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Research Paper

Effect of Heavy Metal Ions on the Growth, Sporulation and Pathogenicty of *Isaria Javanica* = (*Paecilomyces Javanicus*)

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Abstract: *The effect of heavy metal ions (Cd, Cu, Fe, Pb, and Zn) on the growth and sporulation of the entomopathogen fungi Isaria javanica (Synon. Paecilomyces javanicus) was conducted. The metal ions were added into culture media in three concentrations: A- Concentration corresponding to the mean content of that metal in soils of Duhok, Northern of Iraq, B- Concentration 10 – times higher and C- 100 times higher than the mean ones. Investigated metal ions, added to the media at low concentration, didn't affect the growth of entomogenous fungus which was unable to grow on the media containing Cu, Cd, and Zn at the highest (C) concentration. Moreover, Cu and Zn were irestricted fungal growth even at 10 times higher. The ions of Pb , and Fe were also considerably reduced fungal colonies at the highest concentration C. I. Javanica was virulent when resulted a high mortality of two aphid species , but the fungal spores were unable to detect in the media with the presence of Cd , Zn , and Pb at the highest C concentrations , whereas , Fe inhibited fungal sporulation at (A, B, and C) concentrations . Thus, strongly polluted soils with heavy metal ions could be disturb the detection of growth and conidiation of entomogenous fungi.*

Keywords: *Isaria javanica* = (*Paecilomyces javanicus*), heavy metal ions.

Introduction:

Pollutants permitted to the biosphere including heavy metals due to industrial and agricultural, and domestic activities has created a serious problem for the safe utilizes of sod soils (Igwe et al., 2005).

The environment suffers intoxication with industrial products and agronomic application of fertilizers, pesticides, crop preservatives, and metal contaminated sewage contributing to the unusual fluctuations in chemical composition of the soil (Herland et al., 2002). Several experiments demonstrated that ions of heavy metals may prevent or at least restrict the biomass increment or enzymatic activity of some entomopathogenic Hyphomycetales like *Beauveria bassiana, Paecilomyces fumosoroseus* (Jaworska et al., 1996; Tkaczuk, 2003). *P. farinosus,* or *Lacanicillium lecanii* (= *Verticillium lecanii*) (Ropek and Para, 2002).

Worse, the pollution of the ecosystem by heavy metals can't be naturally degraded like organic pollutants and persist or accumulate in different parts of the food chain (Smejkalova et al., 2003).

However, metal toxicity may affect all forms of microorganisms, plants, and animals with different degree. Several literatures proved that heavy metal contamination can cause shifts in microbial populations (Doelman et al., 1994; Roane and Kellogg, 1996).

The toxicity of heavy metals to soil organisms is moderated by metal immobilization by soil colloidal components i.e. trapped as complexions or conversion into insoluble forms. Furthermore, these complexions could be used to protect certain soil microorganisms and soil environment (Ropek and Para, 2003), but may be mobilized by local and global changes in soil conditions i.e. Changes in physical and chemical properties of soil environment, including decrease in pH, potential and enhanced decomposition of organic matter (Hattori, 1996; Kelly et al., 2003).

Entomopathogenic Hyphomycetes fungi commonly reside in Iraq soils, they have found their natural roles in plant protection from most important economic insects (Hassan et al., 2012). However, their pathogenicity is controlled by such factors as temperature, humidity, and activities of antagonists (Farguers et al., 1992). Very little is known about effects of xenobiotics on these fungi, and some heavy metals have atoxic effects, whereas others don't influence them or even stimulate their activity (Bajan et al., 1998) .

This work aimed to determine the influence of five metal ions Zn, Cu, Cd, Fe, and Pb found in the soil samples of Iraq northern on the growth, sporulation and pathogenicity of *Isaria javanica* (Friedrichs & Bally) Samson & Hywel – Jones).

Materials and Methods:

I. javanica pathoigenicity was tested on twenty adults of each two aphid species = *Haylopterus pruni* (Geoff) and *Aphis pomi* (De Greer). This trial was performed by cultivation fungal isolate on PDA plates for 10 days . Sterile water (5 ml.) was powered on each plate containing fungal colony to obtain spore suspension adjusted at 1×10^{8} conidia / ml.

Tested insects were sprayed with 10 ml of spore suspension for 10 seconds and then transferred in 9 cm petri dish with moistened filter paper. Plates sealed with par film to maintain the humidity and then incubated in darkness at 25^{0} C. Infected dead insects were inspected and counted daily. The mean of mortality for both aphid species was calculated after 6 days of infection.

