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EFFECT OF POST-HARVEST ESSENTIAL OILS APPLICATION ON RESISTANCE OF ONION AND GARLIC ON STORAGE ROT DISEASES

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ABSTRACT: *In vitro experiments, growth of Aspergillus niger and Fusarium oxysporum f.sp. cepae was completely inhibited at 200 ppm cinnamon oil. The same result was obtained at 100 ppm for Botrytis allii fungus. However, growth inhibition of the mentioned three pathogens was achieved at 1000 and 2000 ppm when anise and peppermint oils were used, respectively.*

Post harvest application of the all tested oils significantly reduced black mould of onion as compared with control. Soaking onion bulbs in cinnamon and/or clove oils (3000 ppm) for 30 min. resulted 76 and 53% reduction in black mould incidence, respectively.

Basal rot disease was significantly reduced by increasing either oils concentration or the application period. The best results were obtained at 3000 ppm concentration and 30 min. soaking into cinnamon oil (80%), clove oil (66 %) and anise oil (58 % reduction of the disease) 8 months after storage.

On the other hand, soaking garlic bulbs into 3000 ppm of either cinnamon, clove and peppermint oils for 30 min. resulted bulbs free of A. niger infection up to 8 months storage. Anise oil gave the same result, but up to 6 months only.

Garlic bulbs still free of infection with B. allii up to 8 months storage, in response to different oil applications at the concentration of 3000 ppm and 30 minutes soaking.

Key words: *Onion, garlic storage rot diseases , Fusarium oxysporum f.sp. cepae, Botrytis allii, Aspergillus niger.*

INTRODUCTION

Garlic (*Allium sativum*) and onion (*Allium cepa* L.) are very important export crops contributing to the agricultural national income in Egypt. Storage rots, black mould (*Aspergillus niger*), neck rot (*Botrytis allii*) and basal rot (*Fusarium oxysporum* f.sp. *cepae*) occur always on bulbs of garlic and onion greatly reduce quantity of edible bulbs during storage and transit (Radwan, 1980; Chantarasnit and Phanichakaru, 1986 and Martinez and Granda, 1993, *A. niger*

Hayden and Moude, 1992, *B. Allii* Moude and Presly, 1977a and Sharaf El-Din, Azza, 2000).

Storability of onion and garlic bulbs can be improved by pre- and/or post-harvest fungicide treatments. Galben/mancozeb 58 % followed by Dithane M 45 foliar spray on onion plants in field, were the most effective fungicides for controlling post-harvest development of infection with *A. niger* (black mould), *B. allii* (neck rot), and *F. oxysporum* f.sp. *cepae* (basal rot) on bulbs during storage (El-Shehaby *et al.*, 1997). On the other hand, fumigation garlic bulbs after harvest by formalin at the rate 0.03 %, protect garlic bulbs in storage (Sharaf El-Din, Azza, 2000).

Recently, several publications have been concerned with the relationship between volatile oils from aromatic plants and disease. *In vitro* studies, oils obtained from *Eucalyptus globulus* and *Ocimum canum* completely inhibited the fungal growth of *Sclerotium rolfsii* at 4000 ppm (Singh and Dwivedi, 1987). Also, essential oils obtained from *Eucalyptus* spp., *Ageratum conzoides* and *Mentha piperita* inhibited the mycelial growth, sporulation and spore germination of *Aspergillus niger* and *Fusarium oxysporum* (Singh *et al.*, 1992; Tantaoui *et al.*, 1993 and Paran *et al.*, 1996).

The aim of this research was to ascertain the toxicity of some volatile oils, anise (*Pimpinella anisum* L.), eucalyptus (*Eucalyptus rostrata* Schlechtend), peppermint (*Mentha piperita* L.), cinnamon (*Cinnamomum zeylanicum* Breyn) and clove (*Eugenia caryophyllata* Thurb.), to growth of *Aspergillus niger, Botrytis allii* and *Fusarium oxysporum* f.sp. *cepae* fungi, and also the effect of post-harvest applications in improving resistance of onion and garlic bulbs to storage rots.

