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Effect of some Bioagents, Plant Extracts and Gamma Irradiation on the Deterioration and Fumonisin Production in Stored Maize Grains

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Public concern regarding food safety has increased in recent years as mycotoxins have been reported hazards associated with animal feeds in storage. *Fusarium verticillioides* contamination in food grains has been reported worldwide. This fungus produces fumonisins, that harmful to humans and animals.

Three strains of *Trichoderma* spp., *i.e. T. viride, T. harzianum* and *T. hamatum*, plant extracts of halfa barr and thyme as well as different gamma irradiation at doses were tested for their capability to decrease deterioration and fumonisins accumulation in stored maize grains. *In vitro* evaluation of the antagonistic effect of *Trichoderma* spp. against *F. verticillioides* revealed that *T. viride* was the most effective one in reducing the pathgen growth. When they formulated as a powder to study their effect on inhibiting fumonisins toxins produced in artificially inoculated maize grains during storage at 30°C and 90% relative humidity (RH) for up to 4 months, obtained results revealed that *T. viride* recorded the highest reducetion in the frequency of *F. verticillioides* and toxin production during the tested storage period.

Application of different concentrations of *n*-hexane and chloroform – methanol and/or aqueous extracts of halfa barr and thyme caused significant reduction in the *in vitro* growth of *F. verticillioides*. *n*-hexane and chloroform – methanol (2:1 v/v) were more effective than the aqueous extracts of the halfa barr and thyme, respectively. Halfa barr extract at 4000 ppm caused the highest reduction to the fungal growth followed by thyme extract. Moreover, treatment of maize grains with halfa barr extract before storeing for up to 4 months greatly reduced the population of *F. verticillioides* as well as fumonisins production.

Physical treatment of maize grains with different doses of gamma irradiation effectively reduced the incidence of *F. verticillioides* during storage for up to 4 months. Also, in case of artificial inoculation, these treatments greatly reduced fumonisins production in comparison with un-irradiated grains. The highest effect of irradiation was occurred by 10 kGy, which resulted in negligible incidence of *F. verticillioides* and complete inhibition of fumonisins production during the storage period.

Keywords: Biological control, fumonisin, *Fusarium verticillioides*, gamma radiation, halfa barr, maize grains, thyme and *Trichoderma* spp.

Maize (*Zea mays* L.) is considered as one of the most important foods and feed cereal crops world wide. The cultivated area in Egypt annually reached about 1.99 million feddan that yielded about 6.8 million ton of grains, meanwhile it reached about 150 million hectares with annual harvest more than 800 million tons of grain worldwide (Anonymous, 2010). Recent records suggest that 25% of the world's crops are subjected to infect with more than 300 fungal genus, which known to produce metabolites as mycotoxins in their seeds and grains, especially under unsuitable storage conditions (Galvano *et al.,* 2001). Moulds are usually caused by one or more fungus, depending on locality and entries as well as on the environmental factors. In particular, *Fusarium* spp. and *Aspergillus* spp. are the most prevalent through storage (El-Naggar, 2007). These fungi are known to develop more rapidly under bad storage conditions, which also suitable to produce mycotoxins. For example, Fumonisins are produced by *Fusarium verticillioides* and *F. proliferatum* (El-Shabrawi, 2001).

Some trials have been done to evaluate natural means for managing grain deterioration by *F. verticillioides* and fumonisins production (Nayaka *et al.*, 2010). *Trichoderma* spp. are now the most common fungal biological control agents against several fungal pathogens that have been extensively researched and deployed *in vitro* and/or *in vivo* throughout the world. The primary mechanism of antagonism in Trichoderma is mycoparasitism which cause by production of volatile and non-volatile antibiotics and lytic activity (Paavanen-Huhtala *et al.,* 2000).

Nevertheless, *T. viride* isolated from roots of corn plants was able to suppress linear growth of *F. verticillioides* on agar as well as fumonisin B_1 (FB_1) produced on corn kernels; so, it is might be useful in biological control as a pre-harvest agent to prevent disease during plant development and/or as a post-harvest agent to suppress FB_1 production during seed storage (Yates *et al.,* 2000).

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 exhibited antimicrobial properties (EL-Assiuty *et al.,* 2006a; Suleiman, *et al.*, 2008 and Srichana, *et al.*, 2009).

Recently, gamma irradiation was tested by several researchers to protect food stuffs and grains against fungal infection and fumonisins production (Sreenivasa *et al.*, 2009). Moreover, Ferreira-Castro *et al.* (2007) found a possibility to decrease the risk of exposure to fumonisins by irradiating maize grains with 5 or 10 KGy. On the other hand, the levels of mycotoxins produced by irradiated strains were two times greater than those produced by control strains (Ribeiro *et al.,* 2011).

The present investigation aimed to evaluate the efficacy of some bioagents, *i.e. T. viride, T. harzianum* and *T. hamatum*, medicinal plant extracts, *i.e.* herb of halfa barr and Leaves of thyme as well as different gamma irradiation doses, *i.e.* 2.5, 5.0, 7.5 and 10.0 KGy with 60-cobalt gamma source, against the deterioration of stored maize grains caused by *F. verticillioides* and other associated fungi during storing. Also, fumonisins production in stored maize grains was taken in consideration.

Materials and Methods

Source of the tested Fusarium isolate:

In a part of M.Sc. Thesis, the most toxigenic isolate of *F. verticillioides*, chosen from 49 isolates which isolated from stored maize grains in local farmers storages at Kafr-Elsheikh governorate during 2008/2009, was used in the present study.

Preparation of spore suspension:

Spore suspension, of the tested fungus, was empirically prepared from 7-day-old cultures grown on PDA plates (9-cm-diam.) according to the method of Papavizas and Christensen (1960). Spore suspension density was adjusted to 10^6 spore/ml.

Source of Plant materials:

Maize grains (TWC 310 hybrid) were collected at harvest time from farmer fields of Gemmiza Agricultural Experiment Station, Kafr-Elsheikh governorate and artificially inoculated by dipping in the prepared spore suspension for 2.0 min. Final grains moisture content (M.C) was adjusted to 14% according to Anonymous (2000). Uninoculated grains were kept as a control treatment.

Preparation of bioagent inoculum

Three *Trichoderma* spp., *i.e. T. harzianum, T. hamatum* and *T. viride*, kindly provided from Central Lab. of Organic Agric., ARC, Giza, Egypt, were tested in this study. They were maintained refrigerated at 4°C on potato dextrose agar (PDA) medium for further experiments.

