

EFFECT OF MICROELEMENTS AND CALCIUM NUTRITION ON PEANUT YELLOW MOLD AND AFLATOXIN CONTENT

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ABSTRACT

Greenhouse and field studies were conducted in two peanut growing seasons, 2001 and 2002 to determine the effect of micronutrients on the incidence of peanut yellow mold caused by aflatoxigenic fungi (*Aspergillus flavus* and *A. parasiticus*) and aflatoxin content. Iron sulphate, manganese sulphate and zinc sulphate at 0 , 250 and 500 ppm. combined with or without calcium sulphate (Gypsum at 500 Kg/fed. were selected for this study). Dipping peanut seeds in water solution of previous micronutrients showed significant reductions in the incidence of peanut yellow mold and in the frequency of aflatoxigenic fungi. Also an increase in peanut yield. The occurrence of aflatoxigenic fungi and aflatoxin contaminations on shells and seeds increased in zinc sulphate treatments. The additions of Gypsum alone or in combination with the microelements perform a reduction in yellow mold frequency. Gypsum make microelements more effective in reducing the aflatoxin content in shells and seeds.

INTRODUCTION

Peanut, (*Arachis hypogaea* L.) is subjected to yellow mold which is one of peanut pod rots diseases caused by aflatoxigenic fungi *Aspergillus flavus* and *A. parasiticus* (Porter *et al.*, 1990). Aflatoxin contamination may occurs before or after harvesting, but the major concern is preharvest aflatoxin contamination (Payne, 1998). Ample evidence has shown that these two fungi invade pods and produce aflatoxin prior to harvest (Wilson and Payne, 1994 and Widstom, 1996).

A number of investigators (Mostaffa, 1984; Amitava and Basu, 1992; Engelhard, 1993 and Gonzalez *et al.*, 1994) reported the effect of microelements on different infected plants with several pathogens. They found that microelements important role in peanut production. As well as decreasing pod rot incidence and aflatoxin production (Hassan and Frederick, 1995, El-korashy *et al.*, 1997 and Savita and Pareek, 1999).

Calcium is one of the most important nutritional aspects of peanut production (Casinos *et al.*, 1984). It is also reduce the aflatoxin contamination of seeds (Reding and Harrison, 1994, Rocelle *et al.*, 1994 and Hassan and Frederick, 1995).

The aim of this research was designed to study the effect of microelements and calcium nutrition on the incidence of peanut yellow mold, frequency of aflatoxigenic fungi, aflatoxin contaminations and peanut yield.

MATERIAL AND METHODS

A-Green house experiment:

Five seeds of peanut, Giza 6 cv were planted in 50 cm plastic pots, containing sterile sandy clay soil 2:1 and previously autoclaved for 2 hours. Four replications from each treatment were used. The treatments were allocated in the greenhouse following a complete randomized block design. Artificial inoculation of peanut plants by *Aspergillus flavus* and *A. parasiticus* was prepared according to (Salah et al., 1999). The fungi were grown in potato dextrose agar (PDA) medium for 7 days at 27°C. Fungal spores were collected in 0.1% plain agar solution while spore suspension of 4×10^6 spores/ml was furnished the spore suspension was used for artificial inoculation of peanut pots, 45 days after planting.

B-Field experiment:

The field experiment was carried out in a sandy loam soil (77% sand, 11% silt and 12% clay and pH 7.90) located at Ismaillia Experimental Station of Agric. Res.Center. Data of two successive seasons 2001 and 2002 were recorded. A complete randomized block design (1/400 fed.; 3 X 3.5 m) with four replicates in each treatment was furnished. Giza 6 cv of peanut seeds was sown in the first week of May. The yellow mold symptoms, aflatoxin contaminations, and pod yield were recorded when harvesting.

C-Nutrient applied:

Water solution of the microelements, iron (Fe), zinc (Zn) and manganese (Mn) at 0, 250 and 500 ppm as the sulphate form were used for soaking peanut seeds 6 hrs before sowing. Gypsum was applied at a rate of 0 and 500 kg/fed as a soil amendment 50 days after sowing (Filonow et al., 1988).

D-Determination of peanut yellow mold:

Peanut samples were examined and divided into diseased pods and apparently healthy pods. Diseased pods showed different degrees of discoloration ranging from superficial rusting to complete yellowing or yellow greening of the hulls as well as various stages of hull and seed decay. Randomized four samples of 100 pods were collected and investigated to determine the percentage of yellow mold disease of peanut.

