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CONTROL OF POSTHARVEST LIME FRUIT ROTS BY SOME COMPONENTS OF ESSENTIAL OILS

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ABSTRACT: The essential oils of lime fruits peel was extracted after 3,5 and 7 months from fruit set by Gas chromatography / mass spectrum. Lime essential oils contained high concentrations of oxygenated compounds such as α-citral, β-citral, neryl acetate, nerol and α-terpeniol. While, limonene was the main component. Lime fruit peel essential oils inhibited linear growth and spore germination of *Penicillum digitatum*, *P. italicum* and *Geotrichum candidum* the causal organisms of green, blue moulds and sour rot, respectively at 0.25%. Methyl anthranilate, citral, neryl acetate, nerol and terpeniol as essential oils component were the highest effective. Also, imazalil was more effective than thiabendazole in this respect. The most effective components (methyl anthranilate, neryl acetate and nerol) as well as imazalil and thiabendazele were applied on lime fruits under laboratory conditions and methyl anthranilate proved to be the most effective treatment in controlling lime fruit rots.

Key words: Essential oils, Imazalil, Lime, Postharvest, Thiabendazole

INTRODUCTION

Lime fruits are liable to postharvest fruit rots (green and blue moulds as well as sour rot) caused by *Penicillium digitatum*, *P.italicum* and *Geotrichum candidum*, respectively (Mehrotra *et al.*, 1998; Smilanick *et al.*, 1999; Lopez - Garcia *et al.* 2000; Arras and Usai, 2001 and Aly *et al.*, 2003). Lime fruit rots caused great crop losses. These losses could be reduced to a certain extend by controlling the pathogens of lime fruit rots using different fungicides *in vivo* (Mahmoud and Hanafy 1991, Lateef *et al.*, 1994, Agar and Kaska 1995; D'Aquino *et al.*,

1998; Ben-Yehoshua *et al.*, 2000 and Schirra *et al.*, 2000). Green and blue moulds as well as sour rot are controlled by treating the citrus fruits with Imazalil and Thiabendazol fungicides (Javed *et al.*, 1995; D'Aquino *et al.*, 1998; Ben-Yehoshua *et al.*, 2000 and Schirra *et al.*, 2000). However, chemical control increased number of fungicidal resistant strains of pathogens (Eckert, 1990). Also, using fungicides cause harmful effect on environment and human health (Anonymus, 1987). Harmful effect of the fungicides led to find out new materials with no toxic residues in nature's food chains, safe for application and more cheep in cost. Citrus essential oils have biological active effect as antifungal substances against many postharvest citrus fruit rot pathogens (Rodov *et al.*, 1995 and Caccioni *et al.*, 1998). Many plant pathologists detected the chemical composition of lime peel essential oils using Gas Chromatograph (Abdul-Sattar *et al.*, 1992 and Minh *et al.*, 2002). They found that, hydrocarbons were the main components of lime essential oil followed by aldehydes. Many phytopathologests using lime oil components in controlling lime decay *in vitro* and *in vivo* (Singh *et al.*, 1993; Caccioni and Guizzardi. 1994; Rodov *et al.*, 1995; Suprata *et al.*, 1997; Caccuioni *et al.*, 1998 and Vargas *et al.*, 1999).

The objective of this investigation was aimed to extract lime fruit peel essential oils and identify its chemical components. The effect of fungicides (Imazalil and Thiabendazol) and lime essential oils and its constituents on the mycelial growth and spore germination of postharvest lime fruit rot fungi were also undertaken. The control of lime postharvest fruit rot diseases by essential oil components and fungicides under laboratory conditions was also studied.

MATERIALS AND METHODS

1- Citrus fruit rot fungi:

Penicillum digitatum Sacc., *P.itlicum* Whem, *Geotrichum candidum* Link and *Aspergillus niger* Van Tieghem were previoushy isolated from naturally infected lime fruits by Aly *et al.* (2003).

2- Extraction of lime fruit peel essential oils:

Three samples (10kg for each) of lime fruits at 3 different ripening stages, *i.e.* 3, 5 and 7 months after fruit set (Premature. Mature and Ripening stages. respectively) were used. Essential

oils of the fresh lime fruits peel were extracted by steam distillation as mentioned by Clevenger (1928). The essential oils were collected in a cold trap, then separated to its components and finlly dried over anhydrous sodium sulphate. The oils were filtrated, then kept quickly in a dark bottle at -7 °C according to Guenther (1961).

3- Identification and determination of the chemical constituents of lime fruits peel essential oils:

Separation of the resulting lime fruits peel essential oils were accomplished on a Varian Gas Chromatogrophy (Walnut Creek California USA) equipped with Finnigan mat SSQ 7000 (Thermo Inst., USA) mass spectrometer and a 30m X 0.25 mm DB 5 capillary column thickness J & W scientific USA. The column temperature was programmed from 50°C (constant for 3min), at a rate of 7°C to 250°C with 10min isothermal hold. The injector temperature was 220°C and the transition line temperature was 250 °C the carrier gas was helium and the column head pressure was 10-15 pisi. Compounds identifications were based on the following: The detector injector temperature were 200 & 250 °C, respectively. The size of injection was 2 μ and the rate of gas was 15 ml/min.

