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EFFECTS OF PH AND ECW ON GROWTH AND SPORULATION OF INDIGENOUS *TRICODERMA* SPP.

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A B S T R A C T

The influence of three levels of pH and degrees of ECw on the radial growth and sporulation of 34 local isolates of *Trichoderma* spp. were evaluated under laboratory conditions. The pH used were 4, 6 and 8 while, the ECw were 12.92, 14.30 and 17.8 Siemens per meter (ds/m) respectively. The data obtained showed that pH 6 was the optimum degree of growth parameters while pH 8 showed significant reduction ($p< 0.05$) in the growth parameters compared to pH 4. The results also revealed that ECw 17.8 ds/m significantly reduced ($p< 0.05$) the growth parameters compared to ECw 12.92 and 14.30 ds/m. Thus, the results obtained elucidated the importance of reduction in biocontrol activity of fungus in saline-alkali soil.

Keywords: Saline-alkali soil, pH alkaline, Electrical conductivity (ECw), *Trichoderma* isolates.

INTRODUCTION

The genus *Trichoderma* is a soil borne saprophytic fungus that is widely distributed in all soil types (Meraj-ul *et al.,* 2012; Kalaivani *et al.,* 2014; Wu *et al.,* 2014). *Trichoderma* isolates have different potential of antagonistic ability against plant soil borne pathogens and nematodes (Jabara, 2002). The chances of biological control success is still limited to their consistant performance, and one of the most important reasons for this problem is the environmental stress factors such as salinity and pH of the soil. The environmental stress factors of the soil have detrimental effects on *Trichoderma* isolate's growth, antagonistic activity and survival rate (Hafedh, 2001; Mohamed and Haggag 2010; Panahian *et al.,* 2012).

The cultivation of sensitive economic crops in saline soils has been a failure of crop cultivation. The numbers of economic crops resistant to salinity are still limited, and there were differences in their resistance to these conditions. Several studies have stated that the fungal genus *Trichoderma* are sensitive and not tolerant to high levels of pH (Mehrotra *et al.,* 1997; Hafedh, 2001) however, Harman (2000) and Mohamed and Haggag (2010) used protoplast fusion technique between two *T. harzianum* isolates and *T. koningii* and *T. reese* which had high specification tolerances under stress conditions, and obtained a new strain which was considered as highly antagonistic and more tolerant to stress conditions than their parental strains. *Trichoderma* isolates that are good saline-tolerant are more economical for crops cultures than the less tolerant to soil saline, for this reason the incessant search for a new isolates that have good ability to tolerate the soil saline is imperative. so, the objective of this study was to obtain the best antagonist of *Trichoderma* isolates that are more tolerant to soil saline and alkaline pH, the common characters of Iraqi soils.

MATERIAL AND METHODS

***Trichoderma* Isolates:** This study was conducted with 34 isolates of *Trichoderma*, 30 isolates were obtained from the department of biopesticide directorate of agricultural research, three isolates were obtained from the plant protection department college of agriculture University of Baghdad and one isolate was obtained from the public authority of Agricultural research-department of Agriculture.

Effect of pH on Growth Rate: In this assay we selected three levels of pH 4, 6 and 8, and study their biological effect on daily growth rate of (DGR) 34 different *Trichoderma* isolates. A 5 mm diameter discs from

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Trichoderma mycelium of all the isolates were individually applied in the middle of a 9 cm petri dish on potato dextrose agar (PDA). These plates were then incubated at (28±2 ^{0}C) and observed for fungal growth and sporulation over a period of five days, and the pH adjusted by using hydrochloric acid solution (HCL). The daily rate of growth was calculated according to the following equation:

$$DGR = \frac{(D2-D1)}{2(T2-T1)}$$ (Hafedh, 2001).

The sporulation ability of each isolate was calculated by adding 10 ml of distilled water to each plate, and spores collected by using glass rod, and prepared an appropriate dilution of three concentrations of spores for each isolate; and this was calculated using Haemacytometer as described by Hamdia and Kalaivani (2013).

