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Effect of Lead acetate on the mycelial growth of some fungi isolated from the soil of Thi- Qar governorate fields – Iraq

تأثير خلات الرصاص على نمو الغزل الفطري لبعض فطريات التربة المعزولة من حقول محافظة ذي قار – العراق

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Abstract

Four fungi were isolated from the rhizosphere of *Viccia faba l*ocated in the fields of Thi-Qar governorate for estimated their variable resistance to toxic Lead acetate at the different concentration(50,100 , 250 , 500) ppm . These fungi were

Aspergillus niger , Rhizopus stolinifer , Trichoderma harzianum and *Fusarium solani* .

The results were showed that the Lead acetate was inhibited the growth of these fungi with all concentrations of Lead acetate on solid media compared with control , but in the broth media the results revealed that Lead acetate act to decrease the dry weight of *R.stolinifer T. harzianum* and *F.solani* at all concentrations except *R. stolinifer* which increase in 250 ppm as compared with control . At the same time the results showed that the dry weight of *A. niger* was increased with all concentrations of Lead acetate addition .

الخلاصة

أختبرت أربع فطريات معزولة من المنطقة المحيطة لجذور نباتات الباقلاء في حقول محافظة ذي قار وذلك لبيان المقاومة المتباينة لسمية خلات الرصاص بتراكيز 50و100و250و500 جزء في المليون وهذه الفطريات هي :

Aspergillus niger , Rhizopus stolinifer , Trichoderma harzianum , Fusarium solani

إذ وجد أن نمو هذه الفطريات قد ثبط على الوسط الصلب المضاف اليه خلات الرصاص ولجميع التراكيز مقارنة بمعاملة السيطرة . كما أشارت النتائج الى ان خلات الرصاص وفي وسط المرق المغذي Broth قد قللت الوزن الجاف للفطريات . *R stolinifer* و *T . harzianum* و *F. solani* ولجميع التراكيز مقارنة بالسيطرة ، ماعدا الفطر *R . stolinifer* فقد أزداد الوزن الجاف له عند التركيز 250جزء في المليون وفي نفس الوقت أظهرت النتائج أن الوزن الجاف للغزل الفطري للفطر *A.niger* قد أنخفض ولجميع التراكيز عند إضافة خلات الرصاص ، كما أظهر هذا الفطر تحمل عال لخلات الرصاص بينما أظهر الفطر *F.solani* تحمل واطئ لخلات الرصاص .

Introduction

Heavy metals are known as harmful pollutants in soil having anegative effect on soil biota including microorganisms . Some heavy metals e.g. Pb even at low concentrations are toxic ,while others such as zinc and copper at low concentrations are essential for micro organisms (1, 2 , 3). Nevertheless , high metal concentration of the latter metals can exert a harmful effect on micro organisms . the microbes response to heavy metals in naturally polluted environments ,the microbes response to heavy metals toxicity depends on the concentration , the availability of metals and on the action of factors such as the type of metal , the nature of medium and microbial species (4) .Lead (Pb) concentration in normal field soil is in the range of 10 to 100 mg kg^{-1} (5) but in contaminated soils especially in near mines or by sewage sludge applications , its concentration as high as 1000 mg kg^{-1} has been reported 6 , 7) . Toxic effect of heavy metals on the growth of fungi isolated from different places in the world have been will described in(8, 3 , 9, 10 ,11,12,13). Some fungi have been reported to grow in soil contaminated with heavy metals and it is

evident that fungi can withstand the levels of metals higher in exces of those tolerated by higher plants (14) .

As there is some information available on the responses of *A.niger , R . stolinifer , T.harzianum* and *F. solani* isolated from some different soil in Thi-Qar governorate – Iraq to the heavy metals , the present study was designed to determine the toxcity of Lead acetate on these fungi above when applied in different concentrations in solid and liquid media .

Materials and Methods

Four microorganisms Viz . *Aspergillus niger ,Rhizopus stolinifer , Trichoderma harzianum* and *Fusarium solani* . were isolated from the rizosphere of *Viccia faba , in vitro* by using pour plate method(15) and identified depend on macroscopic and microscopic characteristics (shape and color of colony and shape of mycelium and conidial heads radiate as well as the taxonomic references were (16,17,18,19,) .

