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Effect of Gamma Irradiation and Plant Extracts on the Deterioration and Aflatoxin Accumulation in Stored Maize Grains

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Gamma irradiation and medicinal plant extracts were tested for their capability to decrease deterioration and aflatoxins accumulation in stored maize grains. Irradiation of grains with 5 or 10 kGy effectively reduced the infection with *Aspergillus flavus* and other contaminated fungi for up to 3 months of storage. Also, these treatments greatly reduced aflatoxins (B1 & B2) production in comparison to un-irradiated grains. The highest effect of irradiation was occurred by 10 kGy, which resulted in negligible incidence of *A. flavus* and the associated fungi as well as complete inhibition to aflatoxins production until the end of storage period.

Application of different concentrations of chloroform-methanol and/or aqueous extracts of thyme, tooth pick, spider flower as well as tooth-brush caused significant reduction in the *in vitro* growth of *A. flavus*. Chloroform-methanol (2:1v/v) was effective than the aqueous extracts of the other medicinal plants. Tooth pick extract caused the highest reduction to the fungal growth followed by thyme extract. Moreover, treatment of maize grains with chloroform-methanol extract of thyme and tooth pick, before storing for up to 20 to 60 days, greatly reduced the occurrence of *A. flavus* and the other fungi as well as aflatoxins production.

Keywords: Aflatoxins, *Ammi visnaga*, *Aspergillus flavus*, *Cleome arabica*, gamma irradiation, maize, *Salvadora persica* and *Thymus vulgaris*

Maize (*Zea mays* L.) is considered as one of the most important food and feed cereal crops in Egypt. It is well known that maize grains are vulnerable to attack by several fungi in the field which proceed in growth and produce mycotoxins under bad storage conditions. *Fusarium* spp. and *Aspergillus* spp. are the most common fungi that could be recovered from infected ears and stored kernels, which produced mycotoxins at storage (Halloin, 1975 and El-Shabrawi, 2001). Aflatoxins, produced by some strains of *A. flavus* and *A. parasiticus*, are considered the most serious toxic metabolites to human and animal (Alam *et al.*, 2010 and Shah *et al.*, 2010). Therefore, developing effective and efficient method(s) to minimize aflatoxins contamination in maize grains are urgent.

Some trials have been done to evaluate natural means for managing grain rotting and aflatoxin production. Gamma irradiation was tested by several investigators to protect food stuffs and grains against fungal infection and aflatoxin production

(Rustom, 1997 and Rizk and Botros, 2006). They reported that the total number of fungi on maize grains was significantly decreased when treated with 1.5-6 kGy of gamma rays at all storage periods. Aflatoxins B1 & B2 are found to be partially degraded by 2-5 kGy gamma irradiations, while complete degradation was recorded by applying 10 kGy (Aquino *et al.*, 2005).

Plant extracts have been tested by many investigators to protect food stuffs as well as plant products and grains against fungal and bacterial deterioration as well as toxin accumulation. Under laboratorial conditions, many plant extracts exhibit antimicrobial properties (Satish *et al.*, 1999 and Bouamama *et al.*, 2006). Moreover, many investigators reported that extracts of some medicinal plants were effective in inhibition of toxigenic food-borne moulds (El-Assiuty *et al.*, 2006 and Atanda *et al.*, 2007). For instance, tooth pick (*Ammi visnaga*) extract was found to have inhibitory effect on the *in vitro* growth of *Neurospora crassa* (Grange and Davey, 1990). Also, El-Assiuty *et al.* (2006) reported that chloroform-methanol extract of *T. vulgaris, A. visnaga, Cymbopogon proximus* were found to have inhibitory effect, at 2500 ppm, against *A. flavus* growth. Spider flower and tooth-brush were also tested to protect food stuffs against several fungal infections (Badran and Aly, 1995 and Pirzada *et al.*, 2009). However, it was found that *A. visnaga* contains many constituents that may act as fungistatically or fungicidally to plenty of human, animal and plant pathogens (Batanony, 1999). Moreover, Mahmoud (1999) reported that concentration of 2–10 mg/ml aqueous extract of *A. visnaga* fruits inhibited *A. flavus* growth and aflatoxin production.

This investigation aimed to evaluate the efficacy of gamma radiation and some medicinal plant extracts against the deterioration of stored maize grains caused by *A. flavus* and other associated fungi. Also, aflatoxin production in stored maize grains was taken into consideration.

