

Control of Grape Blue Molding *Penicillia* by *Allium sativum*

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Contamination of grapes by mycotoxins due to *Penicillium* infection is one of the most causes of human toxicoses. To minimize the risk of mycotoxin exposure, *Allium sativum* was evaluated as anti-*Penicillium*. Five isolates representing 4 species namely *Penicillium citrinum*, *P. expansum*, *P. puberulum* and *P. verrucosum* were investigated. Mycotoxin production of these isolates was assayed using HPLC and their pathogenicity was also undertaken. Obtained data were statistically analyzed and LSD was used to compare means of the experimental results. All tested *Penicillia* were toxigenic and capable of producing detectable patulin (ranging from 0.4-3.6 ppm) in the culture media, with the highest production from *P. citrinum*. Penicillic acid was produced by all tested isolates except *P. verrucosum* (No. 1) and *P. expansum*, with the highest production rate (26.2 ppm) from *P. puberulum*. All tested isolates were pathogenic but could be inhibited by garlic juice at all concentrations used. *P. citrinum* growth was the most inhibited at the lowest concentration used (1.25%) followed by *P. expansum*. It is concluded that garlic juice inhibited *Penicillium* growth, and can be used as a potential source of natural antifungal compounds which could apply to agricultural commodities to prevent fungal decay. Further studies are needed in order to determine the antifungal compound in garlic juice and to confirm their *in vivo* efficacy against food spoilage fungi.

Key words: Garlic, Grapes, *Penicillium*, HPLC, Mycotoxins.

Grapes are highly perishable fruits greatly affected by fungal infection at harvest period or earlier (Somma *et al.*, 2012). Fungi are the most important and prevalent plant pathogens, cause destructive and economically important loss of fresh grapes. Improper conditions throughout transportation, storage and marketing result in grape rot as well as quantitative and/or qualitative losses affecting grapes based manufactures (Šrobárova and Kakalíková, 2007).

Penicillium species are major cause of deterioration and decay amongst a wide range of post-harvest plant products, particularly fruits such as grape (Guerche *et al.*, 2004; Kim *et al.*, 2007). These fungi are widespread, attack different fruits including grapes particularly in the storage and often produce variety of mycotoxins (Magnoli *et al.*, 2003; Fredj *et al.*, 2007).

Unfortunately, harmful mycotoxins and carcinogenic compounds such as citrinin, patulin, penicillic acid and other secondary metabolites, which affect fruit value and harm the customers' health, could be produced by *Penicillium* spp. (Abrunhosa *et al.*, 2001; Santos *et al.*, 2002; Bragulat *et al.*, 2008).

Effective control of fruit diseases could be achieved by several non-chemical control

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strategies (Mlikota and Smilanicki, 2001; Kanan and Al-Najar, 2008; Sanzani *et al.*, 2010). Meanwhile, the use of synthetic fungicides is very risky resulting in human health and environmental problems. Extracts and essential oils of several herbaceous plants are one of the common non-chemical control strategies that have recently received attention for controlling plant diseases (Wang *et al.* 2004; Soylu *et al.* 2005). Due to their availability, non-toxicity and environmental friendliness, plant extracts have extensively been investigated (Aqil *et al.*, 2011).

Many plant extracts have been found to possess antimicrobial potential and could be used to suppress fruit decaying fungi (Obagwu and Korsten, 2003; Ikeura *et al.*, 2011). Among the natural promising plant substances, garlic has been found to be active as antimicrobial agent (Kyung and Lee, 2001; Onyeagba *et al.*, 2004; Irkin and Korukluoglu, 2007). Antifungal activity of garlic extracts against growth of *Penicillium* spp. and other fungi as well as production of mycotoxin by them has frequently been demonstrated (Ismaiel, 2008; Salim, 2011).

The present study was conducted to evaluate the potency of garlic juice against toxigenic *Penicillium* species involved in grape blue mold.

