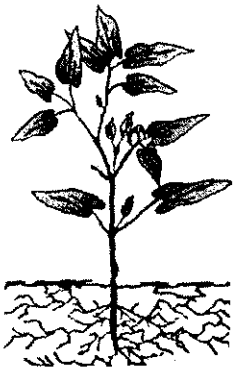


109
Annals
Agric. Sci.,
Ain Shams Univ.,
Cairo, 55(1), 109-119, 2010

EFFECT OF SOME BIOAGENTS ON INHIBITING TOXINS PRODUCED BY *ASPERGILLUS FLAVUS* IN PEANUT PODS

[9]

Maisa L. Abd El-Moneim[1]; A.F. Tolba[1] and A.M. Gomaa[2]

1- Central Lab. Of Organic Agric., ARC, Giza, Egypt
2- Central Lab. of Residue Analysis of Pesticides & Heavy Metals in Food "QCAP Lab", Dokky, Cairo

Keywords: Biological control, *Aspergillus flavus*, Peanut, *Trichoderma harzianum*, *Bacillus subtilis*, Aflatoxin

ABSTRACT

Two different beneficial microorganisms namely *Trichoderma harzianum* and *Bacillus subtilis* were used under laboratory conditions to evaluate their antagonistic effects against *Aspergillus flavus*. *T. harzianum* and *B. subtilis* were formulated as powder each 1 gm contains $30x10^6$ c.f.u, then used as pod dressing treatment to study their effect on inhibiting toxins produced by *Aspergillus flavus* during storage under room condition. Powder formula of both organisms was applied at three different rates and in three different numbers of application. These treatments were done with artificially infested peanut pods stored under room conditions for three months. Obtained data showed that both bioagents inhibit linear growth of *A. flavus*. *T. harzianum* gave better antagonistic effect and gave 77.7 % reduction in linear growth whereas *B. subtilis* gave only 66.66%. All treatments reduced the different four types of Aflatoxins, produced by *A. flavus* in peanut pods. *T. harzianum* gave the best result in reducing the amount of toxins and 87.8% reduction in toxins production was observed whereas *B. subtilis* gave only 76.18% reduction compare with the control treatment. Positive correlation between storage periods of peanut pods and reduction of toxin was observed in case of treatment of peanut pods with both antagonistic agents. Regarding number of applications, treated peanut pods with *T. harzianum* once gave best result when compared with application for two or three times. On the contrary applying *B. subtilis* for two or three times showed more efficacy in reducing amount of toxins produced by *A. flavus*. Data also showed that dose of (20gm) *T. harzianum* or *B. subtilis* formula/1 kg of pods was the most effective dose in reducing the amount of toxins. Positive correlation between increasing period of storage up to three months and percentage of reduction in toxin producing by *A. flavus* was also noticed.

INTRODUCTION

Most isolates of *Aspergillus flavus* produce polypeptide-derived secondary metabolites called aflatoxins (AFs), these toxins contaminate seeds and plant debris of many crops in the field during harvesting, storage and processing. **(Dvorockova 1990, Cvetnic and Pepeljnjak 2007)**, which are highly toxic, mutagenic, teratogenic, immunosuppressive and carcinogenic to animals. **(Hesseltine 1965, and Ainsworth and Austwick 1973).**

Aflatoxins B1, B2, G1 and G2 are classified as group1 human carcinogens **(Eaton and Gallagher 1994).**

As a result for increasing concern about human and animal health bundles of regulations were issued to limit presence of permitted aflatoxins in food or feeds throughout most of the world countries. The carcinogen aflatoxin B1 (AFB1) produced by *A. flavus* is a major food safety concern in crops. **(Cesara Accinelli et al 2008).** American food and pharmacology Administration stipulated the content of (AFB1) should not exceed 20 µg / kg

(Received January 3, 2010)
(Accepted February 24, 2010)

in edible food and 30 µg / kg in animal feed. The permissible content of (AFB1) is also regulated in China: 20 µg / kg for corn, peanut core, and peanut oil, 10 µg/kg for rice, oil and for other food 5µg / kg. Aflatoxins are potent hepatotoxic and carcinogenic metabolites, and their presence in peanuts is heavily monitored and regulated to ensure a safe food supply **(Van Egmond, 1995; Wood and Trucksess, 1998).**

Contaminated lots of peanuts cannot be used for human consumption and therefore represent great economic losses for the peanut industry **(Lamb and Sternitzke, 2001)**. Peanut contamination with aflatoxin can occur in the field (preharvest) when severe late-season drought stress occurs or during storage (postharvest) when improper conditions of moisture and temperature exist **(Cole et al 1995).**

Biological control is a promising approach for reducing both preharvest and postharvest aflatoxin contamination in peanuts **(Dorner et al 1992 and 1998).**

Bacillus subtilis inhibit the growth and aflatoxin produced by *Aspergillus flavus* isolate (NRR 13357) in peanut or corn. **(Norio and Susumu 1988).**, Therefore it was used as an effective biological agent to control post harvest disease caused by *A. flavus* in stored lemon fruits **(Kotan *et al* 2009)**. *B. subtilis* was able to inhibit both *Aspergillus* growth and detoxifying 85% of AFB1 **(Petchkongkaew *et al* 2008).**

Trichoderma harzianum treatment was significantly effective in inhibiting aflatoxin B1 production on soybean seeds by *A. flavus*. **(Krishnamurthy and Shashikala 2006).**

Trichoderma virens and *B. subtilis* were used to control *A. flavus* attacks rice grains and inhibit aflatoxin production. They showed 80% and 68% reduction in *A. flavus* growth and 72% and 58% reduction in aflatoxin (AFB1) when antagonists were used at the rate of 200 ml/kg rice grain respectively **(Reddy *et al* 2009).** The present work was carried out to study the role of *Trichoderma harzianum* and *Bacillus subtilis* in inhibiting either growth of *A. flavus* or synthesis of aflatoxin by this pathogen on peanut pods under normal storage conditions for three months.

