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## Abstract

Mild brown and black spot diseases symptoms were detected on citrus varieties, Valencia and Navel fruits during season 2018 in some citrus orchards at North Egypt. Collected diseased fruit samples revealed isolation of *Alternaria alternata* and *Phyllosticta citricarpa* (McAlpine) van der Aa. Some organic acids, salts and *Saccharomyces cerevisiae* were evaluated against the diseases incidents *in vitro* and *in vivo*. Complete growth inhibition was recorded for fungi tested at 2g/L of salicylic acid, Potassium dihydrogen phosphate, Tri-Sodium polyphosphate and 2.5 g/L of *S. cerevisiae*. All pre-harvest treated Valencia trees, inoculated fruits revealed no diseases symptoms up to 10 days of storage period. Meanwhile, *S. cerevisiae* and salicylic acid treatments had extended protective effect up to 20 days. Control strategy through spraying Valencia orange trees with yeast or some organic acids and salts as pre-harvest approaches should be taken in consideration especially these diseases began to occur in North region of Egypt.

**Keywords:** Black spot. Brown spot. Citrus fruits. Disease control. Organic acids. Organic salts. Pre-harvest approaches. Yeast.

## 1. Introduction

Citrus is considered as one of the most important fruit crops worldwide. However, citrus diseases known as brown and black spot citrus have often been restricted for citrus production and marketing in some regions. Black spot (CBS), of citrus fruits caused by the fungal pathogen *Phyllosticta citricarpa* (McAlpine) van der Aa (*synonym: Guignardia citricarpa* Kiely (asexual and sexual stage, respectively)). In many countries the disease is recorded including Africa, Australia, Southeast Asia and South America (Smith and Charles 1998; CABI/EPPO 2012) and Florida, North America, USA (Schubert et al. 2010; Schubert et al. 2012). It was recorded that the most cultivars of citrus are susceptible to CBS in different degrees with lemons and Valencia oranges which being highly susceptible (Timmer 2000; Spósito et al. 2004). An alert confirming that the Tunisian authorities have officially declared an area infected with *Phyllosticta citricarpa* the causal agent of black spot has issued by (EPPO) the European and Mediterranean Plant Protection Organization (Freshplaza.com 2019). Losses may be essential and usually significant when the affected fruits are unsuitable for the fresh fruit market in addition to chemical control costs (Kotzé 1981; Kotzé 1988). Since black spot (CBS) is absent in the citrus producing European countries, therefore the European Union classified it as first quarantine pest (Paul et al. 2005), and thus is often restricted through the international

trade market of fresh citrus (OEPP/EPPO 2009). Moreover, although CBS was found in Florida and North America, it is still under quarantine regulation by the USA (Schubert et al. 2010; Schubert et al. 2012).

Alternaria brown spot (ABS) is a considerable concern regarding citrus breeding programs, since it is a serious disease affecting susceptible citrus genotypes. Alternaria brown rot of citrus fruit may appear in the field prior to harvest, subsequently it is a significant postharvest problem. Ohtani et al. (2009) reported that fruits of many citrus cultivars affected with brown spot disease (ABS), caused by *Alternaria alternata*. The disease was first described in Australia in 1903 and subsequently in Florida (USA) in 1974 affecting. It was also reported in Israel in 1991, South Africa 1996, Spain 2000, Italy 2001, Brazil and Argentina 2003 and china 2010 (Lengi et al. 2014). In southern São Paulo, southern Minas Gerais states in Brazil, and in Misiones and Corrientes provinces in Argentina, Alternaria brown spot were observed on 'Murcott' tangor (*Citrus reticulata* × *Citrus sinensis*) trees in severe outbreaks infection (Peres et al. 2003). Timmer (2000) reported that many tangerines and their hybrids, affected with Alternaria brown spot causing lesions on leaves, twigs and fruits resulting in both fruits yield and their quality. They added that considerable loss and produce deficiency of citrus fruits caused by this disease result in unacceptable fruits to the consumers and fresh markets.

Although citrus brown and black spot diseases are starting to be significant in Egypt, little attention has been paid to control these diseases. Whereas, without control strategy, losses of marketable fruit would be severe in many citrus orchards. In addition, since the limited number of registered fungicides, producing high quality fruit for the fresh market representing increase challenge to the growers. Therefore, the purpose of the current investigation was to assess the efficacy of some chemical inducers applied as pre-harvest foliar approaches under field conditions to control these diseases incidence at post-harvest storage stage.

