J. Agric. Sci. Mansoura Univ., 29 (11): 6537 – 6547, 2004

EFFECT OF AFLATOXINS ON N_2-FIXER BACTERIA AND RESPONSE OF INFESTED PEANUT SEEDS WITH *Aspergillus flavus* TO BIOLOGICAL CONTROL

Emara, H.A.; A.A. Abo El-Soud and M. Faten Mohamed
Soils, Water and Environment Res. Inst., Agric. Res. Center, Giza.

ABSTRACT

Under lab conditions, an experiment was conducted to evaluate biological effect of aflatoxins on diazotrophs. Also, a greenhouse experiment was executed to study the effect of dose and time of *Bacillus pumilus* as a biocontrol agent application on *Aspergillus flavus* infested peanut yield inoculated with *Bradyrhizobium* sp. Results indicated that *Azotobacter* and *Azospirillum* were more sensitive to aflatoxins B1, B2, G1 and G2. However, *Rhizobium* and *Bradyrhizobium* were only affected slightly by the aflatoxins B1 and B2 but were most resistant against G1 and G2.

Data of pots experiment showed that *Bacillus pumilus* caused a reduction in *Aspergillus* infested peanut. This treatment decreased aflatoxin accumulation in root zone and so enhanced nodulation, growth, yields and crude protein of peanut plants. The reduction effect increased with the increasing of bacterial dose. The maximum reduction of aflatoxin (62%) was obtained when the bacterial dose was 80 ml/pot. Also, results indicated that the highest values of nodulation, growth, pod and straw yields were obtained with *Bradyrhizobium* seed coating plus *Bacillus pumilus* when applied at 40 ml/pot after 20 days and 30 days of planting. Data revealed that the maximum inhibition (70%) of aflatoxin formation was obtained when two doses of *Bacillus pumilus* were added (40 ml/pot of each). The first dose at planting and the second dose after 40 days of planting.

Keywords: *Bradyrhizobium* sp., *Rhizobium*, *Azotobacter*, *Azospirillum*, Aflatoxin, Peanut, *Arachis hypogea* (*Arachis*), *Aspergillus flavus*, *Bacillus pumilus*.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is often invaded before harvested by *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi produce aflatoxins which are potant carcinogens, mutagens, teratogeas and hepatatoxins (Betina, 1989). Soil serves as a reservair for the two *Aspergillus* species (Horn *et al.*, 1995; Horn and Darner, 1998). Since, peanut fruit is formed underground, the pods are in direct contact with soil fungal populations. Pods are most susceptible to invasion by aflatoxigenic fungi and subsequent aflatoxin contamination under conditions of late-season drought and elevated soil temperature (Dorner *et al.*, 1989). *A flavus* is the dominant species in peanuts as well as in aerial crops such as corn and cotton seeds. However, *A. parasiticus* also may be an important contribution to aflatoxin contamination, particularly in peanut (Schroeder and Boller, 1973).

The interactions between plants and microorganisms in the rhizosphere could affect crop yields and improve the biological properties of the soils. Fernando and Linderman (1995) found that *Brevibacterium linens* (DF-3101), *Bacillus thuringiensis* (DF-7107) and *Bacillus pumilus* (DF-1481) could suppress cowpea disease caused by *Pseudomonas vignae*. Significant reduction of chickpea wilt disease caused by *F. oxysporum* was estimated

with soil treated with *Bacillus subtilis* strain AFI (Dileep-Kuman, 1999). Kazmar *et al.* (2000) reported that *Bacillus cereus* exhibited beneficial effects on soybean nodulation and yield, as well as, suppression of damping-off disease of alfalfa.

Various management strategies are necessary to reduce aflatoxin levels to within regulatory limits for animal, losses to peanut growers, shellers and processors (Horn *et al.*, 2000).

The results of peanut plants grown under field conditions by Abo El-Soud *et al.* (2004) demonstrated that *Bradyrhizobium* inoculation alone or combination with *Bacillus pumilus* and/or *Serratia marcescens* increased the nodulation status, as well as, shoot dry weight and nitrogen content also, pods, seeds and straw yields.

This work aimed to study the biological effect of aflatoxin on some diazotrophic bacteria using the modified agar-plate-inhibition-zone technique. Also, a greenhouse experiment was executed to study the effect of dose and time of *Bacillus pumilus* as biocontrol agent application on *Aspergillus flavus* infested peanut yield.