MATERIALS AND METHODS

Different micro organism preparations

Two bio-control agents i.e. *Trichoderma harzianum, Bacillus subtilis* and pathogenic fungus *Aspergillus flavus* isolate P2 were obtained kindly from central lab of organic agriculture ARC. Giza, Egypt.

Trichoderma harzianum was grown in liquid gliotoxin fermentation medium (G.F.M) under complete darkness **(Abd-El-moity and Shatla 1981),** for nine days at 28°C. *B. subtilis* was grown on nutrient glucose broth (Dowson, 1957) for 2 days at 28°C. Bioagents were formulated as powder using method developed by **(Abd-El-moity, 1985).** Prepared powder was adjusted to contain 30 x 10^6 cfu /1gm.

Aspergillus flavus, a Pathogenic isolate P2 for peanut pod was grown on modified czapek's medium **(Hara *et al* 1974)** for 7days at 28°C.

The suspension of *A. flavus* was prepared by adjusting number of Aspergillus propagules in the suspension to be 4X10^6 cfu /ml **(Mahmoud 2004).**

1- Antagonistic effect between bio-agents and pathogenic fungus (*Aspergillus flavus*)

Under laboratory conditions, *Trichoderma harzianum* and *Bacillus subtilis* were used to evaluate their antagonistic effect against pathogenic fungus (*Aspergillus flavus*). Petri dishes 9.0 cm in diameter each contains 15 ml of G.F.M were used to determine the antagonistic effect between *T. harzianum* and pathogenic fungus. On the other hand, plates contained N.G.A medium were used to determine the effect of *B. subtilis* on *A. flavus* radial growth. Plates were inoculated with discs (5mm in diameter) of *A. flavus* obtained from the periphery of 4 days old colony. The pathogenic fungus was inoculated at one side whereas the opposite side was inculcated with either disc of *T. harzianum* (5mm in diameter), obtained from 3 days old colony or with loop full of antagonistic bacteria *B. subtilis* grown on liquid NG medium for 48 hours. Five plates were used for each treatment. Plates only inoculated with Pathogenic fungus (*A. flavus*) served as a control treatment. Inoculated plates were then incubated at 28°C.When mycelial growth covers all medium surface in control treatment, all plates were examined and percentage of reduction in mycelial growth of pathogenic fungus was calculated using the next formula according to **(Abd-El Moneim Maisa, 2005).**

$$X = 100 - [(G_2/G_1) X 100]$$

Where X: % of reduction

G_1: growth of pathogenic fungus in control plates.

G_2: growth of pathogenic fungus in treated plates.

2- Determination the type and quantity of Aflatoxin in infested and free peanut pod samples

The aim of this experiment is to be sure that the used *Aspergillus flavus* isolate P2 is active producer of Aflatoxin and also to identify toxin types. Apparent healthy peanut pods variety Gregory was used. Ten paper bags (39.5 cm high x 27.5 cm diameter) each contained 1 kg of peanuts were used for each treatment. Bags contained peanut pods were divided to two groups. The first groups (10 bags) were sprayed using suspension of *A. flavus* $4x10^6$ cfu/ ml. at the rate of 10ml/ kg peanut pods, while the second group (10 bags) were sprayed only with (10ml water/kg of peanut) served as control. All bags were incubated at room temperature (28-30°C and 70-75 RH). After one month, random samples of each treatment were collected and send to [**Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food, Dokky, Cairo**] to determine the amount and type of toxin in contaminated peanut pods by High Performance Liquid Chromatograph (HPLC) method.

The extraction of peanut pods and determination of aflatoxins were carried out according to **AOAC (2000)**.

Extraction Procedure: Fifty grams of homogenized sample were weighted into blender jar. 200 ml of Methanol/water (80/20) (v/v) solution were added then blended for 2 min to extract the Aflatoxins from solid matrix. Medium fast filter paper was used for filtration.

Partitioning Procedure: 40 ml of filtrate were transferred into 500 ml separatory funnel.40 ml of (10%) sodium chloride solution and 50 ml n-hexane were added to remove soluble fat from solution. Shake gently for 1 min and let phases to separate. The lower aqueous layer which contains Aflatoxins was drain into another 500 ml separatory funnel. 50 ml chloroform was added and the solution was shaked gently for 1min, Aflatoxins will emigrate from aqueous layer to organic layer. After the two phases separate the lower layer was then drained through anhydrous sodium sulphate (15 g) into 250ml flask. The aqueous layer is washed with two portions of 25 ml chloroform and shaked gently for one min. each time. The received chloroform is evaporated by using rotary evaporator till dryness. The sample residue was dissolve in 2 ml dichloromethane.

Column clean-up: Small ball of glass wool loosely placed in bottom of chromatographic column; 0.5 g of anhydrous sodium sulphate was added; 3 ml of dichloromethane was added to the column. 0.5 g of deactivated silica gel was added. After Drain dichloromethane to the top of silica gel, 0.5 g of sodium sulphate was added. The residue was transferred to silica gel column with medium flow rate, Aflatoxins will linked with silica gel. The flask rinsed with two ml dichloromethane to transfer all Aflatoxins from the flask. The column washed with two portions of 5 ml dichloromethane with maximum flow rate to remove interfering matrices, and the washing solvent was discarded.

