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RESEARCH ARTICLE
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GC-mass analysis of some essential oils against mold in orange fruits .caused by Penicillium spp

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ABSTRACT

This study aimed to evaluate the antifungal activity of essential oils against Penicillium digitatum and Penicillium italicum, the fungi that cause green and blue mold on orange fruits. The antifungal activity of essential oils in vitro was evaluated using PDA. Compared to other essential oils, the results demonstrate that the essential oil of Nigella sativa has a 69.92% inhibitory capacity at a concentration of 200 ppm, followed by walnut at a 600 ppm concentration. Results of naturally degraded fruits treated with essential oils in vivo for 7, 10, and 15 days at three different concentrations (600, 800, and 1000 ppm). The essential oil of Nigella sativa significantly inhibits the growth of the two fungi, followed by the ability of walnut oil to stop the growth of P. digitatum and sesame oil to restrict the growth of P. italicum. A phytochemical analysis of EOs using GC-MS revealed the presence of a number of chemical groups, including acetylenes, coumarins, favonoids, terpenoids, and sterols. Oleic acid is followed by linoleic acid, which is responsible for 58.65% of the chemicals in nigella sativa, 40.25% of the chemicals in sesame, and 54.12% of the chemicals in walnut oil. Oleic acid accounts for 60.14% of the primary compounds in almond essential oil.

***Keywords*:** GC-MS, essential oils, Penicillium, phytochemicals.

INTRODUCTION

A significant post-harvest pathogen of citrus fruits is *penicillium*. *Penicillium digitalatum* (Pers.) Sacc (green mold) and *P. italicum* Wehmer (blue mold) can affect any commercial citrus fruit. The most prevalent and harmful post-harvest ailment affecting citrus fruits is blue mold [1] particular *Penicillium* species, including *P. chrysogenum* Thom, *P. digitalatum* (Pers.) Sacc., *P. expansum* (Thom) Fassat., and *P. italicum* Wehmer, can create toxins that can be found in food and fruit and have long-lasting and detrimental (i.e. carcinogenic) consequences.

Because synthetic fungicides are frequently used, postharvest disease populations are evolving greater resistance to chemical pesticides [2,3]. Finding alternatives that consumers will accept as safe and that pose little risk to both people and the environment is therefore necessary to reduce the losses brought on by post-harvest diseases [4]. Aromatic plants convert isoprene molecules into volatile terpenes with carbon chains between C10 and C15. Because of their chemical makeup, these terpenes are an organic defense mechanism against plant diseases. These substances, also known as essential oils, may be extracted from plants using various methods, such as steam distillation.

According to many studies [5–7], a recent study has examined the antifungal qualities of essential oils in preventing post-harvest infections. The first advantage of essential oils is their natural nature, which may increase customers' and the environment's perception of them and their real safety. Second, there is little chance that post-harvest infections will become resistant due to their complex makeup, which includes various oil components with unique methods of antifungal action. However, at low doses, essential oils have limited in vivo activity [8].

Because they are neither damaging to people or the environment, plant extracts have recently gained increased significance for use in managing plant pests and diseases. Citrus trees can be found in tropical and subtropical regions of the world, providing people with important nutrients [9]. In 2020, 158 million tons of citrus were produced worldwide, according to FAO 2020. Plants' secondary metabolism produces essential oils to bolster their defenses against biotic and abiotic stress. Secondary metabolites are crucial components of plants' defenses against pathogens. This may be because the compounds with the strongest anti-fungal activity are volatile.

The objective of the current study was to assess the in vitro effectiveness of plant essential oils against postharvest fungal infections. The antifungal properties of essential oils were also investigated on artificially inoculated orange fruit.

Materials and methods

Fungal pathogens

Penicillium digitatum, which causes green mold, and *Penicillium italicum*, which causes blue mold of citrus fruits, were the fungi used in the current study.

They (*P. digitatum* and *P. italicum*) were supplied by the plant protection laboratory, college of Agricultural engineering sciences.