Materials and Methods

1. Source of Aspergillus flavus:

One toxigenic isolate of *A. flavus*, previously isolated from rotted maize grains collected from farmer`s storage at Behera governorate in 2008, was used in the present study.

2. Preparation of A. flavus spore suspension:

Conidial spores of *A. flavus*, cultured on PDA plates and incubated for 12 days at 27±2°C, were harvested and suspended into 15 ml sterilized water then filtered through folds of filter papers. Spore suspension concentration was adjusted to 10^6 spore/ml by aid of a haemocytometer slide.

3. Plant materials:

3.1. Maize grains:

Maize grains (three way cross 310 hybrid) were collected at harvest time from field of Gemmiza Agricultural Experiment Station and artificially inoculated by dipping for 2 min. in *A. flavus* spore suspension (10^6 spore/ml). Final grain moisture content was adjusted to 14% according to Anonymous (1962). Uninoculated grains were kept as a check treatment.

3.2. Medicinal plants:

Leaves of thyme (*Thymus vulgaris*), seeds of tooth pick (*Ammi visnaga*), herb of spider flower (*Cleome arabica*) and stems of tooth-brush tree (*Salvadora persica*) were collected from local markets, then air dried, grounded to fine powder and subjected to the following extractions:

3.2.1. Aqueous extracts:

Dry plant powder (100 g) was transferred into 500 ml conical flasks containing 200 ml sterilized distilled water, then placed on an orbital shaker (120 rpm) for 24h before filtered through double layers of muslin cloth, then filtered again through Whattman (No.1) filter papers. Collected filtrates were centrifuged at 5000 rpm for 30 min., and then sterilized by Seitz's filter G6 (Ismail *et al.,* 1989). Sterilized extracts were kept in a refrigerator at 5°C for further studies.

3.2.2. Solvent extracts:

Dry plant powder (200 g) was transferred into1000 ml sterilized conical flasks containing 400 ml chloroform + methanol (2:1, v:v) and kept in the dark for 15 days, then filtered through Whattman (No. 1) filter papers. Collected extracts left in the dark at room temperature to be evaporated to the dry-film point (El-Assiuty *et al.,* 2006). Dry films were weighed and kept in a refrigerator at 5°C for further studies.

4. In vitro effect of plant extracts on A. flavus growth:

Different concentrations of the tested medicinal plant extracts were prepared, in sterilized distilled water, to evaluate their efficiency in reducing the *in vitro* radial growth of *A. flavus*. Dilution of any extract was separately incorporated into PDA medium at 45°C and poured into 7-cm-diam. sterilized Petri dishes then left to solidify. Discs (5-mm-diam.), taken from 7-day-old *A. flavus* culture, were placed at the centre of each plate. Five plates were used as replicates for each concentration. Dishes were incubated at 27±2°C for 7 days. Fungal radial growth was measured when the fungal growth covered the plate in the control treatment. Growth inhibition percentage was determined according to the following equation:

$$\text{Growth inhibition (\%)} = \frac{C - T}{C} \times 100$$

Whereas: C= Radial growth in control and T= Radial growth in treatment.

5. Storage experiments:

5.1. Effect of plant extracts on fungal incidence and aflatoxins production in stored maize grains:

Maize grains (TWC 310 hybrid) were artificially inoculated with *A. flavus* spore suspension (10^6 spore/ml) as mentioned before. Solvent extracts of tooth pick and thyme were diluted in distilled water to prepare two concentrations, *i.e.* 2500 and 5000 ppm. Each concentration was thoroughly mixed with inoculated, or not, maize grain samples (250 g) and left to dry on filter papers for 15 min. before packed in sealed polyethylene bags then stored under room conditions (25±2°C & 50-70% RH) for 20, 40 and 60 days. Three samples were used as replicates for each treatment.

5.2. Effect of gamma irradiation on fungal development and aflatoxins production in stored maize grains:

Maize grain samples (250 g each), either neutrally infected or artificially inoculated by the tested fungus, were packed in sealed polyethylene bags for irradiation following the method described by Maity *et al.* (2008). Samples were exposed to the following doses of gamma-irradiation: 5 and 10 kGy (60-cobalt gamma source) at dose rat 1.46 kGy/h, in the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. Three replicates were used for each treatment. Tested samples, irradiated or not, were stored under room conditions (25±2°C & 50-70% RH) for 1, 2 and 3 months.

