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Pre-Harvest Foliar Application for Controlling Green and Blue Molds of Valencia Orange Fruits

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Abstract*-- Pre-harvest spray application to Valencia orange trees with yeast and/or some food preservatives can be an effective approach for controlling the postharvest diseases. Different spray applications of Valencia orange trees against postharvest green and blue molds were evaluated. Eleven different spray treatments with yeast and/or some food preservatives were applied. The harvested Valencia orange fruits were subjected to artificial inoculation with the diseases pathogens under in vivo conditions. Different salts and acids, i.e. Potassium bicarbonate, Potassium dihydrogen Phosphate, Calcium chloride, Trisodiumorthophospate and sorbic acid were evaluated in this study. The applied treatments could reduce molds incidence gradually by increasing according to the used concentrations to reach their minimum at the highest concentration. Treatment of Trisodium orthophosphate + S. cerevisiae proved its highest protection effect to treated Valencia fruits that it has complete inhibition against molds incidence when applied at concentrations of 2 and 4%, meanwhile at 1% reduction calculated ad 66.7%. It is also noticed that the yeast S. cerevisiae enhanced the effect of applied salts in combined treatments comparing with salt application alone. Also, results showed that diseases severity followed similar trend. On the light of the obtained results in the present study it could be conclude that Pre-harvest spray application to Valencia trees with yeast and/or some food preservatives can be an effective approach for controlling such post harvest diseases.

***Index Terms*-Green and blue molds, Valencia orange, postharvest diseases, pre-harvest treatments, yeast, food preservatives.**

I. INTRODUCTION

Valencia orange (*Citrus sinensis* L.) is a late-season fruit, and therefore a popular variety when navel oranges are out of season. Postharvest diseases are an important constraint affecting citrus fruit quality, shelf life, and market values. Several fungal pathogens are capable of causing postharvest diseases on citrus fruits. The most serious diseases include green mold [*Penicilliumdigitatum* (Pers.:Fr.) Sacc], blue mold [*Penicilliumitalicum* Wehmer] [1]. In commercial practice, all registered packinghouses are required to apply a fungicide to fresh fruit for control of postharvest decay [2]. Such fungicides are effective; but with the advent of consumer packaging in which decay losses represent the number of packages with decayed fruit rather than the number of decayed fruit, only a minimum amount of decay can be economically accepted. In an attempt to improve control of postharvest decay in fresh citrus, the use of fungicide applications before harvest has been investigated. Some reduction in postharvest decay was reported with the application with Benlate one week before harvest, which effectively controlled decay in mechanically harvested 'Valencia' oranges [3]. With a growing recognition that many agrochemicals are hazardous to humans, animals and the environment, came the need to substitute these chemical products with biological and physical and/or change agronomic practices that are safe to the environment and human health. These practices have the potential to play an increasingly important role in plant disease control. To achieve this objective on one specific disease, laboratory, field and commercial backhouses trials were conducted to develop effective alternative control measures against green and blue molds of citrus using multiple control measures, independently and in combination. In this regards, many investigations reported the use of potassium salts ($K2HPO_4$ or KNO_3) as a chemical agent for induction of plant resistance [4,5]. Furthermore, there has been considerable interest in the use of sodium bicarbonate, potassium bicarbonate and potassium phosphate for controlling various fungal diseases in plants [6,7]. Also, calcium chloride had been recorded to have antifungal effect. Calcium treatment of apples provided broad-spectrum protection against the postharvest pathogens of *P. expansum* and *B. cinerea* [8]. Moreover, Trisodium phosphate (TSP) is the inorganic compound. Most often found in white powder form, it can also be called trisodium orthophosphate or simply sodium phosphate. Sodium phosphates including monosodium phosphate, disodium phosphate, and trisodium phosphate are approved as food additives in the EU. They are used as antioxidant agents and have the collective E number E339 [9]. In the United States, sodium phosphates are categorized as generally recognized as safe when used in accordance with good manufacturing practice [10].Trisodium phosphate is commonly found in many brands of children's cereal, toothpaste, processed cheese, meats, and canned soups [11]. Biological control has been suggested as one of the alternatives. Yeasts act as natural safety biofertilizer and rich source of phytohormones (especially cytokinins), sugar, vitamins, enzymes, amino acids and minerals [12,13]. It was reported that yeast has stimulatory effects on cell division and enlargement, synthesis of protein and nucleic acid as well as chlorophyll formation [12]. The enhancement effect of yeast might be attributed to its stimulating effect on enzyme activity, production of some phytohormones, improving the uptake of nutrients and convert insoluble form of phosphorous into soluble one to enhance phosphorous availability to plants, all of which increased vegetative growth of plant [14]. It also releases CO_2 which reflected in improving net photosynthesis. Improving growth and productivity of different plant species by application of yeast extract were

