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Antifungal Activity of Essential Oils for Controlling Storage Fungi of Some Medicinal and Aromatic Seeds

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ABSTRACT

The most significant medicinal and aromatic plants in Egypt and the world are caraway (*Carum carvi* L.), anise (*Pimpinella anisum* L.) and cumin (*Cuminum cyminum* L.). *In vitro* trails, completely inhibited of mycelial growth and spore germination was achieved with Thyme and Rosemary at 0.75 % for *A. flavus* and *F. verticillioides* while at 1.0 % for *A. niger*. While in vivo, trails, Thyme and Rosemary essential oils was mixed individually with wheat barn and tested seeds were treated with mixture at rate of 0.00, 5.0,10.0.15.0,20.0 and 25.0 g/kg seeds. Results showed that all tried concentrations of essential oil carrier contact decreased the natural infection of tested seeds. The protective impact of Thyme and Rosemary essential oils carrier against *A. flavus* infection was assessed after 90 days of storage. Results showed that all tried concentrations of essential oil carrier decreased the artificial infection with *A. flavus* of all tested seeds. The highest inhibited was achieved with Thyme and Rosemary carrier at 25.0 g /kg seeds which decreased the infection more than 88.0, 89.0 and 91.0 %, for cumin, anise and caraway respectively. Followed by both essential oils carrier at 20.0g/kg seeds which reduced the infection more than 76.0 % for all tested seeds. Thyme and Rosemary carrier at 25.0 g /kg seeds recorded lower percent of the germination seeds for all tested seeds.

Keywords: Medicinal and aromatic seeds- Essential oils carrier- Storage fungi. *Aspergillus flavus*.

1. Introduction

The most significant medicinal and aromatic plants in Egypt and the world are caraway (*Carum carvi* L.), anise (*Pimpinella anisum* L.) and cumin (*Cuminum cyminum* L.). *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. and *Fusarium* spp. are among the many fungus that attack and harm them after harvest and while they are being stored (Moharram *et al.* 1989 and Regina and Roman 1992). In traditional medicine, cumin is used as a carminative for colic, diarrhoea, and stomach issues (Parthasarathy *et al.,* 2008). The seeds contain 2-5 % essential oil, the major ingredient of which is cuminaldehyde (Hassan *et al.,* 2014). Said and El-Hady Goder (2014) reported to evaluate fungus colonies in anise and cumin seed storage. The total fungal population often displayed erratic monthly changes with certain peaks mainly due to the breakouts in the counts of *A. flavus, A. fumigatus, A. niger, A. sydowii and Mucor hiemalis*. According to Abd-Alla, (2005) assessment of common storage fungi of several aromatic and medicinal seeds, such as fennel, cumin, coriander, and anise, *Aspergillus niger* and *Penicillium* sp. were the most prevalent fungus linked to storage seeds. With the exception of *Penicillium* sp. anise seed, *A. niger* ranked highest among all examined seeds. In traditional medicine, anise seeds are used to alleviate migraines. The essential oils from anise seeds, mainly anethole, were extracted. Antibacterial, antifungal, insecticidal, antiviral muscle relaxant, antispasmodic and smooth muscle relaxant, anticonvulsant, antiulcer, antidiabetic, hypolipidemic, and pain alleviation in dysmenorrhea are only a few of the pharmacological investigations on anise that have been published (Mostafa *et al.,* 2023).

Essential oils (EOs) are aromatic, volatile, sweet-smelling liquid oils that are obtained from flavors and plants by hydrodistillation or supercritical liquid extraction. They are widely available in bioactive

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combinations that have antioxidant and antibacterial properties (Taghavi *et al.* 2018; Mari *et al.,* 2016; Prakash *et al.,* 2015 Danh *et al.,* 2021).

The application of essential oils (EOs) as antifungal agents is one of the practical and efficient methods. EOs are blends of hundreds of naturally occurring volatile plant-based chemicals, primarily oxygenated derivatives of monoterpenes and sesquiterpenes (Tisserand and Young, 2013; Perumal *et al.,* 2016). Strong anti-fungal activity has been shown for several EOs (Rabari *et al.,* 2018). Pathogen resistance to a wide spectrum of chemicals in EOs is unlikely since the antifungal activity of EOs may be primarily connected to their primary components as well as the interaction impact of major and minor components. A large number of EOs are biodegradable and widely accepted as safe (GRAS) and biodegradable. It has been demonstrated that a number of EOs, including ginger and cinnamon oil, can successfully prevent anthracnose, which is caused by different Colletotrichum spp., on mango fruits of different varieties (Sefu *et al.,* 2015 and Danh *et al.,* 2021);peppermint oil on Tommy Atkins variety (de Oliveira *et al.,* 2017); orange, lemon, and mustard oil on Zabdia variety (Abd-Alla and Haggag); lemongrass (Duamkhnmanee 2008); basil oil on Willard variety (Karunanayake *et al.,* 2020; 2013; thyme, clove, and cinnamon oil on Banganapalli and Totapuri varieties (Perumal *et al.,* 2017.