In all storage experiments, tested maize grain samples were subjected, at the end of each storage period, to microbiological analyses and quantitative estimation of produced aflatoxins.

6. Microbiological analyses:

One hundred grains of each treatment were surface sterilized by immersing in 5% sodium hypochlorite solution for 2 minutes, washed thoroughly with sterilized distilled water and left to air dry. Grains were aseptically transferred onto PDA medium and incubated at 27±2°C for 7 days. Emerged fungi were picked up, purified and identified according to Ainsworth and James (1971) and Marasas *et al.* (1984).

7. Determination and quantification of aflatoxins in stored maize grains:

7.1. Aflatoxins extraction and clean up:

The BF method was followed to extract and clean up aflatoxins (Anonymous, 1990). Dried extract was dissolved in 200 µl chloroform. Twenty µl of the extract were spotted on the TLC plate. Twenty µl of the standard solution of B1and B2 as well as of their mixture were also spotted on TLC plates. then developed in a jar 30x10 cm containing the running solvent system of chloroform: acetone: isopropanol: water (88:12:1.5:1 v/v) for 20 min. in darkness. To confirm the presence of aflatoxins, plates were lightly sprayed with 50% sulfuric acid before examined under 365 nm UV.

7.2. Quantification of aflatoxins:

Aflatoxins were quantitatively determined according to Anonymous (1990). TLC plates were scanned under UV (365 nm), using a densitometer. Concentration of mycotoxins was calculated by the following equation:

$$\mu g/kg = \frac{B \cdot Y \cdot S \cdot V}{Z \cdot X \cdot W}$$

Whereas: B = average area of aflatoxin peak in identified sample.
Y = concentration of aflatoxin standard µg / ml.
S = µl spotted aflatoxin.
V = final dilution of extracted sample (µl).
Z = average area of aflatoxin peaks in standard aliquots.
X = µl of spotted sample extract.
W= g sample represent final extract.

Results

1. Effect of plant extracts on radial growth of Aspergillus flavus:

Data presented in Table (1) show that all extract concentrations of the tested medicinal plants resulted in significant reduction in *A. flavus* radial growth, compared with check treatment. In general, growth inhibition (%) was increased with the increasing of concentrations, in both aqueous and/or solvent plant extracts. The 70% efficiency in inhibiting the fungal growth was considered as a reasonable level to consider the extract as a potent inhibitor. However, tooth pick extracts were obviously the most effective in both kinds of tested extracts, when inhibition percentages ranged from 18.57 to 56.29% and from 37.14 to 100% in aqueous and solvent extracts, respectively, followed by thyme extracts (14.86 to 49.71% and 31.43 to 88.29 %, respectively). Aqueous and solvent extracts of tooth-brush recorded the lowest effect, which ranged from 8.29 to 27.14% and 14.57 to 47.43 %, respectively). Generally, recorded data reveal that solvent extract of each medicinal plant was more effective than aqueous extracts. Complete inhibition was recorded at 2500 ppm when solvent extract of tooth pick was used; meanwhile 100% concentration of aqueous extract of the same plant caused 56.29% reduction in the fungal growth.

2. Effect of plant extracts on fungal contamination and aflatoxins accumulation in stored maize grains:

2.1. Effect on fungal contamination:

Data presented in Table (2) show that treating maize grains with plant extracts of tooth pick and thyme, either inoculated or not, before storage effectively reduced the incidence of fungi for up to 60 days, in comparison with untreated grains. In this concern, during all storage periods, it was found that concentration of the tested plant extracts at 5000 ppm was more effective in reducing the percentages of the isolated fungi more than that 2500 ppm in both tested plant materials. Moreover, tooth pick extracts were the most effective in reducing the incidence of the associated fungi, compared with the other tested treatments.

In case of artificially inoculated grains, data cleared that the frequency of *A. flavus* was increased, in all treatments, with increasing storage period from 20 to 60 days, in comparison with zero time. After 60 days of storage, frequency of *A. flavus* was 29.7 and 13.3% in inoculated grains treated with tooth pick extract at 2500 and 5000 ppm, respectively. Meanwhile it was 39.5 and 25.3% in inoculated grains treated with thyme extract at 2500 and 5000 ppm, respectively. Artificially inoculated and untreated treatment (check) recorded 76.5%, 60 days after storage. On the other hand, occurrence of *A. flavus*, in case of uninoculated grains, recorded 18.2 and 5.8% in grains treated with tooth pick extract at 2500 and 5000 ppm, respectively. Meanwhile, it was 26.1 and 14.2% in grains treated with thyme extract at 2500 and 5000 ppm, respectively. Uninoculated and untreated treatment (check) recorded 43.6%, 60 days after storage.

