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Pathological survey at different Egyptian local markets on green and blue moulds of lime and lemon fruits and its management using some mineral salts and organic acids

Atef Mohamed Mohamed, Ahmed abd-elghany Tawfeek and Abdel Radi Taher Bakeer*
Plant pathology, Faculty of Agriculture, Fayoum University, Fayoum, Egypt, 63514.

ABSTRACT :

Survey of decayed lime and lemon fruits of local markets at Giza, Cairo and Fayoum Governorates, Egypt during 2018 and 2019 summer seasons was conducted. Several postharvest diseases was observed *i.e.*, green mold, blue mold and Physical damage are the common on fruits. Results showed that fruit decay incidence in lime and lemon fruits was higher in Cairo Governorate whereas Fayoum Governorate was the lowest one. Routine isolation of rotten samples of lime and lemon fruits yielded mainly three genera of fungi *i.e.*, *Penicillium digitatum, Penicillium italicum* and *Aspergillus* spp. Pathogenicity test indicated that *P. italicum* (No.7) was the most pathogenic fungi caused postharvest diseases on fruits followed by *P. digitatum* (No.6). The effect of two mineral salts; potassium sorbet and potassium silicate and the two organic acids; salicylic and β-amino–butyric acids against *P. digitatum* and *P.italicum* was evaluated for their inhibitory effect *in vitro* and *in vivo* under storage conditions. Under laboratory conditions, complete inhibition of mycelial growth of *P. digitatum* and *P. italicum* was obtained with potassium silicate, potassium sorbet (4.0, 5.0 and 6.0 g/L), as well as salicylic acid and β-amino –butyric acid (1, 1.5 and 2.0 g/L). *In vivo* experiments, in 2018 and 2019 seasons, postharvest-treated fruits by different organic acids at full at a concentration of 4g/L and mineral salts at full at a concentration of 1g/L (w/v) showed various levels of either protective or curative effect for coated lime and lemon fruits against mould infection whatever the time of their artificial inoculation under storage conditions.

All treated fruits showed reduction in green and blue mould diseases when compared with untreated fruits. Also, potassium silicate was the most effective treatment among the tested mineral salts, reducing the disease incidence and severity on lemons and lime fruits at (20±1°C) followed by Potassium sorbate. Salicylic acid was the most effective treatment in reduction ratio in disease incidence and disease severity. Also, the effect of potassium sorbet and potassium silicate as well as salicylic acid and β-amino–butyric on quality characteristics (total soluble solids (TSS %) and total phenolic substances) in lime and lemon fruits was studied. Furthermore, lime and lemon fruits stored and treated with some mineral salts and organic acids as individual treatments increased the activity of peroxidase, polyphenol oxidase and chitinase and achieved the higher content of total soluble solids % (TSS) and total phenolic compounds (Tph) activity % under artificial infection with *P.digitatum* and *P.italicum* stored at 20±1°C.

* Corresponding author Email: bakeer 91@yahoo.com
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The present findings demonstrate that potassium sorbet, potassium silicate, salicylic acid and β-amino–butyric have potential as environmentally friendly products, nontoxic postharvest fungicides against sour rot, green and blue mould incidence of stored lime and lemon fruits and could be suggested for commercial use in packing-houses in consideration to their wide consumption as safely food preservatives.

KEYWORDS: Citrus, lemon and lime, green and blue mould, incidence, severity, mineral salts, organic acids and chitosan.

1.INTRODUCTION:

Citrus is a genus from Rutaceae family, sub family *Aurantoideae* and these are several species in this genus; but there are major species such as sweet orange (*Citrus sinensis* (L.) Osbeck), mandarins group, grapefruits (*Citrus paradisi*), lime (*Citrus aurantifolia)* and sour orange (*Citrus aurantium* L.) **(Salem and Sheta, 2002).** There are different varieties of lemon (*Citrus lemon*) cultivated in Egypt, but acid lime (*Citrus aurantifolia*) also called Egyptian Lime is the main variety depending on cultivated area (about 16324 ha). However, productive area reaches 14461 ha, producing 345929 t. **(Anonymous, 2020)**. Among fungal diseases, post-harvest diseases are also important which result in significant losses. Many pathogens are reported to be associated with post-harvest fungal diseases which drastically reduced yield and quality of citrus **(Plaza et al. 2004)**. Many post-harvest pathogens infecting citrus fruits include *Penicillium digitatum Sacc* and *P. italicum Wehmer, Botrytis cinerea, Alternaria citri, Colletotrichum gloeosporioides, Aspergillus niger, Phytophthora parasitica, Diplodia natalensis, Geotrichum candidum* and *Trichoderma viride* **(Whiteside et al. 1988).** In developing countries, lack of proper handling of freshly harvested fruit resulted in huge losses during transport and storage which exceed sometimes more than 50 percent **(Wisniewski and Wilson, 1992)**. Among post-harvest diseases of citrus, green mould (*Penicillium digitatum)* and blue mould *(P. italicum*) are most commonly observed in all citrus growing areas throughout the world **(Palou et al. 2001 and Skaria et al. 2003).** There are many widely used effective preservatives against yeast and mould. Several inorganic salts and organic lipophilic acids and their salts, some of which are used, in the food-processing industry, have antimicrobial properties and could be useful as postharvest treatment for decay control. Using potassium sorbate or sodium benzoate against postharvest diseases of tomato, apple, carrots and potato have been reported by **Ryu and Hold (1993). Hall (1992)** demonstrated that the food preservatives potassium sorbate or sodium benzoate when applied to citrus fruits inoculated with *Penicillium digitatum* had similar fungicidal activity and are equivalent to the traditional treatment used as a postharvest fungicide for controlling citrus fruit decay. Salicylic acid, as an endogenous signalling molecule e, induces or enhances photosynthesis, stomatal conductance, transpiration, disease resistance, seed germination and, ultimately, crop yield. Salicylates have been shown to delay ripening and maintain postharvest quality of fruit through inhibition of ethylene biosynthesis **(Terry and Joyce, 2004)**. The application of organic acid washes to the surface of fruits and vegetables for the purpose of reducing populations of microorganisms which could be killed or prevented from growing by applying organic acids could be used as a trend. Procedures as simple as applying lemon juice, which contains citric acid as the major acid, to cut fruits have been shown to kill or retard the growth of pathogens **(Escartin et al. 1989)**. The evaluated chemicals such as Salicylic

acid, Sorbic acids, Potassium sorbate, Potassium carbonate led to decrease the mycelial growth of tested fungi in particular both salicylic and sorbic acids at high concentrations. The fungal growth was completely inhibited at concentration of 12.5 mM of both salicylic and sorbic acids. Meanwhile, potassium sorbate and potassium carbonate had inhibitory effect against mycelial growth of all tested fungi *in vitro*. The inhibitory effect increased by the increase of concentration used to reach its maximum at 4% **(El-Mohamedy et al. 2013)**. The chemical elicitor β-aminobutyric acid (BABA) is a non-proteinogenic amino acid that behaves as a safe priming molecule of systemic resistance induction in several crops such as apple, citrus, and strawberry (**Aghdam et al. 2020**).

