

EFFECT OF ANTIOXIDANTS AND FREE RADICAL SCAVENGERS ON AFLATOXIN PRODUCTION IN VIVO

S. Passi¹; C. De Luca¹; M. Picardo¹; E. Finotti¹; A.A. Fabbri²; G. Panfili and C. Fanelli².

¹Istituto S. Galliciano (IFO), Roma, Italy; ²Dip. Biologia vegetale. Università di Roma "La Sapienza", Roma, Italy

ABSTRACT

We demonstrated previously that some antioxidants-butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), cysteamine and sodium thiosulfate (THIO) - were capable of inhibiting, to different extents and depending upon their concentration, the output of aflatoxins induced by lipoperoxides in cultures of Aspergillus parasiticus. However, other antioxidants (vitamin E, vitamin C, reduced glutathione) increased significantly the production of mycotoxins. Following these findings we tested the effect of different concentrations (0.01-0.1% w/v) of the above compounds on wheat, maize and sunflower seeds properly moistened and inoculated with 10^6 conidia of a toxigenic strain of A. parasiticus (NRRL 2999). Uniform distribution of the antioxidants into the seeds was achieved by suspending them in 0.1% alkylamide betaine (an amphoteric detergent). Analysis of aflatoxins was carried out by HPLC on RP18 column. Hexosamine measurements were performed by GLC for the assessment of fungal growth in infected seeds. After 30 days of incubation, fungal growth and aflatoxin levels were much higher in sunflower than in maize or wheat seeds. Among the antioxidants tested, only BHA and BHT, mainly in association with 0.1% THIO, significantly reduced aflatoxin levels ($p < 0.05$). The inhibitory effect was more evident in wheat and maize than in sunflower seeds and was due to three basic reasons: a) more marked reduction of fungal growth in starchy seeds; b) higher content of superficial lipids in the hull of sunflower seeds; c) higher content of phenol-oxidases in the hull of sunflower seeds. The two last factors are capable of lowering the concentrations of BHA and BHT.

INTRODUCTION

It is known that fungal growth need not be visible for toxin production to occur, but certainly fungal growth is a pre-requisite and its inhibition ensures prevention of toxin formation

The best known fungal inhibitors in food and feed include organic acids such as sorbic acid, benzoic acid, propionic acid, butyric acid and their salts. Other aflatoxin inhibitors are trace metals such as barium and vanadium, chelating agents such as ethylenediaminetetraacetate and phytic acid, metabolic inhibitors such as ethionine, dichlorvos, and cysteic acid. Other non specific compounds such as mercaptoethanol, dimethylsulfoxide, citrus oil, gentian violet, ethoxyquin, organophosphates, also show an inhibitory effect (Bennet & Christensen, 1983; Hesseltine, 1972). These chemicals gave results varying from complete inhibition of Aspergillus with no aflatoxin formation, to aflatoxin accumulation in amounts of up to twice that of the control (Hesseltine, 1972), or to formation of other compounds whose toxicity is unknown (Bennet & Christensen, 1983)

Recently we demonstrated that lipoperoxidation plays an important role in stimulating aflatoxin biosynthesis (Fanelli et al., 1980, 1983 a, b, c, 1984, 1985, Passi et al 1984, 1985). "In vitro", the addition of lipoperoxides or carbon tetrachloride (CCl₄) to cultures of Aspergillus parasiticus Speare or A. flavus Link ex Fr highly enhances aflatoxin output (Passi et al, 1985) CCl₄ and other halomethanes are substances capable of inducing lipoperoxidation of endoplasmic reticulum of fungi (Passi et al., 1986).

"In vivo", the aflatoxin production in differently aged seeds inoculated with A. parasiticus paralleled the peroxide number of their oil contents, that is the degree of peroxidation of seeds (Passi et al, 1984). Increased yields of mycotoxins were observed on the sterilized wheat treated with a CCl₄-carbon disulphide mixture (Hesseltine, 1972).

Therefore it was thought worthwhile investigating the effect of the most common antioxidants on growth and aflatoxin production with a view to their possible successful use in the control of aflatoxin formation in vivo.

"In vitro", butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), cysteamine, thiosulfate (THIO), depending upon their concentrations, were capable of reducing or blocking aflatoxin output induced by lipoperoxides or halomethanes in cultures of Aspergillus without affecting significantly fungal growth. In contrast, under the same

cultural conditions, other antioxidants, i e. vitamin C and E, cysteine, and reduced glutathion, actually enhanced aflatoxin biosynthesis (Fanelli et al 1985)

The question as to whether this also applies "in vivo" was the purpose of the following investigation.

