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Effectiveness of *Allium sativum* in Controlling Sorghum Grain Molding Fungi

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Toxin producing fungi are potentially affecting human and animal health and should be eradicated. Toxigenicity of thirteen sorghum gain molding fungi belonging to *Aspergillus, Fusarium* and *Penicillium* species was evaluated in this study using HPLC. Potential of garlic (*Allium sativum)* juice for controlling these fungi was also examined *in vitro*. Obtained results indicated that most of evaluated fungi were toxigenic. *Aspergillus flavus var. columnaris* was the highest producer of aflatoxin-G (2 to 3ppb) and *Aspergillus terreus* was the highest aflatoxin-B (2 to 4ppb) producer. Meanwhile, *Fusarium verticillioides* was the highest producer of fumonisin (19.10 ppb) and zearalenone (21.40 ppb) but *Fusarium nygamai* was the highest producer of vomitoxin (31.30 ppb). On the other hand while *Penicillium chrysogenum* was the highest producer of patulin (38 ppb), *P. oxalicum* produces the highest amount of Citreoviridin (37 ppb). Sorghum grain molding fungi was successfully suppressed *in vitro* by garlic juice. *Aspergillus terreus* was the most sensitive fungus to garlic juice (85.56% inhibition) followed by *Fusarium thapsinum* (77.41% inhibition) and *Penicillium funiculosum* (75.19% inhibition). *Penicillium* isolates were generally the most sensitive to lowest (1.25%) concentration of garlic juice. They exhibited 52.97 and 52.59% inhibition in the growth of *Penicillium chrysogenum* and *Penicillium oxalicum* respectively.

Key words: Garlic, HPLC, Mycotoxins, Seed-borne fungi, Sorghum.

Grain sorghum (*Sorghum bicolor* L.) is one of the main food and feed crops with total world production reached about 63 million ton[1]. In addition; many sorghum grain based manufactures are depending on such grains[2]. Sorghum grain rotting diseases or grain molds are widespread and devastating diseases that quantitatively and qualitatively affect sorghum yield[3,4]. These fungi cause seed deterioration, reduce seed germination capacity, seedling diseases and may result in systemic plant diseases[5]. *Aspergillus, Fusarium* and *Penicillium* have been frequently involved in sorghum grain mold[6]. In addition, such fungi may also produce *in vitro* and/or in *vivo* harmful mycotoxins affect both human and animal health[7,8].

Although synthetic fungicides are effective against many phytopathogenic fungi, their potential effects on human and animal health as well as on the environment should be concern. Nonchemical fungicides however, will be safer and ecofriendly antifungal treatments[9,10].

Several nonchemical strategies including plant-derived products were used to control mycotoxigenic fungi and reduce mycotoxins in agricultural commodities[11, 12]. Many herbaceous and medicinal plants were successfully used against sorghum seed borne mycoflora worldwide[13,14]. Among promising and safer plant

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substances frequently used against seed-borne mycoflora; garlic is very pronounced. It has an abroad antimicrobial properties[15, 16] and efficient against *Aspergillus* and *Penicillium* fungi as well as mycotoxin production[17-19].

The present study aimed to investigate antifungal activity of garlic juice against toxigenic sorghum grain rotting fungi.

MATERIALS AND METHODS

Grain molding isolates

Thirteen isolates belonging to twelve species representing three fungal genera *i.e. Aspergillus, Fusarium* and *Penicillium* were used. Tested grain molding fungi were mainly recovered from sorghum samples collected from different locations (markets) in Riyadh, Saudi Arabia.

Assessment of aflatoxin

Aflatoxin production was examined for *Aspergillii* grown on SMKY liquid medium[20]. Analysis of aflatoxin was performed on HPLC[21]. 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 solution (1:1:3 v/v) and stored in dark vials or subjected immediately to HPLC analysis.