Effect of ECw on Daily Growth Rate: To evaluate the effect of electrical conductivity ECw on the growth and sporulation of 34 *Trichoderma* isolates the medium used include the following components; (5 % of Dextrose, 0.5 % of K_2HPO_4, 0.1 % of $NaNO_3$, 0.2 % of Yeast extract, 0.1 % of $MgSO_4.7H_2O$ and 1.5 % of Agar). Also, the three concentrations of NaCl used were; 1g of NaCl (ECw. 12.92 ds/m) and 2g of NaCl (ECw. 14.30 s/m) and 5g of NaCl (ECw. 17.8 ds/m): The ECw of the salt medium (NaCl) was evaluated using a pH-meter (Handbook, 1954). The medium was sterilized at a temperature of 121 ^{0}C and pressure of 1.5 kg / cm^2 for 15 minutes, after that the medium was poured in petri dishes, and a 5 mm disc of mycelium from each *Trichoderma* isolates were placed in the middle of a 9 cm petri dish, each treatment triplicated.

And later incubated at a temperature of (28 ± 2 ^{0}C) in this test, daily growth rate and sporulation ability were calculated for each *Trichoderma* isolate as mentioned above. The data obtained from the observations were statistically analysis by using a completely randomized block design.

RESULTS

Effect of pH on the growth and sporulation of 34 *Trichoderma* Isolates: The results revealed that the tested isolates of *Trichoderma* spp. exhibited significant differences ($p<0.05$) in their response to tested pH levels (4, 6 and 8) Table 1. The laboratory experiment showed that *Trichoderma* isolates T.2, T.13, T.27 exhibited optimum growth rate at pH4, and isolates T.1, T.6, T.8, T.15 gave optimum growth rate at pH6, while isolates T.2, T.15, T.18, T.33 exhibited optimum growth rate at pH8 (Table 1). The results also showed that isolate T.30 gave the same response growth rate 1 cm at all pH tested Table 1. As for the sporulation the data shows in Table 2 that isolates T.3, T.5, T.13, T.20, T.23, T.26, T.27, T.33 exhibited optimum sporulation rate at pH4, and isolates T.1, T.4, T.8, T.14, T.21, T.22, T.31 revealed optimum sporulation rate at pH6, while isolates T.3, T.9, T.11, T.14, T.19, T.28 exhibited optimum sporulation rate at pH8.

Table 1. The Influence of pH on Daily Growth of 34 *Trichoderma* Isolates under Laboratory Conditions.

Isolates of *Trichoderma* spp.	* Daily Growth Rate		
	* *Mean No. of Daily Growth (cm)		
	PH4	PH6	PH8
T.1	1.5	1.75	0.75
T.2	1.75	1.5	1.5
T.3	1.37	0.75	1
T.4	1.5	1.5	0.5
T.5	0.6	1.37	0.75
T.6	1.12	1.75	1.0
T.7	0.87	1.37	1.0
T.8	0.75	1.87	1.0
T.9	1.25	1.25	1.0
T.10	0.68	1.25	1.0
T.11	0.62	1.25	1.25
T.12	0.75	1.70	0.5
T.13	1.62	1.68	1.0
T.14	1.0	0.81	0.62
T.15	1.37	1.87	1.75
T.16	1.0	1.75	1.12
T.17	0.87	1.37	1.0
T.18	0.62	1.5	1.75
T.19	1.12	1.12	1.5
T.20	0.75	1.37	0.75
T.21	0.5	1.37	0.62
T.22	0.87	1.62	0.62
T.23	0.75	1.62	1.0
T.24	0.87	0.87	1.0
T.25	0.37	1.0	1.0
T.26	1.25	1.12	1.0
T.27	1.87	1.25	1.25
T.28	1.25	1.0	1.0
T.29	0.37	1.62	1.0
T.30	1.0	1.0	1.0
T.31	1.0	0.75	0.75
T.32	0.87	1.5	1.25
T.33	0.37	1.37	1.5
T.34	0.37	1.12	1.25
LSD (P < 0.05) of Growth and Sporulation			0.09
LSD (P < 0.05) of Isolates			0.32
LSD (P < 0.05) of Interaction			NS

*Three replicates of each isolate

* *Mean No. of Daily Growth Rate (DGR) was calculated as following (Hafedh, 2001).

