ESci J. Plant Pathol. 01 (2012) 06-13 DOI: 10.33687/phytopath.001.01.0010

Available Online at EScience Press
ESci Journal of Plant Pathology
ISSN: 2305-106X (Online), 2306-1650 (Print)
http://esciencepress.net/journals/phytopath

EFFECT OF TREATMENT WITH MOLD INHIBITORS ON PLANT GROWTH OF CORN AND SOME NUTRITIONAL COMPONENTS OF STORED GRAINS, INFECTED WITH *A. FLAVUS* AND *F. VERTICILLOIDES*

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A B S T R A C T

Planting sterilized corn grains in soil, treated with the mold inhibitors Fix-a-tox (FAT) or Antitox Plus (AP) resulted, in cases, in dwarfed and malformed corn plants and in the development of deformed, bone-shaped and grain-free corn cobs. Moreover, treating corn grains with the mold inhibitors before storage for one month caused significant changes in some nutritional components of corn grains, i.e. proteins, aminoacids, crude fibers, moisture, fats, ash and carbohydrates. Insignificant differences in protein percetage were detected between control inoculated with *Aspergillus flavus* and those treated with butyl hydroxyanisole (BHA) antioxidant or FAT treatments, whereas ground clove significantly reduced protein content. In corn grains inoculated with *Fusarium verticillioides*, previous treatment with FAT resulted in significant reduction in the content of proline, aspartic acid, cysteine, valine, isoleucine and leucine, whereas treatment with BHA significantly reduced the content of threonine, serine, glutamic, glycine, alanine, phenylalanine and tyrosine. In *Aspergillus flavus* treatments, FAT significantly increased the content of methionine and threonine, whereas, aspartic acid showed 26 percentage decreases, compared to the control. Pronounced reductions in threonine, isoleucine and leucine were also detected in corn grains treated with BHA. Significant increases in fiber content were detected in inoculated corn grains treated with BHA, attaining 1.34 to 2.05-fold over that of control, respectively. Treatment with FAT and BHA led to pronounced reductions in moisture content in corn inoculated with both *F. verticilloides* and *A. flavus* trials. However, treatment with ground clove significantly increased the moisture content in *A. flavus* treatment. FAT treatment led to significant increase in ash and fat contents in both *A. flavus* and *F. verticillioides* treatments, whereas the other tested treatments of *F. verticillioides* significantly reduced ash content. Treatment with ground clove significantly reduced fat content in *A. flavus* treatment. All the tested materials significantly reduced carbohydrate content.

Keywords: *Corn grain molds, Mold inhibitors, BHA antioxidant, Antitox Plus, F. verticillioides, A. niger.*

INTRODUCTION

In order to meet the Egyptian local human and animal consumption of corn, additional 6.0 million. tons of yellow corn is imported annually from different countries (USDA Foreign Agricultural Services, 2010). Imported corn is subjected to infection with many grain-rotting fungi during storage and shipping. The nutritional profile of corn makes it a target of phytopathogenic fungi that cause grain deterioration and subsequent mycotoxins contamination.

The most important grain-rotting fungi are *Aspergillus niger* Van Tieghem and *Fusarium verticilloides* (Sacc.) Nirenberg, which can excrete mycotoxins such as aflatoxins (Youssef *et al.*, 2003; Rahimi *et al.*, 2008; Diedhiou *et al.*, 2011**)** and fumonisins (Youssef *et. al.*, 2003; Afolabi *et al.*, 2007; Schjoth and Tronsmo, 2008; Murillo-Williams and Munkvold, 2008) in corn grains during storage (Martinez and Martinez, 2000; Jaime-arcia and Cotty, 2006). These toxins cause serious diseases to animals and humans (IARC, 1993; Gowda *et al.*, 2004; Proctor *et al.*, 2007).