MATERIALS AND METHODS

Microorganism cultures :

The following bacterial and fungal strains were provided from Microbiology Dept., Soils, Water and Environ. Res. Inst., Agric. Res. Center, Giza, Egypt: *Azotobacter chroococcum* Az1, *Azospirillum brasilense* SP2, *Rhizobium leguminosarum* bv. *viceae* ICARDA 441, *Bradyrhizobium* sp. (*Arachis*) ARC 601, *Asperagillus flavus* NRRL 3251 and *Bacillus pumilus* NRRL NRS 272

Preparation of bacterial inoculum :

Five different broth and agar media were used throughout the current investigation namely: Ashby medium (Hegazi & Niemela, 1976) for *Azotobacter*, malate N-deficient medium (Döberiner *et al.*, 1979) for *Azospirillum*, yeast extract mannitol medium (Vincent, 1970) for *Rhizobium* or *Bradyrhizobium*, Czabek's -Dex medium (Oxoid, 1982) for *Aspergillus flavus* and King's medium (Fernando & Linderman, 1995) for *Bacillus pumilus*.

Bacterial inocula were prepared by inoculating with the tested strain and incubated at 28 °C for 24-48 hours as static culture. One ml of inoculum preparation was added to 100 ml of malted and cooled (50 °C) suitable agar medium for each bacterial type. After inoculation, the agar media were swirled to ensure uniform cell distributed and 10 ml of the seeded agar were then asptically pipetted into sterile petri dishes and allowed to solidify.

Assay procedure :

The method used in the present study (lab. experiment) was as follows: discs of filter paper (diameter of 5 mm) were prepared containing 2, 4, 6, 10, 15 and 20 □g of aflatoxins B1, B2, G1 or G2 solution (aflatoxin-chloroform). Discs containing 10 or 20 □l of only chloroform were prepared and used as control. The discs were allowed to dry for approximately 10 min and then four replicate discs in each case were evenly placed on the agar surface of tested bacterial seeded plate. The plates were then inverted and

pre-incubated at 10 °C for 30 min to allow uniform diffusion into agar. After pre-inocubation, plates were incubated for 24 hours at 28 °C. The inhibition zone between the disc and the bacterial mass were then measured.

The Pot experiments :

Pot experiment under greenhouse conditions was designed during summer growing season of 2002 to study the antagonistic effect of *Bacillus pumilus* against *Aspergillus flavus* infested peanut. Also, *Bacillus pumilus* as a bacterial growth promoter was studied.

Two pot experiments were conducted under wire proof greenhouse experiment at Agricultural Research Center, Giza. The soil used in pot trial was sampled from the top 20 cm layer of the Experimental fields of Ismailia, sieved through 2 mm screen and air dried. The main physico-chemical characteristics (Richard, 1954; Jackson, 1973) of the soil are present in Table (1). Plastic pots (30 cm diameter) were filled with 10 kg of this sandy soil. Superphosphate (15.5% P_2O_5) was applied before sowing at a rate of 100 kg fertilizer/fed.

Table 1:Mechanical and chemical properties of soil used in a greenhouse experiment.

Properties		Values
Sand	%	88.45
Silt	%	4.34
Clay	%	6.22
$CaCO_3$	%	0.57
Textural class		Sandy
pH		7.30
E.C. (dS m^{-1})		0.42
O.C.	%	0.09
T.N.	%	0.026
Soluble cations (meq l^{-1})		
Ca^{++}		1.82
Mg^{++}		0.64
Na^{+}		0.97
K^{+}		0.76
Soluble anions (meq l^{-1})		
CO_3^{--}		0.00
HCO_3^{-}		1.60
Cl^{-}		0.86
SO_4^{--}		1.63

The first experiment aimed to study the influence of different doses of *Bacillus pumilus* (*Bp*) on *Aspergillus flavus* infested peanut seed. The following treatments were conducted:

1 - Control without *Bp* application and seed coating (*Brdy*)
2 - 10 ml of *Bp* /pot and seed coating (*Brdy*)
3 - 20 ml of *Bp* /pot and seed coating (*Brdy*)
4 - 40 ml of *Bp* /pot and seed coating (*Brdy*)
5 - 80 ml of *Bp* / pot and seed coating (*Brdy*)
6 - Control without *Bp* application and soil inoculation (*Brdy*)
7 - 10 ml of *Bp* /pot and soil inoculation (*Brdy*)