Tested *Trichoderma* spp. were individually grown on liquid gliotoxin fermentation medium (G.F.M) [25.0 g dextrose, 2.0 g ammonium tartrate, 2.0 g K_2HPO_4, 1.0 g $MgSO_4$, 20.0 g agar, traces of $FeSO_4.6\ H_2O$ and complete the volume up to 1000 ml distilled water] (Brian and Hemming, 1945) under complete darkness condition at 28°C for 9 days to stimulate toxin production (Abd El-Moity and Shatla, 1981). Bioagents were formulated as a powder form by mixing blended culture media with talc powder at the rate of 1: 1 (v/w) and adjusted to contain 30×10^6 cfu/ 1.0 g using the developed method of Abd El-Moity (1985).

In vitro evaluation of antagonistic ability of Trichoderma spp.:

The antagonistic effect of *Trichoderma* spp. against the tested growth was determined under laboratory conditions. Petri-dishes (9.0-cm- diam.), each contains sterilized PDA medium, were inoculated with discs (5.0-mm-diam.) of *F. verticillioides* obtained from the periphery of 5 days old colony, whereas the opposite side was inoculated with a disc of any of *Trichoderma* spp. (5.0 mm in diameter) obtained from 5 days old colony. Four plates were used as replicates for each treatment. Plates inoculated with *F. verticillioides* only served as a control treatment. Inoculated plates were then incubated at 27±2°C. When mycelial growth covers all medium surface in control treatment, antagonism and/or parasitism occurred between the two tested fungi was examined. *F. verticillioides* mycelial reduction percentages were calculated using the following formula (Srichana *et al.*, 2009):

Mycelial growth reduction (%)= 100 - [(G2 / G1) ×100]

Whereas: G1: mean diameter (mm) of pathogenic fungus growth in control plates;
G2: mean diameter (mm) of pathogenic fungus growth in treated plates in the presence of *Trichoderma* spp.

Medicinal plants:

Herb of halfa barr (*Cymbopogon proximus*) and leaves of thyme (*Thymus vulgaris*) were obtained from the local market and air dried, grinding to fine powder and subjected to by following extractions:

1. Organic solvent extracts:

Dry powder of explants (200 g) was transferred into 1000 ml sterilized conical flasks containing, a) 400 ml N-hexane of halfa barr and b) 400 ml chloroform + methanol (2:1 v:v) of thyme. The extracts were kept in the dark for up to two weeks and then filtered through Whatman (No. 1) filter papers. The filtrates were evaporated to the dry-film at room temperature according to EL-Assiuty *et al.* (2006a) and El-Assiuty *et al.* (2007). Dry films were weighed and kept in a refrigerator at 5°C for further studies.

2. Aqueous extracts

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

In vitro reduction percentage of F. verticillioides growth as affected by plant extracts:

Different concentrations of the tested plant extracts (*i.e.* 500, 1000, 1500 and 2000 ppm in case of solvent extract and 25, 50, 75 and 100% in case of aqueous extracts) were used to study their *in vitro* efficiency on the reducing radial growth of *F. verticillioides.* Dilution of any extract was separately incorporated into PDA medium before solidification, and then dispensed in sterilized Petri-dished. Four dishes (replicates) were used for each concentration, then inoculated at the centre with equal mycelia discs (5.0-mm-diam.) taken from periphery of 7 days old *F. verticillioides* cultures, then incubated at 27±2°C. Fungal radial growth was measured when the fungal growth covers all medium surfaces in the control plates. Reduction (%) in mycelial growth of pathogenic fungus was calculated as mentioned before.

Storage experiments:

Maize grain (500 g) either naturally infected (N_1) or artificially inoculated (A_1) by *F. verticillioides* spore suspension (10^6 spore/ml), first coated with a sticking agent "Sacrust M-455 (Postfach 302, Frankfurt, Germany)", and treated with bioagent, plant extracts and/or gamma irradiation in the following steps. Treated maize grains had been packed in sealed cotton bags, compared with maize grain

without any treatment (control). All bags were stored at 30°C and 90% relative humidity (RH) for up to 4 months (Andrea *et al.*, 2009). Three replicates were used for each treatment and examined periodically every month of the storage period.

1. Trichoderma spp. as bioagent:

Latest maize grains prepared for storage treated with different *Trichoderma* spp., *i.e. T. viride, T. harzianum* and *T. Hamatum*, in powder formulation at the rate of 10.0gm / 1.0 kg maize grains, before start the storage period (Abd EL-Moiety *et al.*, 1990).

2. Plant extracts:

Prepared solvent extracts of halfa barr and thyme were diluted in distilled water to prepare two concentrations, *i.e.* 2000 and 4000 ppm. Each concentration was mixed with previous maize grains prepared for storage and left to dry on filter papers for 15 min before being packed in sealed cotton bags then start the storage period.

3. Gamma irradiation:

Previously prepared maize grains for storage, neutrally infected or artificially inoculated by *F. verticillioides,* were irradiated with gamma irradiation using the method described by Maity *et al.* (2008). Tested samples were exposed to the different doses of gamma irradiation, *i.e.* 2.5, 5.0, 7.5 and 10.0 KGy (60-cobalt gamma source) at dose rat 1.46 KGy/h, in the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Microbiological frequency analysis:

One hundred maize grains, previously stored, was surface sterilized by immersing in 5.0% sodium hypochlorite solution for 3 min, rinsed twice in sterilized distilled water and left to dryness on sterilized filter paper. Grains were aseptically transferred into PDA plates and incubated at 27±2°C for 7 days. Emerged fungi were picked up, purified and identified according to Marasas *et al.* (1984).

Detection and determination of fumonisins (FB_1) in stored maize grains:

1. Extraction and clean up of FB_1:

Extraction and clean up of FB_1 from previously stored maize grains samples were carried out according to the method of Dupuy *et al.* (1993). About 10.0 gm of tested samples were grinded and extracted by soaking in 100 ml of ACN: H_2O (1 : 1, v/v) for 18 h at room temperature. Then, extracts were filtered through Whatman (No.1) filter papers. Collected filtrate was evaporated to dryness under steam of nitrogen. The residues were then dissolved in 100 µl ACN : H_2O (1 : 1, v/v), and 10 µl from sample residue and 10 µl from working standard were spotted on thin layer chromatography (TLC) plates (Merck silica gel 60 without fluorescent, 20×20 cm with layer thickness of 0.25 mm). Separation was carried out using two running systems as follows: a) The plate was developed in glass jar with 1-butanol : acetic acid : water (20 : 10 : 10, v/v/v); after solvent evaporation, the bottom of the plate was cut off approximately 1.0 cm below the expected *Rf* of FB_1 (0.25) to eliminate impurities; b) The upper part of the plate was developed again in the same direction with chloroform : methanol : water : acetic acid (55 : 36 : 8 : 1).