The mineral-based mycotoxin absorber Fix-A-Tox (FAT) (Abdelhamid *et al.*, 2002), AL_2O_3 (Monlinie *et al.*, 2005; Gunes *et al.*, 2007), the detoxifier Antitox-Plus (AP), Florisil and butyl hydroxyanisole (BHA) (Jirovetz *et al*., 2006; Aldred *et al*., 2008; Sidhu *et al*., 2009) are applied to inhibit the growth of corn grain-rotting fungi and prevent mycotoxins production during transportation and storage (Mahmoud *et al*., 1999; Abdelhamid *et al.,* 2002; Youssef *et al*., 2003; Aldred *et al*., 2008). Some medicinal herbal plants and their oils or extracts were also effective in inhibiting corn grains deteriorating fungi and in detoxifying the produced mycotoxins (Youssef *et al*., 2003, 2009; Scherm *et al*., 2005; Akhtar *et al*., 2007).

Treatment of corn grains with mold inhibitors may result in significant changes in plant growth and contents of some important nutritional components of corn grains, which consequently may have direct or indirect effect and threaten the health of our plants and animals. However, according to the available literature, studies on the hazardous effect of mold inhibitors on plant growth and grain components had received insufficient attention.

Many medicinal herbs, especially clove products, showed significant effect in inhibiting growth grains-decaying fungi and detoxification of mycotoxins production (Youssef *et al*., 2003, 2009; Muhammed *et al*., 2005; Scherm *et al.*, 2005; Kokoska *et al.*, 2005; Akhtar *et al*., 2007).

Therefore, this study aimed to investigate the effect of FAT, AP, BHA and ground clove buds on some essential components of corn grains, i.e. proteins and amino acids, fibers, moisture, ash, fats and carbohydrates, during storage. Moreover, effect of planting corn grains in sterilized soil, polluted with these mold inhibitors on the developed corn plants and cobs was also studied.

MATERIALS AND METHODS

Isolation and pathogenicity experiments:

Damaged corn grains were taken from four lots of grains imported from U.S.A. Those grains were tested using Blotter and Agar plate methods (Christensen*,* 1957). Pure cultures of the developing fungi were obtained either by single spore technique (Nelson and Juba, 1994) or hyphal tip technique (Ricker and Ricker*,* 1936) and maintained on potato dextrose agar PDA slants. The isolates were identified to the generic levels (Leslie and Summerell, 2006). The isolated *Fusarium sp.* was sent to Muse'um National d'histoire Naturelle laboratoire de Cryptogamie*,* Paris*,* France and was kindly verified and identified to the species level by Prof. Dr. Marie France Roquebert as *Fusarium verticilloides*. The isolated *Aspergillus sp.* was kindly verified by Prof. Dr. Ebtessam El sheriff*,* Fungal Identification Section, Plant Pathology Institute Giza*,* Agricultural Researche Center**,** Giza, Arabic Republic of Egypt as *Aspergillus flavus*. The sub cultures of the mycotoxin-producing fungi were maintained on autoclaved corn grains and kept at -4°C. These isolates proved to be pathogenic causing decay of the inoculated grains.

Effect of mold inhibitors on plant growth and development:

In order to test the effect of synthetic mold inhibitors on the development of corn plant growth, Sixty three pots 40 x 40 cm for each tested fungus, containing 4 kg autoclaved soil + 2 kg autoclaved sheep manure + 100 gm super phosphate (as organic fertilizer) + 50 g nitrogen (urea) as mineral fertilizer. Soil was treated with 1% FAT (Austrich – Syria Co, city) and 1% AP (company, citry). One week later, ten grains were planted in each pot after surface sterilization with sodium hypochlorite (2 plants per pot). Observations on plant growth and development were recorded throughout the experiment until plants attained the maturity stage on the 110th day after planting.