According to Abd-El-Kareem *et al.* (2022), chitosan at 8.0 g/L and thyme or nerrol at 1.5% each decreased the incidence and severity of green, blue mold, and sour rot diseases by more than 88.0 and 92.0%, respectively.

Haggag- Wafaa *et al.* (2024) reported that Thyme and acetic acid were tested in direct contact assay. Data indicated significant impact on mycelial growth and spore germination inhibition of both *A. flavus* isolates. In the postharvest applications: as vapour and carrier contact assay, Thyme and Acetic acid treatments were effective in inhibition of aflatoxin production in both vapour and carrier assays as they succeeded in reducing AFB1 while they inhibited completely the production of AFB2.

This study aims to investigate the impact of essential oils applied as Carrier Contact Assay against storage fungi of some medicinal and aromatic seeds.

2. Materials and Methods

Cumin (*Cuminum cyminum* L.), caraway (*Carum carvi* L.) and, anise (*Pimpinella anisum* L.) were obtained from commercial markets.

2.1. Storage fungi

One pathogenic isolate of *Aspergillus flavus* (Accession number: OQ135182.1), *Aspergillus niger* (Accession number: OQ135183.1) and *Fusarium verticillioides* (Accession number: OQ135185.1) which was also known as *Fusarium moniliforme* were maintained and supplied by (Dr. Al-Ansary-Noran, (2023). Plant Patholology Department, National Research Centre (NRC) Giza, Egypt.

2.2. Impact of essential oils on mycelia growth of storage fungi.

Thyme and Rosemary essential oils at 0.25,0.50,0.75 and 1.0 ml/L were tested against mycelial growth of storage fungi. Previous concentrations were added to 250 ml conical flasks that held 100 ml of sterilised PDA medium each in order to achieve concentrations of 0.25, 0.50, 0.75, and 1.0 ml/l. After that, it was gently rotated and poured into Petri dishes with a diameter of 10 cm. Equal discs (6 mm in diameter) of fungal growth, extracted from a 10-day-old culture cultivated on PDA media and kept at 20°2°C, were seeded individually in the center of each dish. When the control plates reached full growth, the tested fungal mycelial growth was assessed. For each specific treatment, four replicates were used.

2.3. Impact of essential oils on spore germination of storage fungi.

Thyme and Rosemary essential oils at 0.25, 0.50, 0.75 and 1.0 ml/L were tested against spore germination of storage fungi. As for spore germination, conidia from 10 5 days old tested fungi were collected, then placed in sterile water to create a spore suspension that was adjusted to 106 spores per millilitre. Petri plates were filled with aliquots of the prepared spore suspension. Plates were filled with PDA media containing acetic acid to achieve final concentrations of 0.25, 0.50, 0.75, and 1.0 ml/L. As a comparison, a set of plates with PDA media devoid of acetic acid was utilized. The plates were incubated for 24 hours at 20°C. For both fungi, the spore germination percentage was computed.

2.4. Impact of essential oils applied as carrier contact assay on natural infection of some medicinal and aromatic seeds

Thyme and rosemary essential oils were added separately to sterilize wheat bran at a rate of ½ and then completely mixed. The mixture was added to seeds at rates of 5.0, 10.0, 15.0, 20.0, and 25.0 gm/Kg seeds. For every treatment, five duplicates were employed. The treated seeds were moved to a plate containing Czapek's medium (Difco, Detroit, MI) after 30 days, and they were then incubated for 7 days at 25ºC. It was determined what proportion of seeds had fungal development. Seeds

2.5. Protective effect of essential oils applied as carrier contact assay on artificial infection with *A. flavus* after 90 days of storage

The seeds were disinfected using ethanol alcohol 70% and then rinsed multiple times using sterilized water. Thyme and rosemary essential oils was mixed individually with wheat barn as mentioned before. Seeds were treated with mixture at rate of 0.00, 20.0 and 25.0 g/kg seeds. The protective impact of Thyme and rosemary essential oils was assessed after 90 days of storage. Treated seeds (50 g) were artificially inoculated with 10 ml/g of *A. flavus* spore suspension (10^6 spores/ml) and incubated at 25ºC for 7 days. The percentage of grains with fungal growth was calculated. Seeds treated with wheat bran mixed with sterilized water current acted as the control.

2.6. Impact of essential oils on grains germination

Treated seeds were grown on water agar plates for ten days. Twenty grans of replicate and 10 replicates for each treatments were used.

2.7. Statistical analysis: Neler *et al.* (1985) used the Tukey test for multiple comparison among means.

Results

Impact of essential oils on mycelia growth of storage fungi.

Thyme and Rosemary essential oils at 0.25, 0.50, 0.75 and 1.0 ml/L were tested against mycelial growth of storage fungi. Results in Table (1) indicate that all tried concentrations decreased the mycelial growth of tested fungi. Complete inhibition was achieved with Thyme and Rosemary at 0.75 % for *A. flavus* and *F. verticillioides* while at 1.0 % for *A. niger.* Treated fungi with Thyme and Rosemary at 0.50 % resulted in reducing linear growth more than 70.0, 80.0 and 77.8% for *A. niger*, *A. flavus*, and F. *verticillioides* respectively. Other concentration showed moderate effect.

