European Journal of Plant Pathology **109:** 221–225, 2003.

Control of citrus green and blue molds with garlic extracts

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Accepted 14 October 2002

Key words: Valencia, Shamouti, grapefruit, postharvest, allicin

Abstract

Water and ethanol extracts of garlic cloves were applied to artificially inoculated citrus fruits to test their efficacy in the control of *Penicillium digitatum* and *P. italicum*, the cause of citrus green and blue mold respectively. Extracts were tested either alone, or in combination with vegetable (sunflower) cooking oil or fruit wax at the rate of (0.1% v/v), using two orange cultivars (Valencia and Shamouti), and grapefruit. Treated fruits were stored at $10 \pm 1\,^{\circ}C$, and 90–95% relative humidity for 30 days. All concentrations of extracts were more effective than the water control in inhibiting the growth and development of both pathogens, but were not as effective as the fungicide treatment (imazalil 500 ppm + quazatine 1000 ppm). A remarkable increase in the activity of garlic extracts was observed when extracts were mixed with oil. Consequently, the treatment comprising 1% extract plus oil was as effective (100% control) as the fungicide treatment in controlling both green and blue molds on Valencia oranges.

Introduction

Penicillium digitatum and *P. italicum*, the cause of citrus green and blue mold respectively, are important postharvest pathogens and cause serious losses annually (Palou et al., 2001). These diseases are currently managed with synthetic fungicides. There is, however, a growing concern globally, over the continuous use of synthetic chemicals on food crops because of their potential effects on human health and the environment (Norman, 1988). Pathogen resistance is another factor mitigating against the continuous use of synthetic fungicides (Eckert, 1990). These concerns have resulted in a renewed interest in the search for alternative control measures. Plant extracts are one of several non-chemical control options that have recently received attention. The potentials of plant extracts for control of plant diseases have long been recognized (Ark and Thompson, 1959). The actual use of these products in the control of plant diseases is, however, still limited. This is particularly true for control of postharvest pathogens of fruits generally and citrus pathogens in particular.

Literature on the medicinal values of garlic abound. There are, however, very few references on the use of garlic clove extracts to control plant pathogens. Examples include, Russell and Mussa (1977), with *Fusarium oxysporum* f.sp *phaseoli*; Garcia and Garcia (1990), with *Aspergillus* spp., and Obagwu et al. (1997), with *Colletotrichum capsici* amongst others. The antifungal activity of garlic is attributed to allicin (diallyl thiosulfinate) the main biologically active component of garlic extract (Focke et al., 1990; Miron et al., 2000). The mode of action of allicin is believed to be inhibition of enzymes essential for pathogen infection (Miron et al., 2000).

There is no reference in the literature on the use of garlic clove extracts for control of citrus green and blue molds. The reason(s) for this is unclear, but the economic benefit of using garlic, a relatively expensive spice for plant disease control might be an important factor mitigating against its use. Allicin, the biologically active component of garlic extract, is reported to breakdown easily, a characteristic which could affect the efficacy of extracts. Any additive that helps to slow down this activity would improve its efficacy. The aim

of this study was to evaluate the efficacy of garlic cloves extracts, either alone, or in combination with vegetable cooking oil, or plain fruit wax in the control of citrus green and blue molds.

Materials and methods

Fruit

The orange (*Citrus sinensis* (L.)) cultivars (Valencia and Shamouti) were grown in the Letaba orchard in the northern province of South Africa, while the grapefruits were grown in the IYISSP orchard in Swaziland. No postharvest treatment was applied, and fruits were used immediately or stored at $10 \pm 1\,^{\circ}C$ for future use, but not longer than 2 days after harvest.

Pathogen

Highly aggressive isolates of *P. digitatum* (Q103), and *P. italicum* (JO/1/01), originally isolated from citrus fruits were used. Isolates were grown on potato dextrose agar (PDA; Biolab Diagnostic (PTY), South Africa), at 25 °C for 7 days. Spores were harvested by flooding the media surface with sterile distilled water and gently agitating the plate to dislodge spores. A conidial suspension was prepared in Tween 80 (0.05% w/v) Fluka, Switzerland, and the final inoculum concentration adjusted to 1×10^6 spores ml^{-1}.

