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Use of Gamma Irradiation to Control *Fusarium verticillioides* Producing Two Known Mycotoxins in Infected Corn

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ABSTRACT

Fusarium verticillioides Sacc.(Nirenberg) was isolated from fresh seeds collected from corn fields with ears symptoms in the late 2000. When cultured in liquid media under controlled incubation conditions, found to produce two already known mycotoxins. These two mycotoxins were obtained through the extraction process of the lyophilized culture filtrate under acidic condition using ethyl acetate and detected using thin layer chromatography and high performance liquid chromatography in comparison to authentic from both acids. Mass spectroscopic investigations confirmed the molecular weight of the two toxic compounds which are known as fusaric and 9,10-dehydrofusaric acids. Application of medium doses of gamma irradiation in the range of 0.5 and up to 3 kGy to both buffered saline solution and to corn caused a slight decrease in the count of isolated pathogen while a 5 kGy dose caused a dramatic reduction in fungal count.

Key Words: *Zea mays* L.; *Fusarium verticillioides*; Mycotoxins; Gamma irradiation

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important agriculture crops since it is considered the major ingredient in animal feed throughout the world and the human dietary staple in many regions of Africa, Asia and Central South America (Troxell, 1996).

Among thouands of species infecting different agricultural commodities and dried grains used either as food or as feed staple, arise the *Fusarium* species which are widely distributed and are amongst the fungal species most frequently isolated by plant pathologists.

Most *Fusarium* species invade seeds in the field and after harvest as well as damaging stored grains, they are capable of causing seedling diseases, root rots, stalk rots and ear rots of maize (Davis *et al*., 1989; ApSimon *et al*., 1990). Two known types of *Fusarium* ear rot of corn are world-wide diffused, these are gibberella-ear rot (pink ear rot) caused by *Gibberella zeae* (sexual stage of *F. graminearum*) and which usually spreads from the ear tip down and develops around tunnels made by insects; red ear rot (red fusariosis) which is less common in occurrence and is associated with *F. tricinctum*, *F. equiseti*, *F. avenaceum* and *F. sporotrichioides*. In addition, the ear rot caused mainly by *F. monilforme* and also by other related fungi which occurs during dry weather and is facilitated by insect damage. One of those which are common corn-pathogens world-wide is *Fusarium verticillioides* Sacc. (Nirenberg). This species in addition to other species are seed-borne and seed-transmitted and are associated with some maize development stages and is also among the most common fungi colonizing symptom less maize plant (Kommedahl & Windels, 1981; Leslie *et al*., 1990). *Fusarium* infestation may not only reduce crop yield, germination rate and grain quality but also they are found to deteriorate seeds through their ability to produce secondary metabolites, many of which are toxic either to animal or human and are known as mycotoxins (ApSimon *et al*., 1990; Sugiura *et al*., 1993). The genus *Fusarium* differ from the other genera: *Aspergillus* and *Penicillium*, where only few species can produce mycotoxins, by the ability of almost all the species found under this genus to produce diverse toxic compounds belonging to different chemical groups. Mycotoxins may downgrade the infected grains from food to feed level but remain the health risks like toxicity, carcinogenicity; immunosuppressive activity, which are associated with the consumption of mycotoxin-contaminated grains.

Among a hundred of secondary metabolites the *Fusarium* species produce, some occur in cereals at biologically significant concentrations. These are the fumonisins, zearalenone, trichothecenes and fusaric acids. The International Agency for Research on Cancer (IARC, 1993) working group on carcinogenic risk to humans has classified the toxins derived from *F. moniliforme* (including fumonisins) as possibly carcinogenic to humans. Fusaric acids (chemically 5-butylpicolinic acids) are non-specific toxins which are not only known to play an important role in the plant disease process (Gaumann, 1957; Davis, 1969; Marre *et al*., 1993) but also are known to have different pharmacological activities when tested in laboratory animals. Fusaric acid has been reported to be a potent inhibitor of dopamine-beta- hydroxylase, a key enzyme in the synthesis of norepinephrine in the brain (Nagatsu *et al*., 1970). Fusaric acid caused vomiting to 60% of the dosed

swines as reported by Smith and Macdonald (1990). The lethargy seen in pigs dosed with fusaric acids was aimed to the enhanced action of the serotonergic nervous system resulting from elevated blood and brain tryptophan concentrations and the subsequent synthesis of serotinin as proved by the authors and by Chahaouloff *et al*. (1986). In addition, fusaric acid increased tyrosine concentration in both the brain and the pineal gland in rats, and it was proved that the melatonin synthesis by the pineal gland could be also affected by fusaric acid, this in turn may have pronounced affects on growth, development, maturation and reproduction in tested rats (Porter *et al*., 1995). Prevention of mycotoxin production in food and feed may be achieved by halting the growth and development of the producing fungi in their substrates but if prevention failed and mycotoxins were produced, measures should be taken to destroy or inactivate these compounds. A number of methods including chemical, biological and physical have been investigated (Suttajit, 1989; Visconti *et al*., 1996). Ionizing radiation, particularly medium doses of gamma rays, approved to be an efficient physical method for prevention and/or reduction of fungal growth in food and feed products. Gamma rays from Co-60 are the most widely used technique in irradiating food and feed because of the high penetration power they have and those processed by radiation, in accordance with the recommended doses, are not considered radioactive. This is because gamma rays can kill spoilage organisms without or with slight rising in food or feed temperature in a process known as cold physical process. Studies on the relative radiation-resistant fungal species have illustrated that *Aspergillus* and *Penecillium* species are relatively sensitive to ionizing radiation with a D_{10}-values between 0.25 and 0.65 kGy whereas other species in the genus *Fusarium* are more resistant requiring high but safe D_{10}-values of 0.65 to 1.5 kGy. The FAO (1997) and WHO (1988) committee on the wholesomeness of irradiated foods, after the reviewing of all available data on food safety, have established a safe and toxicless irradiation overall average dose of 10 kGy for any food commodity.

