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Growth and Aflatoxin Production by *Aspergillus parasiticus* in a Medium Containing Plant Hormones, Herbicides or Insecticides

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ABSTRACT

The effect of some widely used plant hormones (indol-3-acetic acid and gibberellic acid), herbicides (gramoxone, stomp and treflan) and insecticides (malathion, actellic and guthion) on *Aspergillus parasiticus* growth and aflatoxin production in a synthetic medium was studied. Addition of indol acetic acid to the medium increased aflatoxin production more than gibberellic acid. Treflan at 5, 10 and 20 ppm levels caused a highly significant stimulatory effect on *A. parasiticus* growth and aflatoxin production. In contrast, stomp at 10 and 20 ppm produced the reverse effect. Guthion, an insecticide, caused a marked decrease in fungal growth and aflatoxin production. The inhibitory effect of insecticides under study on both fungal growth and aflatoxin production in effectiveness followed the sequence: guthion>actellic>malathion. At the recommended application rate (10 ppm), with the exception of indol acetic acid and treflan, all compounds suppressed mold growth and aflatoxin production.

In recent years, there has been an increased interest in mycotoxins which cause many diseases in humans and animals. These mycotoxins are introduced by ingestion of contaminated food and feeds *(1,7)*. Aflatoxins, which are produced by certain strains of *Aspergillus flavus* or *Aspergillus parasiticus,* play a crucial role in human and animal health because of their potential carcinogenic effect. Intensive research was conducted on formation and control of mycotoxin production, particularly aflatoxins, in foods. Some factors as water activity, temperature and atmosphere affect growth and mycotoxin production *(2)*.

Currently some chemical compounds are used to prevent growth of mold and aflatoxin production. These chemicals occur naturally in certain foods, or may appear as a contaminant in foods during production, processing, packaging or storage. Some insecticides, herbicides and plant hormones widely used in agricultural practices for pest control, weed control and growth promotion, might have a secondary effect in controlling fungal growth and aflatoxin production. In fact, residues of pesticides, herbicides and plant hormones are known to carry over into foods. Consequently, in the present work model systems were prepared to study the effect of some of the widely used herbicides, insecticides and plant hormones on *A. parasiticus* growth and aflatoxin production in a synthetic medium.

MATERIALS AND METHODS

Fungus

A. parasiticus (ATCC 120920) was obtained from the Tropical Product Institute, London, England. This strain was checked for purity and identity *(4)*.

Preparation of spore suspension

A. parasiticus was grown on potato-dextrose-agar (Difco) slants for 10 d at 28°C. Spores were harvested by adding sterilized Tween 80 solution (0.1%, v/v), filtered through several layers of sterilized cheese cloth, centrifuged, washed thrice with distilled, sterilized water and suspended in sterilized Tween 80 solution (0.01%, v/v). The number of conidia was estimated by plate count and the suspension was adjusted to contain approximately 10^6 spores/ml.

Sources of insecticides, herbicides and plant hormones

Table 1 lists the trade names, chemical names and sources of insecticides and herbicides used in the present work. Gibberellic and indol acetic acids were purchased from Sigma Chemical Co. and Koch-Light Lab. Ltd., respectively.

Culture conditions

Yeast extract-sucrose medium (YES) was used as a basal medium for aflatoxin production in stationary cultures. One ml portion of 10^6 spores/ml was added to YES medium (50 ml in a 250-ml Erlenmeyer flask). The selected insecticides, herbicides or plant hormones were added to the culture medium to contain 5, 10, 20; 5, 10, 20; and 10, 20, 30 ppm, respectively. After addition of each compound the culture was incubated at 27 ± 1°C for 6 d.

Determination of mycelial dry weight

At the end of the incubation period, fluid cultures were filtered under vacuum through Whatman No. 1 filter paper. The

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TABLE 1. *Trade names, chemical names and sources of insecticides and herbicides.*

Trade name	Chemical Name	Source
	Insecticides	
Malathion	0,0-dimethyl-S-(1,2-dicarbethoxy ethyl)-phosphorodithioate.	American Cyanamide Co., USA
Actellic	0-(2-diethylamino-6-methyl-pyrimidine-4-yl)-0,0-dimethyl phosphorothioate	Imperial Chemical Industries (I.C.I.), U.K.
Guthion	0,0-dimethyl-S-(4-oxo-1,2,3)-benzotriazin-3(4H)-ylmethyl)-phosphorodithioate.	Bayer AG, West Germany
	Herbicides	
Gramoxone	1,1-dimethyl-4,4-bipyridinium	I.C.I., U.K.
Treflan	α,α,α-trifluoro-2,6-dinitro-N,N-diporpyl-p-toluidine.	Elance, Eli-Lilly, USA
Stomp	N-(1-ethyl propyl)-3,4-dimethyl-2,6-dinitrobenzenamine	American Cyanamide Co., USA

TABLE 2. *Effect of some growth regulators on* A. parasiticus *growth and aflatoxin production in YES medium.*