The area of each peak representing the component of essential oils was measured by calculating the area of the triangle formed by drawing two triangles through the infection points of the peak. The base line, being the base of the triangle formed, was calculated by multiplying peak high by the width at half peak height. The separated components were identified by matching them with the NIST mas-spectral library data, published data and according to Adams (1995). The percentage of essential oil composition was calculated by applying the following equation:

$$\%\text{of essential oil} = \frac{\text{area of each peak}}{\text{all area of peaks}} \times 100$$

4-Laboratory studies:

The effect of different fungicides, lime fruit peel essential oils, and citrus essential oils components on the linear growth and percentage of spore germination of the tested pathogenic fungi were studied. Fungistatic and fungicidal effect of citrus essential oils component on pathogenic fungi was also studied.

a) Effect of different tested fungicides on the linear growth of the tested fungi:

Two different fungicides (Imazalil 70% liquid and Thiabendazole (TBZ) 98.% WP) were used at different concentrations, *i.e.* 0.0, 0.10, 0.25, 0.50, 1.0, 2.5, 5,10, 25, 50, 100, 250 and 500 ppm. The different concentrations of the tested fungicides were added separately to PDA medium under aseptic conditions before solidification and shacked vigorously to ensure even distribution of the fungicides, then poured in the dishes (9 cm in diameter) and inoculated in the center with an equal discs (6 mm in diameter) of the different tested pathogenic fungi, *i.e. P.digitatum, P.italicum, G.candidum* and *A.niger* taken from 7 days old culture. Dishes were incubated at 25°C. Fungicides free medium was used as a control. Three Petri dishes were used for each particular treatment. Two perpendicular growth diameters of the tested fungi were measured each two days until the growth in control treatment completely covered the dish, then growth average was calculated and recorded.

b) Effect of different lime fruit peel essential oils on the linear growth of the tested fungi:

Lime fruit peel essential oils, after three fruit ripening stages, were used at different, concentrations, *i.e.* 0.0, 0.05, 0.10, 0.25, 0.50 and 1.00% (V/V) to study their effect on the linear growth of the tested fungi. The different concentrations of the tested lime fruit peel essential oils and 0.3% Tween 80 were added separately to PDA medium under aceptic conditions before solidification and sheked vigorously to insure even distribution of the oils. Oils free medium with 0.3% Tween 80 was used as a control. Inoculation, incubation and growth average calculation were carried out as previously mentioned.

c) Effect of different citrus essential oils components on the linear growth of the tested fungi:

Three hydrocarbon citrus essential oils components and nine oxygenated components presented in Table (1) were used to study their effect on the linear growth of the tested fungi. These components were obtained from Delta Aromatic International Company. 439 Al- Ahram street- Giza- Egypt. These components were

used at different concentrations, *i.e.* 0.0, 0.025, 0.05, 0.10, 0.25 and 0.5% (V/V) to study their effect on the linear growth of the tested fungi. The different concentrations of the tested citrus essential oil components and 0.3% Tween 80 were added separately to PDA medium under aseptic conditions beforc solidification and checked vigorously to insure even distribution of the tested components. Essential oils components free medium with 0.3% Tween 80 was used as a control. Inoculation and estimation of the linear growth of the tested pathogenic fungi were caried out as mentioned before.

5- Spore germination:

Different fungicides, lime fruit peel essential oils and citrus essential oil components were used to study their effect on the percentage of the tested fungi spore germination.

a) Effect of different fungicides:

Imazalil and thiabendazole (TBZ) at different concentrations, i.e. 0.000, 0.005, 0.010, 0.025, 0.050, 0.100, 25, 50, 100, 250 and 500 ppm were used to study their effect on the percentage of tested fungi spore germination.

b) Effect of different lime fruit peel essential oils:

Different aforementioned lime fruit peel essential oils concentrations, *i.e.* 0.00, 0.10, 0.25, 0.50 and 1.00% were used to study their effect on the percentage of tested fungi spore germination.

c) Effect of citrus essential oils components:

Different concentrations of citrus essential oils components mentioned in Table (1) (0.000, 0.025, 0.050, 0.100, 0.250 and 0.500%) were used to study their effect on percentage of the tested fungi spore germination. Percentage of spore germination was assessed using modified slide agar film method described by Pero and Owens (1971). Different concentrations of the aforementioned substances (fungicides, lime peel essential oils and citrus essential oil components) were added to 50 ml PDA medium. PDA medium without any additional treatments was used as control. Tween 80 (0.3%) was added as a detergent in case of lime and citrus essential oils components. Agar film was poured on sterilized slide in Petri dish. Drops of the spore suspention (100µl) of the different fungi (Prepared as mentioned by Aly *et al.*, 2003) were droped on the treated and untreated agar film. The slides were incubated at 20°C for 20 hours. Slides were inspected under light microscope and the percentage of spore germination was calculated.

6- Control of lime postharvest diseases by citrus essential oils components and fungicides under laboratory conditions:

The most effective citrus essential oil components inhibit the linear growth and spore germination of the tested fungi were used. Fresh lime fruits apparently free from physical damage or diseases were used. Fruits were surface disinfested using 5% Clorox for 3 minutes and gently scratched with sterilized needle. Wounded fruits (2) mm depth) were dipped in water wax containing the three effective 1 % of any of the tested fungicides (TBZ) and Imazalil.