Natural products-essential oils

Almond (*Prunus amygdalus*), black caraway (*Nigella sativa*), Sesame (*Sesamum indicum*) and Walnut (*Juglans regia*), different amounts of oils were used in experiments conducted in vitro and in vivo. At 4 ºC, they were kept.

Evaluation of antifungal activity of plant essential oils in vitro

Potato dextrose agar was used for growing *P. digitatum* and *P. italicum*. Each essential oil was added to sterilized water at a concentration of 1000 ppm and thoroughly dissolved after the media had been autoclaved and cooled to 40°C. Adding 0.2% Tween 80 made it easier to distribute the essential oils.

PDA was added to the mixture to create final concentrations of 0, 200, 400, and 600 ppm. The mixtures were then put into Petri dishes where they were cultured with fungus discs (5 mm in diameter) taken from the outer edges of the test fungi colonies that were 7 days old. Growth inhibition was calculated as the percentage of radial growth inhibition compared with the control, using three replicates for each treatment and incubation at 25°C for 7 days.

The inhibition of antagonism of each essential oil was calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Evaluation of antifungal activity of plant essential oils in vivo

Orange fruits that were taken straight from an unsprayed orchard were tested for essential oils.

Fruits that were uniform in size, unharmed, and free of disease symptoms were chosen. The fruits were cleaned with sodium hypochlorite (2%) for 3 minutes, washed three times with water, and then inoculated using two methods: spraying the suspension of *P. digitatum* and *P. italicum* with 10^5 conidia per ml and a drop of Tween 80, and immersing the fruits in it. A hemocytometer slide was used to determine the conidia concentration. Three replicates of each treatment were used to randomly group the inoculated fruits into groups of six in plastic containers.

At concentrations of 200, 400, and 600 ppm, essential oils were dissolved in water without Tween 80 and kept in glass vials. A second group of fruits were submerged in a fungal suspension after the first group of mixtures had been sprayed inside the containers for three hours. Fruits used as controls received a distilled water spray. To fill the space with volatiles, the boxes were sealed. Clementine fruits were checked daily for symptoms, and the proportion of decayed fruits was calculated at intervals of 7, 10, and 15 days.

(GC-MS) stands for gas chromatography-mass spectrometry.

An Agilent 5977A Series gas chromatograph-mass spectrometer system with an auto-sampler was used to perform the GC-MS study. We used an Elite-1 fused silica capillary column (30 mm long, 0.25 mm inside diameter) with a DB-5MS stationary phase to set up the device in a certain way. Helium (99.999%) was used as the carrier gas in the electron impact mode of operation, which ran at 70 electron volts (eV) at a steady flow rate of 1 milliliter per minute. With a split ratio of 10:1, an injection volume of 0.5 microliters (uL) was employed. The ion-source temperature was set at 280 °C, while the injector temperature was kept at 250 °C. The oven's temperature schedule was as follows: two minutes of initial isothermal holding at 60°C, a ramp of 10°C/min up to 270°C, a slower climb of 5°C/min up to 290°C, and nine minutes of final isothermal hold at 310°C. I got mass spectra of pieces with masses between 45 and 450 Da at 70 eV and a scan interval of 0.5 seconds. Sixty minutes were spent on the GC analysis.

Results and discussions

Plant essential oils have an antifungal effect against the development of P. digitatum and Pitalicum in vitro.

According to the results in Table 1, all of the essential oils that were tested had a significant antifungal effect on pathogen growth after 7 days. Nigella sativa EO observed the highest effective mean against *P. digitatum* with 69.92% at concentration 200 ppm, followed by walnut 69.09% at concentration 600 ppm; in contrast, Sesame caused limited inhibition by 27.74% at concentration 400 ppm. Almond EO at a concentration of 400 ppm recorded the highest effectiveness of P. italicum with 66.79%, followed by Sesame EO at 200 ppm with 64.59 %. In comparison, the lowest inhibition of the pathogen was recorded when using sesame at a concentration of 200 ppm, with 20.97%.