Plant hormone	Concentration (ppm)	Mycelial dry weight (g/100 ml)	Aflatoxin production (ppm) B_1	B_2	G_1	G_2	Total
Control	——	2.58 (a)[1]	100 (a)	3	138 (a)	4	245
Indol-3-acetic	10	2.56 (a)	150 (b)	4	173 (b)	4	331
acid	20	2.42 (b)	125 (b)	3	156 (b)	4	288
	30	2.26 (b)	125 (b)	3	143 (b)	4	275
Gibberellic acid	10	2.66 (b)	125 (b)	4	156 (b)	4	289
	20	2.40 (a)	100 (a)	3	138 (a)	4	245
	30	2.28 (b)	100 (a)	3	138 (a)	4	245

[1]Numbers in the column followed by the same letter are not significantly different at $p = 0.01$.

filter paper containing the mycelium was washed several times with distilled water, dried at 65°C under vacuum for 24 h and weighed.

Qualitative and quantitative determination of aflatoxins

A portion from the culture filtrate (10 ml) was extracted twice with chloroform (10 ml each time). The combined chloroform extract was filtered through anhydrous sodium sulfate and the solvent was evaporated to dryness using a nitrogen stream at room temperature *(5)*. A mini-column packed from bottom to the top with glass wool, Drierite (8-10 mm), Floristil (8-10 mm), silica gel (16-20 mm), neutral alumina (8-10 mm), Drierite (8-10 mm) and glass wool was used for holding the aflatoxins. The aflatoxins were eluted with chloroform and quantitatively transferred into volumetric flasks of appropriate size (5, 10, 25 or 50 ml) according to the expected amounts of the aflatoxins in the extract. Aflatoxins were separated by thin-layer chromatographic technique using precoated plates with silica gel 60 (0.2-mm thickness) and developed using a mixture of chloroform-acetone (9:1, v/v). The chromatoplates were air-dried and viewed under UV light (365 nm). Aflatoxins were measured by comparing the fluorescence of the unknown to four standards: B_1, B_2, G_1 and G_2 obtained from Sigma Chemical Co. Aflatoxin production was calculated using the formula: μg aflatoxin/ml $= S \times Y \times \bar{V}/Z \times V$ where: S = μl aflatoxin standard equal to corresponding sample spot.

Y = concentration of specific aflatoxin in standard solution in μg/ml.

$\bar{V}$ = volume in μl to which sample extract is diluted for TLC.

Z = μl sample extract spotted giving fluorescence equal to aflatoxin standard.

V = ml of the original culture medium.

All the chemical determinations were conducted in triplicates and the results are presented as average values.

Statistical analysis

Analysis of variance and least significant difference tests were conducted to determine effects of herbicides, insecticides and plant hormones on fungal growth and aflatoxins B_1 and G_1 production *(6)*.

RESULTS AND DISCUSSION

Effect of plant hormones

It is well established that gibberellic acid (GA_3) and indol-3-acetic acid (IAA) stimulate production of primary metabolites such as proteins, carbohydrates and lipids in higher plants. In the present study, therefore, GA_3 and IAA were added to the fungal medium to promote production of primary substances and suppress production of secondary metabolites such as aflatoxins. The influence of IAA and GA_3 at concentrations of 10, 20 and 30 ppm on *A. parasiticus* growth and aflatoxin production is shown in Table 2. IAA at 10 ppm level had no effect on fungal growth, while the other IAA levels suppressed fungal growth. Treatment with IAA increased the aflatoxin content of the culture medium at all levels.

TABLE 3. *Effect of some herbicides and insecticides on* A. parasiticus *growth and aflatoxin production in YES medium.*

Component	Concentration (ppm)	Mycelial dry weight (g/100 ml)	Aflatoxin production (ppm)				
			B_1	B_2	G_1	G_2	Total
		Herbicides					
Control	——	3.74 (a)[1]	97 (a)	4	128 (a)	6	235
Gramoxone	5	3.24 (b)	113 (b)	4	144 (b)	6	267
	10	2.96 (b)	97 (a)	4	128 (a)	5	234
	20	3.12 (b)	73 (b)	3	80 (b)	5	161
Stomp	5	3.72 (a)	97 (a)	4	128 (a)	6	235
	10	2.98 (b)	73 (b)	4	112 (b)	5	194
	20	2.80 (b)	49 (b)	3	96 (b)	4	152
Treflan	5	4.10 (b)	145 (b)	5	159 (b)	7	316
	10	4.20 (b)	145 (b)	5	151 (b)	7	308
	20	3.92 (b)	129 (b)	4	144 (b)	6	283
		Insecticides					
Control	——	3.44 (a)	102 (a)	3	140 (a)	4	249
Malathion	5	3.06 (b)	122 (b)	3	156 (b)	4	285
	10	2.96 (b)	82 (b)	3	113 (b)	4	202
	20	3.14 (b)	82 (b)	2	99 (b)	4	187
Actellic	5	3.04 (b)	93 (b)	3	113 (b)	4	213
	10	2.52 (b)	82 (b)	3	85 (b)	4	174
	20	2.98 (b)	72 (b)	2	71 (b)	3	148
Guthion	5	2.68 (b)	82 (b)	3	99 (b)	2	186
	10	2.44 (b)	52 (b)	2	21 (b)	1	76
	20	0.98 (b)	21 (b)	ND[2]	8 (b)	ND	29

[1]Numbers in the column followed by the same letter are not significantly different at $p = 0.01$.
[2]ND refers to not detected.