The same trend was observed in case of the other associated fungi, *i.e. A. niger, Penicillium* spp. and *Fusarium* spp. but with low frequency compared with *A. flavus*.

Table 1. Effect of different concentrations of aqueous and solvent extracts of medicinal plants on the radial growth of *A. flavus*, 7 days after incubation at 27±2°C

Tested Plant material	Radial growth (RG) and reduction (R)					
	Aqueous extract			Solvent extract		
	Conc. (%)	RG (cm)	R (%)	Conc. (ppm)	RG (cm)	R (%)
Thyme	0	7.00	0.00	0	7.00	0.00
	25	5.96	14.86	500	4.80	31.43
	50	5.58	20.29	1000	3.92	44.00
	75	4.76	32.00	1500	2.94	58.00
	100	3.52	49.71	2500	0.82	88.29
	Mean	5.36	23.37	Mean	3.90	45.42
Tooth pick	0	7.00	0.00	0	7.00	0.00
	25	5.70	18.57	500	4.40	37.14
	50	4.98	28.86	1000	3.20	54.29
	75	3.58	48.86	1500	2.02	71.14
	100	3.06	56.29	2500	0.00	100.00
	Mean	4.86	30.51	Mean	3.32	52.51
Spider flower	0	7.00	0.00	0	7.00	0.00
	25	6.34	9.43	500	5.78	17.43
	50	6.12	12.57	1000	4.98	28.86
	75	5.62	19.71	1500	4.16	40.57
	100	4.52	35.43	2500	3.02	56.86
	Mean	5.92	15.43	Mean	4.99	28.74
Tooth-brush	0	7.00	0.00	0	7.00	0.00
	25	6.42	8.29	500	5.98	14.57
	50	6.24	10.86	1000	5.20	25.71
	75	5.84	16.57	1500	4.40	37.14
	100	5.10	27.14	2500	3.68	47.43
	Mean	6.12	12.57	Mean	5.25	24.97
L.S.D.$_{(0.05)}$ for:	Treatment (T) = 0.16 Concentration(C) = 0.12 T × C = 0.23			L.S.D.$_{(0.05)}$ for:	T = 0.08 C = 0.12 T × C = 0.23	

Table 2. Effect of different concentrations of the tested plant extracts on the frequency of fungi associated with stored maize grains

Plant extract	Treatment		Frequency (%) of fungi isolated from stored maize grains*							
			Artificially inoculated				Uninoculated			
	Conc. (ppm)	Storage period (day)	*A.* ** *fla.*	*A. nig.*	*F.* spp.	*P.* spp.	*A. fla.*	*A. nig.*	*F.* spp.	*P.* spp.
Tooth pick	2500	20	10.9	1.9	1.3	1.6	4.5	2.9	3.0	3.1
		40	17.3	3.9	2.7	3.1	11.8	4.7	4.6	4.9
		60	29.7	5.7	4.1	5.2	18.2	7.0	8.5	8.3
	5000	20	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		40	8.1	1.3	0.0	1.4	2.0	1.9	1.5	1.8
		60	13.3	1.9	1.0	2.1	5.8	3.9	1.9	3.9
Thyme	2500	20	14.2	2.7	1.9	2.1	6.6	3.2	4.9	4.4
		40	22.7	4.7	3.8	4.0	18.0	6.2	6.3	6.3
		60	39.5	6.9	5.5	8.3	26.1	9.9	11.7	11.2
	5000	20	7.1	1.6	1.0	1.1	3.3	2.7	1.6	2.9
		40	15.6	3.3	2.1	2.0	10.4	3.5	3.8	4.7
		60	25.3	4.9	3.7	4.5	14.2	6.5	6.6	7.4
Check		20	36.7	4.8	6.3	4.0	10.0	6.4	7.0	6.8
		40	61.3	7.7	12.4	6.4	30.3	10.7	13.7	10.9
		60	76.5	13.8	16.7	11.6	43.6	14.3	17.5	17.6

* At zero time, occurrence of *A. flavus* recorded (2.3%), while it was (3%) for *Fusarium* spp.
** *A. fla.*: *A. flavus*; *A. nig.*: *A. niger*; *F.* spp.: *Fusarium* spp.; and *P.* spp.: *Penicillium* spp.