## 2. Material and Methods

### Citrus brown and black spot disease symptoms

Citrus brown spot as reported by Aiello et al (2020) known as Alternaria brown spot is caused by the fungus *Alternaria alternata*. This fungus attacks young fruit, leaves and twigs, producing brown-to-black lesions surrounded by a yellow halo caused by a fungal toxin. Young infected fruitlets are dropped after petal fall. The remaining fruits showing lesions varied in their size from small spot to large pustule appear on the fruit's peel as shown in Figure 1.

Citrus black spot is caused by the fungus *Phyllosticta citricarpa* (previously known as *Guignardia citricarpa*). Disease symptoms as described by Agostini et al. (2006) can be observed on both fruit and leaves and they are clearly seen on mature fruits. Lesions on infected fruits are grouped into several types according to its shape and size to hard spot, false melanoses, freckle spot, cracked spot and virulent spot (Figure 1).

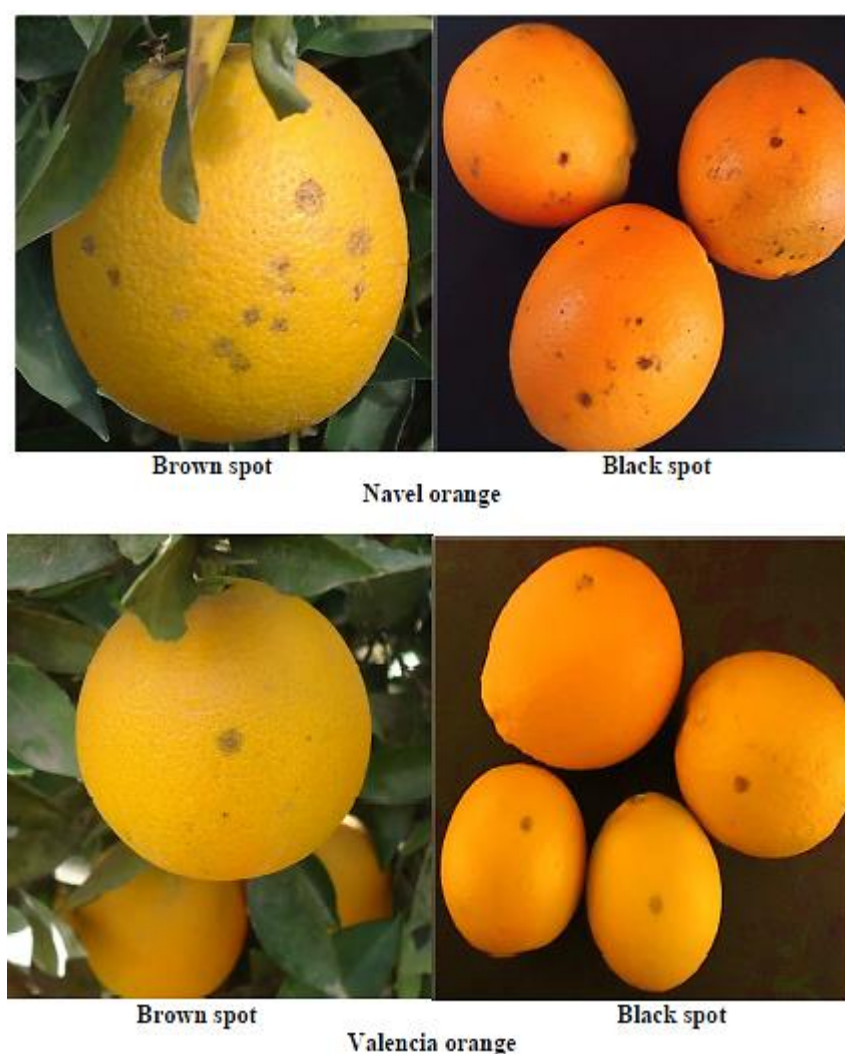
### Isolation and identification of the causal fungi

Several samples of Valencia citrus fruits showing either brown or black spot disease symptoms (Figure 1) were collected from some Valencia orchard located at Nubaria region, Beheira governorate, Egypt and examined in the laboratory. For each disease symptoms the infected areas of fruits were excised, surface sterilized in 2% sodium hypochlorite for one minute, placed on sterile filter paper to dry, and plated on autoclaved water agar. After incubation for 3 days at 25±2°C, advancing mycelium was transferred to potato dextrose agar (PDA) by hyphal tip transfer and maintained for future experiments. All of colony size, color, texture and color of conidial masses were examined microscopically after ten-day incubation on PDA. The isolated fungi were identified based on morphological and cultural characteristics according to Barnett and Hunter (1998) and Simmons (2007).