Aflatoxins were eluted with medium flow rate with 5 ml methanol/chloroform (3/97) (v/v) and received in 10 ml tube. The eluted solvent was evaporated till dryness in water bath adjusted at about (60°C) under air flow.

Derivatization: Aflatoxins were derivatized by adding 50 µl of Tri- Floro Acetic acid TFA and 200 µl hexane to the residue and vortex-mix vigorously for 30 sec. After 5 min, 1.950 ml of acetonitrile - water (1-9) was added and vortex-mix for 30 sec. The sample centrifuged for 3 min at 4000 rpm.The lower aqueous layer used for HPLC determination.

HPLC analysis

Aflatoxins were separated and quantified by reversed phase-HPLC using C18 analytical column. The mobile phase was water-methanol-acetonitrile-(65+22+13), at a flow rate of 1 ml/min. Aflatoxins were detected by fluorescence detector at the excitation and emission wavelengths of 360 nm and 440 nm respectively. The injection volume was 25 µl.

The concentration of aflatoxin was calculated using the formula:

$$\mu g /Kg = (S.Y.V.)/ (X.W)$$

Where

S= volume of aflatoxin standard, in µL of equivalent sample intensity
Y= concentration of aflatoxin standard in µg/ml.
V= volume of solvent required to dilute final extract in µL.
X= volume of sample extract in µL required to give fluorescence intensity comparable to that of S µL of standard.
W= weight of original sample in gram contained in the final extract.

In all follow experiments unless other wise indicated. *Trichoderma harzianum* or *Bacillus subtilis* were used in powder form. All experiments were carried out in the same time under room conditions. Ten replicates were used for each treatment and each replicate contained 1 kg of peanut pod. Control treatment was used for all experiments

3- Effect of bioagents on inhibiting toxin produced by *Aspergillus flavus* in peanut pods

Both antagonistic microorganisms and the method of application were carried out as perversely mentioned [*Trichoderma harzianum* was grown on G.F.M. for 9 days while *Bacillus subtilis* were grown on N.G. broth for 48 hours. *T. harzianum* or *B. subtilis* were formulated in powder form and adjusted to contain $30x10^6$ cfu/ g]. Prepared powder were mixed separately with peanut pods at the rate of 10g of powder/ kg of peanut pods. Thirty paper bags contained peanut pods were used for each treatment, every one contained one kg of peanut pods. All treatments received 10 ml of *A. flavus* suspension $4x10^6$ cfu/ ml. Bags contain peanut pods received only suspension of *A. flavus* acted as control. All bags were incubated at room temperature (28-30°C and 70-75 RH). Every month, different parameters including total amount of toxin, different types of Aflatoxin (B1, B2, G1 and G2) and percentage of reduction in the amount of toxin at the end experimental storage period (after three months from treatment) were measured to evaluate the effect of different biological preparation on inhibition of aflatoxin produced by *A. flavus*.

4- Effect of the number of Applications of biological preparations on inhibiting toxins production by *A. flavus* in peanut pods

To compare between effect of applying different bioagents twice or for three times, 150 paper bags each contains 1kg of peanut pods infested with *A. flavus* suspension were divided to equal 5 batches, each batch contains (30) bags. Each batch received different treatment as follow:-

Treatment 1: bags (30) received 10 gm of *T. harzianum* powder at beginning of storage period and after one month the same bags received the second dose of *T. harzianum* as powder (10) gm/bag.

Treatment 2: bags (30) received one dose of *T. harzianum* at begging storage period (10) gm/1kg of peanut pods, the second dose after one month as powder at the rate of (10) gm while the third dose was applied after two months.

Treatment 3 and 4: The same treatment were repeated by using *B. subtilis* instead of *T. harzianum*

Treatment 5: bags (30) received only suspension of *A. flavus* at the early begging of storage were used as control treatment. Samples from each treatment were taken every month to monitoring toxin and evaluate effect of each treatment on inhibiting toxin production.

5- Effect of bioagents at different doses on inhibition of aflatoxin produced by *A. flavus* in peanut pods

Different bioagent (*T. harzianum* or *B. subtilis)* preparations were used as powder at the rate of 20 or 30gm/1kg of peanut pods. Peanut pods were sprayed by suspension of *A. flavus* contained $4x10^6$ cfu/ ml. Infested pods were divided to 5 batches each batch contain 30kg of infested peanut pods each batches received certain dose (20 or 30gm) *T. harzianum* or *B. subtilis* preparation per each 1kg of infested pods. Each batches was placed in 30 paper bags (replicates)

Bags contain infested peanut pod without antagonist were act as control. All bags were stored at room temperature (28-30 C° and 70-75 RH)

Different treatments were examined periodically every month for three months. Different parameters were measured, amount and type of toxin and % of reduction of toxin) to find out the most effective dose in inhibiting Alfatoxin production.

RESULTS AND DISCUSSION

This work was designed in order to find out a safe method to inhibit toxin production by *Aspergillus flavus*, in peanut pods and to protect human health from harmful effects of Aflatoxin.

Effect of bioagents on liner growth of *A. flavus*

Data in **Table (1)** show effect of the two antagonistic micro-organisms *Trichoderma harzianum* or *Bacillus subtilis* on the liner growth of *Aspergillus flavus* under lab. conditions. *T. harzianum* gave 77.7% reduction where as *B. subtilis* gave 66.6% reduction in mycelial growth of *A. flavus* compared with control treatment. This antagonistic effect may be due to the ability of *T. harzianum* to act through different mechanisms including mycoparasitism **(Abd El Moity and Shatla**

1981), production of antifungal substances (Sanz *et al* 2002) and destruction effect by enzymes i.e chitinase (Padares *et al* 1992 and Bolar *et al* 2000). *B. subtilis* followed *T. harzianum* regarding antagonistic activity. This may be due to that *B. subtilis*, acts only through the production of number of antibiotics (subtilicin, bacteriocin) (Ferreira *et al* 1991 and Asaka and Shoda 1996).

