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Mycotoxins and Non-fungicidal Control of Corn Grain Rotting Fungi

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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 fumonisin, 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; Aqil *et al.*, 2010). Garlic has abroad 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 (5:95 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.

Isolates	Host	Aflatoxin (ppb)			
		B1	B2	G1	G2
<i>A. clavatus</i>	Corn	-	-	-	-
<i>A. flavus</i>	Corn	8.0	2.0	4.0	3.0
<i>A. flavus</i>	Corn	2.0	1.0	0.0	0.0
<i>A. niger</i>	Corn	3.0	-	2.0	2.0
<i>A. terreus</i>	Corn	-	-	-	-
<i>A. clavatus</i>	Popcorn	-	-	-	-
<i>A. clavatus</i>	Popcorn	-	-	-	-
<i>A. f. var columnaris</i>	Popcorn	8.0	6.0	3.0	6.0
<i>A. flavus</i>	Popcorn	6.0	2.0	1.0	3.0
<i>A. fumigatus</i>	Popcorn	2.0	3.0	1.0	2.0
<i>A. niger</i>	Popcorn	5.0	2.0	5.0	-

Table 2: Mycotoxin productivity of corn and popcorn seed borne *Fusarium* sp.

Isolates	Mycotoxin (ppb)			
	Host	Fumonisin	Zearalenone	Vomitoxin
<i>F. proliferatum</i>	Corn	-	-	-
<i>F. proliferatum</i>	Corn	-	-	-
<i>F. proliferatum</i>	Corn	-	5.4	7.4
<i>F. verticillioides</i>	Corn	-	3.3	-
<i>F. verticillioides</i>	Corn	-	-	-
<i>F. verticillioides</i>	Corn	-	-	-
<i>F. subglutinans</i>	Popcorn	-	-	-
<i>F. subglutinans</i>	Popcorn	9.7	1.4	6.3

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

Isolates	Mycotoxin (ppb)		
	Host	Patulin	Citreoviridin
<i>P. funiculosum</i>	Corn	31	20
<i>P. oxalicum</i>	Corn	21	25
<i>P. chermesinum</i>	Popcorn	-	-

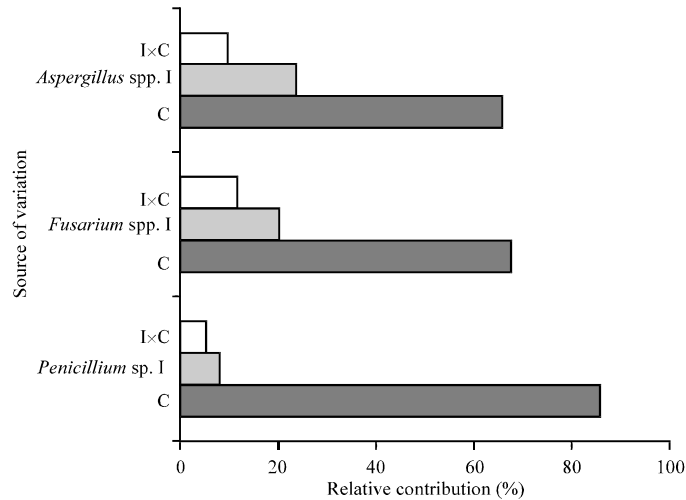


Fig. 1: Relative contribution of concentration (C), Isolates (I) and their interaction (I×C) to linear growth of corn and popcorn seed borne fungi

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

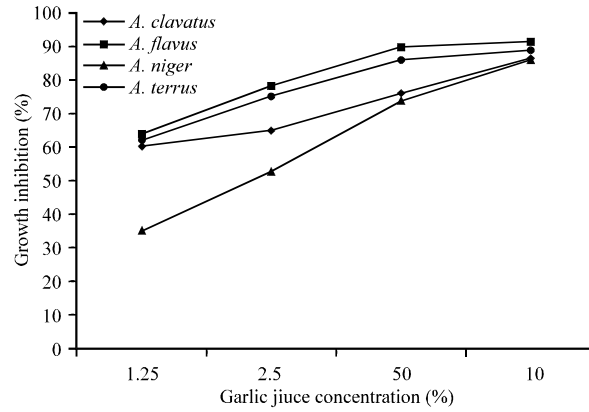


Fig. 2: Antifungal activity of garlic juice against corn seed borne *Aspergillus* species

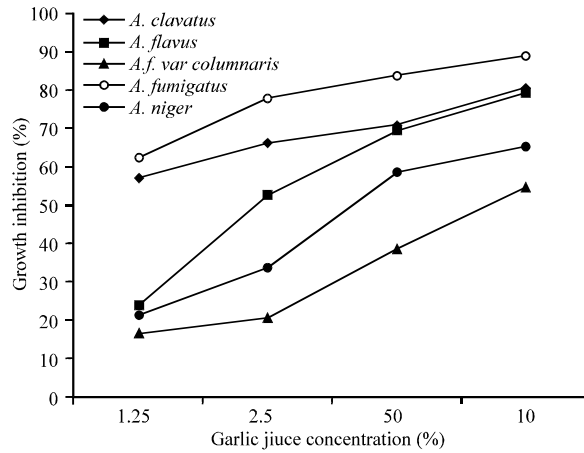


Fig. 3: Antifungal activity of garlic juice against popcorn seed borne *Aspergillus* species

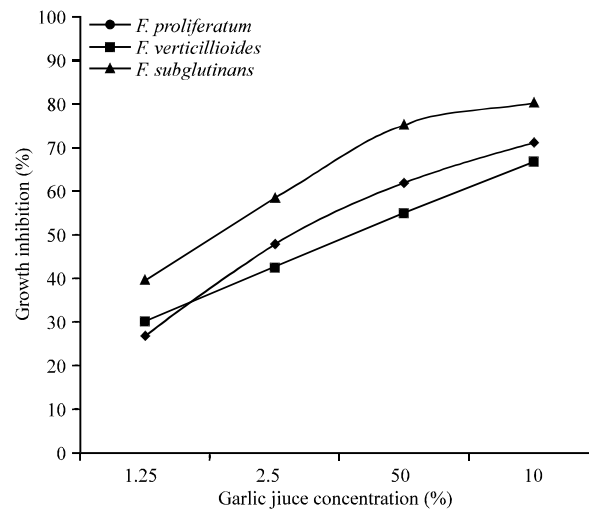


Fig. 4: Antifungal activity of garlic juice against corn and popcorn seed borne *Fusarium* species

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).

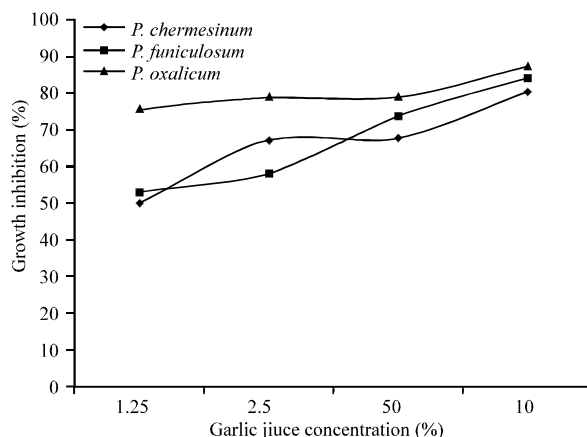


Fig. 5: Antifungal activity of garlic juice against corn and popcorn seed borne *Penicillium* species

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 (Muthomi *et al.*, 2009; Youssef, 2009; Yassin *et al.*, 2011). Fumonisin, 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.

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