8 - 20 ml of *Bp* /pot and soil inoculation (*Brdy*)
9 - 40 ml of *Bp* /pot and soil inoculation (*Brdy*)
10 - 80 ml of *Bp* /pot and soil inoculation (*Brdy*)
Before planting, seeds of peanut were infested with spores of *Aspergillus flavus* (8×10^4/ml).
The second experiment to study time of *Bacillus pumilus* application. The following treatments were conducted:
1 - Control, without (*Bp*) application
2 - 40 ml of suspention (*Bp*) /pot at planting
3 - 40 ml of suspention (*Bp*) / pot, 10 day after planting (DAP)
4 - 40 ml of suspention (*Bp*) / pot, 20 DAP
5 - 40 ml of suspention (*Bp*) / pot, 30 DAP
6 - 40 ml of suspention (*Bp*) / pot, 40 DAP
7 - 40 ml of suspention (*Bp*) / pot, at planting and 10 DAP
8 - 40 ml of suspention (*Bp*) / pot, 10 DAP and 20 DAP
9 - 40 ml of suspention (*Bp*) / pot, 20 DAP and 30 DAP
10 - 40 ml of suspention (*Bp*) / pot, 30 DAP and 40 DAP

Before planting, seeds of peanut were infected with spores of *Aspergillus flavus* and inoculated with *Bradyhizobium* spp. (*Arachis*) strain ARC 601 (seed coating).

After germination, pots were fertilized with ammonium sulphate (20.5% N) at a rate of 20 kg N/fed as starter nitrogen dose and potassium sulphate (48% K_2O) fertilizers at a rate of 50 kg fertilizer/fed.
Pots were arranged in the greenhouse in a complete randomized block design with four replications. During the experimental period, tap water was added to keep soil moisture 70% of water holding capacity.

After 100 days of sowing, plants were carefully uprooted to determine the biological yield as well as pod and straw weight. The percentage of crude protein of seed and straw were determined (Page *et al.*, 1982). Obtained data were subjected to analysis of variance (ANOVA) according to the procedure of Snedecor and Cocharn (1980).

Also, aflatoxins in pods were determined according to method of FAO & UNEP (1989) and FAO (1990).

RESULTS AND DISCUSSION

Laboratory experiment:

The capability of the different diazotrophic preparations to tolerate the antagonistic impact of aflatoxins was investigated using the modified agar-plate-inhibition-zone technique. Different concentration of aflatoxins towards a number of symbiotic and non-symbiotic diazotroph members were monitored.

Results presented in Table (2) indicate that *Azotobacter* and *Azospirillum* were more sensitive to aflatoxins B1, B2, G1 and G2. However, *Rhizobium* and *Bradyrhizobium* were only affected slightly bag the aflatoxins B1 and B2. They were most resistant against G1 and G2. Therefore, the four tested organisms could be arranged according to their sensitivity to the tested aflatoxins as : *Azotobacter* > *Azospirillum* > *Rhizobium* > *Bradyrhizobium*.

Table 2: The inhibition zone (mm*) of associative diazotrophs, rhizobial and bradyrhizobial growth due to aflatoxin (B1, B2, G1 and G2)

Aflatoxin types	Conc. µg/disc	*Azotobacter chroococcum*	*Azospirillum brasilense*	*Rhizobium*	*Bradyrhizobium*
B1	2	5	0	0	0
	4	6	4	0	0
	6	8	5	5	0
	8	8.5	6	6	0
	10	10	8	7	4
	15	12	11	9	5
	20	13	12	10	8
B2	2	0	0	0	0
	4	0	6	0	0
	6	4	4	0	0
	8	6	5	0	0
	10	7	6	4	4
	15	9	8	6	5
	20	10	11	8	7
G1	2	0	0	0	0
	4	0	0	0	0
	6	0	0	0	0
	8	4	0	0	0
	10	5	6	0	0
	15	6.5	7	0	0
	20	8	8	0	0
G2	2	0	0	0	0
	4	0	0	0	0
	6	0	0	0	0
	8	0	0	0	0
	10	4	4	0	0
	15	6	5	0	0
	20	8	7	0	0

*** Values represent means for triplicate assayes.**
0 Negative effect (not detected)

Variable inhibition actions of aflatoxins on the growth of *Azotobacter*, *Azospirillum*, *Rhizobium* and *Bradyrhizobium* were detected. The different responses of the tested bacteria to aflatoxins may be related to the inhibitory mode of action of the toxin on the tested bacteria, which is known to be activated by different exoenzyms derived from bacteria. The varied effects of aflatoxins on bacteria may be also attributed all formation, mRNA transcription inhibition, inhibited incorporation of precursors into DNA, RNA and proteins and blocked induction as well as production of various enzymes at various levels (Betina, 1989). Accordingly, bacteria and other microorganisms could be used for the detection and quantification of aflatoxins production. The present findings as well as their explanations are in agreement with those obtained by Emara (1996) and Munimbozi & Bullerman (1998).