Table 1. Antagonistic effect between different bioagents and pathogenic fungus (*Aspergillus flavus*) under Lab. condition

Bioagent	Length of linear growth of *A. flavus* in cm.	% of reduction in linear growth of *A. flavus*
Trichoderma harzianum	2.0	77.77
Bacillus subtilis	3.0	66.66
Control	9.0	0.00
L.S.D at 5%	0.16	1.63

Activity of *A. flavus* on Aflatoxin types production

Data in Table (2) and (Figs. 1 & 2) illustrate the amount of total and type of toxin in peanut pods (infested with *A. flavus*) compared with non infested pods. Present data show that used *A. flavus* isolate P2 was able to produce 4 types of Aflatoxin (B1 B2, G1 and G2). Regarding amount of different toxin types it ranged from 0.20-7.00 Ppb when peanut was stored under room conditions for one month. The total detected toxin was 9.65 Ppb compare with non detected amount in control treatment (non infested peanut pods).

Table 2. Amount and types of Aflatoxin in infested and non infested peanut pod samples

Sample of peanut pods	Amount of toxin	Type of toxins (ppb)			
		B1	B2	G1	G2
Control	ND	ND	ND	ND	ND
Infested sample	9.65	2.22	0.2	7.00	0.23

Ppb: part per billion ND: Not detect

Efficacy of different used antagonist in inhibiting Aflatoxin production

Data in Table (3) show that *Trichoderma harzianum and Bacillus subtilis* as bioagents can inhibit toxins produced by *Aspergillus flavus* in peanut pods. Both antagonistic microorganisms *Trichoderma harzianum and Bacillus subtilis* gave good reduction in Aflatoxin production compare with control treatment. *T. harzianum* was better than *B. subtilis* in inhibiting Aflatoxin production, it gave 87.8% reduction in total amount of toxin after storage period of three months whereas *B. subtilis* gave 76.18% reduction in toxin production compare with control treatment.

These result can be explain in the light of fact that *Trichoderma harzianum* can spread very quickly and occupy the court of infection preventing most spores of *A. flavus* from germination and producing aflatoxin as secondary metabolites (Abd El-Moity *et al* 2003). Data also can be explained in the light of fact that a *T. harzianum* acts through different mode of actions where as *B. subtilis* acts only through antibiotic products.

Activity of different bioagent on inhibition of different types of Aflatoxin production

Data in Table (4) show that production of different toxin types by *A. flavus* varied in their quantities from one type to another. Aflatoxin type (G1) was the most dominate toxin and 15.3 Ppb was detected in treated peanut pods after 3 months storage (control treatment) where as B2 type was the least one and the amount produced of toxin in three months was out of limit of detection (less than 0.20 Ppb). The other two types (B1&G2) fill in between and give 3.9 and 0.9 Ppb respectively. Positive correlation can also notice between amount of produced toxin (in control treatment) and length of storage period. Using *T. harzianum* or *B. subtilis* as powder formula at rate of 10 gm/1kg of pods led to clear reduction in amounts of all produced toxins. *T. harzianum* was more effective and percentage of reduction through out storage period (3 months) ranged from 100 to 64.3%, at the same time *B. subtilis* only give 100 to 44.3% reduction compare with control treatment. Effect of *T. harzianum* or *B. subtilis* was more clear by increasing storage period. This can be explain in light of work of (Turner 1971) who mention that toxins can be consumed by other microorganisms as food stuff.