MATERIALS AND METHODS

Isolation and identification of tested fungi:

Samples of Chinese garlic bulbs were collected from Giza and Beni-Suef governorates, the samples were transferred onto PDA medium in Petri-dishes and incubated at 20 °C and 25 °C for seven days. *Aspergillus niger, Botrytis allii* and *Fusarium oxysporum* f.sp. *cepae* were purified, and identified by methods of (Brown, 1924 and Singh *et al.*, 1991).

In this study, the following 5 plant oils, listed in Table (1), (obtained from El-Gomhouriya Company for Medicines and Chemicals) were evaluated for their effect on the growth of the tested fungi.

Table (1): Origin plants, scientific names, plant parts and active ingredients of essential oils evaluated in this study.

Origin plant	Scientific name	Plant part	Active ingredient
Anise	*Pimpinella anisum* L.	Seeds	Charicol methyl ether (estragol)
Eucalyptus	*Eucalyptus rostrata* (Schlechtend)	Leaves	Cincole (eucalyptol)
Peppermint	*Mentha piperita* L.	Leaves	Menthol
Cinnamon	*Cinnamon zeylanicumi* Breyn	Bark	Cinnamic aldehyde
Clove plants	*Eugenia carpohyllata* Thurb.	Buds of flowers	Eugenol

Effect of some natural volatile oils on the tested fungi:

Five different volatile oils namely, anise, eucalyptus, peppermint, cinnamon and clove were evaluated for their effect on growth of *A. niger, B. allii* and *F. oxysporum* f.sp. *cepae*. The calculated amounts of each oil were incorporated in separate treatments, into sterilized PDA medium before solidification to obtain five different concentrations 0.0, 100, 200, 500, 1000 and 2000 ppm. The media were then poured into Petri dishes (7 cm diameter). The media were inoculated at the center with 5 mm discs (one/dish) bearing 5 days old culture of *A. niger, B. alli* and *F. oxysporum* f.sp. *cepaei*. Control treatments were PDA medium not supplemented with oils. Four replicates were prepared for each treatment. All treatments were incubated at 28 °C for *A. niger* and *F. oxysporum* f.sp. *cepae*, and 20 °C for *B. allii* for 7 days, after which the linear growth in cm was determined.

Spores of 0.5 cm diameter disc were suspended in 10 ml sterile water and 0.1 ml suspension of each glass slide was placed on moistened filter paper in Petri dish plates. These plates were incubated for 24 hrs at 28 °C for *A. niger* and *F. oxysporum* f.sp. *cepae* and 20 °C for *B. allii*. Slides were microscopically examined and percentages of spore germination were recorded.

Post-harvest treatments:

Bulbs of garlic (Chinese Sedes 40) from Beni-Suef governorate and bulbs of onion (Giza 20) from Gemmeiza (Gharbia governorate) were used for

studying the effect of post-harvest treatments by some volatile oils on bulbs storage rots.

Solutions of each oil were prepared by 2000 and 3000 ppm concentrations, 90 bulbs of garlic or onion were soaked in each solution till 15 or 30 minutes, bulbs were dried on room temperature, so were packed in plastic net containers and stored in uncontrolled room, three replicates were done for each treatment. Garlic and onion bulbs were checked after 2, 4, 6 and 8 months storage for recording percentage of black mould, neck rot and basal rot infection. All results were statistically analyzed using "F" test and L.S.D. to compare significance between treatments (Snedecor and Cochran, 1981).

RESULTS

Screening of oils for mycelial inhibition:

Linear growth of *A. niger, B. allii* and *F. oxysporum* f.sp. *cepae* was significantly reduced or inhibited with specific rates of oils (Table 2). Complete inhibition in mycelial growth of the tested fungi was obtained at 200 ppm of cinnamon. While anise oil inhibited mycelial growth of these fungi at 1000 ppm and peppermint at 2000 ppm. Moreover, eucalyptus at all concentrations only significantly reduced linear growth of tested fungi. On the other hand, complete inhibition in mycelial growth of *B. allii* was obtained at 100 ppm cinnamon and 500 ppm clove.

