USE OF AMMONIA GAS IN THE CONTROL OF
ASPERGILLUS FLAVUS INFECTION AND AFLATOXIN
PRODUCTION IN SUNFLOWER SEED

SHAHNAZ DAWAR AND A. GHAFFAR

Department of Botany,
University of Karachi, Karachi-75270, Pakistan.

Abstract

Sunflower seeds inoculated with Aspergillus flavus reduced seed germination with greater production of aflatoxin during storage. Use of ammonia gas although reduced seed germination but infection of A. flavus decreased with consequent reduction in aflatoxin production. Use of ammonia gas for 15 minutes was found optimum for significant suppression of the production of aflatoxin B₁ and 5 minutes for aflatoxin B₂.

Introduction

Sunflower (Helianthus annuus L.), an important oil seed crop is cultivated over 25, 899 hectares in Pakistan (Anon., 1990). The seeds contain 32-45% oil which is a rich source of polyunsaturated fatty acid used for human consumption. Sunflower seed cake is used in poultry feed. Sunflower seeds are also consumed roasted and a coffee substitute is prepared from roasted seed (Sastri, 1959; Ambasta et al., 1986). Of the mold fungi associated with sunflower seed, Aspergillus flavus was found to be predominant (Dawar & Ghaffar, 1991b), where 54% samples of sunflower seeds were contaminated with aflatoxin B₁ (Dawar & Ghaffar, 1991a). The fungus is known to produce mycotoxins where aflatoxins B₁ and B₂ are carcinogenic, damage liver, reduce growth rate and milk production in animals and man (Goldblatt, 1969). Ammonification is reported to be an effective method for detoxifying animal feed stuff (Cole, 1989). Experiments were therefore carried out on the use of ammonia gas for prevention and control of A. flavus infection and subsequent aflatoxin production in sunflower seed during storage.

Materials and Methods

Moisture content of seed was determined by oven dry method. Moisture content of the seed was adjusted at 15% by adding the required amount of sterilized distilled water to the seed. The seeds were mixed thoroughly and kept in a refrigerator at about 4-5°C for 24 h with frequent mixing to facilitate uniform distribution of moisture throughout the seed (Lutey & Christensen, 1963). Aflatoxin producing strain of A. flavus culture (KUMH 38) isolated for sunflower seed was used. Fifty g of seed sample with 15% moisture content were inoculated with 2 ml suspension of A. flavus @ 8.5x10⁷ conidia/ml. Inoculated seeds were placed in a 30 cm diameter 625 mm sieve lined by a tissue paper. The sieve was put on a wooden stand at the bottom of which a 20 cm diam., Petri plate containing 25 ml of Ammonium hydroxide was placed which was covered with a bell jar to produce a minichamber for the treatment of seeds with
ammonia gas. Seeds treated with ammonia gas for 10, 20, 30 and 40 minutes were placed in plastic bags. A cotton plug was placed at the mouth of each plastic bag and the mouth was tied up with a rubber band. The bags were stored at 30°C in an incubator. Three replicates of each treatment were used for the detection of *A. flavus* infection and aflatoxin production after 0, 15, 30 and 60 days interval. Infection of seed by *A. flavus* was assessed by using Blotter method (Anon., 1976), whereas aflatoxin production was estimated by AOAC method (Anon, 1975).

Fig. 1. Effect of ammonia gas treatment on germination of sunflower seed and seed-borne infection by *Aspergillus flavus* during storage.
A = Control. B = Inoculated with *A. flavus*. C = Inoculated with *A. flavus* + NH₃ gas treatment for 10 min. D = Inoculated with *A. flavus* + NH₃ gas treatment for 20 min. E = Inoculated with *A. flavus* + NH₃ gas treatment for 30 min. F = Inoculated with *A. flavus* + NH₃ gas treatment for 40 min.
In another experiment sunflower seeds were ground in a grinder and inoculated with a toxigogenic strain of A. flavus @ 6x10⁷ conidia/ml. Uninoculated seeds served as control. The seeds were treated with ammonia gas for 5, 10, 15, 20 and 30 minutes and after 10 days of incubation at 30°C production of aflatoxin was detected by the method described above. Data were subjected to Factorial Analysis of variance (FANOVA) following Gomez & Gomez (1984).