Waxed fruits were air dried for 2h. Lime fruits were inoculated citrus essential oil components (Methyl anthranilate, Neryl acetate and Nerol at 0.0, 2 and 4%) and/ or with 200 ml of spore suspension (10^5 cfu) of *P.digitatium*, *P.italicum* and *G.candidum*. Three replicates were used for each particular treatment (each contained 5 fruits). The percentage of disease incidence was recorded after 5, 10 and 15 days. The percentage of the rotted part of fruits was calculated after 15 days and the percentage of the reduction was calculated from the following formula:

$$\textbf{The percentage of reduction} = \frac{\text{\% of control rotted part} - \text{\% of treatment rotted part}}{\text{\% of control rotted part}} \times 100$$

The obtained data were statistically analyzed according to split split plot design (Snedecor and Cochran, 1980). The differences between means were tested by LSD values at 5% .

RESULT AND DISCUSSION

1-Essential oils of lime fruit peel:

Data in Table (2) show the chemical components of lime essential oils extracted from lime fruit peel at 3, 5 and 7 months from fruit setting, respectively. Twelve compounds were identified in lime peel oils as monoterpene hydrocarbons by GC/MS. Limonene appear to be the main compound which recorded 74.989, 79.567 and 80.797 % for fruits of 3, 5 and 7months after fruit setting, followed by Myrcene (5.879, 6.358 and 6.441% after the same periods) and α-Pinene at 3 and 7 months as well as β-phelandrene at 5 months. It is also clear that, Camphene showed the lowest content at 3 and 7 months and P-Cymene at 5 months from fruit setting.

Table(1): Components of different hydrocarbon and oxygenated components of citrus fruit peel essential oils

Chemical. Composition	N0.	Components
Hydrocarbons Components	1	D- Limonene
	2	α - Pinene
	3	Caryophellene
Oxygenated Components	1	Methyl anthranilate
	2	Citral
	3	Neryl acetate
	4	Terpeniol
	5	Nerol
	6	Citronellol
	7	Citronellal
	8	Linalool
	9	Lenalyl acetate

Thirteen light oxygenated compounds were identified from lime fruit peel oils. β- Citral compound showed the highest content followed by α-Terpeniol at 3,5 and 7 months, while β-Terpeniol (trans) showed the lowest content at the same periods fruit setting. Ten sesquterpene hydrocarbon compounds were identified from lime fruit peel oils. Bergamotene (α Cis) showed the highest percentage at 3 and 5 months (2.732 % and 1.989 respectively) and β-Farnesene (Z) at 7 months (2.473%), followed by β-Farnesene (Z) at 3 and 5 months and Bergamotene (α-Cis) after 7 months. While Gurjunen at 3 months and Δ- Elemene at 5 months showed the lowest percentage.

Five heavy oxygenated compounds were identified from lime fruit peel oils. Neryl propanate showed the highest percentage at 3and 5 months (1.934 and 1.460 %, respectively) followed by O-hydroxy bi-phenyl at 3,7 and 5moth (1.281, 1.192 and 1.111%, respectively), while Ledol showed the lowest percentage at 5 months and caryophellen oxid and Spathulenol at 3 and 7 months. These results were in harmony with those obtained by Luigi *et al.*, (1997).

2- Effect of different fungicides:

Results presented in Table (3) indicate that, Imazalil fungicid was more effective on linear growth of the different fungi except *P.digitatum* (thibendazole was more

effective on it than imazalil). Imazalil caused complete inhibition of *P.digitatum* growth at 5 ppm, *P.italcum* at 0.25 ppm, *G.candidum* at 250 ppm and *A.niger* at 10 ppm. Thiabendazole caused complete inhibition of *P.digitatum* at 1.0 ppm, *P.italicum* at 0.5 ppm, *G. candidum* at 500 ppm and *A.niger* at 10 ppm. Using different fungicides for controlling postharvest diseases of citrus fruits was reported by Mahmoud and Hanafy (1991) and Droby *et al* (1999).

The variations obtained in the effect of the different fungicides on the tested fungi could be attributed to one or more of the following factors:

1) Degree of permeability of cell wall and / or plasmalemma of the fungus for the uptake and passage of the fungicides into fungal cell (Ward and Ragsdale, 1977 and Giffine. 1981).

2) Mode of action of the fungal cell to specific fungicides (Watkins *et al.*, 1977).

3) Chemical composition of the fungicides (Carnegi *et al.*, 1990)

The volatile oil from the exocarp of fresh citrus fruit exhibited strong toxicity towards several fungal pathogens being more active than several commercial fungicides (Singh *et al.*, 1993 and Caccioni and Guizzardi, 1994).

3- Effect of essential oils of lime fruit peel:

Results in Table (4) state that, lime essential oil was the highest effective on the linear growth reduction of the tested pathogenic fungi. The affectivity of lime essential oil decreased with increasing the fruit age. It is also clear that, *P. digitatum*, was not affected by fruit ages while, low effect on the other tested fungi was noticed. The antifungal efficacy of lime oils was studied by many investigators (Caccioni *et al.*, 1995, Rodov *et al*, 1995; Neirotti *et al.*, 1996 and Caccioni *et al.*, 1998).

The best results were obtained when orange and lemon oils. *P.digitatum* was found to be more sensitive to the inhibitory action of the oils than *P.italicum*. The efficacy of toxic lime essential oils decreased with the increasing fruit age. The resistance of young fruit to postharvest decay might be related to citral level in lemon flavedo.