E-Frequencies of aflatoxigenic fungi and identification:

Yellow mold fungi were isolated from the harvested peanut pods according to Garren and Porter (1970). Two pods were shelled, 1cm² pieces of shell and seed were surface-disinfested for three minutes in 1% sodium hypochlorite and plated on potato dextrose agar (PDA) medium (4 plates in 4 replicates, 5 seeds or shell pieces per dish). Plates were examined 7 days after incubation at 27 °C, for fungal structure. Identification of the isolates was carried out based on taxonomic criteria for these fungi described by Maren and Johan (1988).

F-Extraction of aflatoxin:

The extraction of aflatoxins was conducted according to A.O.A.C (1998). Samples were blended in 250ml methanol-water (55:45, v/v) and 100ml hexane for 1 min. at high speed. The mixture was transferred to the centrifuge tube and centrifuged for 5 min. at 2000 rpm. An aliquot from the aqueous methanol phase (25 ml) was taken into separator contained chloroform. The separator funnel was shaken (30-60 sec.); the bottom layer (chloroform) was separated and concentrated using rotary evaporator. The residue was quantitatively transferred using small volumes of chloroform. The solvent was completely removed under nitrogen flow.

G-Determination of aflatoxin:

Aflatoxins were determined using Singh *et al.*, (1991) method while thin layer chromatographic technique was followed and the dried film containing the aflatoxins was dissolved in a known amount of chloroform. Aflatoxin standards were spotted along with the samples. The plates were developed using a mixture of acetone-chloroform (1:9, v/v), the chromatoplates were detected under UV light at a wave length of 365nm. The concentration of aflatoxin was calculated using the equation:

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

Where:

S= volume of aflatoxin standard, in μL of equivalent intensity of sample.

Y= concentration of aflatoxin standard in $\mu\text{g/ml}$.

V= volume of solvent required to dilution 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.

H-Data analysis:

Analysis of variance using the Statistical Analysis System (SAS Institute, inc, 1996) was performed. Means were separation by Fisher's protected least significant differences (LSD) at $P \leq 0.05$ level.

RESULTS

A-Green house experiment:

Effect of microelements supplementations and Gypsum soil amendments on yellow mold incidence:

Data in Table (1) indicate that the used microelements gave a significant reduction of peanut yellow mold incidence compared with control. Increases of microelement concentration performed a reduction in yellow mold incidence. Both Fe or Mn at 500 ppm gave the best effect in reducing yellow mold, 13.34 and 14.76 % respectively, while Zn at 250 ppm gave the lowest effect (18.04 %).

Results also indicate that addition of CaSO_4 (Gypsum) as a soil amendment with the same previous treatments led to an increase in the efficiency of the microelements on yellow mold incidence. Fe at 500 ppm under Gypsum soil amendment was the best treatment in reducing the

incidence yellow mold (11.68 %), Zn at 250 ppm plus Gypsum amendment treatment showed the lowest effect on the yellow mold incidence (16.75 %).

Table (1): Effect of microelements supplementation ^{w)} with or without Gypsum amendment ^{x)} on peanut yellow mold under artificial conditions.^{y)}

Micro-elements	Conc.	0.0		Gypsum (500 Kg)	
		Yellow mold ^{z)}	Healthy	Yellow mold	Healthy
Fe	250	15.23	84.77	13.08	86.92
	500	13.34	86.66	11.68	88.32
Mn	250	16.40	83.60	14.84	85.16
	500	14.76	85.24	12.77	87.23
Zn	250	18.04	81.96	16.75	83.25
	500	16.00	84.00	14.83	85.17
Control		21.42	78.58	18.50	81.50
LSD		2.03	-	1.71	-

w) Microelements added as a seed soaking for 6 hr before sowing.

x) Gypsum added after 50 days from sowing.

y) Spore suspension (4×10^6 spores/ml) was used for artificial inoculation of peanut pots after 45 days from planting, 4 replicates with five peanut seed / replicate

z) Means in each column were statistical analyses according to L.S.D. Test ($P \leq 0.05$).

Effect of microelements supplementations and Gypsum soil amendments on occurrence of aflatoxigenic fungi and aflatoxin content:

Data in Table (2) show that the trend in occurrence of aflatoxigenic fungi in the shell was lowered compared with its percentage on seed.