The present study was conducted to survey the information on disease incidence and severity of green and blue molds of citrus at different local markets from different Governorates in Egypt. Besides, and to evaluate the effect of potassium sorbet and potassium silicate in addition to salicylic acid and β-amino–butyric as evaluated on the mycelial growth of fruit rot pathogenic fungi. Moreover, their protective or therapeutic effects on green and blue mould diseases incidence of lemon and lime fruits were examined. Also, Total soluble solids (TSS) and Total phenolic compounds were determined biweekly.

2.MATERIALS AND METHODS

The present research work was done in the laboratory of Plant Pathology, College of Agriculture, University of Fayoum and National Research Center to evaluate the efficacy of mineral salts and organic acids against postharvest diseases of Lime and lemon fruits .

2.1. Survey and collection of diseased samples of Lime and lemon fruits:

Lime and lemon fruits (Healthy and infected) collected from different local markets at Giza, Cairo and Fayoum Governorates during 2018 season. Other samples were collected from wholesale, retail markets, some farms and refrigerators from the above-mentioned locations during autumn and winter growing season; 2018. One hundred fruit from each location were kept in incubator for seven days at 20±1°C, and then were examined for green and blue rot disease. Incidence was calculated as percentage of infected lime and lemon fruits in relation to the total number of fruits in each replicate according to the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected fruits} \times 100}{\text{Total number of fruits examined}}$$

2.2. Isolation and identification of fungi associated with diseased lime and lemon fruits

Samples of diseased fruits from lemon and lime were carefully washed with sterilized water, cut into small pieces, surface sterilized in 70% ethanol, washed several times with sterilized distilled water (SDW) and dried between two sterilized absorbent paper (Whatman filter papers) then transferred to Potato dextrose agar (PDA) plates medium and incubated at 20±1°C for seven days. Developed fungi from diseased samples were purified by using single spore technique described by **Fang et al. (1983)** and hyphal tip transfers method mentioned by **Howard (1981).** Stock cultures of the obtained fungi were maintained onto PDA slants and stored in a refrigerator for further studies. *Penicillium digitatum* (green mould) and *P. italicum* (blue mould) were isolated from naturally infected lemon and lime fruits after storage of several weeks in Plant Pathology Laboratory, Plant Pathology Department, and National Research Center. These isolates were the most aggressive one in our collection and pronounced the largest lesions

on inoculated fruits. Isolated fungi from decayed fruits were identified according to **Ellis (1971)** and **Barnett and Hunter (1972)**. Further identification of *Penicillium digitatum and Penicillium italcum* was achieved according to its morphological characteristics of conidia and conidiophores **(Singh et al., 1991).** Purified fungi were maintained on PDA slant under refrigerator conditions at 5˚C as stock cultures for further studies.

2.3. Pathogenicity test of isolated fungi on lemon and lime fruits.

a – Lime and lemon fruits:

Fresh harvested apparently healthy fruits lemon (*Citrus lemon* L.) and lime (*Citrus aurantifolia* F. Muell) Balady cv., obtained from markets, were washed by sterilized distilled water and surface sterilized in 70% ethanol and then left to dry at sterilized room conditions.

b- Inoculum preparation:

To prepare standard inoculum; the pure fungal isolates of *P. degitatum and P. italcum* were grown separately at 25±2°C for seven days on PDA plates. Spore suspension, from each fungus was obtained by brushing the surface of the culture in the presence of 10 ml sterilized distilled water of each plate, then the spore suspension were filtered through muslin. The concentration of spore suspension was adjusted to about $4x10^6$ spores/ml using haemocytometer slide. Inoculum concentration of *Penicillium degitatum and Penicillium italcum* conidia was prepared and counted with a haemocytometer and adjusted to about $4x10^6$cfu/ml followed the method described by the **Eckert and Brown, 1986** for evaluation of postharvest treatments to control Lime and lemon fruits green and blue molds.

c- Inoculation of lemon and lime fruits.

Referring to the survey, markets of Giza and Fayoum were the most available location for disease occurrence, so, the samples obtained for further studies were taken from these Governorates.

Apparently healthy lemon and lime fruits obtained from markets of Fayoum and Giza Governorates, were surface sterilized in 70 % ethyl alcohol and washed several times with sterilized distilled water then dried at room temperature. Sterilized fruits were artificially wounded by a sterile scalpel at one side of the of fruits top, inoculated with prepared inoculum using an atomizer for each fungus at the rate of 10 ml spore (or growth) suspension ($4x10^6$ spores/ml) per two hundred gram fruits. Healthy wounded lemon and lime fruits were sprayed with the same amount of sterilized distilled water served as control. All inoculated and non-inoculated lemon and lime fruits were kept in foam tray (23×12×4cm), and inserted in polyethylene bags for one day to increase the relative humidity. Three replicates were used for each treatment. Inoculated and non-inoculated fruits were stored at 23- 25˚C, and fruits inoculated with *Penicillium degitatum and Penicillium* were stored at 20±1˚C. After seven days fruits of all treatments were examined. Disease incidence was measured as the percentage of number of infected fruits to the total number of fruits in the treatment.

$$\textbf{Disease incidence (\%)} = \frac{\text{Number of infected fruits} \times 100}{\text{Total number of fruits examined}}$$

	Grade Description
0	No decay development.
1	Decay up 0.5 cm in diameter without sporulation.
2	Decay between 0.5 to1.0 cm in diameter with sporulation.
3	Decay between 1.0 to 2.5cm in diameter.
4	Decay between 2.5 to 4.0 cm in diameter.
5	Fruits completely rotten and heavily covered with mycelium.

While, disease severity was estimated by two dimensions of the external rotted area in the proportion to the total length of the fruit. Rotted navel orange fruits were classified into six

categories (0, 1, 2, 3, 4 and 5) according to decayed area of fruits, and disease severity was calculated as follows (**Embaby et al. 2013):**

The percentage of disease severity was calculated according to the formula suggested by **Townsend and Heuberger (1943)** as follows:

$$\text{Disease severity (D.S). \%} = \frac{\sum (nxr1)\ldots..(nxr5)\ x100}{5N}$$

Where:

D.S. = Disease severity.

N = Number of decayed fruits per category. r1...r5 = Category number.

N = Total examined fruits multiplied by the maximum numerical disease grade, i.e. 5.

2.4. In vitro studies:

a- Source of the pathogens:

The most virulent isolate of *P. degitatum* (Isolate No 6) and *P. italcum* (Isolate No7) causing green and blue mould of lemon and lime fruits were used in the following Laboratory tests.

Effect of different concentrations of some mineral salts and organic acids on the mycelial growth of *Penicillium digitatum* and *Penicillium italcum in vitro*.

