Effect of some Aqueous Plant Extracts on Controlling Orange, Green Mold Disease and Quality of "Washington" Navel Orange

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ABSTRACT

This work was carried out under laboratory conditions at Agricultural Botany and Food Science and Technology Departments, Faculty of Agriculture, Menofiya University Shibin el Kom, Egypt to study the aqueous extracts effect of four plants, i.e., Carnation (*Dianthus caryophyllus*, Chili pepper (*Capsicum annum*), Cinnamon *(Cinnamomum verum*) and Lemongrass (*Cymbopogon citratus*) at three concentrations (2.5%, 5%, and 10 %) on green mold disease caused by *Penicillium digitatum* and the fruit quality of "Washington" Navel orange. Some fruit characteristic i.e., vitamin C, firmness, T.S.S and total acidity were estimated. All treatments significantly reduced the fungal linear growth, disease incidence, and disease severity of the disease and increased the chemical characters of fruits, i.e., Total acidity (TA), vitamin C (Vit. C), and soluble solids content (SSC) compared with the control. Carnation extracts (5 and10 %) and chili pepper (10 %) extract were the best treatments, while the lowest effect was observed by lemongrass (2.5 %) concentration.

Keywords: **Orange, *Penicillium digitatum*, plant extract, fruit quality, soluble solids content, total acidity.**

INTRODUCTION

Citrus (family *Rutaceae*) are major fruit crops that include prominent varieties such as mandarin, orange, and lemon cultivated in many countries of the world. Orange (*Citrus sinensis L Osbeck*) is one of the most important species in citrus. Following skin damage, orange fruit is vulnerable to different diseases during harvest and consumption, especially rot diseases due to their high-water content and nutrient composition (Tripathi and Dubey, 2004). *Penicillium* spp. are the main cause of deterioration and decomposition of plant fruits after harvest, like grapes and oranges (Gende et al., 2008 ; Al-Samarrai et al., 2013). Also, Talibi et al. (2014) reported that the most common and serious diseases that affect citrus fruit are green and blue molds caused by *Penicillium digitatum* Sacc and *Penicillium italicum Wehmer*. The fungal inoculum of *Penicillium* is associated with the surface of fruit during the growing season and, after harvest, can build up to high levels. The fruit rot rate from *Penicillium* spp. ranges from 10 to 30-% in general, but it increases up to 50 % in severe conditions, causing serious economic losses. Moreover, the fungi produce many harmful mycotoxins and carcinogenic compounds like citrinin, patulin, and penicillic acid (Aqil et al., 2010; Ladaniya, 2010). Continuous use of fungicides makes the pathogens resistant and pollute the environment. Moreover, fungicides remain on the citrus peel surface, harm human health. Thence, scientists worldwide devote their time and energy to study the high effect, low toxicity, safety, and inexpensive plant-derived fungicides. Water and alcohol extracts for many plants are a possible option as alternative methods to fungicides. The extracts and essential oils of herbaceous plants are available, low toxic, with environmental friendliness (Aqil et al., 2010). According to El-Samawaty et al. (2021), aqueous extract of cinnamon, cloves, ginger, and turmeric plants were effective against 17 isolates of *Penicillium* spp in vitro. Gupta et al. (2008) compared the antimicrobial activates of cinnamon extracts against ten different isolates of bacteria and seven different isolates of fungi by the agar well diffusion. The extract showed a significant effect against bacteria and fungi, especially *Bacillus cereus* and *Penicillium* spp. (Abd-El-Khair and Hafez, 2006) tested some plant extracts., lemongrass, lantana, and eucalyptus separately or in a mixture against green rot disease on "Washington" Navel orange fruits. They found that all medicinal plant extracts reduced the incidence and severity of green rot compared to control. Al-shemmary et al. (2018) reported that aqueous extract of carnation flowers significantly reduced *Pythium aphanidermatum*, the causal organisms of cucumber damping-off.

The present study aimed to evaluate the efficacy of aqueous extracts four plants. i.e., Carnation (*Dianthus caryophyllus*), Chili pepper (*Capsicum annum*), Cinnamon *(Cinnamomum verum*), and Lemongrass (*Cymbopogon citratus*) at three concentrations 2.5 and 5 and 10 against *Penicillium digitatum* and thein effects on quality of Washington Navel orange fruit under lab condition.