MATERIALS AND METHODS

Non-sterilized sunflower seeds (Helianthus annuus L, cultivar gloriasol), maize grains (Zea mays L, cultivar decalb), and wheat grains (Triticum vulgare Host., cultivar kreso) were used in the experiments. All the seeds were in excellent condition (Table 1). Ten g of seeds from each cultivar were put in Erlenmeyer flasks (100 ml). Moisture content (M.C) was raised in all cases to 20% (wet weight basis) by adding sterile distilled water. After equilibration over night, each flask was seeded with 10^6 conidia of a 15 day old culture of A. parasiticus (strain NRRL 2999). Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), sodium thiosulfate (THIO), ascorbic acid (VIT C), DL- α -tocopherol (VIT E) were purchased from Sigma. All these chemicals were at the highest grade available and were added at different concentrations (0.01 to 0.1%) to the flasks before inoculation. To obtain better distribution of the chemicals with the seeds, each component was suspended in 0.1% ampholitic detergent (alkylamide betaine, AAB). Each flask was capped with aluminium foil and incubated at 30° C for 15 days.

 Table 1. NUMBER OF PEROXIDES, MOISTURE CONTENT, GERMINATION AND FUNGI ISOLATED FROM WHEAT, SUNFLOWER AND MAIZE SEEDS USED FOR THE EXPERIMENTS

SEEDS	N OF PEROXIDES	M.C	GERMINATION	FUNGI ISOLATED
WHEAT	0.6 + 0.1	7.0	100	<u>ALTERNARIA</u> sp. <u>CLADOSPORIUM</u> sp.
SUNFLOWER	0.8 + 0.1	6.3	100	<u>PENICILLIUM</u> sp.
MAIZE	0.7 + 0.1	7.2	100	<u>RHIZOPUS</u> sp. <u>ASPERGILLUS</u> <u>NIGER</u>

The total aflatoxins produced in each flask were

determined by reverse phase HPLC (Fanelli et al, 1980, 1983 a,b ,c, 1985, Passi et al, 1984, 1985). Detection of fungal growth was performed by GLC; the method is based on the quantification of hexosamines, as alditol acetates (Fox, 1983), on a SP 2330 capillary column (15 m x 0.32 mm I.D., Supelco). These aminosugars are a breakdown product of chitin, which is one of the major constituents of fungal cell walls.

Hydrolysis procedures.

Hydrolysis of fungal cell walls was initiated with 0.1 to 1 mg of lyophilized A. parasiticus in 1 ml of 6N HCl in reaction tubes. After flushing with N₂IPP, tubes were capped and heated at 121°C for 2 h. After cooling, the hydrolysates were dried under vacuum in a rotatory evaporator and dissolved in 0.5 ml of water. The pH was raised to 5-6 by 1N NH OH. This solution was applied to a Dowex 50 W X 4 column (prepared with 1 g of resin obtained from Fluka) The column was washed with 20 ml of water, and hexosamines were eluted with 12 ml of 2N HCl. The effluent was dried under vacuum and hexosamines derivatized as alditol acetates (Fox, 1983).

For seeds, the procedure was slightly modified. One mg lyophilized A. parasiticus was added to one g aliquots of seeds inoculated with the fungus and to one g aliquots of non inoculated seeds (control) Seeds were treated with 4 ml 6N HCl for 2 h. Hydrolysed samples were centrifuged at 3000 g for 10 minutes and washed 3 times with 2 ml water. The collected supernatants were dried under vacuum and then derivatized.

The stability of antioxidants was also determined both in the presence and the absence of fungus Every day the flasks were extracted with dichloromethane (3x20 ml) in the case of BHA, BHT, VIT E, or with water (10 ml) in the case of THIO and VIT C. The solvents were filtered, concentrated with N₂ IPP (dichloromethane) or by reduced pressure (water) and injected, with the exception of THIO, into the liquid chromatograph, equipped with a RP18 5 µm column (Passi & Nazzaro-Porro, 1981). THIO was analyzed by 0.01M iodine.

RESULTS

Tables 2, 3 and 4 show fungal growth and aflatoxin content (B1+B2+G1+G2) in sunflower, maize and wheat seeds inoculated with A. parasiticus and treated with different antioxidants after 15 days of incubation at 30° C. As expected, aflatoxin production was higher in sunflower seeds than in maize or in wheat seeds. Fungal growth, which parallels aflatoxin biosynthesis, was uniform on sunflower

TABLE 2 AFLATOXIN AND HEXOSAMINE CONTENTS IN SUNFLOWER SEEDS INOCULATED WITH
 A. PARASITICUS AND TREATED WITH DIFFERENT ANTIOXIDANTS AFTER 15 DAYS OF INCUBATION

<u>ANTIOXIDANT</u>	<u>AFLATOXIN CONTENT</u> µg/10 g	<u>HEXOSAMINE CONTENT**</u> µg/10 g
CONTROL	301.2 ± 38.5	48.6 ± 8.6
AAB (0.1% v/v)	244.2 ± 28.4	46.8 ± 9.5
AAB + THIO (0.1% w/v)	180.8 ± 17.4*	38.1 ± 7.4*
BHA (0.03% w/v) + AAB	107.7 ± 22.6*	24.5 ± 5.3*
BHA (0.03%) + AAB + THIO	41.2 ± 10.5*	10.8 ± 2.1*
BHT (0.03% w/v) + AAB	133.0 ± 20.4*	26.5 ± 4.3*
BHT (0.03%) + AAB + THIO	68.8 ± 14.3*	16.5 ± 4.8*
VIT E (0.05% w/v) + AAB + THIO	289.5 ± 31.6	48.4 ± 9.5
VIT C (0.1%) + AAB + THIO	310.4 ± 50.4	54.5 ± 10

