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RESEARCH ARTICLE

ASSESSMENT OF AFLATOXINS PRODUCED BY CERTAIN ASPERGILLA IN HEAVY METALS CONTAMINATED SOIL TREATED WITH COMMERCIAL CHITOSAN

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ABSTRACT

Heavy metals and aflatoxins contaminated crops are considered a big problem threatening animals and humans. The relationships between heavy metal mixture in contaminated soil and certain fungi produced aflatoxins are studied. The role of commercial chitosan as bi-functional agent was studied too. Chitosan powder 1% realized high heavy metals adsorbance efficacies in both soil and reduces its availability in obtained plants. This study focuses also on developing an effective technology for treatment of aflatoxins and available heavy metals-contaminated soil. Our results indicated that added chitosan1% to seed beads area increased the percentage of germinated seeds, minimized the availability of Cu, Zn, Mn, Ni ,Fe and Al in produced rocket plants. Chitosan significantly reduced aflatoxinG_1 in both tested soils. Meanwhile, the aflatoxin B_1 inhibition was significantly exhibited only in case of sterilized sandy soil. On the other hand, aflatoxin B_1 and G_1were significantly inhibited in plants. The competition between soil flora and the two tested fungi don't affect the chitosan's efficacy.

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INTRODUCTION

Many countries around the world suffer from water shortage problem. Egypt is not able to meet increasing water demand using fresh water from the Nile and has been developing treated waste water (TWW) reuse strategies to increase water availability to meet future demands. The daily volume of available treated wastewater (TWW) in Egypt is expected to increase from 6.3 million m^3 in 2000 to 8.3 million m^3 by 2018 Ayar, (2010) and (USDA) Agricultural Research Service, (2013). Unfortunately, all small-scale farmers in peri –urban areas like Abou rawash region often irrigate corn, fruits, legumes and any edible crops with marginal-quality water, (waste-water**)** because they have no alternative.) Long-term irrigation with Waste water convert sandy soil to contaminated yellow soil which contains a variety of pollutants such as heavy metals, metalloids, pathogens and organic compounds. Any of these components can harm human health and the environment. A lot of researches focused on the harmful effects of heavy metals in the irrigated wastewater on human and animal health (Arora *et al.,* 2008; Githongo, 2010).

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Rather researchers studied heavy metal effects on aflatoxins production in *vitro*. Heavy metal contaminated crops are considered a big problem threatening human (Draghici *et al.,* 2010 and Manju Mahurpawar**,** 2015), animal (Vieira *et al.,* 2011) and plant health (Duxbury *et al.,* 2003). Metal ions(e.g., Zn2+, Cu2+, and Fe2+) enhanced fungal growth and aflatoxin synthesis in *Aspergillus flavus* (Cuero *et al*, 1987 and 2003 and Hartikainen *et al.,* 2012).They found also a relationship between mineral composition (e.g. zinc) of soils and the presence of toxigenic fungi and aflatoxin in maize kernels on plants grown in those soils. Aziz *et al.,* (2000) mentioned that Zn2 (from100 to 300 mg/kg) increased the growth of *A. flavus* and the increase in Cu2 or Fe2 concentration numerically decreased the growth of *A. flavus* (Yuyong *et al.,* 2016). Effect of nickel and aluminium was studied on aflatoxin and lipid production by two strains of *Aspergillus flavus* in a sucrose-asparagine-salts medium. Inclusion of aluminium in the medium established an inverse relationship between aflatoxin and lipid production (Malini *et al.,* 1984). Chitosan is a natural hydrophilic biopolymer derived from chitin, a structural component of fungi, insects and shrimp, which exerts antimicrobial effects against bacteria and fungi (Rabea *et al.,* 2003 and Alburquenque *et al*.,2010) this compound can be easily obtained by N-deacetylation of chitin. Chitosan molecules were shown to inhibit fungal growth. They were reported also to be active against viruses, bacteria and other pests. Chitosan also removes phosphorus, heavy minerals, and