### Pathogenicity test

The pathogenic ability of isolated fungi *Alternaria alternata* and *Phyllosticta mangiferae* were evaluated for their pathogenic ability under laboratory conditions. The isolated fungi were grown on potato

dextrose broth (PDB) medium and incubated at  $20\pm 2^{\circ}\text{C}$  for 14 days. The fungal cultures were filtered through sterile glass wool then the collected spores adjusted to  $10^6$  spore/ml using hemacytometer slide. Apparently healthy Valencia and Navel orange fruits were surface disinfected with 70% ethyl alcohol, allowed to dry and then several wounds/fruit each (0.5-1.0 cm deep) were made up at the fruit body using sterile scalpel. The wounded fruits were inoculated individually by spraying them with the prepared spore suspension of the two tested fungi. For development of disease symptoms, the citrus fruits were placed in sterilized moist desiccators to maintain a high humidity, held for 7-10 days at  $20\pm 2^{\circ}\text{C}$  and examined for typical brown and black spot symptoms. A set of wounded fruits orange fruits were sprayed with sterilized water and kept as control. Twenty fruits of either Valencia or Navel orange fruits were used and the percentage of infected fruits was calculated. The fungi were re-isolated and identified to fulfill Koch's postulates.



**Figure 1.** Citrus brown and black spot diseases of Navel and Valencia orange fruits.

### Laboratory test

The inhibitory effect of antagonistic yeast *Saccharomyces cerevisiae* at concentrations of 2.5, 5.0 and 10g/L as well as Salicylic acid, Ascorbic acid, Calcium chloride, Potassium dihydrogen phosphate, Tri-Sodium polyphosphate at concentrations of 1, 2 and 3g/L against the linear growth of *Alternaria alternata* and *Phyllosticta citricarpa* was evaluated under *in vitro* conditions. Different tested agents were added individually to conical flasks containing 100 ml of sterilized PDA medium before its solidification in consideration of the previously mentioned concentrations. The supplemented media were poured into Petri dishes (9 cm) about 20 ml each. PDA plates free of tested materials were used as control treatment. Five plates were used as replicates for each particular treatment as well as control. Discs (5 mm diameter) of cultures of *Alternaria alternata* or *Phyllosticta citricarpa* were placed on to the Centre of Petri dishes with

PDA, then incubated at 25±2°C. When the fungal growth in the control filled the whole Petri dish, then all treatments were examined and the mean linear fungal growth was measured and recorded.

### Pre-harvest field experiment

Since brown and black spot diseases affecting both citrus varieties Navel and Valencia orange fruits, the present field study conducted with only Valencia orange orchards which it was available to carry out this work. Some foliar approaches using fungicide alternatives, *i.e.* the yeast *Saccharomyces cerevisiae*, organic acids (salicylic & ascorbic) and organic salts (potassium dihydrogen phosphate, trisodium orthophosphate) were applied for the purpose of managing brown and black spot diseases, the postharvest disease that cause losses during handling and storage. This experiment was applied as foliar spray with different treatments at Experimental and Production Station, National Research Centre, Neubaria region, Behiera Governorate, at 2018. Under Valencia orchard conditions, some Valencia trees were chosen in random with account of five replicate trees for each particular treatment. Foliar spray was carried out individually according to certain treatment twice to Valencia orange trees with two weeks' interval between each. All trees include their leaves and fruits were sprayed with respect to the rain full point. All organic acids and salts treatments were applied at concentration of 3g/L, meanwhile *S. cerevisiae* at concentration of 10g/L.

The fruits picking was done after two weeks from the second treatment.

The sprayed treatments were as follows:

1. yeast *Saccharomyces cerevisiae* (commercial product)
2. salicylic acid
3. ascorbic acid
4. potassium dihydrogen phosphate
5. calcium chloride
6. trisodium orthophosphate
7. untreated (Control).

