Mycotoxins and Non-fungicidal Control of Corn Grain Rotting Fungi

Mohamed A. Yassin, Mohamed A. Moslem and Abd El-Rahim M.A. El-Samawaty

Department of Botany and Microbiology, Faculty of Science, King Saud University, Riyadh, Saudi Arabia
Agricultural Research Center, Plant Pathology Research Institute, Giza, Egypt

Corresponding Author: Mohamed A. Yassin, Department of Botany and Microbiology, Faculty of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

ABSTRACT
To avoid fungicide risks, efficacy of Allium sativum was evaluated against corn grain rotting fungi. Mycotoxigenicity of tested fungi was also investigated using HPLC. All Aspergillus species were toxigenic except corn and popcorn isolates of Aspergillus clavatus as well as corn isolate of Aspergillus terreus. Highest Aflatoxin was produced by corn Aspergillus flavus and popcorn Aspergillus flavus var. columnaris. Meanwhile, three of eight Fusarium isolates were toxigenic and varied in the kind and amount of toxin produced. Although some Fusaria failed to produce any toxins, Popcorn isolate No. 8 of Fusarium subglutinans produced fumonisins, vomitoxin and zearalenone. Corn isolate of Penicillium funiculosum was produced more Patulin and less citreoviridin than corn isolate of P. oxalicum. Garlic juice was effectively inhibited the fungal growth at all concentrations used. All tested isolates were responded to garlic juice regardless of the concentration used. The most sensitive fungi to all garlic juice concentrations used were corn isolates of A. flavus and Penicillium oxalicum. They exhibited significant inhibitory effects of about 63.70 and 75.56%, respectively, at 1.25% concentration. Popcorn isolate of F. subglutinans was the most sensitive Fusaria to all concentrations used with maximum inhibition of about 81%. Efficacy of garlic juice against mycotoxin producing fungi suggests its possible use in minimizing the risk of mycotoxins as well as fungicides exposure.

Key words: HPLC, mycotoxins, seed-borne fungi, Zea mays L.

INTRODUCTION
Grain rotting Aspergillus, Fusarium and Penicillium fungi are generally invading corn (Zea mays L.) at pre-harvest period (Bigirwa et al., 2007; Alakonya et al., 2008). Field infection by these fungi may be continued and worsen throughout the post-harvest handling, marketing and storage periods (Bigirwa et al., 2006; Jimoh and Kolapo, 2008). Other than yield losses, they are able to reduce grain quality polluting food and feedstuffs with hepatocarcinogens mycotoxins that harm animals and humans (Hussein and Brasel, 2001; Mokhles et al., 2007; Iheshiulor et al., 2011).

Chemical control of phytopathogens preventing rot development results in environmental pollution, health hazard and affects the natural ecological balance. Thus, early detection of grain rotting fungi and/or nonchemical fungicides application would result in more efficient control and may lead to improved storage (Onyeagba et al., 2004; Yassin et al., 2011).

Among several alternative strategies used to control phytopathogenic fungi and reduce mycotoxins in agricultural commodities; great success has been achieved using plant-derived products (Centeno et al., 2010; Reddy et al., 2010). Antifungal activity of many plant-derived products against wide range of phytopathogens had frequently been documented (Hasan et al.,
2005; Hadizadeh et al., 2009; Ikeura et al., 2011). Moreover, efficacy of some herbaceous and medicinal plants against corn seed-borne mycoflora was proved (El-Samawaty et al., 2011; Kiran et al., 2010).

Among many plant substances generally used against seed-borne mycoflora, garlic is very promising and safer, particularly for food preservation (Haciseferogullari et al., 2005; Aql et al., 2010). Garlic has broad antimicrobial properties (Obagwu and Korsten, 2003; Irkin and Korukluoglu, 2007) and its activity against Aspergillus and Penicillium fungi and mycotoxin production had frequently been documented (Pereira et al., 2006; Ismaiel, 2008; Salim, 2011). The present study aimed to investigate antifungal activity of garlic juice against toxigenic fungi causing corn grain rot disease.

**MATERIALS AND METHODS**

This study was initiated in 2011 to investigate the efficacy of garlic juice against mycotoxin producing fungi isolated from corn grains.

**Fungi:** Twenty two isolates belonging to twelve species representing three fungal genera were used. Grain rotting Aspergillus, Fusarium and Penicillium fungal isolates were mainly recovered from commoditized corn and popcorn samples collected from different locations (markets) in Riyadh City of Saudi Arabia.

**Garlic juice:** Garlic juice was obtained by blending and homogenizing fresh garlic bulbs in enough quantity of distilled water (1 mL water/1 g garlic bulb v/w), for 5 min using a blender (Braun CombiMax 700 Vital, Germany). Obtained juice was then filtered through one layer sheath clothes and used immediately or stored at 4°C until used (Ismaiel, 2008).