Preparation of extracts

The garlic cultivars used in this study were chosen based on their performance in a preliminary study (J. Obagwu, Plant Pathology, University of Pretoria, South Africa, pers. comm). Extracts were prepared from either fresh or dry samples. In preparing extracts from fresh samples, the outer, dry peel of cloves was first removed, surface-sterilized for 2 min in 70% ethanol, and washed in three changes of sterile distilled water. One, 3, 5, 7 and 10 g cloves were weighed out and crushed into a pulp in sterile porcelain mortar using a pestle. The pulp was suspended in half the required quantity of solvent (50 ml water or 50 ml of 20% ethanol) in 250 ml Erlenmeyer flask. The suspension was agitated for 1 min and filtered through sterile cotton wool into a 200 ml Erlenmeyer flask. The volume of the filtrate made up to 50 ml with either sterile distilled water or 20% ethanol.

To prepare extracts from dry samples, cloves sterilized as described above were cut into small pieces (approx. 1 cm^3), and dried at 25 °C for 10 days. Dry samples were ground into powder using a waring blender. Different quantities of powder were suspended in solvent (water or 20% ethanol) to obtain different concentrations of extract. Extracts were treated as described above. A preliminary trial (results not shown) to determine the effects of extract source (fresh or dry samples) on efficacy of extracts showed that a comparatively higher concentrations of both water and ethanol extracts of powder were required from dried samples compared to fresh samples, to achieve the same level of inhibition of pathogen growth. Only extracts obtained from fresh samples were evaluated further.

In vitro *screening*

Preparation of garlic amended potato dextrose agar

Extracts were incorporated into 50 ml of sterile, molten PDA of double concentration (wt/vol), which had been cooled to about 45 °C. The flasks were gently agitated for 2 min to allow for a proper mixing of extract with media. Twenty milliliter aliquots of the amended media were dispensed into 90-mm Petri-plates. Chloramphenicol (250 mg l^{-1}) was added to the medium to prevent bacterial growth.

Effect of extracts on pathogen growth

One-hundred microliters of *P. digitatum* and *P. italicum* spore suspension (1×10^6 spores ml^{-1}) were pipetted onto the center of garlic amended PDA. Inoculated plates were incubated at 25 °C for 10 days. Colony diameter was determined by measuring the average radial growth. There were five plates/replicates per concentration. The control consisted of pathogen grown on unamended PDA.

In vivo *screening*

Effect of extracts on disease control

In vitro results (Table 1) showed no significant difference in colony diameter between the two cultivars of garlic assayed. Only Rose du var was evaluated further. Treatments included; extracts alone, extracts combined with sunflower cooking oil (black cat), and extracts combined with fruit wax (0.1% v/v). The choice of 'black cat' was based on results of an earlier study to test the effects of different sources of

Table 1. Effect of water extracts of two garlic cultivars incorporated into potato dextrose agar on the growth of *P. digitatum* and *P. italicum* after 10 days of incubation at 25 °C

Quantity of clove (g/100 ml solvent)	Cultivar/colony diameter (mm)*			
	Penicillium digitatum		*Penicillium italicum*	
	Nootka Rose	Rose du var	Nootka Rose	Rose du var
0	73 f	76 d	65 e	62 d
1	51 d	49 c	46 d	42 c
3	43 c	45 c	39 c	41 c
5	22 b	20 b	18 b	15 b
7	2 a	1 a	0 a	0 a
10	0 a	0 a	0 a	0 a

*Mean of five replicates.
Means having the same letter in the same column are not significantly different at 5% level.

vegetable oil on germination of *P. digitatum* spores (J. Obagwu, unpublished data). Although all oils tested were effective in inhibiting spore germination, 'black cat' was cheaper, and more readily available. Fresh, healthy fruits were surface-sterilized with 70% ethanol for 1–2 min, and wound-inoculated with *P. digitatum* or *P. italicum* (1×10^6 spores ml^{-1}), by pricking, using sterile dissecting needle. Four wounds, each approximately 1 mm wide and 5 mm deep were made in the middle of each fruit. Four to six hours after inoculation, fruits were sprayed to run-off with one of three treatments prepared as described above using a spraying bottle (Efekro, (Pty), South Africa). This was to simulate packhouse condition where fungicide may be incorporated into wax before application on fruits. The run-off was collected in sterile containers and re-used where necessary. Treated fruits were stored in cardboard boxes at 10 ± 1 °C, and 90–95% relative humidity (RH) for 30 days, and assessed thereafter for decay symptoms. Disease assessment was based on a scale of 0 and 1; where 0 = healthy fruits and 1 = diseased fruits. A fruit was considered diseased as long as there was a visible sign of decay at the inoculation point irrespective of the diameter of symptom. This is because the entire fruit is normally destroyed in a few days following initial symptomsinfection especially if fruits are kept at temperatures around 25 °C. There were 30 fruits per treatment and each treatment was replicated three times. The control consisted of fruits immersed in sterile distilled water or treated with a mixture of commercial fungicides, which included Fungazil (imazalil 500 ppm). Janssen, plus Decotine (guazatine 1000 ppm) (Aventis). The experiment was repeated three times.