2. Quantitative determination of FB1 by TLC:

TLC plates were dried and then sprayed with a developing solvent of 0.5% p-anizaldehyde in methanol. Plates were then heated for 5 min at 110°C, and visually inspected under Ultra Violate (UV 365 nm). FB_1 appear as a reddish-purple spotting comparing with standard spot colour and R*f* (0.25) according to Dupuy *et al.* (1993).

Quantification of FB_1 was performed according to Anonymous (1990) by scanning the TLC plates with a spectrophotodensitometer (No. CS930; Shimadzu Corp., Kyoto, Japan) set at 600 nm to identifying sample peak area comparing with the FB_1 standard concentration area peaks. Sample concentrations were calculated by the following equation:

$$\mu g/kg = (B.Y.S.V) / (Z.X.W)$$

Whereas: B = Average area of peak in identified sample.
Y = Concentration of FB1 standard (µg / ml).
S = µl spotted FB1.
V = Final dilution of extracted sample (µl).
Z = Average area of FB1 peaks in standard aliquots.
X = µl of spotted sample extract.
W= Weight (g) sample represents final extract.

Data analysis and statistics:

Data were analyzed using analysis of variance (ANOVA), and the means were compared by the least significant differences (LSD) at $P \geq 0.05$ described by Snedecor and Cochran (1980), the significant mean differences between treatment means were separated by Duncan's Multiple Range Test (Duncan, 1955).

Results

1. Inhibition effect of bioagents and plant extracts:

1.1. Antagonistic potential of Trichoderma spp. against F. verticillioides:

Data presented in Table (1) indicate that all tested strains of *Trichoderma* spp. significantly inhibited mycelial growth of *F. verticillioides* with variable antagonistic potentials. Among the three evaluated *Trichoderma* strains, *T. viride* recorded the most antagonistic potential (76.66%) followed by *T. harzianum* (64.44%), while *T. hamatum* showed the lowest antagonistic potential (50.00%).

1.2. Minimum inhibition concentration of plant extracts:

The two tested plant extracts recorded reduction in linear growth of *F. verticillioides* under laboratory conditions. Results presented in Table (2) demonstrate that all tested plant extracts, concentrations, resulted in significant reduction in radial growth of *F. verticillioides*, compared with control treatment. In addition, growth inhibition (%) was increased with increasing of plant extract concentrations, in case of both solvent and/or aqueous plant extracts. However, halfa barr extracts were obviously the most effective in both kinds of tested extracts, when percentages of inhibition ranged from 42.22 to 100% and from 37.77 to 75.55% in case of solvent and aqueous extracts, respectively, followed by thyme extracts (36.33 to 89.11% and 33.55 to 64.00%, respectively).

Table 1. *In vitro* antagonistic ability of *Trichoderma* spp. against *F. verticillioides*, grown on PDA, 7 days after incubation at 27±2°C.

Tested bioagent	*F. verticillioides* growth (cm)	Reduction (%)
Control	9.0	0.00 ± 0.00 [d]
T. viride	2.1	76.66 ± 0.83 [a]
T. harzianum	3.2	64.44 ± 0.72 [b]
T. hamatum	4.5	50.00 ± 1.51 [c]
L.S.D at 5%		1.75

- Each value represents the mean of three replicates.
- Values with the same letters in column are not significantly different by Duncan's Multiple Range Test at ($P \leq 0.05$).

Table 2. Effect of different concentrations of aqueous and solvent extracts of medicinal plants on the linear growth of *F. verticillioides*, 7 days after incubation at 27±2°C.

Tested Plant material	Mean ± SD					
	Solvent extract			Aqueous extract		
	Conc. (ppm)	Linear growth (cm)	Reduction (%)	Conc. (%)	Linear growth (cm)	Reduction (%)
Halfa barr	0	9.00	0.00 ± 0.00[e]	0	9.00	0.00 ± 0.00[e]
	500	5.20	42.22 ± 0.46[d]	25	5.60	37.77 ± 0.41[d]
	1000	4.30	52.22 ± 0.58[c]	50	4.95	45.00 ± 0.50[c]
	1500	2.00	77.77 ± 2.34[b]	75	3.02	66.44 ± 2.00[b]
	2000	0.00	98.24 ± 2.07[a]	100	2.20	75.55 ± 1.59[a]
	Mean	4.10	54.21	Mean	4.95	44.95
L.S.D. (0.05)	2.00			1.71		
Thyme	0	9.00	0.00 ± 0.00[e]	0	9.00	0.00 ± 0.00[e]
	500	5.70	36.33 ± 0.39[d]	25	5.98	33.55 ± 0.36[d]
	1000	4.90	45.55 ± 0.51[c]	50	5.49	39.00 ± 0.43[c]
	1500	2.50	72.22 ± 2.17[b]	75	4.40	51.11 ± 1.54[b]
	2000	0.98	89.11 ± 1.88[a]	100	3.24	64.00 ± 1.35[a]
	Mean	4.62	48.64	Mean	5.62	37.53
L.S.D. (0.05)	1.84			1.33		

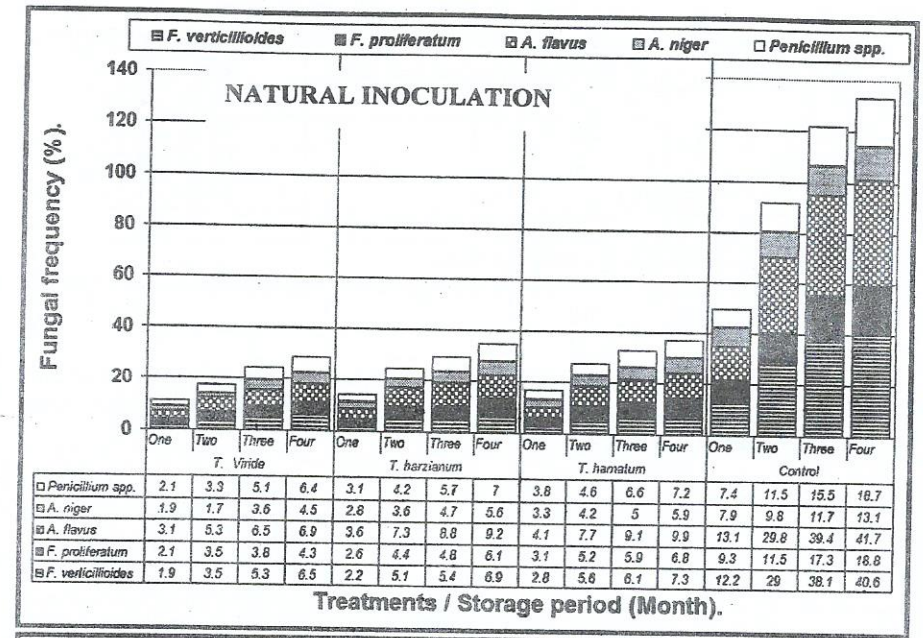
- Each value represents the mean of three replicates; SD: standard deviation.
- Mean values with the same letters in column are not significantly different by Duncan's Multiple Range Test at ($P \leq 0.05$).