Toxicity to spore viability:

Spores produced on PDA poisoned with essential oils, when stimulated for germination in water, exhibited levels of germination lower than control (Table 3). Complete loss in spore viability was detected as spores were unable to germinate in water. Rates of essential oils that completely inhibited viability of resultant spores were 200 ppm cinnamon and 1000 ppm clove and anise on *A. niger, B. allii* and *F. oxysporum* f.sp. *cepae.*

Effect on onion:

A. Black mould:

All oils post-harvest treatments significantly reduced black mould of onion compared with control (Table 4).

However, percentages of the disease varied according to oil used, concentration and soaking period applied. Among all oil treatments, soaking onion bulbs till 30 min. with cinnamon at 3000 ppm was the most effective on reducing black mould disease till 6 and 8 months storage.

However, difference between 2000 ppm and 3000 ppm concentrations and 15 or 30 min. soaking periods was significant. Anise and peppermint oils were less effective than other tested oils. Average reduction in black mould incidence obtained with cinnamon (74 %) at 3000 ppm and 30 min. soaking period. Clove was less effective than cinnamon by 56 % reduction.

Table (2): Effect of some volatile essential oils on the linear growth of *A. niger, B. allii* and *F. oxysproum* f.sp. *cepae* fungi.

Treatments essential oils	Conc. (ppm)	Linear growth (cm) at rate of oils		
		A. niger	*B. allii*	*F. oxysporum* f.sp. *cepae*
Cinnamon	0	7	7	7
	100	4.7	0	5
	200	0	0	0
	500	0	0	0
	1000	0	0	0
	2000	0	0	0
Clove	0	7	7	7
	100	5.4	1.4	4.8
	200	3.6	1.4	5
	500	4.3	0	4
	1000	0	0	0
	2000	0	0	0
Anise	0	7	7	7
	100	5.1	2.8	4
	200	4.3	2.1	4
	500	4.7	2	4.3
	1000	0	0	0
	2000	0	0	0
Peppermint	0	7	7	7
	100	5.7	2.5	7
	200	4.4	2	6.8
	500	4.1	1.1	6.8
	1000	1.3	0	2.3
	2000	0	0	0.6
Eucalyptus	0	7	7	7
	100	5.2	5	5
	200	3.2	4.6	4.4
	500	4.6	4	4.4
	1000	5.5	3.2	4.2
	2000	5.7	2.8	3.5
L.S.D. at 5% treatments:	Cinnamon:	0.21	N.S.	0.04
	Clove:	0.48	0.13	0.34
	Anise:	0.54	0.78	0.30
	Peppermint:	0.67	0.14	0.51
	Eucalyptus:	0.50	0.43	0.40

Table (3): Effect of essential oils on germination of resultant spores of *A. niger, B. allii* and *F. oxysproum* f.sp. *cepae* in water.

Fungus	Essential oils	Percentage of spore germination at concentration (ppm) of essential oils:					
		0	100	200	500	1000	2000
A. niger	Cinnamon	90	7	0	0	0	0
	Clove	85	18	15	9	0	0
	Anise	85	25	19	20	0	0
	Peppermint	90	17	25	30	7	0
	Eucalyptus	85	35	30	22	18	8
L.S.D. at 5% for: Treatments (T) = 1.13; Concentrations (C) = 1.03; T x C = 2.52							
B. allii	Cinnamon	82	0	0	0	0	0
	Clove	83	36	28	0	0	0
	Anise	83	38	5	2	0	0
	Peppermint	82	21	17	0	0	0
	Eucalyptus	90	32	29	19	21	13
L.S.D. at 5% for: Treatments (T) = 1.35; Concentrations (C) = 1.23; T x C = 3.02							
F. oxysporum f.sp. *capae*	Cinnamon	100	60	0	0	0	0
	Clove	100	80	60	0	0	0
	Anise	100	80	80	65	0	0
	Peppermint	100	86	74	70	85	35
	Eucalyptus	100	89	85	78	70	0
L.S.D. at 5% for: Treatments (T) = 1.63; Concentrations (C) = 1.49; T x C = 3.65							

Table (4): Effect of post-harvest volatile essential oil applications on percentage of black mould and basal rot of onion at different storage periods.