Potassium sorbate ($C_6H_7KO_2$) and potassium silicate ($Sio_3K_2o_2$) as mineral salts (Sigma-Aldrich) while salicylic acid ($C_7H_6O_3$) and β-amino–butyric acid ($C_{12}H_{13}NO_2$) as organic acids were purchased from El-Nasr pharmaceutical chemicals company and used in the present work. The two mineral salts were evaluated for their capability to inhibit *P. digitatum* and *P. italcum* fungal growth *in vitro*. Certain weights of each salt were dissolved in to PDA medium before it's solidifying to obtain the proposed concentrations of 1.0, 2.0, 4.0 and 0.5, 1.0, 2% for mineral salts and 0.125, 0.25, 0.5, 1.0 and 2% for organic acids then poured into Petri-plates. After medium solidification, Petri dishes were inoculated in its center with 5 mm discs of 7 days old culture of *P. digitatum* and *P. italcum* separately and then incubated at 25±1°C for 5 days. Five plates of each treatment were used as replicates per each concentration. Five replicates were prepared for each treatment as well as five plates without mineral salts and organic acid served as control. Linear growth of *P.digitatum* and *P. italcum* was measured when the check plates reached full growth and the average mycelial growth of fungi was calculated. Each treatment was represented by five replicates. The reduction in the fungal growth due to effect of mineral salts and organic acids was calculated using the following formula suggested by **Mansour (2012)** as follows:

$$\text{Growth reduction (\%)} = \frac{\text{Growth diam. in check - growth diam. in treatment}}{\text{Growth diam. in check}} \text{ x100}$$

2.5. In vivo studies:

Disease management, chemical properties and quality measurements of Lime and lemon fruits studies:-

Effect of postharvest dipping treatments on development of postharvest rots of lime and lemon fruits *in vitro*:

On the base of the *in vitro* experiment results obtained from laboratory experiment, two mineral salts i.e. potassium sorbet and potassium silicate (4.0gm/l con.) and two organic acids i.e salicylic acid and β-amino–butyric acid (1.0gm/l) were evaluated for their negative effect on green and blue molds disease incidence of lime and lemon fruits. This experiment was carried out on apparently healthy Lime and lemon fruits cv. Balady under laboratory conditions during the growing season 2018 and 2019. This experiment was designed to study the effect of the most effective concentration of the treated material on disease incidence and severity as post-harvest treatments.

Fresh samples of lemon and lime fruits were washed with tap water and then sterilized with 70% ethanol for one minute. The sterilized wounded fruits or not were inoculated separately with the mycelia suspension of *P. digitatum* and *P. italcum* (artificial infection), and the other unsterilized samples were left for the treated with water (control). After 24 hours of incubation at (20±1°C), the artificially inoculated lemon and lime fruits were dipped individually in salt and organic acid solutions treatment for three minutes, while control treatment was dipped in sterilized distilled water, then left to dry at aseptic condition **(Naffa and Rabie, 2006)**. Three replicates were assigned for each treatment (10 fruits for each was placed on foam tray and then, packed in polyethylene bags, retained high humidity (about 85-90%) and stored at 20±1°C for three weeks.. The treated fruits were air dried, after each individual treatment, for 2 hours in a laminar flow. This protocol was repeated in a separate trial and conducted for two seasons (2018 and 2019). Percentage of disease incidence and severity percentages on fruits were calculated after the storage period as mentioned before.

1- Effect of postharvest dipping treatments on quality characteristics:-

a- Total soluble solids content (TSS) %:

Total Soluble Solids (TSS %) in fruit juice was determined by using a hand refractometer. The percentages of TSS were recorded according to **(Sharman et. al. 1991, Embaby et. al. 2007 and Omayma et al. 2010)**.

b- Total phenols %:

The same extract prepared for measuring of total and reducing sugars was used for estimating of total phenols. Total phenolic content was caculated according to the Folin Ciocalteau procedure **(Shilpa et al. 2018)** .The absorbance was measured at 725 nm after 1 h against a reagent blank. Standard curve was prepared using different concentration of tannic acid. Total phenol value was expressed as mg tannic acid equivalents (TAE) / g dry weight (DW).

Statistical analysis:

Experiments were setup in a complete randomized design. Data were subjected to one way analysis of variance (ANOVA) using Statistica Software (Ver. 6.0) and means were separated by **Duncan (1955)** test at $P<0.05$ for *in vitro* experiment, the significant differences were indicated with a different letter. For other experiments Fisher's protected least significant difference (L.S.D.) was used at $P\leq0.05$ levels according to **Snedecor and Cochran (1980)** to distinguish the differences among various treatments.

3. RESULTS AND DISCUSSION:

3.1. Survey of lime and lemon decay:

Samples of Lime and lemon fruits were collected from different local markets at Giza, Cairo and Fayoum Governorates and were stored at room temperature under laboratory conditions and examined for decayed ones. Other samples were collected from wholesale, retail markets, some farms and refrigerators from the same locations mentioned above during seasons 2018-2019. The collected fruits were classified into three groups according to natural decay incidence.

Survey of some Egyptian markets in Giza, Cairo and Fayoum Governorates, Egypt during summer seasons 2018 and 2019 showed that postharvest diseases of Lime and lemon fruits *i.e.*, green mold, blue mold and Physical damage are the common on fruits. Results presented in table 1 indicated that green and blue molds are the most important and the most frequently diseases affecting lime and lemon fruits with average of 21.10, 20.06% and 13.43, 15.30%, respectively. Meanwhile**,** Physical damage recorded the lowest incidence of decayed fruits on lime and lemon fruits nearly 3.8% of the decayed fruits with average of 4.53 and3.11%, respectively. Isolation from decayed fruits and pathogenicity test proved

that the causal agents associated with the green and blue mold are *Penicillium digitatum* and *P. italicum,* respectively.

Results in Table (1) indicate that Fruit decay incidence % in lime and lemon fruits were higher in Cairo Governorate with average of (21.61%) followed by Giza (12.0%) whereas Fayoum Governorate was the lowest one (11.72%).

Table 1. Incidence post-harvest diseases and physical damages of lime and lemon fruits at different local markets from different Governorates in Egypt (Cairo-Giza-Fayoum).

