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Integrated Field Approaches of Yeast and Some Food Preservatives Treatments for Controlling Postharvest Green and Blue Molds of Eureka Lemon

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Abstract-Postharvest green and blue molds, caused by Penicilliumdigitatum and Penicilliumitalicum, respectively, can be severe problems to Eureka lemon during handling and storage processes. The potential of lemon trees spray application two weeks before harvest for control of postharvest green and blue molds was evaluated. Eleven different spray treatments with yeast Saccharomyces cerevisiae and/or some food preservatives, i.e. Ascorbic acid, Citric acid, Sodium benzoate, Sodium bicarbonate and Potassium sorbate were sprayed with respect to the rain full point two weeks before harvest.The harvested Eureka lemon fruits were subjected to artificial inoculation with the diseases pathogens under laboratory conditions. Reduction in molds incidence and severity increased in parallel with the increase in the used treatment concentrations to reach their maximum reduction at the highest concentration. Treatment of Sodium benzoate + S. cerevisiae proved its highest protection effect to treated lemon fruits including inhibition (100%) against molds incidence when applied at all concentrations of 1, 2 and 4%, meanwhile application of sodium benzoate alone could reduce molds incidence (66.7%) at the same concentrations. The yeast Saccharomyces cerevisiae alone or combined with potassium sorbate and sodium bicarbonate caused the highest protective effect against molds incidence recorded as 33.4, 66.7 and 66.7% at concentrations of 1, 2 and 4%, respectively.

***Index Terms*- Eureka lemon, Green and blue molds, Pre-harvest treatments, post-harvest diseases, Yeast, food preservatives.**

I. INTRODUCTION

Eureka lemon (*Citrus limon* L. Burm. f.) grows year-round and abundantly. This is the common supermarket lemon [1] also known as 'Four Seasons' (*QuatreSaisons*) because of its ability to produce fruit and flowers together throughout the year. Fungal infections are the main cause of postharvest rots of fresh fruit and vegetables during storage, transport and cause significant economic losses in the commercialization phase [2]. Infections caused during postharvest conditions lowers the shelf life and adversely affect the market value of fruits [3].

Green and blue molds caused by *Penicilliumdigi-tatum*and*Penicilliumitalicum*, respectively, are common postharvest diseases of fruits and vegetables [2,3,4]. Chemical fungicides provide the primary means for controlling postharvest fungal decay of fruit and vegetables. Continuous use of fungicides has faced two major obstacles; increasing public concern regarding contamination of fruits and vegetables with fungicidal residues, and proliferation of resistance in the pathogen populations [5]. There is a growing need to develop alternative approaches for controlling postharvest diseases of citrus fruits. Food safety is one of the major issues related to fresh fruit and vegetables [6]. The development of natural crop protective products as alternatives to synthetic fungicides is currently in the spotlight [7]. The natural and bio-control fungicides might be a good alternative to the use of synthetic fungicides, and in turn to fulfil consumer requirements for more natural and healthy foods [8,9].Under this concept, all possible modes of plant pests and disease control methods should be integrated to minimize the excessive use of synthetic pesticides [10].

The objective of the present study was to evaluate the pre-harvest spray application to Eruka lemon trees with Yeast *Saccaromyses cerevisiae* individually or combined with some food preservatives against post-harvest fruit molds incidence under artificial inoculation with thefungal pathogens *in vivo* conditions.