oils from the water. (El Hadrami *et al.,* 2010). Kamari *et al.,* 2011 investigated the potential of chitosan, a fishery waste-based material, as a soil amendment to clean-up metal contaminated Soil. FTIR analysis confirmed that N and O atoms served as binding sites for the metal ions. Chitosanand treated chitosans were able to bind metal ions, even in the presence of K+, Cl− and NO3−, which are dominant ions in soil. Therefore, They mentioned that remediation of metal contaminated soil using chitosan and cross-linked treated chitosans as soil amendments is feasible. The aim of this study is to investigate the effect of heavy metal mixture (which naturally occurred in abou rawash soil) on aflatoxin production and its bioavailability to plant and consequently to human. This study revealed also to investigate the effect of commercial chitosan on bioavailability of heavy metals and aflatoxins in contaminated soil. Furthermore, this work revealed to study the role of heavy metals on certain fungi produced mycotoxins during occurrence and absence of natural flora in soil. This study aimed also to investigate the role of commercial chitosan on aflatoxins detoxification and heavy metal adsorbance to prevent or at least to minimize these contaminants to reach food chain during the absence and existance of natural flora of the both tested soils on aflatoxins production and on the effectiveness of chitosan remediation process under field conditions.

MATERIALS AND METHODS

Materials used during this study

- Rocket seeds bring from Agriculture researches Center (Ghiza-Cairo).
- The tested soils: Sandy and contaminated soil (from region abu Rawash).
- Fresh Shrimp shells.

- **Fungal isolates:** *Aspergillus flavus* and *Aspergillus parasiticus* previously isolated from sewage water (bring from dekhila station) with hair bait analysis technique according to Shtayeh and Jamous, (2000) and (Ulfig, 2003), then purified and identified according to Raper and Fennel 1965 and finely tested for aflatoxin production capability using plug agar method according to Abbas *et al.,* 2004).then aflatoxin was re-determined using HPLC (Mahoney and. Molyneux, 2010). Both isolates are aflatoxin producers.
- **Preparation of sterile soils:** Plots containing tested soils (500g) each were sterilized using 1% formalin according to (Thulin *et al.,* 1958) and the pots are left covered for one month until formalin odor was completely evaporated.

Preparation of commercial chitosan

Extraction of chitin and chitosan: The chitosan preparation process was carried out according to Shah *et al.,* 2011 and Younes and Rinaudo, 2015, the chitin and chitosan sequence involves washing of crushed shrimps shells (5kg) were placed in 2000 ml beakers and soaked in boiling sodium hydroxide (2 and 4% w/v) for one hour in order to dissolve the proteins and sugars thus isolating the crude chitin. 4% NaOH is used for chitin preparation (Lertsutthiwong *et al.,* 2002 and Abdou *et al*.,2008). After the samples are boiled in the sodium hydroxide, the beakers containing shrimp shell samples are removed from the hot plate, and allowed to cool for 30 minutes at room temperature (Lamarque, 2005). Then they are further crushed to pieces of 0.5-5.0 mm using a meat tenderizer.

Demineralization: The grounded shrimp shells is demineralized using 1% HCl with four times its quantity. The samples were allowed to soak for 24 h to remove the minerals (mainly calcium carbonate) (Trung, 2006). The demineralized shrimp shell samples were then treated for one hour with 50 ml of a 2% NaOH solution to decompose the albumen into water soluble amino-acids. The remaining chitin is washed with deionized water, which is then drained off. The chitin was further converted into chitosan by the process of deacetylation (Huang, 2004).

Deacetylation: The deacetylation process is carried out by adding 50% NaOH and then boiled at 100°C for 2 h on a hot plate. The samples are then placed under the hood and cooled for 30 min at room temperature. After-wards the samples are washed continuously with the 50% NaOH and filtered in order to retain the solid matter, which is the chitosan. The samples were then left uncovered and oven dried at 110°C for 6 h. The chitosan obtained will be in a creamy-white form (Muzzarelli and Rochetti, 1985).