Lead acetate was added to the agar media of Potato Dextrose Agar (P.D.A) while still hot, aseptically poured in sterile 9 cm diameter Petri dishes . The final Lead acetate concentration would be 50 , 100 , 250 , 500 ppm . 4mm diameter disc from the perfery of 7 day old culture of each of the four fungi were centrally inoculated on the agar media and daily measurements the colony diameter were carried out after incubation5 days at 25 ^{o}C in light and stoped the experiment when the colony of control reached to 9cm in petri dishes. This was performed on 3 replicate dishes for each Lead a cetate concentration as well as controls .

Similar experiments were carried out using 50 ml aliquots of liquid media(Trace salts solution) containing the same concentrations of the Lead a cetate in order to obtain the dry weight of the produced mycelial felts at 7 day . Flasks were incubated at 25^{o}C and after 7 days the mycelial felts were washed with distilled

water and dried in a continous hot air at 120^{o}C for 30 min. and then at 80^{0}C until constant weight . Three replicate samples were used in each treatment . The liquid medium has the following constituents : $MgSO_4$. $7H_2O$: 0.1g , $ZnSO_4$.$7H_2O$: 0.1 g,

$MnCl_2$. $4H_2O$: 0.1 g , $FeSO_4$. $7H_2O$: 0.1 g . all these components dissolved in 1 liter distilled water and the determination of the index of tolerance . The index is defined as the ratio of the mycelium dry weight of the treated medium to that of the untrea-ted medium.

Statistical analysis

The present study conducted an Anova (analysis of variance) which was performed on all the treatments and done using the SPSS (version 10.0) package to determine whether or not , a significance difference .

Results and Discussion

1- **Daily Linear Growth Rate** : -

Fig (1) shows that during treatments of Lead acetate , the growth of *Aspergillus niger , Rhizopus stolinifer , Trichederma harzianum* and *Fusarium solani* started to decrease on solid media with the added Lead acteate 50, 100 , 250 , 500 ppm concentration when comparison with control . The inhibition percentage of *T.harzianum* reached to (68.8 %) with Lead acetate in 50ppm concentration , but the inhibition percentage was (87.7 % , 100 % , 100 %) in 100 , 250 , 500ppm concentration during the 5 day Table (1) .At the same time , Table (1) shows that the inhibition percentage of *F. solani* reached to (56.6 % , 78.8 % , 84.4 % , 100 %) with Lead acetate in 50 , 100 , 250 , 500ppm respectively . As well as , Table (1) shows that the inhibition percentage of *R .stolinifer* reached to (23.3%,75%,78. 8 % , 100 %) with Lead acetate in 50,100, 250, 500ppm respectively , but the inhibition percentage of *A .niger* reached to (46.6%,56.6 % ,83.3%, 73.3 %) with Lead acetate in 50 , 100,250,500ppm respectively. The statistical methods showed highly significant differences between the concentrations of Lead acetate and very high significant differences between these fungi and Lead acetate concentrations

and during the period, Table (2).These results were similar to results reported by (20, 21) when found that Cadium chloride and Copper sulphate were more toxic in solid media ,and the same time (22) found that inorganic Tin in solid media can be more toxic than inorganic Tin in liquid medium. (23) suggested that the toxicity of a heavy metal pollutant depend , in part , on the anionic composition of the environment in to which the pollutant is deposited . In assessing the toxicity of pollutants to the biota , a ttention must therefore be focused on the specific physiochemical a biotic factors of the recipient environment , which my mediate or potentiate the toxicity of the environment (23) .

Toxic effect of metals include blocking of functional groups of biologically important molecules , the displacement and substitution of essiential metal ions from biomolecules,con formation, modification, denaturation and inactivation of enzymes and disruption of cellular and organellar membrane integrity(24,25).Similar results were reported by(26) who found that Cadium at a conce ntration of 1mM showed the strongest inhibition towards isolated from the genera *Aspergillus , Fusarium , Alternaria* and *Geotrcihum* , only *Penicillium* isolates were able to grow . (27) reported that the differences in resistance levels were probably due to the potential variation in the mechanism of resistance. At the same time (26) reported that the variation in the metal tolerance may be due to the presence of different types of tolerance processes or resistance mechanisms exihibited by different isolates. In the present study the results were similar with (28) who found that *A .niger* was resistant a gainst Pb when cultured in SDA(Sabouraud dextrose agar) amended with this heavy metal . At the same time(10) reported that Cobalt and Lead inhibited the growth of *Aspergillus candidus* , *Penicillium notatum , Ulocladium atrum* at 3000µg /ml , but (14) reported that the growth of *A .niger* isolateds on agar media containing a high Lead concentration was accompanied with the removel of white colouration of PDA medium around the colony .