This work describes the detection of two known mycotoxins named fusaric and 9,10-dehydrofusaric acids produced in liquid culture filtrates of *F. verticillioides* Sacc. (Nerinberg) isolated from maize fields by two different chromatographic techniques and the results of application of calculated doses of gamma irradiation for the halting of the producer pathogen and the subsequent prevention of mycotoxin production.

MATERIALS AND METHODS

Isolation of the fungus *F. verticillioides* associated with *Zea mays* grains. Fifty grams of fresh maize seeds collected at random from infected corn fields with typical ears symptoms of *Fusarium* kernel rot were surface disinfected by soaking in 0.5% sodium hypochlorite. Seeds were washed several times with sterile distilled water and allowed to sink into petridishes of potato dextrose agar media supplemented with Rose Bengal and Chloramphenicol as antibacterial agents. Plates were then incubated at 26±1°C under alternating light periods (12 h on/12 h off) for 10–14 days. Following incubation, the numbers of fungi growing from the seeds were counted and isolates were initially identified to the genus level whereas isolates of *Fusarium* to the species level. Identification of different *Fusarium* species was carried out by examining the morphological characteristics of cultures grown on potato dextrose agar (PDA) and on sabouraud's media using the key of (Booth, 1977; Nelson *et al*., 1983; 1994). Isolates of *F. verticillioides* Sacc. (Nirenberg) were stored at 4°C and re-cultured every four weeks.

Production and extraction of the two mycotoxins, fusaric and dehydrofusaric acids. Three Erlenmeyer flasks containing 50 mL culture media were inoculated with single agar plugs (1.5 mm^3) containing the isolate actively growing mycelia. The following culture medium was used:

0.74 g $MgSO_4.7H_2O$; 0.045 g $MnSO_4.4H_2O$; 0.018 g $ZnSO_4.7H_2O$; 5.0 µg $CuSO_4.5H_2O$; 0.084 g $FeSO_4.7H_2O$; 0.11 g Na_2EDTA; 0.1 g myo-inositol; 2.0 mg glycine; 0.5 mg nicotinic acid; 0.5 mg pyridoxine hydrochloride; 0.1 mg thiamine hydrochloride; 2.0 g L-asparagine; 10.0 g glucose; 5.0 µg biotin; 1.0 g KH_2PO_4; 1 L distilled water. Flasks were incubated in the dark at 26±1°C for 2–4 weeks, cultures were filtered through cheesecloth and the resulted filtrates were lyophilized.

Lyophilized culture filtrates (correspond to 150 mL) was dissolved in suitable amount of ultra pure water, extracted four times (4x150) using ethyl acetate under acidic condition (pH 2, formic acid 1M). The resulted organic extracts were combined, dried then evaporated under reduced pressure. Preliminary screening of the organic extract on 0.25 silica gel plates (Kieselgel 60 F_{254}, Merck, Germany) and on 0.20 mm reversed phase plates (Stratocrom, KC-18, Whatman, Cliffort, New Jersey, USA) using ethyl acetate:methanol:water, 85:20:10 (eluent 1) and acetonitrile:water, 1:1 (eluent 2), respectively and in comparison to authentic of the two fusaric acids, revealed the presence of fusaric and 9,10-dehydrofusaric acids. Thin layer chromatography (TLC) spots were visualised by exposure to ultraviolet radiation. An aliquot of the organic extract which seems to contain both toxic acids was prepared for further analyses using High Performance Liquid Chromatography (HPLC) to confirm the presence of the two acids.

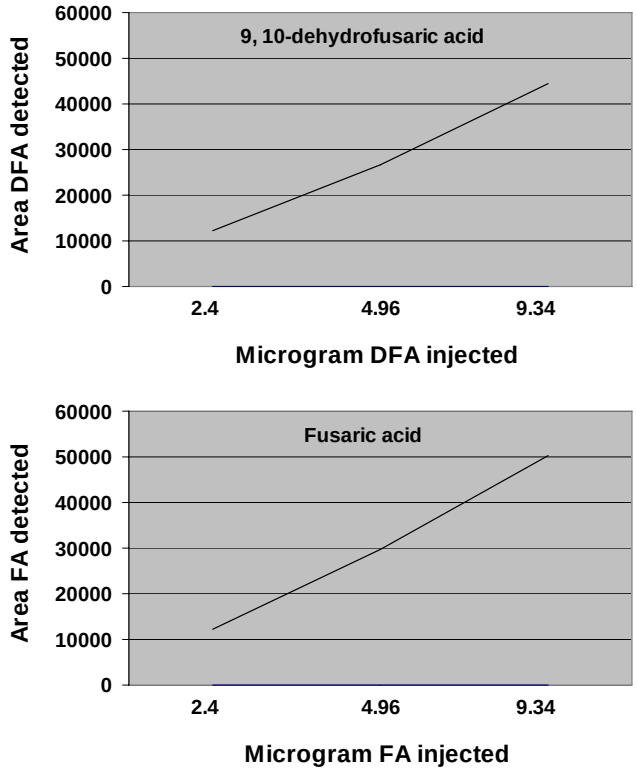
Analysis of fusaric acids using HPLC. An aliquot of 20 µL of methanol-soluble organic extract of lyophilized culture filtrate of *F. verticillioides* passed through a disposable filter was prepared for HPLC injection, triplicates were performed. The HPLC system used consisted of a series LC-10 *VP* pump to which a high density reversed phase C18 100-5 Nucleosil column chromatography of (5 µm, 250 x 4.6 mm) was fixed.

