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 10⁶ 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 10⁶ 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
PLANT CHEMICALS AFFECT ASPERGILLI

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

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

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

<table>
<thead>
<tr>
<th>Plant hormone</th>
<th>Concentration (ppm)</th>
<th>Mycelial dry weight (g/100 ml)</th>
<th>Aflatoxin production (ppm)</th>
<th>B₁</th>
<th>B₂</th>
<th>G₁</th>
<th>G₂</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indol-3-acetic acid</td>
<td>10</td>
<td>2.58 (a)</td>
<td>100 (a)</td>
<td>3</td>
<td>138 (a)</td>
<td>4</td>
<td>245</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.56 (a)</td>
<td>150 (b)</td>
<td>4</td>
<td>173 (b)</td>
<td>4</td>
<td>331</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2.42 (b)</td>
<td>125 (b)</td>
<td>3</td>
<td>156 (b)</td>
<td>4</td>
<td>288</td>
<td></td>
</tr>
<tr>
<td>Gibberellic acid</td>
<td>10</td>
<td>2.26 (b)</td>
<td>125 (b)</td>
<td>3</td>
<td>143 (b)</td>
<td>4</td>
<td>275</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.66 (b)</td>
<td>125 (b)</td>
<td>4</td>
<td>156 (b)</td>
<td>4</td>
<td>289</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2.40 (a)</td>
<td>100 (a)</td>
<td>3</td>
<td>138 (a)</td>
<td>4</td>
<td>245</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.28 (b)</td>
<td>100 (a)</td>
<td>3</td>
<td>138 (a)</td>
<td>4</td>
<td>245</td>
<td></td>
</tr>
</tbody>
</table>

'Numbers in the column followed by the same letter are not significantly different at p = 0.01.

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 B₁ and G₁ production (6).

RESULTS AND DISCUSSION

Effect of plant hormones
It is well established that gibberellic acid (GA₃) 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, GA₃ 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 GA₃ 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.

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (ppm)</th>
<th>Mycelial dry weight (g/100 ml)</th>
<th>Aflatoxin production (ppm)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B₁</td>
<td>B₂</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>3.74 (a)¹</td>
<td>97 (a)</td>
<td>4</td>
</tr>
<tr>
<td>Gramoxone</td>
<td>5</td>
<td>3.24 (b)</td>
<td>113 (b)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.96 (b)</td>
<td>97 (a)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.12 (b)</td>
<td>73 (b)</td>
<td>3</td>
</tr>
<tr>
<td>Stomp</td>
<td>5</td>
<td>3.72 (a)</td>
<td>97 (a)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.98 (b)</td>
<td>73 (b)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.80 (b)</td>
<td>49 (b)</td>
<td>3</td>
</tr>
<tr>
<td>Treflan</td>
<td>5</td>
<td>4.10 (b)</td>
<td>145 (b)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4.20 (b)</td>
<td>145 (b)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.92 (b)</td>
<td>129 (b)</td>
<td>4</td>
</tr>
<tr>
<td>Insecticides</td>
<td></td>
<td></td>
<td>102 (a)</td>
<td>3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>3.44 (a)</td>
<td>82 (b)</td>
<td>3</td>
</tr>
<tr>
<td>Malathion</td>
<td>5</td>
<td>3.06 (b)</td>
<td>82 (b)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.96 (b)</td>
<td>82 (b)</td>
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<tr>
<td></td>
<td>20</td>
<td>3.14 (b)</td>
<td>113 (b)</td>
<td>3</td>
</tr>
<tr>
<td>Actelic</td>
<td>5</td>
<td>3.04 (b)</td>
<td>82 (b)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.52 (b)</td>
<td>72 (b)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.98 (b)</td>
<td>72 (b)</td>
<td>2</td>
</tr>
<tr>
<td>Guthion</td>
<td>5</td>
<td>2.68 (b)</td>
<td>82 (b)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.44 (b)</td>
<td>52 (b)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.98 (b)</td>
<td>21 (b)</td>
<td>ND²</td>
</tr>
</tbody>
</table>

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

IAA to the fungal medium increased the aflatoxin production more than GA₃. One would expect this result since IAA but not GA₃ 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, B₁, B₂, G₁, and G₂, were found in all treatments. G₁ and B₁ were the most abundant toxins while G₂ and B₂ 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...
A. parasiticus
ings. This suggests that the inhibitory effect could be
presence of the aromatic nucleus. Malathion contains no
(3).
The inhibitory effect of these insecti­
centration
ence is mainly in the functional groups attached to the
insecticides on Gj and B, production was more pronounced
increased with the increase in insecticide concentration.
In general, the inhibitory effect of these insecticides has a thiophosphate ester group. The differ­
ion at different concentrations markedly reduced mold
growth and aflatoxin yield, the reduction being propor­
tional 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 signi­fically inhibits production of aflatoxins at the 100 ppm
concentration (3). The inhibitory effect of these insec­tics on Gj and B, production was more pronounced
in the presence of 20 ppm, and the Bj:Gj ratio generally
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 con­
trol 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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Prot. 41:375.

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A. parasiticus
ings. This suggests that the inhibitory effect could be
presence of the aromatic nucleus. Malathion contains no
(3).
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 con­
trol pests and/or weeds or to increase plant growth and yield, depending on the nature of the compound. At the
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