Ellagic acid, flavonoids, polyphenols, phenols, ketones, and alcohols are abundant in N. sativa oil and have medicinal and antibacterial activities [10].

Thyme, sage, and sesame were found to be effective against *B. cinerea* as well as *Penicillium expansum*, another post-harvest pathogen [11–13]. The investigations. [14,15] that were previously reported and included in the current antimicrobial surveys of Nigella oil. The antifungal properties of Nigella oil were discovered to include thymohydroquinone, thymoquinone, pcymene (monoterpene), and longifolene (sesquiterpene).

Note: $\% \text{ Inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$

Table 1: Plant essential oils ability to inhibit fungus growth in vitro

Essential oils	Conc. (ppm)	% inhibition of mycelial growth *P. digitatum*	*P. italicum*
Almond	200	49.15 bc	48.81 c
	400	41.17c	58.64 b
	600	41.80 c	66.97 a
Black caraway	200	69.92 a	54.94 b
	400	48.54 c	31.79 d
	600	54.22 b	62.13 ab
Sesame	200	37.22 cd	64.59 a
	400	27.74 de	62.08 ab
	600	60.71 ab	56.22 b
Walnut	200	30.55 d	20.97 de
	400	57.65 b	32.64 cd
	600	69.09 a	43.92 c

*Means followed by the same letter(s) in each column are not significant different at ≤ 0.05.

The results in (Table 2) demonstrated that treatment of orange fruits with essential oils by spraying and immersion methods at three concentrations (600, 800, and 1000) ppm in addition of control treatment, and three hours after treating the fruits with essential oils, they were contaminated with a spore suspension of two species of *Penicillium* fungus.

Nigella sativa essential oil has a significant effect on inhibiting growth of the two fungi in its three concentrations and the two methods of treatment, followed by walnut oil could inhibit the fungus *P. digitatum* and did not affect the other type of fungi when used at concentrations of 800 and 1000 ppm, while sesame oil could inhibit growth of the fungus *P. italicum* when used at concentrations of 600 and 800ppm by spraying method and on fungus *P. digitatum* by immersion method and same concentrations. No effect was observed when using almond oil on the pathogenic fungi. Additionally, Lopez-Reyes *et al*. [16] showed that the postharvest pathogens *B. cinerea* and *Penicillium expansum* were

significantly suppressed by the essential oils from oregano, savoury (Saturega montana), and thyme. Citrus essential oils, [17], stop the growth of the pathogens *Penicillium chrysogeum*, *Aspergillus niger*, and *Alternaria alternata.*

- = non-infected fruits

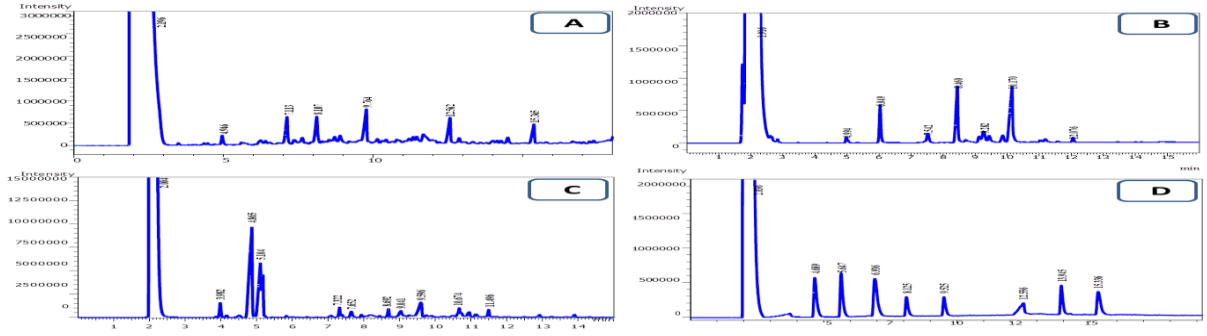
+ = infected fruits.