Effect of mold inhibitors on some corn grain components:

Synthetic mold inhibitors, i.e. Fix–A –Tox (FAT) (introduced from Austrich – Syria Co.), the antioxidant BHA (from Sigma – Aldrich Co.) and flower buds extract of clove (*Eugenia caryophyllata),* as herbal mold inhibitor were applied to test their effect on some corn grain components. A total of 20 g of freshly collected flower buds of clove were sprayed with 20 ml alcohol 70% for surface sterilization, placed between sterilized filter paper and then dried at 40°C overnight. Sample was finally ground in 100 ml sterilized water at 4°C for 16 hours after that the aqueous suspension was resuspended in 100 ml methyl alcohol overnight. The suspension was centrifuged at 3000 rpm for 20 min and filtered through Whatman no.1 filter paper. The supernatant fluid was evaporated to dryness under reduced pressure on a rotary evaporator*,* resuspended in distilled water (4-5 ml) and sterilized by filtration using nitrocellulose membranes (0.45 Mm Millipore). Samples were stored at -20°C until used (Youssef *et al*., 2003). Surface sterilized grains were dipped in extracts of the tested mold inhibitors.

Aspergillus flavus Link and *F. vericilloides* were used in corn grains inoculation trials. Experimental treatments included: (1) healthy untreated grains; (2) untreated grains inoculated with *A. flavus*; (3) untreated grains inoculated with *F. verticillioides*; (4) grains inoculated with *A. flavus* and treated with BHA at conc. 500 µg/g; 5 grains inoculated with *F. verticilloides* and treated with BHA at conc. 500 µg/g; 6 grains inoculated with *A. flavus* and treated with FAT at conc. 1%; (7) grains inoculated with *F. verticilloides* and treated with FAT at conc. 1%; (8) grains inoculated with *A. flavus* and treated with ground clove 0.5% and (9) grains inoculated with *F. verticilloides* and treated with ground clove 0.5%.

All samples were homogenized and then incubated in an incubator at optimum temperature for each fungus (at 28°C for *A. flavus* and 25°C for *F. verticilloides*) for one month. At the end of incubation, samples were taken and finely ground, then refrigerated at -4°C until proximate analysis was done for each sample (AOAC, 1990). Samples were triplicated to indicate the levels of protein, fiber, moisture, ash and fats.

After protein determination (AOAC, 1990), samples (150 g each) were taken for analysis of amino acids (Moore and Stein, 1958). Amino acids were determined by using a Beckman amino acid analyzer (Model 7300) in the Central Laboratory, Faculty of Agriculture, Alexandria University. Moisture of each sample was determined by official method for drying sample in oven at 132°C for three hours (AOAC, 1990) and the sample was weighed before and after drying process. Plates containing samples were reweighed after one hour until stability of sample weight. Ash content was determined according to AOAC (1990), using muffle model F6010 at 600°C for 2 hours and half. Fat ratio was analyzed using Sockslet apparatus. The carbohydrate levels were determined by subtracting the other components.

The experiment of proximate analysis was statistically analyzed as factorial design according to Steel and Torrie (1984).

RESULTS

Effect of synthetic mold inhibitors on plant growth and development:

Soil treatment with FAT and AP prior to planting resulted in drastic effect on the developed corn plants and cobs (Fig. 1-a &1-b).

Symptoms on foliage mostly begin with the development of water-soaked areas, in most cases near leaf tips, which turned into necrotic areas, enlarged to cover most of the leaf area. These symptoms may appear first on lower leaves and may lead to complete burning of the foliage. The developed symptoms characterized both synthetic mold inhibitors FAT and AP, however, symptoms were more pronounced in FAT treatment. Developed corn plants appeared dwarfed and malformed (Fig.1a).

The developed corn plants had deformed lobe–shaped cobs; each was divided into three deformed small cobs at the lower parts of the plant. Moreover, treatment with AP resulted in the development of deformed, bone-shaped and grain-free cobs at the upper part of the plant (Fig. 1b).