Markets Locations		%Frequency of Fruit decay incidence of lime and lemon fruits						
		Lime			Lemon			Mean
		Green mold	Blue mold	Physical damage	Green mold	Blue mold	Physical damage	
	Obour	19.00	23.00	5.00	15.00	13.00	3.00	
	Elslaam	22.00	20.00	3.00	14.00	14.00	2.00	
	Ain shams	22.0 0	22.00	4.00	13.00	12.00	2.00	**21.61**
Cairo	**Mean**	21.00	21.60	4.00	14.00	12.60	2.33	
	Doki	17.00	18.00	6.00	12.00	10.00	3.00	
	Moneeb	20.00	21.00	5.00	15.00	12.00	4.00	
Giza	**Imbabah**	21.00	22.00	4.00	12.00	12.00	2.00	**12.00**
	Mean	19.30	20.30	5.00	13.00	11.30	3.00	
	Fayoum	18.00	20.00	5.00	12.00	11.00	3.00	
	Ibshwai	19.00	17.00	4.00	15.00	14.00	4.0k	**11.720**
Fayoum	**Snouras**	16.00	18.00	5.00	13.00	12.00	5.0k	
	Mean	17.60	18.30	4.60	13.30	12.30	4.0	
Average		21.10	20.06	4.53	13.43	15.30	3.11	

As seen in table (1) and figure (1), results reveal that blue and green molds diseases incidence% and disease severity% in lemon fruits was higher in Obour in Cairo Governorate and in Fayoum Governorate and Moneeb and Imbabah markets in Giza Governorate (15.0, 14.0% and 18.0%), respectively. While Doki and Fayoum markets were the lowest one in the disease incidence % and disease severity% (10.0 and 12.0 %) and (12.0 and 14.0, %) for blue and green %), respectively A Results Figure (2) indicate that blue and green molds diseases incidence % and disease severity % in lime fruits were higher in Obour and Ain shams in Cairo Governorate and Ain shams and Elslaam markets in Cairo Governorate (23.0, 22.0% and 17.0 and 16.0%), respectively. While Ibshwai and Fayoum markets in Governorate Fayoum were the lowest one in the disease incidence % and disease severity% (17.0, 16.0%) and (9.0 and 10.0 %) for blue and green molds diseases, respectively.

3.2. Isolation and frequency occurrence of pathogenic fungi: Different fungi were isolated and purified from collected Lime and lemon samples, showing rot symptoms at different local markets in Cairo, Giza and Fayoum Governorates during 2018 and 2019 through harvesting and storage seasons.

Remarkably, cultural characteristics and microscopic examination revealed that the obtained isolates were identified as *Penicillium digitatum P.italicum* and *Aspergillus* spp. Results presented in Table (2) and figure (2) that, *Penicillium digitatum* and *P. italicum* were the were the most prevalent fungi followed by *Aspergillus* spp. Also, the data showed that *Penicillium digitatum, P.italicum* were more frequently occurred than *Aspergillus* spp. where they ranged in average at the different Governorates from (31.81%), to (28.75%)

and (11.99%),respectively. While the remaining 28.36 % was made up of other fungi. There was a significant difference between the fungi isolated from the infected Lime and lemon samples. No significant differences were recorded between lime and lemon varieties.

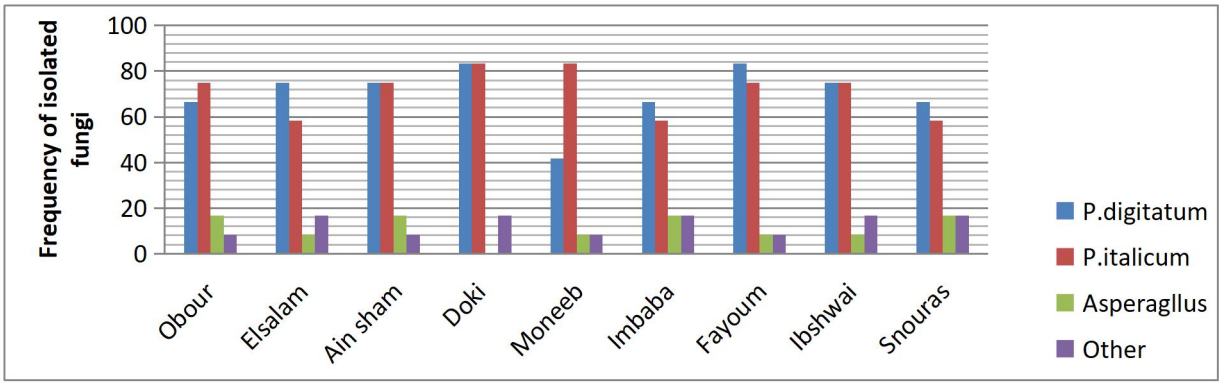

Fig. 1. Frequency of isolated fungi of diseased *P.digitatum, P.italicum* and others fungi in lime fruits collected from different locations

3.3. Pathogenicity test of the fungal isolates on lime and lemon fruits

Results in Table (3) indicate that, all tested fungi showed a significant percentage of pathogenic capability to infect Lime and Lemon fruits (Balad cv.) at (20±1°C) for 10 days of time course decay development.. All isolates of *P.digitatum and P.italicum* showed the highest level of both disease incidence and disease severity on both fruits with an average of (60.45 and 62.49%) and (46.26 and50.56%), respectively. While *Aspergillus* sp. showed the lowest level of disease incidence and disease severity on both fruits with an average of 6.50% and 23.42%, respectively. Significant differences were recorded between *P.digitatum and P.italicum.*

Table 2. Frequency occurrence of isolated fungi of diseased lemon and lime fruits collected from different locations.

Governorate	Location markets	Cultivar	Frequency of the isolated fungi % *P.digitatum*	*P.italicum*	*Asperagillus sp*	Others	Means
Cairo	Obour	Lime	43.3	35.0	8.3	8.3	22.29a
		Lemon	43.3	35.0	8.3	8.3	
	Elslaam	Lime	35.0	35.0	8.3	16.6	
		Lemon	43.3	35.0	16.6	8.3	
	Ain Shams	Lime	26.6	18.3	16.6	15.6	
		Lemon	26.6	18.3	16.6	15.6	
Giza	Doki	Lime	26.6	35.0	16.6	8.3	20.93a
		Lemon	26.6	26.6	8.3	8.3	
	Moneeb	Lime	35.0	18.3	8.3	16.6	
		Lemon	35.0	18.3	16.6	16.6	
	Imbabah	Lime	35.0	35.0	16.6	8.3	
		Lemon	26.6	35.0	16.6	8.3	
Fayoum	Fayoum	Lime	43.3	43.3	0.0	15.6	21.26 a
		Lemon	43.3	35.0	0.0	8.3	
	Ibshwai	Lime	11.6	43.3	8.3	8.3	
		Lemon	18.3	43.3	8.3	15.6	
	Snouras	Lime	26.6	18.3	16.6	16.6	
		Lemon	26.6	18.3	16.6	16.6	
	Means		31.81a	28.75b	11.99d	28.36c	
	Cultivar Mean :- Lime = 20.98 a & Lemon= 21.11 a						

Mean values within columns followed by the same letter are not significantly different (P < 0.05).