2- **Mycelial Dry Weight :-**

Fig (2) shows that the dry weight of mycellium of *A .niger* were dcreased with all concentrations to Lead a cetate in liquid media , but the dry weight of

mycelium of *R .stolinifer* were increased with Lead acetate in 250ppm concen-tration only and decreased with 50 , 100 , 500 ppm concentration . At the same time , Fig (2) shows that the dry weight of mycelium of *T. harzianum* and *F.solani* were decreased with all concentrations of Lead acetate in liquid medium. Table (3) shows that highly significant differences between these fungi .

The observed tolerance or sensitivity of these fungi does not apper to inherent to the organisms but may also be due to other facters prevailing such as $_{pH}$ and ionic strength in medium . However, the toxicity of a metal depends on the physiochemical characteristics of the environment were it is deposited (29) . A biotic factors such

as $_{pH}$, temperature , pressure , and ionic strength affect the a bility of metals to complex with various ligands (30 , 31) . Although the effect of heavy metals on

the growth of soil fungi was generally toxic, but some fungi appeared to benefit from some treatments(20;21).Similar results were reported by(32) who found that the higher concentration of Cobalt and Lead inhibition growth of *Fusarium moniliform* and *Ulochladium alrum*(3000µg / ml) , but (4) reported that *Aspergillus niger* and *Penicillium chrysogenum* could survive in a liquid media containing up to 500µg/ml Cu .

In the present study the results were similar with results reported by (12) when found that *Aspergillus* species isolated from different soils in Saudi Arabia can grow in media containing higher concentration of Cd , Co , Pb and Zn and with results reported by (6,7) when found that *A. niger , R . stolinifer , T . harzianum* and *F . solani* were decreased with Cadium Chloride in 100 , 250 ppm concentration and the dry weight of mycelium of *F . solani* inhibited with 50 , 100 ppm concentration of Copper sulphate . In the present study the survival of *A . niger* at higher concentration of Lead acteate was due to an adaptation of the test fungus to this new habitate and some studies have shown that fungi accumulate

heavy metals from dilute background concentrations (11 , 33, 34, 35). It is a property which could be utilized to monitor heavy metals pollution (26) . Table (4) showes that *A. niger* has a higher tolerance of Lead acetate , this result were similar with results reported by (14) who found that the *Aspergillus* and *Penicillum* isolates were the most resistant to the Pb , Cr , Cu and Zn tested .

Conclusions :

The data obtained in the present investigation a dvance our knowledge of Lead resistance in *Aspergillus niger* isolated from Iraqi soils and may make this promising candidates for further investigations regarding their ability to remove metals from contaminated environments .