Table (2): Effect of microelements supplementation with or without Gypsum soil amendment on frequency of *Aspergillus flavus*, *A. parasiticus* and aflatoxin content in shell and seed under artificial conditions.

Micro-elements	Conc.	Pod	0.0				Gypsum (500 Kg)				
			% of aflatoxigenic fungi		Content of aflatoxin (ppb)		% of aflatoxigenic fungi		Content of aflatoxin (ppb)		
			<i>A. flavus</i>	<i>A. parasiticus</i>	B ₁	B ₂	<i>A. flavus</i>	<i>A. parasiticus</i>	B ₁	B ₂	
Fe	250	Shell	20	10	0	0	10	10	0	0	
	500		15	10	0	0	10	5	0	0	
Mn	250		25	15	95	65	15	10	0	0	
	500		15	10	75	35	10	10	0	0	
Zn	250		20	15	120	115	15	10	75	0	
	500		30	20	220	167	25	15	110	45	
Control			20	15	111	98	15	10	0	0	
Fe	250		Seed	25	15	0	0	15	10	0	0
	500			15	10	0	0	10	10	0	0
Mn	250			30	20	210	115	20	15	0	0
	500	20		10	130	70	15	10	0	0	
Zn	250	30		20	185	130	25	15	125	63	
	500	45		30	335	200	40	20	235	110	
Control		30		20	250	145	20	15	150	105	

The frequency of *A. flavus* was higher than *A. parasiticus* on shells and seeds. Increase the concentrate of microelement led to a decrease in the occurrence of aflatoxigenic fungi in shells and seeds except in zinc sulphate treatment which recorded the highest frequency of aflatoxigenic fungi in shells and seeds. Iron sulphate treatment recorded the lowest frequency of aflatoxigenic fungi. Data also show that Gypsum showed its positive effect on the frequency of the fungi in shells and seeds.

Each value is mean of four replicates (4 plates / replicate, five seeds or shell pieces per dish) were incubated on PDA medium for 7 days at 27 °C.

The highest content of aflatoxin in shells and seeds associated with zinc sulphate and the content of aflatoxin increased with increasing its concentration, while iron sulphate treatment gave pods (shell and seed) free from aflatoxin contaminations. Additions of Gypsum to the previous treatments increased the reducing of aflatoxin contaminations.

B-Field experiment:

Effect of microelements supplementations and Gypsum soil amendments on yellow mold incidence:

When microelements concentration increased a significant reduction of yellow mold incidence increased with an increase in total peanut yield during the two successive seasons (Table 3). In this respect the highest effect to the microelements during two seasons 2001 and 2002 was in iron sulphate treatment at 500 ppm 8.30 and 8.10% respectively while, zinc sulphate at 250 ppm recorded the lowest effect on the yellow mold during the two growing seasons 14.90 and 13.10% compared with control.

Table (3): Effect of microelements supplementation ^{w)} with or without Gypsum amendment ^{x)} on peanut yellow mold under field conditions. ^{y)} during two seasons 2001 and 2002.

Micro-elements	Conc.	0.0				Gypsum (500 Kg)			
		Yellow mold		Yield (Ton/fed)		Yellow mold		Yield (Ton/fed)	
		2001	2002	2001	2002	2001	2002	2001	2002
Fe	250	10.70	10.20	0.998	0.976	7.31	8.10	1.051	1.019
	500	8.30	8.10	1.023	1.007	5.10	5.31	1.112	1.082
Mn	250	13.10	11.11	0.989	0.968	10.00	9.44	1.039	1.015
	500	11.70	9.90	1.009	1.000	8.71	8.60	1.082	1.073
Zn	250	14.90	13.10	0.976	0.966	11.60	11.12	1.013	0.998
	500	12.10	11.40	0.991	0.990	9.33	9.25	1.008	1.010
Control		18.75	16.64	0.968	0.952	13.97	12.61	0.997	0.989
LSD		1.93	1.43	0.012	0.022	2.36	1.99	0.016	0.018

w) Microelements added as a seed soaking for 6 hr before sowing.

x) Gypsum added after 50 days from sowing.

y) Four replicates for each treatment, the field plot was 2.0 × 2.5 m (5 m² = 1/800 Fed.).

z) Means in each column were statistical analyses according to L.S.D. Test (P ≤ 0.05).

