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Combination Between Citral and Chitosan for Controlling Sour rot Disease of Lime Fruits

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Abstract: Sour rot caused by *Giotrichum candidum* is the most important disease affecting lime fruits. Effect of citral and chitosan as single or in combination for controlling sour rot disease of lime fruits was studied. Complete inhibition of linear growth and spore germination of *G. candidum* was obtained with citral at concentrations of 5.0 ml / l and chitosan at 8.0 g / l. The promising concentrations of citral *i.e* 4.0 & 5.0 ml / l and chitosan at 6.0 & 8.0 g / l were applied as single or in combination against sour rot disease. Fresh lime fruits were artificially wounded using sterilized scalpel and inoculated with spore suspension (10^6 spores/ml) of *G. candidum* then treated with citral and /or chitosan.Results indicate that the most effective treatments are combined treatments between citral at 4.0 or 5.0 ml / l and chitosan at 6.0 or 8.0 g / l which reduced the disease incidence and rotted part tissue more than 89.5 and 93.5% respectively.While single treatments of each concentration of citral and chitosan reduced the sour rot incidence and rotted part tissue more than 81.5 and 84.5 %. respectively. It could be suggested that combination between citral and chitosan as fruit coating considered as one of applicable safely for controlling postharvest diseases of lime fruits.

Key words: lime fruits - Sour rot - citral – chitosan- postharvest disease

INTRODUCTION

Sour rot caused by *Giotrichum candidum* is the most important disease affecting lime fruits[1,2,3,4]. There is a growing need to develop alternative approaches for controlling postharvest diseases of citrus fruits.

Essential oils of citrus or their constituents are shown to have fungicidal activities against postharvest pathogens of citrus[5,6,7]. In this regard, French, *et al*.,[5] stated that citral is the most effective constituent of citrus essential oil. Rodov *et al*., [8] reported that resistance of young lemon fruit to decay development is related to citral level in lemon flavedo. They added that flavedo of green lemon contained 1.5-2 times higher levels of citral comparing with the yellow fruit. The inhibitory effect of citral on postharvest pathogens was reported by Asthana *et al*., [9]. Using of citral for controlling postharvest diseases of navel orange was reported by Abd-El-Kareem and Abd-Alla[10].

Chitosan, a natural biopolymer with antifungal and eliciting properties, also, a common food additive with antifungal properties, able to reduce postharvest decay of table grapes was reported by Gianfranco *et al*., [11]. The antifungal activity of chitosan were examined at various concentrations against fungi including Penicillium digitatum, Penicillium italicum, Botrydiplodia lecanidion and Botrytis cinerea. The effectiveness of these chitosans to control the post-harvest quality of Tankan fruit [12]. The effect of chitosan coating on disease incidence was also evaluated. The application of chitosan coating reduced respiration rate and weight loss, delayed the increase in PPO activity and the changes in colour, and eating quality, and partially inhibited decay of fruit during storage [13]. They added that integration of preharvest spray with *C. laurentii* and postharvest chitosan coating treatment may be a promising management strategy for decay control and quality maintenance of table grapes. Chitosan is seafood industry was reported by many investigators as a protective safe material against many pathogens [14,15]. Coating fruits with chitosan decreased postharvest diseases of tomato, strawberry and lime fruits [16,17,18,19]. Du *et al*., [20] reported that coating fruits with chitosan reduced the respiration rate, ethylene production, interval O_2 levels and increased the interval CO_2 of peach and pear fruits.They added that coated fruits were markedly firmer and less mature of the end of storage.

Moreover, chitosan, is able to extend storage life and to control decay of strawberries, apples, peaches, pears, kiwfruit, cucumbers sweet cherries, and citrus fruit [21,22,23,24].

The objective of this study is to evaluate the effect of combination between citral and chitosan for controlling sour rot disease of lime fruits.

