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Molecular Identification of Green Mold Pathogen and Impact of Formulation of Polyvinyl Alcohol and Some Organic Acid On Disease and Quality of Washington Navel Orange Fruits During Storage

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ABSTRACT

Pathogenicity test showed that every tested isolate significantly caused the green mold disease on orange fruits. *Penicillium* .2, was the most virulent isolate; it induced disease incidence and severity by 90.0 and 92.0%, respectively. At the National Center for Biotechnology information (NCBI) alignment showed the percentage of identity (93.75%) of *Penicillium digitatum* between our isolates and Gene bank isolate. The effects of Benzoic acid (BA) and Salicylic acid (SA) at 0.0, 0.5, 1.0, 1.5, and 2% against *Penicillium digitatum* mycelial growth were assessed. The linear growth of *Penicillium digitatum* was significantly inhibited by all tested concentrations, according to the results. With SA and BA at 2.0%, linear growth was completely inhibited. The greatest reduction was achieved by using SA and BA at 1.5%, which, respectively, lowered the linear growth by 86.7 and 85.6%.The linear growth of *Penicillium digitatum* was strongly suppressed in all formulations involving SA or BA and PVA. Complete suppression of linear growth was achieved by combining PVA at 10.0% with either SA or BA at 2.0%. The greatest reduction of 84.4 and 83.3%, respectively, in linear growth was obtained with SA or BA at 1.5% and PVA at 10.0%. The effects of PVA at 10% combined with SA and BA at 1.5% and 2.0% were evaluated in vivo in order to examine their impact on Washington navel orange fruits green mold disease. The incidence and severity of green mold disease were shown to have been greatly decreased by all formulations, according to the results. The disease incidence and severity were lowered by 90.0 and 88.0 percent and 92.0 and 91.0 percent, respectively, when SA or BA at 2.0% and PVA at 10.0% were combined. SA or BA at 1.5% and PVA at 10.0% came next, which reduced the disease incidence by 80.0 and 78.0 % and disease severity by 80.0 and 82.0 % respectively. Previous formulations had no negative effect on fruit quality of orange fruits. All tested formulations reduced weight loss more than 38.5% and fruit disorders more than 80.0%. As for total soluble solids (TSS) there was no significant effect on total soluble solids percentage (TSS).

Keywords: Orange fruits, salicylic acid, benzoic acid, polyvinyl alcohol, postharvest disease, *Penicillium digitatum* .

1. Introduction

Numerous diseases that infect citrus fruits and cause significant losses after harvest. According to Zhang *et al.* (2005), unpleasant postharvest disease of citrus is green mold, caused by *Penicillium digitatum* (Pers.: Fr.) Sacc. It lives on healthy citrus fruit and attacks it through handling injuries sustained during harvesting, transit, and storage.

Fruit deterioration and tissue maceration are frequent outcomes of *P. digitatum* infection in citrus fruits. For citrus producers and packers around the world, green mold deterioration could result in significant financial losses. Fungicides such thiabendazole and imazalil are frequently applied in packing houses to control fruit rot caused by *P. digitatum* (Liu *et al.,* 2013). On the other hand, frequent

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exposure to hazardous chemicals may cause the pathogen to develop fungicide-resistant strains and raise the risk to human health.

Green mold, blue mold, and sour rot are the primary citrus postharvest diseases that pose a risk to human health, respectively, and are caused by *Penicillium digitatum, P. italicum, and Geotrichum citri-aurantii(*Cheng *et al.,* 2020; Bhatta,2021 ; Shawky , Heba, *et al.,* 2023 and Abd-El-Kareem *et al.,* 2022). Just *Penicillium digitatum* only is causing 90% of fruit postharvest losses (Zhu *et al.,* 2017 and Bhatta, 2021). According to Chen *et al.* (2019), Youssef and Hussien (2020), and Shawky *et al.* (2023), citrus fruits are spreading disease due to pre- or post-harvest damage.

Polyvinyl alcohol (PVA) is a biocompatible and biodegradable polymer with excellent mechanical, optical, physical, and film-forming properties in addition to strong chemical resistance, according to Zanela *et al.* (2018). Effective research has been done on the potential use of PVA and its composites in food packaging systems (Youssef, *et al.,* 2019). Because of its safe and nontoxic profile, it has been designated as generally recognized as safe (GRAS), meaning that it can be utilized to make edible films (Keller and Heckman, 2018). The introduction of bioactive substances such phenolic compounds in food packaging could have a significant positive impact (Andrade, *et al.,* 2021).

Gallic acid is one phenolic compound that dissolves in water and has been associated with potent antibacterial and antioxidant properties. It has been demonstrated that PVA films with gallic acid enhance their antioxidant properties (Awad, *et al.,* 2017). Therefore, it is expected that adding an extract rich in gallic acid to PVA coatings will assist delay the ripening of bananas based on the research that is now available. It has been found that the leaf extract of Ficus auriculata is high in gallic acid and exhibits strong antioxidant activity (Baite *et al.,* 2021). PVA is an environmentally benign synthetic polymer that is hydrophilic, nontoxic, biocompatible, and biodegradable (He *et al.,* 2019 and Candéo *et al.,* 2020). PVA is commonly utilized as a hot and cold water soluble film for food packaging, detergents, pharmaceuticals, and agricultural chemicals, among other packaging uses. Additionally, the FDA has cleared it for close contact with food items.