Table (2): Chemical components of lime fruit peel essential oil

Components	R.T. * (min)	Concentration % Fruit age /mon. from fruit setting		
		3	5	7
A- Monoterpene hydrocarbons:				
1- Thujene	7.43	0.011	0.021	0.014
2- Tricyclene	7.58	0.093	0.092	0.137
3- α-Pinene	9.35	0.311	0.087	0.406
4- α- Fenchene	9.53	0.093	0.021	0.005
5- Camphene	9.57	0.001	0.080	0.006
6- Sabinene	10.12	0.010	0.051	0.013
7- β-Pinene	10.16	0.014	0.116	0.015
8- Myrcene	11.13	5.879	6.358	6.441
9- γ- Carene	11.25	0.021	0.026	0.024
10- P-Cymene	11.33	0.011	0.015	0.129
11- Limonene	13.31	74.989	79.567	80.797
12- β- Phelandrene	13.57	0.040	0.158	0.298
Total		**81.473**	**86.592**	**88.285**
B- Light Oxygenated compounds				
1- Linalool	14.06	0.287	0.332	0.191
2- β-Terpeniol (Trans)	14.26	0.016	0.092	0.057
3- β- Terpeniol (Cis)	15.49	0.827	0.306	0.629
4- α- Terpeniol	16.23	1.075	0.772	0.651
5- Decanone	16.25	0.004	0.151	0.339
6- Nerol	16.46	0.475	0.382	0.288
7- Citronellol	17.01	0.644	0.449	-
8- Neral	17.25	0.811	0.707	0.225
9- β-Citral	17.30	1.821	1.434	0.931
10- Geraniol	17.43	0.864	0.310	0.327
11- α- Citral	18.00	0.222	0.122	0.080
12- Neryl acetate	18.18	0.549	0.409	0.166
13- Geranyl acetate	18.56	-	-	0.135
Total		**7.595**	**5.466**	**4.019**

Table (2): cont.

C- Hydrocarbons Sesquterpene				
1- Elemene	18.12	0.420	0.004	0.256
2- Elemene	19.14	0.647	0.043	0.345
3- Bergamotene (α-Cis)	20.10	2.732	1.989	1.572
4- Caryophellene (E)	20.33	0.681	0.483	0.263
5- Aromadendrene	20.38	0.009	0.013	0.005
6- β- Gurjunene	20.56	0.003	0.191	0.145
7- β- Farnesene (Z)	21.31	1.891	1.335	2.473
8- - Gurjunene	21.45	0.003	0.167	0.015
9- γ- Muurolene	21.52	0.025	0.087	0.058
10- Selenene	23.07	0.245	0.388	0.116
Total		**6.656**	**4.696**	**5.248**
D- Heavy Oxygenated compounds				
1- Neryl Propanate	21.39	1.934	1.460	0.938
2- O-Hydroxy bi-phenyl	22.30	1.281	1.111	1.192
3- Spathulenol	23.31	0.429	0.297	0.037
4- Caryophellene oxid	23.50	0.301	0.253	0.144
5- Ledol	24.13	0.331	0.125	0.137
Total		**4.276**	**3.246**	**2.448**

*Retention time /min

Table (3): Effect of different concentrations of the tested fungicides on the linear growth (mm) of the tested fungi(12 days after incubation at 25°C).

Fungicides	Conc. (ppm)	*P.digitatum*	*P.italicum*	*G.candidum*	*A.niger*
Imazalil	0.00	90.0	90.0	90.0	90.0
	0.10	61.3	22.0	90.0	90.0
	0.25	54.7	0.0	90.0	80.7
	0.50	47.3	0.0	90.0	68.0
	1.00	39.0	0.0	90.0	55.7
	2.50	34.3	0.0	90.0	41.3
	5.00	0.0	0.0	90.0	28.0
	10.00	0.0	0.0	87.7	0.0
	25.00	0.0	0.0	66.7	0.0
	50.00	0.0	0.0	46.3	0.0
	100.00	0.0	0.0	28.0	0.0
	250.00	0.0	0.0	0.0	0.0
	500.00	0.0	0.0	0.0	0.0
Average		**25.1**	**8.6**	**66.1**	**34.9**
Thiabendazole (TBZ)	0.0	90.0	90.0	90.0	90.0
	0.10	85.7	47.3	90.0	90.0
	0.25	74.3	35.7	90.0	90.0
	0.50	63.7	0.0	90.0	90.0
	1.00	0.0	0.0	90.0	79.7
	2.50	0.0	0.0	90.0	67.3
	5.00	0.0	0.0	90.0	55.0
	10.00	0.0	0.0	86.7	0.0
	25.00	0.0	0.0	73.0	0.0
	50.00	0.0	0.0	60.3	0.0
	100.00	0.0	0.0	55.7	0.0
	250.00	0.0	0.0	42.3	0.0
	500.00	0.0	0.0	0.0	0.0
Average		**24.1**	**13.3**	**72.9**	**43.2**

L.S.D at 5 % level for					
Fungicides	**(A):**	**0.1**	**Conc. (C):**	**0.3**	
Fungi	**(B):**	**0.1**	**(AC):**	**0.4**	
	(AB):	**0.2**	**(BC):**	**0.5**	
			(ABC):	**0.7**	

Table (4): Effect of different concentrations of lime fruit peel essential oils on linear growth (mm) of tested fungi(12 days after incubation at 25 C).