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recorded by Khalil and Ismael, [14,15,16]. Therefore, the objective of this study was to evaluate the potential of spray application of some single or combined treatments to trees two weeks before harvest and their effectiveness to control green and blue molds on artificially inoculated fruits was determined.

II. MATERIALS AND METHODS

To manage green and blue molds, the primary postharvest disease that causes losses during handling and storage, some foliar approaches were applied to Valencia orange trees two weeks before fruits picking. 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 experiment, certain Valencia trees were chosen in random with consideration of three replicate trees for each particular treatment. Valencia trees were sprayed individually according to certain 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. Yeast *Saccharomyces cerevisiae*
2. Potassium bicarbonate ($KHCO_3$).
3. Potassium bicarbonate + *S.cerevisiae.*
4. Sorbic acid ($C_6H_8O_2$).
5. Sorbic acid + *S. cerevisiae.*
6. Potassium dihydrogen Phosphate (KH_2PO_4).
7. Potassium dihydrogen Phosphate + *S. cerevisiae*
8. Calcium chloride ($CaCl_2$).
9. Calcium chloride + *S. cerevisiae*
10. Trisodiumorthophospate (Na_3PO_4)
11. Trisodium orthophosphate + *S. cerevisiae*
12. Untreated (Control)

All treatments were applied at three concentrations, 1, 2, 4%. Then after the harvested treated Valencia orange fruits were subjected to artificial infestation with green and blue mold pathogens under *in vivo* conditions at laboratory of Plant Pathology Dept. All the fruits were disinfected (Lopez-Reyes *et al.*, 2010) in sodium hypochlorite solution (2.5%) for 2 min., then air dried. Valencia orange fruits were arranged by groups according to the previous field spray application treatments. Valencia orange 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 [17]. Conidia of fungal pathogens either *Penicilliumdigitatum*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 10^5 conidia per ml and a drop of Tween 80 was added to the suspension. Each fruit group was inoculated individually either *P. digit tum*or *P. italicum* and stored at 20±2°C. 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 (52x23x28 cm) with a capacity of 36 fruits/box and stored in a cold room at 20±2°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 Valencia fruits was also calculated. Infected Valencia 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 Valencia fruit area. The formula suggested by [18] 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 *in vivo* experiments were repeated twice. The results of two experiments were averaged.

Statistical analysis

Tukey test for multiple comparisons among means was utilized as described by [19].

III. RESULTS AND DISCUSSION

Green and blue molds are commercially controlled through postharvest handling processes and applications of fungicides. However, it will be beneficial if a fungicide applied on citrus trees before harvest can also reduce postharvest diseases incidence and severity. It was reported that, an effective pre-harvest fungicides with continuing activity that persists to provide postharvest decay control after harvest would be a useful management tool for citrus growers [20,21]. In this regards, the present study conducted with evaluation of different treatments applied as foliar Valencia trees spray two weeks before harvesting against postharvest diseases. The harvested Valencia 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 treatments of sorbic acid and sorbic acid + *S. cerevisiae* had no protected effect against green and blue molds incidence. In general, the other applied treatments could reduce molds incidence gradually by increasing according to the used concentrations to reach their minimum at the highest concentration. Treatment of Trisodium orthophosphate + *S. cerevisiae* proved its highest protection effect to treated Valencia fruits that it has complete inhibition against molds incidence when applied at concentrations of 2 and 4%, meanwhile at 1% reduction calculated ad 66.7% (Fig. 1).