Soaking period	Treatment (ppm) / Storage periods	2 months		4 months		6 months		8 months	
		% *A. niger*	% *Fusarium* sp.	% *A. niger*	% *Fusarium* sp.	% *A. niger*	% *Fusarium* sp.	% *A. niger*	% *Fusarium* sp.
15 minutes	Cinnamon (2000)	13.3	6.6	26.66	16.6	30.0	20.0	33.0	23.33
	Cinnamon (3000)	13.3	0.0	13.33	10.0	23.3	13.33	23.33	13.33
	Clove (2000)	13.3	10.0	26.6	13.3	23.33	20.0	43.33	30.0
	Clove (3000)	6.6	10.0	23.3	10.0	50.0	20.0	30.0	23.33
	Anise (2000)	20.0	3.3	40.0	20.0	26.66	20.0	50.0	26.66
	Anise (3000)	3.3	3.3	10.0	10.0	53.3	16.66	36.6	20.0
	Peppermint (2000)	16.6	10.0	46.60	26.6	30.0	26.66	53.3	30.0
	Peppermint (3000)	13.3	10.0	26.66	13.33	53.3	20.0	30.0	23.33
	Control	36.6	30.0	53.3	38.8	55.0	50.0	63.33	50.0
30 minutes	Cinnamon (2000)	6.6	0.0	10.0	10.0	16.66	16.66	30.0	20.0
	Cinnamon (3000)	0.0	0.0	10.0	10.0	10.0	13.33	16.66	16.66
	Clove (2000)	20.0	3.33	23.33	13.33	30.0	23.33	36.66	23.33
	Clove (3000)	16.6	0.0	23.33	10.0	23.33	16.66	30.0	20.0
	Anise (2000)	6.6	3.3	23.33	16.6	38.0	20.0	40.0	23.33
	Anise (3000)	6.6	3.3	36.66	10.0	23.33	13.3	26.66	16.66
	Peppermint (2000)	6.6	13.33	23.33	16.66	30.0	16.66	33.33	36.66
	Peppermint (3000)	3.3	0.0	30.0	6.66	23.33	13.33	30.0	16.66
	Control	36.6	30.0	53.3	38.8	55.0	50.0	63.0	50.0
L.S.D. 5% for: Treatments (T)		3.71	0.64	0.35	0.41	0.35	0.39	0.35	0.37
Periods (P)		1.75	0.30	0.17	0.19	0.23	0.18	0.17	0.18
T x P		5.25	0.90	0.50	0.57	0.57	0.55	0.50	0.52

B. Effect on basal rot:

Post-harvest, oil treatments, also reduced basal rot checked on bulbs during storage (Table 4). Reduction in disease incidence mostly increased as oil concentrations and soaking period increased. Best reduction in basal rot obtained with 3000 ppm cinnamon was 80 % followed by clove and anise (66% and 58 %), respectively.

Effect on garlic:

The effect of oils on black mould incidence varied greatly and was apparently accompanied by different concentrations and soaking periods of oils (Table 5). According to the statistical analysis, cinnamon and clove oils reduced black mould infection by not less than 100 % compared with the control, till 8 months storage, followed by anise and peppermint, which showed 100 % reduction in black mould till 6 months storage at 30 min. soaking period.

Application of different tested oils gave reduction in basal rot disease. Clove at 3000 ppm and 30 min. soaking period showed resulted bulbs free of infection with basal rot pathogen till 6 months storage.

Cinnamon was less effective. While, anise and peppermint almost behaved equally with respect to reduction of the disease.

Neck rot disease appeared just after 8 months storage on garlic bulbs treated by different oils. Cinnamon showed 100 % reduction in neck rot disease incidence during 8 months storage.

DISCUSSION

The five different natural volatile oils tested in this study have been shown to be effective against *A. niger, B. allii* and *F. oxysporum* f.sp. *cepae*. Anise, and peppermint could inhibit fungal growth completely at concentrations more than 1000 ppm against *A. niger* and *F. oxysporum* f.sp. *cepae* while cinnamon oil gave the same result at 200 ppm concentration but at 100 ppm against *B. allii.* In addition, eucalyptus in the testing experiments did not inhibit the growth of tested fungi, while anise and peppermint oils had varying inhibitory effects. The most effective oil against *B. allii* was cinnamon (100 ppm), followed by clove (500 ppm), while a higher concentration (1000 ppm) of peppermint oil was necessary to inhibit the pathogen. At the highest concentration tested (2000 ppm) peppermint oil; the growth of *A. niger* and *F. oxysporum* f.sp. *cepae* had been inhibited.