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MATERIAL AND METHODS

Plant materials:

Mature "Washington" Navel orange fruits (*Citrus sinensis L Osbeck*) were obtained from a private orchard at Ashmon, Menofiya governorate. Uniform size and appearance of fruits were used. Plant samples from Carnation (*Dianthus caryophyllus*) flower buds, Chili pepper (Capsicum *annum*) fruits, Cinnamon *(Cinnamomum verum*) dried bark and flowers, and Lemongrass (Cymbopogon *citratus*) leaves were collected from Faculty of Agriculture farms, Shibin El-Kom.

Preparation of plant extracts:

Aqueous plant extracts were obtained as the method described by Ismaiel (Ismaiel, 2008). The sample of each plant was washed with distilled water then left for two weeks for drying. Then each sample was ground separately by using sterile pestle and mortar. After that, one hundred grams of each ground plant materials were homogenized in 100 ml of distilled water (1:1 w/v) for 3 min using a blender. The obtained extract was filtered through a sterilized double layered muslin cloth to remove the debris of plant material. Then, this extract was again filtered through a filter paper (Whatman No.1). The filtered extract was centrifuged at 4000 rpm for 5 minutes to get a homogenous aqueous solution. After centrifuging, the supernatant of each solution was filtrate through a 0.45 µm membrane filter to avoid any microbial contamination. Concentrations (2.5-5-10) were prepared and stored at the dark glass at 5 ºC until used

Pathogen:

Isolation from rotted orange fruits was carried out on a PDA. Small pieces (about2 cm in diameter) of rotted fruit were disinfested by dipping into 2% sodium hypochlorite solution for 5 min., then washed several times with sterilized water, and dried between sterile filter paper. The sterilized pieces were placed onto the surface of the medium in sterilized Petri-dishes. Inoculated plates were incubated at 25ºC for 6 days. The hyphal tip technique was followed for the purification of the isolated fungi. Fungal cultures were identified Using taxonomic and morphological characteristics. The identified pathogen was *Penicillium digitatum*.

Effect of plant extracts on Penicillium digitatum mycelium growth.

The effect of plant extracts on P digitatum mycelium growth was studied in Petri dishes filled with PDA media. After media preparation flasks were cooled down to about 40ºC. 2.5,5,10 ml of aqueous extracts aided to 97.5,95 and 90 ml of media to get the concentrations then media gently agitated by hand for 2 min to allow extract mixes with it. Petri dishes, 9.0 cm in diameter, were used. *Penicillium digitatum* (4 mm in diameter disk) was inoculated at the center. Three replicates were used for each treatment. Plates were inoculated with pathogenic fungus only were used as control treatment. The plates were incubated at 26 ºC. Linear growth of the fungus was measured when pathogenic fungi completely covered the medium surface in the control treatment. Percentage inhibition was calculated as:

$$\mathbf{PI = (C\text{-}T / C) \times 100.}$$

PI = The percent inhibition over control

C = Mycelial radial growth in control plate

T = Mycelial radial growth in treatment

Table 1. Plant materials used in aqueous extracts for control *Penicillium digitatum*

Plant extracts				
English name	**Scientific name**	**Family**	**Appreciation**	**Used part**
Carnation	*Dianthus caryophyllus*	Caryophyllaceae	CDC	**Flower buds**
Chili Pepper	*Capsicum annum*	Solanaceae	CCA	**Fruits**
Cinnamon	*Cinnamomum verum*	Lauraceae	CCV	**Dried bark and Flowers powder**
Lemongrass	***Cymbopogon citratus***	**Poaceae**	**LCC**	**Leaves**

Effect of plant extracts on green mold disease on orange fruits.

Healthy fresh citrus fruits were washed with tap water and then air-dried and disinfested by immersion in 70% ethanol for 1 min. Fruits were treated by dipping for 3 minutes in the aqueous solution of each plant mentioned above extract treatments, and benomyl fungicide (50,100,200 ppm) separately excepting control treatment then air-dried. In the control treatment, fruits were dipped in distilled water. One wound, approximately 3 mm depth, was made in each fruit cortex. After that, fruits were inoculated by the spraying with aqueous spore suspension 0.1 ml of spores ($1x10^6$ mL), then treated fruits were put inside carton boxes and stored under cold conditions at 5ºC for one week. Ten fruits were used for each treatment. Incidence and severity of rotted disease and quality of fruits were recorded. Rotted fruits were removed and counted. Disease incidence was calculated about the initial number of stored fruits by the following equation:

(The number of rotted fruits / total number of fruits) × 100.