Effect of chitosan on germination process: 20g of rocket Seeds (Arugula) or (*Eruca Sativa*) were implanted on cotton cushion and dampened with distilled water and added to sterile de-ionized water 10 ml and chitosan powder 1% for 7days under room temperature. The chitosan had been previously ground to a fine powder in an electrical mill. Seeds implanted on cotton pad with chitosan powder1% and other implanted on non treated pad are used as controls (Ohta *et al.,* 1999). After one week, germinated seed were counted then germination ratio was calculated, shoot and root lengths are registered to tested the chitosan effects on germinated seeds growth rate as illustrated in table n°= (1a&b).

Determination of available heavy metals in Abou rawash (sample of contaminated soil) and Sandy soil: The available heavy metal in Abou rawash as a sample of contaminated soil and sandy soil as a non contaminated soil were determined in DTPA extract for both soils using flame atomic absorption spectrometry (contrAA 300) according to Lindsay and Norvell, 1978 . The resulted data are registered as shown in table n°=2).

Estimation of aflatoxins in both soil samples: Aflatoxins were estimated in both soil samples (20g) each according to Abbas *et al.,* 2004. Ethanol and water 70:30 were add to soil after blending , mixture was filtered then aflatoxin was determined in the supernatant containing fungal spores and hyphae. Results were registered.

Effect of chitosan on heavy metals concentration in produced fresh plant: For the soil treatment, 1.0% chitosan (by weight of rhizosphere area (200g/kg) was mixed with a sterile contaminated soil and /or sandy soil and two replicates were used for each treatment. The same process was repeated with non sterilized soils. The pots were placed at 25 °C day/18 °C night in natural daylight in a greenhouse. Starting 2 weeks after germination, the seedlings were watered daily the seedlings were thinned to two per plot at the two-pair leaf

stage. Rocket plants were collected after one month of the last mentioned stage. Plants are purified gently with sterilized water, weighted then divided into two groups and kept freeze .Heavy metals are estimated in the first group of plants collected from each treatment according to Issac *et al*.,1975 . Data were registered as explained by table n°=3.

Effect of chitosan on aflatoxins concentration in produced fresh plant: The second group of plants was been allocated for aflatoxins estimation. The results were registered as illustrated in table n°=4

Determination the effects of obtained metal ions mixtures on the synthesis of aflatoxins in both tested soils during the absence and presence of natural mycoflora: In order to determine the role of soil natural mycofora in induction or suppressing the both tested functions of chitosan as detoxifying agent and heavy metals (H.M.) adsorbent agent, this experiment was carried out during occurrence and absence of soil natural mycoflora. Aflatoxins determination process was caring out using HPLC apparatus according to Fern´andez-Cruz *et al.,* 2010. The resulted data are registered as shown in table n°=5.

RESULTS

Effect of chitosan on germination process: The effect of chitosan on germination process and growth rate of germinates seeds efficacies was tested. Data in table n=1&b illustrated that chitosan increase germination process and growth parameters including germination percentage and radical length. Whereas, no significant difference between treated and untreated seeds in hypocotyl length Our findings coinceeded with those of Oha *et al.,* 1999, El Tawala *et al.,* 2013 which indicated that there were significant differences in germination percentage, hypocotyl length and radical length between treated and nontreated seeds. The highest germination percentage, hypocotyl length, radical length was proportionel with the concentration of chitosan. Our results also are closely in agreement with Sheikha and Al-Malki (2011) who indicated that chitosan enhanced bean shoot and root length, fresh and dry weights of shoots, root and leaf area as well as thelevel of chlorophylls. Our findings are in harmony with those of Salachna and Zawadzińska, (2014) which found that chitosan influence on the length of the main inflorescence shoots.

Determination of Heavy metal availability in Abou rawash (sample of contaminated soil) and Sandy soil: Heavy metal availability in Abou rawash as a sample of contaminated soil and sandy soil as a non contaminated soil were determined in DTPA extract for both soils using flame atomic absorption spectrometry (contrAA 300) according to Lindsay and Norvell, 1978. The resulted data as shown in table n°=(2) illustrated that copper (Cu),mangnesium (Mn), lead (Pb), zinc (Zn), iron(Fe) and aluminum(Al) metal concentrations in DTPA-soil extract were significantly higher than non contaminated soil except manganese (Mg) and chrome (Cr). Nickel (Ni) concentrations difference between the two tested soils is not significant. These resulted data were highly in agreement with Khouri *et al.,* 1994; Arora, *et al.,* 2008; Karanja and Githongo., 2010 and Githongo, 2010.