Essential oil	Fruit age/ month	Conc. (%)	Different fungi			
			P.digiltaum	*P.italicum*	*G.candidum*	*A.niger*
Lime oil	3	0.00	90.0	90.0	90.0	90.0
		0.05	32.0	27.3	65.7	20.3
		0.10	0.0	0.0	44.0	0.0
		0.25	0.0	0.0	0.0	0.0
		0.50	0.0	0.0	0.0	0.0
		1.00	0.0	0.0	0.0	0.0
	Average	-	**20.3**	**19.6**	**33.3**	**19.4**
	5	0.0	90.0	90.0	90.0	90.0
		0.05	90.0	54.7	90.0	73.3
		0.10	81.3	32.0	87.0	63.3
		0.25	0.0	0.0	63.3	44.3
		0.50	0.0	0.0	33.7	35.7
		1.00	0.0	0.0	0.0	0.0
	Average	-	**43.6**	**29.5**	**60.7**	**51.1**
	7	0.0	90.0	90.0	90.0	90.0
		0.05	90.0	88.7	90.0	80.0
		0.10	85.3	76.0	90.0	67.7
		0.25	0.0	0.0	83.3	53.3
		0.50	0.0	0.0	53.0	40.0
		1.00	0.0	0.0	0.0	0.0
	Average	-	**44.2**	**42.5**	**67.7**	**55.2**
	Grand av.	-	**36.0**	**30.5**	**53.9**	**41.6**

L.S.D at 5 % level for:				
	Fungi	(A)	:	0.2
	Age	(B)	:	0.3
		(A B)	:	0.2
	Conc.	(C)	:	0.4
		(A C)	:	0.4
		(B C)	:	0.7
		(A B C)	:	0.7

The flavedo of green lemon contained 1.5-2 times higher levels of citral comparing with the yellow fruits (Rodov *et al.*, 1995). Citrus essential oils have several components which are differed in their antifungal activities (Caccioni *et al.*, 1995 and Neirotti *et al.*, 1996).

4-Effect of different citrus essential oil components:

Results in Table (5) show that, citrus essential oils components differed in their inhibitory effect on the linear growth of lime fruit rot pathogenic fungi. The oxygenated components (Methyl anthranilate, Citral, Neryl acetate, Terpeniol and Nerol) were more toxic than the hydrocarbons component, i.e. Limonene, α-Pinene and Caryophellene. The antifungl activity of different citrus essential oil components was reported by Tripathi *et al.* (1984), Caccioni *et al.*, (1995) and Caccioni *et al* (1998).

Difference among components of each other against postharvest fungi might be due to their structures, solubility, and capability of penetrate cell wall and to interfere with the enzymatic reaction (Knobloch *et al.*, 1989 and Suprapta *et al.*, 1997).

5-Effect of different fungicides on spore germination:

Results shown in Table (6) show that, imazalil and thiabendazole affected spore germination of tested fungi with different degrees. Spore germination was severely affected with low concentration of imazalil, while higher concentration of thiabendazole were needed for the same effect of imazalil. *P.italicum* and *A.niger* were the most sensitive fungi to imazalil as their spore germination was completely inhibited at 0.01 ppm. On the other hand, spores of *P.digitatum* completely inhibited at 0.025 ppm. On the other hand, spores of *G.candidum* were the most resistant ones which their germination was completely inhibited at 100 ppm.

Thiabendazole was less toxic to spore of the tested fungi than imazalil except in case of *P.digitatum* spores.

The explanation of such results might be due to the differences in the fungicide mode of action, their selective harmful effect on the pathogens metabolism or killing the pathogen (Okuno, *et al.*, 1989).

6-Effect of lime essential oil:

Data presented in Table (7) show that, lime essential oil was the most effective one the percentage of spore germination of all the tested fungi. The effectivity of lime essential oil was decreased by increasing fruit age. Lime essential oils obtained from fruits of three months age caused complete inhibition to *P.digitatum, P. italicum* and *A.niger* at 0.25%, while *G.candidum* was completely inhibited at 0.5%. Lime essential oils extracted from fruits of 5 and 7 months age completely inhibited the germination spores of *P.digitatum*, *P.italicum* and *G.candidum* at 0.5 %, while *A.niger* was completely inhibited at 0.25%.

7-Effect of different components of citrus essential oils on spore germination:

Results in Table (8) indicate that, hydrocarbon compounds had no effect on spore germination.

As for oxygenated compounds, the most effective treatments were Citral and Methyl anthranilate. They caused complete inhibition of spore germination of the tested fungi at 0.05%followed by Terpeniol, Nerol , Neryl acetate and citronellol at 0.25% conc. In this respect the other components were less effective.

Methyl anthranilate, Neryl acetate and Nerol (the most compatible components) as well as different fungicides, (Imazalil and TBZ) were applied on lime fruits to control green and blue mould, as well as sour rot diseases under artificial inoculation.

8-Effect of citrus essential oil components and fungicides on lime postharvest diseases:

Results presented in Table (9) reveal that, all the tested treatments reduced the percentage of postharvest disease incidence of lime fruits.

Complete protection of both moulds was obtained with methyl anthranilate 4% for 15 days storage. The highest protection of green and blue moulds was obtained with methyl anthranilate at 2%, and Imazalil as well as TBZ at 1 %. The same trend was obtained in case of rotted part. These results are in harmony with those obtained by Rodov *et al.*, (1995); Neirotti *et al.*, (1996); Suprapta *et al.*, (1997); Caccioni *et al.*, (1998); Duccio *et al.*, (1998) and Vargas *et al.*, (1999).

The protective effect of methyl anthranilate might be due to its structures, solubility and capacity to penetrate cell wall and to interfere with the enzymatic reaction (Suprapta *et al.*, 1997).

Table (5): Effect of lime essential oil components at different concentrations on the linear growth (mm) of the tested fungi (12 days after incubation at 25°C).

