysporum.*



A B C

Fig. 1. Cross sections in lintel roots (Giza 4 cultivar) shows the changes of anatomical structures by root rot fungi(X 100)
A: Control B: *R. solani* C: *F. oxysporum*



A B C

Fig. 2. Cross sections in lintel roots (Giza 9 cultivar) shows the changes of anatomical structures by root rot fungi(X 100)
A: Control B: *R. solani* C: *F. oxysporum*

Field experiments

Disease assessment

The percentage of damping-off (pre- and post-emergence) as well as dead plants (resulted from root rot and/or wilt) during two successive seasons under natural infection in field conditions were recorded in Table 2. Data show that Giza 4 cultivar was more tolerant for infected with damping-off and root rot diseases than Giza 9 cultivar. The application of both species of *Trichoderma* and both levels of kinetin decreased significantly damping-off and dead plants of lentil in both seasons compared with control. *T.harzianum* was the most effective in this respect followed by *T. viride* then kinetin at 100 ppm in both lentil cultivars.

Physiological activities

Photosynthetic pigments

The photosynthesis bulk of higher plants takes place in the green leaves, it contains chloroplasts that green due to presence of chlorophyll. The light energy used is taken up by chlorophyll. The photosynthetic process occurs in chloroplasts. Photosynthesis which called carbon assimilation consists in the synthesis of certain carbohydrates from CO_2 and water by green cells in the presence of light. All living organisms requires energy for growth and reproduction. This energy comes from the chemical energy in the food consumed. So., chlorophyll is a good parameter reflecting the health condition of plants.

Data in Table 3 show that Giza 4 was the best cultivar in photosynthetic pigments (chl a, b and carotenoids) as compared with Giza 9. All treatments increased significantly the concentration of photosynthetic pigments in lentil leaf blade except Rizolex-T50 which inverse this. The high chlorophyll concentration occurred under the application of kinetin at 100 ppm followed by kinetin at 50 ppm then *T. harzianum*. On the other side, the highest values of carotenoids obtained from kinetin at 50 ppm followed by *T. harzianum*.

Table 2.Effect of kinetin and *Trichoderma* on damping-off and dead plants of lentil plant under field conditions

Treatment		Pre-emergence damping-off				Post-emergence damping-off				Dead plants (Resulted from root rot or wilt			
		Season 1		Season 2		Season 1		Season 2		Season 1		Season 2	
Variety													
Giza 4		9.55	B	11.72	b	6.72	b	7.83	B	6.00	b	7.11	b
Giza 9		18.50	A	15.72	a	9.39	a	8.17	A	7.72	a	9.00	a
Treatments													
Control		30.17	A	29.50	a	15.50	a	15.83	A	14.00	a	14.50	a
Kinetin 50		16.50	B	16.83	b	9.33	b	9.50	B	7.83	b	10.33	b
Kinetin 100		13.17	C	13.33	c	7.83	c	7.67	C	6.67	c	8.33	c
Trichoderma harzianum		8.50	E	8.00	e	5.33	e	5.17	E	4.50	d	5.17	e
Trichoderma viride		10.33	D	9.83	d	6.50	d	6.67	D	5.33	d	6.83	D
Fungicide		5.50	F	4.83	f	3.83	f	3.17	F	2.83	e	3.17	F
Interaction													
Giza 4	Control	20.33	B	24.00	b	12.67	b	15.33	A	11.67	b	12.00	B
	Kinetin 50	12.00	E	15.00	d	8.00	de	9.33	B	7.67	cd	10.00	C
	Kinetin 100	9.33	F	12.33	e	7.00	e-g	7.67	C	6.67	c-e	8.33	D
	T.harzianum	5.67	H	7.00	gh	4.33	i	5.00	D	3.33	gh	4.00	F
	T.viride	7.00	gh	8.33	fg	5.67	g-i	6.67	C	4.33	fg	5.67	E
	Fungicide	3.00	I	3.67	i	2.67	j	3.00	E	2.33	h	2.67	F
Giza 9	Control	40.00	A	35.00	a	18.33	a	16.33	A	16.33	a	17.00	A
	Kinetin 50	21.00	B	18.67	c	10.67	c	9.67	B	8.00	c	10.67	Bc
	Kinetin 100	17.00	C	14.33	d	8.67	d	7.67	C	6.67	c-e	8.33	D
	T.harzianum	11.33	E	9.00	f	6.33	f-h	5.33	D	5.67	ef	6.33	E
	T.viride	13.67	D	11.33	e	7.33	d-f	6.67	C	6.33	de	8.00	D
	Fungicide	8.00	Fg	6.00	h	5.00	hi	3.33	E	3.33	gh	3.67	F