2.2. Effect on aflatoxins production:

Data presented in Table (3) show that no aflatoxins were detected at zero time, while increasing storage period from 20 to 60 days led to considerable increase in the amount of total aflatoxins (B1 & B2) from 0.00 to 29.05 ppb, in untreated (check) grains. Meanwhile, it increased from 16.55 to 65.68 ppb in untreated and artificially inoculated grains, respectively.

Plant extracts of tooth pick and thyme markedly reduced the amount of aflatoxins after storage for 60 days. Moreover, the highest efficiency in reducing aflatoxins by both plant extracts was observed at 5000 ppm. However, tooth pick extract reduced aflatoxins production more than thyme extract at any tested

Table 3. Effect of different concentrations of plant extracts on aflatoxin production by *A. flavus* in stored maize grains

Plant extract	Treatment		Aflatoxin level (ppb)*					
			Artificially inoculated			Uninoculated		
	Conc. (ppm)	Storage period (day)	B1	B2	Total	B1	B2	Total
Tooth pick	2500	20	0.56	0.00	0.56	0.00	0.00	0.00
		40	2.98	1.12	4.10	1.00	0.14	1.14
		60	7.64	1.81	9.45	3.13	1.22	4.35
	5000	20	0.00	0.00	0.00	0.00	0.00	0.00
		40	0.00	0.00	0.00	0.00	0.00	0.00
		60	1.46	0.00	1.46	0.00	0.00	0.00
Thyme	2500	20	2.54	1.20	3.74	0.00	0.00	0.00
		40	5.07	2.48	7.55	3.38	0.98	4.36
		60	9.98	3.61	12.59	7.49	1.20	8.69
	5000	20	0.00	0.00	0.00	0.00	0.00	0.00
		40	2.98	0.00	2.98	0.39	0.00	0.39
		60	6.94	0.00	6.94	2.24	0.00	2.24
Check		20	14.41	2.14	16.55	0.00	0.00	0.00
		40	39.05	4.89	43.93	8.55	2.38	10.93
		60	56.18	9.50	65.68	25.79	3.26	29.05

* No aflatoxin was detected at zero time.

concentration. It was also observed that no aflatoxins produced 20 days after storage at 2500 ppm of both plant extracts in uninoculated grains, while slight amount was produced in artificially inoculated grains. On the other hand, aflatoxins were gradually increased with increasing storage period either in uninoculated or artificial inoculated grains.

3. Effect of gamma irradiation on deterioration and aflatoxins production:

3.1. Effect on mould incidence:

Results presented in Table (4) show that gamma irradiation effectively reduced the incidence of *A. flavus* and the other associated fungi, either in artificially inoculated or in uninoculated maize grains. It was also observed that dose of 10 kGy was more effective than that of 5 kGy in this regard.

Table 4. Effect of different doses of gamma irradiation on the occurrence of fungi in the stored maize grains

Gamma dose (kGy)	Storage period (month)	Fungal occurrence*							
		Artificial inoculation				Uninoculated			
		A. fla. **	*A. nig.*	*F.* spp.	*P.* spp.	*A. fla.*	*A. nig.*	*F.* spp.	*P.* spp.
5	One	2	0	0	0	0	0	0	0
	Two	11	6	0	0	3	3	0	0
	Three	23	9	2	0	11	3	2	0
10	One	0	0	0	0	0	0	0	0
	Two	0	0	0	0	0	0	0	0
	Three	5	2	0	0	2	1	0	0
Check (un-irradiated)	One	55	8	9	2	11	3	9	5
	Two	62	12	15	8	33	10	18	9
	Three	83	14	21	11	49	16	28	17

* At zero time, occurrence of both *A. flavus* and *Fusarium* spp. recorded 3%.
** As described in footnote of Table (2).

At zero time, occurrence of both *A. flavus* and *Fusarium* spp. reached 3%. Meanwhile, two months after storage, exposing the grains to 10 kGy resulted in complete absence of *A. flavus* and other associated fungi, either in uninoculated and artificially inoculated grains. While, 5 kGy prevented the incidence of the associated fungi up to one month in both un-inoculated and inoculated grains.