A request for approval to use PVA as a component of an edible film that dissolves in water and contains ingredients for dry food was recently submitted to the FDA (Keller and Heckman, 2018; GRAS Notice No. 676, 2018). PVA films have a high tensile strength, are translucent, and are flexible. They are also well-suited as an aroma and oxygen barrier. Numerous publications claim that the formation of interpenetrated polymer networks in mix films consisting of starch and PVA enhanced the mechanical and water barrier properties of the composite films, offering several advantages over pure starch films (Cano, *et al.,* 2015). According to Sapper *et al.* (2021), depending on the distribution and content of carvacrol in the films, different PVA coating formulated with starch or carvacrol when applied to apple fruits demonstrated that highly suppression of disease incidence and the growth of both black and green mold fungi. Furthermore, according to Baite *et al.* (2022), *Ficus auriculata* leaf extract high in gallic acid an antioxidant was mixed with poly (vinyl alcohol) (PVA) and used as a coating to stop green bananas from ripening too quickly.

According to El-Mougy *et al.* (2008), Abd-El-Kareem *et al.* (2015), Abd-El-Kareem and Saied (2015), and Elshahawy *et al.* (2015) A variety of inorganic salts, organic acids, and their salts, some of which are utilized in the food processing industry, have antibacterial characteristics and may be useful as postharvest therapies for decay management.

Significantly more affordable, efficient, and human health-safe alternative management techniques have been employed to manage post-harvest infections. Gray mold fungus was dramatically reduced in a lab setting by treating pepper fruits with (SA) and abscisic acid as chemical inducers (Kamara *et al.,* 2016).

Plants' ability to withstand stress is greatly influenced by the phenolic hormone salicylic acid (SA). Better control against infections in pears and sweet cherries has been attained by pre- and post-harvest SA spraying (Jiankang *et al.,* 2006). This is due to the induced defense resistance (Yao and Tian, 2015) and stimulated of antioxidant enzymes (Xu and Tian, 2008). According to Abdel-latif *et al.* (2011), guava fruits treated with salicylic or citric acids prior to harvesting exhibited the greatest reduction in disease incidence and disease severity of rot when stored at room temperature. However, after 15 days of cold storage, no infection was found in the fruits treated with salicylic acid.

The examination of various chemical food preservatives to inhibit gray mold disease in strawberry fruits was reported by El-Fawy *et al.* (2020). The pathogen's ability to proliferate mycelially was examined at doses of 10, 20, 40, and 80 mM for six compounds: acetic acid, potassium citrate, sodium

benzoate, benzoic acid, citric acid, and sodium citrate. Data from in vitro experiments indicated that every treatment significantly suppressed mycelial growth of the pathogen.

The goal of this study are, evaluation of formulated of polyvinyl alcohol with salicylic and benzoic acids to control gray mold disease of Washington navel orange fruits during storage.

2. Materials and Methods

2.1. Pathogenicity test

2.1.1. Isolation of fungi from navel orange fruits infected with green mold disease

Navel orange that were infected with green mold disease were collected from the Ellewaa, and October markets. Fruits exhibiting disease were meticulously cleaned with sterile water, sliced into tiny pieces, subjected to a one-minute surface sterilization in 70% ethanol, and then repeatedly rinsed with sterile distilled water. Following that, they were dried using sterilized filter paper, put onto Petri plates with Potato Dextrose Agar (PDA) medium, and cultured for seven days at 20±2°C. For future research, stock cultures of the isolated isolates were kept on PDA slants and refrigerated.

2.1.2. Inoculum preparation

For these tests, three isolates of Penicillium spp. from diseased orange fruits collected from Ellewaa and October markets were employed. The pure isolates of Penicillium spp. were cultivated individually on PDA plates for 10 days at 20±2°C in order to prepare the standard inoculum. Each isolate's spore suspension was made by brushing the culture's surface with 10 mL of distilled water that had been sterilized for each plate. The spore suspensions were then filtered through muslin. Using a hemocytometer slide, the spore suspension concentration was brought down to roughly 10^6 spore/mL.

2.1.3. Inoculation of navel orange fruits.

Sterilized needles caused artificial damage to sterile navel orange fruits of the cultivar Washington. Using an atomizer, damaged orange fruits were infected with a prepared inoculum for each isolate at a rate of 100 mL spore (10^6 spore/mL) per 10 fruits. As a control, the same volume of sterile distilled water was sprayed on a portion of the injured and unscathed fruits. All inoculated and un- inoculated fruits, were stored in a foam tray measuring 25 by 15 by 15 cm, which was then placed within polyethylene bags to raise the relative humidity. Four repetitions for every treatment and five fruits per replicate were used. All fruits were stored at 20 ^{o}C, for 15 days. Disease incidence was assessed as infected fruits to total fruits While, the disease severity was calculated as follow: -

$$\text{Disease severity \%} = \frac{\text{Rotted part weight of fruit}}{\text{Fruit weight}} \text{ x100}$$

2.2. The pathogen

Penicillium sp. isolate no.2 (October) was isolated from naturally diseased orange fruits and proved to be pathogenic to healthy fruits according to pathogenicity tests conducted in this study. Preliminary identification of this isolate was done using morphological characters

2.3. Morphological identification

Penicillium sp. was identified at the species level by key to Penicillium of Pitt and Hocking (2009), and standard parameters of Frisvad and Samson(2004), and Raper and Thom(1949).

Molecular identification utilizing the internal transcribed spacer region of rRNA (ITS) Trimmed sequences (ITS 573 bp) (Staats *et al.* 2004) was carried out as follows:

2.4. DNA extraction

DNeasy® Plant Mini Kit used to extraction DNA from fungal growth was carried out according to (Fan *et al.* 2015).