The obtained results also indicate that *Bradyrhizobium* was more resistant nitrogen fixing bacteria among the tested microorganisms in the present work against aflatoxins. This hypothesis led to use *Bradyrhizobium* as a nitrogen fixer in the present investigation, which aimed to examine the suitable inoculum dose and suitable time of inoculation for *Bacillus pumilus*

as a biological agent for controlling *Aspergillus flavus* infection and its production of aflatoxins.

The pot experiments:

The complementation of bradyrhizobial inoculation and bacterial biocontrol treatment was investigated under two pot experiments. The first one, where the different dose of *Bacillus pumilus* were applied to *Aspergillus flavus* infested peanut seeds either by seed coating or over head soil addition of *Bradyrhizobium* was introduced into peanut soil system in two different methods. The second experiment was conducted to find out the suitable time of application of *Bacillus pumilus* on the yield of peanut.

Data of the first experiment presented in Table (3) show the effect of inoculation with *Bradyrhizobium* sp. (*Arachis*) and *Bacillus pumilus* doses application on nodulation of *Aspergillus* infested peanut. Low number of nodules and dry weight being 16 nodules/plant and 217.8 mg nodule/plant, respectively, were recorded with inoculated and infested seeds without applying *Bacillus* (control). Significant increases of nodules number and dry weight for seed coating treatment with *Bradyrhizobium* sp inoculation combined with applied *Bacillus* (80 ml/pot) as compared with control. These increases reached to 131.3% for nodule number and 83.7 % for nodule dry weight over the control. Under soil inoculation with *Bradyrhizobium* and *Bacillus pumilus* applied, increases of nodules number and dry weight were also detected. These increases were 157.1 and 83.5% for number and dry weight of nodule, respectively over the control. There were non-significant differences with *Bacillus pumilus* 40 ml or 80 ml doses applied on nodulation were recorded.

Concerning the growth of peanut, data presented in Table (3) showed that unapplied *Bacillus* (control) recorded low shoot dry weight and nitrogen content (9.6 g/plant and 208.9 mg N/plant, respectively). Applied of *Bacillus pumilus* in dose of 80 ml/pot produced dry weight and nitrogen content of shoot of 21.3 g/plant and 509.2 mg N/plant, respectively for the seed coating *Bradyrhizobium* inoculation. Also, *Bradyrhizobium* over head soil addition and applied the 80 ml/pot produced more pod and straw yields of 20.7 g/plant and 502.5 mg N/plant, respectively.

Inoculation with *Bradyrhizobium* and applying *Bacillus* at different doses recorded highly significant increases in shoot dry weight and N-content as compared to control. These increases were 117.3 or 115.6% under seed or soil bradyrhizobial inoculation for shoot dry weight and were 104.6 or 118.4% under seed or soil bradyrhizobial inoculation for shoot N-content, respectively. While, there were insignificant increases in shoots dry weight with *Bacillus pumilus* inoculation at different doses.

To study the suitable addition of *Bacillus* pumilus in the second experiment, results in Table (4) revealed that the highest values on number and dry weight of nodules (26 nodule/plant and 359.5 mg/plant, respectively) were obtained with *Bradyrhizobium* seed coating plus *Bacillus pumilus* applied at two doses 40 ml/pot after 20 days of planting and 40 ml/pot after 30 days of planting.

Concerning the growth of peanut, data presented in Table (4) showed that control recorded the lowest values of dry weight and nitrogen

Table 3: Effect of inoculated *Bradyrhizobium* sp. (*Arachis*) and *Bacillus pumilus* doses application on biological parameters of pot-grown *Aspergillus* infested peanut.