Effect of Metal Ions on the Fungal Growth and Sporulation:

Metals used in this study included, Zinc chloride $ZnCl_2$, lead nitrate Pb $(NO_3)_2$, Cupric chloride $CuCl_2.H_2O$, Iron sulfate $FeSO_4$ $.7H_2O$ and Cadmium bromated ($Cd\ Br_2.\ 4H_2O$). Thirteen samples of natural forest and agricultural soil were collected around Gara mountain, Duhok, Northern of Iraq. Physical and chemical properties of the soil texture, % soil separates, pH, Ec. and % carbonates were determined using the methods described by Gupta (2004).

Heavy metal salts were added separately to the PDA medium and autoclaved (121^0C) for 15 min.

The metal ions were used at three concentrations:

A- Concentration corresponding to the mean content of that metals in Duhok soils with the following quantities: Zn 3.3mg/L., Pb 2.5 mg /L. , Cu 2.0 mg/L., Cd 2 mg/ L.and Fe 2.75 mg /L.
B- Concentration 10 times higher and
C- 100 times higher than mean ones.

Sterilized medium was applied to petri plates and inoculated in their centers with *I. javanica* using 4mm of the fungal plugs**.**

The dishes with fungus growing on a medium devoid of heavy ion metals provided the control. The plates were incubated at 25 ^{0} C and the colony diameter was measured after 5, 10, 15, and 20 days. The fungal spores was collected after 4 weeks with a scalpel and placed in a test tube with 10 ml of distilled water. Spore concentrations in the initial suspension were counted using Haemocytometer for each treatment. The experiment was replicated four times in Completely Randomized Design CRD. Analysis of variance ANOVA and Duncan test were used to determine the statistical differences between means of treatments at $P \leq 0.05$ with SAS software (SAS, 1999).

Results and Discussion:

Isaria javanica (Frieder & Bally) Samson & Hywel-Jones. Mycol. Res. 109, 588 (2005) formerly known as *Paecilomyces javanicus* (Frider & Bally) A. H. S. Brown & Smith was the first record from Iraq as an entomopathogenic fungus during 2008 - 2012 (Hassan et al., 2012).

The soil samples collected was sandy loam, clay, and silt clay in texture with 0.55 – 24.62 % of $Caco_3$, pH 6.01-7.52, and Ec. Of 0.2 – 1.2 (Table 1).

Table (1): Crop vegetation and physio-chemical properties of the examined soil samples

Sample No.	**% Soil Separates**			**Crop-vegetation**	**Texture**	**pH**	**$CaCo_3$**	**Ec ds.m^{-1}**
	Clay	Sand	Silt					
1	13.9	73.9	12.2	Natural forests	Sandy loam	7.52	22.93	0.31
2	66.1	14.9	19	=	Clay	6.58	2.5	0.31
3	41.1	30.9	28	=	Clay	6.01	2.04	1.20
4	61.1	18.4	20.5	=	Clay	6.55	1.33	1.01
5	71.1	14.9	14	Vegetables	Clay	6.91	2.51	0.71
6	63.6	21.9	14.5	=	Clay	6.70	1.8	0.68
7	58.6	23.4	18	=	Clay	6.75	2.55	0.41
8	50.1	29.9	20	=	Clay	6.97	0.55	0.47
9	36.1	16	47.9	Oka shrubs	Silt-clay loam	7.15	24.62	0.22
10	54	18.5	27.5	=	Clay	6.95	1.5	0.26
11	54	8.5	37.5	=	Clay	7.41	11.51	0.20
12	51.5	11	37.5	Grapevines	Clay	7.22	10.21	0.20
13	59	13.5	27.5	=	Clay	7.12	4.43	0.20

The fungus displayed low sensitivity to metal ions present in the medium at the concentration A (Table 2). The low toxic effect of Cu, Cd, Pb and Zn ions, added to the medium in the above – mentioned concentrations on the growth of *Beauveria brassiana, Paecilomyces fumosoroseus* was reported by Tkaczuk et al., 2003; Tkaczuk, 2005).

However, some heavy metal ions such as lead or Zinc could stimulate the growth and fungal biomass (Bajan et al., 1998). Results represented that the fungus was unable to grow on the media containing Cu, Zn, and Cd at the highest (C) concentration. In deed Cu and Zn strongly inhibited fungal growth even at 10 times higher level of individual metals in the soil. Apparent toxic effects of Cu, Cr, and Zn on the growth of *P. fumosoroseus* was published (Jaworska et al. , 1996) .In the meamtime , *Verticillium lecanii* was found by (Ropek and Para, 2002) that didn't grow on media contaminated with more than 400 mg Cu / L among the other tested metal ions including Cd , Ni , Pb, or Zn . Metals of Cu and Zn present in the medium at 100 – times higher concentration confirm stongly inhibited mycelial growth of *B. bassiana , Metarhizium anisopliae , M. flavoviride* (Tkacznk et al. , 1998), but didn't affect their growth at 10 times higher concentration . This revealed that *I. javanica* seems to be more sensitive to the Cu and Zn ions.