The percentage of disease severity (D.S.) was calculated after 15 days as the following equation:

(The infected rotted area of treatment / the infected rotted area of control) × 100.

Fruits quality.

Fruits were moved to the laboratory of Food Science and Technology Department to study their quality by assessing some fruit characteristics:

Vitamin C

Immediately after taking the juice, Vitamin C was evaluated by titrating 3 mL of juice in 3 mL trichloroacetic acid (TCA, 5% w/v) with 2, 6-dichlorophenolindophenol (DCPIP, 0.03 percent w/v) until the colour changed to a stable pink. Using a standard curve made up of varying concentrations of ascorbic acid, the results were represented as mg 100 g1 of ascorbic acid on a fresh weight (AOAC, 1990).

Firmness

The firmness of the orange was determined using a penetrometer and an eight mm diameter probe that was gently pressed into the surface of each orange. The result was calculated in kilos per square centimeter (Adetunji et al., 2019).

Measurements of total soluble solids

Using a hand refractometer, the total soluble content (TSS) in pulp was determined and expressed as °Brix (Ramful et al., 2011).

Measurements of titratable acidity in the pulp

Sayyari et al. (2009) established methodologies for determining the titratable acid in pulp juice. First, 50 mL of distilled water was mixed with 5.0 g of orange pulp juice, which was then titrated with 0.1 M NaOH and expressed as a percentage of citric acid.

Statistical Analysis

The obtained data were subjected to analysis of variance (ANOVA) using Costat software, version 6.4. Duncan multiple range tests (DMRT) at $p < 0.05$ level was used for means separation (Gomez and Gomez, 1984). Experimental data (Vitamin C, Firmness, total soluble solids, titratable acidity, and Sensory evaluation) were subjected to two-way ANOVA analysis. MSTAT-C was used to conduct all of the analyses. Concentrations (%) and treatments were the sources of variance. The mean values were calculated and the mean ± standard error (n = 4) was reported. Mean comparisons were performed using the least significant difference (LSD) test to examine if differences were significant at $P \leq 0.05$.

RESULTS

Data presented in Table (2) and Figure (1) showed that all treatments significantly reduced the linear growth of *Penicillium digitatum* in vitro with varying concentrations compared to control. The concentrations 5 and 10% of carnation,10 % of chili pepper and benomyl fungicide (100 and 200 ppm) were superior with 100% reduction (no linear growth found), while 50 ppm of benomyl gave 97.63 % reduction. Lemongrass extract (2.5%) gave the lowest effect with a 30.32 % reduction followed by cinnamon (2.5%) 40.02 %.

Dealings of aqueous plant extracts and fungicides significantly reduced the disease severity and incidence of green mold on orange fruits, as shown in Table (3) and Figure (2). The heights effect was observed by carnation 5% and 10 %,10% of chili pepper and 200 ppm of benomyl concentrations with 100 % reduction in disease severity (no mold was found on fruits) and 0 % of disease incidence (all fruits were healthy). On the other hand, lemongrass 2.5% concentration had the lowest effect with a 40 and 62.06 % reduction in disease incidence and severity.

Table 2. In vitro effects of aqueous plant extracts at different concentrations on the linear growth (mm) of *Penicillium digitatum*

Plant extract	Concentration (%)	Linear growth (mm)	Growth reduction (%)
Chili Pepper	2.5	8.26^{h}	90.77
	5	2.03^{ij}	97.73
	10	0.00^{j}	97.47
Cinnamon	2.5	53.68^{c}	40.02
	5	39.50^{e}	54.86
	10	12.98^{g}	85.49
Carnation	2.5	11.24^{g}	87.44
	5	00.00^{j}	100
	10	00.00^{j}	100
Lemongrass	2.5	62.35^{b}	30.32
	5	44.31^{d}	50.48
	10	17.36^{f}	80.96
Benomyle (ppm)	50	2.12^{i}	97.63
	100	0.00^{j}	100
	200	0.00^{j}	100
Control	-	89.50^{a}	-

Duncan's Multiple Range Test shows that values with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range Test.