The highest peanut yield in the two seasons was recorded in iron sulphate treatment at 500 ppm followed by manganese sulphate at 500 ppm, while zinc sulphate at 250 ppm recorded the lowest total peanut yield.

Results also indicate that addition of Gypsum as a soil amendment with the same previous treatments perform an increase in the efficiency of microelements on yellow mold incidence. Gypsum treatment increased the

total peanut yield and decreased of yellow mold. The best treatment in reducing yellow mold incidence and increase of yield during the two seasons was iron sulphate at 500 ppm under Gypsum soil amendment.

Effect of microelements supplementations and Gypsum amendments on occurrence of aflatoxigenic fungi and aflatoxin content:

Data obtained in Tables (4 and 5) state that occurrence of aflatoxigenic fungi were affected by the microelements supplementation in both shells and seeds. Increase the concentrations of microelement led to a decrease in the occurrence of aflatoxigenic fungi in shells and seeds. The highest occurrence of these fungi was in zinc sulphate treatments and its frequency increased by increasing the concentration of zinc sulphate from 250 to 500 ppm.

The highest content of aflatoxin in shells and seeds was shown when zinc sulphate was applied. The content of aflatoxin increased with increasing its concentration. Additions of Gypsum to previous treatments increased the reduction of aflatoxin contaminations in shells and seeds during the two seasons (2001 and 2002).

Table (4): Effect of microelements supplementation with or without Gypsum amendment on frequency of *Aspergillus flavus*, *A. parasiticus* and aflatoxin content in shell under field conditions. during two seasons 2001 and 2002.

Gypsum	Micro-elements	Conc.	Season 2001				Season 2002			
			% of aflatoxigenic fungi		Content of aflatoxin (ppb)		% of aflatoxigenic fungi		Content of aflatoxin (ppb)	
			<i>A.flavus</i>	<i>A.parasiticus</i>	B ₁	B ₂	<i>A.flavus</i>	<i>A.parasiticus</i>	B ₁	B ₂
0.0	Fe	250	10	10	0	0	15	15	0	0
		500	5	5	0	0	10	10	0	0
	Mn	250	10	10	0	0	15	10	0	0
		500	10	5	0	0	5	0	0	0
	Zn	250	15	15	0	0	20	15	0	0
		500	20	10	95	0	25	20	123	85
Control	15	10	0	0	20	15	110	65		
500 kg	Fe	250	10	5	0	0	10	10	0	0
		500	5	0	0	0	5	5	0	0
	Mn	250	10	10	0	0	10	5	0	0
		500	5	5	0	0	5	5	0	0
	Zn	250	15	10	0	0	15	10	0	0
		500	10	10	0	0	20	20	75	25
	Control	10	5	0	0	10	10	45	0	

Each value is mean of four replicates (4 plates / replicate , five seeds or shell pieces per dish) were incubated on PDA medium for 7 days at 27 °C.

Table (5): Effect of microelements supplementation with or without Gypsum amendment on frequency of *Aspergillus flavus*, *A. parasiticus* and aflatoxin content in seed under field conditions. during two seasons 2001 and 2002.

Gypsum	Micro-elements	Conc.	Season 2001				Season 2002			
			% of aflatoxigenic fungi		Content of aflatoxin (ppb)		% of aflatoxigenic fungi		Content of aflatoxin (ppb)	
			<i>A.flavus</i>	<i>A.parasiticus</i>	B ₁	B ₂	<i>A.flavus</i>	<i>A.parasiticus</i>	B ₁	B ₂
0.0	Fe	250	15	10	0	0	20	20	0	0
		500	10	10	0	0	20	10	0	0
	Mn	250	20	15	0	0	25	20	0	0
		500	15	10	0	0	15	10	0	0
	Zn	250	20	20	108	45	25	20	140	96
		500	30	30	120	60	35	30	200	110
Control		20	15	112	40	25	25	153	100	
500 kg	Fe	250	10	5	0	0	15	10	0	0
		500	5	5	0	0	10	10	0	0
	Mn	250	15	10	0	0	20	20	0	0
		500	10	5	0	0	15	10	0	0
	Zn	250	15	15	0	0	20	15	70	35
		500	20	15	65	0	25	20	105	78
Control		15	10	45	20	20	15	95	60	

Each value is mean of four replicates (4 plates / replicate , five seeds or shell pieces per dish) were incubated on PDA medium for 7 days at 27 °C.