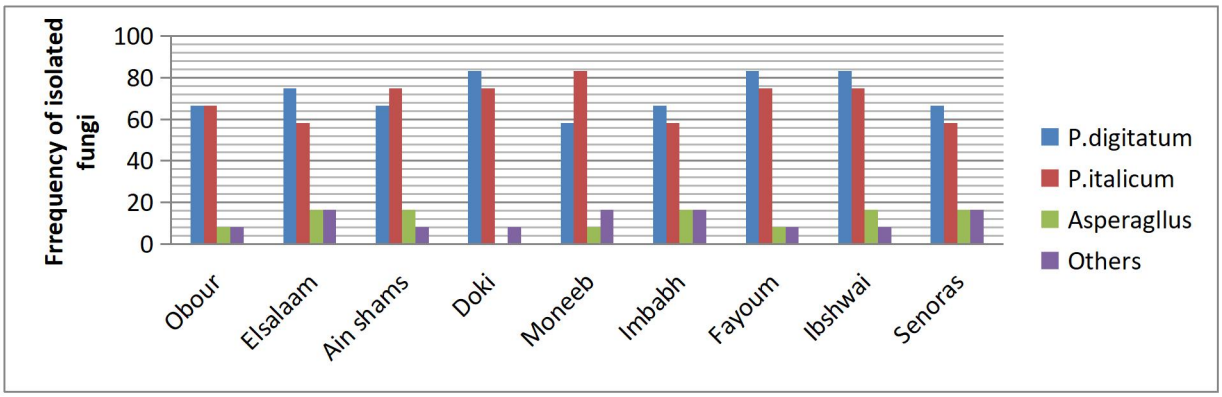

Fig. 2. Frequency of isolated fungi of diseased *P.digitatum* ,*P.italicum* and others fungi in lemon fruits collected from different locations.

3.4. Alternative methods to control post-harvest green and blue molds caused by *Penicillium digitatum* and *P.italicum* in lime and lemon fruits.

The experiment was repeated three times and the results were found to be reproducible. Some alternatives to chemical control regime *i.e.* mineral salts and organic acids as postharvest treatments were selected.

3.4.1. *In vitro* experiments:

3.4.1.1. The inhibitory efficacy of different concentrations of some mineral salts on the mycelial growth of *Penicillium digitatum* and *P.italicum*.Two mineral salts; potassium sorbet and potassium silicate were evaluated for their effect on linear growth of *Penicillium digitatum* and *P.italicum* under *in vitro* conditions. Data in Table (3) and figure (3) indicated that the two mineral salts can inhibit mycelial growth on *Penicillium digitatum* and *P.italicum* as compared to the control but the rate of inhibition varied with concentrations. The effects of different concentrations of potassium sorbet and potassium silicate also differed significantly. The results also indicate that the complete inhibition in the mycelial growth was obtained on *P. digitatum* and *P.italicum* treated with 4.0, 5.0 and 6.0 g/L concentrations of potassium sorbet and potassium silicate.

Table 3. Effect of different concentrations of some mineral salts on the average of mycelial growth of *P. digitatum* and *P.italicum* grown on PDA medium incubated at 25°C and calculations have been performed after the full hyphal growth of the control.

		P.digitatum		*P.italicum*			
***Treat. Salts**	**Conc. g/L**	**mycelia growth (cm)**	**Reduction %**	**mycelial growth (cm)**	**Reduction%**	**Salts means**	**%Reduction Mean**
	1.0	4.46c	**40.5**	0.5e	**44.4**		
K.Si	**2.0**	0.83d	**90.7**	0.75d	**91.6**		
	4.0	0.0f	**100.0**	0.0f	**100.0**	**15.68b**	**86.7**
	5.0	0.0f	**100.0**	0.0f	**100.0**		
	6.0	0.0f	**100.0**	0.0f	**100.0**		
	1.0	5.25b	**41.6**	5.6c	**42.6**		
	2.0	1.25d	**86.11**	1.0d	**88.8**	**16.0a**	**85.9**
K.S	**4.0**	0.0f	**100.0**	0.0f	**100.0**		
	5.0	0.0f	**100.0**	0.0f	**100.0**		
	6.0	0.0f	**100.0**	0.0f	**100.0**		
Control	**0.0**	9.00a	**0.0**	9.00a	**0.0**	**9.00 c**	**0.00**
Fungi mean		**1.60a**		**15.56b**			
Con. Mean :-		**0= 9.0a**	**1= 3.62b**	**2= 1.05c**	**3= 0.0d**	**4=0.0d**	**5=0.0d** **6=0.0d**

Mean values within columns followed by the same letter are not significantly different ($P < 0.05$). K.si = potassium silicate K.s = potassium sorbet *Treat. =Treatments, Conc. =Concentration

Fig. 3. Growth of *P.italicum* on PDA amended with varying concentration potassium silicate salt; Control = unamended medium incubation at 25°C in 9-cm-diameter Petri dishes.

3.4.1.2. The inhibitory efficacy of different concentrations of some organic acids on the mycelial growth of *P. digitatum* and *P.italicum*.

Salicylic acid and β-amino–butyric acid at five concentrations, *i.e.* 0.25, 0.5, 1.0, 1.5, and 2.0 %, were tested for their inhibitory effect on the mycelial growth of *Penicillium digitatum* and *P.italicum*. Data in Table (4) indicate that all of tested organic acids significantly reduced the mycelial growth of the tested fungi in varying level comparing to the control. On the contrary, a significant reduction was obtained by salicylic acid and β-amino–butyric acid on the mycelial growth of *P. digitatum* and *P.italicum* in which the activity was increased when the concentration increase. Among two tested organic acids (1.0, 1.5and 2.0 g/L) of Salicylic and β-amino–butyric acids had a high adverse effect on growth of *Penicillium digitatum* and *P.italicum* where they have been completely inhibited. On the other hand, lower concentrations of the above mentioned salts (0.5g/L) or even less hampered the fungal growth but the inhibition was not fully occurred. So, the only effective treatment could confer a resistance against these decay fungi was acting in a dose-dependent manner.

Table 4. Effect of different concentrations of some organic acids on the average of mycelial growth of *P.digitatum* and *P.italicum* grown on PDA medium incubated at 25°C and calculations have been performed after the full hyphal growth of the control.

Organic acids	Conc. g/L	*P.digitatum* growth (cm)	*P.digitatum* Reduction %	*P.italicum* mycelial growth (cm)	*P.italicum* Reduction %	Organic acid means	%Red. Mean
S.A	0.25	5.25b	41.6	5.6b	42.6		
	0.5	1.25d	86.11	1.0d	88.8		
	1.0	0.0f	100.0	0.0	100.0	**1.61a**	**85.9**
	1.5	0.0f	100.0	0.0	100.0		
	2.0	0.0f	100.0	0.0	100.0		
BABA	0.25	4.3	38.7	4.46	40.5		
	0.5	0.5	94.4	0.5	94.4		
	1.0	0.0	100.0	0.0	100.0	**1.58b**	**86.8**
	1.5	0.0	100.0	0.0	100.0		
	2.0	0.0	100.0	0.0	100.0		
Control	0.0	9.00	0.0	9.00	0.0	**9.00 c**	**0.00**
Fungi mean		**15.94a**			**15.96a**		
Con. Mean :-		**0= 90.0a & 1=4.90b & 2=0.81c & 3=0 .0d & 4=0.0d &5=0.0d**					

Mean values within columns followed by the same letter are not significantly different (P < 0.05). *Treatments: S.A = Salicylic acid BABA = β-amino–butyric acid * Conc.=Concentration Red. =Reduction

3.5. Disease management and Chemical Characteristics of Lime and lemon fruits studies:

4.5.1. Effect of postharvest dipping treatments of *in vitro* on development of postharvest rots of lime and lemon fruits.