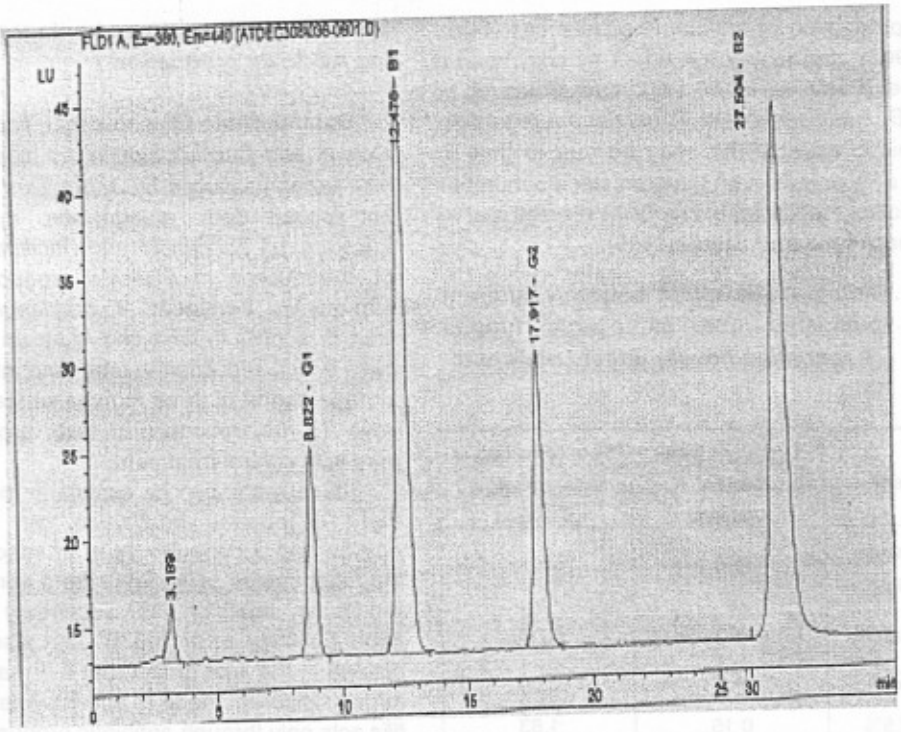


Fig. 1. Injection stander of different types of aflatoxin in 50 Ppb

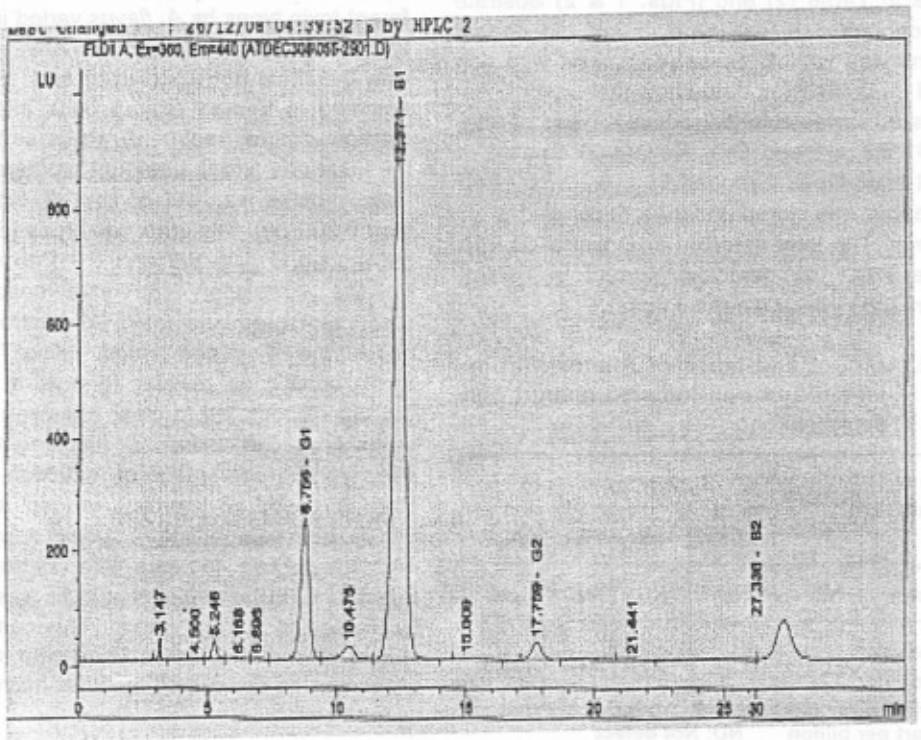


Fig. 2. Types of Aflatoxin in infested peanut pod sample

Table 3. Effect of some bioagents on inhibiting toxins produced in peanut pods

Period in months / Treatments	1	2	3	%of reduction in toxin amount after 3 mounts
Trichoderma harzianum	3.5	2.6*	2.43	87.8
Bacillus subtilis	5.1	7.73	4.77	76.18
Control	9.65	17.21	20.03	0.00

*Amount of toxin in Ppb

Table 4. Effect of some bioagents on inhibiting different types of toxin produced by *A. flavus* in peanut pods

Type of toxins produced during different periods													
Per.* / Treatments		B1			B2			G1			G2		
		1	2	3	1	2	3	1	2	3	1	2	3
Trichoderma harzianum	A	0.6	0.5	ND	ND	ND	ND	2.5	2.1	2	ND	ND	ND
	P	72.9	87.1	100	--	--	--	64.3	82.5	86.9	--	100	100
Bacillus subtilis	A	1.2	1.3	ND	ND	ND	ND	3.9	6.2	4.5	ND	ND	ND
	P	45.9	66.6	100	--	--	--	44.3	48.3	70.6	--	100	100
Control	A	2.22	3.9	3.6	ND	ND	ND	7.0	12.6	15.3	ND	0.55	0.9
	P	0.0	0.0	0.0	--	--	--	0.0	0.0	0.0	--	0.0	0.0

A: Amount of toxin in Ppb P: % of reduction ND: Not detected * Period in months

Relation between number of applications and toxin production

Data in **Table (5)** show the effect of applying antagonist for two or three times on inhibiting toxin produced by *A. flavus* in stored peanut pods during storage under room condition (28-30 ^{0}C) for three months. Present data indicate that negative correlation between numbers of application by *T. harzianum* and its inhibitory effect. Applying *T. harzianum* twice was more effective when compare with three times. On the contrary applying *B. subtilis* two times or three times gave more inhibition effect for toxin production.

These results can be explain in the light of work of **(Turner, 1971)** who stated that toxin production increase under shortage of nitrogen or phosphorus in the medium. Spraying *T. harzianum* for more than one time led to increase nitrogen and phosphorus in the court of infection due to presence of these elements in the formula of *T. harzianum*. As a result of repeating applying *T. harzianum* formula, Nitrogen and Phosphorus were increased in the medium consequently *T. harzianum* stop digesting and consume aflatoxin as food stuff, eventually toxins of *A. flavus* are accumulate in stored peanut pods.