Each result represents the mean ± SD of five experiments. AAB: alkylamide betaine; THIO :sodium thiosulphate; BHA; butylated hydroxyanisole; BHT; butylated hydroxytoluene; VIT E : DL-α-tocopherol; VIT C. ascorbic acid.
 *: p < 0.05; **: glucosamine + galactosamine. Values of hexosamines were corrected on the basis of levels of glucosamine found on seeds before inoculation of A. parasiticus and probably due to pre-existent fungi.

TABLE 3 AFLATOXIN AND HEXOSAMINE CONTENTS IN MAIZE SEEDS INOCULATED WITH A. PARASITICUS AND TREATED WITH DIFFERENT ANTIOXIDANTS AFTER 15 DAYS OF INCUBATION

ANTIOXIDANT	AFLATOXIN CONTENT μg/10 g	HEXOSAMINE CONTENT** μg/10 g
CONTROL	120.2 ± 17.6	24.6 ± 4.7
AAB (0.1% v/v)	96.5 ± 20.4	20.3 ± 6.2
AAB + THIO (0.1% w/v)	58.3 ± 17.4*	11.8 ± 3.2*
BHA (0.03% w/v) + AAB	22.2 ± 10.3*	5.8 ± 1.4*
BHA (0.03%) + AAB + THIO	1.2 ± 1.4*	tr *
BHT (0.03% w/v) + AAB	30.3 ± 6.6*	8.4 ± 2.3*
BHT (0.03%) + AAB + THIO	2.0 ± 1.5*	tr.*
VIT E (0.05% w/v) + AAB + THIO	106.5 ± 22.1	22.7 ± 8.2
VIT C (0.1%) + AAB + THIO	118.4 ± 32.3	26.3 ± 9.2

Each result represents the mean ± of five experiments. AAB alkylamide betaine; THIO: sodium thiosulfate; BHA butylated hydroxyanisole; BHT. butylated hydroxytoluene; VIT E: DL-α-tocopherol; VIT C. ascorbic acid
 *. p<0.05; **. glucosamine + galactosamine. Values of hexosamines were corrected on the basis of levels of glucosamine found on seeds before inoculation of A. parasiticus and probably due to pre-existent fungi

TABLE 4 AFLATOXIN AND HEXOSAMINE CONTENTS IN WHEAT SEEDS INOCULATED WITH A. PARASITICUS
AND TREATED WITH DIFFERENT ANTIOXIDANTS AFTER 15 DAYS OF INCUBATION

<u>ANTIOXIDANT</u>	<u>AFLATOXIN CONTENT</u> $\mu\text{g}/10 \text{ g}$	<u>HEXOSAMINE CONTENT**</u> $\mu\text{g}/10 \text{ g}$
CONTROL	68.8 \pm 16.0	16.2 \pm 5.4
AAB (0.1% v/v)	50.9 \pm 18.5	12.6 \pm 3.4
AAB + THIO (0.1% w/v)	35.6 \pm 10.4*	8.3 \pm 2.5*
BHA (0.03% w/v) + AAB	15.3 \pm 7.8*	4.2 \pm 2.1*
BHA (0.03%) + AAB + THIO	5.1 \pm 3.2*	2.1 \pm 0.8*
BHT (0.03% w/v) + AAB	22.8 \pm 12.4*	7.2 \pm 2.4*
BHT (0.03%) + AAB + THIO	9.0 \pm 4.5*	2.8 \pm 0.9*
VIT E (0.05% w/v) + AAB + THIO	59.4 \pm 10.3	16.3 \pm 4.7
VIT C (0.1%) + AAB + THIO	63.2 \pm 15.4	19.6 \pm 5.5

Each result represents the mean + of five experiments. AAB: alkylamide betaine; THIO: sodium thiosulphate; BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene; VIT E: DL- α -tocopherol; VIT C: ascorbic acid.
*: p<0.05; ** glucosamine + galactosamine. Values of hexosamines were corrected on the basis of levels of glucosamine found on seeds before inoculation of A. parasiticus and probably due to pre-existent fungi.

seeds, while in maize and wheat it started from the germ and then spread over the grains.

BHA, BHT and THIO both alone and in association were capable of inhibiting significantly ($p < 0.05$) both aflatoxin output and fungal growth as compared to controls. The inhibition is dose dependent: concentrations lower than those reported in the tables, viz., 0.01% or 0.005 % of BHA and BHT, 0.05% or 0.01% of THIO and AAB showed a lesser effect (data not reported). Furthermore, VIT C and VIT E failed to affect both aflatoxin production and fungal growth. The latter was measured as increased concentrations of chitin. It is interesting to underline that, by our method, we have quantified, in addition to glucosamine, conspicuous amounts of galactosamine in the fungal wall (Fig 1B). The contents of glucosamine and galactosamine in 1 mg of lyophilised A. parasiticus were 31.5 μ g and 21.2 μ g respectively.