4.5.1.1 Effect of some Mineral salts as post-harvest treatments on disease incidence and severity of green and blue mold diseases of lime and lemon fruits artificially infected with *P.digitatum* and *P.italicum* under storage conditions.

The influence of postharvest application of one promising concentration (4gm/L) was selected from all tested mineral salts i.e. potassium silicate and potassium sorbet were evaluated against the development of green and blue mold diseases of lime and lemon fruits **Table (5)** and the overall effect of the treatments on each cultivar was compared.

Data in Table (5) of season (2018) illustrated that, all tested mineral salts significantly reduced the disease incidence and disease severity % of artificially infected lime and lemon fruits with *P.digitatum* and *P.italicum* under storage condition (20±1°C) comparing with control treatment. On the other hand, the results in Table (5) showed that lime and lemon fruits treated with potassium silicate mineral salts at (4gm/l) revealed the highest reduction among tested substances compared to control where it reduced disease incidence by (91.7%) and reduced the disease severity by (91.6%) for *P.digitatum* and *P.italicum,* respectively under storage condition (20±1°C). Meanwhile, potassium sorbet recorded good effect against *P.digitatum* and *P.italicum* with about (72.8%) in diseases incidence and (78.6%) disease severity, respectively.

Regarding the second season, 2019, in the same table also indicated the data illustrated that, all tested mineral salts significantly reduced the disease incidence and disease severity % of artificial infected under storage condition (20±1°C) lime and lemon fruits with *P.digitatum* and *P.italicum* comparing with control treatment. The data presented in Table (5) showed that the incidence of decay on lime and lemon fruits was restricted by applying 4.0g/L of Potassium silicate, hence the incidence was significantly reduced by (91.7%) and reduced disease severity by (87.6%) for *P.digitatum* and *P.italicum*, respectively under different storage condition at (20±1°C). In a similar manner, treating fruits with (4gm/l) of potassium sorbet provided a compromised protection against the tested fungi, where significantly reduced disease incidence by (70.8%) and disease severity by (76.7%) for the *P.digitatum* and *P.italicum* respectively under the same storage conditions.

Significant differences between cultivars of disease incidence and disease severity % in lime and lemon fruits was found while, the greatest reductions of disease incidence and severity were observed on cultivar lime when mineral salts were applied in 2018 and 2019 seasons.

Table 5. Effect of some mineral salts as post-harvest treatments on green and blue mold disease severity of lime and lemon fruit artificial infected with *P.digitatum* and *P.italicum* under storage conditions during 2018 and 2019 seasons.

Disease assessment (Diseases incidence% and Diseases severity %)																
Season 2018																
Mineral Salts 4gm/L	Diseases incidence%								Diseases severity %							
	P. d.			P. i		Mean	Over mean	Red. %	P. d		Mean	P. i		Mean	Over mean	Red. %
	Lime	Lemon	Mean	Lime	Lemon				Lime	Lemon		Lime	Lemon			
K.si	8.30	8.30	8.30	8.30	8.30	8.30	8.30	91.7	8.30	8.63	8.46	8.40	8.30	8.35	8.40	91.6
K.s	25.33	33.30	29.31	25.0	25.0	25.0	27.15	72.8	19.53	19.40	19.26	20.80	25.0	22.9	21.08	78.9
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	1000.0	100.0	100.0	100.0	100.0	100.0	0.0
Fungi	45.87		-------	44.43		------	-------	------	42.64		------	43.75		-------	-----	------
Cultivar	Mean Lime= 44.49			Mean Lemon=45.82					Mean Lime= 42.84			Mean Lemon=43.56				
Season 2019																
K.si	8.30	8.30	8.30	8.30	8.30	8.30	8.30	91.7	8.30	16.50	12.4	16.60	8.40	12.5	12.4	87.6
K.s	25.0	25.0	25.0	33.30	33.30	33.30	29.15	70.8	19.40	19.40	19.40	20.80	33.30	27.05	23.22	76.7
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0
Fungi	44.43		------	47.20		------	-----	------	43.93		------	46.52		------	------	-------
Cultivar	Mean Lime= 42.49			Mean Lemon=48.82					Mean Lime= 42.49			Mean Lemon =48.82				

K.si = potassium silicate K.s = potassium sorbet *Treat=Treatments Conc. =Concentration

L.S.D at	Diseases incidence%			Diseases severity%		
0.05	Salts	Fungi	Cultivar	Salts	fungi	cultivar
2018	**0.77**	**0.63**	**0.63**	**0.68**	**0.56**	**0.56**
2019	**0.96**	**0.78**	**0.78**	**0.79**	**0.65**	**0.65**

3.5.1.2 Effect of some organic acids as post-harvest treatments on disease incidence and severity of green and blue mold diseases of lime and lemon fruits artificially infected with *P.digitatum* and *P.italicum* under storage conditions.

The results presented in Table (6) clearly revealed that lime and lemon fruits treated with Salicylic acid at (1gm/l) provided the highest reduction among tested substances compared to control where it reduced disease incidence by (95.4%) and reduced the disease severity by (88.5%) for *P.digitatum* and *P.italicum,* respectively under storage condition (20±1°C).,). Also, it is obvious that application of β-amino–butyric acid conferred the fruits a protection against *P. italicum* and *P. digitatum* at its concentration (1 gm/L), where disease incidence was reduced by (89.6%) and disease severity (91.7%) respectively. Noteworthy, the gradual minimizing of doses of salts, the lowering of control on decay attained. In other word, when conc. of Salicylic acid or β-amino–butyric acid decreases, the inhibitory impact against rotted fungi decreases as noted with the lower concentrations, consequently the size of rotten area expands through the time course of storage. Data cited in Table (6) in the second season (2019) showed the significance impact of using Salicylic acid at 1.0 g/l against the decay causing fungi, reducing diseases incidence and disease severity % in treated fruits. All tested organic acids reduced the diseases incidence and disease severity under artificial infection of Lime and lemon fruits with *P.digitatum* and *P.italicum* under different storage condition (20±1°C).

On the other hand, the results in the same Table clarified that lime and lemon fruits treated with β-amino–butyric acid at (1gm/l) revealed the highest reduction among tested substances compared to control where it reduced disease incidence by (91.7%) and reduced the disease severity by (91.7%) for

P.digitatum and *P.italicum,* respectively under storage condition (20±1°C)., Meanwhile, Salcylic acid recorded good effect in diseases incidence (91.7%) and disease severity(88.5%) respectively.