Table 5. Effect of number of Application of different biological preparation on inhibiting toxins produced by *A. flavus* in peanut pods

Different treatments	Number of Applica-tion	Toxin / Period*	B1			B2			G1			G2		
			1	2	3	1	2	3	1	2	3	1	2	3
Tricho-derma harzianum	Two time	A	0.6	0.8	0.8	ND	ND	ND	2.5	3.7	3.6	ND	ND	ND
		P	72.9	79.5	77.7	--	--	--	64.3	69.2	76.5	--	100	100
	Three time	A	0.6	0.8	2.8	ND	ND	ND	2.5	3.7	0.31	ND	ND	ND
		P	72.9	79.5	22.2	--	--	--	64.3	69.2	97.9	--	100	100
Bacillus subtilis	Two time	A	1.2	1.0	0.61	ND	ND	ND	3.9	4.5	3.2	ND	ND	ND
		P	45.9	74.4	83.0	--	--	--	44.3	62.5	79.1	--	100	100
	Three time	A	1.2	1.0	3.2	ND	ND	ND	3.9	4.5	ND	ND	ND	ND
		P	45.9	74.4	11.11	--	--	--	44.3	62.5	100	--	100	100
Control		A	2.22	3.9	3.6	ND	ND	ND	7.0	12.0	15.3	ND	0.55	0.9
		P	0.00	0.00	0.00	--	--	--	--	--	--	--	0.0	0.0

A: Amount of toxin P: % of reduction ND: Not detected *period in months

Table 6. Effect of different doses of different bioagents in inhibited toxins produced by *A. flavus* in peanut pods

Different treatments	Different doses	Type*	B1			B2			G1			G2		
		Per**	1	2	3	1	2	3	1	2	3	1	2	3
Trichoderma harzianum	20g/kg	A	0.72	ND	ND	ND	ND	ND	1.9	1.8	1.3	ND	ND	ND
		P	67.5	100	100	--	--	--	72.9	85.7	91.5	--	100	100
	30g/kg	A	0.6	1.8	2.1	ND	ND	ND	2.3	6.0	6.1	ND	ND	ND
		P	72.9	53.8	41.6	--	--	--	67.1	52.4	60.1	--	100	100
Bacillus subtilis	20g/kg	A	1.2	1.3	0.73	ND	ND	ND	3.6	5.0	3.4	ND	ND	ND
		P	45.9	66.6	79.7	--	--	--	48.6	60.3	77.7	--	100	100
	30g/kg	A	1.2	2.2	1.8	ND	ND	ND	3.8	10.4	10.6	ND	ND	0.5
		P	45.9	43.58	50	--	--	--	45.7	17.5	30.7	--	100	44.4
Control		A	2.22	3.9	3.6	ND	ND	ND	7.00	12.6	15.3	ND	0.55	0.9
		P	0.00	0.00	0.00	--	--	--	0.00	0.00	0.00	--	0.00	0.00

A: Amount of toxin P: % of reduction ND: Not detected * Type of different aflatoxin ** Period in months

Regarding *B. subtilis* treatment, different types of aflatoxin varied in their reaction against *B. subtilis* whereas the most effective number of application to inhibit B1 was two time, all the other toxin (B2, G1 and G2) show that increasing number of application led to increase reduction. This can be explain relaying on fact that *B. subtilis* works, through antifungal substance products **(Ryder *et al* 1999)**. Increasing number of application led to increase antifungal substance consequently increase inhibition of toxin production. B1 produced by *A. flavus* was deviated and increasing number of application led to increase toxin this may be due to that this type of toxin is produced by the fungus (*A. flavus*) as secondary metabolites under unsuitable growth condition such as increases *B. subtilis* exudates in medium **(Turner, 1971)**.

Comparison between using different doses of antagonist on aflatoxin production

Data in **Table (6)** show that different doses (20 or 30gm/kg) of different bioagent per each kg of peanut pods led to different degrees of reduction in aflatoxin produced by *A. flavus* during storage.

Dose of (20gm bioagent /kg of peanut pods) gave the best result and reduction of toxins were detected either in *T. harzianum* or *B. subtilis*, treatment.

This might be due to that treatment with bio preparation at 20gm/kg of peanut pods increase secondary metabolites, enzymes and antifungal substances **(Hayes 1992, Rodriguez and Cotes 1999)**. Regarding *B. subtilis*, it produces some antibiotics as Iturine and Surfactine **(Asaka and Shoda 1996; Hwang *et al* 1996 and Ryder *et al* 1999)**. These products are responsible for antagonistic effect of this bioagent against *A. flavus*. Increase the dose of *T. harzianum* or *B. subtilis* to be 30gm led to increase nutrient substance which led to inhibit toxin digestion and consumption.

REFERENCES

Abd El-Moity, T.H. (1985). Effect of single and mixture of *Trichoderma harzianum* isolates on controlling three different soil borne pathogens. **Egypt. J. Microbiol., Special Issue, pp. 111-120.**

Abd El-Moity, T.H. and M.N. Shatla, (1981). Biological control of white rot disease of onion *Sclerotium cepivorum* by *Trichoderma harzianum*. **Phytopathology Z.; 100: 29-35.**

Abd El-Moity, T.H.; L. Maisa, Abd-EL-Moneim; M.M.M. Atia; A.Z. Aly and M.R.A. Tohamy (2003). Biological control of some cucumberdiseases under organic agriculture. **In: Proc. of the International Symposium on the Horizons of Using Organic Matter and Substrates in Horticulture, pp. 227-236, Cairo.** Ed. Abou Hadid A.F. **Published by ISHS.**

Abd El-Moneim, Maisa, L. (2005). Effect of some non-chemical, seed and foliage applications on cotton damping off disease incidence and yield characteristics. **Zagazig J. Agric. Res., 32: 1829-1853.**

Ainsworth, G.C. and P.K.C. Austwick, (1973). Fungal Diseases of Animals. 2nd Ed. pp. 429-434. (commonwealth Agriculture Bureau) Slough, U.K.

AOAC. Official Methods of Analysis (2000). Natural toxins p. 49. Washington, D.C.