Effects of treatment on pathogen behavior in vivo
To determine if treatment has any effect on pathogen development, peel pieces were taken from the inoculation point at 24 h after inoculation with a no. 1 cork borer. Six peel pieces, two per fruit were taken and bulked. The peel macerate prepared in 10 ml sterile distilled water was sieved through two layers of sterile cheesecloth. Fifty spores were observed under the light microscope for germination.

Statistical analysis

Data were statistically analyzed using SAS software by analysis of variance and the significance of the treatments was determined using Duncan's multiple range test ($P = 0.05$).

Results

*Effects of extract on pathogen growth (*in vitro*)*

Results presented in Table 1 show that extracts had a significant effect on the growth of both *P. digitatum* and *P. italicum.* The rate of growth was influenced by concentration of extracts, as indicated by the decrease in colony diameter with increasing concentration. There were no significant differences in the levels of control between the two cultivars. Only the media incorporated with 10% garlic extract completely inhibited the growth of both pathogens.

Effect of extracts on disease control

All concentrations of extracts alone were significantly better than the water control, but were not as effective as the fungicide treatments, which gave a 100% control (Table 2). The results also show that better control was achieved on Valencia than on Shamouti and on grapefruit. A significant increase in the biological activity of extracts was observed when treatments were combined with oil. Consequently, the treatments comprising 1%, 3%, and 5% extracts combined with oil were as effective as fungicide in the control of green mold. The increased activity was more obvious on Valencia. The same level of control was, however, not achieved when extracts were combined with wax (Table 2). A comparatively higher level of disease incidence was recorded on treatments involving ethanol extracts compared to water extracts (Table 2). Results presented in Table 3

Table 2. Effect of garlic extracts alone or combined with vegetable cooking oil (black cat), or fruit wax on control of citrus green mold

Treatment	Percentage disease control*					
	Valencia		Shamouti		Grapefruit	
	WE	EE	WE	EE	WE	EE
1% garlic	83 b	50 d	70 c	44 c	50 d	60 e
3% garlic	83 b	50 d	60 d	44 c	50 d	83 c
5% garlic	83 b	65 c	75 c	26 e	67 c	90 b
1% garlic + oil	100 a	93 a	85 b	44 c	100 a	83b c
3% garlic + oil	100 a	85 b	60 d	50 c	100 a	70 d
5% garlic + oil	100 a	65 c	50 d	39 d	80 b	100 a
Oil alone (0.1% v/v)	67 c	65 c	23 e	22 e	50 d	70 d
1% garlic + wax	67 c	61 c	65 c	60 b	80 b	82b c
3% garlic + wax	55 d	56 d	55 d	58 b	80 b	80 c
5% garlic + wax	83 b	80 b	60 d	57 b	80 b	80 c
Wax alone (0.1% v/v)	55 d	55 d	60 d	62 b	50 d	58 e
20% ethanol	—	25 e	—	37 d	—	29 f
Water (negative control)	17 e	—	15 e	—	20 e	—
Fungicide (positive control)	100 a	—	100 a	—	100 a	—

*Mean of three replicates. Means followed by the same letter in the same column are not significantly different at 5% level.
WE = water extracts; EE = ethanol extracts; Fungicide = Fungazil (imazalil 500 ppm) + Decotine (quazatine 1000 ppm).

Table 3. Effect of garlic extracts alone or combined with vegetable cooking oil (black cat), or fruit wax on control of citrus blue mold

Treatment	Percentage disease control*			
	Valencia		Shamouti	
	WE	EE	WE	EE
1% garlic	80 c	72 c	70 c	76 c
3% garlic	91 b	87 b	84 b	81 b
5% garlic	92 b	83 b	80 b	82 b
1% garlic + oil	91 b	77 c	89 b	80 b
3% garlic + oil	100 a	85 b	100 a	82 b
5% garlic + oil	100 a	85 b	100 a	80 b
Oil alone (0.1% v/v)	52 e	46 e	44 e	52 e
1% garlic + wax	71 d	65 d	69 c	60 d
3% garlic + wax	88 b	82 b	88 b	70 c
5% garlic + wax	92 b	84 b	85 b	81 b
Wax alone (0.1% v/v)	50 e	47 e	52 d	31 f
20% ethanol	—	42 e	—	37 f
Water (negative control)	31 e	—	27 f	—
Fungicide (positive control)	100 a	—	100 a	—

*Mean of three replicates. Means followed by the same letter in the same column are not significantly different at 5% level.
See Table 2.

show that treatments were more effective in controlling blue mold than green mold. As a result, the treatments comprising 3% and 5% extracts plus oil were as effective (100% control) as fungicide in the control of blue mold on both Valencia and Shamouti. This is in contrast to green mold where complete control with the above treatments was achieved only on Valencia.