Assessment of *Fusarium* toxins

Fumonisin, zearalenone and vomitoxin production of *Fusarium* isolates were analyzed using HPLC (22). 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 ml of the filtrate was diluted with 40 ml of wash buffer and filtered again through 1 µm micro-fiber filter prior to HPLC analyses.

Assessment of *Penicillium* toxins

Citreoviridin and patulin production were evaluated by HPLC for *Penicillium* isolates, grown on malt extract broth. Cultured isolates were incubated at 27±2°C for 7–10 days, after which mycotoxins were extracted using acetonitrile: water solution (5:95v: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 subjecting to HPLC analyses[23].

Garlic juice preparation

Fresh garlic bulbs were blended and homogenized for 5 minutes in enough quantity of distilled water (1mL water/1gm garlic bulb v/w), by the aid of electric blender. Obtained juice was then filtered through one layer sheath clothes and used immediately or stored at 4°C until used[17].

Evaluation of garlic antifungal activity

Four concentrations *i.e:* 1.25, 2.5, 5.0 and 10.0% of garlic juice were examined. Crude solution of garlic juice was added to sterilized, PDA just before solidification to obtain required concentrations. 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[17]. 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

Assessment of mycotoxins

Results of the present study revealed that sorghum grain molding *Aspergillus flavus var. columnaris* and *Aspergillus terreus* were aflatoxigenic. While, *Aspergillus flavus var. columnaris* was the highest producer (2-3 ppb) of aflatoxin G, *Aspergillus terreus* was the highest aflatoxin-B producer (2-4 ppb). Meanwhile, *Aspergillus niger* failed to produce any detectable aflatoxin (Table 1).

All tested fusaria except one were toxigenic with variable productivities. *Fusarium verticillioides* was the highest producer of fumonisin (19.10 ppb) and zearalenone (21.40 ppb). On the other hand, *Fusarium nygamai* was the highest producer of vomitoxin (31.30 ppb) although it failed to produce zearalenone (Table 2).

In respect of *Penicillium* mycotoxins; *P. chrysogenum* was the highest producer of patulin

Table 1. Aflatoxin productivity of sorghum grain molding *Aspergillus* species

S. No	Isolates	Mycotoxin (ppb)			
		B1	B2	G1	G2
1.	*A. flavus var columnaris*	2.00	1.00	2.00	3.00
2.	*Aspergillus niger*	-	-	-	-
3.	*Aspergillus terreus*	4.00	2.00	1.00	2.00

Table 2. Mycotoxin productivity of sorghum grain molding *Fusarium* species

S. No	Isolates	Mycotoxin (ppb)		
		Fumonisin	Vomitoxin	Zearalenone
1.	*Fusarium verticillioides*	3.50	-	-
2.	*Fusarium verticillioides*	19.10	12.10	21.40
3.	*Fusarium semitectum*	4.60	23.40	17.90
4.	*Fusarium nygamai*	11.70	31.30	-
5.	*Fusarium thapsinum*	-	-	-
6.	*Fusarium sp.*	2.10	14.10	12.30

Table 3. Mycotoxin productivity of sorghum grain molding *Penicillium* species

S. No	Isolates	Mycotoxin (ppb)	
		Citreoviridin	Patulin
1.	*P. chrysogenum*	-	38
2.	*P. funiculosum*	-	12
3.	*P. oxalicum*	37	25
4.	*P. griseofulvum*	10	-

(38 ppb) and *P. oxalicum* was produced the highest amount of citreoviridin (37 ppb). Meanwhile, neither *P. chrysogenum* nor *P. funiculosum* could produce any citreoviridin as well as no detectable patulin could produce by *P. griseofulvum* (Table 3).

Garlic antifungal activity

Figure (1) indicated that garlic concentrations, fungal isolates and their interaction were highly significant sources of variation in fungal linear growths. Garlic concentration was the most important as source of variation in linear growth of the tested fungi.