Asaka, O. and M. Shoda (1996). Biocontrol of *Rhizoctonia solani* damping off of tomato with *Bacillus subtilis* RB 14. **Applied and Environmental Microbiology, 62: 4081-4085.**

Bolar, J.P.; J.L. Norelli; K.W. Wong; C.K. Hayes; Q.E. Harman and H.S. Aldwinkle (2000). Expression of endochitinase from *Trichoderma harzianum* in transgenic apple increase resistance to apple scab and rduce vigour. **Phytopathology 90: 72-77.**

Cesara Accinelli, H.K.; R.M. Zablotowicz and J.R. Wilkinson (2008). *Aspergillus flavus* aflatoxin occurrence and expression of aflatoxin biosynthesis genes in soil. **Can. J. Microbiol., 45: 371-379.**

Cole, R.J.; J.W. Dorner; C.C. Holbrook (1995). Advances in mycotoxin elimination and resistance. In: Stalker, H.T.; H.E. Pattee, (eds.), **Advances in Peanut Science. pp. 456-474.** American Peanut Research and Education Society, Stillwater.

Cvetnic, Z. and S. Pepeljnjak (2007). Interaction between certain molds and aflatoxin B1 producer *Aspergillus flavus* NRRL 3251. **Arh Hig Reda Takiskal 58: 429-434.**

Dorner, J.W.; R.J. Cole and P.D. Blankenship (1992). Use of a biocompetitive agent to control preharvest aflatoxin in drought stressed peanuts. **J. Food Prot., 55: 888–892.**

Dorner, J.W.; R.J. Cole and P.D. Blankenship (1998). Effect of inoculum rate of biological control agents on preharvest aflatoxin contamination of peanuts. **Biol. Control 12: 171–176.**

Dowson, W.J. (1957). Plant Diseases Due to Bacteria. Second Ed. p. 23. Cambridge University Press, London.

Dvorockova, I. (1990). **Aflatoxins and Human Health. pp. 469-474.** CRC Press, Boca Raton, Fla.

Eaton, L.D. and E.P. Gallagher (1994). Mechanisms of aflatoxin carcinogenesis. **Annual Rev. Pharmacol. 34: 135-172.**

Ferreira, J.H.S.; F.N. Matthee and A.C. Thomas (1991). Biological control of *Eutpa lota* on grapevine by antagonistic strain *Bacillus subtilis*. **Phytopathology 81: 283-287.**

Hara, S.; D.L. Fennel and C.W. Hesseltine (1974). Aflatoxin producing strains of *Aspergillus flavus* detected by fluorescence of agar medium under ultraviolet light current. **Advance of Applied Microbiology 72: 1118-1123.**

Hayes, C.K. (1992). **Improvement of *Trichoderma* and *Gliocladium* by Genetic Manipulation. pp. 227-266.** In: Biological Control of Plant Diseases Progress and Challenges for the Future. Thamos, E.C.; G.C. Papuvizas and R.J. Cook (eds.), Plenum Press, New York.

Hesseltine, C.W. (1965). Amillennium of fungi, food and fermentation. **Mycologia 57: 149-197.**

Hwang, S.F.; K.F. Chang; R.J. Howard; B.A. Deneka and G.D. Turnbull (1996). Decrease in incidence of Pythium damping off of field pea by seed treatment with *Bacillus sp.* and metalaxyl. **Zeitschrift-fur- pflanzenenkrankheiten Und Pflanzanschutz. 10(1): 31-34, (c.f. C.A.B 1996-1998).**

Kotan, R.; W. Dikbas and H, Bostan (2009). Biological control of post harvest disease caused by *Aspergillus flavus* on stored lemon fruits. **African Journal of Biotechnology 8: 209-214.**

Krishnamurthy, Y.L. and J. Shashikala (2006). Inhibition of aflatoxin B production of *Aspergillus flavus*, isolated from soybean seeds by certain natural plant products. **Let. App Microbial. 43: 469-474.**

Lamb, M.C. and D.A. Sternitzke (2001). Cost of aflatoxin to the farmer, buying point, and sheller segments of the southeast United States peanut industry. **Peanut Sci., 28: 59-63.**

Mahmoud, E.Y. (2004). **Integrated Control of Pod Rot Disease of Peanut. pp. 22-36.** Ph.D. Thesis, Fac. of Agric., Ain Shams Univ., Cairo.

Norio, K. and H. Susumu (1988). Inhibitory strains of *Bacillus subtilis* for growth and Aflatoxin production of Aflatoxigenic fungi. **Agricultural and Biological Chemistry 52: 1173-1179.**

Padares, D.F.; J. Hockenhull; D.F. Jensen and S.B. Marthur (1992). In vivo screening of *Trichoderma* isolated for antagonism against *Sclerotium rolfsii* using rice seedlings. **Bulletin oil B/SROP, 15: 33-35. (c.f. Rev. Pl. Pathol., 72: 208).**

Petchkongkaew, A.; P. Taillandier; P. Gasaluck and A. Lebrihi (2008). Isolation of *Bacillus* spp. from Thai fermented soybean (Thuanao): Screening for aflatoxin B1 and ochratoxin A detoxification. **J. App. Microbiol. 104: 1495-1502.**

Reddy, K.R.N.; C.S. Reddy and K. Muralidharan (2009). Potential of botanicals and biocontrol agents on growth and Aflatoxin production by *Aspergillus flavus* infecting rice grains. **Food Control 20: 173-178.**

Rodriguez, R.F. and A.M. Cotes (1999). Biological control of the bean root rot (*Rhizoctonia solani* Kuhn) using pre fermentation control in the presence of *Trichoderma koningii* Oudemans. **Scolfi-informa. 25: 3-4 (c.f. CAB. 1998-1999).**