2. Effect of bioagents, plant extracts and gamma irradiation on fungal contamination and fumonisins accumulation in stored maize grains:

2.1. Effect of Trichoderma spp. as bioagent:

2.1.1. Effect of bioagent on fungal contamination:

Data illustrated in Fig. (1) show that treating maize grains with *Trichoderma* spp., either (N_1) or (A_1), before storage effectively reduced the frequencies of the associated fungi up to 4 months, in comparison with untreated (control) grains.



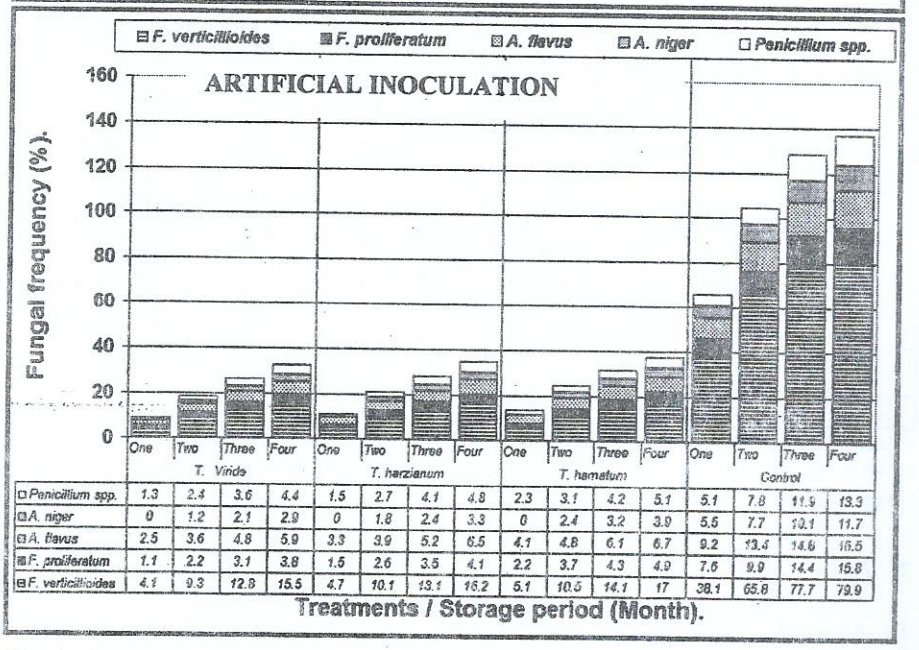


Fig. 1. Effect of *Trichoderma* spp. on the frequency of fungi associated with stored maize grains up to 4 months at 30°C / 90 RH. Where, at zero time, frequency of *F. verticillioides* recorded (1.0%), meanwhile it was (2.2%) for *A. flavus*.

Moreover, *T. viride* was the most effective in reducing the frequency of the associated fungi, compared with the other tested bioagent. The frequency of *F. verticillioides* was increased in all treatments of (A_1) grains, with increasing storage period from 1 to 4 months, in comparison with zero time. After 4 months of storage, frequency of *F. verticillioides* was 15.5, 16.2 and 17.0% in (A_1) grains treated with *T. viride*, *T. harzianum* and *T. hamatum*, respectively. Artificially inoculated and untreated treatment (control) recorded 79.9% after 4 months of storage. One the other hand, frequency of *F. verticillioides*, in case of (NI) grains, recorded 6.5, 6.9 and 7.3% in grains treated with *T. viride*, *T. harzianum* and *T. hamatum*, respectively. Uninoculated and untreated treatments (control) recorded 40.6%, after 4 months of storage.

2.1.2. Efficacy of different bioagent in inhibiting fumonisins production:

Data presented in Fig. (2) show that no fumonisins were detected at zero time, while increasing storage period from 1 to 4 months led to considerable increasing in the amount of fumonisins from 0.00 to 37.37 ppb, in untreated grains (control). Meanwhile, fumonisins level increased from 33.55 to 79.09 ppb, in control treatment with untreated and artificially inoculated grains, respectively. Also, *Trichoderma* spp. as bioagents can inhibit fumonisins toxins produced by *F. verticillioides* in maize grains. Both of *Trichoderma* spp. gave remarkable reduction in fumonisins production compare with untreated grains (control). *T. viride* was better than *T. harzianum* and *T. hamatum* in inhibiting fumonisins production; it was recorded 95.6, 90.1 and 85.7% of reduction, respectively, in case of (AI) after 4 months of storage in comparison with inoculated control treatment.

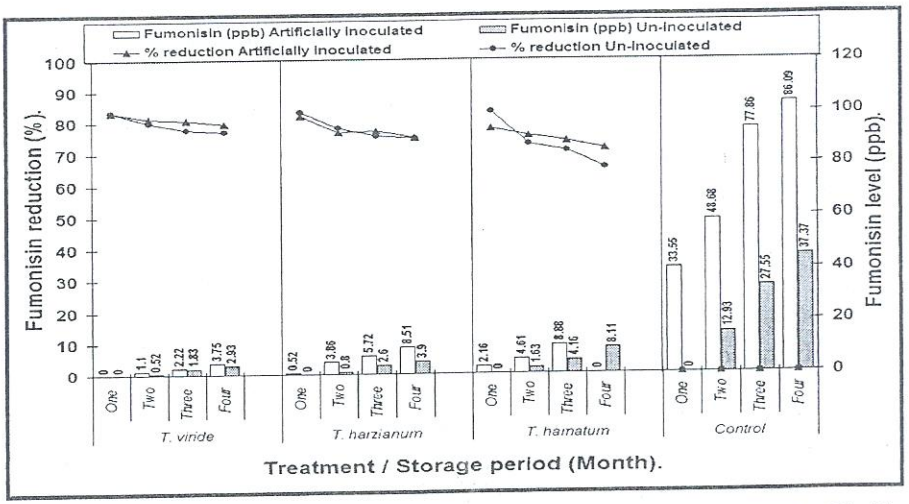


Fig. 2. Effect of *Trichoderma* spp. on fumonisin production by *F. verticillioides* in stored maize grains up to 4 months at 30°C / 90 RH. Where, no fumonisins were detected at zero time.