II. MATERIALS AND METHODS

A. *Field experiment*

This experiment was initially carried out as foliar application with different treatments at Experimental and Production Station, National Research Centre, Neubaria region, Behiera Governorate. Under field conditions, certain Eureka lemon trees were chosen in random with consideration of three replicate (trees) for each particular treatment. Lemon trees were sprayed individually for each treatment two weeks before harvest. All trees include their fruits were sprayed with respect to the rain full point two weeks before harvest. The sprayed treatments were as follows:

1. *Saccharomyces cerevisiae*
2. Ascorbic acid ($C_6H_8O_6$)
3. Ascorbic acid + *S. cerevisiae.*
4. Sodium benzoate.
5. Sodium benzoate + *S. cerevisiae.*
6. Potassium sorbate ($C_6H_7KO_2$)
7. Potassium sorbate + *S. cerevisiae*
8. Sodium bicarbonate ($NaHCO_3$)
9. Sodium bicarbonate + *S. cerevisiae*
10. Citric acid ($C_6H_8O_7$)
11. Citric acid + *S. cerevisiae*
12. Untreated (Control)

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Laboratory tests

Harvested Eureka lemon fruits were used *in vivo* experiments. All the fruits were disinfected [11] in sodium hypochlorite solution (2.5%) for 2 min. After abundant washing with distilled water three times the fruits were placed in sterile commercial packages. Eureka lemon fruits were arranged by groups according to the previous field spray application treatments in carton package having moistened blotters at the bottom. Each group containing a number of nine fruits was randomly distributed into three replicates. Lemon fruits were wounded (0.5 cm deep and 1.0 cm long -three wounds per fruit) using sterile scalpel. The wounds were inoculated with spore suspension [11]. Conidia of fungal pathogens either *P. digitatum*or *P. italicum* were recovered from 2-week old cultures by adding 10 ml of sterile water to each plate. The conidia suspension was filtered through three layers of sterile cheesecloth. The concentration of the conidial suspension was adjusted to 10^5conidia per ml and a drop of Tween 80 was added to the suspension. Each fruit group was inoculated individually with either *P. digitatum*or *P. italicum.* The fruits were inoculated by dipping the wounded fruits into the prepared fungal suspensions. The treated fruits were air dried, after each individual treatment, for 2 hours in a laminar flow. The inoculated treated fruits were placed into carton box (40x40x22 cm) with a capacity of 30 fruits/box and stored in a cold room at 20±2^{o}C for 3 weeks. Three boxes were used as replicates for each particular treatment. Percentage of infected fruits was calculated after the storage period.

Moreover, the percentage of disease severity of mold infection of Eureka lemon fruits was also calculated. Infected lemon fruits were classified into five categories according to the infected area, *i.e.* healthy fruits, lesion area up to 25%, between 25 and 50%, between 50 and 75% and more than 75% of Eureka fruit area. The formula suggested by [12] was modified and was used as follows:

$$S = \frac{\sum (n \times c)}{N}$$

Where: S = severity of mold disease infection
n = number of infected lemon fruits per category
c = category number
N = total examined fruits.

The experiments were repeated twice. The results of two experiments were averaged. The percentage of mold infection and severity was calculated.

Statistical analysis

Tukey test for multiple comparisons among means was utilized as described by[13].

III. RESULTS AND DISCUSSION

The present study conducted with evaluation of different treatments applied as foliar Eureka lemon trees spray two weeks before harvesting against postharvest diseases. The harvested Lemon fruits were artificially inoculated individually with either green or blue molds pathogens under laboratory conditions. Molds incidence and severity were recorded after 3 weeks of incubation. Presented data in Table (1and 2) and Figures (1 and 2) revealed that the used treatments had protected effect against green and blue molds incidence and. In general, reduction in molds incidence and severity increased in parallel with the increase in the used treatment concentrations to reach their maximum reduction at the highest concentration. Treatment of Sodium benzoate + *S. cerevisiae* proved its highest protection effect to treated lemon fruits. It had complete inhibition (100%) against molds incidence when applied at all concentrations of 1, 2 and 4%, meanwhile application of sodium benzoate alone could reduce molds incidence (66.7%) at the same concentrations (Fig. 1).