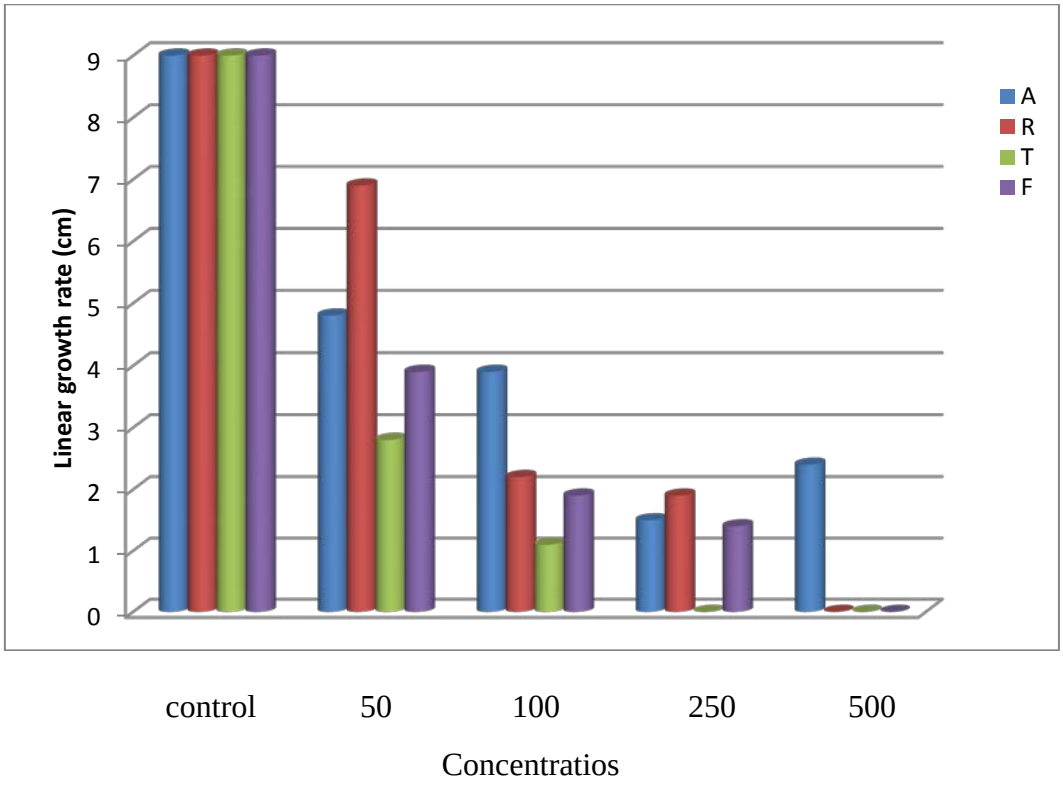

Fig . 1. Effect of lead acetate on growth of fungi : Aspergillus niger Rhizopus stolinifer , Trichoderma harzianum and Fusarium solani in solid media.

A : Aspergillus niger , R : Rhizopus stolinifer
T : Trichoderma harzianum , F : Fusarium solani

Table .1 Inhibition percentage of isolated fungi with different concentrations of lead acetate in solid media after 5 days from treatment .

Isolated fungi	Inhibition Percentage (%)				Mean of colony diameter
	Concentrations				
	50	100	250	500	
A.niger	46.6	56.6	83.3	73.3	9.0
R.stolinifer	23.3	75.5	78.8	100	9.0
T.harzianum	68.8	87.7	100	100	9.0
F.solani	56.6	78.8	84.4	100	9.0

Table .2 Analysis of variance to compare the growth of fungi in solid media at different concentrations of Lead acetate .

Isolated fungi	concentratios	Mean	Std. Deviation	Numbers
A.niger	0.0	4.7250	3.1383 *	4
	50	2.6000	2.0331	4
	100	2.2750	1.6860	4
	250	1.3250	0.1708	4
	500	1.7000	0.5715	4
	Total	2.5250	2.0476	20
R.stolinifer	0.0	6.0750	3.1983	4
	50	4.1750	2.9792	4
	100	1.3250	0.9430	4
	250	0.4750	0.9500	4
	500	0.0000	0.0000	4
	Total	2.4100	3.0070	20
T.harzianum	0.0	2.9250	4.1096	4
	50	0.7000	1.4000	4
	100	0.2750	0.5500	4
	250	0.0000	0.0000	4
	500	0.0000	0.0000	4
	Total	0.7800	2.0746	20
F.solani	0.0	4.0500	3.8596	4
	50	1.7500	2.0469	4
	100	0.4750	0.9500	4
	250	0.4500	0.6608	4
	500	0.0000	0.0000	4
	Total	1.3450	2.3471	20
Total	0.0	4.4438	3.4293	16
	50	2.3063	2.3572	16
	100	1.0875	1.2863	16
	250	0.5625	0.7201	16
	500	0.4250	0.8021	16
	Total	1.7650	2.4667	80