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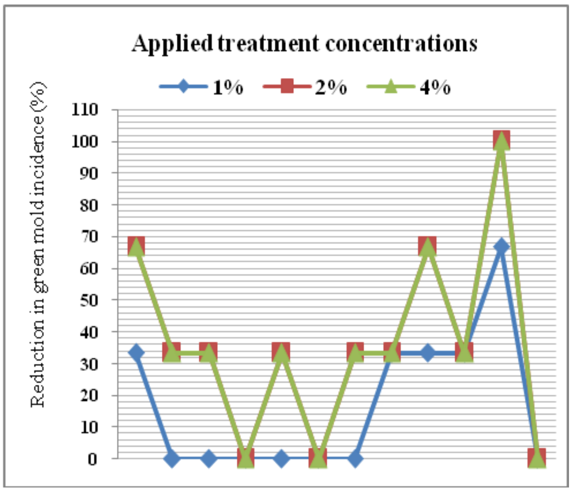
Table 1. Efficacy of Pre-Harvest treatments on Green and Blue molds incidence of Valencia orange under artificial infection with Penicilliumdegitatum and P. italicum pathogens

Treatments	Disease incidence % *					
	Green mold			Blue mold		
	Concentration %					
	1	2	4	1	2	4
Saccharomyces cerevisiae	66.6 b	33.3 c	33.3 c	66.6 b	33.3 c	33.3 c
Potassium bicarbonate	100 a	66.6 b	66.6 b	66.6 b	66.6 b	66.6 b
Potassium bicarbonate + *S. cerevisiae*	100 a	66.6 b	66.6 b	100 a	33.3 c	33.3 c
Sorbic acid	100 a	100 a	100 a	100 a	100 a	100 a
Sorbic acid + *S. cerevisiae*	100 a	66.6 b	66.6 b	66.6 b	66.6 b	66.6 b
Potassium dihydrogen Phosphate	100 a	100 a	100 a	100 a	100 a	100 a
Potassium dihydrogen Phosphate + *S. cerevisiae*	100 a	66.6 b	66.6 b	100 a	66.6 b	66.6 b
Calcium chloride	66.6 b	66.6 b	66.6 b	100 a	66.6 b	66.6 b
Calcium chloride + *S. cerevisiae*	66.6 b	33.3 c	33.3 c	33.3 c	33.3 c	33.3 c
Trisodiumorthophospate	66.6 b	66.6 b	66.6 b	66.6 b	66.6 b	66.6 b
Trisodium orthophosphate + *S. cerevisiae*	33.3 c	0 d	0 d	33.3 c	0 a	0 a
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 calcium chloride occupied the second protective treatment against molds incidence. They could reduce both green and blue molds by 33.4% when applied at concentration of 1% and 66.7 when applied at 2 and 4%. 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. It is clear from presented data in Table (2) andillustrated in Figures (2) that the previously sprayed Valencia fruits 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. Similar conclusions regarding these results are reported.



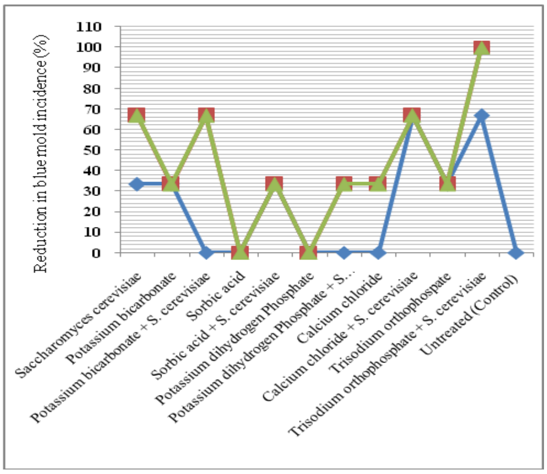


Fig. 1.Reduction in Green and Blue molds incidence in response to Pre-Harvest treatments on Valencia orange under artificial infection with *Penicilliumdegitatum* and *P. italicum* pathogens