Sorghum grain molding fungi was significantly inhibited by all tested concentration of garlic juice. Garlic antifungal activity was generally increased as concentration increased. Isolates responses to garlic juice were existed regardless of the concentration used. The most inhibited *Aspergillii* by all tested concentrations of garlic juice was *Aspergillus terreus* with about 85.56% inhibition (Fig. 2). Inhibitory effects of garlic were significantly differed among concentrations used as well as from *Aspergillus* isolate to the other within the same concentration. Similar trend was observed with tested *Fusarium* isolates. *Fusarium thapsinum* was the most sensitive followed by *Fusarium verticillioides* with about 77.41% and 70% inhibition respectively (Fig. 3). Meanwhile, *Penicillium* isolates were generally the most sensitive even to the lowest (1.25%) concentration of garlic juice. They exhibited 52.97 and 52.59% inhibition for, *Penicillium chrysogenum* and *Penicillium oxalicum* respectively (Fig. 4).

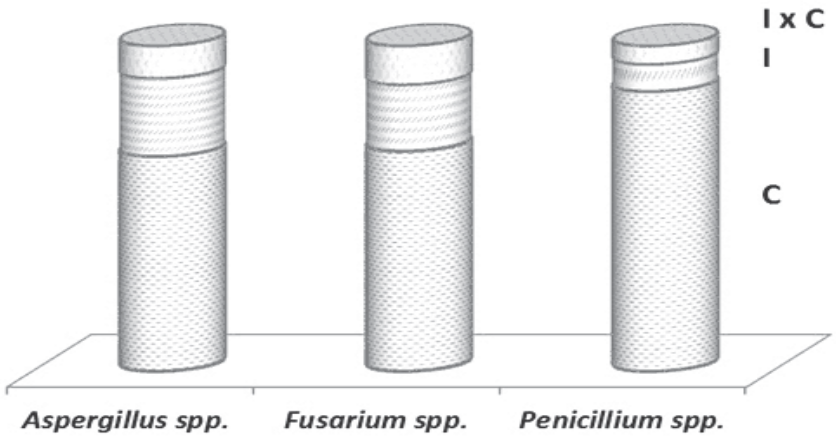

Fig. 1. Relative contribution of concentration (C), Isolates (I) and their interaction (IxC) in variation of fungal growth

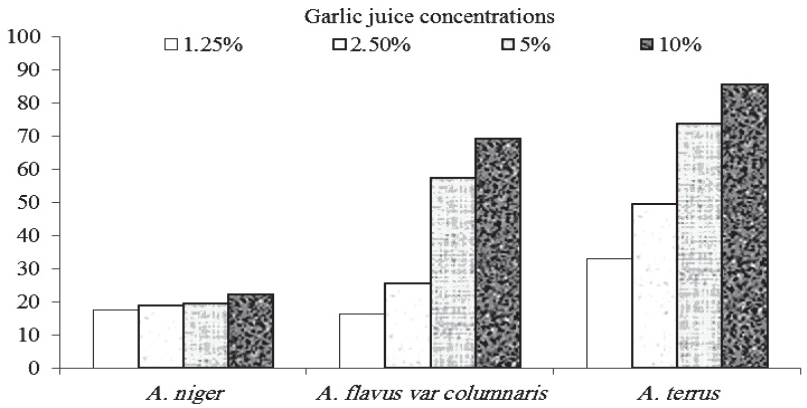

Fig. 2. Antifungal activity of garlic juice against sorghum grain molding *Aspergillii*

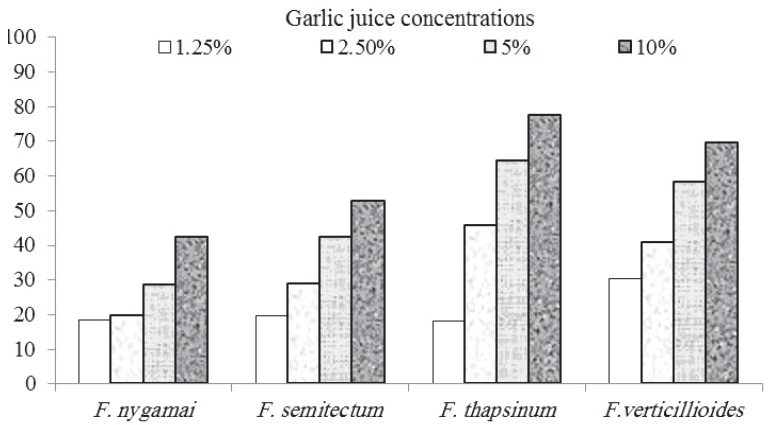

Fig. 3. Antifungal activity of garlic juice against sorghum grain molding *Fusarium*