Table (5): Effect of post-harvest volatile essential oil applications on percentage of black mould and basal rot of garlic bulbs at different storage periods.

Soaking period	Treatment (ppm) / Storage periods	2 months		4 months		6 months		8 months		
		% *A. niger*	% *F. oxysporum*	% *A. niger*	% *F. oxysporum*	% *A. niger*	% *F. oxysporum*	% *A. niger*	%*B. allii*	% *F. oxysporum*
15 minutes	Cinnamon (2000)	0.0	6.66	3.33	13.33	6.66	13.33	13.33	6.66	13.33
	Cinnamon (3000)	0.0	0.0	0.0	0.0	3.33	10.0	3.33	0.0	6.66
	Clove (2000)	3.33	3.33	3.33	16.66	3.33	16.66	16.66	3.33	33.33
	Clove (3000)	0.0	3.33	3.33	3.33	3.33	3.33	3.33	3.33	20.33
	Anise (2000)	0.0	6.66	0.0	13.33	3.33	13.33	13.33	3.33	20.0
	Anise (3000)	0.0	0.0	0.0	6.66	3.33	6.66	6.6	0.0	10.0
	Peppermint (2000)	3.33	3.33	3.33	20.0	3.33	16.66	20.0	6.66	20.0
	Peppermint (3000)	0.0	3.33	0.0	13.33	0.00	13.33	13.33	0.0	16.33
	Control	6.66	23.33	16.66	20.0	16.66	26.66	26.66	6.33	43.33
30 minutes	Cinnamon (2000)	3.33	3.33	3.33	3.33	3.33	3.33	3.33	0.0	23.33
	Cinnamon (3000)	0.0	0.0	0.0	3.33	0.0	3.33	0.0	0.0	13.33
	Clove (2000)	6.66	0.0	6.66	3.33	6.66	3.33	6.66	6.66	10.0
	Clove (3000)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.33	6.66
	Anise (2000)	0.0	3.33	0.0	10.0	0.0	13.33	3.33	3.33	16.66
	Anise (3000)	0.0	0.0	0.0	6.66	0.0	6.33	3.33	3.33	16.66
	Peppermint (2000)	0.0	6.66	3.33	10.0	3.33	10.0	3.33	0.0	20.0
	Peppermint (3000)	0.0	0.0	0.0	3.33	0.0	6.66	0.0	0.0	10.0
	Control	6.66	23.33	16.66	26.66	16.6	20.66	16.66	6.33	43.33
L.S.D. 5% for: Treatments (T)		0.89	1.68	0.59	1.24	0.66	1.616	0.025	0.98	2.56
Periods (P)		N.S	N.S	0.28	0.58	0.31	0.55	N.S	N.S	1.21
T x P		1.25	2.37	0.83	1.75	0.94	1.64	0.10	1.39	3.62

The fungicidal effects of these oils on *A. niger, B. allii* and *F. oxysporum* f.sp. *cepae* fungi were emphasized as the fungal growth has noticed by transferring fungal discs from the plates of treatments to oil-free medium. These oils probably exerted their action on fungal enzymes responsible for metabolism of some materials inside hyphal cells and this should correlated with degree of permeability and/or penetration into cell wall or more accurately cell membranes (Ismail *et al.*, 1989; Sharaf-El-Din (Azza), 2000 and Moursy (Maysa) *et al.*, 2001).

Onion or garlic bulbs, treated with cinnamon oil, showed a marked decline in percentage of infection with *A. niger* and *F. oxysporum* f.sp. *cepae* fungi after 4 and 6 months storage. In the samples treated with clove oil, an increase in *A. niger* and *F. oxysporum* f.sp. *cepae* appeared to occur after 8 months storage, but this increase was not significant. Furthermore, the counts declined during 2 and 4 months storage. The clove and cinnamon oils were significantly more effective against *F. oxysporum* f.sp. *cepae* when onion and garlic bulbs soaked for 30 minutes in clove oil.