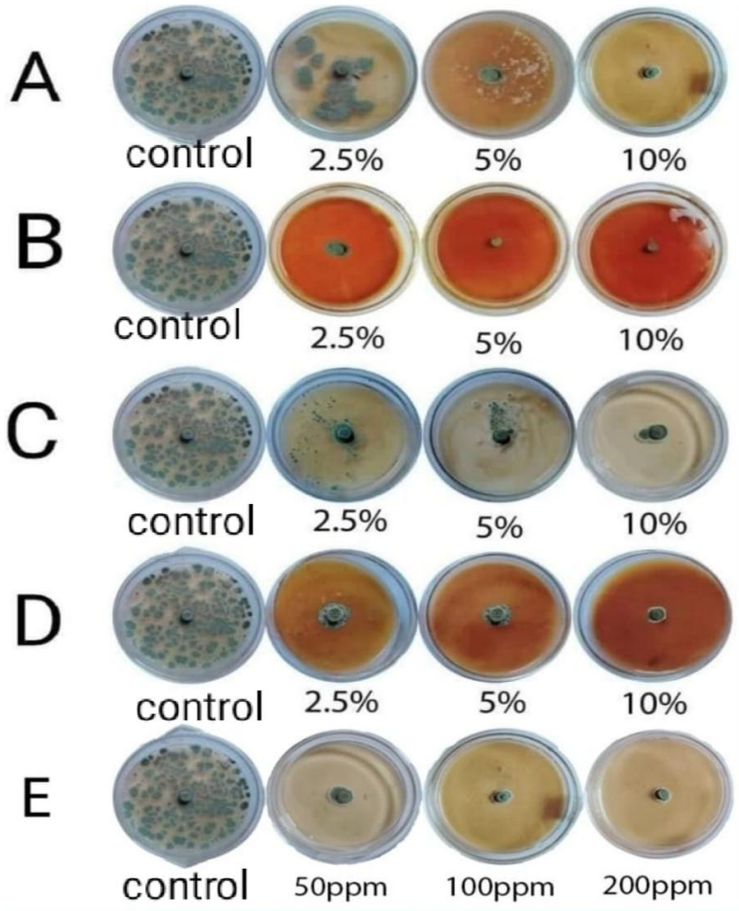

Figure 1. In vitro effects of aqueous plant extracts with a different concentration on the linear growth (mm) of *Penicillium digitatum*. A: Cinnamon B: Carnation C: Lemongrass D: Chili pepper E: Benomyle (Fungicide)

Table 3. In vivo effects of aqueous plant extracts at different concentrations on the disease incidence and disease severity of *Penicillium digitatum* on "Washington" Navel orange fruits

Plant extracts	Conc. (%)	N. of rotted fruits	Disease incidence (%)	Lesion diameters (mm)	Disease severity reduction (%)
Chili Pepper	2.5	4.0^{b}	40.0	9.00^{f}	82.04
	5	2.0^{cd}	20.0	3.00^{hi}	86.15
	10	0.0^{e}	00.0	0.00^{j}	93.58
Cinnamon	2.5	3.0^{bc}	30.0	14.5^{c}	77.69
	5	3.0^{bc}	30.0	12.50^{d}	80.76
	10	2.0^{cd}	20.0	5.00^{g}	92.30
Carnation	2.5	2.0^{cd}	20.0	3.00^{hi}	95.15
	5	0.0^{e}	00.0	0.00^{j}	97.44
	10	0.0^{e}	00.0	0.00^{j}	98.20
Lemon grass	2.5	4.0^{b}	40.0	24.66^{b}	62.04
	5	3.0^{bc}	30.0	15.00^{c}	76.92
	10	2.0^{cd}	20.0	10.66^{e}	84.09
Benomyle (ppm)	50	1.0^{de}	10.0	$4.03f^{gh}$	93.8
	100	0.0^{e}	0.00	1.67^{ij}	97.43
	200	0.0^{e}	0.00	0.00^{j}	0.00
control	-	10^{a}	100	65.00^{a}	-

Values with the same letter are not significantly different at the 5% probability level by Duncan's Multiple Range Test.

All experimental extractions of Vitamin C contents of orange treated samples and control showed no significant difference ($p>0.05$), according to the data (Table 4). The oranges treated with carnation, chili pepper, cinnamon, lemongrass had significantly higher Firmness than the untreated oranges (Table 4). Moreover, it was observed that the oranges treated with carnation had a significantly higher ability to preserve firmness than other samples. In all of the different treatments that were carried out on orange fruits, no difference in vitamin C values was noticed between the concentrations and some of them. Oranges treated with both coatings from Carnation and Cinnamon contained significantly higher levels of ascorbic acid than those coating with Chili Pepper.