2.2. Effect of halfa barr and thyme as plant extract

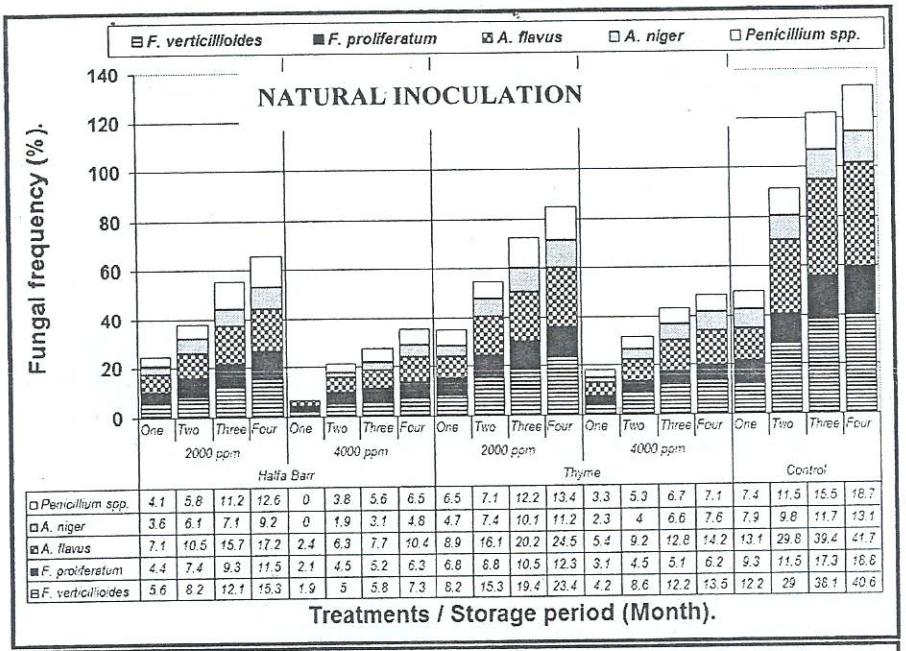
2.2.1. Effect of plant extract on fungal contamination

Data illustrated in Fig. (3) show that treating maize grains with halfa barr and thyme extracts, either (A_1) inoculated and uninoculated (N_1), before storage effectively reduced the frequencies of fungi up to 4 months, in comparison with untreated grains. In this concern, during all storage periods, it was found that concentration of the tested plant extracts at 4000 ppm was more effective in reducing the percentages of the isolated fungi more than that of 2000 ppm in both tested halfa barr and thyme extracts. Moreover, halfa barr extracts were the most effectively in reducing the frequency of the associated fungi, compared with the other tested treatments. Also, obtained data clearly indicated that frequency of *F. verticillioides* was increased, in all treatments of inoculated (AI) grains with increasing storage period from 1 up to 4 months, in comparison with zero time as a control.

After 4 months of storage, frequency of *F. verticillioides* recorded 32.3 and 16.5% in inoculated (A_1) grains treated with halfa barr extract at 2000 and 4000 ppm, respectively. Meanwhile, it was 37.9 and 25.0% in inoculated (A_1) grains treated with thyme extract at 2000 and 4000 ppm, respectively. Inoculated (A_1) and untreated treatment (control) recorded 79.9 and 40.6%, respectively, after 4 months of storage. One the other hand, frequency of *F. verticillioides*, in case of uninoculated (N_1) grains, recorded 15.3 and 7.3% in grains treated with halfa barr extract at 2000 and 4000 ppm, respectively. Meanwhile, it was 23.4 and 13.5% in grains treated with thyme extract at 2000 and 4000 ppm, respectively. The same trend was observed in case of the other associated fungi, *i.e. F. proliferatum, A. flavus, A. niger* and *Penicillium* spp., but with lowest frequencies compared with *F. verticillioides*.

2.2.2. Activity of different plant extracts concentrations in inhibiting fumonisins production:

Data illustrated in Fig. (4) show that no fumonisins were detected at zero time in both (N_1) and (A_1), while during storage period from 1 up to 4 months led to considerable increase in the amount of fumonisins by 0.00 to 37.37 ppb, respectively, in (N_1) grains as control treatment. Meanwhile, it was increased from 33.55 to 86.09 ppb, respectively, in (A_1) grains as control treatment. Plant extracts of halfa barr and thyme markedly reduced the amount of fumonisins after storage up to 4 months. Moreover, the highest efficiency in reducing fumonisins by both plant extracts was observed at 4000 ppm. However, halfa barr extract was better than thyme extract in inhibiting fumonisins production; it was gave 84.4 and 94.9% reduction in total amount of toxin after storage period up to 4 months of concentrations 2000 and 4000 ppm, respectively, in case of (A_1). Whereas, thyme extract was recorded 85.0 and 88.8% reduction in toxin production of concentrations 2000 and 4000 ppm, respectively, in case of (A_1); compare with control treatment. Also, it was observed that no fumonisins produced after 1 months of storage at 2000 ppm of both plant extracts in case of (N_1) grains, while, slight amount of fumonisins was produced in case of (A_1) grains. On the other hand, fumonisins were gradually increased by increasing storage period either in (N_1) and / or (A_1) grains.



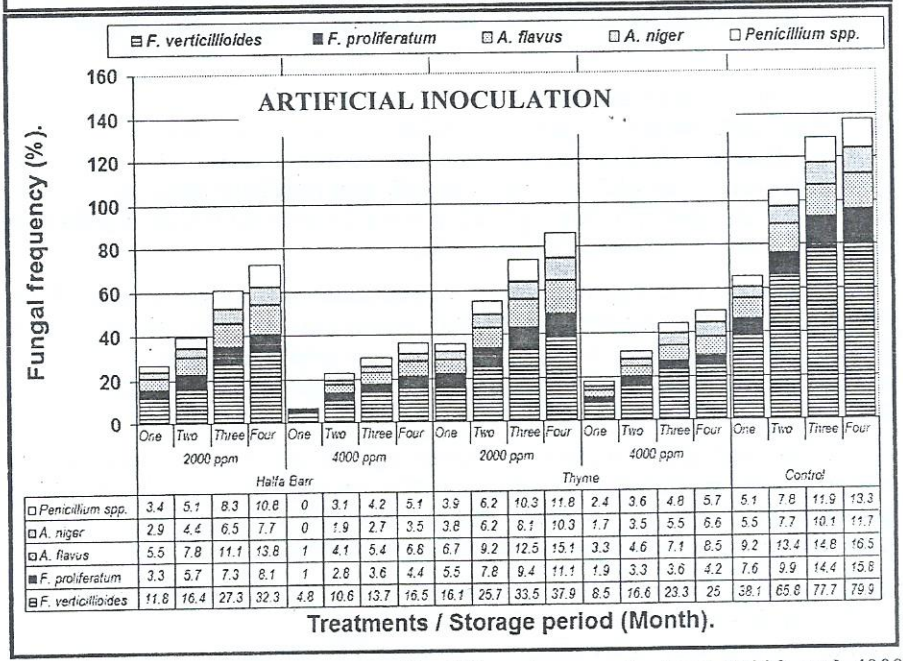


Fig. 3. Effect of plant extracts with different concentrations (2000 and 4000 ppm) on the frequency of fungi associated with stored maize grains up to 4 months. Where: At zero time, frequency of *F. verticillioides* recorded (2.9%), Meanwhile it was (4.0%) for *A. flavus*.