Treatments	Nodulation		Shoots	
	Number /plant	DW (mg/plant)	DW (g/plant)	N-content (mg/plant)
Seed inoculated with *Bradyrhizobium*				
Control	16	217.8	9.8	208.9
Bacillus pumilus (10 ml/pot)	26	306.1	16.4	392.2
Bacillus pumilus (20 ml/pot)	28	375.8	15.2	357.9
Bacillus pumilus (40 ml/pot)	33	382.4	18.7	455.0
Bacillus pumilus (80 ml/pot)	37	400.1	21.3	509.2
Soil inoculated with *Bradyrhizobium*				
Control	14	215.9	9.6	230.1
Bacillus pumilus (10 ml/pot)	24	263.6	12.4	292.5
Bacillus pumilus (20 ml/pot)	24	325.9	15.9	379.1
Bacillus pumilus (40 ml/pot)	32	383.3	19.3	468.7
Bacillus pumilus (80 ml/pot)	36	396.1	20.7	502.5
L.S.D. 0.05	5.0	50.9	3.3	80.9

content of shoot. Applied the *Bacillus pumilus* in the double doses after 30 and 40 days of planting (40 ml/pot of each) recorded the highest shoot dry weight (20.5 g/plant) and its nitrogen content (663.3 mg N/plant).

There are non-significant difference between applied the bacteria in the double doses at different times, at planting, 10, 20, 30 or 40 days of planting.

At harvest, regarding to pod and straw yields, they were significantly affected by *Bacillus pumilus* doses treatments (Table 5). Untreated peanut produced the lowest pod yield (2.3 g/pot and 4.0 g/pot for seed coating or over head soil addition, respectively).

Applied of *Bacillus pumilus* in dose of 80 ml/pot (Table 5) produced pod and straw yields of 7.6 and 44.7, respectively for the seed coating of *Bradyrhizobium* inoculation. Also, *Bradyrhizobium* over head soil addition and applied the 80 ml/pot produced more pod and straw yields of 7.7 and 35.7, respectively.

Peanut contained variable quantities of proteins in their seed (16.2 – 20.2%) and straw (7.4 – 8.5). Significant increases in protein contents were attributed to different doses of *Bacillus pumilus* application. Under seed coating of *Bradyrhizobium* inoculation, the 80 ml/pot was the superior among the other doses of bacteria applied 20.0 and 8.5% crude protein was estimated for seed and straw yield, respectively. Untreated plants showed the lowest amount of protein (16.2% and 7.4% for seed and straw yield, respectively).

Regardless to doses of application of *Bacillus pumilus*, data in Table (5) also acheived significant increases of pods straw yields due to *Bradyrhizobium* over head soil inoculation than seed coating.

Results in Table (6) showed high values of pods, and straw yields (6.4 and 47.9 g/pot, respectively) were obtained with *Bradyrhizobium* seed coating plus *Bacillus pumilus* applied at two doses 40 ml/pot after 20 days of planting and 40 ml/pot after 30 days of planting.

Applied *Bacillus pumilus* after 20, 30 or 40 days of planting recorded

Table 4: Effect of inoculated *Bradyrhizobium* sp. (*Arachis*) and time of application *Bacillus pumilus* on biological parameters of pot-grown *Aspergillus* infested peanut.

Treatments	Nodulation		Shoots	
	Number /plant	DW (mg/plant)	DW (g/plant)	N-content (mg/plant)
Seed inoculated with *Bradyrhizobium*				
Control	16	217.8	9.8	208.9
40 ml/pot *Bp* at planting	23	241.2	12.9	385.1
40 ml/pot *Bp* 10 DAP	21	262.8	13.7	427.4
40 ml/pot *Bp* 20 DAP	22	310.8	16.5	502.1
40 ml/pot *Bp* 30 DAP	24	281.7	16.7	514.2
40 ml/pot *Bp* 40 DAP	22	289.7	17.2	531.7
40 ml/pot *Bp* at planting and 10 DAP	22	265.9	17.6	555.3
40 ml/pot *Bp* 10 DAP and 20 DAP	25	333.7	20.3	650.2
40 ml/pot *Bp* 20 DAP and 30 DAP	26	359.5	20.4	652.8
40 ml/pot *Bp* 30 DAP and 40 DAP	25	345.0	20.5	663.3
L.S.D. 0.05	4	42.2	3.4	50.9

significant increases of pods and straw peanut yields over the control, applied at planting or 10 days of planting.

In the single or the second dose, there are non-significant differences between applying the bacteria at different time, except the control, applying at planting or 10 days of planting.