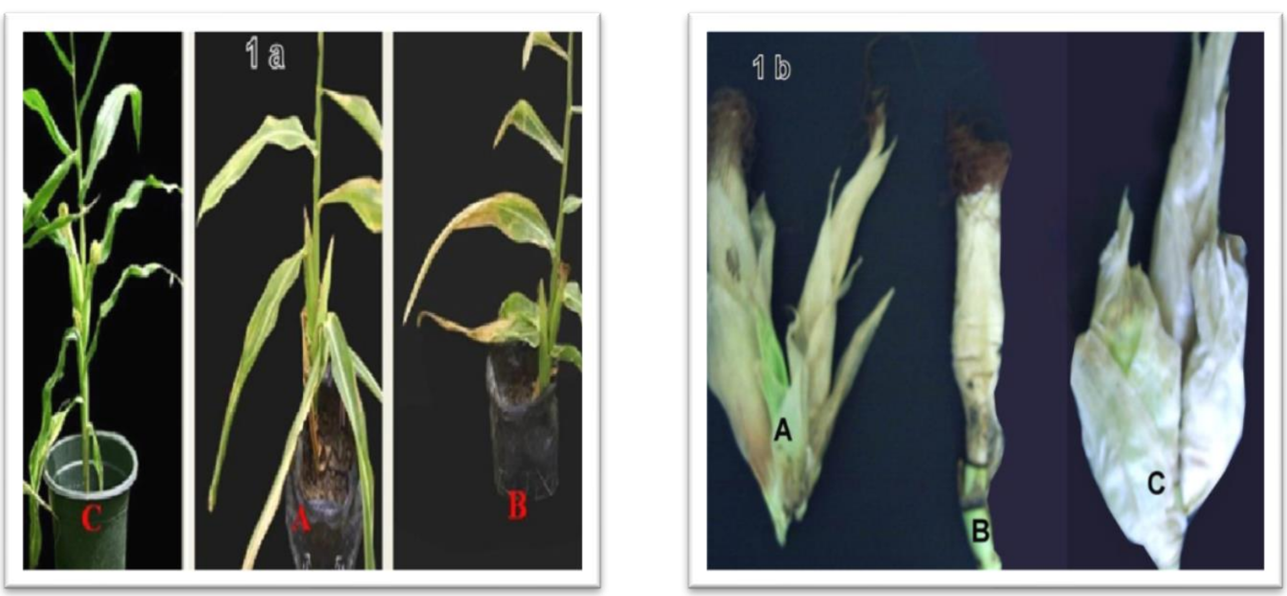

Fig.1. Effect of treatment with Anti Plus and Fix-A-Tox on maize foliage and cobs. Where: 1a: Effect of treatment with AP (A) and FAT (B) on plant foliage, compared with control (C) and 1b: Effect of treatment with AP (A and B) and FAT (C) on maize cobs.

 DOI: 10.33687/phytopath.001.01.0010

Table (1) Effect of treatment with some mold inhibitors and ground clove on some nutritive of corn grains, previously inoculated with *A. flavus* or *F. verticilloides* under stroge conditions.

Treatment	Nutritive Components											
	Proteins		Fibers		H_2O		Ash		Fats		CHO	
C1	7.90 b	7.90 b	3.10 c	3.10 c	11.2 h	11.2 h	1.03 c	1.03 c	2.68 d	2.68 d	74.09 b	74.09 b
C2	8.20 ab	7.75 b	1.71 g	1.68 g	14.9 c	16.1 c	0.98 d	1.19 b	2.46 f	2.32 g	71.75 c	70.91 e
BHA	7.70 b	8.41 a	4.21 a	3.45 b	13.7 g	13.3 g	1.06 c	1.14 b	2.85 c	3.00 a	70.48 f	71.36 d
Ground Clove	7.00 c	7.65 bc	1.18 h	2.62 f	19.3 a	17.6 b	1.07 c	0.94 d	1.62 b	2.55 c	69.83 g	69.00 b
FAT	7.70 b	7.65 bc	2.50 e	2.65 d	14.4 c	11.1 h	1.38 a	1.16 b	2.93 b	2.83 c	71.09 de	74.61 a
L.S.D 0.05	0.66		0.07		0.1		0.05		0.04		0.3	
C1: Non-Inoculated Control		C2: Inoculated Control			BHA: Butylated Hydroxy Anisole				FAT: Fix-A-Tox			

* Values followed by the same letter in each vertical column don't differ significantly according to LSD test (P≤ 0.05).

Table (2). Effect of treatment with the tested mold inhibitors on amino acids content in inoculated stored grains.