The stability of antioxidants added to the seeds was also taken into account. Figs 2-5 show the rate of decomposition of 0.05% BHA, 0.05% VIT E, 0.1% VIT C, 0.1% THIO added to the seeds, whose M. C. was 20%. BHT decomposition paralleled that of BHA. The seed M. C. significantly affected the stability of antioxidants added to them: the drier the seeds, the lower the rate of decomposition of antioxidants (Fig. 6).

Fig.7 shows the formation of dopachrome following the addition of 20 ml of a solution of l-dopa (0.1%, w/v in 0.1 M phosphate buffer, pH 7.0) to 10 g of sunflower seeds. Dopachrome becomes detectable after 2-3 minutes, reaches a maximum after 20-25 minutes of incubation at 30 C and then disappears slowly, while a melanoid polymer becomes evident. Consequently the level of l-dopa decreases. This phenomenon, which is more remarkable in sunflower than in maize or wheat seeds, is probably due to non-specific oxidases present on the surface of healthy seeds capable of oxidizing l-dopa to dopachrome and then to a melanoid polymer. The oxidation of l-dopa does not take place when seeds are sterilized at 121° C; sterilization, in fact, destroys oxidases.

DISCUSSION

Following infection with A. parasiticus, both aflatoxin output and fungal growth were higher in sunflower than in maize or wheat seeds, probably because of the larger amount of lipids on the external surface of sunflower as compared with the two other starchy seeds. Also Lillehoy et al. (1974), found that full fat corn germ favored aflatoxin production in comparison with whole corn, when infected with A. flavus, while defatted germ was the poorest substrate for

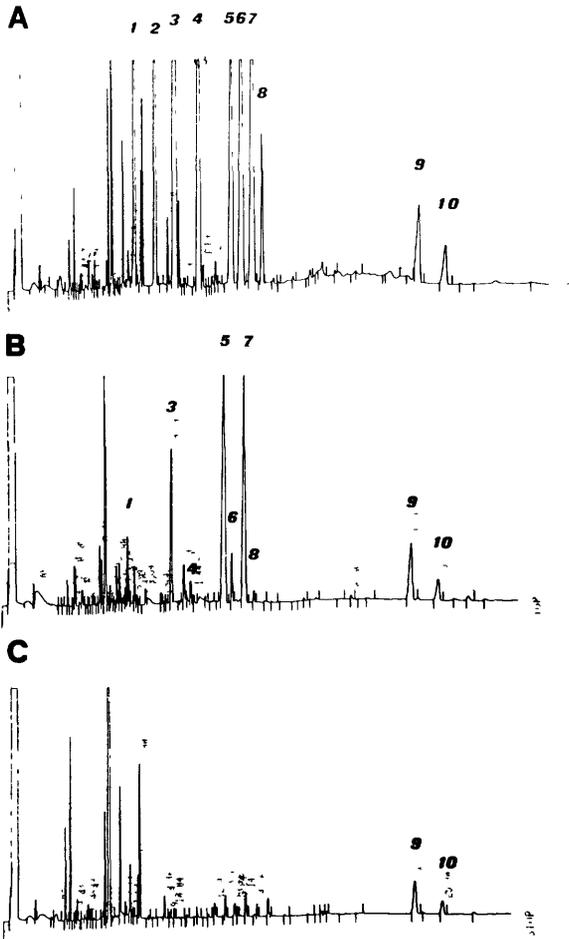


FIG 1. GLC separation of alditol acetates of neutral and amino sugars on fused silica SP 2330 capillary column (15 m x 0.32 mm I.D.). Temperature: 50-200°C at 25°C/min, maintained at 200 °C for 1 min and increased at 250 C at 4 °C/min. Injector: 275°C; flame ionization detector: 300°C; helium at flow rate ca. 2 ml/min; helium pressure: 16 p.s.i.. Injection, splitless.

A: Standard sugars. 1:erythritol; 2:D-ribitol; 3:L-arabinitol; 4 D-xylitol; 5:D-mannitol; 6:glucitol, 7:D-galacticol; 8:myo-inositol; 9: D-glucosaminitol; 10 :D-galactosaminitol B: Neutral and amino sugars found in lyophilized A. parasiticus (strain NRRL 2999) C Hexosamines found in a sample of sunflower seeds infected with A. parasiticus The most part of neutral sugars formed during 6 N HCl hydrolysis were eliminated by purification on Dowex 50 W-X4 resin.