MATERIALS AND METHODS

Fungi

Grape blue molding *Penicillium* isolates, identified by Assiut University Mycological Centre, Egypt, according to Pitt, (1988) were investigated. Five isolates representing 4 species recovered from blue moldy grape fruit samples, collected from different locations (markets) in Riyadh, Saudi Arabia were used in this study.

Pathogenicity

Fresh table grape fruits obtained from a commercial storage were used. Single berries cutting from the rachis with the pedicel attached or detached were placed on sterilized plastic racks in triplicates. Suspensions that contained 12.5×10^3 spores/mL, were sprayed on the berries surfaces for a few seconds to each replicate until they were evenly coated. Inoculated berries on a plastic racks were placed inside plastic boxes humidified with paper tissue soaked with water on

the bottom of each box. Inoculated berries were incubated for 7 days at $15 \pm 1^\circ\text{C}$, then berries that developed mold were counted and the incidence and severity were calculated (Mlikota and Smilanicki, 2001).

Mycotoxigenicity

Penicillium isolates were aseptically cultured onto malt extract broth and incubated at $27 \pm 2^\circ\text{C}$ for 7–10 days (Moslem *et al.*, 2011), after which mycotoxins were extracted using acetonitrile-water solution (5:95 v:v). Solvent was then evaporated under vacuum at 35°C . Dried residues were dissolved in 1 ml of the same solution and then filtered through a $0.45 \mu\text{m}$ filter just before analyses (O'Brien *et al.*, 2011). Mycotoxin production was assayed using high performance liquid chromatography (HPLC).

In vitro control of *Penicillium* isolates

Fresh garlic bulbs were blended in distilled water (1ml water/1gm garlic bulb v/w), and homogenized in a domestic juicer (Braun Combimax 700 Vital, Germany) for 5 minutes at average speed to obtain the total juice (Ismaiel, 2008). Different volumes of filter-sterilized 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 *Penicillium* colonies were placed in the center of these plates (Benkeblia, 2004). Plates without garlic juice were maintained as controls. Cultures were incubated at $28 \pm 2^\circ\text{C}$ and radial growth measured daily for seven days. Three replicate plates were used for each treatment. Inhibition of fungal growth was measured using comparisons with the control plates.

Statistical analysis

Analysis of variance (ANOVA) performed with the MSTAT-C statistical package (Michigan State Univ., USA) was applied. Least significant difference (LSD) was used to compare means.

RESULTS

Pathogenicity

ANOVA of table (1) showed that *Penicillium* isolates were highly significant source of variation in disease incidence and disease severity on grape fruits. All tested isolates were

virulent compared with the control. Significant differences were found in disease incidence between tested isolates belonging to different species. Disease severity was also significantly different between all tested isolates (Table 2).

Mycotoxigenicity

Results of Table (3) revealed that all *Penicillium* isolates were mycotoxigenic and varied in kind and concentration of mycotoxin produced. All tested *Penicillia* were capable of

producing detectable amounts of patulin (ranging from 0.4-3.6 ppm) in the culture media, with the highest production from *P. citrinum*. None of the tested isolates could produce any citrinin except *P. citrinum*. Penicillic acid was produced by all tested isolates except *P. verrucosum* (No. 1) and *P. expansum*, with the highest production rate (26.2 ppm) from *P. puberulum*.

Antifungal activity of garlic juice

ANOVA (Table 4) indicates that

Table 1. Analysis of variance of disease incidence and disease severity on grape fruits caused by *Penicillium* species

	Source of variation	D.F	M.S	F. value	P>F
Disease incidence (D.I)	Replication	2	87.687	3.305	0.079
	Fungi	5	3215.091	121.181	0.000
	Error	10	26.531		
Disease severity (D.S)	Replication	2	0.225	3.103	0.089
	Fungi	5	3.5568	48.993	0.000
	Error	10	0.073		

Table 2. Effect of *Penicillium* species on Grape fruits

<i>Penicillium</i> isolates	Disease incidence(D.I)	Disease severity (D.S)
1. <i>P. verrucosum</i>	69.07 c*	2.05 b*
2. <i>P. verrucosum</i>	61.87 c	1.47c
3. <i>P. puberulum</i>	38.07 d	0.91 d
4. <i>P. citrinum</i>	90.43 a	2.89 a
5. <i>P. expansum</i>	78.60 b	2.62 a
6. Control	0.00 e	0.00 e

D.I= Disease incidence(percentage of infected grape fruits in sample consist of 15 fruits).