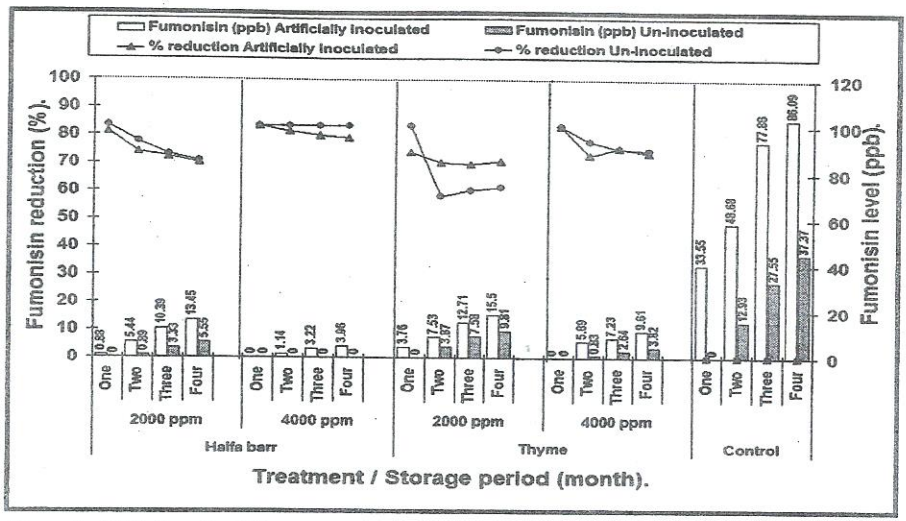


Fig. 4. Effect of different concentrations of plant extracts on fumonisin production: by *F. verticillioides* in stored maize grains up to 4 months at 30°C / 90 RH. Where: no fumonisins were detected at zero time.

2.3. Effect of gamma irradiation:

2.3.1. Effect different doses of gamma irradiation on fungal occurrence:

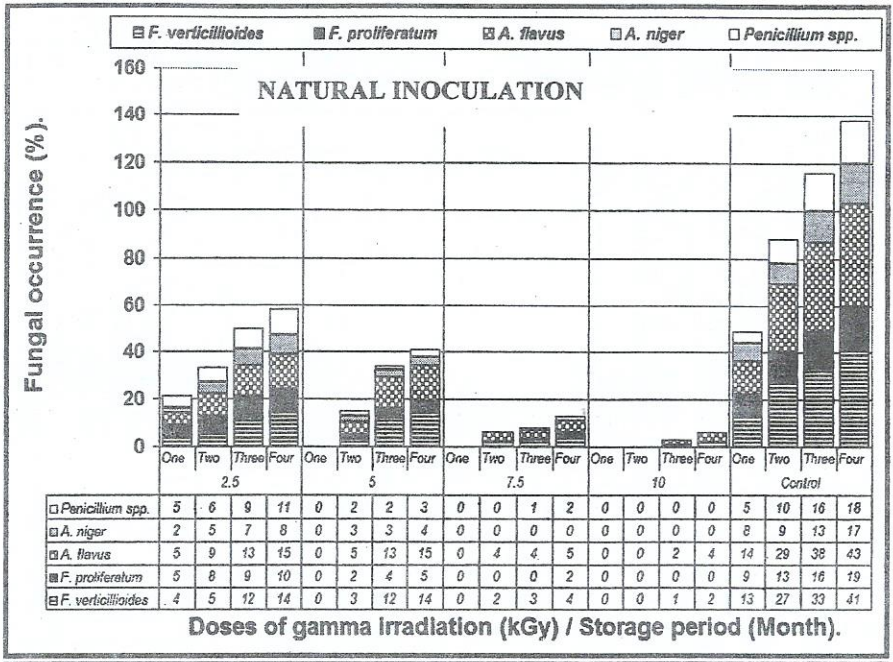
Data illustrated in Fig. (5) indicate that gamma irradiation has effectively reduced the occurrence of *F. verticillioides* and other associated fungi, either in (A_1) or in (N_1) maize grains. In this regarded, it was also observed that dose of 10 KGy was more effective than that of 2.5, 5, 7.5 KGy.

At zero time, occurrence of both *F. verticillioides* and *A. flavus* reached 3.5%. Meanwhile, exposing the grains to 10 KGy, before store up to two months resulted in complete absence of *F. verticillioides* and other associated fungi, either in (N_1) and (A_1) grains. While, 2.5 KGy prevented the occurrence of the associated fungi up to one month in both (N_1) and (A_1) grains.

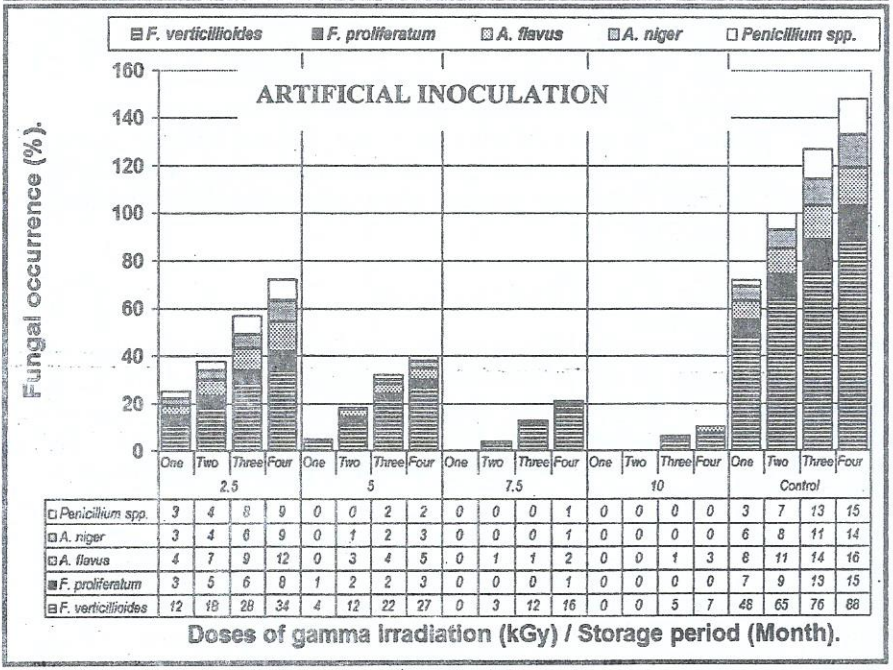
Un-irradiated grains (control) recorded high incidence for *F. verticillioides* compared with irradiated ones. Also, it was increased with increasing of storage period, where the higher incidence was recorded after four months of storage in either (A_1) or (N_1) grains, being 88 and 41%, respectively.