Table 2. The Influence of pH on Sporulation of 34 *Trichoderma* Isolates under Laboratory Conditions.

Isolates of *Trichoderma* spp.	Sporulation *Mean No. of Sporulation × 10^8 spore/cm^3		
	PH4	PH6	PH8
T.1	30.08	2050	33.3
T.2	29.08	51.83	28
T.3	82.50	80.42	82.5
T.4	26.33	310.8	28.75
T.5	58.33	60.00	28.42
T.6	30.08	33.30	28.75
T.7	32.26	31.25	27.42
T.8	27.66	125.42	27.75
T.9	27.33	42.50	47.83
T.10	29.16	29.17	28.17
T.11	30.73	41.00	37.66
T.12	27.50	52.50	26.33
T.13	42.58	27.42	27.16
T.14	31.15	115.9	34.83
T.15	32.40	40.83	28.3
T.16	30.83	29.16	29.66
T.17	27.25	157.3	26.08
T.18	26.16	44.16	26.08
T.19	26.60	26.66	33.16
T.20	66.60	29.66	27.42
T.21	39.75	114.23	27.42
T.22	57.50	134.75	27.42
T.23	55.83	83.75	27.75
T.24	00.87	52.50	28.42
T.25	30.42	38.33	31.15
T.26	34.06	70.83	28.00
T.27	54.33	51.66	26.03
T.28	59.17	39.16	40.42
T.29	36.66	51.66	26.08
T.30	26.66	31.25	25.00
T.31	31.75	125.41	28.00
T.32	41.00	50.83	26.08
T.33	27.50	54.16	26.60
T.34	51.00	66.60	27.08
LSD (P < 0.05) of Sporulation			1.73
LSD (P < 0.05) of Isolates			4.1
LSD (P < 0.05) of Interaction			7.15

* Mean No. of three replicates of each isolate.

The effect of the degree of electrical conductivity (ECw) on the growth and sporulation of the 34 *Trichoderma* isolates: The results of the study showed significant differences ($p<0.05$) of the three degrees of ECw, 12.92, 14.30 and 17.8 ds/m on the growth and sporulation of the 34 *Trichoderma* isolates Table 3 and 4. Isolates T.7, T.15, T.18, T.21, and T.28 exhibited optimum growth rate at 12.92, and isolates T.9, T.13, T.18, T.19, T.28 gave optimum growth rate at 14.39, while isolates T.9, T.13, T.18, T.19, T.21, T.28 exhibited optimum growth rate at 17.69 Table 3.

Table 3. The Influence of ECw on Daily Growth of 34 *Trichoderma* Isolates under Laboratory Conditions.

Isolates	*Daily Growth Rate * *Mean No. of Daily Growth (cm)			
	Control	12.92 ds/m NaCl	14.30 ds/m NaCl	17.80 ds/mNaCl
T.1	0.34	0.57	0.61	0.62
T.2	0.41	0.50	0.68	0.73
T.3	0.32	0.35	0.59	0.67
T.4	0.40	0.44	0.61	0.61
T.5	0.36	0.43	0.60	0.62
T.6	0.32	0.35	0.67	0.69
T.7	0.31	0.36	0.78	1.00
T.8	0.40	0.48	0.68	0.75
T.9	0.58	0.69	0.73	0.95
T.10	0.49	0.51	0.68	0.70
T.11	0.38	0.59	0.69	0.73
T.12	0.48	0.57	0.70	0.80
T.13	0.56	0.65	0.72	0.81
T.14	0.52	0.59	0.73	0.83
T.15	0.54	0.62	0.76	0.80
T.16	0.55	0.62	0.66	0.82
T.17	0.37	0.45	0.57	0.58
T.18	0.61	0.67	0.77	0.78
T.19	0.64	0.68	0.72	0.78
T.20	0.41	0.46	0.71	0.76
T.21	0.65	0.71	0.75	0.83
T.22	0.20	0.66	0.73	0.75
T.23	0.39	0.41	0.48	0.78
T.24	0.42	0.71	0.61	0.82
T.25	0.07	0.65	0.70	0.81
T.26	0.22	0.34	0.73	0.86
T.27	0.36	0.47	0.67	0.72
T.28	0.62	0.71	0.77	0.84
T.29	0.49	0.69	0.68	0.82
T.30	0.41	0.47	0.41	0.74
T.31	0.31	0.36	0.72	0.75
T.32	0.37	0.42	0.65	0.69
T.33	0.40	0.63	0.69	0.67
T.34	0.53	0.63	0.73	0.79
LSD (P < 0.05) of Growth and Sporulation				0.009
LSD (P < 0.05) of Isolates				0.027
LSD (P < 0.05) of Interaction				0.054