Similarity, metal ions of Cd, Pb, and Fe added at the highest level considerably reduced the size of fungal colonies (Table 2).On the contrary the same ions didn't limit fungal growth significantly when used at 10 times higher than the mean ones.

It is known that entomopathogenic fungi are able to accumulate or tolerate significant amounts of metals (Gaad, 1992); (Trevors et al., 1986), since the composition of fungal cell walls of polysaccharides, proteins and lipids (Farkas, 1985) which contain functional groups with potential metal complexity capacities. Moreover, the resistance or tolerance of entomopathogenic fungi to soil contents of heavy metals, e.g. *B. bassiana, M. anisopliae and P. farinosus* (Nordgren et al., 1985) attributed to their increased accumulation of high metal contents in their fruiting bodies and mycelium that serve to survival in the contaminated habitats (Popowska – Nowak, 2004).

Table (2): Effect of heavy metal ions at different concentrations on the radial mycelial growth of *I. javanica*

Metal ions	Conc.	Colonies growth (mm) of fungus after 5- 20 days			
		5	10	15	20
Cd	A	6.3 a	18.4 a	40.5 a	53.8 a
	B	5.6 b	12.4 bc	33.4 b	53.1 a
	C	0 d	0 d	0 d	0 d
Cu	A	6.0 a	17.3 ab	37.4 a	53.4 a
	B	2.4 c	4.8 cd	10.3 cd	14.0 cd
	C	0 d	0 d	0 d	0 d
Fe	A	4.5 b	12.8 b	33.0 ab	56.4 a
	B	7.3 a	16.0 ab	37.9 a	51.7 ab
	C	5.0 b	7.2 cd	16.6 cd	39.0 c
Pb	A	6 a	16.2 ab	38.0 a	53.0 a
	B	5.6 a	15.3 ab	38.2 a	52.6 a
	C	4.2 b	9.6 c	18.8 c	29.8 c
Zn	A	6.8 a	13.7 b	37.8 a	52.2 a
	B	4.9 b	11.4 c	19.0 c	31.1 c
	C	0 d	0 d	0 d	0 d
Cont.		6.4	18.8	39.0 a	57.6 a

Means followed by the same letters in each column are n't significant ($P \leq 0.05$)

Effects on Heavy Metal Ions on the Fungal Sporulation:

Not all examined ions limited sporulation of *I. javanica*. The fungal spores were unable to detect density in the presence of Cd, Zn, Fe and Pb at the highest C concentration of these metals in the

media. Cd also prevented or inhibited the conidiation of the entomogenous fungus at the A and B concentrations (Table 3).

References documented that the ions of Al, Cd, Cr, Cu, Li, Ni, Pb, and Zn added to the medium in the form of nitrates at 100 time's higher concentration, completely inhibiting the spore germination of *P. fumosoroseus*. Similar results were obtained by strongly limited spore germination of *Paecilomyces* spp. , even at 10 times concentration of the mean ones and afew spores detected in the media contained each of Cu at 10 times and Zn at 100 times concentration of those metals (Tkaczuk , 2003).

A wide spectrum of potentially toxic interactions between metals and fungi in their metabolism, growth, and sporulation may change depending on the fungal species, metal concentration and soil properties (Tobin et al., 1994; Babich and Stotzky, 1977). However, studies under laboratory conditions have shown that some heavy metals, especially ahigh concentration can considerably limit growth and pathogenicity of entomogenous fungi (Jaworska and Gorczyca, 2004).

I. javanica caused a total mortality of two aphid species *(H. pruni*, and *A. pomi* within 6 days when sprayed with spore suspension (Table 3). Its pathogencity was most limited by the Cd ion. The results implied that the Cu, Pb, Cd were the most toxic for the investigated *I. javanica*, the Cu ions which had very strong toxic effect on the mycelia growth of fungus influenced neither sporulation nor pathogenicity when used at lower A concentration. Therefore, we conclude that strong pollution of soil by some heavy metals could be restrictive and crucial factor of development and pathogenicity of entomopathogenic fungi in the environment.

Table (3): Effect of heavy metal ions on the sporulation and pathohenicity of *I. javanica*

Metal ions	**Conc.**	**Sporulation 1×10^6 conidia / ml**	**% aphids mortality ***
Cd	A	7.7 b **	30 c
	B	6.2 b	50 b
	C	2.7 c	60 b
Cu	A	8.9 a	70 b
	B	8. 32 ab	100 a
	C	8.0 ab	100 a
Fe	A	3.37 c	90 a
	B	1.15 c	100 a
	C	2.23 c	100 a
Pb	A	9.2 a	80 a
	B	10.0 a	100 a
	C	0.37 c	100 a
Zn	A	6.42 b	100 a
	B	6.25 b	100 a
	C	1.25 c	100 a
Cont.		6.67 b	100

*Average of mortality for two aphid species.
**Means followed by the same letters in each column are n't significant ($P \leq 0.05$)

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