Pathogen	Mold Inhibitor	Amino Acid Content (%)							
		Aspartic Acid	Threonine	Serine	Glutamic Acid	Proline	Glycine	Alanine	Cystine
Aspergillus flavus	C1	8.30 g	4.71 b	5.03 b	20.45 b	11.64 b	4.05 c	6.64 c	0.26 a
	C2	8.38 f	4.28 c	4.82 c	15.90 c	11.47 c	4.31 b	7.73 b	0.05 d
	FAT	9.00 e	2.45 h	2.74 d	11.95 g	7.64 h	2.34 d	4.39 d	0.17 bc
	G.Clove	8.12 h	2.79 f	3.86 d	17.51 c	7.51 i	2.96 d	6.43 c	0.14 c
	BHA	7.87 i	2.29 i	3.11g	14.83 f	8.27 g	2.69 f	5.95 c	0.13 c
Fusarium verticilloides	C1	8.30 g	4.71 b	5,03 h	20.45 b	11.64 b	4.05 c	7.64 b	0.26 a
	C2	12.69 a	5.17 a	5.39 a	20.45 b	12.74 a	4.32 b	8.73 a	0.28 a
	FAT	10.89 b	3.96 d	5.45 a	23.46 a	10.25 f	4.72 f	8.98 a	0.17 bc
	G.Clove	9.13 d	2.88 e	3.65 c	17.01 d	10.75 e	2.79 e	6.45 c	0.11 c
	BHA	9.77 c	2.65 g	3.44 f	16.90 d	11.04 d	2.71 ef	6.17 c	0.19 b

Pathogen	Mold Inhibitor	Amino Acid Content (%)								
		Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine
Aspergillus flavus	C1	5.96 c	1.67 c	7.51 b	12.55 b	4.26 c	4.52 d	3.66 a	3.61 b	5.56 a
	C2	6.95 b	1.77 bc	7.15 c	11.96 c	4.87 b	4.98 c	1.77 d	3.23 c	3.32 c
	FAT	3.92 b	1.95 a	3.62 g	8.49 g	2.45 e	3.04 h	1.19 f	2.56 c	1.23 h
	G.Clove	4.93 e	1.30 e	4.13 e	7.71 h	2.16 g	4.05 f	2.63 c	1.76 h	1.33 g
	BHA	4.75 f	1.76 bc	3.58 g	7.02 i	1.89 h	3.49 h	1.51 e	1.88 g	1.65 e
Fusarium verticilloides	C1	5.96 c	1.67 c	7.51 b	12.55 b	4.26 c	4.52 d	3.66 a	3.61 b	5.56 a
	C2	7.08 a	1.68 c	8.45 a	13.65 a	5.84 a	5.54 a	3.35 b	3.96 a	5.33 b
	FAT	6.91 b	1.83 b	7.24 c	10.81 d	3.36 d	5.46 a	1.84 d	2.94 d	1.72 d
	G.Clove	4.57 g	1.49 d	3.95 f	8.80 f	2.28 f	4.23 c	1.48 e	1.67 i	1.41 f
	BHA	5.29 d	1.61 d	4.32 d	9.42 c	2.11 g	3.52 g	1.44 c	2.30 f	0.92 i
C1: Untreated-Non-inoculated Control				C2: Untreated Inoculated Control						

* Values followed by the same letter in each vertical column don't differ significantly according to LSD test (P≤ 0.05).

Effect of corn grains treatment with mold inhibitors on some nutritional components of grains:

According to the obtained results in Table (1), there were insignificant differences in protein % between untreated control, inoculated with *A. flavus* and those treated with BHA or FAT. However, treatment of inoculated grains with ground clove resulted in significant reduction in protein % (14.63% less than control). On the other hand, differences in protein% among *F. verticilloides* treatments and inoculated control were insignificant.

According to the obtained results in Table (2), it was evident that treatment of grains, inoculated with *A. flavus*, with the tested mold inhibitors resulted in significant reduction in most of the individual amino acids content, compared with those of inoculated untreated grains. However, pronounced significant increases in cystine were detected in inoculated grains treated with FAT, ground clove and BHA antioxidant, compared with control (3.4, 2.8 and 2.6 times more than control, respectively).