Separation was performed (according to the procedure established by Amalfitano *et al*. (2002) using the following HPLC grade solvents: methanol (eluent 3); water (eluent 4) and 1% dipotassium hydrogen phosphate in water with pH 7.4 using concentrated phosphoric acid (eluent 5). Elution started with 3:4:5 (50:10:40) which was changed gradually within 20 min to 3:4:5 (75:10:15) then back to the starting column condition 3:4:5 (50:10:40) in 5 min. The column was stored in 3:4 (80:20) then only in 3 (100%) where it is equilibrated after additional 20 min before a further run was proceeded.

The flow rate was 1 mL/min and the absorption was detected with a SPD-10 *VP* ultraviolet spectrophotometric detector set at wavelength 268 nm. Standard curves were established with pure fusaric acids dissolved in methanol (Fig. 1).

Phytotoxicity of fusaric acids. Phytotoxicity on test plants was also measured by using 4-week-old tomato plants (*Lycopersicon lycopersium* Karsf.). Stems were cut then immersed into glass test tubes containing a solution of the organic extract containing mixture of the two fusaric acids (1 mg/mL methanol) in comparison to the control (2% methanol/water). Test tubes were all incubated at 27°C for 48 h then cuttings of tomato were transferred to other test tubes containing distilled water. Wilt symptoms were evaluated and rated on a scale index of 0-4 where 0 = no symptoms and 4 = 100% wilt. The experiment was repeated twice.

Fig. 1. Standard curves of authentic fusaric and 9,10-dehydrofusaric acids



Preparation of fungal spore suspension. *F. verticillioides* was grown on 250 mL Erlenmeyer flasks containing 100 mL of the previously described medium for mass production of spores at 26±1ºC. Following an incubation period of 10–14 days, the spores were harvested according to the method described by Applegate and Chipley (1974) by adding sterile distilled water containing 0.1% Tween 80 and by gentle scrapping of the surface. Suspensions were allowed to stand for 2 h and the supernatant was separated from hyphal fragments. One mL of a previously prepared fungal spore suspension was added either to 9 mL of buffered saline solution (0.85% sodium chloride) or to 9 g of corn. The tubes were then exposed to various doses of gamma radiation (0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 kGy) in comparison to a control one (0.0 kGy) triplicates were used for each dose. Following the irradiation process, survival counts were determined by plate count technique and dose-response curve was graphed by plotting the log survival counts against irradiation doses used and the D_{10}-value was calculated from the resulted curve and by using the following mathematical equations:

$$y = a + bx \qquad D10 = -1/b$$

$$b = \frac{\sum x\,y - n\,\bar{x}\,\bar{y}}{\sum x^2 - n\,\bar{x}^2}$$

where, a: log of conidial count when x equal to zero; b: regression factor; n: number of treatments; x: dose level (kGy); y: log of survival count and Σ: summation.

Artificial inoculation. Moisture content of the used corn was adjusted at 25% by adding a known volume of sterile water then corn was first exposed to a radiation dose of 8 kGy to eliminate the natural existing fungal flora. Under the new sterile conditions, corn samples (250 g for each) were packaged in polyethylene bags and 5 ml of the prepared spore suspension was added to each bag then all bags were sealed. The inoculated bags were divided into six groups where the first group was left without being radiated (control) and the other five groups (2–6) were irradiated at 2.5, 5.0, 7.5, 10.0 and 12.5 kGy, respectively.

Irradiation process and storage. Irradiation process was achieved by using Russian Gamma Cell, Model Issledovatel Cobalt-60. The average dose rate from this source at the time of D_{10} experiment was 6.2 kGy/h while the dose rate at the time of storage experiment was 5.56 kGy/h.

Both irradiated and unirradiated corn samples were stored at room temperature for 12 weeks and total fungal counts as well as visual observation of mold growth were followed at zero time of storage and at 3, 6 and 12 weeks.

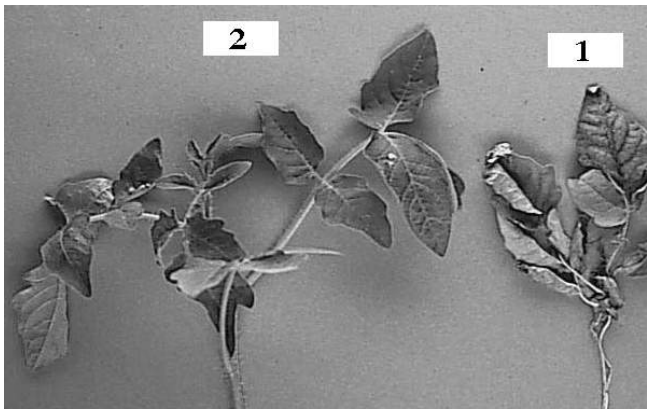
RESULTS AND DISCUSSION

The lyophilized culture filtrate (150 mL) obtained from both filtration and lyophilization of liquid cultures of the pathogenic fungus *F. verticillioides* isolated from infected corn seeds, when was dissolved in water and exhaustively extracted with ethyl acetate under acidic condition then organic extracts were combined and brought to dryness under vacuum, a deep brown organic residue with a strong characteristic odor was obtained. When the residue was applied on both silica gel and reversed phase chromatographic plates using eluent system (1 and 2), respectively, in comparison to authentic of fusaric and 9,10-dehydrofusaric acids, two large spots referring to the two main compounds with retention factor (R*f*) value of 0.40 and 0.33 on silica gel plates and R*f* value of 0.30 and 0.51 on reversed phase plates were proved to be identical to those of authentic samples.