Chemical structure	Treatment	Conc.	*P.digitatum*	*P.italicum*	*G.candidum*	*A.niger*
Oxygenated Compunds	Methyl anthranilate	0.00	90.0	90.0	90.0	90.0
		0.025	27.7	34.7	60.7	59.0
		0.50	0.0	0.0	0.0	0.0
		0.100	0.0	0.0	0.0	0.0
		0.250	0.0	0.0	0.0	0.0
		0.500	0.0	0.0	0.0	0.0
	Average		19.6	20.8	25.1	24.8
	Citral	0.00	90.0	90.0	90.0	90.0
		0.025	90.0	90.0	31.7	84.3
		0.050	90.0	90.0	17.7	68.3
		0.100	0.0	0.0	0.0	0.0
		0.250	0.0	0.0	0.0	0.0
		0.500	0.0	0.0	0.0	0.0
	Average		45.0	45.0	23.2	40.4
	Neryl acetate	0.00	90.0	90.0	90.0	90.0
		0.025	90.0	90.0	79.7	90.0
		0.50	71.3	68.3	55.3	87.3
		0.100	35.7	46.0	0.0	0.0
		0.250	0.0	0.0	0.0	0.0
		0.500	0.0	0.0	0.0	0.0
	Average		47.8	49.1	37.5	44.6
	Terpeniol	0.00	90.0	90.0	90.0	90.0
		0.025	90.0	85.0	78.7	73.3
		0.050	77.3	63.7	24.0	57.7
		0.100	57.7	41.3	24.0	0.0
		0.250	0.0	0.0	0.0	0.0
		0.500	0.0	0.0	0.0	0.0
	Average		52.5	46.7	32.1	36.8
	Nerol	0.000	90.0	90.0	90.0	90.0
		0.025	90.0	90.0	80.7	42.3
		0.050	80.3	61.3	57.7	17.0
		0.100	65.7	43.0	0.0	0.0
		0.250	0.0	0.0	0.0	0.0
		0.500	0.0	0.0	0.0	0.0
	Average		54.3	47.4	38.1	24.9
	Citronellol	0.000	90.0	90.0	90.0	90.0
		0.025	90.0	90.0	63.3	65.7
		0.050	81.7	90.0	37.7	43.3
		0.100	633	77.7	12.3	21.3
		0.250	41.3	29.0	0.0	0.0
		0.500	0.0	0.0	0.0	0.0
	Average		61.1	62.8	33.9	36.7

Table (5): Cont.

	Citronellal	0.000	90.0	90.0	90.0	90.0
		0.025	90.0	90.0	90.0	90.0
		0.050	86.3	90.0	66.3	87.7
		0.100	69.7	87.7	39.7	75.3
		0.250	44.3	63.3	13.3	51.7
		0.500	0.0	0.0	0.0	0.0
	Average		63.4	70.2	49.9	65.8
	Linalool	0.000	90.0	90.0	90.0	90.0
		0.025	90.0	90.0	90.0	90.0
		0.50	90.0	90.0	90.0	74.3
		0.100	90.0	76.7	85.3	51.7
		0.250	75.3	55.3	68.0	27.3
		0.500	0.0	0.0	0.0	0.0
	Average		72.6	67.0	70.6	55.6
	Lenalyl acetate	0.000	90.0	90.0	90.0	90.0
		0.025	90.0	90.0	90.0	90.0
		0.50	90.0	90.0	90.0	90.0
		0.100	90.0	79.3	90.0	76.7
		0.250	73.3	53.7	78.0	55.3
		0.500	57.7	24.0	53.0	23.0
	Average		81.8	71.2	81.8	70.8
Hydrocarbons compounds	Limonene	0.000	90.0	90.0	90.0	90.0
		0.025	90.0	90.0	90.0	90.0
		0.050	90.0	90.0	90.0	90.0
		0.100	90.0	90.0	90.0	90.0
		0.250	86.3	90.0	90.0	90.0
		0.500	77.0	87.0	90.0	87.3
	Average		87.2	84.5	90.0	89.6
	α- Pinene	90.0	90.0	90.0	90.0	90.0
		0.025	90.0	90.0	90.0	90.0
		0.050	90.0	90.0	90.0	90.0
		0.100	90.0	90.0	90.0	90.0
		0.250	88.0	90.0	90.0	90.0
		0.500	72.3	82.3	85.0	90.0
	Average		86.7	88.7	89.2	90.0
	Caryophellene	0.000	90.0	90.0	90.0	90.0
		0.025	90.0	90.0	90.0	90.0
		0.050	90.0	90.0	90.0	90.0
		0.100	90.0	90.0	90.0	90.0
		0.250	85.7	90.0	90.0	82.7
		0.500	75.0	79.0	85.3	69.3
	Average		86.8	88.2	84.2	85.3

L.S.D at 5 % level for :	Fungi.	(A)	:	0.1
	Components	(B)	:	0.2
		(A B)	:	0.4
	Conc.	(C)	:	0.1
		(A C)	:	0.2
		(B C)	:	0.4
		(A B C)	:	0.9

Table (6): Effect of different fungicides on spore germination percentage of the tested pathogenic fungi (after 24h at 25°C).