### Post-harvest storage experiment

The harvested apparently healthy treated Valencia orange fruits were subjected to artificial infestation with brown and black spot pathogens under *in vivo* conditions at laboratory of Plant Pathology Dept., NRC, Egypt. 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. The after, Valencia orange fruits were wounded (0.5 cm deep and 1.0 cm long -three wounds per fruit) using sterile scalpel. The wounds were artificially inoculated with spore suspension (Lopez-Reyes et al. 2010). Conidia of fungal pathogens either *Alernaria alternata* or *Phyllosticta citricarpa* 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<sup>6</sup> conidia per ml and a drop of Tween 80 was added to the suspension. Each fruit group was inoculated individually with either *Alernaria alternata* or *Phyllosticta citricarpa* and stored at 20±2°C. The fruits were inoculated by dipping the wounded fruits individually 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 24 fruits/box and stored in a cold room at 20±2°C for 20 days. Three boxes were used as replicates for each particular treatment. Percentage of infected fruits with either brown or black spot diseases was calculated after the storage period. This experiment was repeated twice. The results of the two experiments were averaged.

The severity of Valencia brown and black fruit infection 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 modified formula suggested by Chastanger and Ogawa (1979) was used as follows:

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

Where: S = severity of infected fruits; n = number of infected Valencia fruits per category; c = category number and N = total examined fruits.

### Statistical analysis

The obtained data were subjected to IBM SPSS software version 14.0. Analysis of variance was determined and the mean values were compared by Duncan's multiple range test at  $P < 0.05$ .

## 3. Results

### Isolation, identification and pathogenic ability of the causal fungi

Isolation and examination trails revealed that the causal agent of brown spot identified as *Alternaria alternata*, meanwhile the identified causal agent of black spot was *Phyllosticta citricarpa*. The tested pathogenic ability of isolated fungi revealed typical symptoms of brown and black spot diseases on Valencia and Navel orange fruits artificially infested with *Alternaria alternata* and *Phyllosticta mangiferae*, respectively, which can be seen in Figure 1. The re-isolated fungi revealed the same two fungal isolates used for the artificial inoculation of citrus fruits.

### Laboratory test

Presented data in Table 1 obviously showed the inhibitor effect of tested organic acids and yeast on the linear fungal growth of both *Alternaria alternata* and *Phyllosticta citricarpa*. It was observed that the fungal linear growth is decreased by increasing concentration of tested materials. At concentrations of 1, 2 and 3g/L *Alternaria alternata* and *Phyllosticta citricarpa* showed the lowest growth measured as 30mm, 25mm; 40mm, 30 mm and 40mm, 35mm at salicylic acid followed by *Potassium Dihydrogen Phosphate* and *Trisodium orthophosphate*, in respective relevant treatment. At Ascorbic acid followed by Calcium chloride treatments moderate inhibitor effect was recorded for the growth of *Alternaria alternata* and *Phyllosticta citricarpa*. Growth of the two fungi was inhibited at concentrations of 2 and 3g/L. they revealed linear growth measured as 63mm, 52mm; 66mm, 51mm and 74mm, 57mm, 78, 56mm, in relative respect for chemical tested and their concentrations. The yeast *Saccharomyces cerevisiae* caused complete reduction for both fungi at the lowest concentration used 2.5g/L.

### Post-harvest storage experiment

The incidence of brown and black spot diseases of harvested Valencia orange fruits previously sprayed with some organic acids, salts and the antagonist *S. cerevisiae* as pre-harvest approaches under field conditions was evaluated under artificial conditions. The apparently healthy harvested Valencia fruits were artificially inoculated individually with either brown or black pathogens, and stored for 10 and 20 days, diseases incidence and severity were recorded. It is obviously from data presented in Table 2 and Figure 2 that *S. cerevisiae* and salicylic acid showed announced effect for protecting the treated fruits against fungal invasion throughout the 20 days of storage. Meanwhile, temporary protective effect was observed at the other tested treatments. They could provide protection to treated fruits for 20 days only. Furthermore, illustrated data in Figure 2 revealed that after 20 days of storage ascorbic acid treatment reduced brown and black spot disease incidence by 66.6%. Meanwhile reduction by 66.6, 33.3%, 33.3, 66.6% and 66.6, 66.6% in brown and black spot incidence were recorded at Potassium dihydrogen phosphate, Calcium chloride and Trisodium orthophosphate treatments, in respective order. Disease severity of the infected Valencia fruits with brown and black spot diseases followed the same trend.