The oily organic residue obtained from the extraction process with ethyl acetate and which contain the two fusaric acids in mixture proved to be highly phytotoxic when tested on 4-weeks-old tomato cuttings in a concentration not exceeding 1mg/mL. In fact, the test plant showed complete wilt symptoms within 48 h in comparison to the control (Fig. 2).

The presence of the two compounds in the phytotoxic organic extract was also evaluated by their detection using HPLC analysis. In fact, three different injections of an aliquot of 20 µL of the methanol-soluble organic extract confirmed the presence of the two toxic compounds. Fig. 3

Fig. 2. Phytotoxicity of the organic extract on cuttings of tomato plants (1) in comparison to the control (2)



is an HPLC pattern in which it is easy to detect the two peaks corresponding to fusaric and 9,10-dehydrofusaric acids by the coincidence of retention times with those of standards (Figs. 4, 5). As shown in Fig. 3, the used chromatographic procedure separated the two compounds and in sequence 9,10-dehydrofusaric acid (DFA) and fusaric acid (FA) at retention time (R*t*) 6.50 ± 0.50 and 10.55 ± 0.50 min, respectively.

Table I. Effect of irradiation on the viability of *F. Verticillioides* in buffered saline solution and in corn

Irradiated dose (kGy)	*Saline solution*		*Corn*	
	Count (cfu/mL)	Log count	Count (cfu/mL)	Log count
0.0	1.6×10^7	7.3	2.5×10^6	6.4
0.5	1.0×10^7	7.0	1.9×10^6	6.0
1.0	9.8×10^6	6.7	4.7×10^5	5.7
1.5	7.9×10^5	6.2	4.0×10^5	5.4
2.0	2.0×10^5	5.7	3.2×10^5	5.1
3.0	1.6×10^5	5.2	3.4×10^4	4.5
4.0	3.8×10^4	4.5	1.1×10^4	4.0
5.0	6.4×10^3	3.8	3.2×10^3	3.5

Table II. Effect of gamma irradiation on the growth of *F. verticillioides* inoculated in corn

Irradiated dose (kGy)	*Storage period (weeks)*			
	0	3	6	12
0.0	1.5×10^6	1.5×10^7	ND	ND
2.5	1.0×10^5	2.2×10^5	1.0×10^6	ND
5.0	1.0×10^2	6.8×10^2	3.1×10^4	1.2×10^6
7.5	< 10	< 10	< 10	1.8×10^4
10.0	< 10	< 10	< 10	1.8×10^2
12.5	< 10	< 10	< 10	< 10

ND = Not determined due to full mold growth

The identification of FA and DFA was further completed by the molecular ions observed in their electron ionization mass spectrum (Fig. 6) detected at *m*/*z* 179.7 and 178, respectively and in agreement with those in the literature data reported by Capasso *et al*. (1996).

Table I shows that irradiation generally decreased the counts of *F. verticillioides* in both buffered saline solution and in corn, this reduction was proportional with irradiation dose applied. An irradiation dose of 5 kGy caused a dramatic decrease in the fungal count reaching 99.9% from the initial count.

The radiation survival curve of *F.verticillioides* (Fig. 7) shows a simple relationship with constant slop (a straight line) between the irradiation dose and log count. The D_{10}-value (defined as irradiation dose required to reduce the count of fungal spores by one log cycle) was calculated from the curve and was deduced to be 1.5 and 1.4 kGy in saline solution and in corn, respectively. From the calculated D_{10}-value it is obvious that the fungal spores were more radio-resistant in corn than in saline solution, these results are in agreement with those described in a previous work by Hammad and El-Baza (1988) who found that the D_{10}-value of *Aspergillus flavus* was 0.43 and 0.5 kGy in buffered saline solution and in smoked herrings, respectively. This may be attributed to the indirect effect of primary water free radicals (OH^o, H^o, e^-) resulting from water radiolysis which are, certainly, much more in saline solution than in dried corn. In addition, corn as a suspending medium may contain

Fig. 3. HPLC pattern of the organic extract showing two well separated peaks representing fusaric and 9,10-dehydrofusarc acids at R*t* =7.08 and 11.12, respectively