Fongicides	Conc. (ppm)	*P. digitatum*	*P. italicum*	*Gcandidum*	*A. niger*
Imazalil	0.000	100	100	100	100
	0.005	16	22	100	6
	0.010	11	0	100	0
	0.025	0	0	100	0
	0.050	0	0	100	0
	0.100	0	0	100	0
	25.000	0	0	100	0
	50.00	0	0	76	0
	100.00	0	0	0	0
	250.000	0	0	0	0
	500.000	0	0	0	0
	Average	**11.5**	**11.1**	**70.5**	**9.6**
Thiabendazole (TBZ)	0.000	100	100	100	100
	0.005	12	52	100	76
	0.010	0	34	100	49
	0.025	0	17	100	21
	0.050	0	0	100	0
	0.100	0	0	100	0
	25.000	0	0	94	0
	50.00	0	0	77	0
	100.00	0	0	56	0
	250.000	0	0	24	0
	500.000	0	0	0	0
	Average	**10.2**	**18.5**	**77.4**	**22.4**

L.S.D at 5 % level for :				
	Fungicides.	(A)	:	0.2
	Fungi.	(B)	:	0.3
		(A B)	:	0.5
	Conc.	(C)	:	0.6
		(A C)	:	0.8
		(B C)	:	1.1
		(A B C)	:	1.6

Table (7): Effect of lime fruit peel essential oils on the spore germination percentage of the tested fungi (after 24h at 25°C).

Treatment	Fruit age (mon.)	Conc. (%)	*P.digitatum*	*P. italicum*	*G.candidum*	*A. niger*
Lime oil	3	0.00	100	100	100	100
		0.10	57	50	94	39
		0.15	0	0	11	0
		0.50	0	0	0	0
		1.00	0	0	0	0
	Average	-	**31.4**	**30.0**	**41.0**	**23.2**
	5	0.00	100	100	100	100
		0.10	60	54	100	94
		0.25	34	29	78	0
		0.50	0	0	0	0
		1.00	0	0	0	0
	Average		**38.8**	**36.6**	**55.6**	**38.8**
	7	0.00	100	100	100	100
		0.10	71	66	100	100
		0.25	38	35	100	0
		0.50	0	0	0	0
		1.00	0	0	0	0
	Average		**41.8**	**40.2**	**60.0**	**40.0**
	General av.		**37.3**	**35.6**	**52.5**	**34.0**

L.S.D at 5 % level for :	Fungi	(A)	: 1.1
	Age.	(B)	: 0.9
		(A B)	: 1.8
	Conc.	(C)	: 1.2
		(A C)	: 2.3
		(B C)	: 2.0
		(A B C)	: 4.1

Table (8): Effect of different concentrations of citrus essential oils components on the percentage of spore germination of the tested fungi (after 24h at 25°C).

Chemicals	Treatment	Conc. %	*P.digitatum*	*P.italicum*	*G.candidum*	*A.niger*
Oxygenated Compounds	Methyl anthranilate	0.000	100	100	100	100
		0.025	65	61	51	41
		0.050	0	0	0	0
		0.100	0	0	0	0
		0.250	0	0	0	0
		0.500	0	0	0	0
	Average		**27.5**	**26.8**	**25.2**	**23.5**
	Citral	0.000	100	100	100	100
		0.025	35	31	58	22
		0.050	0	0	0	0
		0.100	0	0	0	0
		0.250	0	0	0	0
		0.500	0	0	0	0
	Average		**22.5**	**23.8**	**26.3**	**20.3**
	Neryl acetate	0.000	100	100	100	100
		0.025	100	100	100	72
		0.050	100	95	91	44
		0.100	82	60	0	0
		0.250	0	0	0	0
		0.500	0	0	0	0
	Average		**63.7**	**59.2**	**48.5**	**36.0**
	Terpeniol	0.000	100	100	100	100
		0.025	95	100	100	73
		0.050	71	95	97	20
		0.100	0	0	19	0
		0.250	0	0	0	0
		0.500	0	0	0	0
	Average		**44.3**	**49.2**	**52.7**	**32.3**

Table (8): Cont.

	Nerol	0.000	100	100	100	100
		0.025	100	100	100	96
		0.050	94	94	99	74
		0.100	0	61	0	0
		0.250	0	0	0	0
		0.500	0	0	0	0
	Average		**49.0**	**59.2**	**49.8**	**45.0**
	Citronellol	0.000	100	100	100	100
		0.025	81	72	77	54
		0.050	49	34	42	22
		0.100	21	10	12	0
		0.250	0	0	0	0
		0.500	0	0	0	0
	Average		41.8	36.0	38.0	38.5
	Citronellal	0.000	100	100	100	100
		0.025	100	100	100	46
		0.050	92	93	91	12
		0.100	73	50	74	0
		0.250	20	0	52	0
		0.500	0	0	0	0
	Average		64.2	57.2	69.5	26.3
	Linalool	0.000	100	100	100	100
		0.025	76	100	100	100
		0.050	59	96	100	95
		0.100	49	82	89	78
		0.250	39	32	65	0
		0.500	0	27	14	0
	Average		53.8	72.8	78	62.2
	Lenalyl acetate	0.000	100	100	100	100
		0.025	100	100	100	100
		0.050	99	100	100	100
		0.100	95	100	100	98
		0.250	92	100	100	96
		0.500	87	100	100	89
	Average		95.5	100.0	100.0	97.2
Hydrocarbon components	Limonene	0.000	100	100	100	100
		0.025	100	100	100	100
		0.050	100	100	100	100
		0.100	100	100	100	100
		0.250	100	100	100	100
		0.500	100	100	100	100
	Average		100.0	100.0	100.0	100.0

Table (8): Cont.