D.S= Disease severity assessed in sample of 15 fruits on 5 grade scal (from 0-4), where grade 0 indicated healthy fruits and grade 4 the most infected one.

* Same letter indicates no significant differences between means

Table 3. Mycotoxins productivities of *Penicillium* isolates recovered from grapes

	Mycotoxins (ppm)					
	Citrinine		Patulin		Penicillic acid	
	R. C (%)	PPM	R. C (%)	PPM	R. C (%)	PPM
1. <i>P. verrucosum</i>	-	0.00	20.112	1.5	-	0.0
2. <i>P. verrucosum</i>	-	0.00	17.322	2.8	3.416	7.8
3. <i>P. puberulum</i>	-	0.00	16.965	1.6	1.702	26.2
4. <i>P. citrinum</i>	0.705	0.95	12.705	3.6	0.740	2.2
5. <i>P. expansum</i>	-	0.00	27.507	0.4	-	0.0

Penicillium isolates, garlic juice concentrations and their interaction were all highly significant sources of variation. Concentration was the most important source of variation (Fig. 1). Due to significance of interaction, the responses of *Penicillium* isolates to garlic juice varied among and within the applied concentrations (Table 5). For example response of *P. citrinum* isolate was higher to 1.25% concentration of garlic juice compared with *P. puberulum* and vice versa at 2.5%. Meanwhile, *P. verrucosum* isolate No. 1 was more respond to 1.25% concentration compared with

isolate No. 2 and vice versa at 2.5%. *P. citrinum* growth was the most inhibited at the lowest concentration used (1.25%) followed by *P. expansum*. Potency of 2.5% concentration of garlic juice against *P. citrinum*, *P. expansum* and *P. puberulum* was significantly different although it was insignificantly different at 1.25% concentration. Efficacy was increased as concentration increase reaching the maximum suppression at 10% concentration against all tested isolates (Table 6).

Table 4. Analysis of variance of effect of isolates, Garlic juice concentration and their interaction on linear growth of *Penicillium* isolates

Source of variation	D.F	M.S	F. value	P>F
Replication	2	0.653	0.176	
penicillium(P)	4	100.587	27.120	0.000
concentration(C)	4	14071.020	3793.864	0.000
P X C	16	74.287	20.029	0.000
Error	48	3.709		

D.F.= Degrees of freedom

M.S.= Mean square

Table 5. Effect of concentration on linear growth of *Penicillium* isolates grown on Garlic juice amended medium

Fungus	Linear growth (mm)					Mean
	Garlic	control	1.25%	2.5%	5%	10%
<i>P. verrucosum</i>	85.00	32.33	26.33	12.33	5.00	32.20
<i>P. verrucosum</i>	85.00	38.00	20.67	9.67	5.00	31.67
<i>P. puberulum</i>	85.00	28.33	12.33	5.00	5.00	27.13
<i>P. citrinum</i>	71.87	22.67	21.33	16.33	5.00	27.40
<i>P. expansum</i>	85.00	25.33	28.00	16.33	5.00	32.07
Mean	82.33	29.33	21.73	11.93	5.13	
LSD for I x C interaction		P≤ 0.05 3.16	P≤ 0.01 4.22			

Table 6. Efficacy of garlic juice on linear growth of *Penicillium* isolates.