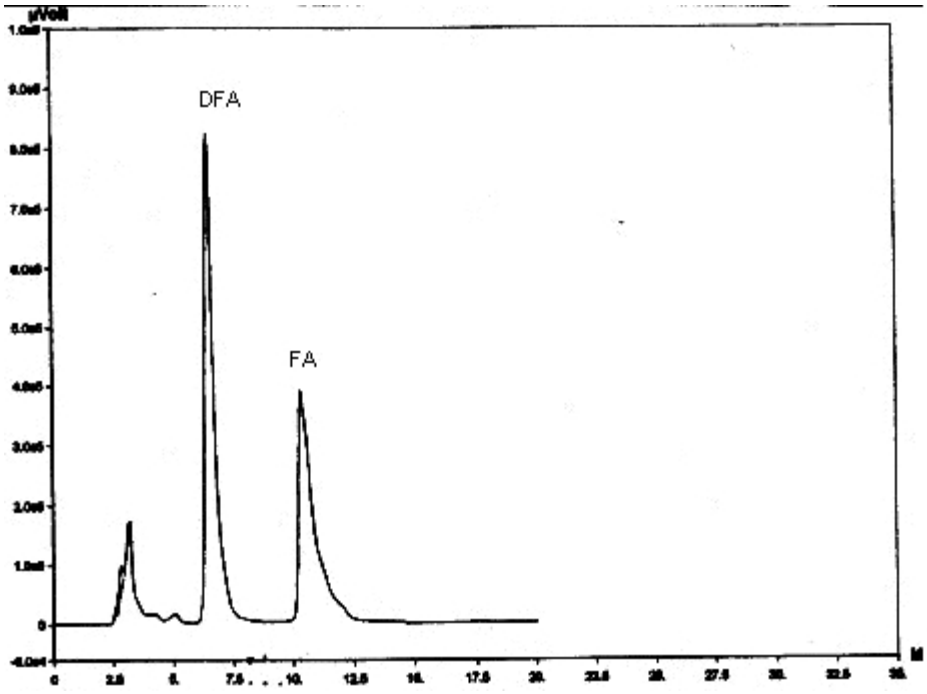
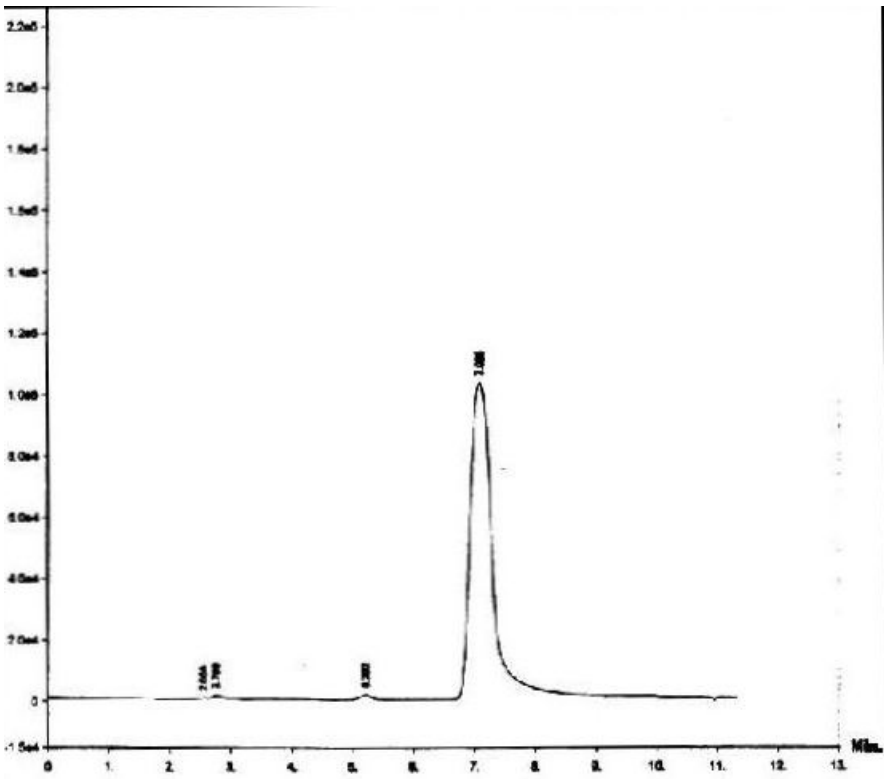


Fig. 4. HPLC pattern of standard 9,10-dehydrofusaric acid showing a well defined peak at R*t* =7.08 min