Table 2: Effect of essential oils on decay orange fruits by spraying and immersion methods

Essential oils	Conc.	Spraying method						Immersion method					
	(ppm)	*P. digitatum*			*P. italicum*			*P. digitatum*			*P. italicum*		
		7 D	10 D	15 D	7 D	10 D	15 D	7 D	10 D	15 D	7D	10 D	15 D
Control	0	+	+	++	+	+	+	-	-	-	-	-	-
Almond	600	-	-	-	-	-	-	+	+	+	+	++	++
	800	-	-	-	++	+++	+++	-	-	-	+	+	+
	1000	-	+	+	+	+	++	-	-	-	-	+	+
Black caraway	600	-	+	++	+	+	+	-	-	-	-	-	-
	800	-	-	-	-	-	-	-	-	-	-	-	-
	1000	-	-	-	-	-	-	-	-	-	-	-	-
Sesame	600	++	+++	+++	-	-	-	-	-	-	++	+++	+++
	800	++	++	+++	-	-	-	-	-	-	+	++	++
	1000	-	+	++	+	++	++	+	++	++	-	+	+
Walnut	600	+	++	++	-	-	+	-	-	-	+	++	++
	800	-	-	-	-	+	++	-	-	-	-	+	++
	1000	-	-	-	-	+	+	-	-	-	-	-	-

Essential oil chemical components as determined by gas chromatography-mass spectrometry (GC- MS)

The chemical composition of almond, nigella sativa, sesame, and walnut EOs is shown in Table (3) along with chromatograms of these EOs from GC/MS that can be seen in Fig. (1. A, B, C, and D). Different compounds from various chemical groups were examined in great detail. Sterols, favonoids, terpenoids, coumarins, cafeoylquinic acids, and acetylenes are some of the main chemical components of plant EOs, which support their potential use in the food and pharmaceutical industries as well as in the management of pests and diseases [18].



- Figure: 1Chromatography of GC- MS of EOs from (A) Almond, (B) Black caraway (C) Sesame and (D) Walnut.

Oleic acid, 60.14%, linoleic acid, 20.15%, and palmitic acid, 5.49%, were generally the main compounds in almond EOs (Table 3). The two main compounds in Nigella sativa, linoleic acid (58.65%) and oleic acid (15.99%, can be identified as separate substances. Additionally, this oil had a moderate amount of palmitic acid (12.25%) and stearic acid (5.98%).

Linoleic acid (40.25%) and oleic acid (33.01%) were the two most prevalent compounds in sesame. Linoleic acid, which comprised 54.12% of the walnut oil, was followed by oleic acid (24.11%), and palmitic acid (6.35%). The powerful antifungal properties of benzaldehyde and some of its derivatives have been confirmed by numerous studies [19–21].

Table 3: Major chemical composition, retention time and percentage of essential oils obtained with GS-MS analysis.

Essential oils	Compound	R. t. (min.)	Molecular weight	Molecular formula	Composition (%)
Almond	Arachidonic acid	4.949	304.478	C20H32O2	0.47
	Palmatic acid	7.113	256.424	$C_{16}H_{32}O_2$	5.49
	Linoleic acid	8.107	280.433	C18H32O2	20.15
	Oleic acid	9.764	280.445	C18H34O2	60.14
	Stearic acid	12.562	284.480	C18H36O2	3.25
	Linolenic acid	15.365	280.446	C18H32O2	0.58
Black caraway	Arachidonic acid	4.994	304.478	C20H32O2	1.00
	Palmatic acid	6.049	256.424	$C16H_{32}O_2$	12.25
	Stearic acid	7.542	284.480	C18H36O2	5.98
	Oleic acid	8.460	280.445	C18H34O2	15.99
	Linolenic acid	9.282	280.446	C18H32O2	0.88
	Linoleic acid	10.170	280.433	C18H32O2	58.65
	Lauric acid	12.076	200.315	$C12H_{24}O_2$	0.25
Sesame	Palmatic acid	3.982	256.424	$C_{16}H_{32}O_2$	3.55
	Linoleic acid	4.865	280.433	C18H32O2	40.25
	Oleic acid	5.104	280.445	C18H34O2	33.01
	Eicosapentaenoic acid	7.322	302.459	$C_{20}H_{30}O_2$	2.58
	Arachidonic acid	7.652	304.478	C20H32O2	1.05
	Linolenic acid	8.692	280.446	C18H32O2	0.35
	Nervonic acid	9.041	366.623	$C_{24}H_{46}O_2$	0.25
	Stearic acid	9.596	284.480	C18H36O2	11.12
	Lauric acid	10.674	200.315	$C_{12}H_{24}O_2$	0.17