DISCUSSION

The results of this study present that, the microelements nutrients and their concentrations play an important role in the reduction of yellow mold disease, frequency of aflatoxigenic fungi and aflatoxin content and increase of total peanut yield. This results is in agreement with Hassan and Frederick, (1995) El-korashy *et al.*, (1997), Savita, and Pareek, (1999). Data also suggested that microelements may increase the level of phenols in peanut plant and decrease total and reducing sugars (Murugesan and Mahadevan, 1987). Ascorbic acid oxidase, peroxidase, and phenolase increased markedly in treated plant with microelements (Walker and Webb, 1981 and Murugesan and Mahadevan, 1987). Moreover, microelements interacts with N metabolism and is intimately involved in carbohydrate synthesis, photosynthesis, coenzymes to many of plant enzymes and synthesis of other compounds associated with the defense of plant against pathogens like phytoalexins and lignin (Walker and Webb, 1981 and Engelhard, 1993).

Data also show clearly that, Gypsum had an important effect in reducing of yellow mold disease incidence, frequency of aflatoxigenic fungi and aflatoxin content and increasing of peanut yield and when Gypsum addition to microelements treatments increased their efficiency. This results is in agreement with Casinos and Gaines (1986), Reding and Harrison, (1994), Rocelle *et al.*, (1994), and Hassan, and Frederick, (1995). This is due to the role of calcium in regular the uptake and absorb of ions in peanut plants,

which make pods not predisposition to microorganism's invasion (Frank, 1972; Garcia and Mitchell, 1975 and Porter *et al.*, 1990). While Casinos and Gaines (1986) suggested that, the role of Gypsum in controlling the peanut pod rot depend on increase the calcium concentration in pods and that, make it more resistance to the invasion with pathogens. Moreover, calcium can be effected of peanut aflatoxin contaminations by reducing the percentage of aflatoxigenic fungi colonies in peanut seed (Wilson and Walker, 1981 and Davidson *et al.*, 1983).

On the other hand, results indicated that, zinc sulphate make peanut pods (shells and seeds) more susceptible to fungul attack and increased their aflatoxin content. This suggestion is in agreement with Reding and Harrison (1994) and Payne and Brown (1998) who stated that Zn play an important role in the regulation of aflatoxin production and supplemented of Zn perform the activity of fatty acid synthetase (FAS) which, corresponded to aflatoxin B₁ biosynthesis.

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تأثير العناصر الصغرى ، والجبس الزراعي علي حدوث عفن ثمار الفول السوداني الأصفر وتلوثها بالأفلاتوكسين

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أجريت هذه الدراسة تحت ظروف العدوى الصناعية بالصوبة والحقل في موسمي ٢٠٠١ ، ٢٠٠٢ لتقدير تأثير العناصر الصغرى والتي تشمل كبريتات الحديد ، كبريتات المنجنيز ، كبريتات الزنك بجرعات صفر ، ٢٥٠ ، ٥٠٠ جزء في المليون ، واستخدامها مع الجبس الزراعي (بمعدل ٥٠٠ كجم/إدان) أو بدونه علي حدوث عفن الأصفر لثمار الفول السوداني والمتسبب عن الفطريات المنتجة للأفلاتوكسين (أسبرجلس فلافس ، أسبرجلس باراستيكس) وكذا محتوى الثمار من الأفلاتوكسين .

أدي غمر بذور ثمار الفول السوداني بصفة عامة في محاليل العناصر إلي ظهور نقص معنوي في كل من العفن الأصفر لثمار الفول السوداني ونسبة تكرار عزل فطريات الأفلاتوكسين وكذلك تلوث الثمار بالأفلاتوكسين . بينما ازداد محصول ثمار الفول السوداني بمقارنته . ازداد تواجد الفطريات المنتجة للأفلاتوكسين في بذور وقشور ثمار الفول السوداني فسي معاملة كبريتات الزنك ، كما ارتبط محتوى ثمار الفول السوداني من الأفلاتوكسين بزيادة تركيز الزنك .

أدي إضافة الجبس الزراعي بمفرده أو مع العناصر الصغرى المغذية إلي نقص في حدوث عفن ثمار الفول السوداني الأصفر وفي تكرار عزل الفطريات المنتجة للأفلاتوكسين . أدي إضافة الجبس الزراعي إلي المعاملات بالعناصر الصغرى إلي زيادة فعاليتها في خفض محتوى القشور والبذور من الأفلاتوكسين .