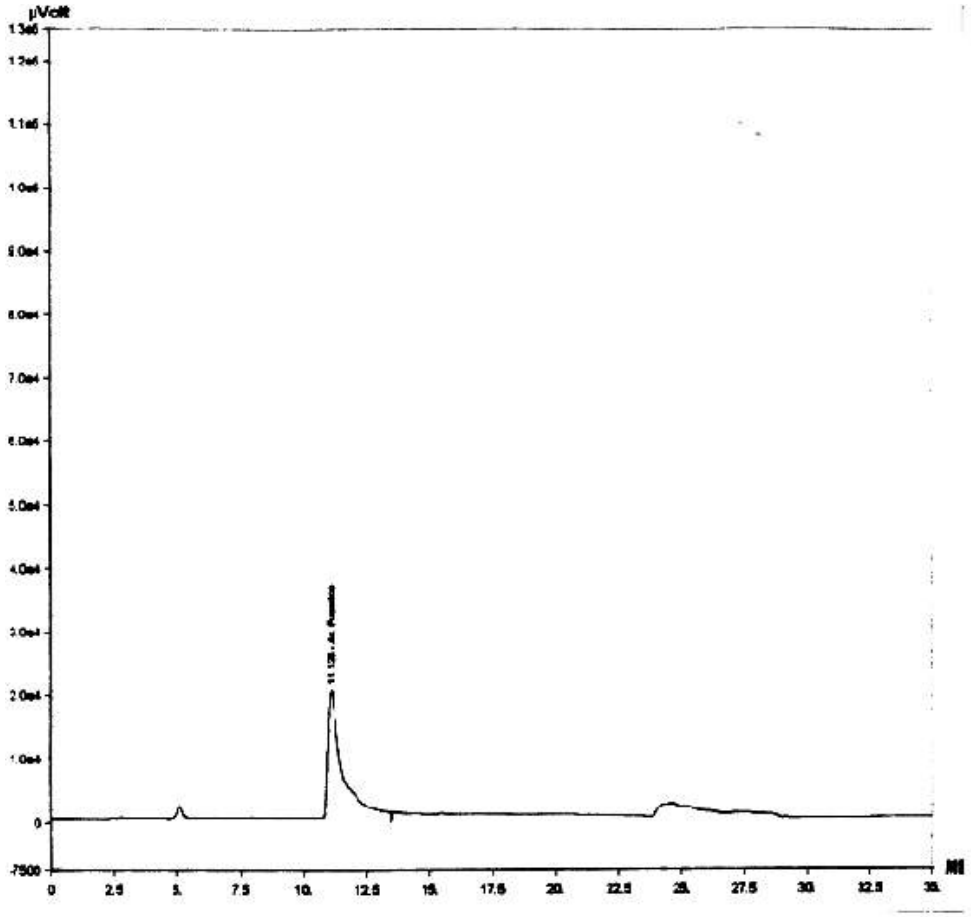


certain compounds that act as protective agents (scavengers) which give protection against irradiation damage of the fungal spores. Rowley and Brynjolfsson (1980) reported that radiation resistance of any particular fungus is influenced by many factors among which is the availability of water in suspending medium. Schubert (1981) mentioned that the protective agents (scavengers) may react with free radicals liberated from water radiolysis hence protecting or reducing the radiation damage to the cell normally attacked by these radicals.

Determination of D_{10}-value for any microorganism is considered important before the practical application of ionizing radiation. By knowing the D_{10}-value of any microorganism and its level in a product (food, medicinal, pharmaceutical), one can predict the exact irradiation dose required to eliminate a contaminant microorganism from that product. Hammad *et al.* (1995) determined the D10-values of *A. flavus* and *A. ochraceous* as 0.5 and 0.4 kGy, respectively while Hammad (1989) found that D_{10}-values of *Penicillium chrysogenum* and *A. fumigatus* were 0.5 and 0.57 kGy, respectively.

The effect of radiation doses on the growth of *F. verticillioides* was

Fig. 5. HPLC pattern of standard fusaric acid showing a well defined peak at R*t* =11.12 min



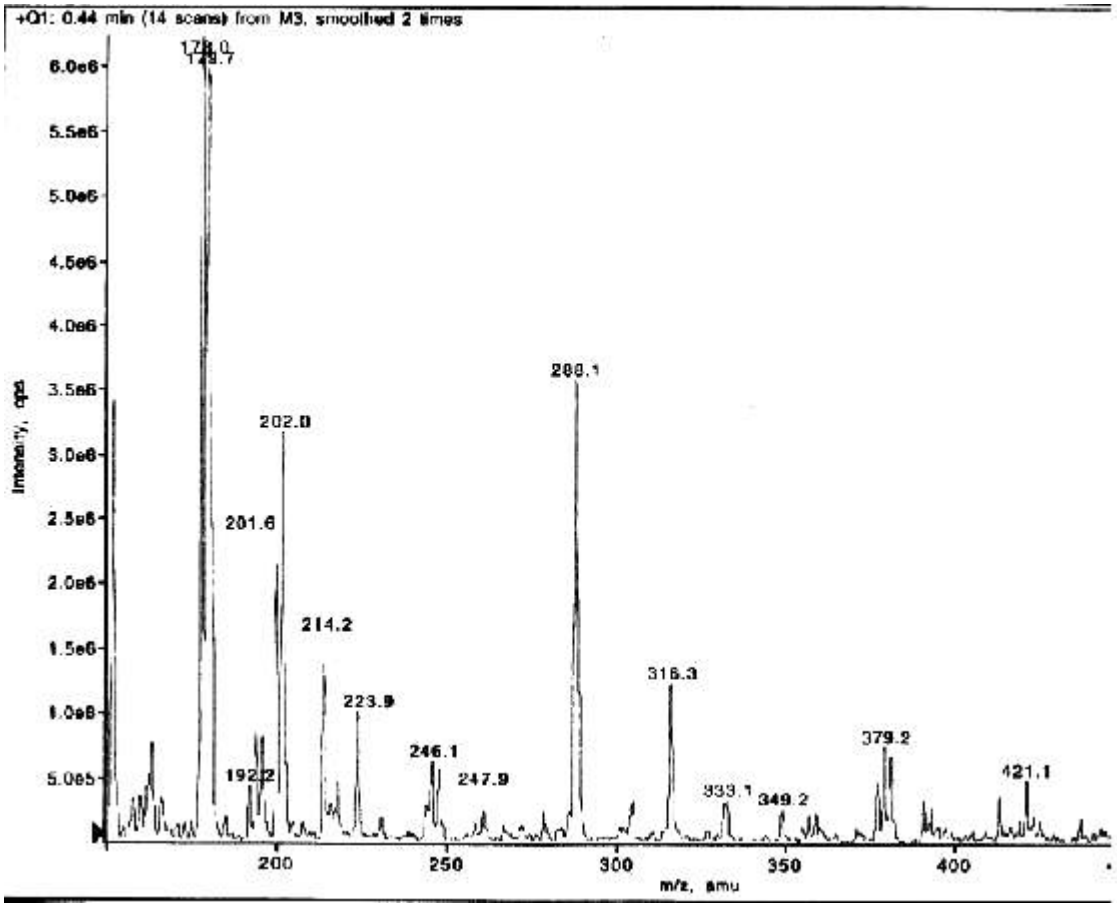
followed during storage by enumerating the viable counts of the fungal spores. The results shown in Table II indicate that irradiation caused a great reduction on the viable fungal counts and this reduction was parallel to irradiation doses. An irradiation dose of 5 kGy reduced the viable counts by 3.4 log cycle meanwhile, the viable counts in corn samples exposed to higher doses were less than the detectable level {<10 colony forming unit (cfu)/g}. These results are in agreement with the results recorded by Kiss and Farkas (1977) who found that irradiation doses of 4 and 8 kGy greatly reduced the fungal count of wheat with 23% moisture content and of corn with 23-31% moisture content.