Un-irradiated grains recorded high incidence for *A. flavus* compared with irradiated ones. Also, it was increased with increasing of storage period, where the higher incidence was recorded after three months of storage in either artificial inoculated or un-inoculated grains, being 83 and 49%, respectively.

A. niger was found in un-irradiated grains with low incidence compared with *A. flavus*. Meanwhile, *Penicillium* spp. was observed in un-irradiated grains only.

3.2. Effect on aflatoxins production:

Results in Table (5) show that no aflatoxins were detected at zero time, whereas one month after storage of un-irradiated grains, no aflatoxins (B1 & B2) were detected in uninoculated grains and were 35.3 ppb in artificially inoculated grains, then reached to 30.1 and 70.6 ppb, after three months of storage respectively. On the other hand, exposing to gamma radiation was very effective in reducing aflatoxins production during storage for three months, where no aflatoxins produced in uninoculated grains exposed to both doses. In case of artificially inoculated grains, no aflatoxins produced when 10 kGy was applied, while slight amount of only B1 was recorded in grains exposed to 5 kGy, after two and three months of storage, (being 0.6 and 4.9 ppb, respectively).

Table 5. Effect of different doses of gamma irradiation on aflatoxins (B1 &B2) produced by *A. flavus* in stored maize grains

Gamma dose (kGy)	Storage period (month)	Aflatoxin concentration (ppb)*					
		Artificial inoculation			Uninoculated		
		B1	B2	Total	B1	B2	Total
5	One	0.0	0.0	0.0	0.0	0.0	0.0
	Two	0.6	0.0	0.6	0.0	0.0	0.0
	Three	4.9	0.0	4.9	0.0	0.0	0.0
10	One	0.0	0.0	0.0	0.0	0.0	0.0
	Two	0.0	0.0	0.0	0.0	0.0	0.0
	Three	0.0	0.0	0.0	0.0	0.0	0.0
Check (un-irradiated)	One	29.8	5.5	35.3	0.0	0.0	0.0
	Two	36.2	9.8	46.0	7.9	0.0	7.9
	Three	54.9	15.7	70.6	26.4	3.7	30.1

* No aflatoxins were detected at zero time.

Discussion

In current study, an attempt was done to mange maize grain deterioration caused by the associated fungi as well as accumulation of aflatoxins during different storage periods under controlled conditions. Two methods of application were tested, *i.e.* plant extracts and gamma irradiation.

Plant extracts used in the present study caused an inhibitory effect on *in vitro* radial growth of *A. flavus*. As all experimented extracts caused significant reduction to the fungal growth, Chloroform-methanol was superior to the aqueous extract in this respect. Extracts of tooth pick (*Ammi visnaga)* followed by thyme (*Thymus vulgaris*) caused the highest effect on the fungal growth. Treating maize grains with thyme and tooth pick extracts resulted in great reduction in grain deterioration and obvious reduction in aflatoxins accumulations. These results are in accordance with the findings of El-Assiuty *et al.* (2006) who reported that chloroform-methanol extracts obtained from *A. visnaga* and *T. vulgaris* out of various medicinal and indigenous plants, were found to have highly potent effect on the growth of *A. flavus*. Batanony (1999) reported that *A. visnaga* contains many constituents that may have fungitoxic effect on plant pathogens. Khellin, visnagin, khellol, khellenin and khellenol are the most identified substances reported to be involved in this extract. As reported by Gouda (2001), Khellin was responsible for the potent effect on the growth of root-rot fungi of sugar beet and regarded the toxicity to this substance.

Thyme extract was extensively studied for its antimicrobial effect on different pathogens as reported by Baranauskiene *et al.* (2003). Some phenolic compounds were identified by Schvartz *et al.* (1996) from hexane extract of thyme. Thymol had higher inhibitory action which might be due to the presence of a phenolic –OH group (Farag *et al.*, 1989). It is well known that plenty of phenols were reported to be responsible for the fungitoxic effect of many medicinal plants (Davidson, 2001).

Maize grains were reported to be satisfactory protected from infection with some ear and kernel rots and mycotoxin production by treating with various plant extracts as reported by El-Assiuty *et al.* (2007). They found that maize kernel rots caused by *A. flavus* and *F. verticillioides* could be significantly reduced by treating maize ears with *Cymbopogon proximus*-extract. Also, they observed that accumulation of aflatoxins B1 & B2 were obviously reduced due to the treatment. Similarly, on rice grains, Reddy *et al.* (2009) stated that aflatoxins could be completely inhibited with the extract of *Syzigium aromaticum* and there is a possibility of its being used to protect rice grains against Aspergillus contamination.