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MATERIALS AND METHODS

Inhibitory Effect of Citral and Chitosan on the Linear Growth of *G. candidum in vitro*: Citral solutions at concentrations of 0.0, 2, 3, 4 and 5 ml/l and chitosan at 2, 4, 6 and 8 g / l were tested against linear growth of *G. candidum in vitro*. Citral and chitosan solutions were added to conical flasks containing PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri plates (9 cm – diameter). Plates were individually inoculated at the center with equal disks (6-mm- diameter) of 10 days old culture of *G. candidum*.

Inoculated plates were incubated at 20±2C°.The average linear growth of fungus was calculated after 10 days. Each treatments was represented with 5 replicates and 5 plates for each replicate were used.

Inhibitory Effect of Citral and Chitosan on Spore Germination of *G. candidum*: Citral solutions at concentrations of 0.0, 2, 3, 4 and 5 ml/l and chitosan at 2, 4, 6 and 8 g / l were tested to study their inhibitory effect on spore germination of *G. candidum*. Spores of 10-days -old cultures of *G. candidum* were harvested in sterilized water (containing 0.01% Tween 80) then adjusted to reach concentration of 10^6 spores / ml. One ml of spore suspension was placed in Petri plates. PDA media containing previous concentrations of both treatments were poured before solidifying into the previous inoculated plates and rotated gently to ensure even distribution of fungal spores. Inoculated plates were incubated at 20°C for 24 h. Germinated spores were counted microscopically and percentage of spore germination was calculated.

Testing of citral and chitosan against sour rot disease of lime fruits

Different concentrations of Citral and chitosan were tested to study their effect on sour rot disease of lime fruits. Fresh lime fruits apparently free from physical damage and diseases were artificially wounded using sterilized scalpel. Inoculation of wounded fruits was carried out by spraying fruits with spore suspension (10^6 spores/ml) of *G. candidum* then air dried at room temperature, 23-25C°. Inoculated fruits were dipped in citral solutions at concentrations of 0.0, 1.0,2.0, 3.0, 4.0 and 5.0 ml / l or chitosan at concentrations 2.0, 4.0, 6.0 and 8.0 g / l containing 0.01% Tween 80 for 3 min, then air dried. All treated or un-treated (control) lime fruits were placed into carton boxes at the rate of 30.0 fruits / box. Each particular concentration as well as control treatment were represented by one carton box. All boxes were stored at 20±2C° for 15 days. Percentage of infected fruits (disease incidence) and rotted parts of fruits (disease severity) were recorded.

Testing of citral and chitosan applied as single or in combination against sour rot disease of lime fruits

The promising concentrations of citral and chitosan were applied as single or in combination treatments as follow :-

Treatments	
Combined	Single
Citral 4.0 + Chitosan 6.0	Citral 4.0 m/ l
Citral 4.0 + Chitosan 8.0	Citral 5.0 ml / l
Citral 5.0 + Chitosan 6.0	Chitosan 6.0 g / l
Citral 5.0 + Chitosan 8.0	Chitosan 8.0 g / l
Control	

Fresh lime fruits apparently free from physical damage and diseases were artificially wounded using sterilized scalpel. Inoculation of wounded fruits was carried out by spraying fruits with spore suspension (10^6 spores/ml) of *G. candidum* then air dried at room temperature, 23-25C°. Inoculated fruits were dipped previous treatments. Storage and disease assessment were carried out as mentioned before.

Assessment of Diseases: Disease incidence expressed as percentage of infected fruits

The percentage of rotted part of fruit was calculated from the following formula:-

$$\%\text{of rotted part of fruit} = \frac{\text{Rotted part weight}}{\text{fruit weight}} \times 100$$

Statistical Analysis: Tukey test for multiple comparison among means was utilized[25].

Results:

Inhibitory Effect of Citral and Chitosan on the Linear Growth and Spore Germination of *G. candidum in vitro*: Citral solutions at concentrations of 0.0, 2, 3, 4 and 5 ml/l and chitosan at 2, 4, 6 and 8 g / l were tested to study their inhibitory effect against linear growth and spore germination of *G. candidum in vitro*. Results in Table (1) indicate that complete inhibition of linear growth and spore germination of *G. candidum* was obtained with citral at concentrations of 5.0 ml / l and chitosan at 8.0 g / l. The highest reduction was obtained with citral at 3.0 ml / l which reduced the linear growth and spore germination by 76.7 and 100.0 respectively. Meanwhile other treatments showed moderate effect.