During storage, the total fungal count inoculated in corn was increased in unirradiated control samples and in those exposed to 2.5 and 5 kGy however, remains the rate of increase lower in irradiated corn than in unirradiated ones. This indicates that 2.5 and 5 kGy doses retarded the growth of the fungal spores. Also, from visual observation, it was clear that the control corn samples became full moldy after three weeks with offensive odor while corn samples irradiated at 2.5 kGy became full moldy after 12 weeks. Irradiation doses of 7.5 and 10 kGy delayed fungal growth up to six weeks and after 12 weeks storage period, the fungal counts of these irradiated samples were much lower than the initial count before irradiation. Irradiation dose of 12.5 kGy completely inhibited the growth of the fungus in corn throughout the storage period (12 weeks).

CONCLUSIONS

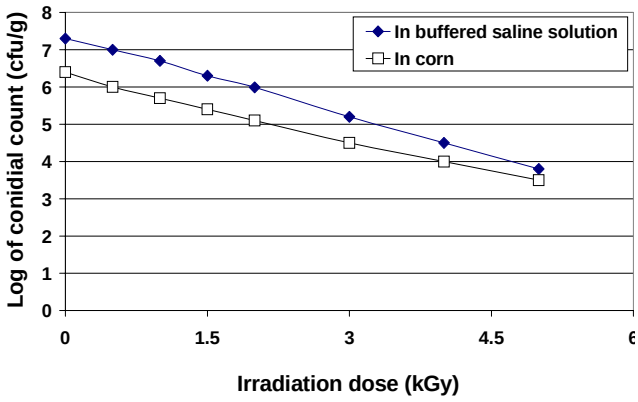
In conclusion, Wheat, barley and maize together account for about two-thirds of the world production of cereals and unfortunately are the crops most susceptible to *Fusarium* fungal disease and mycotoxin production. *Fusarium* infection of cereal ears causes at least three undesired effects: (1) reduction in grain yield and quality; (2) economic losses in livestock fed with contaminated cereals and the consequent reduced animal production; (3) mycotoxins carry-over to food products associated with potential toxicity to human. The number of *Fusarium* metabolites occurring in maize is significantly higher than the number encountered in wheat and other small-grain cereals. For instance, amounts of some *Fusarium* metabolites accumulating in maize kernels are significantly (near 10 times) higher than amounts present in kernels of wheat or barley infected by the same fungal species.

Fig. 6. Electron ionization mass spectrum of the organic extract showing the two main molecular ions representing FA and DFA



Moreover, health risks associated with the consumption of cereal products contaminated with *Fusarium* mycotoxins are widely recognized and depend on the extent to which they are consumed in a diversified diet. The hazard of *Fusarium* mycotoxins are responsible for and the fact that their production is associated with fungal growth pushed researchers in several countries to assess methods for halting the growth of *Fusarium* species. Irradiatioin was proved to be an efficient method (following the recommended doses) in preventing food and feed contamination with mycotoxin-producing fungi and results illustrated by Halasz *et al.* (1989) proved that neither growth nor toxin production could be detected in corn and rice inoculated with *F. tricinctum* when exposed to 9 kGy. The results illustrated before may have importance when applied on agricultural products and on storage ones since species under the genus *Fusarium* are considered the most powerful among other fungal species responsible for economic losses in the field of agriculture.

Fig. 7. Effect of different irradiation doses on the viability of F. verticillioides in buffered saline solution (0.85 Nacl) and in corn



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REFERENCES

Amalfitano, C., R. Pengue, A. Andolfi, M. Vurro, M.C. Zonno and A. Evidente, 2002. HPLC analysis of fusaric acid, 9,10-dehydrofusaric acid and their methylesteres, toxic metabolites produced by weed pathogenic *Fusarium* species. *Phytochemical Analysis,* 13: 277–82

Applegate, K.L. and T.R. Chipley, 1974. Daily variations in the production of aflatoxins by *Aspergillus flavus* NRRL' 3145 following exposure to co-60 irradiation. *J. Appl. Bacteriol.*, 37: 359–72

ApSimon, J.W., B.A. Blackwell, L. Blais, D.A. Fielder, R. Greenhalgh, R. Kasitu, J.D. Miller and M. Savard, 1990. Mycotoxins from *Fusarium* species: detection, determination and variety. *Pure Appl. Chem*., 62: 1339–46

Booth, C., 1977. *Fusarium*, *Laboratory Guide to the Identification of the Major Species*. p. 237. Commwealth Mycological Institute, Kew, England.