2.5. PCR amplification

Penicillium cultures were identified molecularly using the conserved ribosomal internal transcribed spacer (ITS) region (White, *et al.,* 1990).

2.6. Sequencing

Using the Basic Local Alignment Search Tool for Nucleotide Sequences, the ITS nucleotide sequences for isolate (*Penicillium* sp.no.2 October) were compared to those in the public domain databases NCBI (National Center for Biotechnology Information; www.ncbi.nih.gov) (BLASTN). The Clustal W tool was used to align ITS DNA sequences. CLC Sequence Viewer Version 6.3 was used to generate a phylogenetic tree based on UPGMA (unweighted pair group method for arithmetic analysis). Bootstrap analysis was used to determine the branching's confidence (Fan *et al.* 2015).

2.7. Washington navel orange fruits

National Research Farm provided the mature Washington navel oranges used in this investigation. They were selected based on the absence of fungal diseases and their homogeneity in size, color, and shape.

2.3. In Vitro, trails

2.3.1. Evaluating the effect of some organic acids on the linear growth of the fungus *Penicillium digitatum*

Salicylic acid (SA) and benzoic acid (BA) at 0.0, 0.5, 1.0, 1.5, and 2% were tested for their effects on *Penicillium digitatum* mycelial growth on PDA medium at 25 ± 2°C. Divided into 100 ml parts, the PDA medium was put into 250 ml Erlenmyer flasks to be sterilized. Next, for 15 minutes, the flasks were autoclaved at 121°C.

To obtain the final concentrations, the final concentrations were produced separately, added to the PDA medium just before it hardened, and then progressively mixed in 0.1% Tween 80 (Sigma) to improve solubility. Before the medium in each flask solidified, it was first broken up in a sterile, 9-cm-diameter Petri plate. Disks (6.0mm) of *Penicillium digitatum* cultures that were ten days old were used to inoculate individual plates.

For the cultures, a temperature of 25 ±C was maintained. Once the control plates had reached their full size and the average growth diameter had been determined, the fungus's linear mycelial development was assessed. Five plates were utilized for each treatment.

2.3.2. Effect of polyvinyl alcohol formulated with some organic acids on linear growth of *Penicillium digitatum*

Against the linear growth of *Penicillium digitatum*, 10% polyvinyl alcohol was combined with salicylic and benzoic acids at0.0,0.5,1.0,1.5, and 2%. To obtain the final concentrations, separately generated prior concentrations mixed with 10% PVA were added to the PDA medium just before it solidified. Then, 0.1% Tween 80 (Sigma) was progressively mixed in to improve solubility. Prior to each flask's media solidifying, it was first broken up in a sterile, 9-cm-diameter Petri plate. Inoculation of individual plates was done using disks (6-mm) of *Penicillium digitatum* cultures that were ten days old.

The cultures were kept at a constant temperature of 25 ±C. After determining the average growth diameter and the control plates' full growth, the fungus's linear mycelial growth was measured. There were five plates used as duplicates for each treatment.

2.4. In Vivo trails

2.4.1. Impact of formulation of polyvinyl alcohol containing some organic acids on the green mold disease of Washington navel orange fruits during storage

Inoculum preparation

To create the standard inoculum for these assays, a pure isolate of *Penicillium digitatum* was grown on PDA plates for 10 days at 20±2°C. To make the isolates spore suspension, 10 ml of sterilized distilled water were brushed over the surface of each plate. After that, muslin was used to filter the spore suspensions. The concentration of the spore suspension was lowered to about 106 spore/mL using a hemocytometer slide.

2.4.2. Inoculation of orange fruits

An in vivo evaluation of the effects of SA and BA at 1.5 and 2.0% mixed with PVA at 10% was conducted on Washington navel orange fruits. The fruit was repeatedly washed in sterile water

following a two-minute application of 70% ethanol to its surface. Using a sterile scalpel, fake wounds were created on the fruits. After being individually dipped in the earlier concentrations, the fruits were let to air dry. To inoculate treated fruits, a suspension of 106 *Penicillium digitatum* spores/ml was sprayed on them, and then they were left to air dry. As control, a group of fruits that had only been inoculated with Penicillium digitatum were used. In order for the fruits to be evaluated, the four carton boxes used for each treatment, each holding five fruits, were filled with either treated or untreated (control) fruits. The boxes were then stored for 15 days at 20–2°C and 90–95% relative humidity. Fruits were routinely examined for green mold diseases. Calculations were made to determine the severity and occurrence of the diseases.

Disease incidence was assessed as infected fruits to total fruits While, the disease severity was calculated as follow:

$$\text{Disease severity \%} = \frac{\text{Rotted part weight of fruit}}{\text{Fruit weight}} \text{ x100}$$

2.4.3. Effect of Effect of polyvinyl alcohol formulation with some organic acids on fruit quality

Washington navel orange fruit quality during storage without artificial infection were applied to determine how SA, BA, and PVA at 1.5 and 2.0% combined with 10% PVA affected the fruit weight loss, total soluble solids (TSS), and fruit decay, an in vivo evaluation of the effects was conducted on Washington navel orange fruits. Three carton boxes (60 x 40 x 15 cm) with one layer weighing around 10 kg each made up each treatment. The experimental boxes were maintained in an artificial infection-free environment at 20±2˚C and 90% relative humidity for a period of 21 days.