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

Table 3. Effect of kinetin and *Trichoderma* on photosynthetic pigments of lentil plant under field conditions

Treatment		Ch a				ch b				Total chls				Carotenoids			
		Season 1		Season 2		Season 1		Season 2		Season 1		Season 2		Season 1		Season 2	
Variety																	
Giza 4		1.117	a	1.244	a	0.703	a	0.854	a	1.820	a	2.098	a	0.323	a	0.375	a
Giza 9		1.055	a	1.156	b	0.650	b	0.716	b	1.705	b	1.871	b	0.283	b	0.311	b
Treatments																	
Control		0.920	e	1.007	e	0.543	e	0.600	e	1.463	e	1.607	e	0.232	d	0.258	e
Kinetin 50		1.198	b	1.332	b	0.763	b	0.900	b	1.962	b	2.232	b	0.390	a	0.450	a
Kinetin 100		1.253	a	1.380	a	0.860	a	0.973	a	2.113	a	2.353	a	0.342	b	0.368	c
Trichoderma harzianum		1.168	c	1.285	c	0.712	c	0.847	c	1.880	c	2.132	c	0.300	c	0.333	d
Trichoderma viride		1.130	d	1.228	d	0.673	d	0.810	d	1.803	d	2.038	d	0.353	b	0.417	b
Fungicide		0.847	f	0.967	f	0.507	f	0.580	e	1.353	f	1.547	f	0.202	e	0.230	f
Interaction																	
Giza 4	Control	0.957	e	1.077	f	0.570	f	0.647	g	1.527	f	1.723	h	0.250	e	0.273	e
	Kinetin 50	1.233	b	1.360	b	0.797	b	0.977	b	2.030	b	2.337	b	0.413	a	0.497	a
	Kinetin 100	1.290	a	1.410	a	0.920	a	1.030	a	2.210	a	2.440	a	0.367	b	0.400	b
	Trichoderma harzianum	1.207	b	1.317	c	0.720	cd	0.930	c	1.927	c	2.247	c	0.320	c	0.360	c
	Trichoderma viride	1.160	c	1.270	d	0.680	e	0.907	c	1.840	d	2.177	d	0.380	b	0.483	a
	Fungicide	0.857	fg	1.030	g	0.530	g	0.637	g	1.387	g	1.667	i	0.210	f	0.237	f
Giza 9	Control	0.883	f	0.937	h	0.517	g	0.553	h	1.400	g	1.490	j	0.213	f	0.243	f
	Kinetin 50	1.163	c	1.303	c	0.730	c	0.823	d	1.893	c	2.127	e	0.367	b	0.403	b
	Kinetin 100	1.217	b	1.350	b	0.800	b	0.917	c	2.017	b	2.267	c	0.317	c	0.337	c
	Trichoderma harzianum	1.130	cd	1.253	d	0.703	d	0.763	e	1.833	d	2.017	f	0.280	d	0.307	d
	Trichoderma viride	1.100	d	1.187	e	0.667	e	0.713	f	1.767	e	1.900	g	0.327	c	0.350	c
	Fungicide	0.837	g	0.903	i	0.483	h	0.523	i	1.320	h	1.427	k	0.193	f	0.223	f

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

Phenol content

The effects of kinetin and *Trichoderma* as well as Rizolex-T50 on average of total phenols content (mg/100 g fresh weight) during two successive growing seasons are presented in Table 4. It was observed that total phenols content increased significantly in Giza 4 cultivar compared to Giza 9 in both seasons. There is a significant differences ($p \leq 0.05$) in cultivar response to different treatments. *T. harzianum* application gave the highest values of total phenols followed by kinetin at 50 ppm then*T. viride*. However, Rizolex-T50 fungicide had no significant effect in this respect.