**Aflatoxins:** SMKY liquid medium (Diener and Davis, 1966) was used to examine aflatoxin productivity of Aspergillus. Aflatoxins were extracted from homogenized culture filtrates using methanol solution (80:20 methanol/isolate filtrates). Solvents were then evaporated under vacuum, dried residues containing aflatoxin were dissolved in 1 mL of methanol: acetic acid: water (1:1:3 v/v) solution and stored in dark vials. Aflatoxin analysis was performed on HPLC (Stroka et al., 2000).

**Penicillium toxins:** Patulin and citreoviridin productivity were studied using HPLC. Penicillium isolates were aseptically cultured onto malt extract broth and incubated at 27±2°C for 7-10 days, after which mycotoxins were extracted using acetonitrile: water solution (6:95 v/v). Solvent was then evaporated under vacuum. Dried residues were dissolved in 1 mL of the same solution and then filtered through a 0.45 μm micro-filter prior to subject to HPLC analyses (O'Brien et al., 2006).

**Fusarium toxins:** Isolates were grown on sterilized SMKY liquid medium for 10 days at 27±2°C. Fungal culture of each treatment was blended with 5 g sodium chloride and 100 mL of methanol: water (80:20) solution at a high speed for one min, then filtered through glass micro-fiber filter. Ten milliliter of the filtrate was diluted with 40 mL of wash buffer and filtered again through 1 μm micro-fiber filter. Fumonisin, zearalenone and vomitoxin concentration were performed on HPLC (Mazzani et al., 2001).

**Antifungal activity of garlic juice:** Crude solution of garlic juice were added to conical flasks containing 100 mL of sterilized PDA medium just before solidification to obtain concentrations of
1.25, 2.5, 5.0 and 10.0%. The supplemented media was immediately poured into 9 cm Petri plates. Five mm diameter plugs cutting from the margin of 7 days old fungal colonies were placed in the center of such plates (Benkeblia, 2004). Three replicate plates were used for each treatment and untreated plates were served as control. Cultures were incubated at 27±2°C and radial growth measured daily for 7-10 days. Obtained data were statistically analyzed.

Statistical analysis: Analysis of variance (ANOVA) was performed with the MSTAT-C statistical package, Michigan State Univ., USA. Least Significant Difference (LSD) was used to compare means.

RESULTS
Mycotoxin productivity: Aflatoxin assay revealed that most of Aspergillus were toxigenic and varied in the kind and amount of aflatoxins produced. Corn and popcorn isolates of A. clavatus as well as corn isolate of A. terreus were non toxigenic. B1 aflatoxin was generally the most dominant toxin. A. flavus isolate No. 2 and Aspergillus flavus var. columnaris isolate No. 8 were the highest producers of aflatoxin (Table 1).

Three of eight Fusarium isolates were toxigenic with varied production ability. While some isolates of Fusarium proliferatum and Fusarium verticillioides failed to produce any toxins, corn isolate of F. proliferatum (No. 3) produced both vomitoxins and zearalenone. On the other hand corn isolate of F. verticillioides (No. 4) produced only zearalenone. Popcorn isolate of F. subglutinans (No. 8) produced fumonisin, vomitoxin and zearalenone. However, F. subglutinans isolate No. 8 was the highest producer of fumonisin, F. proliferatum isolate No. 3 was the highest producer of vomitoxins and zearalenone (Table 2).

Corn isolate of P. funiculosum was produced more Patulin and less citreoviridin than corn isolate of P. oxalicum. Popcorn isolate of Penicillium chermesinum failed to produce any detectable amount of mycotoxins (Table 3). Moreover, while P. funiculosum isolate was the highest producer of patulin, P. oxalicum isolate was the highest producer of citreoviridin.

Antifungal activity of garlic juice: Statistical analysis indicated that garlic concentrations, fungal isolates and their interaction were highly significant sources of variation. Concentration was