The following findings were used to assess the impact of the tested treatments on mango fruits:

2.4.5. Fruit weight loss percentage

Weights of Washington navel orange fruits were first noted for every treatment. The percentage of fruit weight loss was then determined by weighing the identical fruits at the conclusion of the cold storage period and applying the following formula:

$$\text{Weight Loss \%} = \frac{\text{Initial Weight} - \text{Weight at end experiment}}{\text{Initial Weight}} \text{ x100}$$

2.4.6. Total soluble solids percentage (TSS)

According to Chen and Mellenthin (1981), the TSS% in orange fruit juice was measured using a hand refractometer.

2.4.7. Fruit decay percentage

Fruits impacted by physiological or pathological decay were counted visually and their percentage to the starting fruit count for each therapy was determined.

2.5. Statistical analysis

Tukey test for multiple comparison among means was utilized (Neler *et al.,* 1984).

3. Results

3.1. Pathogenicity test

For this test, three isolates of *penicillium* spp. from disease Washington navel orange fruits collected from Ellewaa and October markets were employed. Table (1 and Fig 1) results show that every tested isolate significantly caused the green mold disease on orange fruits. *Penicillium* .2, which was isolated from the October market, was the most virulent isolate; it induced disease incidence and severity by 90.0 and 92.0%, respectively.

Table 1: Pathogenicity test of different isolates of *Penicillium* spp on Navel orange fruits collected from commercial markets in Egypt

Commercial markets	No. of isolate	Disease incidence	Disease severity
Ellewaa	*Penicillium* sp.1	85.0 b	76.0b
	Penicillium sp.2	70.0d	62.0c
	Penicillium sp. 3	85.0b	74.0b
October	*Penicillium* sp.1	80.0c	77.0b
	Penicillium sp.2	90.0 a	92.0 a
	Penicillium sp.3	80.0c	74.0b
Control		0.0e	0.0d

Figures with the same letter are not significantly different (P=0.5)

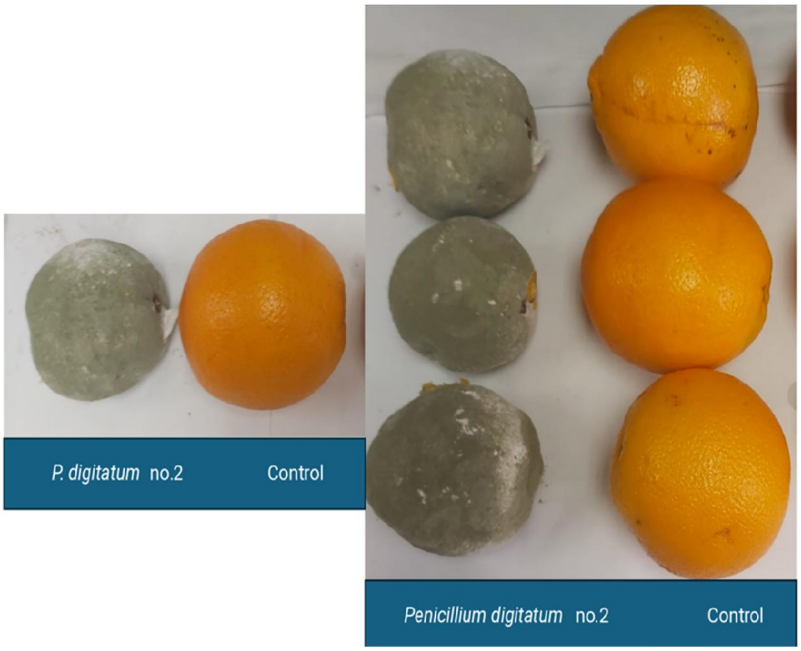

Fig. 1: Pathogenicity test of different isolates of *Penicillium* spp on Navel orange fruits.

3.2. Identification of *Penicillium* sp. using molecular biology

Penicillium sp. internal transcribed spacer (ITS) genes, including the 5·8S ribosomal rRNA, were amplified and DNA sequences were determined. A new polymerase chain reaction (PCR) primer pair was designed for specific amplification of DNA based on a comparison of sequence information. This primer pair successfully amplified a 700-bp DNA sequence 98-100 percent of the time.

Results in (Fig 2) indicate that the NCBI (National Center for Biotechnology information) alignment showed the percentage of identity (93.75 %) of *Penicillium digitatum* between studied isolates and Gene bank isolate. While, results in (Fig 2) indicated that the phylogenic tree showed Convergence between our isolates (Yellow color) and Gene bank isolate. Our isolates showed in separated cluster which mean it's had diversity.

3.3. Isolate 2 (*Penicillium digitatum*)

TGGGTATATTGATCTCTGGTTTACCTGTTGTTTATGGTTTATCATTATAACATATGTTATA
ATGATAAGT
AGTAGAACACTACGAGCATAATTGTTATCTTTAACAATTAATAGGCTTTGGTTGTAAGAA
TGAAAACATT
TTGAGATATAAAGTACTAATTTTTAAAAATAGTATACTAAATATTATTTAATATCATTATA
ATATTTTAT