The success of antagonists (non-disinfested fruits) than individual one (disinfested fruits) referred to different mode of action of mixture numerous modes of actions have been postulated and demonstrated for antagonist effective in controlling postharvest diseases, including nutrient competition, antibiotic production, enzymes that act on fungal cell wall components such as chitinases and ß-1,3 glucanase and induced host resistance [22,23]. The mechanisms by which yeast exert their biocontrol activity have not been fully elucidated. Biological activity of antagonistic yeasts may involve nutrient competition [24], site exclusion [22], direct parasitism, and perhaps induced resistance [25,26,27]. Furthermore, several antagonistic yeasts have been isolated and shown to protect a variety of fruit against postharvest pathogens [28,29].

Table 2.Efficacy of Pre-Harvest treatments on Green and Blue molds diseases severity of Valencia orange under artificial infection with Penicilliumdegitatum and P. italicum pathogens

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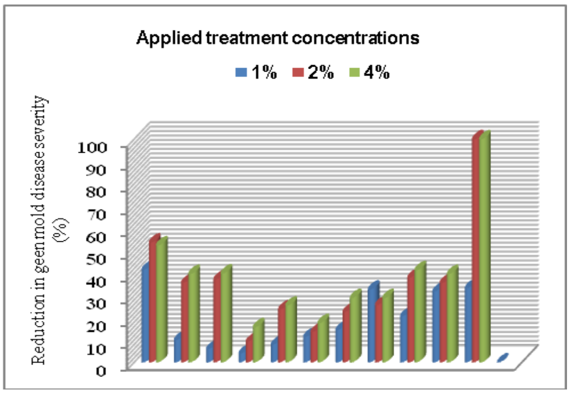
Treatments	Disease severity % *					
	Green mold			Blue mold		
	Concentration %					
	1	2	4	1	2	4
Saccharomyces cerevisiae	58.4 f	46.3 g	44.6 g	81.2 c	54.3 f	52.2 f
Potassium bicarbonate	90.0 b	64.3 e	60.4 e	83.4 c	80.2 c	78.8 d
Potassium bicarbonate + *S. cerevisiae*	94.0 b	62.8 e	60.2 e	93.4 b	59.4 f	56.2 f
Sorbic acid	96.0 b	90.7 b	84.3 b	94.6 b	92.4 b	90.2 b
Sorbic acid + *S. cerevisiae*	92.0 b	76.3 d	74.6 d	62.2 e	60.2 e	58.8 f
Potassium dihydrogen Phosphate	88.6 bc	86.4 c	82.4 bc	94.2 b	92.6 b	90.2 b
Potassium dihydrogen Phosphate + *S. cerevisiae*	85.4 b	77.4 d	71.2 d	93.2 b	72.4 d	68.4 e
Calcium chloride	77.4 d	74.3 d	71.2 d	94.6 b	73.4 d	70.2 d
Calcium chloride + *S. cerevisiae*	79.3 d	62.1 e	58.7 f	52.4 f	50.6 f	48.8 g
Trisodiumorthophospate	68.2 e	64.4 d	60.4 d	72.3 d	70.1 d	67.4 e
Trisodium orthophosphate + *S. cerevisiae*	66.8 e	0 h	0 h	46.2 g	0 h	0 h
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).

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 addition, microbial biocontrol agents will be expected to display curative activity comparable to that observed with synthetic fungicides. Currently available antagonistic microorganisms do not appear to be able to control previously established infections and are most effective when applied prior to infection by the pathogen [27,30]. Moreover, beneficial effect of two food additives, ammoniummolybdate and sodium bicarbonate on antagonistic yeasts for control of brown rot in sweet cherry was evaluated [31]. They found that application of additives improved biocontrol of brown rot on sweet cherry fruit under various storage conditions. It is postulated that the enhancement of disease control is directly because of the inhibitory effects of additives on pathogen growth, and indirectly because of the relatively little influence of additives on the growth of antagonistic yeasts.