Table 4. Effect of kinetin and *Trichoderma* on total phenol contents of lentil plant under field conditions

Treatment		Total phenol Season 1		Season 2	
Variety					
Giza 4		130.89	a	123.67	a
Giza 9		118.33	b	105.22	B
Treatments					
Control		105.00	e	97.17	e
Kinetin 50		137.50	b	124.83	b
Kinetin 100		124.67	d	114.83	d
Trichoderma harzianum		144.00	a	132.33	a
Trichoderma viride		131.17	c	120.33	c
Fungicide		105.33	e	97.17	e
Interaction					
Giza 4	Control	112.33	h	85.00	g
	Kinetin 50	142.67	b	85.67	g
	Kinetin 100	129.67	e	108.67	f
	Trichoderma harzianum	150.67	a	108.67	f
	Trichoderma viride	135.67	c	109.33	ef
	Fungicide	114.33	h	111.67	e
Giza 9	Control	97.67	i	118.33	d
	Kinetin 50	132.33	d	121.00	cd
	Kinetin 100	119.67	g	122.00	c
	Trichoderma harzianum	137.33	c	129.00	b
	Trichoderma viride	126.67	f	131.33	b
	Fungicide	96.33	i	142.67	a

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

Growth parameters

It is clear from Table 5 that seed soaking with kinetin at both levels, each of *Trichoderma* isolates followed by foliar spraying of the growing lentil plants with the same treatments increased significantly the growth parameters (plant height and branches number $plant^{-1}$). Giza 9 cultivar was the best in plant height while, Giza 4 was the best in branches number. In this respect, kinetin at high level gave the highest values compared with other treatments. The low level of kinetin came after high level followed by *T. harzianum*. However, the fungicide had not any significant effect on growth parameters.

Yield and its components

Data in Table 6 of two growing seasons show that Giza 4 cultivar recorded the highest values of yield components (pods number $plant^{-1}$, 100- seed weight and seed yield (kg/fed)). All tested treatments increased significantly lentil yield components in both cultivars. The high level of kinetin (100 ppm) gave the highest average of pods number $plant^{-1}$, weight of 1000 seeds and seed yield. On the other side, *T. harzianum* came after followed by kinetin at 50 ppm.

Seed quality

Lentil seed quality was estimated as seed protein and seed phosphorus percentage. As shown in Table 7 that Giza 9 cultivar was the best in seed quality (protein % and P %) as compared with Giza 4 in both seasons. Moreover, all of treatments used increased significantly protein and phosphorus percentage in lentil seeds. The highest % in protein occurred under *T. viride* followed by *T. harzianum* then kinetin 50 ppm. On the other side, kinetin at 100 ppm came first in increasing phosphorus percentage followed by kinetin at 50 ppm then *T. harzianum*.

Table 5.Effect of kinetin and *Trichoderma* on growth parameters of lentil plant under field conditions

Treatment		Plant height Season 1		Season 2		No. of branches Season 1		Season 2	
Variety									
Giza 4		47.17	b	51.11	b	10.72	a	10.61	a
Giza 9		54.94	a	60.44	a	8.5	b	8.5	b
Treatments									
Control		44.33	e	49.00	e	7.67	d	7.33	e
Kinetin 50		57.00	b	61.17	b	9.83	c	10.17	c
Kinetin 100		60.17	a	66.50	a	12.00	a	12.00	a
Trichoderma harzianum		52.50	c	56.67	c	11.00	b	11.33	b
Trichoderma viride		47.83	d	52.00	d	9.33	c	9.17	d
Fungicide		44.50	e	49.33	e	7.83	d	7.33	e
Interaction									
Giza 4	Control	40.33	g	44.00	h	8.67	d	8.33	e
	Kinetin 50	53.67	cd	57.67	d	11.00	bc	11.67	bc
	Kinetin 100	55.33	c	61.33	c	13.67	a	13.00	a
	Trichoderma harzianum	48.67	e	51.67	f	12.00	b	12.33	ab
	Trichoderma viride	44.33	f	48.00	g	10.33	c	10.33	d
	Fungicide	40.67	fg	44.00	h	8.67	d	8.00	e
Giza 9	Control	48.33	e	54.00	ef	6.67	e	6.33	f
	Kinetin 50	60.33	b	64.67	b	8.67	d	8.67	e
	Kinetin 100	65.00	a	71.67	a	10.33	c	11.00	cd
	Trichoderma harzianum	56.33	c	61.67	c	10.00	c	10.33	d
	Trichoderma viride	51.33	de	56.00	de	8.33	d	8.00	e
	Fungicide	48.33	e	54.67	e	7.00	e	6.67	f

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

Table 6. Effect of kinetin and *Trichoderma* on yield components of lentil plant under field conditions