Table 1. Efficacy of Pre-Harvest treatments on Green and Blue molds incidence of Eureka Lemon verity under artificial infection with *Penicilliumdegitatum* and *P. italicum* pathogens

Treatments	Disease incidence % *					
	Green mold			Blue mold		
	Concentration %					
	1	2	4	1	2	4
Saccharomyces cerevisiae	66.6 b	66.6 b	66.6 b	66.6 b	66.6 b	66.6 b
Ascorbic acid	100 a	66.6 b	66.6 b	100 a	66.6 b	66.6 b
Ascorbic acid + *S. cerevisiae*	66.6 b	66.6 b	66.6 b	66.6 b	66.6 b	66.6 b
Sodium benzoate	33.3 c	33.3 c	33.3 c	33.3 c	33.3 c	33.3 c
Sodium benzoate + *S. cerevisiae*	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d
Potassium sorbate	66.6 b	66.6 b	66.6 b	100 a	66.6 b	66.6 b
Potassium sorbate + *S. cerevisiae*	66.6 b	33.3 c	33.3 c	66.6 b	33.3 c	33.3 c
Sodium bicarbonate	100 a	66.6 b	66.6 b	100 a	66.6 b	66.6 b
Sodium bicarbonate + *S. cerevisiae*	66.6 b	33.3 c	33.3 c	66.6 b	33.3 c	33.3 c
Citric acid	100 a	66.6 b	66.6 b	100 a	66.6 b	66.6 b
Citric acid + *S. cerevisiae*	66.6 b	66.6 b	33.3 c	66.6 b	66.6 b	33.3 c
Untreated (Control)	100 a			100 a		

* Disease incidence was recorded two weeks after artificial infection
Figures with the same letter are not significantly different (P≤ 0.05).

Illustrated data in Figure (1) showed that application of the yeast *Saccharomyces cerevisiae*alone or combined with potassium sorbate and sodium bicarbonate caused the highest protective effect against molds incidence recorded as 33.4, 66.7 and 66.7% at concentrations of 1, 2 and 4%, respectively. Moreover, data in Table (1) showed that the yeast *S. cerevisiae* enhanced the effect of applied salts in combined treatments comparing with salt application alone. Similar trend was also observed concerning the recorded diseases severity.

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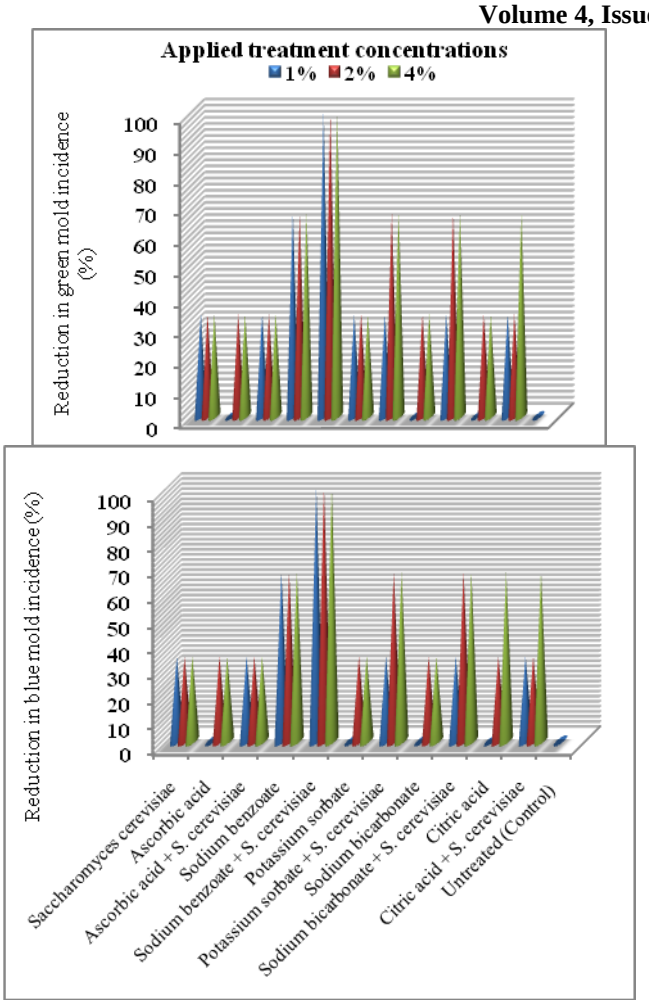