Effect of Citral and Chitosan on Sour Rot Disease of Lime Fruits: Citral solutions at concentrations of 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 ml / l or chitosan at concentrations 2.0, 4.0, 6.0 and 8.0 g / l were tested to study their effect on sour rot disease of lime fruits.

Table 1: Effects Citral and chitosan on linear growth (mm) and spore germination (%) of *G. candidum*

Salts	Conc./ l	*G. candidum*			
		Linear growth	Reduction %	Spore germination	Reduction %
Citral	2.0	45.0 c	50.0	19.0 c	80.0
	3.0	21.0 d	76.7	0.0 d	100.0
	4.0	5.0 e	100.0	0.0 d	100.0
	5.0	0.0 e	100.0	0.0 d	100.0
Chitosan	2.0	70.0 b	22.2	43.0 B	54.7
	4.0	40.0 c	55.6	22.0 c	76.8
	6.0	7.0 e	100.0	0.0 d	100.0
	8.0	0.0 e	100.0	0.0 d	100.0
Control	0.0	90.0 a	—	95.0 a	

Figures with the same letter are not significant (P=0.05).

Results in Table (2) indicate that all treatments significantly reduced the disease incidence. The most effective treatments are citral ant concentrations 4.0 & 5.0 ml / l and chitosan at 6.0 & 8.0 g / l which reduced the sour rot incidence more than 82.0 %. Followed by citral at 3.0 ml / l and chitosan at 4.0 g / l which reduced the disease incidence by 69.0 and 75.5 respectively. Other treatments showed moderate effect.

Effect of citral and chitosan applied as single or in combination on sour rot disease of lime fruits

The promising concentrations of citral i.e 4.0 & 5.0 ml / l and chitosan at 6.0 & 8.0 g / l were applied as single or in combination for controlling sour rot disease of lime fruits.

a- Disease Incidence: Results in Table (3) indicate that all treatments significantly reduced the sour rot disease. The most effective treatments are combined treatments between citral at 4.0 or 5.0 ml / l and chitosan at 6.0 or 8.0 g / l which reduced the disease incidence more than 89.5 %. Wile single treatments of each concentration of citral and chitosan reduced the sour rot incidence more than 81.5 %.

b- Rooted Part Tissue: Results in Table (3) indicate that all treatments significantly reduced rotted part tissue of lime fruits. The most effective treatments are combined treatments between citral at 4.0 or 5.0 ml / l and chitosan at 6.0 or 8.0 g / l which reduced the rotted tissue part more than 93.5 %. Wile single treatments of each concentration of citral and chitosan reduced the rotted tissue part more than 84.5 %.

Discussion: Lime fruits is the most important exportation fruits.Postharvest decay of lime fruits caused by *Giotrichum candidum* (sour rot)is the most important factor affecting harvested lime fruits during handling, transportation, exportation and storage [2,4].

There is a growing need to develop alternative approaches for controlling postharvest diseases of lime fruits.

Essential oils of citrus or their constituents are shown to have fungicidal activities against postharvest pathogens of citrus [5, 6]. In present study citral essential oils at 5.0 ml / l caused complete inhibition of linear growth and spore germination of *G. candidum In Vitro.* Moreover, citral at the same concentrations controlled the sour rot disease of lime fruits when applied as single or combined with chitosan treatment. In this regard, Rodov *et al*., [8] reported that young mature – green lemon fruit manifests a significant lower level of postharvest decay compared with older yellow fruit because resistance of young fruit to decay development is related to citral level in lemon flavedo. They added that the flavedo of green lemon contained 1.5-2 times higher levels of citral comparing with the yellow fruit. Also during long – term storage of lemon fruit, citral concentrations decreased in parallel with the decline of antifungal activity in the peel which reflected in the increase of decay incidence. The inhibitory effect of citral on postharvest pathogens was also reported by Asthana *et al*., [9]. Using of citral for controlling postharvest diseases of navel orange was reported by Abd-El-Kareem and Abd-Alla [10].