Treatment		Pods number/plant Season 1		Pods number/plant Season 2		1000 seeds weight (g) Season 1		1000 seeds weight (g) Season 2		Seed yield (Kg fed) Season 1		Seed yield (Kg fed) Season 2	
Variety													
Giza 4		68.28	a	64.89	a	28.04	a	26.55	A	503.67	a	486.11	a
Giza 9		61.72	b	56.94	b	26.21	b	25.33	B	465.67	b	453.22	b
Treatments													
Control		54.33	d	49.83	e	26.05	e	24.88	E	463.17	e	445.00	e
Kinetin 50		66.67	c	63.83	c	27.52	c	26.18	C	488.50	c	474.33	c
Kinetin 100		79.00	a	72.50	a	27.95	b	26.58	B	503.00	b	492.33	b
Trichoderma harzianum		71.33	b	69.00	b	28.32	a	27.13	A	513.83	a	499.50	a
Trichoderma viride		64.33	c	59.17	d	26.80	d	25.77	D	475.50	d	461.83	d
Fungicide		54.33	d	51.17	e	26.13	e	25.10	E	464.00	e	445.00	e
Interaction													
Giza 4	Control	56.67	g	52.00	e	27.20	d	25.83	Ef	482.00	f	462.33	e
	Kinetin 50	71.00	cd	69.67	b	28.20	b	26.60	b-d	508.67	c	493.00	c
	Kinetin 100	83.00	a	75.33	a	28.77	a	26.87	B	521.33	b	504.00	b
	Trichoderma harzianum	73.33	bc	72.00	b	29.00	a	27.43	A	533.33	a	514.00	a
	Trichoderma viride	68.00	d	66.67	c	27.83	c	26.37	b-e	494.00	d	481.00	d
	Fungicide	57.67	fg	53.67	e	27.27	d	26.20	d-f	482.67	ef	462.33	e
Giza 9	Control	52.00	h	47.67	f	24.90	g	23.93	H	444.33	i	427.67	g
	Kinetin 50	62.33	e	58.00	d	26.83	e	25.77	F	468.33	g	455.67	e
	Kinetin 100	75.00	b	69.67	b	27.13	de	26.30	c-f	484.67	e	480.67	d
	Trichoderma harzianum	69.33	d	66.00	c	27.63	c	26.83	Bc	494.33	d	485.00	cd
	Trichoderma viride	60.67	ef	51.67	e	25.77	f	25.17	G	457.00	h	442.67	f
	Fungicide	51.00	h	48.67	f	25.00	g	24.00	H	445.33	i	427.67	g

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

Table 7. Effect of kinetin and *Trichoderma* on protein and phosphorus percentage of lentil seeds

Treatment		Protein percentage Season 1		Protein percentage Season 2		Phosphorus percentage Season 1		Phosphorus percentage Season 2	
Variety									
Giza 4		27.01	b	27.26	b	0.487	b	0.494	B
Giza 9		27.55	a	27.60	a	0.518	a	0.516	A
Treatments									
Control		26.53	e	26.63	e	0.478	e	0.481	E
Kinetin 50		27.40	c	27.62	c	0.526	b	0.522	B
Kinetin 100		27.00	d	27.20	d	0.537	a	0.532	A
Trichoderma harzianum		27.95	b	28.15	b	0.502	c	0.514	C
Trichoderma viride		28.30	a	28.35	a	0.495	d	0.501	D
Fungicide		26.53	e	26.63	e	0.477	e	0.480	E
Interaction									
Giza 4	Control	26.16	e	26.43	h	0.458	i	0.457	E
	Kinetin 50	27.09	d	27.31	e	0.511	de	0.522	B
	Kinetin 100	26.89	d	27.15	f	0.524	c	0.531	A
	Trichoderma harzianum	27.77	c	28.06	c	0.490	g	0.510	C
	Trichoderma viride	28.00	b	28.18	bc	0.482	h	0.490	D
	Fungicide	26.17	e	26.42	h	0.456	i	0.454	E
Giza 9	Control	26.89	d	26.84	g	0.497	f	0.506	C
	Kinetin 50	27.71	c	27.92	d	0.542	b	0.522	B
	Kinetin 100	27.10	d	27.25	ef	0.550	a	0.532	A
	Trichoderma harzianum	28.12	b	28.24	b	0.514	d	0.518	B
	Trichoderma viride	28.59	a	28.51	a	0.508	e	0.511	C
	Fungicide	26.89	d	26.84	g	0.498	f	0.507	C