Effects of treatment on spore germination

Twenty-four hours after fruit inoculation and treatment, only the fungicide treatment completely inhibited spore germination (Figure 1). The percentage of spores germinated in all other treatments was, however, lower than in the water control. Less than 14% of spores recovered from all treatments combining extracts with oil germinated, and none of these spores germinated when plated on PDA. A delayed growth on PDA, relative to the control was observed for the other treatments.

Discussion

Results obtained from the present study indicate that garlic extracts have a significant effect on the growth (*in vitro* and *in vivo*) of both *P. digitatum* and *P. italicum*. This finding agrees with earlier reports (Russell and Mussa, 1977; Garcia and Garcia, 1990; Obagwu et al., 1997), on the antifungal properties of garlic clove extracts. This is, however, the first report where garlic clove extracts were evaluated alone or in combination with vegetable oil and plain fruit wax. Extracts were more effective in inhibiting *P. italicum* than *P. digitatum*. A remarkable improvement in biological activity was observed when extracts were mixed with oil. As a result, the treatment comprising 1% extract plus oil was as effective as fungicides in controlling both green and blue molds on Valencia oranges and grapefruit. The reason(s) for this observation is not well understood. However, unlike spores recovered from fruits treated with extracts alone, less than 14% of spores recovered from fruits treated with extracts mixed with oil germinated (Table 1). Also, none of these spores were capable of further growth when plated on PDA, thus indicating that the treatment affected both spore germination and further pathogen development.

Garlic is edible and has not been reported to have any harmful effects. The crushing and extraction of cloves is simple and does not require sophisticated machine. This approach therefore presents a promising alternative to synthetic fungicides for control of citrus green

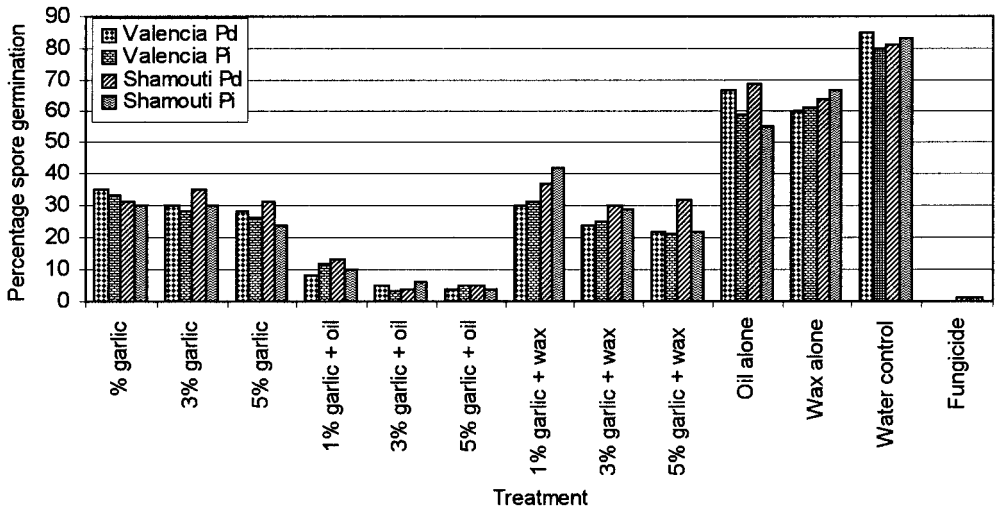

Figure 1. Effect of garlic extracts alone or combined with vegetable cooking oil (black cat), or fruit wax on the germination of *P. digitatum* and *P. italicum* spores extracted from the peel of Valencia and Shamouti orange cultivars. Pd = *Penicillium digitatum*; Pi = *Penicillium italicum*.

and blue molds. But some issues need further examination before the technology can be recommended for commercial adoption. For example, the identification of an acceptable fragrance capable of suppressing the so-called 'bad smell' of garlic without interfering with its biological activity for instance needs further research.

Acknowledgements

The financial assistance received from the German Educational Exchange Program (DAAD), the South African National Research Foundation (NRF), and the South African Technology and Human Resources for Industry Programme (THRIP) is gratefully acknowledged. We thank Letaba Estates, South Africa, for providing the fruit used in this trial.

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