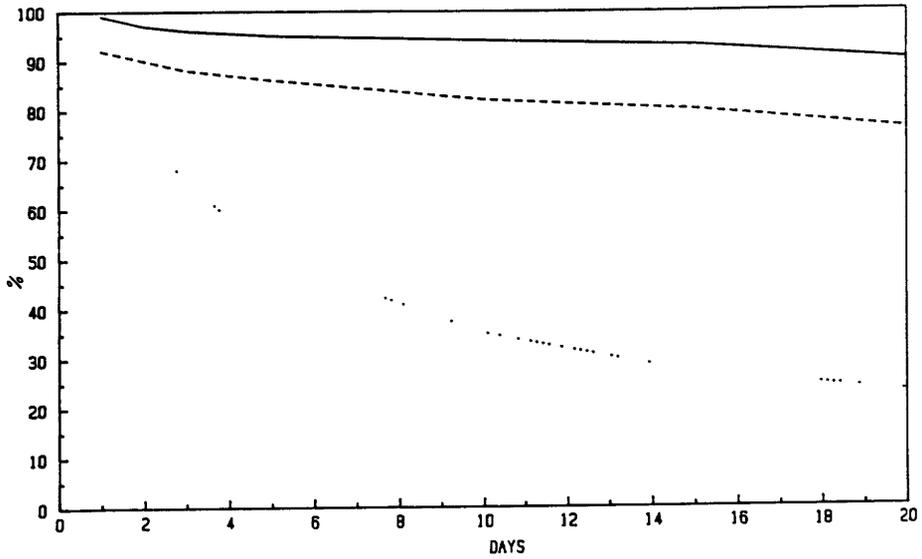


FIG 2. Decomposition of 0.03% BHA added to seeds (Humidity 20%) ——— Wheat, - - - - - Maize, Sunflower. For details see text.

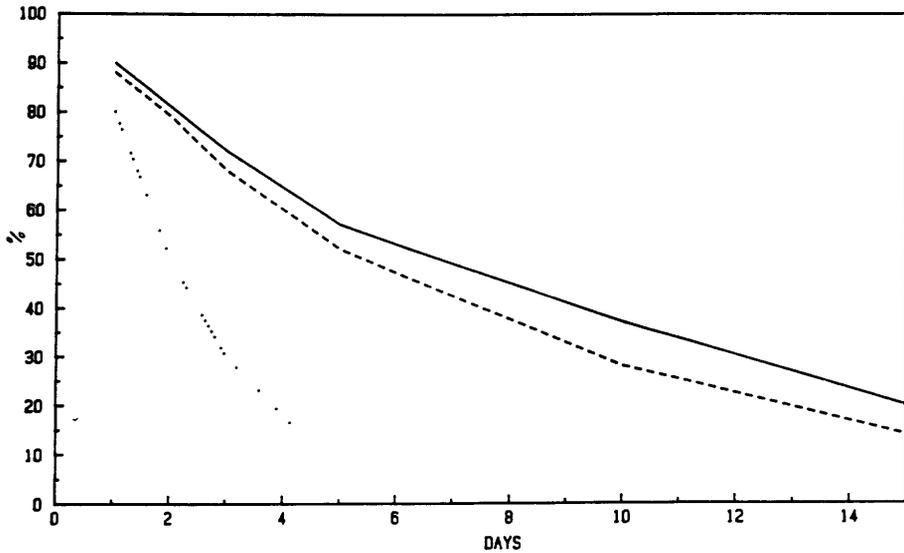


FIG. 3. Decomposition of 0.1% VIT C added to seeds (Humidity 20%). ——— Wheat, - - - - - Maize, Sunflower. For details see text.

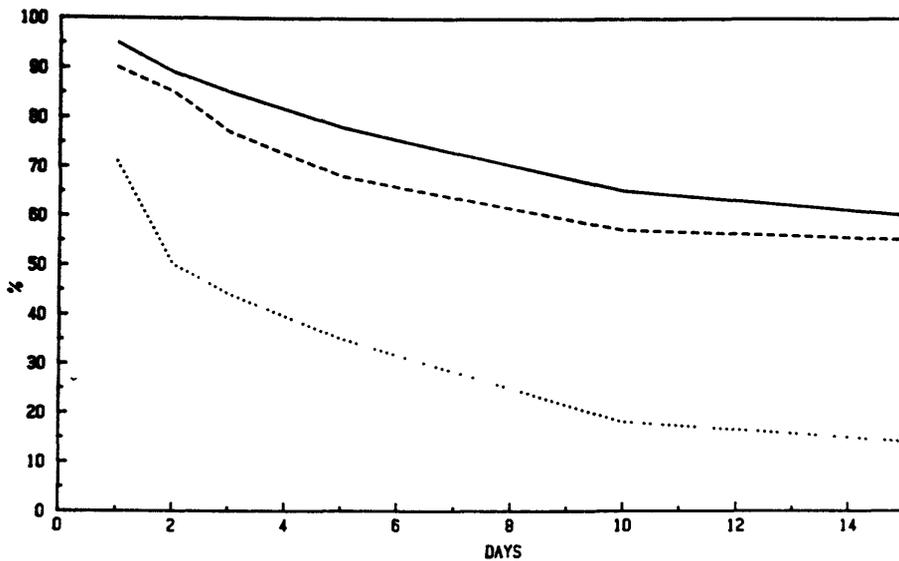


FIG. 4. Decomposition of 0.05% VIT E added to seeds (Humidity 20%). — Wheat, - - - Maize, Sunflower. For details see text.