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

DISCUSSION

Damping-off and root rot diseases are caused by a complex fungi i.e. *Rhizoctonia solani*, *Fusarium* spp., *Macrophomina phaseolina* and *Sclerotinia sclerotiorum* (Abou-Zeid *et al*., 1997 and El-Shennawy *et al*., 2010). These diseases lead to high reduction in lentil yield, sometimes total loss in yield (Hamdi *et al*., 1991 and Abdel-Monaim and Abo-Elyousr, 2012). In this investigation, two fungal genera were obtained from diseased lentil roots and identified as *R. solani* and *F. oxysporum*. Both fungi were pathogenic and causing damping-off and root rot diseases in both cultivars of lentil (Giza 4 and Giza 9). Giza 4 was the most tolerant than Giza 9 due to genetic variance between both cultivars.

The harmful effects of the both tested pathogenic fungi on growth and yield of lentil may be due to seed rot as well as killing and damage of root system that reduced absorption surface and uptake of essential nutrients and water (Hussain *et al*., 1989 and Porter *et al*., 1990). Moreover, the infection with root rot fungi cause chlorosis of leaves (Saleh, 1997), causing a reduction in photosynthetic capacity and net photosynthesis. In the present study both tested pathogenic fungi causes destruction in the root anatomy structure (Fig. 1), in turn causes damping-off.

The use of chemical fungicide to control the plant diseases leads to menace to the human and animal health as well as reducing the populations of beneficial microorganisms in soil. In turn, the present investigation was planning to study the possibility of improving lentil growth and yield through controlling damping-off and root rot diseases using kinetin and *Trichoderma* compared with fungicide Rizolex-T50.

The results in the present investigation showed that both levels of kinetin and two species of *Trichoderma* significantly reduced the percentage of pre- and post-emergence damping-off as well as dead plants of lentil. The positive effect of kinetin in decreasing the infection of lentil with damping-off and root rot might be due to the inhibitory effect on fungal growth (Musa, 2016). The same author found that kinetin increased the activities of the four resistance related enzymes i.e. peroxidase, polyphenol oxidase, catalase and phenylalanine ammonia lyase as well as total phenol content. Theses enzymes lead to protect plants against pathogen stress (Chowdhury, 2003). While, infection of mung bean with *F. oxysporum* or *R. solani* inhibited the above enzymes. In this investigation, kinetin increased total phenols content (Table 4). The rapid accumulation of phenols at the infection site is considered the first step of defense mechanism in plants, which lead to restricts or slows pathogen growth due to its action as antioxidant, antimicrobial and photoreceptor (Lamba *et al*., 2008). Moreover, polyphenol oxidases (PPO) have some mechanisms for the pathogen defense include; general toxicity of PPO-generated quinones, alkylation and reduced bioavailability of cellular proteins to the pathogen, cross-linking of quinones with protein or other phenolics forming a physical barrier to pathogen in the cell and H_2O_2which resulting from quinones redox cycle, are known to be important factor in plant pathogen interaction and defense signaling (Raj *et al*., 2006).

Our results showed that the use of both *Trichoderma* species i.e. *T. harzianum* and *T. viride* significantly reduced the percentage of damping-off and root rot diseases under greenhouse and field conditions. These results are in agreement with those of Abou-Zeid *et al*., (2003), Abd-El-Kareem *et al*., (2004), Gonzalez *et al*., (2005), Malik *et al*., (2005) and Abd-El-Khair *et al*., (2011). They found that *T. harzianum* and *T. viride* protected the germinating seedlings against soil-borne fungi i.e. *Fusarium* spp. and *R. solani* infection and significant reduction to incidence of damping-off diseases. This may be due to *Trichoderma* spp. can produce lytic enzymes, antifungal antibiosis and they can also be competitors of fungal pathogens as well as promote plant growth (Almassi *et al*. 1991, Inbar *et al*., 1994, Haran *et al*., 1996 and Gajera *et al*., 2013). Finally, *Trichoderma* can work in several strategies i.e. competition for space and nutrients, antibiosis, induce resistance and parasitism (Monte, 2001 and Gajera *et al*., 2013).