Chitosan is seafood industry was reported by many investigators as a protective safe material against many pathogens [12,15].

The mechanism by which chitosan affects the growth of several phytopathogenic fungi has not been fully elucidated, but several hypotheses have been postulated, first: its polycationic nature, it is believed that chitosan interferes with negatively charged residues of macromolecules exposed on the fungal cell surface. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents [26]. Second the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of

Table 2: Effects citral and chitosan on linear sour rot incidence of lime fruits

Salts	Conc.	Sour rot incidence %	
		Disease incidence	Reduction %
Citral ml / l	2.0	42.0 b	58.0
	3.0	31.0 cd	69.0
	4.0	17.5 ef	82.5
	5.0	14.0 f	86.0
Chitosan g / l	2.0	34.0 bc	66.0
	4.0	24.5 d	75.5
	6.0	18.4 ef	82.0
	8.0	16.5 ef	83.5
Control	00	100.0 a	----

Figures with the same letter are not significant (P=0.05).

Table 3: Sour rot incidence of lime fruits as affected with citral and chitosan applied as single or in combination

Application	Disease incidence	Reduction %
Single treatments		
Citral 4.0 m/ l	. 18.5 b	81.5
Citral 5.0 ml / l	16.0 b	84
Chitosan 6.0 g / l	20.0 b	80
Chitosan 8.0 g / l	17.0 b	83
Combined treatments		
Citral 4.0 + Chitosan 6.0	10.5 c	89.5
Citral 4.0 + Chitosan 8.0	9.0 c	91
Citral 5.0 + Chitosan 6.0	9.0 c	91
Citral 5.0 + Chitosan 8.0	8.0 c	92
Control	100.0 a	-----

Figures with the same letter are not significant (P=0.05).

Table 4: Percent of rotted tissue part caused sour rot disease on lime fruits as affected with citral and chitosan applied as single or in combination

Application	Rotted part tissue	Reduction %
Single treatments		
Citral 4.0 m/ l	15.5 b	84.5
Citral 5.0 ml / l	14.0 b	86
Chitosan 6.0 g / l	16.0 b	84
Chitosan 8.0 g / l	14.5 b	85.5
Combined treatments		
Citral 4.0 + Chitosan 6.0	6.5 c	93.5
Citral 4.0 + Chitosan 8.0	6.5 c	93.5
Citral 5.0 + Chitosan 6.0	5.0 c	95
Citral 5.0 + Chitosan 8.0	4.0 c	96
Control	100.0 a	-----

Figures with the same letter are not significant (P=0.05).

mRNA and protein synthesis[27], third : the interaction of chitosan with fungal DNA and RNA[27]. Fourth : Malformation of fungal mycelial in this respect Cheah *et al.,*[28] reported that *Sclerotinia sclerotiorum* was treated with chitosan resulted in excessive mycelial branching, abnormal shapes, swelling, and hyphae size reduction. Also, chitosan is not only effective in halting the growth of the pathogen, but also induces marked morphological changes, structural alterations and molecular disorganization of fungal cells[17,29,30]. Effect of chitosan for inhibition of pathogenic fungi was reported by several investigators [20,28,29].

Coating fruits with chitosan decreased postharvest diseases of tomato, strawberry and lime fruits [11,16,17,18,19]. In present study chitosan at concentration 6.0 and 8.0 g / l controlled sour rot disease when applied as single or combined with citral treatments. In this respect Du *et al*., [19] reported that coating fruits with chitosan reduced the respiration rate, ethylene production, interval O_2 levels and increased the interval CO_2 of peach and pear fruits.They added that coated fruits were markedly firmer and less mature of the end of storage. Moreover, Chitosan, is able to extend storage life and to control decay of strawberries, apples, peaches, pears, kiwfruit, cucmbers, litchi sweet cherries, and citrus fruit[20,21,22,23].

It could be suggested that combination between citral and chitosan as fruit coating considered as one of applicable safely for controlling postharvest diseases of lime fruits.

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