CCGAAGAATTGTGGTAAGTTAGTGAAAGACAAAACTGACTAGTATAGCTGGTTTTCCGC
GAAACCTATGT
AAGTAGGTAATTTAATTAACATCTTATGATTAAATATTTAGGAGAAAACGCCGAAAATTT
AACGGATCTA
AAATAATATACCGAAACCTTGTACACATTTATATGTGGGGTAGCGGAACATTGGATAATA
ATCATATGAT
TTACATTTATTCTAAATGTAAAAATATAAATTTGAAATAAGGTTATAGTAGTATTAATAC
TATAGCAATA
CTAGATCTCAACGTAGAACTAAATATAAAGGAAATTTAGAAAATGTTTATTCTTCATTTA
AAGGTTATCC
ATAATAGATATCTCTAGATATCTATTATTTATTTTATTTAATTTAAATTTATTTATATTTTT
TATTCAGA
TATGCTTTTATCTTCAATCTTCTGTTTAATATTATCTAATGCATTGTCTTTTAGACGTGATA
CAGCTATT
CTTTATTCAAGAATAGGTATTATAATATTATTTTACTGTATTTATTTATGTTATAATAATTT
ATTTATAA
CATATTTAGATAATGGTATAGGGTTGTTTGGTGGTTTATTTTATACATCACCTATAACACA
AACATTTCA
CATATTAATATTTATTATAACATTATTAATACTGAATTTAACTGGGTTTTATCCTAGAAAA
CTTATATCT
AGTCAGTACTTAAGTTTATCTAAATTATTATTTACAAAATTACAATTTGTTAAAAACATTG
CTGTATCTA
ATATAATTTTAAAAAAAGGAGAACAATATACAATATTAGAATATACATTAATGTTATTAT
TTATTGTAAT
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CAGGATGTA
TGATCAGATTCGTTCTGATCATATCATACATAATAATTACTTTATACATATGAGAGGTAT
AGATAATGTT
GATTACCGTATAGCTTATGAGTATATAGTAGAATGTTTAGATTATCTAGACAGATTAATA
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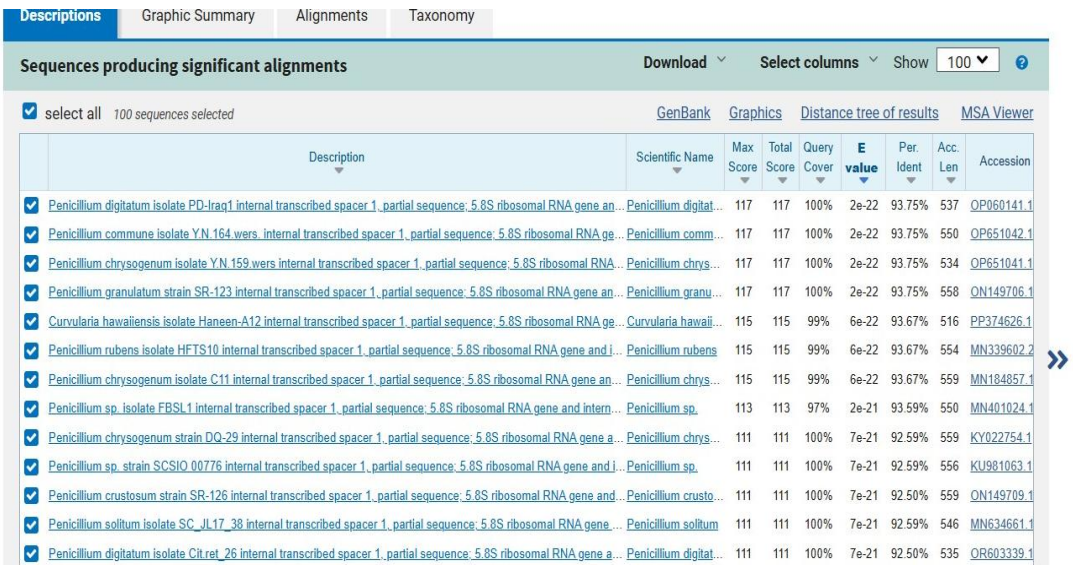

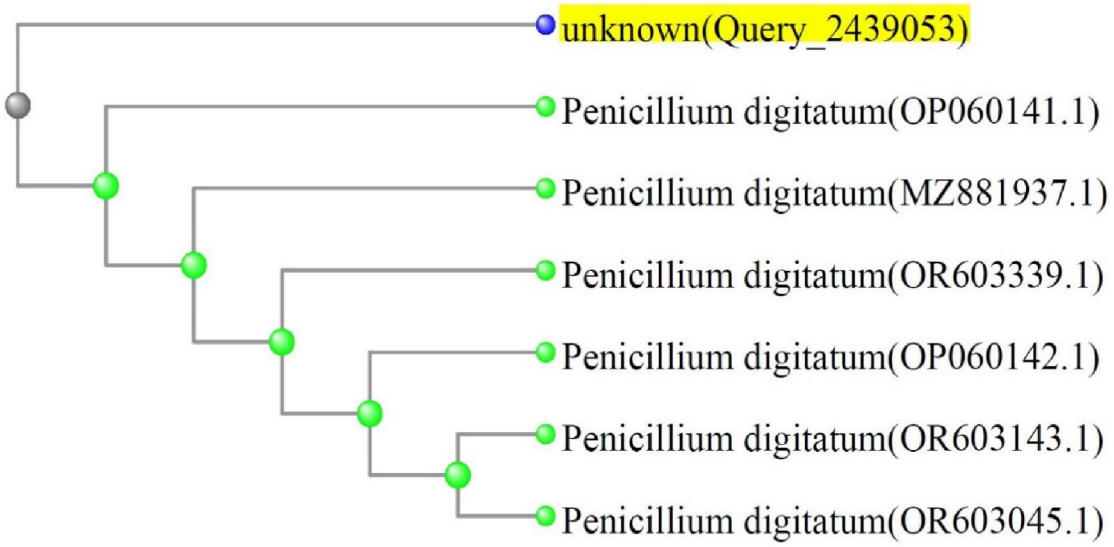

Fig. 2: The phylogenic tree showed Convergence between our isolated shaded area and Gene bank isolate. Our isolate showed in separated cluster which mean it's had diversity.