* siginificant $p < 0.05$

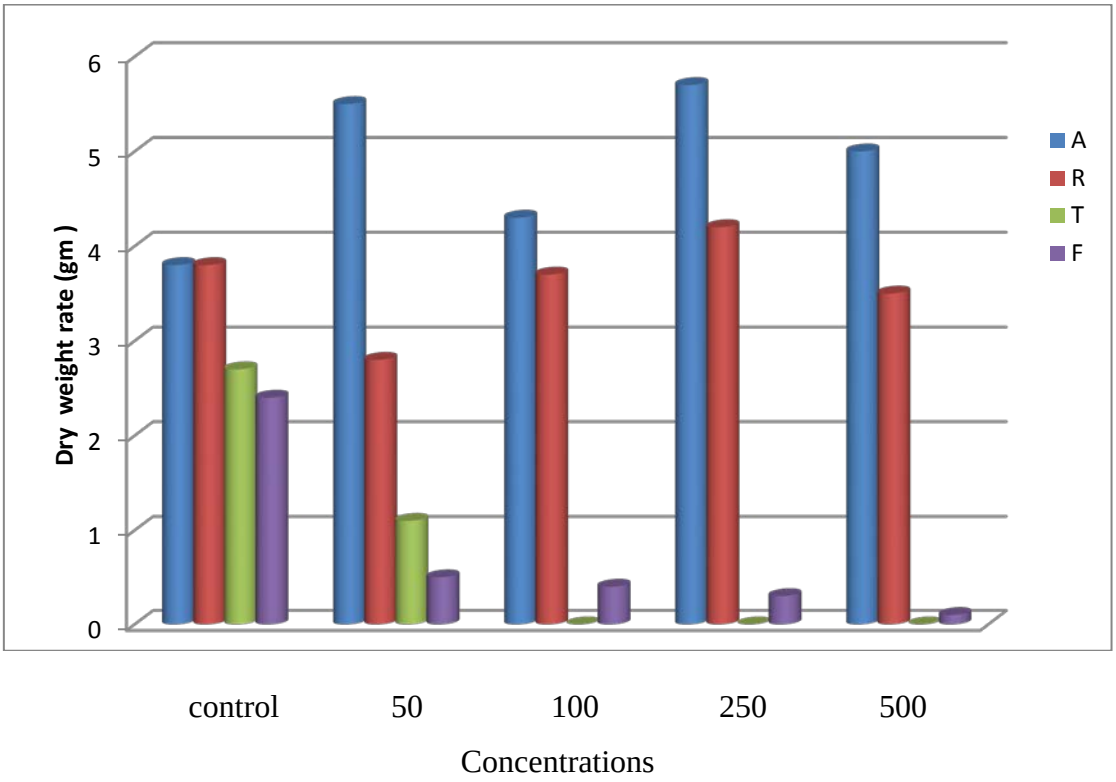

Fig.2.Effect of Lead acetate on mycelial dry weight to fungi : Aspergillus niger Rhizopus stolinifer,Trichoderma harzianum and Fusarium solani in the liquid media in 7th day after treatment .

A : Aspergillus niger , R : Rhizopus stolinifer
T : Trichoderma harzianum , F : Fusarium solani

Table .3 Analysis of variance to compare the mycelial dry weight of fungi in liquid media in the 7th day after treatment with different concentrations of Lead a cetate .

Isolated fungi	concentrations	Mean	Std . Deviation	Numbers
A.niger	0.0	3.8000	0.6986 *	3
	50	5.5933	0.5361	3
	100	4.3600	0.6528	3
	250	5.7100	1.0573	3
	500	5.0500	2.1616	3
	Total	4.9027	1.2526	15
R.stolinifer	0.0	3.8000	0.3606	3
	50	2.8833	0.8401	3
	100	3.7000	0.3606	3
	250	4.2667	5.774E-02	3
	500	3.5333	0.9292	3
	Total	3.6367	0.6909	15
T.harzianum	0.0	2.7333	0.6429	3
	50	1.667	0.2082	3
	100	0.0000	0.0000	3
	250	0.0000	0.0000	3
	500	0.0000	0.0000	3
	Total	0.7800	1.1428	15
F.solani	0.0	2.4667	0.4041	3

	50	0.5667	0.1528	3
	100	0.4333	0.1528	3
	250	0.3667	0.1528	3
	500	0.1667	5.774E-02	3
	Total	0.8000	0.8920	15
Total	0.0	3.2000	0.7872	12
	50	2.5525	2.0841	12
	100	2.1233	2.0387	12
	250	2.5858	2.6091	12
	500	2.1875	2.4808	12
	Total	2.5298	2.0655	60

* significant $p < 0.05$

Table . 4 . Tolerance of mycelial dry weight (gm) with different concentrations of Lead acetate after 7^{th} day growth of isolated fungi .

Concentratios	Isolated fungi			
	A.niger	R.stolinifer	T.harzianum	F.solani
50	+	+	+	+
100	+	-	-	-
250	+	+	-	-
500	+	-	-	-

(+) Tolerance .
(-) Non tolerance .

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