In the present study, the increase of soaking period appeared to enhance the inhibitory effect of clove, cinnamon and anise against *F. oxysporum* f.sp. *cepae*. Similarly, Singh *et al.* (1992) found that essential oils obtained from *Eucalyptus* spp., *Ageratum conzoides* and *Mentha piperita* inhibited the mycelial growth, sporulation and spore germination of *A. niger* and *F. oxysporum* f.sp. *cepae*. Also Moursy (Maysa) *et al.* (2001) found that common smut disease incidence was reduced by foliar spraying with oils of soybean and maize.

Moreover, in Pharmaceutical Codex (1979) reported that cinnamon oil contains about 70 % w/w of cinnamic aldehyde has antibacterial and antifungal properties similar to those of benzoic acid. Anise oil contains charicol methyl ether, which also known as estragol. The chief constituent of clove oil is eugenol (about 85 to 90 % v/v), eucalyptus oil contains chiefly cincole, which also known as eucalyptol and peppermint oil contains chiefly menthol.

Thus, essential oils of cinnamon, clove, anise, peppermint and eucalyptus could be used to control storage rots of garlic and onion bulbs, as antifungal post-harvest applications, especially at 30 min. soaking period and 3000 ppm concentration.

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تأثير معاملات ما بعد الحصاد بزيوت عطرية وطبية على إصابة الثوم والبصل بأعفان المخزن

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معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة

الملخص العربى

أثبتت التجارب المعملية أن زيت القرفة بتركيز ٢٠٠ جزء فى المليون يوقف تماماً نمو الفطرين أسبرجلس نيجر وفيوزاريوم أوكسيسبورم شكل النوع سيبا. فى حين كان تركيز ١٠٠ جزء فى المليون من نفس الزيت كافياً لوقف نمو الفطر بوترايتس أليای. وتم التوصل لوقف تام لنمو جميع الفطريات الممرضة المذكورة عند تركيزى ١٠٠٠ و ٢٠٠٠ جزء فى المليون من زيت الينسون والنعناع على التوالى.

وقد أدت معاملات الأبصال بعد الحصاد بأى من الزيوت المختبرة إلى النقص المعنوى لمرض العفن الأسود فى البصل ، مقارنة بتلك الغير معاملة. فقد أدى نقع أبصال البصل فى تركيز ٣٠٠٠ جزء فى المليون من أى من زيت القرفة أو القرنفل لمدة ٣٠ دقيقة إلى تقليل حدوث هذا المرض بنسبة ٧٦ و ٥٣% على التوالى.

كما لوحظ نقص معنوى لحدوث مرض عفن القاعدة فى البصل إما بزيادة تركيز الزيت المستخدم أو فترة المعاملة به. وتم التوصل إلى أفضل النتائج عند غمر الأبصال لمدة ٣٠ دقيقة فى تركيز ٣٠٠٠ جزء فى المليون من أى من زيت القرفة (٨٠%) ، زيت القرنفل (٦٦%) ، زيت الينسون (٥٣% أقل فى مرض عفن القاعدة) بعد ٢ ، ٤ ، ٦ و٨ أشهر من التخزين.

ومن جهة أخرى ، أدى نقع أبصال الثوم فى تركيز ٣٠٠٠ جزء فى المليون من أى من زيت القرفة أو القرنفل أو النعناع لمدة ٣٠ دقيقة إلى بقاء الأبصال خالية من الإصابة بالفطر أسبرجلس نيجر بعد تخزينها لمدة ثمانية أشهر. وكانت نفس النتيجة عند استخدام زيت الينسون ، ولكن لمدة ستة أشهر فقط.

وقد لوحظ خلو أبصال الثوم من الإصابة بالفطر بوترايتس أليای لمدة ثمانية أشهر كاستجابة لمعاملتها بأى من الزيوت تحت الإختبار بتركيز ٣٠٠٠ جزء فى المليون ولمدة ٣٠ دقيقة.