Concerning crude protein percentage, data showed high crude protein of seed (20.2%) was recorded in the two equal doses 40 ml/pot after 20 days of planting and 30 days of planting treatment (Table 6).

High crude protein of straw (8.6%) was recorded in 40 ml/pot after 10 days of planting and 40 ml/pot after 20 days of planting treatment.

It could be concluded that *Bacillus pumilus* application at rate of 40 ml/pot at two doses caused a reduction in *Aspergillus* infested peanut, with a decrease in aflatoxin accumulation in root zone and so enhanced nodulation, growth, yields and crude protein of peanut plants.

For aflatoxins, the present results indicate that *Bacillus pumilus* decreased aflatoxin accumulation in seed and these reduction effect increased with the increasing of bacterial dose (Table 5). The maximum reduction of aflatoxin (62%) was obtained when the bacterial dose was 80 ml/pot. The results also, indicate that no-significant differences between the two treatments of *Bradyrhizobium* (seed inoculated or soil application).

The addition time of *Bacillus pumilus*, results in Table (6) revealed that the maximum reduction (70%) of aflatoxin was obtained when two doses of *Bacillus pumilus* were added (40 ml/pot of each). The first dose at planting and the second dose after 40 days of planting.

The inhibiting of aflatoxin by *Bacillus pumilus* was likely due to extracellular metabolites produced by the bacterium and possible competition for nutrients and/or space between the bacterium and the mold. However, inhibitory effect due to possible organic acids such as lactic acid which may have been produced in the growth medium can not be excluded. Also, *Bacillus pumilus* may produce antifungal compound(s) inhibiting mycelial growth and mycotoxin. More investigations in these point are required. These results and explanations are in agreement with those of Kimura & Hirano

(1988) who reported that *Bacillus subtilis* inhibited aflatoxin in corn and peanuts by more than 95%. Also, Munimbozi & Bullerman (1998) reported that 99.9% of aflatoxin was inhibited by *Bacillus pumilus* in yeast extract sucrose broth medium.

Table 5: Effect of inoculated *Bradyrhizobium* sp. (*Arachis*) and *Bacillus pumilus* doses application on pods, straw yields and its crude protein (%) of pot-grown *Aspergillus* infested peanut.

Treatments	Weight (g/pot)		Crude protein (%)		Aflatoxin µg/g pods
	Pods	Straw	Seed	Straw	
Seed inoculated with *Bradyrhizobium*					
Control	2.3	13.2	16.2	7.4	80.0
Bacillus pumilus (10 ml/pot)	5.4	26.4	19.4	8.0	80.0
Bacillus pumilus (20 ml/pot)	5.2	33.8	19.6	8.2	72.0
Bacillus pumilus (40 ml/pot)	6.8	40.6	19.9	8.4	58.0
Bacillus pumilus (80 ml/pot)	7.6	44.7	20.0	8.5	32.0
Soil inoculated with *Bradyrhizobium*					
Control	4.0	31.3	17.0	7.6	80.0
Bacillus pumilus (10 ml/pot)	5.1	43.8	19.7	8.2	78.0
Bacillus pumilus (20 ml/pot)	7.4	40.9	19.9	8.4	70.0
Bacillus pumilus (40 ml/pot)	7.1	42.0	20.0	8.5	80.0
Bacillus pumilus (80 ml/pot)	7.7	35.7	20.2	8.5	30.0
L.S.D. 0.05	2.1	11.9	1.7	0.4	-

Table 6: Effect of inoculated *Bradyrhizobium* sp. (*Arachis*) and time of application of *Bacillus pumilus* on pods, straw yields and its crude protein (%) of pot-grown *Aspergillus* infested peanut.

Treatments	Weight (g/pot)		Crude protein (%)		Aflatoxin µg/g pods
	Pods	Straw	Seed	Straw	
Control	2.3	13.2	16.2	7.4	80
40 ml/pot *Bp* at planting	4.0	36.7	19.5	8.1	58
40 ml/pot *Bp* 10 DAP	3.9	43.8	19.8	8.4	55
40 ml/pot *Bp* 20 DAP	6.3	46.9	19.9	8.4	59
40 ml/pot *Bp* 30 DAP	6.4	45.3	19.8	8.4	65
40 ml/pot *Bp* 40 DAP	6.3	39.8	19.7	8.4	77
40 ml/pot *Bp* at planting and 10 DAP	6.4	34.9	20.0	8.6	24
40 ml/pot *Bp* 10 DAP and 20 DAP	5.0	32.6	20.1	8.6	62
40 ml/pot *Bp* 20 DAP and 30 DAP	6.4	47.9	10.2	8.0	50
40 ml/pot *Bp* 30 DAP and 40 DAP	6.1	29.5	19.6	8.5	64
L.S.D. 0.05	1.7	11.3	1.4	0.43	-