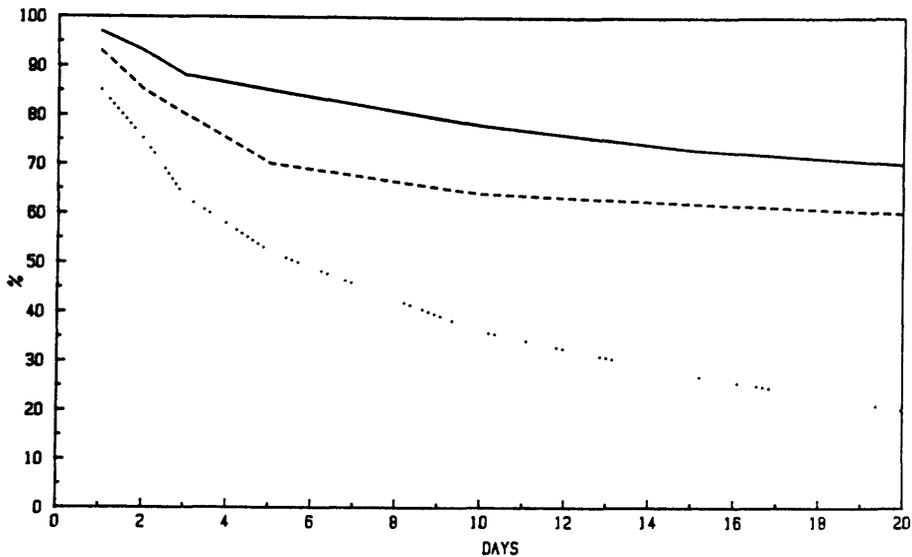


FIG. 5. Decomposition of 0.1% THIO added to seeds (Humidity 20%). — Wheat, - - - Maize, Sunflower. For details see text.

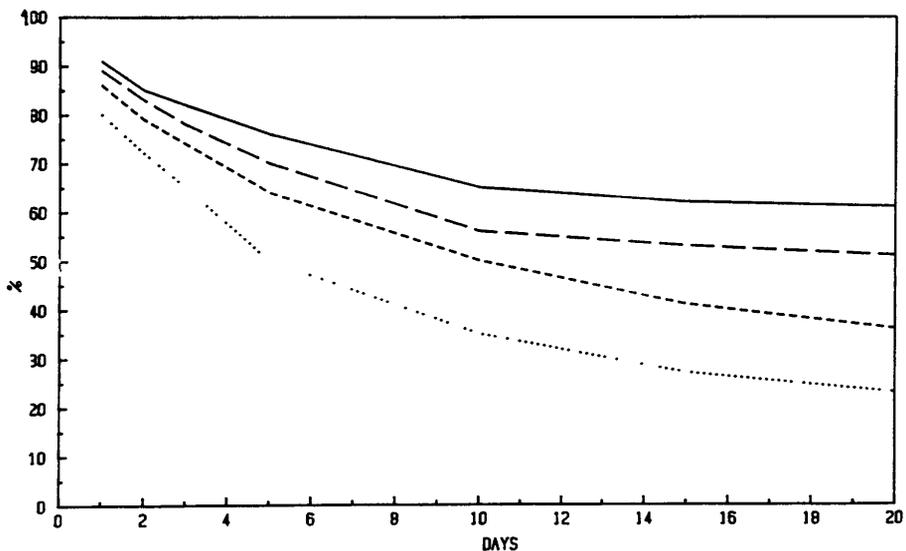


FIG. 6. Water dependent decomposition of 0.03% BHA added to sunflower seeds. For details see text
 — 5% H₂O, - - - 10% H₂O, - . - . - 15% H₂O, 20% H₂O.

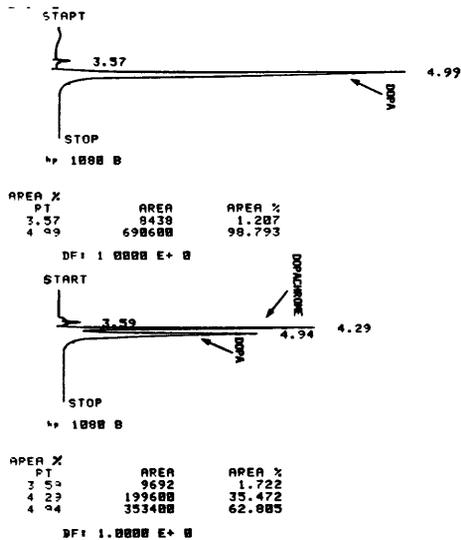


FIG. 7. Formation of dopachrome following addition of 20 ml of a solution of l-dopa to sunflower seeds. For details see text

toxin biosynthesis. It is interesting to underline, however, that lipids, viz. triglycerides and free fatty acids, support very well fungal growth by acting as carbon sources (Fanelli et al., 1980), but they do not affect aflatoxin formation. This depends upon other factors available in the environment in which Aspergillus is growing, such as trace elements, mainly zinc, free radicals, etc (Fanelli et al., 1984; Lillehoj et al., 1974).