| Table 1: Aflatoxin productivity of corn and popcorn seed borne Aspergillus sp. |
|------------------|------|---|---|---|---|
| Aflotoxin (ppb)  |      |   |   |   |   |
| Isolates         | Host | B1 | B2 | G1 | G2 |
| A. clavatus      | Corn | -  | -  | -  | -  |
| A. flavus        | Corn | 8.0| 2.0| 4.0| 3.0|
| A. flavus        | Corn | 2.0| 1.0| 0.0| 0.0|
| A. niger         | Corn | 3.0| -  | 2.0| 2.0|
| A. terreus       | Corn | -  | -  | -  | -  |
| A. clavatus      | Popcorn | - | - | - | - |
| A. clavatus      | Popcorn | - | - | - | - |
| A. f. var columnaris | Popcorn | 8.0 | 6.0 | 3.0 | 6.0 |
| A. flavus        | Popcorn | 6.0 | 2.0 | 1.0 | 3.0 |
| A. funigatus     | Popcorn | 2.0 | 3.0 | 1.0 | 2.0 |
| A. niger         | Popcorn | 5.0 | 2.0 | 5.0 | -  |
Table 2: Mycotoxin productivity of corn and popcorn seed borne *Fusarium* sp.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Host</th>
<th>Fumonisin</th>
<th>Zearalenone</th>
<th>Vomitoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. proliferatum</em></td>
<td>Corn</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>F. proliferatum</em></td>
<td>Corn</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>F. verticillioides</em></td>
<td>Corn</td>
<td>-</td>
<td>5.4</td>
<td>7.4</td>
</tr>
<tr>
<td><em>F. verticillioides</em></td>
<td>Corn</td>
<td>-</td>
<td>3.3</td>
<td>-</td>
</tr>
<tr>
<td><em>F. subglutinans</em></td>
<td>Popcorn</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>F. subglutinans</em></td>
<td>Popcorn</td>
<td>-</td>
<td>9.7</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Table 3: Mycotoxin productivity of corn and popcorn seed borne *Penicillium* sp.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Host</th>
<th>Patulin</th>
<th>Citreoviridin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. funiculosum</em></td>
<td>Corn</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td><em>P. oxalicum</em></td>
<td>Corn</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td><em>P. chermesinum</em></td>
<td>Popcorn</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 1: Relative contribution of concentration (C), isolates (I) and their interaction (I×C) to linear growth of corn and popcorn seed borne fungi.

the most important as source of variation followed by isolates but the (I×C) interaction was the least in importance (Fig. 1).

Garlic juice was effectively inhibited the fungal growth at all concentrations and its activity was increased as concentration increased. Moreover, all tested isolates were responded to garlic juice regardless the concentration used. Corn isolates of *A. flavus* (Fig. 2) and *P. oxalicum* (Fig. 5) were the most sensitive isolates to all tested concentration of garlic juice. They exhibited significant inhibitory effects of 63.70 and 75.56%, respectively, at 1.25% concentration. Meanwhile, popcorn isolate of *A. fumigatus* was the most sensitive isolate to all concentrations by significant inhibition
Fig. 2: Antifungal activity of garlic juice against corn seed borne *Aspergillus* species

Fig. 3: Antifungal activity of garlic juice against popcorn seed borne *Aspergillus* species

Fig. 4: Antifungal activity of garlic juice against corn and popcorn seed borne *Fusarium* species of 62.22-88.89% (Fig. 3). On the other hand, popcorn isolate of *F. subglutinans* was the most sensitive Fusaria to all concentrations with maximum inhibition of about 80.37% (Fig. 4).
Fig. 5: Antifungal activity of garlic juice against corn and popcorn seed borne Penicillium species

DISCUSSION

Mycotoxigenicity of corn grain rotting fungi; Aspergillus, Fusarium and Penicillium have frequently been documented (Kumar et al., 2008; Trung et al., 2008; Amadi and Adeniyi, 2009). Aflatoxins; the most widespread toxins frequently produced by Aspergillus sp. isolated from corn grains (Muthoni et al., 2009; Youssef, 2009; Yassin et al., 2011). Fumonisins, vomitoxin and zearalenone were also produced by corn grain rotting Fusaria (Yazar and Omurtag, 2008; Makun, 2007). Production of patulin and citreoviridin by corn grain isolates of P. funiculosum and P. oxalicum were also demonstrated (Dombrink-Kurtzman and Blackburn, 2005; El-Samawaty et al., 2011). It was proved that phytopathogenic fungi may produce these toxins in culture media and in agricultural commodities as well as in food waste (Abramson et al., 2009).

In vitro screening of garlic juice activity against corn grain rotting fungi revealed that it was effectively inhibited mycelial growth of all tested fungi. This result was agreed with the finding of Afzal et al. (2010), who stated that A. sativum has a wide antifungal spectrum, reached about 60-82% inhibition in the growth of seed borne Aspergillus and Penicillium fungi. Efficacy of garlic in controlling toxigenic fungi had also proved by Salim (2011) who concluded that aqueous extracts of garlic could be used as a good natural food preservative against mycotoxigenic fungi. Many literatures worldwide also indicated that garlic had potential antifungal properties (Irkin and Korukluoglu, 2007; Rashid et al., 2010; Tagoe et al., 2011). Such antimycotic activity might be attributed to phytochemical properties of garlic plant (Obagwu and Korsten, 2003; Ogita et al., 2009). Garlic allicin however, decomposes into several effective antimicrobial compounds such as diallyl sulphide, diallyl disulphide, diallyl trisulphide, allyl methyl trisulphide, dithiins and ajoene (Harris et al., 2001; Jabar and Al-Mossawi, 2007).

It is concluded that efficacy of garlic juice against mycotoxin producing fungi suggests its possible use in minimizing the risk of mycotoxins as well as fungicides exposure. Meanwhile, water base usage of garlic juice provides an alternative of chemical solvents.

ACKNOWLEDGMENTS

This study was supported by the research Center, College of Science, King Saud University.

REFERENCES