Estimation of aflatoxins in both soil samples: Total aflatoxins were estimated in both soil samples (20g) each according to Abbas *et al.,* 2004. The total amount of aflatoxins in contaminated soil sample was 2.89 ppb and 1.57ppb in non contaminated soil sample , these results are in harmony with those of Falih,1997.who mentioned that Apergillus genus is most tolerant to heavy metals exposure than other genera specially genus Fusarium and Curvularia.

Effect of chitosan on heavy metals concentration in produced fresh plant: Total heavy metals concentration are estimated individually in the first group of plants according to Isaac *et al.,* 1975. The registered data as explained by table n°=(3) illustrated the following:

- In case of contaminated soil:
- Chitosan efficacy as adsorbant and antifungal agent was not the same for all metal ions.

Chitosan reduced the bioavailability and plant uptake of Cu, Mn, Ni, Fe and Al. This findings are in harmony with those of Eric Guibal, 2003 which declared that Metal cations can be adsorbed by chelation on amine groups of chitosan. Our findings are also in agreement with those of Tripathi *et al.*,2016 who evaluated the effects of pure and modified chitosan beads on the remediation of zinc (Zn) polluted soils and compared to the non-amended soils, chitosan bead amended soils had greater plant biomass, reduced plant metal uptake and increased immobilisation of Zn in soil and pore water. The application of modified chitosan beads to Zn contaminated soil could significantly decrease Zn bioavailability and toxicity Furthermore. These findings were in harmony with those of Burke *et al.,* (2002) who mentioned that in vitro, adsorption experiments showed that chitosan is capable of adsorbing excess iron and The equilibrium studies showed that chitosan powder has the highest sorption capacity for the iron.

On the other hand, the fungi infected soil release more metal ions which caused their concentration in plant tisuses. *Aspergillus flavus* release Cu, Mn, Fe and Al ions more than *A.parasiticus*, this phenomen can be explained by Karcprzak and Malina (2005) and Arora *et al.,* (2008) which have stated that filamentous fungi have the capacity to form chemical complexes between the metal ions and extracellular enzymes, besides the ability to bind the metals ions to their cell walls. Fungi are accumulated broad range of metals due to the composition of their cell walls that are obtained of polysaccharides, protein and lipids are accumulated substantial amounts of metals, so, they can provide an array of binding sites for metals ions which cause a sorte of heavy metal accumulation during their plant invasion. Our finding are also in harmony with Siddiquee *et al.,* (2013) who mentioned that the resistance levels of different concentrations of heavy metals are diverse because the uses of different strains of filamentous fungi. The absence of nickel (Ni) in case of *A.parasiticus* may be due to the ability of the fungus to removing Ni .these findings are coinceeded with those of Iram *et al.,* (2009) who ranged *Aspergillus* sp> *Pencillium* sp> *Fusarium* sp regarding their abilities to removing metals from contaminated soil.

In case of non contaminated (Sandy) soil: The process of plant production was failed in case of sandy soil .The treatment with chitosan ameliore the cultivation process this fact was closely coincided with those of Salachna and Zawadzińska, (2014) which revealed that chitosan is used as a biostimulator in the cultivation of potted freesia.

Table (1a): Effect of chitosan on rocket seeds germination ratios

Treatment	Germination ratio after 3days (%)	Germination ratio after 6 days (%)
Control	44.374b	62.736b
Control+chitosan 1%	76.730a	83.386a
L.S.D $_{0.05}$	2.266957	2.266957

Table 1b. Effect of chitosan on growth rate of germinated rocket seeds

Treatments		Growth rate average of germinated seeds after 6days			L.S.D 0.05
		Average ofgroup1	Average of group 2	Average of group 3	
Control	hypocotyl length	2.015 a	2.3226 a	3.140 a	0.30637
	radical length	0.309 b	0.3655 b	0.4467b	
Control+ Chitosan1%	hypocotyl length	2.212a	1.4818 a	1.292 a	0.30637
	radical length	1.138a	0.6870 a	0.6881 a	