**Table 1.** Growth inhibition of *Alternaria alternata* and *Phyllosticta citricarpa* in response to some chemical inducers and yeast *in vitro*.

Treatment	Fungal growth mm.					
	<i>Alternaria alternata</i>			<i>Phyllosticta citricarpa</i>		
	Chemical concentration g/L					
	1	2	3	1	2	3
Salicylic acid	30	0	0	25	0	0
	±3.0 g	±0.0 h	±0.0 h	±1.0	±0.0 h	±0.0 h
Ascorbic acid	90	63	52	90	66	51
	±0.0 a	±2.0 cd	±3.0 de	±0.0 a	±1.0 c	±3.0 de
Potassium dihydrogen Phosphate	40	0	0	30	0	0
	±4.0 f	±0.0 h	±0.0 h	±3.0 g	±0.0 h	±0.0 h
Calcium chloride	90	74	57	90	78	56
	±0.0 a	±3.0 b	±2.0 d	±0.0 a	±1.0 b	±2.0 d
Trisodium orthophosphate	40	0	0	35	0	0
	±2.0 f	±0.0 h	±0.0 h	±2.0 g	±0.0 h	±0.0 h
	Yeast g/L					
<i>S. cerevisiae</i>	2.5	5.0	10.0	2.5	5.0	10.0
	0	0	0	0	0	0
	±0.0 h	±0.0 h	±0.0 h	±0.0 h	±0.0 h	±0.0 h
Untreated (Control)		90			90	
		±0.0 a			±0.0 a	

Means ± standard deviations within a column followed by the same letter are not significantly different Duncan Multiple Range Test at  $P < 0.05$ .

**Table 2.** Efficacy of Pre-harvest treatments against brown and black spot disease on Valencia orange under artificial infection during storage for 10 and 20 days.