Capasso R., A. Evidente, A. Cutignano, M. Vurro, M.C. Zonno and A. Bottalico, 1996. Fusaric and 9,10-dehydrofusaric acids and their methyl esters from *Fusarium Nygamai*. *Phytochem.,* 41: 1035–9

Chahaouloff, F.D., D. Laude, D. Merino, B. Serrurrier and J.L. Elghozi, 1986. Peripheral and central short-term effects of fusaric acids, a dopamine beta-hydroxylase inhibitor on tryptophan and serotinin metabolism in the rat. *J. Neural Transm*., 65: 219

Davis, R.M., F.R. Kegel, W.M. Sills and J.J. Farrar, 1989. *Fusarium* ear rot of corn. *Calif. Agric*., 43: 4–5

Davis, D., 1969. Fusaric acid in selective pathogenicity. *Phytopathol.,* 59: 1391–5

FAO, 1997. Worldwide regulation for mycotoxins. A compendium. Food and Nutrition Rome, Italy. *Paper No.,* 64: 46

Gaumann, E., 1957. Fusaric acid as a wilt toxin. *Phytopathol.,* 47: 342–57

Halasz, A., A. Badaway, J. Sawinsky, E. Kozma-Kovacs and J. Beczner, 1989. Effect of gamma-irradiation on F-2 and T-2 toxin production in corn and rice. *Folia Microbiol*., 34: 228–32

Hammad, A.A. and Z. El-Baza, 1988. Mold contaminating smoked herrings and their control by gamma irradiation. *Az. J. Microbiol*., 4: 10–23

Hammad, A.A., 1989. Effect of irradiation, water activity and propionic acid on growth of *Penicillium chrysogenum* and *Aspergillus fumigatus*, *7th Conf. Microbiol*., pp: 105–115. Cairo. November 19.

Hammad, A.A., M.M. Attalla, Zeinat El-Shayeb, M. Neffesa and A. Abeir, 1995. Effect of gamma irradiation on growth and aflatoxin production by certain local aflatoxigenic isolates of *Aspergillus flavas*. *Acta Pharmaceutica Twecica*, XXXVII: 43–52

IARC, 1993. International Agency for Research on Cancer, Lyon, France. Monographs on the evaluation of carcinogenic risks to humans. Some naturally occurring substances: food items and constituents: heterocyclic aromatic amines and mycotoxins, 56: 599

Kiss, I. and J. Farkas, 1977. The storage of wheat and corn of high moisture content as affected by ionizing radiation. *Acta Alimentaria*, 6: 193–214

Kommedahl, T. and C.E. Windels, 1981. Root-, stalk-, and ear-infecting *Fusarium* species on corn in the USA. *In:* Nelson, P.E., T.A. Toussoun and R.J. Cook (eds.), *Fusarium*: *Diseases, Biology, and Taxonomy,* pp: 94–103. Pennsylvania State Univ., University Park, USA.

Leslie, J.F., C.A.S. Pearson, P.E. Nelson and T.A. Toussoun, 1990. *Fusarium* spp. from corn, sorghum, and soybean fields in the central and eastern United States. *Phytopathol.,* 80: 343–50

Marre, M.T., P. Vergani and F.G. Albergoni, 1993. Relationship between fusaric acid uptake and its binding to cell structures by leaves of *Egeria densa* and its toxic effects on membrane permeability and respiration. *Physiol. Molec. Plant Path.,* 42: 141–57

Nagatsu, T., H. Hidaka, J. Kuzuya, K. Takeya, H. Umezawa, T. Takeuchi and H. Suda, 1970. Inhibition of dopamine beta-hydroxylase by fusaric acids (5-butylpicolinic acid) *in vitro* and *in vivo*. *Biochem. Pharmacol*., 19: 35–44

Nelson, E.P., T.A. Tousson and W.F.O. Marasas, 1983. *Fusarium* species. *An Illustrated Manual for Identification. p. 193.* The Pennsylvania State University Press. U.S.A.

Nelson, E.P., M.C. Dignani and E.J. Anaissie, 1994. Taxonomy, biology, and clinical aspects of *Fusarium* species. *Clin. Microbiol. Rev*., 7: 479–504

Porter, J.K., C.W. Bacon, E.M. Wray and Hagler, 1995. Fusaric acid in *Fusarium moniliforme* cultures, corn, and feeds toxic to livestock and the neurochemical effects in the brain and pineal gland of rats. *J. Nat. Toxins,* 3: 91–100

Rowley, D.B. and A. Brynjolfsson, 1980. Potential uses of irradiation in the processing of food. *Food Technol*., 34: 75–7

Schubert, J.C., 1981. *Introduction to Radiation Chemistry.* Lecturer 31 at the third. IFFIT-Training course, Wagenigene, The Netherlands.

Smith T.K. and E.J. Macdonald, 1990. Effect of fusaric acid on Brain regional neurochemistry and vomiting behavior in swine. *J. Anim. Sci*., 69: 2044–9

Sugiura. Y., K. Fukasaku, T. Tanaka, Y. Matsui and Y. Ueno, 1993. *Fusarium poae* and *Fusarium crookwellense*, fungi responsible for the natural occurrence of nivalenol in Hokkaido. *Appl. Environ. Microbiol*., 59 :3334–8

Suttajit, M., 1989. Prevention and control of mycotoxins. *In:* Semple, R.L., Firo, A.S., P.A. Hicks and J.V. Lozare (eds.), *Mycotoxin Prevention and Control in Food Grains.* FAO, Vialedelle Termedi Caracalla, Rome, Italy

Troxell, T.C., 1996. Regulatory aspects of fumonisins in the United States. *In:* Jackson, L.S., J.W. Devries and L.B. Bullerman (eds.), *Fumonisins in Food. pp: 355–62.* Plenum Press, New York, USA.

WHO, 1988. Food Irradiation: A technique for preserving and Improving the safety of food. World Health Organization. Geneva.

Visconti, A., M. Solfrizzo, M.B. Doko, A. Boenke and M. Pascale, 1996. Stability of fumoinisins different storage periods and temperatures in gamma irradiated maize. *Food Additives and Contaminants*, 13: 929–38

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