Table 6. Effect of some organic acids as post-harvest treatments on green and blue mold

Disease assessment (Diseases incidence% and Diseases severity %)																
Season 2018																
organic acids 1gm/L	**Diseases incidence%**								**Diseases severity %**							
	P. d.			**P. i**		**Mean**	**Over mean**	**Red. %**	**P. d**		**Mean**	**P. i**		**Mean**	**Over mean**	**Red. %**
	Lime	**Lemon**	**Mean**	**Lime**	**Lemon**				**Lime**	**Lemon**		**Lime**	**Lemon**			
S A	16.60	8.30	12.45	16.60	16.60	16.60	14.52	95.4	12.50	12.50	12.5	12.50	8.30	10.4	11.45	88.5
BABA	8.30	16.60	12.45	8.30	8.30	8.30	10.37	89.6	8.30	8.30	8.30	8.30	8.30	8.30	8.30	91.7
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.	100.0	100.0	100.0	100.0	0.0
Fungi	41.63		-------	41.63		------	-------	------	40.27		------	39.57		-------	-----	------
Cultivar	Mean Lime= 41.63			Mean Lemon=41.63					Mean Lime= 40.27			Mean Lemon=39.57				
Season 2019																
S A	8.30	8.30	8.30	16.60	0.0	8.30	8.30	91.7	12.50	8.30	10.4	12.50	12.50	12.50	11.45	88.5
B.A.B.A	8.30	8.30	8.30	8.30	8.30	8.30	8.30	91.7	8.30	8.20	8.25	8.30	8.30	8.30	8.27	91.7
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.	100.0	100.0	100.0	100.0	100.0	100.0	0.0
Fungi	38.87		------	38.87		------	-----	------	39.25		------	40.27		------	------	------
Cultivar	Mean Lime= 40.25			Mean Lemon=37.48					Mean Lime= 39.25			Mean Lemon=40.27				

disease severity of stored lime and lemon fruit artificial infected with *P.digitatum* and *P.italicum* under storage conditions during 2018 and 2019 seasons.

S A= Sallcylic acid =β-amino–butyric acid

3.5.2.. Effect of some mineral salts and organic acids on total soluble solids % of lime and lemon fruits under artificial infection with P.digitatum and P.italicum stored at 20 +1oC.

a- Application of mineral salts.

The results in Table (7) revealed that different level of TTS% content found to be increased in treated fruits with different mineral salts, it was obviously sort and dose dependent. Where, all tested mineral salts increased the content of (TSS %) amongst the treated and even compared with the not treated fruits. As shown in the above-mentioned table, The highest increase of TTS % content was obtained at potassium silicate treatment by (13.45 and 13.65) under artificial infection respectively, at storage 20±1°C, followed by potassium sorbet by (11.60 and 11.70) in lime and lemon stored at 20+1oC under artificial infection conditions, respectively.

b**- Application of organic acids.**

Data presented in Table (7) indicated that, under storage temperature of at 20+1oC, all tested organic acids increased the content of (TSS) in artificially infected lime and lemon fruits compared with control treatment. It was observed that the increase of TTS % differs among organic acids treatments. However, salicylic acid recorded the highest increase in (TSS%), followed by β- amino – butyric acid recorded the lowest increase in (TSS %) in artificially infected lime and lemon with *P.digitatum and P.italicum* under storage condition

All fruits treated with organic acids showed an increase of (TSS) content comparing with control treatment and even between the treatments themselves. In this concern, salicylic acid showed the highest increase in (TSS %) of lime and lemon (12.90 &13.0) by *P.digitatum and P.italicum* comparing with control under artificial infection conditions respectively. On the other hand, β- amino–butyric acid showed an increase in the (TSS%) estimated as (12.65 &12.85) in fruits under artificial infection by *P.digitatum and P.italicum,* respectively.

Treatments	Total phenolic compounds %						
	Storage at 20±1^{0}C					Mean	Over Means
	P.degitatum		Mean	*P.italicum*			
	Lime	Lemon		Lime	Lemon		
Effect mineral salts(4gm/L)							
K.si	2.55	2.43	2.49	2.50	2.41	2.45	2.47
K.s	1.30	1.32	1.31	1.25	1.29	1.27	1.29
Control	1.61	1.34	1.47	1.50	1.23	1.36	1.41
Fungi mean	1.76		----	1.70		-----	
cultivar mean	Lime=1.79			Lemon=1.67			
Effect of organic acids(1gm/L)							
S.A	2.53	2.26	2.39	2.39	2.15	2.27	4.33
BABA	1.61	1.34	1.47	1.50	1.23	1.36	1.41
Control	1.61	1.34	1.47	1.50	1.23	1.36	1.41
Fungi mean	1.78		----	1.67		------	
cultivar mean	Lime=1.86			Lemon=1.59			

Treatments	Total soluble solids (TSS) %						
	Storage at 20_+1C					Mean	Over Means
	P.degitatum		Mean	*P.italicum*			
	Lime	Lemon		Lime	Lemon		
Effect mineral salts(4gm/L)							
K.si	13.40	13.50	13.45	13.70	13.60	13.65	13.55
K.s	11.50	11.70	11.60	11.90	11.50	11.70	11.65
Control	11.10	11.0	11.5	11.30	11.20	11.25	11.15
Fungi mean	12.03		--------	12.20		---------	
cultivar mean	Lime=12.15			Lemon=12.08			
Effect of organic acids(1gm/L)							
S.A	13.0	12.80	12.90	13.0	13.0	13.0	12.95
BABA	12.70	12.60	12.65	12.90	12.80	12.85	12.75
Control	11.10	11.0	11.5	11.30	11.20	11.25	11.15
Fungi mean	12.20		-------	12.37		--------	
cultivar mean	Lime=12.33			Lemon=12.23			

Table 7. Effect of various postharvest treatments on Total soluble solids % and Total phenolic compounds activity% of lime and lemon fruits under artificial infection with *P.digitatum* and *P.italicum* stored at 20 +1oC.

Postharvest diseases caused high losses during storage, transportation and marketing before consumption worldwide, 10-30% of the total yield of crops and in some perishable crops especially in developing ountries, they destroy more than 30% of the crop yield **(Agrios, 2005).** Green and blue molds caused by *Penicillium digitatum* and *Penicillium italicum*, respectively, are the most economically important postharvest diseases of citrus in all production areas **(Palou et al., 2008).**

Pathogenicity test of different isolated fungi on lemon and lime fruits were carried out. Results indicate that, all tested fungi showed a significant percentage of pathogenic capability to infect lime and lemon fruits at (20±1ºC) for 10 days. All isolates of *P. digitatum* and *P. italicum* showed the highest level of both disease incidence and disease severity. Variation in the pathogenicity among fourteen isolates of *P. digitatum* and *P. italicum* on Balady cultivar fruits were observed. Isolate (No.6) of *P. digitatum* and isolate (No.7) *P.italicum* were the most

virulent isolates and recorded highest disease incidence and disease severity percentage. *P. digitatum* and *P. italicum* pathogenicity on citrus fruits have extensively been reported **(Palou et al. 2001**).