The titratable acidity in the orange-treated of all treatments compared with the control samples in (Table 4). The titratable acidity in the Orange-treated with carnation, chili Pepper, cinnamon was higher than that of the Lemongrass, and fungicide. The TSS in the treated fruit was significantly higher than in the control ones (Table 4). The treated orang with carnation, chili Pepper, and cinnamon had significantly higher TSS values compared to the oranges treated with FB and lemongrass. The 10% concentration produced the best effects in the all-orange treatments compared to the other concentrations.

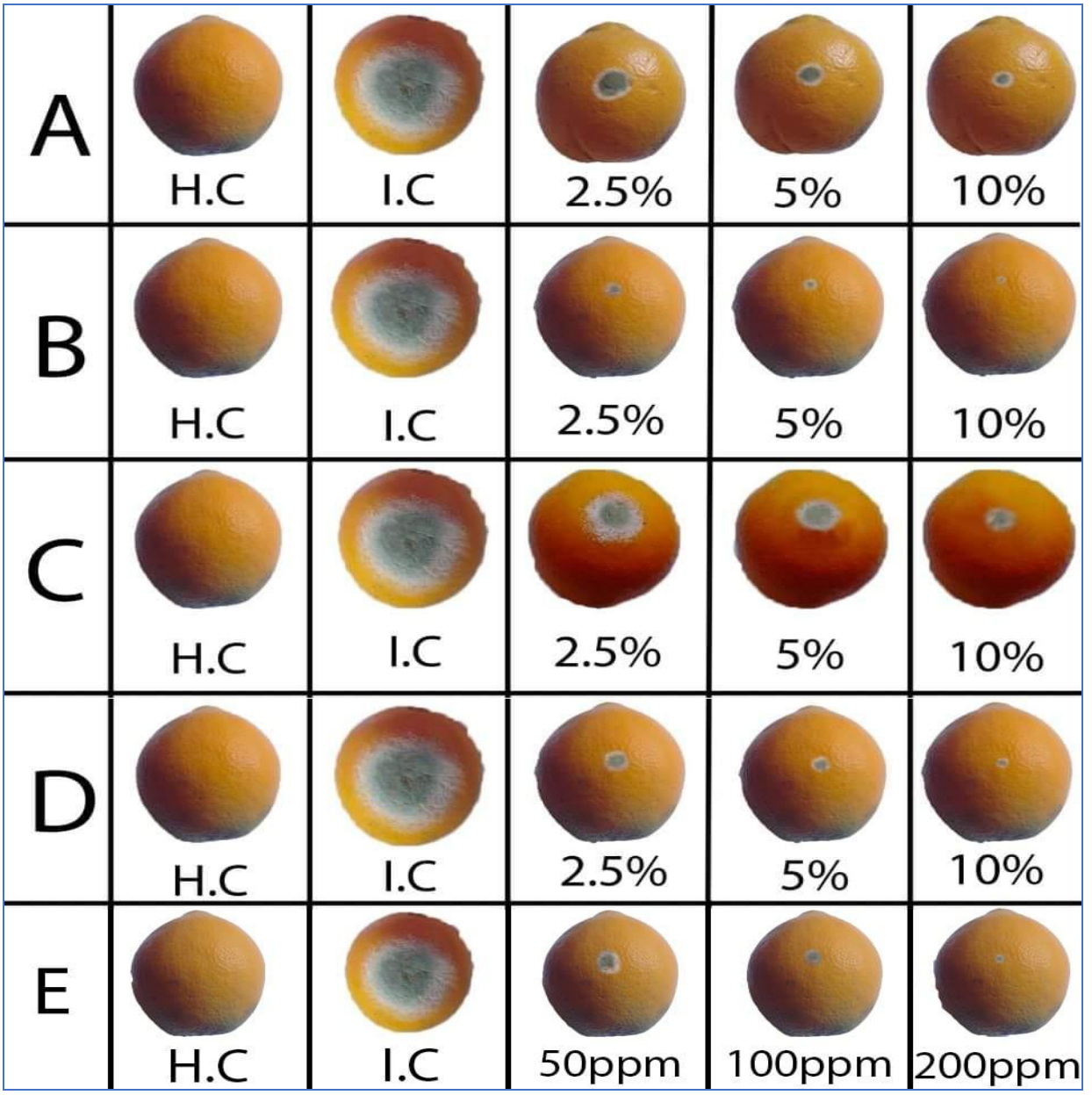

Figure 2. In vivo effects of aqueous plant extracts with a different concentration on the disease incidence and disease severity of *Penicillium digitatum* on "Washington" Navel orange fruits. A: Cinnamon; B: Carnation; C: Lemongrass; D: Chili pepper; E: Benomyle (*Fungicide*); H.C: Healthy control; IC: Infected control