3.4. In vitro, trails

3.4.1. Impact of some organic acids on the fungus *Penicillium digitatum* linear growth

A comparison was made between the mycelial growth of *Penicillium digitatum* and the effects of salicylic acid (SA) and benzoic acid (BA) at 0.0, 0.5, 1.0, 1.5, and 2% respectively. Results in Table (1) show that *Penicillium digitatum* linear growth was significantly inhibited at all tested concentration. Use of SA and BA at 2.0% resulted in complete suppression of linear growth. Reducing the linear growth by 86.7 and 85.6%, respectively, at a 1.5% of SA and BA produced the most reduction. Modest effects were seen with other concentrations.

Table 1: Linear growth of *Penicillium digitatum* in response to different concentrations of organic acids

Organic acids	Concentration (%)	*Penicillium digitatum*	
		Linear growth (mm)	Reduction %
Salicylic	0.0	90.0a	0.0
	0.5	41.0b	54.4
	1.0	22.0c	75.6
	1.5	12.0d	86.7
	2.0	00.0e	100.0
Benzoic	0.0	90.0a	0.0
	0.5	44.0b	51.1
	1.0	25.0c	72.2
	1.5	13.0d	85.6
	2.0	0.0e	100.0

Figures with the same litter are not significantly different (P =0.05)

3.4.2. Effect of polyvinyl alcohol formulated with some essential oils on linear growth of *Penicillium digitatum*

Penicillium digitatum was evaluated for linear growth using a mixture of 10% polyvinyl alcohol (PVA) with salicylic and benzoic acids at0.0,0.5,1.0,1.5, and 2%. The results shown in Table (2) indicate a significant reduction in the linear growth of *Penicillium digitatum* for all formulations containing either SA or BA and PVA. Complete suppression of linear growth was achieved by combining 10.0% PVA with either SA or BA at 2.0%. The greatest reduction of 84.4 and 83.3%, respectively, in linear growth was obtained with SA or BA at 1.5% and PVA at 10.0%. Another formulation had a middling impact.

Table 2: Effect of polyvinyl alcohol formulation with some organic acids on linear growth of *Penicillium digitatum*

Penicillium digitatum		
Treatments (%)	**Linear growth (mm)**	**Reduction %**
PVA 10 + Salicylic acid 0.0	90.0a	0.0
PVA 10 + Salicylic acid 0.5	40.0	55.6
PVA 10 + Salicylic acid 1.0	24.0	73.3
PVA 10 + Salicylic acid 1.5	14.0	84.4
PVA 10 + Salicylic acid 2.0	00.0	100.0
PVA only	90.0a	0.0
PVA 10 + Benzoic acid 0.0	90.0a	0.0
PVA 10 + Benzoic acid 0.5	38.0	57.8
PVA 10 + Benzoic acid 1.0	22.0	75.6
PVA 10 + Benzoic acid 1.5	15.0	83.3
PVA 10 + Benzoic acid 2.0	00.0	100.0
PVA only	90.0a	0.0

Figures with the same litter are not significantly different (P =0.05)

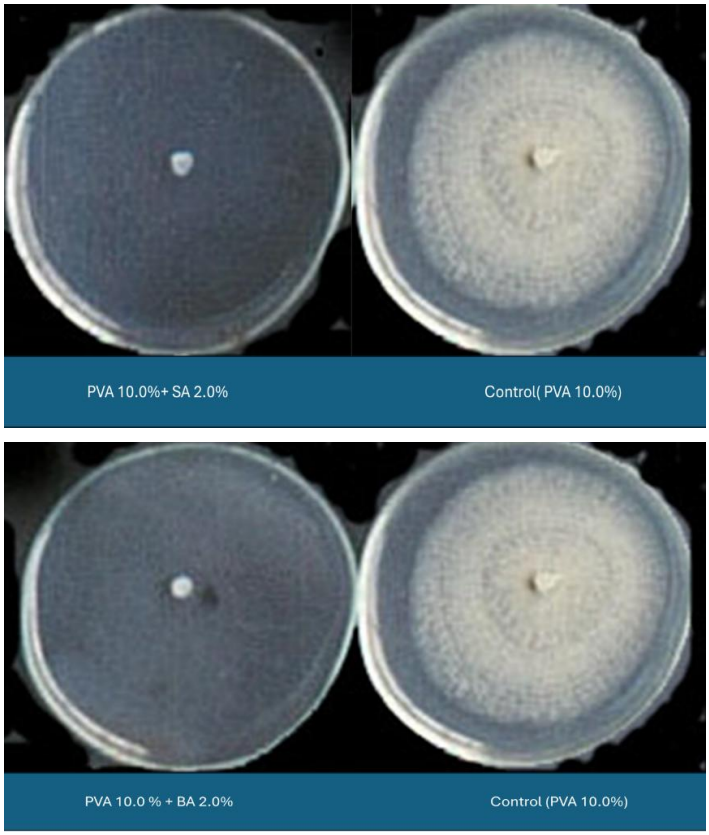

Fig. 3: Effect of polyvinyl alcohol formulation with some organic acids on linear growth of *Penicillium digitatum* (PVA= polyvinyl alcohol, SA= salicylic acid, BA= benzoic acid)