Table 2. Heavy metal contents in both tested soils

Abou Rawash (contaminated Sandy Soil with WW)	Conc mg/L (ppm	(Non contaminated Soil) Sandy Soil)	Conc mg/L (ppm	L.S.D$_{0.05}$
Cd	0.00	Cd	0.00	---
Cu	9.045a	Cu	3.5 b	2.26695
Mn	752.5a	Mn	99.5625b	160.361
Ni	5.39a	Ni	6a	1.6028
mg	0.0b	mg	1028.125a	6.411925
Pb	33.6a	Pb	6.0825b	5.06907
Zn	35.6625a	Zn	16b	4.5339
Fe	5024.375a	Fe	2311.25b	817.3633
Al	4500 a	Al	4098.75a	1602.99
Cr	0.00b	Cr	9.00a	3.20596

Table 3. Effect of chitosan on heavy metal concentration in produced fresh plant

Modified Treatments		AV. Of one Plant Fresh. Weight.	Heavy metal concentration in plant parts(shoot and root) mg/L (ppm)							
			Cd	Cu	Mn	Ni	Pb	Zn	Fe	Al
Abou Rawash (contaminated Sandy Soil with WW)	Healthy grains (H.G)	0.803^{a}	N.D	95.13^{d}	122.24	20.05	N.D	456.95	2577.42	1620.74 ^{a}
	(H.G) +Chitosan	1.777^{ab}	N.D	71.74^{e}	66.09	10.25	N.D	257.93	2099.88	938.1^{b}
	Infected grains with *A.flavus*(I.P.A.f)	1.310^{bc}	N.D	146.67^{b}	208.91	44.81	N.D	196.6	15528.84	2478.21^{c}
	(I.G.A.f)+ chitosan	1.523^{c}	N.D	79.33^{g}	122.62	0.04	N.D	104.13	2587.3	1389.29 ^{d}
	Infected grain with *A.parasiticus* (I.P.A.p)	0.773 ^{d}	N.D	116. ^{c}	196.96	0.00	N.D	247.72	2249.53	1670.11 e
	(I.G.A.p)+ chitosan	1.150 ^{d}	N.D	53.83	122.98	0.00	N.D	243.76	1389.32	1526.10 f
(Non contaminated Soil) Sandy Soil)	Healthy grains (H.G)	0.00^{e}	N.D	0.00	0.00	0.00	N.D	0.00	0.00	0.0 i
	(H.G) +Chitosan	0.990^{d}	N.D	164.46a	108.69	5.77	N.D	352.52	4836.21	1070.29 g
	Infected grains with *A.flavus*(I.P.A.f)	0.00^{e}	N.D	0.00	0.00	0.00	N.D	0.00	0.00	0.0 i
	(I.G.A.f)+ chitosan	0.737^{d}	N.D	65.65h	208.24	16.76	N.D	553.78	7102.71	1135.17h
	Infected grain with *A.parasiticus* (I.P.A.p)	0.00^{e}	N.D	0.00	0.00	0.00	N.D	0.00	0.00	0.0 i
	(I.G.A.p)+ chitosan	0.723d	N.D	81.73f	604.31	13.97	N.D	452.82	3971.43	4438.66
L.S.D $_{0.05}$		0.291	----	4.3671	4.62179	4.62179	--	4.77803	6.5556	9.7037

Where:
H.G = Healthy grains in non sterilized soil
I.G.A.f = Infected grains with *A.flavus*
I.P.A.p = Infected grain with *A.parasiticus*

Table 4. Estimation of aflatoxins in produced plant (pp) (in sterilized soil before and after infection with tested isolated Aspergilla)