Previous studies by Abo El-Soud *et al.* (2004) indicated that both *Serratia marcescens* and *Bacillus pumilus* caused a reduction ranged from 37 to 42% in spores infested seeds and from 15 to 21% with aflatoxin infested seed, respectively. In the field experiment the reduction of aflatoxin by either *Bacillus* or *Serratia* were 43.00 and 28.35%, respectively.

From afrementioned results, it could be concluded that combined inoculation with *Bradyrhizobium* and *Bacillus pumilus* (in the two equal doses 40 ml/pot at planting and after 10 days of planting) reduced the aflatoxin effects and enhanced nodulation, growth, yields and crude protein of peanut in *Aspergillus flavus* infested peanut plants.

REFERANCE

Abo El-Soud, A.A.; H.A. Emara and Elham I. El-Khatib (2004). Biological control of *Aspergillus flavus* infested peanut seeds and their effected on nitrogen fixation by *Bradyrhizobium*. Egypt. J. Appl. Sci., 19: 76-87.

Betina, V. (1989). Mycotoxins: Chemical, Biological and Environmental Aspects. Elsevier, pp. 75-79.

Dileep-Kuman, B.S. (1999). Fusarial wilt suppression and crop improvement through two rhizobacterial strains in chickpea growing in soils infested with *Fusarium oxysporum*. Biol. Fertil. Soils, 29: 87-91.

Dőbereiner J., Reis V.M.‹ Paula M.A. and Obivases F. (1993) Endophytic diazotrophs in sugar cane, cereals and tuber plants. In: New Horizon in Nitrogen Fixation. Palacios, R.‹ Mora, J. and Newton, W.E. (Eds). Dordrecht, Kluwer Academic Publishers, pp. 671-676.

Dorner, J.W.; R.J. Cole; T.H Sanders and P.D. Blankenship (1989). Interrlationship of kernel water activity, soil temperature naturity and phytoalexin production in preharvest aflatoxin contamination of drought stressed peanuts. Mycopathologia, 105: 117-128.

Emara, H.A. (1996). Production of aflatoxin by *Aspergillus parasiticus* and its control. Ph. D. Thesis, Univ. of Striling, Scotland, U.K.

FAO & UNEP (1989). Sampling of agricultural products and their analysis for aflatoxin determination. Center of International Project. The USSR State Committee for Environment Protection, Mosscow.

FAO (1990). Manuals of food quality control. 10- Training in Mycotoxins Analysis. FAO food and nutrition paper 14/10. FAO, Roma.

Fernando, W.G.D. and R.G. Linderman (1995). Inhibition of phytophthora vignae and stem and root rot of cowpea by soil bacteria. Biol. Agric. and Horticul., 12: 1-14.

Hegazi N.A. and Nemiela S. (1976). A note on the estimation of *Azotobacter* densities by membrane filter technigue. J. Appl. Bacteriol., 41, 311-313.

Horn, B.W. and J.W. Dorner (1998). Soil population of *Aspergillus* species from section flavi along a transect through peanut-growing redions of the utiled states. Mycologia, 90: 797-776.

Horn, B.W.; R.L. Greene and J.W. Dorner (1995). Effect of corn and peanut cultivation on soil populations of *Aspergillus flavus* and *A. parasiticus* in southwestern gieorgia. Appl. Environ. Microbiol., 61: 2472-2475.

Horn, B.W.; R.L. Greene; R.B. Sorensen; P.D. Blankenship and J.W. Dorner (2000). Conidial movement of nontoxigenic *Aspergillus flavus* and *A. parasiticus* in peanut fields following application to soil. Mycopathologia, 151: 81-92.

Jackson, M.L. (1973). Soil Chemical Analysis. Constable Co. London, Prentice Hall Inc., Englwood Cbiffs, New Jersy.