Fig.1. Reduction in Green and Blue molds incidence of Eureka Lemon verity in response to Pre-Harvest treatments under artificial infection with *Penicilliumdegitatum* and *P. italicum* pathogens

It is clear from presented data in Table (2) and illustrated in Figures (2) that the previously sprayed lemon trees with different treatments resulted in reduction in disease development as percentage of rotted tissues compared with untreated fruits which expressed as 100% rot appearance. Also application of *Saccharomycescerevisiae* alone or combined with different salts and acid reduce diseases development expressed as percentage of rotted fruit tissue. These results might be attributed to the increasing the number of yeast persistence on the fruit's surface and/or synergistic effect between them which may probably occur. Although the use of antagonistic yeasts to control postharvest diseases have been demonstrated with several commodities, their commercialization will be depend on whether they are capable of effectively controlling decay of fruit from different locations with variable inoculum loads, types of infection, and levels of mechanical injury. In this concern, the mechanisms by which yeast exert their biocontrol activity have not been fully elucidated. Biological activity of antagonistic yeasts may involve nutrient competition [14], site exclusion [15], direct parasitism, and perhaps induced resistance [16,17]. Furthermore, several antagonistic yeasts have been isolated and shown to protect a variety of fruit against postharvest pathogens [18,19]. Also, [20] found that application of sodium bicarbonate improved biocontrol of brown rot on sweet cherry fruit under various storage conditions.

Table. 2. Efficacy of Pre-Harvest treatments on Green and Blue molds disease severity of Eureka Lemon verity under artificial infection with *Penicilliumdegitatum* and *P. italicum* pathogens

Treatments	Disease severity % *					
	Green mold			Blue mold		
	Concentration %					
	1	2	4	1	2	4
Saccharomyces cerevisiae	52.4 e	43.3 f	38.4 g	81.2 b	54.3 e	52.2 e
Ascorbic acid	88.2 b	54.6 e	50.4 e	86.8 b	71.2 c	68.6 d
Ascorbic acid + *S. cerevisiae*	84.4 b	54.8 e	48.2 f	82.2 b	53.6 e	46.8 f
Sodium benzoate	54.0 e	52.7 e	51.3 e	58.6 e	52.4 e	50.2 e
Sodium benzoate + *S. cerevisiae*	0.0 h	0.0 h	0.0 h	0.0 h	0.0 h	0.0 h
Potassium sorbate	84.2 b	80.0 b	72.8 c	84.2 b	77.6 c	62.2 d
Potassium sorbate + *S. cerevisiae*	82.4 b	73.4 c	64.2 d	83.2 b	72.2 c	67.2 d
Sodium bicarbonate	72.4 c	67.3 d	61.4 d	74.6 c	63.4 d	60.4 d
Sodium bicarbonate + *S. cerevisiae*	71.3 c	64.4 e	58.2 e	62.2 d	54.2 e	48.6 f
Citric acid	70.2 c	62.4 d	58.4 e	71.3 c	66.4 d	60.2 d
Citric acid + *S. cerevisiae*	66.8 d	60.2 d	54.6 e	46.2 f	42.2 f	40.4 f
Untreated (Control)	100 a			100 a		

* Disease severity was recorded two weeks after artificial infection
Figures with the same letter are not significantly different (P≤ 0.05).

Similar conclusions regarding these results are reported. Spraying plants with either sodium bicarbonate or potassium bicarbonate solution provided good control of several plant diseases [21,22,23].The food preservatives potassium sorbate or sodium benzoate, had antifungal activities against postharvest decaying fungi [24,25]. Sorbic acid and its salts derivatives are the most widely used antimicrobial agents for food preservation worldwide [26]. Using potassium sorbate or sodium benzoate against postharvest diseases of tomato, apple, carrots and potato was reported [27,28,29]. The food preservatives potassium sorbate or sodium benzoate were applied to citrus fruits inoculated with *Penicilliumdigitatum* have similar fungicidal activity and are equivalent to the traditional treatment used as a postharvest fungicide for controlling citrus fruit decay [30].