2.3.2. Effect of different doses of gamma irradiation in inhibiting fumonisins production:

Fig. (6) show that no fumonisins were detected at zero time and after one month of storage with un-irradiated grains (control); meanwhile, it reached 33.55 ppb in (AI) grains after one month of storage. Also, no fumonisins were detected when grains treated with 7.5 and 10 KGy in case of (N_1) and (A_1) grains up to 2 months of storage; moreover, it was reached to 5.6 and 0.00 ppb, respectively, of both treatments after four months of storage.



	2.5 One	2.5 Two	2.5 Three	2.5 Four	5 One	5 Two	5 Three	5 Four	7.5 One	7.5 Two	7.5 Three	7.5 Four	10 One	10 Two	10 Three	10 Four	Control One	Control Two	Control Three	Control Four
Penicillium spp.	5	6	9	11	0	2	2	3	0	0	1	2	0	0	0	0	5	10	16	18
A. niger	2	5	7	8	0	3	3	4	0	0	0	0	0	0	0	0	8	9	13	17
A. flavus	5	9	13	15	0	5	13	15	0	4	4	5	0	0	2	4	14	29	38	43
F. proliferatum	5	8	9	10	0	2	4	5	0	0	0	2	0	0	0	0	9	13	16	19
F. verticillioides	4	5	12	14	0	3	12	14	0	2	3	4	0	0	1	2	13	27	33	41



	2.5 One	2.5 Two	2.5 Three	2.5 Four	5 One	5 Two	5 Three	5 Four	7.5 One	7.5 Two	7.5 Three	7.5 Four	10 One	10 Two	10 Three	10 Four	Control One	Control Two	Control Three	Control Four
Penicillium spp.	3	4	8	9	0	0	2	2	0	0	0	1	0	0	0	0	3	7	13	15
A. niger	3	4	6	9	0	1	2	3	0	0	0	1	0	0	0	0	6	8	11	14
A. flavus	4	7	9	12	0	3	4	5	0	1	1	2	0	0	1	3	8	11	14	16
F. proliferatum	3	5	6	8	1	2	2	3	0	0	0	1	0	0	0	0	7	9	13	15
F. verticillioides	12	18	28	34	4	12	22	27	0	3	12	16	0	0	5	7	48	65	76	88

Fig. 5. Effect of different doses of gamma irradiation (KGy) on the occurrence of fungi associated with stored maize grains up to 4 months.

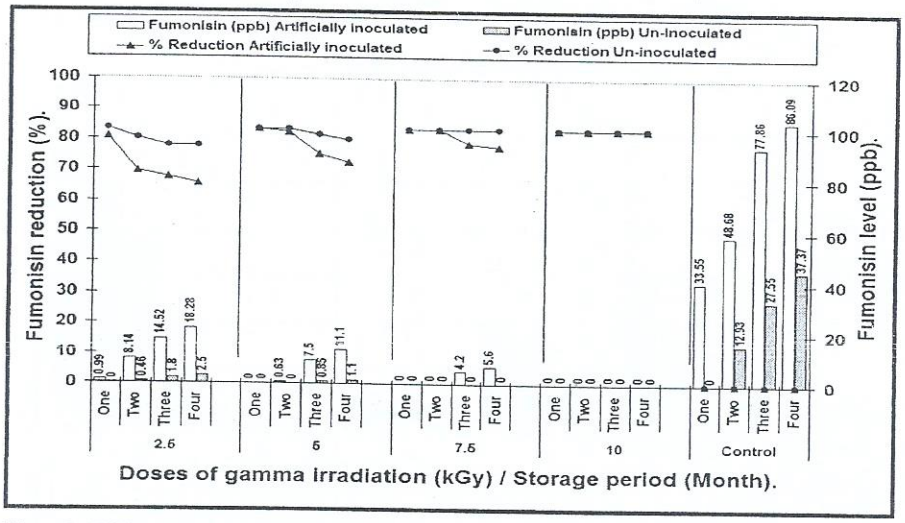


Fig. 6. Effect of different doses of gamma (KGy) irradiation on fumonisin production by *F. verticillioides* in stored maize grains up to 4 months. Where: no fumonisins were detected at zero time.

On the other hand, exposing to gamma radiation was very effective in inhibiting fumonisins production during storage up to 4 months of (A_1), being 78.8, 87.1, 93.5, and 100% of reduction in case of treated grains with 2.5, 5.0, 7.5 and 10 KGy, respectively. Whereas, (N_1) grains recorded 93.3, 96.0, 100 and 100% of reduction in case of treated grains with 2.5, 5.0, 7.5 and 10 KGy, respectively. In case of (A_1) and (N_1) grains, no fumonisins were recorded when grains treated with 10 KGy and stored for up to four months.

Discussion

In this study, an attempt was done to mange maize grain deterioration caused by *Fusarium verticillioides,* as well as accumulation of fumonisins toxin during storage up to 4 months. Three methods of application were tested under controlled conditions, bioagent (*Trichoderma viride, T. harzianum* and *T. hamatum*), plant extracts (halfa barr and thyme) and physical treatment (gamma irradiation at 2.5, 5.0, 7.5 and 10 KGy doses).

Obtained results could be explained in the light of fact that *Trichoderma* spp. have antagonistic effect, due to their ability to act through different mechanisms, including: 1) mycoparasitism (Abd EL-Moiety , 1985), 2) production of antifungal substances (Sanz *et al.*, 2002) and 3) destructive effect by enzymes activity, *i.e.* chitinase (Bolar *et al.*, 2000). In the light of fact that Trichoderma has mycoparasitism.