Several inorganic(mineral) salts and organic lipophilic acids and their salts , some of which are used , in the food – processing industry , have antimicrobial properties and could be useful as postharvest treatment for decay control **(El-Mougy, et al. 2008).** Results in the present study indicated that potassium sorbet and potassium silicate significantly inhibit mycelial growth on *Penicillium digitatum* and *P.italicum* under laboratory trails and significantly reduced the disease incidence and severity when applied postharvest treatments on lime and lemon fruits artificially infected with *P.digitatum* and *P.italicum* under storage conditions. This is in agreement with **El-Mohamedy et al. (2013)**. Who proved that, potassium sorbate and potassium carbonate had inhibitory effect against mycelial growth of all tested fungi *in vitro*. Potassium sorbate (PS) as postharvest treatment against *Penicillium digitatum* and *P. italicum*, the cause of Valencia orange fruits green and blue mold, respectively. *In vitro* results showed that, complete inhibition of both linear mycelial growth and conidial germination of both *P. digitatum* and *P. italicum* was obtained with (SB) at 20 g/ L and (PS) at 15 g/ L. Also coated peel disks of Valencia orange with (SB) or (PS) showed antifungal activity against *P. digitatum* and *P. italicum* by formation inhibition zone in agar assays, with increase in salt concentrations, the radius of the inhibition zone was increased **Elshahawy et al**. **(2015).** The reduction in disease severity with the exogenous application of SA may be attributed to their fungitoxic and fungistatic effect, respectively. Additionally, an exogenous spray application of elicitors may induce resistance in host plants consequently reducing chances of development of secondary infections. Exogenous applications of different elicitors have been reported to enhance induction of resistance in the fruits against aggressive pathogens **(Malamy et al. 1990).**

Soluble potassium silicate completely suppresses mycelial growth of *Phytophthora cinnamomi* and *Phytophthora capsicii* at concentrations of 40 and 80 ml $^{-1}$ potassium silicate. The direct effect overrides the effect of pH. The inclusion of KOH as a control treatment eliminated any potential role potassium may play in enhancing or suppressing mycelial fungal growth. It is proposed that silicon may act as the first protecting barrier in silicon treated plants, and may inhibit pathogen colonization and consequential infection by inhibiting fungal growth on the plant surface **(Bekker,et al. 2009).**

The inducer of disease resistance β-aminobutyric acid have also shown activity against citrus penicillium moulds **(Porat et al., 2003)**. On the other hand, it has been repeatedly observed that fumigation with the ethylene inhibitor 1-methylcyclopropene to prolong postharvest life of stored citrus fruit increased the incidence of postharvest decay **(Porat et al. 1999)**. In plants, BABA acts on various post-harvest fungi **(Cheng et al. 2019)** with multiple biochemical and physical defense mechanisms, including the creation of physical barriers (callose; lignin, and papillae), hypersensitivity reaction, accumulation of phytoalexins, induction of pathogenesis-related (PR) proteins; biosynthesis of terpenoids, generation of reactive oxygen species (ROS) with H_2O_2 and activation of defense pathways mediated by abscisic acid, SA, and Jasmonic acid **(Walters et al. 2013).**

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الملخص العربى

المسح المرضي على العفن الأخضر والأزرق علي ثمار الليمون الاضاليا والبنزهير في الأسواق المحلية المصرية المختلفة وإلتحكم فيه باستخدام بعض الأملاح المعدنية والأحماض العضوية

تعتبر أمراض ما بعد الحصاد التي يسببها *Penicillium digitatum* (العفن الأخضر) و *Penicillium. italicum* (العفن الأزرق) من أهم العوامل السلبية والخطيرة التي تؤثر على تداول وتسويق الموالح في مصر. الهدف من هذه الدراسة هو تقييم طرق مكافحة العفن الأخضر والأزرق ما بعد الحصاد على ثمار الموالح (الليمون الاضاليا والبنزهير) الناجم عن فطرى *P. digitatum* و *P. italicum*، على التوالي باستخدام بدائل لمبيدات الفطريات الكيميائية (الاملاح المعدنية والاحماض العضوية) بتركيزات مختلفة على النمو الميسليومى للفطرين فى المعمل و على الثمار تحت ظروف العدوى الصناعية. من نتائج الحصر لتلك الأعفان التي تصيب ثمار الليمون الاضاليا والبنزهير (صنف بلدى) في الاسواق المحلية لبعض المحافظات وهى القاهرة (العبور والسلام وعين شمس) والجيزة (الدقى والمنيب وامبابة) والفيوم (الفيوم وابشواى وسنورس)، وتبين فى العموم ان اعلى نسبة تكرار للضرر واصابة للثمار كانت فى محافظة القاهرة ويليها الجيزة.أشارت اختبارات القدرة المرضية أن جميع عزلات *P. digitatum* و *P. italicum* أحدثت إصابة على ثمار الليمون الاضاليا والبنزهير، وتحت ظروف المعمل تم الحص

ول على تثبيط كامل لنمو فطريات *P. digitatum* و *P. italicum* مع سيليكات البوتاسيوم، سوربات البوتاسيوم (4.0 ، 5.0 و 6.0 جم / لتر) ، حمض الساليسيليك وبيتا-أمينو بيوتريك اسيد (1 ، 1.5 و 2.0 جم / لتر). أظهرت تجارب ما بعد الحصاد أن المعاملة بسيليكات البوتاسيوم هي المعاملة الأكثر فاعلية بين الأملاح المعدنية المختبرة حيث نجحت في تقليل حدوث المرض وشدته على ثمار الليمون الاضاليا والبنزهير عند درجة حرارة (20 ± 1 درجة مئوية) تليها سوربات البوتاسيوم. وكان حمض الساليسيليك العلاج الأكثر فعالية في خفض نسبة الإصابة بالمرض وتقليل شدة المرض على ثمار الليمون الاضاليا والبنزهير المعاملة بأعلى تركيز بعد الحصاد حيث قللت من حدوث المرض وشدة العفن الأخضر والأزرق أثناء التخزين عند (20 + 1 درجة مئوية). كما تمت دراسة تأثير بدائل مبيدات الفطريات على الخواص الكيميائية (نشاط الإنزيمات المتعلقة ببيروكسيديز الدفاع، بوليفينول أوكسيديز وكيتيناز، وعلى خصائص الجودة (المواد الصلبة الذائبة الكلية (TSS٪) والمواد الفينولية الكلية) في ثمار الليمون الاضاليا والبنزهير المخزنة والمعالجة ببعض الأملاح المعدنية والأحماض العضوية حيث أدت العلاجات الفردية إلى زيادة نشاط الانزيمات المختبرة وحققت نسبة أعلى من المواد الصلبة الذائبة الكلية (TSS) وإجمالي نشاط المركبات الفينولية (Tph)٪ تحت العدوى الاصطناعية بـ *P.digitatum* و *P.italicum* المخزنة عند 20 ± 1 درجة مئوية. يمكن استخدام بعض وسائل التحكم البديلة ضد مسببات الأمراض الفطرية لإطالة العمر الافتراضي للمنتجات الزراعية. بتطبيق مثل هذه العلاجات الآمنه والرخيصة والسهلة التطبيق والتى لا تترك آثار ضارة بالبشر والبيئة.

الكلمات المفتاحية: الحمضيات، ثمار الليمون الاضاليا والبنزهير، العفن الأخضر والأزرق، الإصابة، الشدة، الأملاح المعدنية، الأحماض العضوية .