Tested soil	Treatments	$AflaB_1$ (ppb)	$AflaG_1$ (ppb)	ER%
Abou Rawash (contaminated Sandy Soil with WW)	Healthy pp (from H.G) in sterilized soil	0.00 d	0.00	----
	pp(from H.G) +Chitosan in sterilized soil	0.00 d	0.00	-----
	Infected pp with *A.flavus* (I.G.A.f)	13.9b	0.0c	-----
	Infect. pp from (I.G.A.f)+ chitosan	12.5b	0.0c	10.0719
	Infected pp with *A.parasiticus* from (I.G.A.p)	0.00	17a	----
	Infected pp (I.G.A.p)+ chitosan	0.00	13b	23.529
(Noncontaminated Soil) Sandy Soil)	Healthy pp from(H.G)	0.0	0.0	----
	pp(from H.G) +Chitosan	0.0	0.0	----
	Infected pp with *A.flavus*(I.G.A.f)	17.5a	0.0c	-----
	Infect.pp from (I.G.A.f)+ chitosan	10c	0.0c	42.857
	Infected pp with *A.parasiticus* from (I.G.A.p)	0.0 d	18a	----
	Infected pp (I.G.A.p)+ chitosan	0.0 d	14b	22.223
$L.S.D_{0.05}$		2.206467	2.447856	

Where:

Pp= produced plants from healthy grain(H.G)

I.G.A.f= infected grains with *Aspergillus flavus*.

I.G.A.p= infected grains with *Aspergillus parasiticus.*

Table 5. Role of chitosan and fungal competition between soil fungal flora and the two tested fungi on aflatoxin production during natural contamination with heavy metal mixture

Tested soil	During Absence of competition	$AflaB_1$(ppb)	Afla G_1 (ppb)	ER% of detoxification.	During Occurrence of competition	$AflaB_1$ (ppb)	$AflaG_1$ (ppb)	ER% of detoxification.
Abou Rawash (contaminated Sandy Soil with WW)	Sterilized soil (Ss)	1.3c	0.6c	----	Non sterilized soil (NSs)	0.9c	0.42c	----
	(Ss) +Chitosan	0.0c	0.0c	100	(NSs)+Chitosan	0.0c	0.0c	100
	Infected (Ss) with A.flavus (I.Ss.A.f)	18.6a	----	-----	Infected(N Ss) with A.flavus (I.NSs.A.f)	15b	-----	-----
	(I.G.A.f)+ chitosan	14.5ab	----	22.043	(I.G.A.f)+ chitosan	11bc	-----	26.667
	Infected(Ss) with A.parasiticus (I.Ss.A.p)	----	18a	----	Infected(NSs) with A.parasiticus (I.Ss.A.p)	-----	16 b	-----
	(I.Ss.A.p)+ chitosan	----	8.5bc	52.778	(I.NSs.A.p)+ chitosan		10bc	37.5
(Non contaminated Soil) (Sandy Soil)	Sterilized soil (Ss)	0.0c	0.0c	----	NonSterilized soil (NSs)	0.0c	0.0c	-----
	(Ss) +Chitosan	0.0c	0.0c	-----	(NSs) +Chitosan	0.0c	0.0c	-----
	Infected(Ss) with A.flavus(I.Ss.A.f)	21a	-----	----	Infected(Ss) with A.flavus (I.NSs.A.f)	23a	-----	-----
	(I.G.A.f)+ chitosan	14 ab	-----	33.334	(I.G.A.f)+ chitosan	20b	------	13.043
	Infected(Ss) with A.parasiticus (I.Ss.A.p)	----	23a	----	Infected (NSs) with A.parasiticus (I.SNs.A.p)		31a	----
	(I.Ss.A.p)+ chitosan	-----	10bc	56.522	(I.NSs.A.p)+ chitosan		18b	41.935
$LSD_{0.05}$	8.02648				8.14110798			

Our findings are also in agreement with those of Tripathi *et al.,* 2018 who mentioned that chitosan addition ameliorate the nutrient-deficient soil. The metal ions concentration varied in produced plants that may be due to the infected fungus specie and its availability to release or to be abiosorbent agent of metal ions from soil, this fungal act as a transporter of these metal ions and concentrated them during its plant invasion process**.** These findings are in harmony with those of Tangahu *et al*., (2011) and Siddiquee *et al.,* (2015) who announced that Filamentous fungi are included as a biosorption agent of metal ions including Copper, Zinc, Cadmium , Iron, Nickel and lead.