Also, results could be explained in the light of fact that Trichoderma has mycoparasitism, which cause by production of volatile and non-volatile antibiotics and lytic activity (Paavanen-Huhtala *et al.*, 2000). Effect of *Trichoderma* spp. was clear by increasing storage period, this can be explaining in light of works of (Abd El-Moneim *et al.*, 2010), who mentioned that toxins can be consumed by other micro-organisms as food stuff. On the other hand, this fact was explained by Abd EL-Moiety *et al.* (2003), who stated that some types of *Trichoderma* spp. could be spread very quickly and occupied the court of infection preventing most spores of *F. verticillioides* from germination and producing fumonisins as secondary metabolites.

The previous data of plant extract action might be due to the presence of the phenolic –OH group in solvent extract of thyme (Schvartz *et al.*, 1996). On the other hand, the *n*- hexane extract of halfa barr contain the most potent compounds, *i.e.* 2-methyl undec-2-en-10-al & eudesm-11-ol and elemol, against *F. verticillioides* growth (El-Assiuty *et al.*, 2006a). Also, EL-Assiuty *et al.* (2006b) found that *n*- hexane extract of halfa barr had eight compounds which fractionated by preparative TLC chromatography and identified as sesquiterpenesm, five of them are derivatives of eudesmol, one as ketonic monocyclic sesquiterpens, one as alcoholic monocyclic sesquiterpens and the last one is straight chain aliphatic unsaturated aldhyde. All of them are potent inhibitors to the growth of *F. verticillioides* in growing media, indicating the potential of this fraction in disease control.

Generally, obtained results revealed that solvent extract of each medicinal plant was more effective than aqueous extracts. Complete growth inhibition of *F. verticillioides* was recorded at 2000 ppm when solvent extract of halfa barr was applied. Meanwhile, 100% concentration of aqueous extract of the same plant caused 75.55% reduction of the fungal growth. Because of this extract contains many constituents that may have fungitoxic effect which suggested the highly potential inhibition highly growths of the *F. verticillioides* (El-Assiuty *et al.*, 2007 and Srichana *et al.*, 2009).

It is well known that plenty of phenols were reported to be responsible for the fungitoxic effect of many medicinal plants (Davidson, 2001and Baranauskiene *et al.*, 2003).

Treating maize grains with halfa barr and thyme extracts resulted in a significant reduction in grains deterioration and reduced fumonisins accumulation. These results are in harmony with that of El-Assiuty *et al.* (2006b and 2007), who reported that *n*-hexane and chloroform-methanol extracts obtained from *C. proximus* (halfa barr) and *T. vulgaris* (thyme), out of various medicinal and indigenous plants, were found to have highly potent effect on the growth of *F. verticillioides* and *A. flavus*.

Obtained results revealed that gamma irradiation was sufficiently effective in reducing deterioration of grains treated with 2.5, 5, 7.5 and 10 KGy and stored for up to 4 months. This application caused obvious reduction in infection by *F. verticillioides* as well as the associated fungi, compared with control (un-irradiated) treatment. As found in the present study, increasing of irradiation

dose was reported by Sreenivasa *et al.* (2009) to increase the reduction in fungal growth. Also, these results are matching with those obtained by many investigators (Ferreira-Castro *et al.*, 2007).

Results indicated, also, that gamma irradiation caused great reduction in accumulation of fumonisins compared with the control treatment. The effect of irradiation on fumonisins production was increased obviously by increasing the radiation dose from 2.5 to 10 KGy. In this respect, the higher dose of irradiation caused complete inhibition of toxins production as reported by (Ferreira-Castro *et al.*, 2007 and Sabet *et al.*, 2010), whose found that mycotoxin production decreased by increasing gamma irradiation dose. Also, gamma irradiation may be causes cellular inactivation (Kim and Thayer, 1996). These mechanisms could be summarized as: 1) Damage of DNA, 2) Inhibition of protein synthesis, 3) Damage of cell membrane and 4) Inactivation of critical metabolic enzymes. Findings of the current study emphasized that the effect of gamma irradiation on controlling maize grain-borne pathogens is proposed to maximize grain safety.

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تأثير بعض الكائنات المضادة والمستخلصات النباتية وأشعة جاما علي تدهور وإنتاج سموم الفيومونيسين في حبوب الذرة المخزنة

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تمت دراسة تجربة مقاومة تخزين حبوب الذرة الشامية لمحاولة تقليل تكرار الأصابة بالفطر *Fusarium verticillioides* وكذا إنتاج وتراكم الفيومونيسين. حيث تم إختبار فعالية بعض عوامل المكافحة المتكاملة (أشعة جاما ، فطريات المقاومة الحيوية *Trichoderma* spp. ، مستخلصات بعض النباتات الطبية) ضد تدهور حبوب الذرة الشامية المخزنة ومدى تراكم الفيومونيسين، والتي أثبتت قدرتها على تثبيط النمو الفطري للفطر *F. verticillioides* في المعمل.

أدت معاملة الحبوب بفطريات المقاومة الحيوية *Trichoderma* spp. وتخزينها لمدة أربعة أشهر إلى خفض ملحوظ في نسب الإصابة بالفطر *F. verticillioides* والفطريات المصاحبة الأخرى ، كما أدت إلى انخفاض كبير في إنتاج الفيومونيسين بالمقارنة مع الحبوب غير المعاملة بهذه الفطريات.

عند إستخدام أشعة جاما ، وجد أن معاملة الحبوب بالجرعة ١٠ كيلو جراي أدت إلى التثبيط التام في إنتاج الفيومونيسين في نهاية مدة التخزين مقارنة بالجرعات ٢,٥ ، ٥ ، ٧,٥ كيلو جراي.

وقد وجد أيضاً أن المعاملة بمستخلصات الهكسان والكلوروفورم- ميثانول والمستخلصات المائية وبتركيزات مختلفة لكل من الحلفا بر والزعتر قد سببت إنخفاضاً معنوياً في نمو الفطر *F. verticillioides* حيث أظهرت النتائج أن الهكسان ومستخلصات الكلوروفورم – ميثانول كانت الأكثر فعالية عن المستخلصات المائية، كما وجد أن مستخلص الحلفا بر كان له التأثير الأعلى في نسب إنخفاض النمو الفطري مقارنة بمستخلص الزعتر.

وجد أن معاملة الحبوب بمستخلصات الهكسان والكلوروفورم – ميثانول لكل من المستخلصين بتركيز ٢٠٠٠ ، ٤٠٠٠ جزء في المليون أظهرت إنخفاضاً كبيراً في نسب الظهور للفطر *F. verticillioides* والفطريات المصاحبة له بالإضافة إلى إنخفاض ملحوظ في إنتاج الفيومونيسين في نهاية مدة التخزين.