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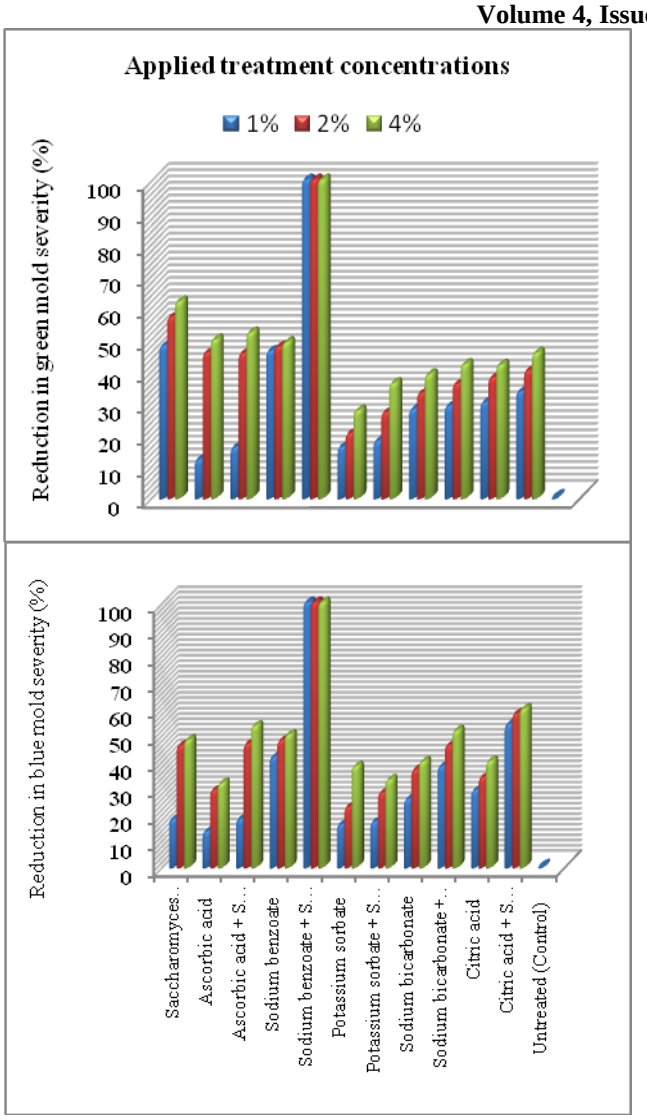

Fig. 2. Reduction in green and blue molds diseases severity of Eureka lemon in response to pre-harvest treatments under artificial infection with *Penicilliumdegitatum* and *P. italicum* pathogens

The use of sodium bicarbonate alone to control postharvest decays of fruit has its limitations [31], but it can be combined with other alternative treatments to synthetic fungicides, resulting in the control that is superior to individual treatments alone. For example, sodium bicarbonate was successfully used in combination with bacterial and yeasts biocontrol agents to enhance control of postharvest decays on citrus, pome, and stone fruits [21,32]. These reports are clearly demonstrated in the present study and show that the application of *S. cerevisiae* enhanced the control of lemon fruits molds when combined with either of sodium bicarbonate, potassium sorbate or citric acid spray. Furthermore, Sodium and ammonium bicarbonate were shown to inhibit fungal pathogens of fruits, field crops, vegetables, and ornamentals [33]. Also, sodium bicarbonate applied at room temperature at 2 to 4% reduced blue mold caused by *Penicilliumitalicum*in Citrus fruits [31].

IV- CONCLUSION

The results presented in this work highlight the use of biological and inorganic salts singly or in combination as an alternative control strategy against citrus postharvest green and blue molds. This study also provides an insight into expanding these strategies, partly or fully, for the control of other postharvest infections.

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