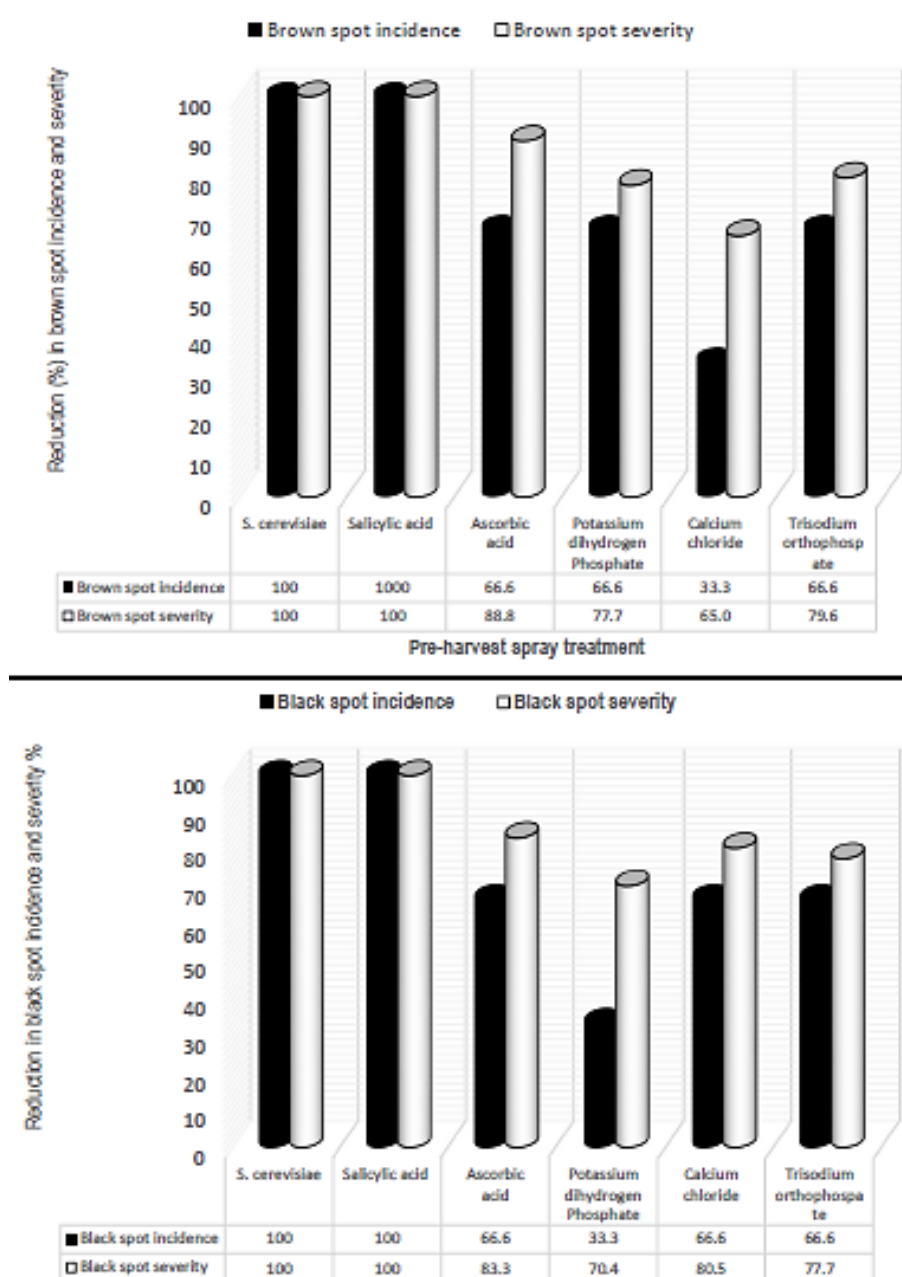
Tree spray treatment	Av. diseases incidence %*				Av. diseases severity %*			
	Brown spot		Black spot		Brown spot		Black spot	
	10 days	20 days	10 days	20 days	10 days	20 days	10 days	20 days
<i>S. cerevisiae</i>	0	0	0	0	0	0	0	0
	±0.0 j	±0.0 j	±0.0 j	±0.0 j	±0.0 j	±0.0 j	±0.0 j	±0.0 j
Salicylic acid	0	0	0	0	0	0	0	0
	±0.0 j	±0.0 j	±0.0 j	±0.0 d	±0.0 j	±0.0 j	±0.0 j	±0.0 j
Ascorbic acid	0	33.3	0	33.3	0	10.0	0	15.0
	±0.0 j	±0.05 c	±0.0 j	±0.05 c	±0.0 j	±0.5 i	±0.0 j	±0.05 h
Potassium dihydrogen Phosphate	0	33.3	0	66.6	0	20.0	0	26.6
	±0.0 j	±0.5 c	±0.0 j	±0.11 b	±0.0 j	±1.15 g	±0.0 j	±0.11 f
Calcium chloride	0	66.6	0	33.3	0	31.5	0	17.5
	±0.0 j	±0.11 b	±0.0 j	±0.05 c	±0.0 j	±0.86 e	±0.0 j	±0.11 h
Trisodium orthophosphate	0	33.3	0	33.3	0	18.3	0	20.0
	±0.0 j	±0.05 c	±0.0 j	±0.05 c	±0.0 j	±0.52 h	±0.0 j	±0.05 g
Untreated (Control)	66.6	100	66.6	100	78.5	90.0	52.5	90.0
	±0.11 b	±0.0 a	±0.11 b	±0.0 a	±0.76 b	±5.0 a	±6.0 c	±0.0 a

\* Average percent of diseases Valencia fruits for the two carried experiments. Means ± standard deviations within a column followed by the same letter are not significantly different

#### 4. Discussion

Isolation trails revealed two fungi identified as *Alternaria alternata* and *Phyllosticta mangiferae* which proved their pathogenic ability to cause brown and black spot of Valencia and Navel orange fruits. The sporulation characteristics of the two fungi were compared with those reported in the identification manuals of Barnett and Hunter (1998) and Simmons (2007). *Alternaria* conidia is easily identified by its shape, which are large, ovoid to obclavate, dark-colored, multicellular with longitudinal and horizontal septations. Near the base, they are wide becoming narrow to the top, providing a club-like appearance. Conidia are produced in single or branched chains on short conidiophores. The causal agent of citrus Brown Spot *A. alternata* is reported by many researchers (Timmer 2000; Peres et al. 2003; Lengia et al. 2014). As for the *Phyllosticta* Genus include about 330 known species. Many species in this family and genus are known as plant endophytic fungi and among them, the causal agent of Citrus Black Spot *G. citricarpa* which reported by