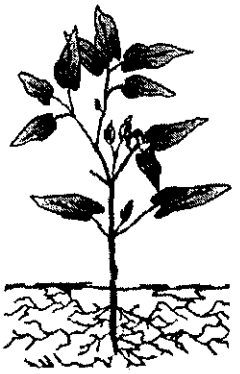
Ryder, M.H.; Z.H. Yan; T.E. Terrace; A.D. Rovira; W. Tang; R.I. Correll; Z. Yan and W. Tang (1999). Use of strain of *Bacillus* isolated in China to suppress take all and Rhizoctonia root rot and promote wheat seedling growth of glasshouse grown wheat in Australian soil. **Soil Biology and Biochemistry. 31: 19-29.**

Sanz, L.M. Montero; I. Grondona; A. Loiobell and E. Monte (2002). *In vitro* antifungal activity of *Trichoderma harzianum*, *T. longibrachiatum*, *T. asperellum* and *T. atroviride* against *Botrytis cinerea* pathogenic to strawberry. **Bulletin-oil B/SROP. 25: 253-256.**

Turner, W.B. (1971). **Fungal Metabolites. pp. 234-320.** Academic Press, New York.

Van Egmond, H.P. (1995). Mycotoxins, regulations, quality assurance and reference materials. **Food Addit. Contam. 12: 321-330.**

Wood, G.E. and M.W. Trucksess (1998). Regulatory control programs for mycotoxin-contaminated food. In: Sinha, K.K. and D. Bhatnagar, (eds.), **Mycotoxins in Agriculture and Food Safety. pp. 459-481**. Marcel Dekker, New York.



حوليـــات العلـــوم الزراعية
جامعة عين شمس ، القاهرة
مجلد(٥٥)، عدد (١)، ١٠٩-١١٩، ٢٠١٠

تأثير بعض الكائنات الحيوية على تثبيط السموم المفرزه بواسطة فطر *Aspergillus flavus* فى قرون الفول السودانى

[٩]

مايسة لطفى عبد المنعم[١] - عبد الرحمن فرحات طلبه[١] - احمد ممدوح جمعة[٢]

١- المعمـــل المركـــزى للزراعـــة العضويـــة - مركـــز البحـــوث الزراعية - جيزة - مصر

٢- المعمل المركزى لتحليل متبقيات المبيدات و العناصر الثقيلة فى الاغذية - الدقى - القاهرة

الموجـــز

درست القـــدره التـــضاديه لكـــائنين نـــافعين وهمـــا *Trichoderma harzianum, Bacillus subtilis* ضد فطر *A. flavus* تحت ظروف المعمل. كما تم استخدام كـــلا منها على شكل مسحوق جاف يحتوى كل جرام منـــه على $30x10^6$ وحدة تكاثر مـــن اى مـــن الكائنـــات المذكوره وعوملت بها قرون الفول السودانى المعدى بالفطر الممرض *A. flavus* وذلك لدراســـة تأثيرهمـــا على تثبيط انتاج السموم فى قرون الفـــول الـــسودانى تحت ظروف التخزين العاديه.

استخدمت مساحيق الكائنات المضادة بمعـــدلات استخدام مختلفة وعدد مرات استخدام مختلفة للوقوف على احسن جرعه وعلى احسن عدد مرات اســـتخدام فى تثبيط انتاج المواد السامه اثناء فترة التخزين تحت ظروف المعمل لمدة ثلاثة اشهر.

أوضـــحت النتـــائج المتحـــصل عليهـــا وجـــود تأثيرتضادى لكل من كائنى المقاومـــة علـــى النمـــو الخطى لفطـــر *A. flavus*. أظهرفطـــر *T. harzianum* تأثير افضل وأعطي نسبة خفض فى النمـــو الخطـــى للفطر *A. flavus* قدرت ٧٧,٧ %. اوضحت النتائج أن كل المعاملات أدت الى خفض انتاج السموم الفطريه المختلفة (وهى اربعة انواع ينتجها فطـــر *A. flavus*) فى قرون الفول السودانى وكـــان *T. harzianum* هـــو الافضل فى خفض كميات السموم الفطريـــه المنتجـــة بواسطة فطر *A. flavus*. واعطى نسبة خفض تـــصل الى ٨٧,٨ % وذلك بعد ثلاثة شهور مـــن المعاملـــة بينما اعطت بكتيريا *B. subtilis* نسبة خفض تصل الى ٧٦,١٨ % اذا ما قورنت بمعاملة الكنتـــرول. كمـــا اظهرت النتائج ان هناك علاقة طرديـــة بـــين مـــدة التخزين لقرون الفـــول السودانى و زيادة انخفـــاض افراز السموم. وقد وجـــد ان القرون المعاملة بفطـــر *T. harzianum* مرة واحدة اعطت افضل النتائج وذلك مقارنة بالمعاملة مـــرتين او ثـــلاث مـــرات . بينمـــا المعاملة *B. subtilis* مرتين او ثـــلاث مـــرات كانـــت الافضل فى خفض كمية السمـــوم المنتجة بواسطـــة *A. flavus* .

استخدمت جرعـــات مختلفة من *T. harzianum* او *B. subtilis* و قد اظهرت النتائج ان المعاملة بالجرعة ٢٠جم / كجم قرون كانت افضل جرعة فى خفـــض كمية السموم المنتجة مقارنة باستخدام الجرعات ١٠ ا و ٣٠جم / كجم قرون.

كما اظهرت النتائج ان هناك علاقة موجبة بـــين مدة التخزين لقرون الفول السودانى و زيادة التـــأثير المثبط لانتاج السموم الفطريه.

تحكيـــم: أ.د مصطفى حلمى مصطفى
أ.د توفيق حافظ عبد المعطى