Gamma irradiation was sufficiently effective in reducing deterioration of grains treated with 5 and 10 kGy and stored for up to 3 months. This application caused obvious reduction in infection by *A. flavus* as well as the associated fungi, compared with check treatment. These results are in accordance to those obtained by previous investigators (Aziz and Abd El-Aal, 1990 and Aquino *et al.*, 2005). As we found in the present work, increasing of irradiation dose was found by Rustom (1997) to increase the reduction of fungal growth.

Aflatoxin production was also found to be affected by irradiation. Results indicated that gamma irradiation caused great reduction in accumulation of total aflatoxins (B1 and B2) compared with the control. The effect of this treatment on aflatoxins production was increased obviously by increasing the radiation dose from 5 to 10 kGy. In this respect, the higher dose of irradiation caused complete inhibition of toxins production. Aflatoxin production was found to be affected by gamma radiations as reported by some investigators working on some crop seeds rather than maize (Aquino *et al.*, 2005 and Mahrous, 2007). On maize grains, Aquino *et al.* (2005) stated that aflatoxins production was degraded by gamma irradiation. Aziz *et al.* (2005) reported that mycotoxin production was decreased by increasing gamma irradiation dose. Some of mechanisms by which irradiation may cause cellular inactivation were summarized by many researchers (Hurst, 1977; Gould, 1989 and Kim and Thayer, 1996). These mechanisms are: i) Damage of DNA, ii) Inhibition of protein synthesis, iii) Damage of cell membrane, iv) Inactivation of critical metabolic enzymes. Findings of the current study emphasized that the effect of irradiation on controlling maize grain-borne pathogens is proposed to maximize grain safety.

In a conclusion, usage of pesticides to minimize grain deteriorations seriously affects human and animal health and pollutes the environment. Thus, it is recommended to follow some safe methods, responsive with the environment, to protect food and feed stuffs. Current investigation suggesting the usage of gamma irradiation and some extracts of medicinal plants, such as thyme and tooth pick, to mange infection by rotting fungi and decrease aflatoxin production in stored grains.

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تأثير المعاملة باشعة جاما والمستخلصات النباتية على أعفان حبوب الذرة الشامية المخزنة وتراكم الأفلاتوكسينات

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تم اختبار فعالية أشعة جاما ومستخلصات بعض النباتات الطبية ضد تدهور حبوب الذرة الشامية المخزنة وتراكم الأفلاتوكسينات بها. وقد أدت معاملة الحبوب بأشعة جاما بجرعتي ٥ و ١٠ كيلوجراي وتخزينها لمدة ثلاثة أشهر إلى خفض ملحوظ فى نسب الإصابة بالفطر *Aspergillus flavus* والفطريات المصاحبة الأخرى، كما سببت انخفاض كبير في إنتاج الأفلاتوكسينات (B1& B2) وذلك بالمقارنة مع الحبوب غير المعاملة بالأشعة. ولوحظ أن التأثير الأعلى لهذه المعاملة ظهر عند الجرعة ١٠ كيلوجراي حيث أدت إلى تثبيط تام لإنتاج الأفلاتوكسينات في نهاية مدة التخزين.

وجد أيضاً أن المعاملة بتركيزات مختلفة من مستخلصات الكلوروفورم - ميثانول والمستخلصات المائية لكل من الزعتر والخلة والسموة والسواك قد سببت انخفاض معنوي فى نمو الفطر *A. flavus* . وقد أظهرت النتائج أن مستخلصات الكلوروفورم - ميثانول كانت أكثر فعالية من المستخلصات المائية لهذه النباتات. كما وجد أن مستخلصات الخلة كان لها التأثير الأكبر على خفض النمو الفطري يليها مستخلصات الزعتر. كذلك وجد أن معاملة حبوب الذرة الشامية بمستخلصات الكلوروفورم - ميثانول لكل من الزعتر والخلة بتركيز ٢٥٠٠ و ٥٠٠٠ جزء بالمليون أظهرت انخفاضاً كبيراً في نسبة ظهور الفطر أسبرجلس فلافس والفطريات المصاحبة بالإضافة إلى انخفاض ملحوظ في إنتاج الأفلاتوكسين بعد التخزين لمدة ٢٠ إلى ٦٠ يوم.