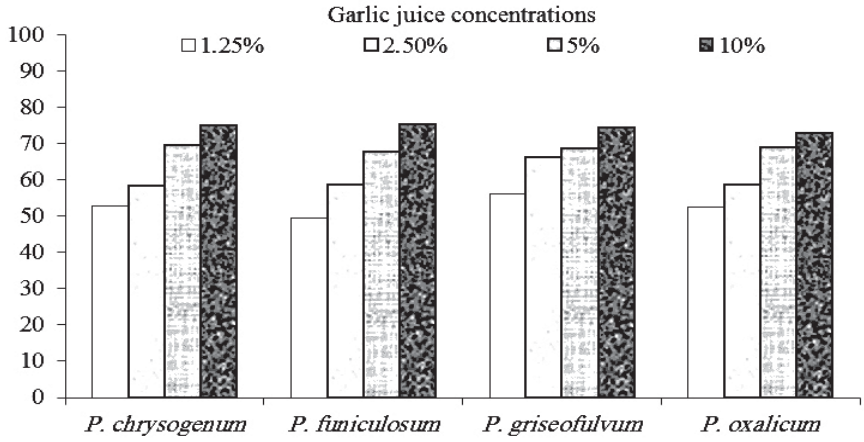

Fig. 4. Antifungal activity of garlic juice against sorghum grain molding *Penicillium*

DISCUSSION

Mycotoxins occurrence both *in vitro* and in *situ* had frequently been discussed[27]. Association of aflatoxigenic *Aspergillii* as well as other toxigenic genera with sorghum grains had frequently documented[28,29]. Mycotoxigenic seed-borne *Aspergillii* had previously been isolated from both freshly harvested and stored Brazilian sorghum[30]. Fumonisin, vomitoxin and zearalenone were also exerted by *Fusarium* isolates recovered from sorghum grains[31,32]. Production of these mycotoxins by sorghum grain molding *Fusarium* has frequently been documented[33, 34]. Citreoviridin and/or patulin were proved to produce by sorghum grain *Penicillium* isolates[35].

Sorghum grain molding fungi in this study were *in vitro* inhibited by all concentrations of garlic juice. The role of garlic in controlling seed borne phytopathogenic fungi had frequently been discussed[36,37]. It was also reported that *Allium sativum* was effectively inhibited seed borne infection (by about 88-97%) of sorghum grains in Bangladesh[13]. In addition, garlic were potentially inhibited mycotoxigenic fungi in many studies over countries and its aqueous extracts could be applied as a good natural food preservative against mycotoxin producing fungi [5,19,38].

It was reported that composition as well as phytochemical properties of *Allium sativum* functional groups might explain its important role as potent antifungal[16,26]. Garlic allicin, decomposes into several effective compounds such as diallylsulphide, dialyldisulphide, diallyltrisulphide, allyl methyl trisulphide, dithiins and ajoene serve as antimicrobial agents[39,40]. Moreover, observed variation in fungal sensitivity to garlic juice could be attributed to differences in the rate of garlic constituent's penetration through the fungal cell wall and cell membrane structures[41]. Furthermore, application methods and strains variation may lead to variable results[42].

CONCLUSION

Garlic juice effectiveness against sorghum grain molding fungi particularly mycotoxigenic genera, suggest its possible use in controlling cereal spoilage fungi. Meanwhile, the use of water base juice provides an alternative to chemical solvents minimizing the risk of chemical exposer.

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