	α- pinene	0.000	100	100	100	100
		0.025	100	100	100	100
		0.050	100	100	100	100
		0.100	100	100	100	100
		0.250	100	100	100	100
		0.500	100	100	100	100
	Average		100.0	100.0	100.0	100.0
	Caryophellene	0.000	100	100	100	100
		0.025	100	100	100	100
		0.050	100	100	100	100
		0.100	100	100	100	100
		0.250	100	100	100	100
		0.500	100	100	100	100
	Average		100.0	100.0	100.0	100.0

L.S.D at 5 % level for :	Fungi	(A)	:	0.2
	Components	(B)	:	0.4
		(A B)	:	0.8
	Conc.	(C)	:	0.3
		(A C)	:	0.6
		(B C)	:	1.0
		(A B C)	:	1.9

Table (9): Effect of different citrus essential oil components on the percentage of green and blue moulds as well as sour rot incidence and the percentage of rotted part of lime fruits after 15 days storage at room temperature.

Teatments	Conc. %	Percentage of disease incidence (after days)									Percentage of rotted part of fruit					
		Green mould			Blue mould			Sour rot			Green mould		Blue mould		Sour rot	
		5	10	15	5	10	15	5	10	15	% rotted part	% reduction	% rotted part	% Reduction	% rotted part	% reduction
Methyl anthranilate	2	0.0	0.0	6.7	0.0	0.0	13.3	0.0	0.0	6.7	5.6	94.4	8.6	90.6	5.1	94.5
	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	0.0	100.0
Neryl acetate	2	20.0	40.0	80.0	33.3	60.0	80.0	0.0	26.7	40.0	70 9	29.1	67.8	25.7	32.4	64.9
	4	13.3	33.3	66.7	26.7	46.7	60.0	0.0	20.0	26.7	53.4-	46.6	48.1	47.3	19.6	78.8
Nerol	2	40.0	60.0	100.0	40.0	66.7	80.0	26.7	53.3	600	88.6	11.4	70.1	23.2	51.9	43.8
	4	33.3	46.7	86.7	40.0	53.3	73.3	20.0	40.0	46.7	81.2	18.8	57.4	37.1	37.8	59.1
Imazalil	1	0.0	6.7	20.0	0.0	0.0	13.3	0.0	13.3	20.0	15.4	84.6	10.0	89.0	12.2	86.8
Thiabendazole	1	6.7	13.3	33.3	0.0	6.7	20.0	13.3	33.3	40.0	32.7	76.3	14.5	84.1	31.7	65.7
Water wax	-	46.7	73.3	100.0	40.0	66.7	100.0	33.3	46.7	100.0	100.0	0.0	91.3	4.6	92.4	3.9
Control	-	46.7	86.7	100.0	46.7	66.7	100.0	33.3	53.3	100.0	100.0	0.0	95.7	0.0	96.1	0.0

L.S.D at 5 % level for :	Disease incidence			Green Mould	Blue Mould	Sour Rot
	Interval	(A)	:	5.8	5.2	5.7
	Components	(B)	:	7.5	6.8	7.0
		(A B)	:	13.0	11.7	12.1
	Conc.	(C)	:	4 7	N.S.	N.S.
		(A C)	:	N.S.	N.S.	N.S.
		(B C)	:	N.S.	N.S.	9.9
		(A B C)	:	18.4	N.S.	N.S.
L.S.D at 5 % level for :	Fruit rotted part			Green Mould	Blue Mould	Sour Rot
	Components	(A)	:	7.5	7.2	5.0
	Conc.	(B)	:	N.S	4.5	N.S.
		(A B)	:	10.6	10.2	7.0

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مقاومة أعفان ثمار الليمون المالح بعد الجمع باستخدام بعض مكونات الزيوت الطيارة

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**** قسم أمراض النبات – المركز القومي للبحوث**

تعد ثمار الليمون المالح من أهم محاصيل الفاكهة التصديرية في مصر وكذلك للاستهلاك المحلي وتصاب هذه الثمار بعدد من الأمراض بعد الجمع وأثناء النقل والتسويق ويمكن تلخيص النتائج التى تم الحصول عليها في النقاط الآتية:

تم تحليل زيت الليمون الطيار الذى تم استخلاصه من ثمار ذات أعمار ٥،٣،٧ شهور من العقد وذلك بطريقة GC/MS وأوضحت النتائج أن مادة الليمونين هي المادة الرئيسية في تركيب الزيت وتمثل المركبات الفا-سترال ، بيتا-سترال ، نيريل أسيتات ، نيرول ، تربينول نسب متفاوته في تركيب الزيت.

أدى استخدام زيت الليمون الطيار بتركيز ٠,٢٥ % إلى التثبيط الكامل لنمو الفطريات المختبرة وكذلك إنبات جراثيم.

تم اختبار ١٢ مادة من مكونات زيت الليمون المالح على نمو الفطريات المختبرة و إنبات جراثيمها وأوضحت النتائج أن مثيل أنثرانيليت ، سترال ، نيريل أسيتات ، نيرول ، تربينول ، كانت مواد الأكثر كفاءة فى تثبيط النمو وإنبات الجراثيم.

تم معاملة ثمار الليمون المالح بكل من ميثيل انثرانيليت ، نيريل أسيتات، نيرول بالإضافة إلى المبيدين إيمازاليل، الثيابندازول كمقارنة وأوضحت النتائج أن مادة ميثيل أنثرانيليت كانت أفضل المعاملات في مقاومة أعفان ثمار الليمون.

وتشير النتائج إلى إمكانية استخدام مادة ميثيل أنثرانيليت كبديل آمن للمبيدات في مقاومة أمراض ما بعد الجمع في ثمار الليمون المالح على نطاق تجارى.