Table 4. Vitamin C, firmness, soluble solids content (%) and titratable acidity in orange fruit untreated or treated with aqueous plant extracts with a different concentration on the disease incidence and disease severity of *Penicillium digitatum* on "Washington" Navel orange fruits

Treatments	Concentration (%)	Vitamin C (mg/kg)	Firmness	Soluble solids content (%)	Titratable acidity (%)
Control	**0**	**55.14 ± 0.55 ^{Aa}**	**17.97 ± 0.17 ^{Ea}**	**19.44 ± 0.18 ^{Ga}**	**19.33 ± 0.54 ^{Ea}**
Chili Pepper	**2.5**	55.44 ± 0.45 ^{Aab}	19.26 ± 0.15 ^{Cc}	21.11± 0.18 ^{Ba}	22.36 ± 0.44 ^{Bb}
	5	54.57 ± 0.44 ^{Aab}	20.64 ± 0.19 ^{Bb}	21.25± 0.17 ^{BCa}	22.74 ± 0.48 ^{Bab}
	10	54.38 ± 0.57 ^{Aab}	20.99 ± 0.21 ^{Aa}	21.27± 0.19 ^{BCa}	22.98 ± 0.41 ^{Bab}
***L.S.D.**		**1.55**	**0.22**	**0.31**	**0.25**
Cinnamon	**2.5**	54.62 ± 0.56 ^{Ab}	21.55 ± 0.33 ^{Ba}	20.84± 0.22 ^{Da}	21.45± 0.44 ^{Cac}
	5	55.14 ± 0.45 ^{Aab}	21.45 ± 0.35 ^{Ba}	20.98± 0.25 ^{Da}	21.81 ± 0.41 ^{Cbc}
	10	55.31 ± 0.61 ^{Aa}	21.64 ± 0.31 ^{Ba}	21.25± 0.29 ^{CDa}	21.95± 0.39 ^{Cab}
***L.S.D.**		**0.55**	**0.44**	**0.33**	**0.44**
Carnation	**2.5**	55.14 ± 0.66 ^{Aa}	22.45 ± 0.36 ^{Aa}	22.98 ± 0.28 ^{Aa}	20.33 ± 0.25 ^{Dc}
	5	55.36± 0.74 ^{Aa}	22.15 ± 0.39 ^{Aa}	23.44 ± 0.24 ^{Aa}	20.55 ± 0.33 ^{Dbc}
	10	55.94 ± 0.56 ^{Aa}	21.98 ± 0.31 ^{Aba}	23.55 ± 0.19 ^{Aa}	20.87± 0.36 ^{Dab}
***L.S.D.**		**1.22**	**0.55**	**0.65**	**0.22**
Lemongrass	**2.5**	54.88 ± 0.46 ^{Ab}	19.66 ± 0.18 ^{Ca}	20.44± 0.11 ^{Fa}	23.45 ± 0.41 ^{Aa}
	5	55.01 ± 0.55 ^{Aab}	18.55 ± 0.15 ^{Db}	20.55± 0.21 ^{Fa}	23.54 ± 0.44 ^{Aa}
	10	55.09 ± 0.31 ^{Aab}	18.01 ± 0.19 ^{Dc}	20.81± 0.18 ^{Fa}	23.98 ± 0.41 ^{Aa}
***L.S.D.**		**0.61**	**0.35**	**0.45**	**0.61**
Fungicide	**2.5**	54.14 ± 0.36 ^{Aa}	19.55 ± 0.22 ^{Cb}	21.48± 0.33 ^{DEa}	20.44 ± 0.31 ^{Da}
	5	54.36± 0.44 ^{Aa}	19.88 ± 0.21 ^{Ca}	21.36± 0.29 ^{Ea}	20.61 ± 0.29 ^{Da}
	10	54.94 ± 0.36 ^{Aa}	19.05 ± 0.26 ^{Cc}	20.92± 0.35 ^{Ea}	20.87 ± 0.22 ^{Da}
***L.S.D.**		**0.88**	**0.22**	**0.69**	**0.55**
****L.S.D.**		**1.84**	**0.55**	**1.55**	**0.64**