several investigators (Marques et al. 2012; Carvalho et al. 2014; Tran et al. 2017). All previous reports confirmed that *Alternaria alternata* and *Phyllosticta citricarpa* are the causal agents for citrus brown and black spot diseases, respectively. Under *in vitro* conditions, the inhibitory effect of some organic acids and antagonistic yeast on the linear fungal growth of both *Alternaria alternata* and *Phyllosticta citricarpa* were evaluated. In this regard, various antioxidants which had antifungal inhibitory effect on fungal growth *in vitro* were reported by many investigators. Salicylic and ascorbic acids have inhibitory effect against the fungal growth which contaminates food and feed. Organic acid application causes acidic medium which affect the fungal cell membrane as well as the enzymes activity which responsible for degrading the substrate (Kristiansen and Sinclair 1979). Furthermore, Dalie et al. (2010) reported that changing the electrochemical properties of the plasma membrane and increasing its permeability caused by organic acids action. Increasing the diffusion of acid across the plasmic membrane and cytoplasm is resulted due to the reduction of pH at greater concentration of protons. Abd El-Hai et al. (2009) tested the inhibitor effect of organic acids, citric and salicylic against the linear growth of *Macrophomina phaseolina* and *Rhizoctonia solani* *in vitro*. They found that salicylic acid and citric acid and their combination significantly reduced the linear growth of both fungi.



**Figure 2.** Influence of some organic acids, salts and yeast as pre-harvest foliar application on reduction of brown and black spot disease incidence and severity under artificial inoculation during storage for 20 days.

Furthermore, a complete reduction in the two fungal growth was observed at 10 mM of salicylic acid or combined salicylic plus citric acids treatment. Qi et al. (2012) reported that in the presence of increasing concentration of salicylic acid in both liquid and solid media the mycelial growth and conidial germination *Fusarium graminearum* were significantly inhibited and ultimately suppressed. Also, Verghese et al. (2017) demonstrated the ability of Vitamin C (ascorbic acid) to inhibit pathogenic bacteria and inhibit biofilms of *Escherichia coli* and *Klebsiella pneumoniae*. Bacterial growth was inhibited at all Vitamin C concentrations of 5,10,20 mg/ml. At concentrations 1.5% and 2.0% of calcium chloride and calcium hydroxide mycelial growth, spore germination and germ tube significantly decreased of *Colletotrichum acutatum*, *C. gloeosporioides*, *Alternaria alternata*, and *Penicillium expansum* fungal isolates (Stosic et al. 2014). Mecteau et al. (2014) stated that the mycelial growth of *Fusarium solani* var. *coeruleum* significantly inhibited when exposed to several salts. Trisodium phosphate, aluminium acetate, aluminium chloride, sodium benzoate, sodium metabisulfite, potassium sorbate at 0.2 mM resulted in complete growth reduction of the tested fungus. Also, Boumaaza et al. (2015) recorded complete growth reduction of *Botrytis cinerea* at 300ppm concentration of Calcium chloride *in vitro*. On the other hand, complete growth reduction of both tested fungi was observed in growth medium supplemented with the yeast *S. cerevisiae*. Similarly, remarkable antagonistic properties by both *Candida guilliermondii* and *Saccharomyces cerevisiae* strains against *P. expansum* on apple was reported by Scherm et al. (2003). Also, utilization of antagonistic yeasts as an alternative appears to be a promising technology (Fan et al. 2002). Further, six different isolates of *S. cerevisiae* were evaluated against *Colletotrichum acutatum* the causal agent of post-bloom fruit drop disease which occurs during pre-harvest stage of citrus growth (Lopes et al. 2015). They found that all six *S. cerevisiae* isolates had the ability to produce antifungal compounds which inhibit the pathogen germination, compete for nutrients, as well as produce killer activity and hydrolytic enzymes when contacted the fungus cell walls.