In the present investigation, both kinetin and *Trichoderma* increased significantly lentil growth, productivity and yield as well as seed quality. These results may be due to the positive effect of kinetin and *Trichoderma* on photosynthetic pigments (Table 3). This is in agreement with El-Zawily *et al*., (1994)who stated that cytokinin causes an increase in the number of chloroplasts in leaves by increasing both intensity of cell growth phytohormones and the activity of cytoplasm ribosomes, thus stimulation of chlorophyll synthesis. Photosynthesis process consists in the synthesis of carbohydrates that used as a source of energy. There is a relationship between photosynthesis and protein content, hence induces sucrose translocated from leaf to seeds, which metabolized to precursor for protein (Smith *et al*., 1989). In turn, any treatment causes increase photosynthetic pigments will be expected to stimulate yield and protein content in seeds.

In the present study, the chemical control with recommended dose of Rizolx-T50 as seed coating was more effective and fast therapy for damping-off and root rot diseases in lentil. This is may be due to plant seeds always requires treatment with fungicides to assure an adequate plant stand in the field. Similar findings were obtained by Abd-El-Kareem *et al*., (2004), Ali *et al*., (2009) and Abd El-Hai *et al*., (2010).

It could be concluded that the application of kinetin at 100 ppm and/or *Trichoderma harzianum* as seed soaking is recommended for integrated management of damping-off and root rot diseases as well as enhancing growth and productivity of lentil.

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تنظيم خفض أمراض موت البادرات وعفن الجذور فى العدس باستخدام الكينيتين والترايكوديرما

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تؤدى أمراض موت البادرات وعفن الجذور والتى يسببها العديد من فطريات التربة إلى حدوث نقص كبير فى محصول العدس.ولقد صمم هذا البحث بغرض دراسة وتقييم فعالية الكينيتين والترايكوديرما فى مقاومة هذه الأمراض واثرها على زيادة انتاجية العدس مقارنة بالمبيد الفطرى ريزولكس-تى 50.و قد تم عزل الريزوكتونيا سولانى والفيوزاريوم اوكسيسبورم كمسببات مرضية من نباتات العدس المصابة بعفن الجذور والتى تم تجميعها من حقول مختلفة بمحافظة الدقهلية. وأوضحت الدراسة ما يلى: فى الصوبة: وجد ان كلا المسببان أحدثا الاصابة بموت البادرات وعفن الجذور. وكان الصنف جيزة 9 أكثر حساسية للإصابة بكلا المسببان المرضيان. وكان فطر الفيوزاريوم الأكثر عدوانية فى احداث مرض موت البادرات بعد ظهورها فوق سطح التربة. وعلى الجانب الاخر سجل مبيد الريزولكس-تى 50 أعلى نقص فى مرض موت البادرات فى كلا صنفى العدس تلاه الريكوديرما هاريزيانم. تشريحياً: سبب فطر الريزوكتونيا سولانى فى تحطم كامل لخلايا البشرة والقشرة فى جذور نباتات العدس وكان الصنف جيزة 9 الأكثر تأثراً. فى الحقل: وجد أن صنف جيزة 4 أكثر تحملا للإصابة بأمراض موت البادرات وعفن الجذور تحت ظروف العدوى الطبيعية. المعاملة بكلا نوعى الترايكوديرما وكلا تركيزى الكينيتين أدت لحدوث نقص معنوى فى موت البادرات وكذلك موت النباتات (الناتج من عفن الجذور او الذبول). وكانت الترايكوديرما هاريزيانم الأكثر فعالية. وفى القياسات الفسيولوجية وجد أن الصنف جيزة 4 الأفضل فى محتوى صبغات البناء الضوئى وفيما عدا مبيد الريزولكس-تى 50 أدت جميع المعاملات لزيادة صبغات البناء الضوئى وكان الكينيتين بتركيز 100 جزء فى المليون الأكثر تأثيراً. فى حين أعطت معاملة الترايكوديرما هاريزيانم أعلى قيم لمحتوى الفينولات الكلية. أعطى الكينيتين 100 جزء فى المليون أعلى قيم لصفات النمو والمحصول. سجل صنف جيزة 4 أعلى قيم للمحصول بينما كان صنف جيزة 9 الأفضل فى صفات الجودة بالبذور. وكانت أعلى نسبة للبروتين بالبذور تحت المعاملة بالترايكوديرما فيردى. بينما أعطى الكينيتين 100 جزء فى المليون أعلى نسبة مئوية للفوسفور بالبذور.توصى الدراسة بمعاملة بذور العدس بالكينيتين 100 جزء فى المليون أو الترايكوديرما هاريزيانم لمقاومة أمراض موت البادرات وعفن الجذور بالإضافة لتحسين النمو والإنتاجية.