In this work fungal growth was measured by gas chromatography on capillary columns. By using alditol acetates derivatives of hexosamines we were able to detect considerable levels of galactosamine, in addition to glucosamine, among the breakdown products of chitin, which is one of the major constituents of fungal cell wall. Colorimetric or HPLC methods (Ride & Drysdale, 1971; Lin & Cousin, 1985) developed for detection of hexosamines in fungal cell wall chitin were not capable of differentiating glucosamine from galactosamine.

BHA and BHT (which are widely added as antioxidants to some fat containing processed foods) significantly reduced ($p < 0.05$) aflatoxin production and fungal growth in seed inoculated with A. parasiticus (Tables 2 to 4). The inhibitory effect was more marked in starchy than in sunflower seeds, and was enhanced by the presence of sodium thiosulfate and alkylamidebetaine, which are known to be weak antifungal agents. In contrast, under the same cultural conditions, VIT C and VIT E proved quite ineffective. These results are in agreement with those obtained "in vitro" (Fanelli et al., 1985). However, it must be pointed out that while "in vitro" BHA, BHT and THIO really act as antioxidants or free radical scavengers, by inhibiting aflatoxin output induced by CCL_4 without affecting significantly fungal growth, "in vivo" on healthy seeds they exert their action against aflatoxin formation essentially by preventing mold growth (Tables 2 to 4).

In this connection several studies have been published on the "in vitro" inhibition of bacterial and fungal growth by BHA (Ahmad & Branen, 1981). BHT was found ineffective (Benchet & Jones, 1978) but probably, the lack of effect of BHT is attributable to its extreme insolubility in the media, as compared with BHA. BHA and BHT as well as VIT E are capable of preventing, but not eliminating or lowering the levels of pre-existent lipoperoxides, when added to rancid seeds.

Does lipoperoxidation actually play a role in aflatoxin accumulation in vivo?. We have at least three arguments supporting such a suggestion :

1) Although lipoperoxides and their breakdown products are extremely toxic substances for most microorganisms (Cortesi & Privett, 1972; Del Maestro, 1980), A. parasiticus and A. flavus grow very well in their presence (Panelli et al., 1983 a, b; Passi et al., 1984);

2) "In vitro", the addition to cultures of A. parasiticus or A. flavus of lipoperoxides (synthetic hydroperoxides, epoxides, UV peroxidated sterols and UV peroxidated unsaturated free fatty acids and triglycerides) or components such as halomethanes capable of inducing lipoperoxidation of endoplasmic reticulum of fungi, highly stimulate aflatoxin production (Panelli et al. 1983 a, b; Passi et al. 1984, 1985, 1986);

3) "In vivo" the aflatoxin accumulation in rancid aged seeds inoculated with A. parasiticus or A. flavus paralleled the peroxide number of their oil content (Passi et al., 1984).

As a matter of fact, lipoperoxidation is a complex phenomenon which depends upon several physicochemical factors in the environment, i.e., degree of unsaturation and structure of lipids, humidity, temperature, radiation, trace metals, aging, chelating agents, antioxidants, etc. The same factors affect also aflatoxin production. It was suggested that the peroxidation and the relevant production of organic free radicals of seed lipids leads to loss of viability of stored and aged seeds (Stewart & Bewley, 1979; Buchevaros & Gautheff, 1984). Following deterioration of seeds, their enzymatic systems become altered, probably trace elements, particularly zinc and phosphorous, become biologically available for the development of the Aspergilli, because of the splitting of the bond with phytate (Lillehoj et al., 1974).

An important aspect of the subject (which has not been duly considered in the case of other antifungal agents) is that "healthy seeds" are capable of degrading antioxidants (Figs. 2 to 6) added to them, to different degrees. The decomposition of antioxidants may be due to two main reasons: 1) presence of autooxidable lipids on the external surface of seeds; 2) presence of phenol-oxidase enzymes, as demonstrated by the formation of dopachrome in seeds incubated with l-dopa.

On the other hand oxidases such as peroxidase, cytochrome oxidase and phosphoglycerate dehydrogenase, have been found in the testa of some seeds, and were considered to be involved in oxygen consumption by the hull, which is attributed to the oxidation of various phenolic compounds such as phlorizin, chlorogenic acid, para-coumarylquinic acid, known to be present in the testa (Beweley & Black, 1982).

Since both the amount of external lipids and the activity of oxidase enzymes are much more conspicuous on sunflower than on starchy seeds, this may account for the more noticeable degradation of antioxidants added to sunflower as compared with starchy seeds.