Results and Discussion

Inoculation of seeds with A. flavus showed a gradual decline in seed germination as compared to control (p<0.001). Treatment of seeds with ammonia gas significantly reduced seed germination (p<0.001) and none of the seeds showed germination when exposed to ammonia gas for 40 minutes (Fig.1). A change in seed colour from black to green was also observed.

Seed infection by A. flavus was greater in inoculated seeds than in uninoculated control. Seeds inoculated with A. flavus when exposed to ammonia gas showed a significant reduction in infection by A. flavus with gradual increase in treatment time (p<0.001). However, none of the treatments showed complete elimination of A. flavus infection after 15, 30 & 60 days of storage (Fig.1). Pongsawat & Chinanwate (1991) reported that A. flavus and A. parasiticus used @ 10 spores/g was completely inhibited by ammonium benzoate in the control of aflatoxin production on peanut and maize. In the present study, a higher number of spores/g of seed used for inoculation could be the reason for the failure of ammonia gas in complete elimination of A. flavus infection. It is interesting to note that ammonia gas released from the decomposing oats and barley tissues in soil was also found lethal to soilborne fungi (Lewis, 1976). It might also be possible that the spores of A. flavus used for inoculation were killed by ammonia gas treatment and A. flavus infection observed in the inoculated seeds treated with ammonia gas was internally seed borne since non-inoculated seeds also showed infection by A. flavus.

Production of aflatoxin B₁ in uninoculated control was zero at 0-days and in traces at subsequent intervals. Seeds inoculated with toxigogenic strain showed higher production of aflatoxin B₁ at different interval of storage. No aflatoxin production was observed at 0-days in seed treated with ammonia gas. Amount of aflatoxin B₁ and B₂ significantly decreased with the increase in time (p<0.001). Production of aflatoxin B₁ was observed in ammonia gas treated seeds also after 60 days period which approached to the tolerance limit of 20 μg/kg (Anon, 1977) whereas production of aflatoxin B₂ was either zero or in traces (Table 1). It may be mentioned that Ammonium hydroxide showed 60% reduction in aflatoxin production in peanut and maize (Napaporn et al., 1991).

In another experiment, sunflower seeds substrate was inoculated with spore suspension of A. flavus and then treated with ammonia gas for 5, 10, 15, 20 and 30 minutes. A 5 minutes treatment was not effective whereas treatment for 10 minutes reduced the production of aflatoxin B₁ very close to the tolerance limit of 20 μg/Kg whereas treatment for 15 minutes or more completely prevented the production of aflatoxin B₁. Use of ammonia gas treatment for 5 minutes or more significantly suppressed the production
Table 1. Effect of ammonia gas treatment on production of aflatoxins in sunflower seeds during storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days</th>
<th>Aflatoxin B₁ (µg/kg)</th>
<th>Aflatoxin B₂ (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>Traces</td>
<td>Traces</td>
</tr>
<tr>
<td>Inoculated with toxigenic strain of <em>A. flavus</em> and treated with ammonia gas for 0 minute</td>
<td>73</td>
<td>20</td>
<td>54</td>
</tr>
<tr>
<td>10 minute</td>
<td>0</td>
<td>Traces</td>
<td>36</td>
</tr>
<tr>
<td>20 minute</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>30 minute</td>
<td>0</td>
<td>Traces</td>
<td>4</td>
</tr>
<tr>
<td>40 minute</td>
<td>0</td>
<td>0</td>
<td>Traces</td>
</tr>
</tbody>
</table>

Table 2. Detoxification of aflatoxins by ammonia gas treatment on sunflower seed substrate.

<table>
<thead>
<tr>
<th></th>
<th>Aflatoxin B₁ (µg/kg)</th>
<th>Aflatoxin B₂ (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (uninoculated)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inoculated with <em>A. flavus</em> and treated with ammonia gas for 0 minutes</td>
<td>80</td>
<td>36</td>
</tr>
<tr>
<td>5 minutes</td>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>10 minutes</td>
<td>22</td>
<td>Traces</td>
</tr>
<tr>
<td>15 minutes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20 minutes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30 minutes</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
USE OF AMMONIA IN THE CONTROL OF ASPERGILLUS FLAVUS IN SUNFLOWER SEED

References


(Received for publication 21 December 1997)