Nots:

- ✓ **Each value represents the mean ±standard deviation of three triplicate determinations.**
- ✓ **Data represent the means ± SD, n = 4.**
- ✓ *** L.S.D. values of concentrations (%); values indicated by different small letters (a, b, c..) difference significantly ($p \leq 0.05$).**
- ✓ **** L.S.D. values of treatments; values followed by different capital letters (A, B, C) different significantly ($p \leq 0.05$).**

DISCUSSION

The green mold of citrus fruit caused by *Penicillium digitatum* is an important postharvest pathogen that causes serious annual losses. The extensive use of fungicides to manage fruit disease has undesirable effects on human health. Different strategies are employed to control the disease worldwide to reduce fungicides application. One important approach is using plant extracts (El-Samawaty et al., 2013). Aqueous or organic solvent extracts of plants from different origins are sources of antifungal activity against citrus postharvest pathogens under different experimental conditions (Askarne et al., 2012). *Penicillium* spp. showed highly significant impacts from exposure to some plant extracts (Askarne et al., 2012). Cinnamon (*Cinnamomum zeylanicum*) extract has good activity against *Penicillium* spp. These activities could be attributed to the presence of antifungal compounds; cinnamaldehyde, eugenol, cinnamic acid and flavonoids, alkaloids, tannins and saponins (Mahmoud, 2012). According to Daferera et al. (2000), crude extracts of lantana and lemongrass gave high antifungal activity against fungal diseases development under storage conditions. Carnation extracts riches with antifungal compounds such as eugenol can be used to manage postharvest diseases (Daferera et al., 2000). Chili pepper extract has a good antimicrobial property. The possible explanation for this may be due to the inhibitory effect of hydroxycinnamic and capsaicin compounds. In general, the mode of action of phytochemicals against mycotoxigenic fungi is

facilitated through the following mechanisms; disruption of the fungal cell membrane, inhibition of ergosterol biosynthesis, a major sterol that regulates plasma membrane biogenesis and production of reactive oxygen species (ROS), which results in oxidative stress (Xu et al., 2021). In conclusion, the obtained results showed the antifungal effect of aqueous plant extracts and their value in controlling orange, green mold disease and improved the quality of fruits. So, these extracts could be recommended to control green mold disease for reducing fungicides application.

CONCLUSION

Overall, postharvest application of carnation, chili pepper, cinnamon and lemongrass can be regarded of as a promising treatment for preserving the quality of cold-stored oranges without causing harm.

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الملخص العربي

تأثير المستخلص المائي لبعض النباتات على مقاومة مرض العفن الأخضر وجودة ثمار البرتقال أبو سرة صنف واشنطون

سحر حسن الهلالي، أيمن يونس علام ، محمد زكي الشناوي

أجريت الدراسة تحت ظروف المعمل في قسم النبات الزراعي و قسم علوم وتكنولوجيا الأغذية – كلية الزراعة جامعة المنوفية – شبين الكوم – مصر وذلك لدراسة تأثير بعض المستخلصات المائية بثلاث تركيزات مختلفة (٢,٥% ، ٥% ،١٠%) لأربعة نباتات وهى الفلفل الحار والقرفة والقرنفل بالإضافة إلى حشيشة الليمون على النمو الفطري ونسبة الإصابة وشدة الإصابة المرضية لفطر *Penicillium digitatum* المسبب لمرض العفن الأخضر على ثمار البرتقال "أبو سرة" وكذلك دراسة بعض صفات الجودة للثمار مثل نسبة فيتامين سي و صلابة الثمار و نسبة المواد الصلبة الذائبة الكلية و نسبة الحموضة. وقد أوضحت النتائج أن جميع المعاملات كان لها تأثير معنوي في خفض وإيقاف النمو الفطري ونسبة الإصابة بالمرض وشدة الإصابة وتحسين صفات الجودة للثمار وكانت أفضل المعاملات هي مستخلص القرنفل بتركيزي (٥% و١٠%) وأيضا مستخلص الفلفل الحار بتركيز (١٠%) وكان تركيز (٢,٥%) لحشيشة الليمون هو أقل المعاملات تأثيرا على النمو الفطري للفطر ونسبة الإصابة وشدة الإصابة بالمرض.

الكلمات المفتاحية: البرتقال أبو سرة ، العفن الأخضر، المستخلصات النباتية، صفات جودة الثمار.