	Decanoic acid	11.496	172.269	$C_{10}H_{20}O_2$	0.18
	Palmatic acid	4.689	256.424	$C_{16}H_{32}O_2$	6.35
	Linoleic acid	5.647	280.433	C18H32O2	54.12
	Oleic acid	6.936	280.445	C18H34O2	24.11
	Myristic acid	8.125	228.37	C14H28O2	0.68
Walnut	Arachidonic acid	9.525	304.478	C20H32O2	1.00
	Linolenic acid	12.556	280.446	C18H32O2	2.65
	Stearic acid	13.945	284.480	C18H36O2	3.12
	Eicosapentaenoic acid	15.336	302.459	$C_{20}H_{30}O_2$	3.88

R. T. = Retention Time

Conclusions

Results from both in vitro and in vivo tests revealed that the essential oils of almond, black caraway, sesame, and walnut inhibited the growth of the mycelium and the spore germination of the postharvest pathogens *P. digitatum* and *P. italicum*. Both studies revealed a dosage effect, with increased antifungal activity as the essential oil content increased. As a result, essential oils could be used to treat fruits and vegetables to prevent postharvest illnesses.

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التحليل الكتلي لبعض الزيوت العطرية ضد العفن في ثمار البرتقال المتسبب عن الفطر *Penicillium spp*.

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لخلاصة

هدف الدراسة هو تقييم النشاط المضاد للفطريات للزيوت العطرية ضد *Penicillium digitatum* و*Penicillium italicum* وهي الفطريات المسببة للعفن الأخضر والأزرق على ثمار البرتقال. تم تقييم النشاط المضاد للفطريات للزيوت الأساسية في المختبر باستخدام *PDA*. بالمقارنة مع الزيوت العطرية الأخرى؛ أظهرت النتائج أن الزيت العطري لحبة البركة لديه قدرة مثبطة بنسبة 69.92% بتركيز 200 جزء في المليون، يليه زيت الجوز بتركيز 600 جزء في المليون. نتائج الفواكه المتحللة طبيعيا المعالجة بالزيوت العطرية في الجسم الحي لمدة 7 و 10 و 15 يوما بثلاثة تراكيز مختلفة (600، 800، و 1000 جزء في المليون). يمنع الزيت العطري لحبة البركة بشكل كبير نمو الفطريات، تليها قدرة زيت الجوز على وقف نمو *P. digitatum* وزيت السمسم لتقييد نمو *P. italicum*. كشف التحليل الكيميائي النباتي لـ *EOs* باستخدام *GC–MS* عن وجود عدد من المجموعات الكيميائية، بما في ذلك الأسيتيلين والكومارين والفافونويدات والتيربينويدات والستيرول. يلي حمض الأوليك حمض اللينوليك، وهو المسؤول عن 58.65% من المواد الكيميائية الموجودة في حبة البركة، و40.25% من المواد الكيميائية الموجودة في السمسم، و54.12% من المواد الكيميائية الموجودة في زيت الجوز. ويشكل حمض الأوليك 60.14% من المركبات الأولية في حبة البركة. زيت اللوز الأساسي.

. الكلمات المفتاحية : *GC–MS* :، الزيوت العطرية، البنسليوم، المواد الكيميائية النباتية.