*Three replicates of each isolate

**Mean No. of Daily Growth Rate (DGR) was calculated as mentioned in Table (1).

The results also showed that isolates T.30 gave the same response growth rate 1 cm at all pH tested Table 3. As for the sporulation the data shows that isolates T.10, T.17, T.20, T.28 exhibited optimum sporulation rate at 12.92, and isolates T.1, T.4, T.8, T.14, T.21, T.22, T.31 showed optimum sporulation rate at 14.39, while isolates T.3, T.9, T.11, T.14, T.19, T.28 exhibited optimum sporulation rate at 17.69 (Table 4).

Table 4. The Influence of ECw on Daily Growth of 34*Trichoderma* Isolates under Laboratory Conditions.

Isolates	Sporulation			
	*Mean No. of Sporulation × 10^8 spore/cm^3			
	Control	12.92 ds/mNaCl	14.30 ds/mNaCl	17.80 ds/mNaCl
T.1	511.33	82.33	30.00	12.50
T.2	122.00	113.00	22.50	21.83
T.3	245.00	51.50	9.50	7.17
T.4	142.33	117.50	29.67	7.00
T.5	450.00	51.33	45.00	14.00
T.6	143.67	27.50	15.00	11.50
T.7	220.50	32.50	25.67	12.50
T.8	160.50	102.00	35.00	13.67
T.9	151.50	62.33	38.67	20.00
T.10	198.33	197.00	40.50	20.00
T.11	622.33	33.67	32.50	27.00
T.12	623.67	30.83	30.00	15.83
T.13	114.00	40.00	32.17	28.50
T.14	222.67	35.00	24.00	23.67
T.15	123.67	75.00	22.67	13.00
T.16	209.00	35.83	28.33	12.33
T.17	571.00	222.33	31.50	27.67
T.18	111.50	98.67	31.00	22.67
T.19	63.33	74.00	27.67	19.67
T.20	640.50	310.83	42.33	28.50
T.21	594.83	38.33	36.33	28.67
T.22	282.33	82.67	24.00	12.00
T.23	322.00	22.50	21.00	18.00
T.24	90.83	65.83	37.67	28.67
T.25	60.50	37.50	25.83	23.33
T.26	144.83	60.00	24.00	20.33
T.27	282.00	42.50	22.33	18.33
T.28	294.50	284.83	31.00	29.00
T.29	345.17	44.67	31.67	12.00
T.30	303.50	42.50	36.50	23.67
T.31	832.33	106.67	34.00	28.00
T.32	615.83	50.50	40.67	15.50
T.33	145.17	92.67	40.67	17.83
T.34	137.33	82.67	33.33	14.00
LSD (P < 0.05) of Growth and Sporulation				0.56
LSD (P < 0.05) of Isolates				1.64
LSD (P < 0.05) of Interaction				3.28

DISCUSSION

The aim of this study was to evaluate the response of 34 *Trichoderma* isolates. Besides, the results of this study explained the variation of the *Trichoderma* isolates performance. The data Table 1 and 2 showed that pH 6 was the best for the growth and sporulation in most of the isolates studied. While the pH 8 showed high reduction of colony diameter growth rate and sporulation, compared to the pH 4 (Table 1). Table 1 and 2 also showed lack of correlation between the growth rates and the ability of sporulation, for example the isolate T.14 Table 1 showed the lowest rate of growth of diameter 0.81 cm when the pH was 6, while the average sporulation 115.9 × 10^8 spores / cm^3 Table 2 was high at the same pH 6. However, the isolate T.15 showed the highest daily growth rate of 1.87 when the pH was 6, but showed sporulation rate of 40.83 ×10^8 spores / cm^3 at the same pH of 6. On the other hand, some isolates such as T.1 Table 1 achieved the highest growth rate of 1.75 cm, and sporulation rate of 205 × 10^8 spores / cm^3 Table 2 at the same pH 6.