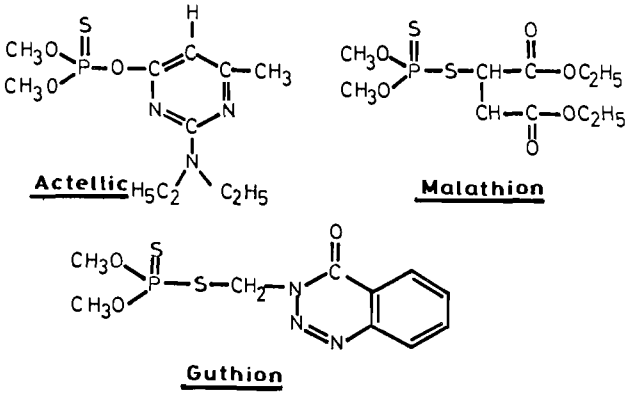

Figure 1. *Chemical structures of insecticides.*

Variable results were obtained from the GA_3 treatment. For example, GA_3 at the recommended level had no effect on either fungal growth and aflatoxin production. GA_3 at the 10 ppm level caused a highly significant increase in both fungal growth and aflatoxin production. In contrast, GA_3 at 30 ppm produced a highly significant decrease in fungal growth, while it had no effect on aflatoxin production. The ratio of B_1/G_1 was also altered by using these growth regulators at different concentrations.

Generally, introduction of plant hormones to the fungal culture medium decreased *A. parasiticus* growth and increased aflatoxin production. This finding is not in line with the fact that plant hormones are widely used to increase vegetative growth of higher plants. Addition of IAA to the fungal medium increased the aflatoxin production more than GA_3. One would expect this result since IAA but not GA_3 contains two functional groups, i.e. acetate and an indol ring in its moiety and both are precursors of aflatoxin synthesis.

Effect of some herbicides and insecticides

The effect of gramoxone, stomp and treflan at different concentrations on *A. parasiticus* growth and aflatoxin production is shown in Table 3. The four aflatoxins, B_1, B_2, G_1 and G_2, were found in all treatments. G_1 and B_1 were the most abundant toxins while G_2 and B_2 occurred as minor components and G-group content was greater than B-group content in all instances. It seems that each herbicide at the various levels under study behaved differently towards fungal growth and aflatoxin production. For instance, treflan produced a highly significant stimulatory effect on both fungal growth and aflatoxin production. Gramoxone caused a highly significant decrease in mycelial dry weight at all concentrations. This herbicide at 5 and 20 ppm produced a highly significant increase and decrease, respectively, on total aflatoxin content while at 10 ppm had no significant effect. Stomp at low concentration did not significantly alter the fungal growth or aflatoxin production. This herbicide at or above the recommended level produced a highly significant decrease in the aforementioned parameters.

The organophosphorous insecticides suppressed *A. parasiticus* growth and aflatoxin production (Table 3). However, the degree of activity was dependent upon the

type and concentration of insecticides. For instance, guthion at different concentrations markedly reduced mold growth and aflatoxin yield, the reduction being proportional to the guthion concentration. Actellic exhibited the same effect as guthion but to a lesser degree. Malathion at 5 ppm showed a highly significant stimulatory effect on aflatoxin production and an inhibitory effect at 10 and 20 ppm levels. It has been reported that malathion significantly inhibits production of aflatoxins at the 100 ppm concentration *(3)*. The inhibitory effect of these insecticides on G_1 and B_1 production was more pronounced in the presence of 20 ppm, and the B_1:G_1 ratio generally increased with the increase in insecticide concentration. In general, the inhibitory effect of these insecticides on *A. parasiticus* growth and aflatoxin production followed the sequence: guthion>actellic>malathion.

The chemical structures of the three insecticides are shown in Fig. 1. The formulas show that each of these insecticides has a thiophosphate ester group. The difference is mainly in the functional groups attached to the thiophosphate ester group. The extent of the inhibitory effect of the tested insecticides could be attributed to the presence of the aromatic nucleus. Malathion contains no aromatic nucleus, while actellic and guthion have heterocyclic rings, the latter has two fused heterocyclic rings. This suggests that the inhibitory effect could be increased by increasing the aromaticity since guthion with two fused rings showed the most powerful inhibitory effect towards aflatoxin production.

In conclusion, compounds in the present study possess dual functions, i.e., each compound can be used to control pests and/or weeds or to increase plant growth and yield, depending on the nature of the compound. At the recommended application rate, with the exception of IAA and treflan, all compounds suppressed mold growth and aflatoxin production.

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