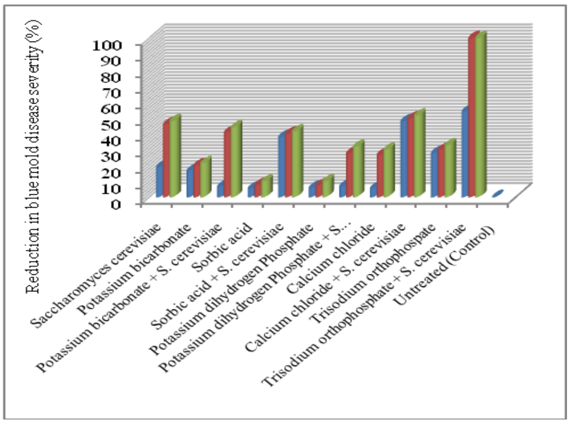


Fig. 2.Reduction in green and blue molds diseases severity of Valencia orange in response to pre-harvest treatments under artificial infection with *Penicilliumdegitatum* and *P. italicum* pathogens

Furthermore, there has been considerable interest in the use of sodium bicarbonate, potassium bicarbonate andpotassium phosphate for controlling various fungal diseases in plants [6,7]. Spraying plants with either sodium bicarbonate or potassium bicarbonate solution provided good control of several plant diseases [7,32,33].The food preservatives potassium sorbate or sodium benzoate, had antifungal activities against postharvest decaying fungi [34,35]. Sorbic acid and its salts derivatives are the most widely used antimicrobial agents for food preservation worldwide [36]. Using potassium sorbate or sodium benzoate against postharvest diseases of tomato, apple, carrots and potato was reported [37,38,39]. 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 [40]. The use of sodium bicarbonate alone to control postharvest decays of fruit has its limitations [41], 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 [32,42]. These reports are clearly demonstrated in the present study and show that the application of *S. cerevisiae* enhanced the control of foliar vegetables diseases when combined with either sodium bicarbonate or calcium chloride spray. It was reported that *S. cerevisiae* required an inoculum of 105 cfu/g to inhibit the growth of *Penicilliumroquefortii*n non-sterile high-moisture wheat grains [43]. Many researchers have shown that calcium plays an im-portant role in the inhibition of postharvest decay of fruits [44,45], and in enhancing the efficacy of postharvest bio-control agents [46,47]. Postharvest calcium treatment of apples provided broad-spectrum protection against the postharvest pathogens of *Penicilliumexpansum* and *Botrytis cinerea*. The addition of CaCl2 (2% w/v) to the formulation of the yeast biocontrol agent, *Candida oleophila*, enhanced the ability of this yeast to protect apples against postharvest decay [47]. The efficacy of controlling grey mould and blue mould rots in apples was enhanced when *Trichosporon*sp. was applied in the presence of CaCl2 (2% w/v) in an aqueous suspension [48]. In the present study calcium chloride application either alone or combined with other plant resistance inducers and/or *S. serevisiae* was able to reduce foliar disease incidence. Also, [49] recorded that incidence and severity of white mold on dry bean were significantly reduced with application of calcium chloride and calcium silicate. Selected organic and inorganic salts are active antimicrobial agents and have been widely used in the food industry. Many of these salts are effective against a range of micro-organisms; most of them have low mammalian toxicity and therefore have potential for postharvest disease control. Salt treatments can inhibit plant pathogens or suppress my cotoxin production [51,52]. Also,[53] found that from field experiments, that spraying cantaloupe plants three times with fungicides in alternation with another three sprays with any of calcium chloride or salicylic acid resulted in significant reduction in the disease severity with significant increase in the fruit yield when compared with unsprayed (check) plants.

Furthermore, Sodium and ammonium bicarbonate were shown to inhibit fungal pathogens of fruits, field crops, vegetables, and ornamentals [54]. Also, sodium bicar-bonate applied at room temperature at 2 to 4% reduced blue mold caused by *Penicilliumitalicum*in Citrus fruits [41].

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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