Significant increases in threonine and methionine were also observed in FAT treatment (7.4% and 10.17% more than control, respectively). Similar increases were detected in glutamic and histidine in ground clove treatment (10.19% and 48.59% more than control, respectively).

The highest reduction rates in amino acids content in *A. flavus* treatments, compared with control, were detected in FAT treatment, in which reduction rates ranged from 7.4% in aspartic and attained maximum values (62.95%) in arginine. Moreover, BHA treatment showed significantly pronounced reductions in threonine (46.5%), isoleucine (49.93%), leucine (41.3%), attaining maximum reduction rates in tyrosine (61.19%).

Treatment of grains, inoculated with *F. verticilloides*, with BHA resulted in the highest reduction rates in the content of arginine (70.37%), tyrosine (62.62%), isoleucine (49.63%), threonine (47.67%), histidine (42.58%), glycine (37.35%), serine (35.88%), leucine (35.78%), phenylalanine (33.46%) and alanine (26.37%), arranged in descending order. Treatment with ground clove buds significantly decreased the content of all the analyzed amino acids, however, the highest reduction rates among all treatments were detected in lysine (52.37%), valine (32.24%), methionine (19.19%) and aspartic (18.14%), in descending order.

Treatment with FAT, except for proline, realized the least reduction rates, compared with the other tested treatments.

From Table (1) it was evident that treatment of grains inoculated with *A. flavus* with BHA antioxidant led to a pronounced increase in fiber content (2.46-fold more than control), followed by FAT (1.46-fold of that of control). Treatment with ground clove resulted in significant reduction in fiber content, compared with inoculated control (31% less than control). However, BHA treatment was the only treatment, among the other tested treatments that improved fiber content (1.35 times more than control). In *F. verticilloides* treatments, BHA, ground clove and FAT significantly increased fiber content (2.05, 1.34 and 1.42-fold more, respectively).

From results in Table (1), treatment of grains, inoculated with *A. flavus* or *F.verticilloides*, with ground clove buds significantly increased moisture in grains (1.1 to 1.3-fold more control). On the other hand, BHA and FAT resulted in significant reduction in moisture in both of the tested pathogens (68% to 68.49% of control). Ash content in *A.flavus* treatments (Table 1), significantly increased by FAT (1.41-fold), whereas BHA and clove resulted in significant reduction in ash content in *F. verticilloides*, attaining highest rates in ground clove treatment (79% less than control).

Significant increases in fat content were detected in all treatments; however increasing rates were not pronounced. Drastic reductions in fat content were occurred only in ground clove treatment (34.15% less than control), at the end of storage period. Significant reductions in carbohydrates content were detected by all the tested mold inhibitors; however, reduction rates were not pronounced.