Kazmar, Elizbeth R.; R. M. Goadman; G.R. Grau; D.W. Johnson; E.V. Nordheim; Daniel J. Undersonder and J. Handelsman (2000). Regression analysis for evaluating the influence of *Bacillus cerus* on

alfalafa yield under varaible disease intenstiy. Amer. Phytopathol. Soc., 90: 657-665.

Kimura N. and S. Hirano (1988). Inhibitory strains of *Bacillus subtilis* for growth and aflatoxin production of aflatoxigenic fungi. J. Agric. Biol. Chem., 52: 1173-1179.

Munimbozi, C. and L.B. Bullerman (1998). Inhibition of aflatoxin production of *Aspergillus parasiticus* NRRL2999 by *Bacillus pumilus*. Mycopathologia, 140: 163-169.

Oxoid, (1982). The oxoid manual of culture media, ingredients and other lab. Services. Oxoid, Limited Turnergraphic Ltd, England, Fifth ed.

Page, A.L.; R.H. Miller and D.R. Keeney (1982). Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties, Second Edition, Amer. Soc. Agron. Inc., Soil Sci. Sco. Amer., pp. 403-430.

Richard L.A. (1954) Diagnosis and improvement of saline and alkaline soils. USDA., Handbook No. 60, US. Gavt. Print Office, Washington D.C.

Schroeder, H.W. and R.A. Boller (1973). Aflatoxin production of species and strains of the *Aspergillus flavus* group isolated from field crops. Appli. Microbiol., 25: 885-889.

Snedecor, G.W. and W.G. Cochrn (1980). Statistical Methods. Seventh ed., Iows Statte Univ. Press, Ames, Iowa, USA., pp. 255-269.

Vincent, J.M. (1970). A Manual for Practical Study of Root-Nodule Bacteria. IBP Handbook, No. 15, Black Well Sci., Pub. Oxford.

تأثير الأفلاتوكسين على بكتريا المثبته للنيتروجين الجوى وأستجابة بذور فول السودانى المصابة بفطر الاسبرجيلس فلافس للمقاومة الحيوية

حمدى على عمارة ، علاء الدين عبد الحميد أبوالسعود ، فاتن محى الدين محمد

قسم بحوث الميكروبيولوجيا الزراعية ــ معهد بحوث الأراضى والمياه والبيئة ــ مركز البحوث الزراعية ــ الجيزة.

أجريت تجربة معملية لدراسة مدى تأثير الأفلاتوكسين على البكتريا المثبتة لنيتروجين الهواء الجوى ، وكذلك تجربة أصص على مستوى الصوبة خلال الموسم الصيفى ٢٠٠٢ لدارسة تأثير إضافة بكتريا المقاومة الحيوية *Bacillus pumilus* على فطر الـ*Aspergillus flavus* فى نباتات الفول السودانى المصابة والملقحة بالـ*Bradyrhizobium* sp.. أوضحت الدراسة أن *Azotobacter* and *Azospirillum* أكثر حساسية لانواع الأفاتوكسين B1, B2, G1 and G2 مع أن *Rhizobium* and *Bradyrhizobium* يتأثر فقط لنوعين الأفلاتوكسين B1 and B2 وأكثر مقاومة للنوعين الاخرين G1 and G2.

أوضحت النتائج فى تجربة الصوبة أن الـ *Bacillus pumilus* فى معدل أضافة ٨٠ ملى /أصيص اعطى أعلى اعداد واوزان عقد جذرية وأعلى مجموع خضرى وكذلك محصول قرون وقش عن بافى المعدلات وكذلك أعلى نسبة فى محتواهما من البروتين. وقد ظهر أيضا أن *Bacillus pumilus* ثبط من تأثير الأفاتوكسين وهذا التثبيط يزداد بزيادة معدل أضافة البكتريا. وكان أعلى تثبيط للأفاتوكسين (٦٢%) حدث عند معدل اضافة ٨٠ ملى /أصيص، أظهرت النتائج أيضاً أن أعلى اعداد واوزان عقد جذرية ومجموع خضرى وكذلك قيم محصول القرون والقش وجدت فى المعاملة الملقحة بالـ*Bradyrhizobium* المضافة على جرعتين (٤٠ ملى/أصيص) بعد ٢٠ ، ٣٠ يوم من الزراعة . وتشير النتائج أيضا أن أعلى تثبيط للأفاتوكسين (٧٠%) حدث عند أضافة جرعتين بواقع٤٠ ملى/أصيص الأولى عند الزراعة والثانية بعد ٤٠ يوم من الزراعة،