Fungus	Inhibition %			
Garlic	1.25%	2.5%	5%	10%
<i>P. verrucosum</i>	61.69	69.02	85.49	94.11
<i>P. verrucosum</i>	55.29	75.68	88.62	94.11
<i>P. puberulum</i>	66.67	85.49	94.11	94.11
<i>P. citrinum</i>	68.45	74.90	77.27	93.04
<i>P. expansum</i>	70.2	67.03	80.79	94.11

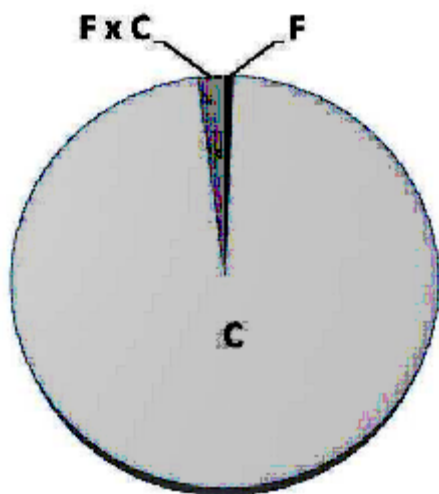


Fig. 1. Relative contributions of fungus (F), Garlic juice concentration (C) and their interaction (F x C) on linear growth of *Penicillium* isolates

DISCUSSION

Results of this study indicated that all tested *Penicillia* were capable of producing typical blue mold symptoms on grape fruits. Involvement of *P. citrinum*, *P. expansum* and *P. verrucosum* in the grape blue mold has previously been documented (Bragulat *et al.*, 2008; Mikusová, 2010). Moreover, *Penicillium* spp. is highly damaging species for the post harvest conservation of numerous fruits (Garcia *et al.*, 2006). *Penicillium* infections resulted in fruit tissue decay but the quality effects depends on the ripening stage at which the infection occurs (Maulani *et al.*, 2012). *Penicillium* species have previously been found to be associated with the grape berry surface from the earliest stages of its development; and/or at all maturation stages (Serra *et al.*, 2005). Significant differences showed in the virulence of tested isolates between and/or within different species have also been discussed (Moslem *et al.*, 2012).

Results also showed that all examined *Penicillia* were mycotoxigenic and varied in the kind and concentration of mycotoxin produced (Serra *et al.*, 2006, Abramson *et al.*, 2009). Occurrence of mycotoxigenic fungi in grapes have frequently been documented (Battilani *et al.*, 2003; Bellí *et al.*, 2006). The differences between and within tested fungi in mycotoxin production could

be attributed to genotypic differences (White *et al.*, 2006; Di Conza *et al.*, 2007). Patulin, the characteristic mycotoxin of *Penicillium* species, could be produced by all tested *Penicillia* in the culture media with the highest production from *P. citrinum* (Andersen *et al.*, 2004). This finding was in agreement with many previous reports (Abrunhosa *et al.*, 2001; Santos *et al.*, 2002). The ability of several *Penicillium* spp. involved in postharvest decay of fruits to produce *in vitro* and/or *in vivo* such mycotoxins, has previously been reported (Bragulat *et al.*, 2008; Aydogdu and Gucer, 2009).

Effectiveness of garlic juice against tested isolates of *Penicillium* spp. showed in the present study is in agreement with many previous studies (Kanan and Al-Najar, 2008; Ikeura *et al.*, 2011). Antifungal potential of *Allium sativum* against many plant pathogenic fungi have frequently been discussed worldwide (Mari *et al.*, 2003; Irkin and Korukluoglu, 2007). Potency of *Allium sativum* against *Penicillium* spp. might be due to its phytochemical properties and contents of aromatic sulphur based compounds such as diallyltrisulfide, diallyl disulfide, allicin and ajoene that have fungistatic activities (Harris *et al.*, 2001; Haciseferogullari *et al.*, 2005; Ogita *et al.*, 2009). *In vitro* inhibition of *Penicillium* growth by garlic juice may indicate the possibility of *in vivo* application of garlic materials to prevent fruit fungal decay (Obagwu and Korsten, 2003; Ikeura *et al.*, 2011).

CONCLUSION

Garlic juice was successfully suppressed the growth of *Penicillium* spp. isolates and could be promising as a source of natural applicable anti-fruit decay compounds. Determination of garlic bioactive molecules and confirmation of their *in vivo* efficacy against food spoilage microorganisms are needed.

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