In current study, reduction postharvest diseases incidence and severity could be achieved by pre-harvest management strategy. Application of an efficacious pre-harvest fungicides can supply satisfaction control for postharvest decay after harvest was reported (Ritenour et al. 2004; Yildiz et al. 2005). In the same way several investigators followed the same strategy using some antioxidants and chemical resistance inducers as pre-harvest application to achieved the same target avoiding agro-chemical intensive use. El-Mougy et al. (2015) recorded that successful approach for controlling the postharvest diseases could be achieved through utilization of yeast and/or some food preservatives as pre-harvest spray application to Valencia orange trees. The successful attempts following eco- friendly approach which able to suppress or delay the development of disease incidence known in the last few decades lead to the use of natural plant defense inducers, *i.e.* salicylic and jasmonic acids (Pluskota et al. 2007; Tamaoki et al. 2008) fulfill in the acquired systemic resistance and obvious a long constant protection against a wide spectrum of plant pathogens. Therefore, the present study conducted with evaluation of some organic acids and salts as well as yeast as foliar spray to Valencia trees two weeks before harvesting time against brown and black spot the postharvest diseases. Comparable researches outcome to our results were reported by various investigators. Many investigators stated that application of antioxidants may control foliar fungal diseases. Moreover, the plant phenols which play a major role in plant disease defense were enhanced by the level of antioxidants (Hahlbrock and Sceil 1989). Salicylic acid is a small phenolic compound is reported to play an important role in plant immunity (An and Mou 2011), plant growth and development (Rivas-San Vicente and Plasencia 2011). In majority of studies up to recent it has been reported that salicylic acid classified as defense-related plant hormones which play remarkable role in signal transduction and acquired disease resistance (Hao et al. 2018). Abd El-Hai et al. (2009) reported significant reduction in the incidence of damping-off and charcoal rot of sunflower was obtained by foliar spray with citric acid and salicylic acid at 10 mm (antioxidants) as well as manganese and zink at 2g/l (microelements). Moreover, Ascorbic acid as an antioxidant and in combination with other modules of the antioxidant system, protects plants from oxidative damage which could be resulted from aerobic metabolism, photosynthesis as well as a wide range of pollutants such as ozone, heavy metal and saline stress (Mazid et al. 2011). Also, results of Barth et al. (2004) demonstrate that greater disease resistance came after the deficiency of ascorbic Acid which positively modulates plant biotic defense sequences. Katay et al. (2011) reported that ascorbigen and 1-methyl ascorbigen the derivatives of ascorbic acid affect disease resistance in bean against the rust fungal pathogen *Uromyces phaseoli* and also suggests that efficacy of plant protection depended on the dosage and the time interval between the



chemical pre-treatment of applied 1-methylascorbigen and inoculation with the pathogen. In addition, Belide et al. (2011) also suggested that using ascorbic acid singly and along with L-cysteine and iota-carrageenan effectively controlled hyper-hydration of and necrosis caused by the bacterium *Agrobacterium*. On the other hand, applications of potassium bicarbonate were efficient approaches that reduce the disease severity of powdery mildew on *Eriobotrya japonica* and pumpkin trees (Ziv and Hagiladi 1993). Also, Reuveni et al. (1996) reported that pre-inoculation foliar sprays of using mono- or dipotassium phosphate, sodium bicarbonate significantly suppressed powdery mildew colonies on diseased foliage of greenhouse-grown cucumber. They added that highly protective effect against powdery mildew fungus was obtained by application of mono- or dipotassium phosphates and potassium nitrate solutions under natural infection in the greenhouse. Also, research by Reuveni et al. (1994) demonstrated the reduction in powdery mildew severity on rose through phosphate and potassium salts foliar applications. Significant reduction in the damping off and the unconventional malformed seedlings of cotton plants was reported using trisodium orthophosphate (Elwakil et al. 2015). The potential role of calcium supplementation in the postharvest period for reducing fruit decay have been examined in small numbers of studies. Pre- and postharvest calcium applications supply an effective broad-spectrum protection against apple fruit rots caused by *Penicillium expansum*, *Botrytis cinerea* and *Alternaria alternata* rots (Biggs et al. 2000; Maouni et al. 2007). Reduction in the development of postharvest diseases of fruit has been attributed mainly to the potentiality of calcium to form calcium cross-linkages in the cell wall which resulting in reduced efficacy of cell wall-lytic enzymes release by the pathogen (Conway et al. 1992).

## 5. Conclusions

The achieved results in this study spotlight the use of yeast and organic acids and salts as an alternative tool for control strategy against brown and black spot the citrus postharvest diseases. This strategy could be partly or fully developed the control of other postharvest infections by a new vision supplied in this study and could become effective module of an integrated management system for such postharvest diseases.

**Authors' Contributions:** KHALIL, M.S.A.: conception and design, and drafting the article; EL-GAMAL, N.G.: acquisition of data, analysis and interpretation of data, and critical review of important intellectual content EL-MOUGY, N.S.: acquisition of data, analysis and interpretation of data; ABDEL-KADER, M.M.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article. All authors have read and approved the final version of the manuscript.

**Conflicts of Interest:** The authors declare no conflicts of interest.

**Ethics Approval:** Not applicable.

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