The data obtained from the present study agreed with some previous studies which found that pH of soil to had significant antagonistic effect on biological effectiveness of *T. harzianum*, and also indicated about 32% decreased in antagonistic effect of *T. harzianum* when bean seeds was treated with spores suspension at the pH 3.5. However, the antagonism increased to 65 % when the pH was 5.6 % (Marshall, 1982). Hafedh (2001) recorded high decline in the antagonistic ability of some *Trichoderma* isolates when used under field conditions.

The results of the study on the effect of the three degrees of ECw., 12.92, 14.30 and 17.8 s/m on the growth and sporulation of the 34 *Trichoderma* isolates Table 3 and 4 showed that degree of ECw12.92 s/m was the best for the growth and sporulation of most tested isolates, while the degree of ECw17.8 s/m, exhibited significant reduction in daily growth rates and sporulation Table 3 and 4 of most *Trichoderma* isolates as comparison to the degrees 12.92 and 14.30 s/m (Table 3). The results of this study also indicated the lack of correlation between growth rates and the ability of sporulation as denoted in (Table 3 and 4). The isolate T. 25 in Table 3 showed the lowest growth rate of 0.07 cm at the degree of ECw17.8 s/m, and showed high rate of sporulation of 23.33 × 10^8 spores / cm^3 Table 4, while T.19 isolate showed the highest rate of daily growth of 0.64 cm Table 3, and the

rate of sporulation was 19.67 × 10^8 spores / cm^3 Table 4 at that degree of ECw of 17.8 s/m.

Moreover, the present study documented on the low performance of *Trichoderma* spp. under environmental conditions even though this fungus has high capacity to grow in a wide range of soil as well as the biogenic properties of *Trichoderma* species. Furthermore, there are biotic conditions that play important role in reduction of the antimicrobial activity of this fungus (Danielson and Davey, 1973; Prabavathy *et al.,* 2006). On the other hand, the environmental conditions provide other soils suppressive which accentuates the effectiveness of *Trichoderma* spp. antagonism. The weak correlation between the daily growth of colony of each isolate and the ability to sporulation may be referred to the *Trichoderma* isolates behavior when provided the appropriate nutritional conditions leading to reduce sporulation ability of *Trichoderma* fungus. Additionally, the stress conditions encourage sporulation ability of *Trichoderma* isolates and reduce the normal growth rate in order to keep the species survive.

There is paucity of studies on *Trichoderma* isolates and their tolerance to the different concentrations of salt (NaCl). However, there were few studies carried out under field conditions, which indicated that some *Trichoderma* isolates had less activity in alkaline soils (Kumar, 1995). Besides he found that the accumulation of salts in the rhizospher area as a result of irrigation with saline water leads to the changes in the pH soil, as well as to produced large amounts of CO_2 which negatively affected the growth and sporulation of this fungus. Likewise, dissolving of the CO_2 in the water, resulted in the built up of bicarbonates (HCO_3) that played a role in changing the pH in the root zone (Macauley and Griffin, 1969), and all of these combined factors negatively affect the enzymatic activity of *Trichoderma* fungus which is considered one of the important antagonistic mechanisms of this fungus.

CONCLUSION

In this study, effect of pH and ECw on the daily growth rate and sporulation ability of the 34 *Trichoderma* isolates individually under laboratory conditions found that each isolate of *Trichoderma* varied in its inhibitory degree base on each pH levels and ECw degrees. Therefore the results showed that some *Trichoderma* isolates that had good ability to grow on salt media can be used successfully when a suitable formulation is used under field conditions as biocontrol agents.

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