In conclusion, BHA, BHT and, to a lesser degree THIO can be considered extremely useful tools in the war against aflatoxins in agricultural crops, mainly in starchy seeds. They in fact couple the capacity of inhibiting the growth of toxigenic fungi to their antioxidant power, which allows them to prevent accumulation of aflatoxin stimulating lipoperoxides. The legality of adding these antioxidants at concentrations greater than those at present permitted has to be determined by the regulatory agencies.

This study was supported by European Community-grant n. TSD .A.111.I. (S)

REFERENCES

- Ahmad S. and Branen A.L. (1981) Inhibition of mold growth by butylated hydroxyanisole. J. Food Science 46, 1059-63.
- Benchet L.R. and Jones W.K. (1978). Effect of food preservatives and antioxidants on colony formation by heated conidia of *Aspergillus flavus*. Acta Alimentaria 7, 373-378.
- Bennet J.W. and Christensen S.B. (1983) New perspective on Aflatoxin biosynthesis. Adv. Appl. Microbiol. 29, 53-92.
- Beweley J.D. and Black M. (1982) Physiology and Biochemistry of seeds in relation to germination Vol. II Springer-Verlag. Berlin Heidelberg. New York.
- Buchevaros P. and Gautcheff T. (1984) Influence of accelerated and natural aging of free radical levels in soybean seeds. Physiol. Plant 60, 53-56.
- Cortesi R. and Privett O.S. (1972) Toxicity of fatty ozonides and peroxides. Lipids 7, 715-719.
- Del Maestro R.F. (1980) An approach to free radicals in medicine and biology. Acta Physiol. Scand. (Suppl.) 492, 153-168.
- Fanelli C., Fabbri AA. and Passi S. (1980) Growth requirements and lipid metabolism of *Aspergillus flavus*. Trans. Brit. mycological Soc. 75, 371-375.
- Fanelli C., Fabbri AA, Finotti E. and Passi S. (1983a) Stimulation of Aflatoxin by lipophilic epoxides. J. Gen. Microbiol. 129, 1721-1723.
- Fanelli C., Fabbri AA., Passi S. and Fasella P. (1983b) Control mechanisms of Aflatoxin biosynthesis. Micotoxin Symposium in Sydney 12-15/8/83 abstr P9.
- Fanelli C., Fabbri AA., Finotti E., Panfili G. and Passi S. (1983c) Cerulenin and tetrahydrocerulenin: stimulating

- factors of Aflatoxin biosynthesis Trans. Br. mycol. Soc. 81, 201-204.
- Fanelli C., Fabbri AA., Finotti E., Fasella P. and Passi S (1984) Free radicals and Aflatoxin biosynthesis. Experientia 40, 191-193.
- Fanelli C., Fabbri AA., Pieretti S., Finotti E., and Passi S. (1985) Effect of different antioxidants and free radical scavengers on Aflatoxin production. Mycotoxin Research 1, 65-69
- Fox A. (1983) Capillary Gas-chromatographic analysis of alditol acetates of neutral and amino sugars in bacterial cell wall J. Chromatography 256, 429-438.
- Hesseltine C.W. Recent research for the control of mycotoxin in cereal Proc Symp. Control Mycotoxins Goteborg 1972, pp 251-258 Butterworths, London. Aflatoxin biosynthesis. Adv. Appl. Microbiol. 29, 53-92.
- Lillehoj E.B., Garcia W.J. and Lambrow M. (1974) Aspergillus flavus infection and Aflatoxin production in corn: influence of trace elements. Applied Microbiology 28, 763-767.
- Lin H.H. and Cousin M.A. (1985) Detection of mold in processed foods by high performance liquid chromatography. J. Food Protection 48, 671-678.
- Passi S. and Nazzaro-Porro M (1981) Molecular basis of substrate and inhibitory specificity of tyrosinase: phenolic compounds. BR. J. Dermatol. 104, 659-665.
- Passi S., Nazzaro-Porro M., Fanelli C., Fabbri AA. and Fasella P. (1984) Role of lipoperoxidation in Aflatoxin production. Appl. Microbiol. Biotechnol. 19, 186-190.
- Passi S., Fanelli C., Fabbri AA., Finotti E., Panfili G. and Nazzaro-Porro M. (1985) Effect of halomethanes on Aflatoxin induction in cultures of Aspergillus parasiticus. J. Gen. Microbiol. 131, 687-691.
- Passi S., Nazzaro-Porro M., Picardo M., Finotti E., Fabbri AA. and Fanelli C. (1986) Microsomal and mitochondrial involvement in production of Aflatoxins induced by carbon tetrachloride and hydroperoxide in cultures of Aspergillus parasiticus. Trans. Br. mycol. Soc. 87, 451-456.
- Ride J.P. and Drysdale R.B. (1971). A chemical method for estimating Fusarium oxysporum f. lycopersici in infected tomato plants. Phys. Pl. Path. 1, 409-420.
- Stewart R.R.C. and Bewley J.D. (1979) Lipid peroxidation associated with accelerated aging of soybean axes. Plant Physiol 65, 245-248.