3.5. In Vivo trails

3.5.1. Effect of polyvinyl alcohol formulation with some organic acids on green mold disease of Washington navel orange fruits during storage

The effects of PVA at 10% combined with SA and BA at 1.5% and 2.0% were evaluated in vivo in order to examine their impact on orange fruit green mold disease. The results shown in Tables (3 and 4) demonstrate that every formulation considerably decreased the disease incidence and severity of orange fruit green mold disease. Formulation of SA or BA at 2.0% with PVA at 10.0% reduced the disease incidence by 90.0 and 88.0 % and disease severity by 92.0 and 91.0 % respectively. Followed

by SA or BA at 1.5% with PVA at10.0% which reduced the disease incidence by 80.0 and 78.0 % and disease severity by 80.0 and 82.0 % respectively.

Table 3: Effect of polyvinyl alcohol formulation with some organic acids on green mold incidence of Washington Navel orange fruits during storage

Treatments (%)	Green mold disease			
	Disease incidence %			
	Days after storage			Reduction after 21 days
	7	14	21	
PVA 10 + Salicylic acid 1.5	10.0c	20.0c	20.0c	80.0
PVA 10 + Salicylic acid 2.0	5.0d	8.0d	10.0d	90.0
PVA 10 + Benzoic acid 1.5	11.0c	18.0c	22.0c	78.0
PVA 10 + Benzoic acid 2.0	4.0d	7.0d	12.0d	88.0
PVA 10	25.0b	65.0b	70.0b	30.0
Control (un treated)	65.0a	100.0a	100.0a	0.0

Figures with the same letter are not significantly different (P=0.5)

Table 4: Effect of polyvinyl alcohol formulation with some organic acids on green mold severity of Washington Navel orange fruits during storage

Treatments (%)	Green mold disease			
	Disease severity %			
	Days after storage			Reduction after 21 days
	7	14	21	
PVA 10 + Salicylic acid 1.5	10.0c	17.0c	20.0c	80.0
PVA 10 + Salicylic acid 2.0	6.0d	8.0d	8.0d	92.0
PVA 10 + Benzoic acid 1.5	11.0c	16.0c	18.0c	82.0
PVA 10 + Benzoic acid 2.0	5.0d	9.0d	9.0d	91.0
PVA 10	25.0b	60.0b	65.0b	35.0
Control (un treated)	60.0a	100.0a	100.0a	0.0

Figures with the same letter are not significantly different (P=0.5)

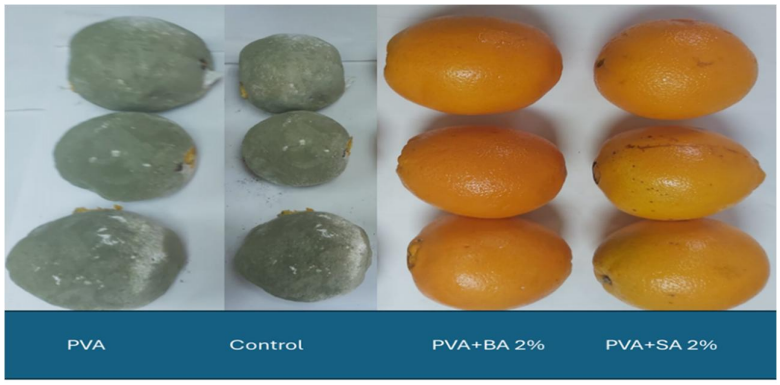

Fig. 4: Impact of polyvinyl alcohol formulation with some organic acids on green mold severity of Washington Navel orange fruits during storage

3.5.2. Effect of Effect of polyvinyl alcohol formulated with some organic acids on fruit quality

The effects of SA and BA at 1.5 and 2.0 % in combination with PVA at 10 % without artificial infection were tested to find out how they affected the quality of the orange fruits. Results in Table (5) reveal that All formulations had no negative effect on fruit quality of orange fruits. All tested

formulations reduced weight loss more than 38.5% and fruit decay more than 80.0%. As for total soluble solids (TSS) there was no significant effect on total soluble solids percentage (TSS).

Table 5: Effect of polyvinyl alcohol formulation with some organic acids on fruit quality of Washington Navel orange fruits after 21 days of storage without artificial infection

Treatments (%)	Fruit quality		
	Weight loss (%)	Total soluble solids (TSS)	Fruit decay %
PVA 10 + Salicylic acid 1.5	3.0	15.9a	2.5
PVA 10 + Salicylic acid 2.0	3.0	16.0a	2.5
PVA 10 + Benzoic acid 1.5	3.2	15.9a	2.6
PVA 10 + Benzoic acid 2.0	3.1	15.8a	2.6
PVA 10	3.2	16.0a	8.0
Control (un treated)	5.2	16.0a	13.0

Figures with the same letter are not significantly different (P=0.5)

4. Discussion

Based on research conducted by Cheng *et al.* (2020), Bhatta (2021), Shawky *et al.* (2023), and Abd-El-Kareem *et al.* (2022*), Penicillium digitatum, P. italicum*, and *Geotrichum citri-aurantii* are the causal organisms of green mold, blue mold, and sour rot, respectively.