Determination of the chitosan effect on aflatoxins concentration in produced fresh plant: Aflatoxins estimation was carried out in the second group of the collected plants using HPLC apparatus according to Fern´andez-Cruz *et al.,* 2010. The registered results in table n°= 4 illustrated that chitosan effect in reducing AFB_1 is significant in plant cultivated in sandy soil but not significant in contaminated soil, whereas, AFG_1 reduction is significant in plant cultivated in both soils. Our results are may be explained by the findings of Malini *et al*.,(1984). Who announced that aluminium and nickel induces the aflatoxin production by *Aspergillus flavus*. Contaminated soil contained aluminium and nickel more than sandy soil Furthermore, *Aspergillus parasiticus* producing AFG_1 could be less sensitive than *Aspergillus flavus to* aluminium and nickel. Results indicated that chitosan was more effective in reducing aflatoxin G_1 in contaminated soil than aflatoxin B_1 that is may be due to the difference in sensitivity of both tested fungi against high concentration of heavy metals. This finding was closely in agreement with those of Valix *et al.,* 2001 who mentioned that *Aspergillus spp* are varied in their sensitivety against the same treatment. Furthermore, our results also were in harmony with those of Kumar *et al*.,2011 who mentioned that *Aspergillus spp* are the most metal tolerant species, showing enhanced growth even at high concentrations (2000 mg^{-1}) of heavy metal. Minewhile, in sandy soil, the efficacy of $aflaB_1$ reduction was higher to fold than afla G_1 that may be due to fungus behavior toward drought amelioration caused by the chitosan addition, these results are relatively in agreement with those of rabea *et al.,* 2003 and João *et al.,* 2008 who mentioned that 129 references describes the biological activity of chitosan derivatives and their mode of actions varied as an antimicrobial agent against fungi, as an elicitor of plant defense mechanisms. The absence of resulted plant in sandy soil without treatments is logic, these results are coinceeded

with those of O'Danu, (2011) who mentioned that sandy soils can be difficult environments for seed germination; It was very important thing to remember with sandy soil that organic matter is essential to retaining water and nutrients for plants. It improves soil texture by settling between the large particles of and plugging the spaces in sandy soil. This notice explained why plants resulted in case of contaminated soil and treated soils with chitosan.

Effects of obtained metal ions mixtures on the synthesis of aflatoxins in both tested soils during the absence and presence of natural mycoflora: In order to investigate the effectiveness of chitosan during the natural occurrence of soil mycoflora, in induction or suppressing the chitosan function as aflatoxins detoxifying agent. Aflatoxins determination process was carried out using HPLC apparatus according to Fern´andez-Cruz *et al.,* 2010. The registered data as shown in table n°=5 illustrated that the natural occurred mycoflora in both tested soil don't antagonized the role of chitosan as aflatoxins detoxifying agent so, we can applied chitosan addition in seed beds to minimize the aflatoxins availabity to plants.our results are in agreement with those of Campaniello *et al.,* 2008; Munoz, *et al.,* 2009 and Xianghong *et al.,* 2010 who mentioned that chitosan inhibited fungal growth and mycotoxins production especially aflatoxins produced by *Aspergillus flavus* .Chitosan significantly ($p < 0.05$) inhibited the growth of the Aspergillus and its effect was increased with increased concentration.

Conclusion

Sandy soil enhanced the aflatoxin B_1 and G_1 production, but the addition of chitosan ameliorate the texture of sandy soil particles and minimize the fast loosing of ground water which conduct to minimize the drought stress which considered the main inducer agent of aflatoxin production in plants. Chitosan can be applied in contaminated soil to minimize the aflatoxin production and the available heavy metals to cultivated plants. Chitosan can act as an amendment for remediation of such heavy metal toxicity in Contaminated soils, its efficiency was not affected by the soil microflora, this fact was very important for chitosan application in amended soils. The auther wants to thanks Assitant Prof. Mohamed Lotfy for his kind help in determination of heavy metals in soils and plants.

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