DISCUSSION

The hazardous effect of treatment with synthetic mold inhibitors; i.e. Antitox Plus (AP) and Fix-A-Tox (FAT) and antioxidant mold inhibitors, e.g., butyl hydroxyanisole (BHA) was extensively investigated in human and animals (Madhavi and Salunkhe, 1996; IARC, 1999; JECFA, 2000; Hell *et al.,* 2000; Singh *et al.*, 2006), however, according to the available literature, studies on the effect of mold inhibitors on plant growth and development are very rare.The present study showed that planting sterilized corn grains in soil, treated with FAT or AP resulted in many cases in malformed corn plants and in the development of deformed corn cobs. Aluminosilicate compound FAT attacks cell membranes and DNA, causing considerable damage, mutagenicity and carcinogenic effect in many animal and plant tissues (Morikowa and Saigusa, 2002 and Gunes *et al*., 2007). Treatment of corn grains with these substances may expose grain tissues to similar damage of cell membranes and DNA, leading to serious mutagenicity and the development of deformed corn plants. This concept had been realized throughout the present work through the drastic effect of such mold inhibitors on the development of corn plants and the developed cobs. According to the available literature, this was the first record of the deleterious effect of corn grains treatment with synthetic mold inhibitors, in particular FAT and AP. Gunes *et al*. (2007) found that Silicon mediates changes to some physiological and enzymatic parameters symptomatic for oxidative stress in spinach (*Spinacia oleracea* L.) grown under B toxicity. Morikowa and Saigusa (2002) showed that Si ameliorates the toxicity of Al in barley. They concluded that aluminum is reported to interfere with the uptake, transport, and metabolism of several essential nutrients (Gleen *et al.,* 2002; Gunes *et al.,* 2007). Data showed that differences in protein % among most of the tested treatments were insignificant. However, pronounced changes in amino acids contents were detected. FAT caused drastic reduction in aspartic acid (74%) and parallel increase in methionine (76.4%). This pronounced increase may be attributed to the inhibition of the enzyme aspartic protease by specific inhibitor, produced by *A. flavus*. Such explanation was adopted by Chandravanu *et al.* (2001), who found that *Bacillus sp*. can produce a novel bifunctional inhibitor (ATBI) exhibiting an activity against phytopathogenic fungi, including *Alternaria, Aspergillus, Curvularia, Colletotrichum, Fusarium* and Phomopsis species, as it was found to inhibit xylanase and aspartic protease.In FAT treatment, glycine and alanine content showed pronounced increase. This was in agreement with Igarashi *et al*. (2006) who concluded that in photorespiration, peroxisomal glutamate: glyoxylate aminotransferase (GGAT) catalyzes the reaction of glutamate and glyoxylate to produce 2-oxoglutarate and glycine. Reductions in proline content, observed after treatment the inoculated grains with FAT were also recorded by Morikowa and Saigusa (2002), Gunes *et al*. (2007) and Charlotte *et al*. (2008) who indicated that the supply of Si decreased the proline concentration.Treatment with both of BHA and FAT induced drastic reductions in moisture content in grains (80-90% less than control), whereas ground clove resulted in 30% increase in moisture content. Alluminosilicate compounds are known to serve as good adsorbent and enhanced the absorption and retention of plant nutrients and water and supplemented micronutrients (Burriesci *et al.,* 1984).

The tested synthetic mold inhibitors are known to be effective in fungal growth suppression and in reducing toxin content (Youssef *et al*., 2003 and 2009). However, these compounds are proved to be very harmful to plants, humans and animals (IARK, 1999; Morikowa and Saigusa, 2002; Gunes *et al*., 200; Youssef *et al*., 2009). Hell *et al.* (2000) and Singh *et al.* (2006) mentioned that BHA has demonstrated to cause hyperplasia and hyperkeratosis in the fore stomachs of a variety of test animal species, but although beginning tumors were reported in the hamster fore stomach. No carcinomas were induced in species other than rats.

Moreover, treatment corn grains with the tested synthetic mold inhibitors induced undesirable changes in the nutritional components of the grain. In addition, application of such chemicals is very expensive, and they are not applicable under field conditions (Nesci *et al*., 2008).

Ground clove buds and other herbal plant oils and extracts were found to be highly effective as mold inhibitors (Moussa *et al*., 2005; Ghasemi *et al*., 2007; Marin *et al*., 2008; Reddy *et al*., 2009 and Youssef *et al*., 2009), locally available, cheaper, safe, applicable both under storage or field conditions and may enhance many growth parameters of corn plants. They also characterized by the natural synergism of their active components. Application of herbal plants products with corn grains during transportation, shipping, storage or under field conditions may serve as good alternative to the synthetic unsafe mold inhibitors (Magan and Aldred, 2007).

More efforts should be exerted to determine the efficacy of large scale of herbal plants in protecting corn plants from the deleterious effect of *A. flavus* and *F. verticilloides* and their mycotoxins, as alternative of synthetic mold inhibitors, under field and storage conditions.

Thereby, our findings might partially contribute to better understanding of the side effects of using synthetic mold inhibitors on plant growth and development or on the nutritional components of corn grains and the significance of using herbal plant products as a better alternative.

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