Just *Penicillium digitatum* only is causing 90% of postharvest losses of citrus fruits (Zhu *et al.,* 2017 and Bhatta, 2021). According to Chen *et al.* (2019), Youssef and Hussien (2020), and Shawky *et al.* (2023), citrus fruits are spreading disease due to pre- or post-harvest damage.

Numerous inorganic salts, as well as organic acids and their salts, some of which are employed in the food processing sector, possess antibacterial qualities and may prove beneficial as a postharvest intervention to manage deterioration (El-Mougy *et al.,* 2008; Abd-El-Kareem *et al,* 2015 ; Abd-El-Kareem and Saied 2015 and Elshahawy *et al.,* 2015).

Results in the present study, the effects of benzoic acid (BA) and salicylic acid (SA) at 0.0, 0.5, 1.0, 1.5, and 2% against *Penicillium digitatum's* mycelial growth were assessed, according to present study's results. The linear growth of Penicillium digitatum was significantly inhibited by all tested concentrations, according to the results. With SA and BA at 2.0%, linear growth was completely inhibited. The greatest reduction was achieved by using 1.5% SA and BA, which, respectively, lowered the linear growth by 86.7 and 85.6%. In this regards, El-Fawy *et al.* (2020) reported on the assessment of certain chemical food preservatives in relation to the management of gray mold illness in strawberry fruits. At concentrations of 10, 20, 40, and 80 mM, six compounds—acetic acid, potassium citrate, sodium benzoate, benzoic acid, citric acid, and sodium citrate—were examined for their ability to prevent the pathogen's mycelial development. According to results from in vitro experiments, every therapy considerably ($P < 0.05$) reduced the pathogen's ability to growth mycelial. Significantly more affordable, efficient, and human health-safe alternative management techniques have been employed to manage post-harvest infections. Gray mold development was dramatically reduced in a lab setting by treating pepper fruits with (SA) and abscisic acid as resistance inducers (Kamara *et al.,* 2016).

One phenolic hormone that is essential to plants' ability to withstand stress is salicylic acid (SA). By inducing the defensive resistance system (Yao and Tian, 2015) and stimulating antioxidant enzymes (Xu and Tian, 2008), pre- and post-harvest SA spraying has improved control against pathogens in pears (Jiankang *et al.,* 2006). It has also improved control against infections in sweet cherries.

According to Abdel-latif *et al.* (2011), guava fruits treated with salicylic or citric acids prior to harvesting exhibited the greatest reduction in disease incidence and disease severity of rot when stored at room temperature. However, after 15 days of cold storage, no infection was found in the fruits treated with salicylic acid.

Adding an extract rich in gallic acid to PVA coatings will help delay the ripening of bananas. *Ficus auriculata* leaf extract has been discovered to have strong antioxidant activity and to be rich in gallic acid (Baite *et al.,* 2021). Results in the present study indicated that all formulations between SA or BA with PVA significantly reduced the linear growth of *Penicillium digitatum.* Complete suppression of linear growth was obtained with formulation of SA or BA at 2.0 %with PVA at 10.0%. While, in vivo

assessment of the effects of SA and BA at 1.5 and 2.0 % in combination with PVA at 10 % were applied to study their effect on green mold disease of orange fruits. Results indicated that all tested formulation significantly reduced the disease incidence and severity. Also, previous formulations had no negative effect on fruit quality of orange fruits.

PVA is commonly utilized as a hot and cold water soluble film for food packaging, detergents, pharmaceuticals, and agricultural chemicals, among other packaging uses. Additionally, the FDA has cleared it for close contact with food items. PVA has recently been submitted to the FDA to request approval for use in an edible film that comprises ingredients for dry food and dissolves in water (Keller and Heckman, 2018; GRAS Notice No. 676, 2018).

PVA films have a high tensile strength, are translucent, and are flexible. They are also well-suited as an aroma and oxygen barrier. Numerous publications claim that the formation of interpenetrated polymer networks in mix films consisting of starch and PVA enhanced the mechanical and water barrier properties of the composite films, offering several advantages over pure starch films (Cano, *et al.,* 2015).

Polyvinyl alcohol (PVA) is a biocompatible and biodegradable polymer with excellent mechanical, optical, physical, and film-forming properties in addition to strong chemical resistance, according to Zanela *et al.* (2018).

Effective research has been done on the potential use of PVA and its composites in food packaging systems (Youssef, *et al.,* 2019). Because of its safe and nontoxic profile, it has been designated as generally recognized as safe (GRAS), meaning that it can be utilized to make edible films (Keller and Heckman, 2018).

The introduction of bioactive substances such phenolic compounds in food packaging could have a significant positive impact (Andrade, *et al.,* 2021). Gallic acid is one phenolic compound that dissolves in water and has been associated with potent antibacterial and antioxidant properties. It has been demonstrated that PVA films with gallic acid enhance their antioxidant properties (Awad, *et al.,* 2017).

PVA is an environmentally benign synthetic polymer that is hydrophilic, nontoxic, biocompatible, and biodegradable (He *et al.,* 2019 and Candéo *et al.,* 2020).

Furthermore, according to Sapper *et al.* (2021), depending on the distribution and content of carvacrol in the films, different polyvinyl alcohol (PVA) coating formulations with starch and carvacrol as the active agent when applied to Golden Delicious apples demonstrated a highly effective disease control against the growth of both black and green mold. Furthermore, according to Baite *et al.* (2022), Ficus auriculata leaf extract high in gallic acid an antioxidant was mixed with poly(vinyl alcohol) (PVA) and used as a coating to stop green bananas from ripening too quickly.

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