Table 1: Impact of essential oils on mycelial growth of associated fungi with some medicinal and aromatic seeds.

Essential oils	Conc. %	Linear growth (mm)					
		Aspergillus niger		***Aspergillus flavus***		***Fusarium verticillioides***	
		Growth	**Reduction %**	**Growth**	**Reduction %**	**Growth**	**Reduction %**
Thyme	0.25	55.0 b	38.9	44.0b	51.1	42.0b	53.3
	0.50	23.0 c	74.4	15.0 c	83.3	18.0 c	80.0
	0.75	14.0 d	84.4	0.00 d	100.0	0.00 d	100.0
	1.0	0.00 e	100.0	0.00 d	100.0	0.00 d	100.0
Rosemary	0.25	58.0 b	55.6	46.0b	48.9	43.0 b	52.2
	0.50	27.0 b	70.0	18.0 c	80.0	20.0c	77.8
	0.75	14.0 d	84.4	00.0 d	100.0	00.0 d	100.0
	1.0	100.0 e	100.0	00.0 d	100.0	00.0 d	100.0
Control	90.0	90.0 a	0.0	90.0 a	0.0	90.0	0.0

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

Impact of essential oils on spore germination of storage fungi.

Thyme and Rosemary essential oils at 0.25,0.50,0.75 and 1.0 ml/L were tested against spore germination of storage fungi. Results in Table (2) indicate that all tried concentrations decreased the spore germination of tested fungi. Complete suppression was achieved with Thyme and Rosemary at 0.75 % for *A. flavus* and *F. verticillioides* while at 1.0 % for *A. niger.* Treated fungi with Thyme and

Rosemary at 0.50 % resulted in reducing spore germination more than 77.7, 85.3 and 83.3 % for *A. niger*, *A. flavus*, and *F. verticillioides* respectively. Other concentration showed moderate effect.

Table 2: Impact of essential oils on spore germination of associated fungi with some medicinal and aromatic seeds

Essential oils	Conc. %	Spore germination %					
		Aspergillus niger		*Aspergillus flavus*		*Fusarium verticillioides*	
		Germination	Reduction %	Germination	Reduction %	Germination	Reduction %
Thyme	0.25	42.0b	55.3	38.0b	60.0	40.0b	58.3
	0.50	18.0c	80.9	12.0c	87.4	14.0 c	85.4
	0.75	10.0d	89.4	0.00d	100.0	0.00 d	100.0
	1.0	0.00e	100.0	0.00d	100.0	0.00 d	100.0
Rosemary	0.25	44.0b	53.2	37.0b	61.1	36.0 b	62.5
	0.50	21.0c	77.7	14.0c	85.3	16.0 c	83.3
	0.75	12.0d	87.2	00.0d	100.0	00.0 d	100.0
	1.0	00.0e	100.0	00.0d	100.0	00.0 d	100.0
Control	0.0	94.0a	0.0	95.0a	00.0	96.0a	00.0

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

Impact of essential oils applied as carrier contact assay on natural infection of some medicinal and aromatic seeds

The mixture of thyme and rosemary with wheat bran was used at rates of 5.0, 10.0, 15.0, 20.0, and 25.0 gm/Kg seeds to study their effect on natural infection and germination percent of tested seeds. Results in Table (3 and Fig.1) indicate that all tried concentrations of essential oil carrier decreased the natural infection of tested seeds. The highest inhibited was achieved with Thyme and Rosemary carrier at 25.0 g /kg seeds which decreased the natural infection by 88.0, 90.0 &91.0 and 89.0, 90.0 and 92.0 for cumin, anise and caraway respectively. Followed by both essential oils carrier at 20.0g/kg seeds which reduced the natural infection more than 76.0 % for all tested seeds. Other concentrations showed moderate effect.

Table 3: Impact of essential oils applied as Carrier contact assay on natural infection of some medicinal and aromatic seeds.

Essential oil carrier (g/kg seeds)	% Fungal natural infection					
	Cumin		Anise		Caraway	
	Infection	Efficacy %	Infection	Efficacy %	Infection	Efficacy %
			Thyme treatment			
5.0	64.0b	36.0	60.0 a	40.0	58.0 b	42.0
10.0	52.0c	48.0	49.0 b	51.0	46.0 c	54.0
15.0	34.0d	66.0	29 .0 c	71.0	32.0 d	68.0
20.0	22.0 e	78.0	19.0 d	81.0	20.0 e	80.0
25.0	12.0 f	88.0	10.0 e	90.0	9.0 f	91.0
			Rosemary treatments			
5.0	64.0 b	36.0	58.0 b	42.0	60.0 b	40.0
10.0	50.0 c	50.0	42.0c	58.0	46.0c	54.0
15.0	35.0 d	65.0	32.0d	68.0	30.0d	70.0
20.0	24.0 e	76.0	21.0 e	79.0	19.0e	81.0
25.0	11.0 f	89.0	10.0 f	90.0	8.0f	92.0
Control	100.0a	0.0	100.0 a	0.0	100.0 a	0.0

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

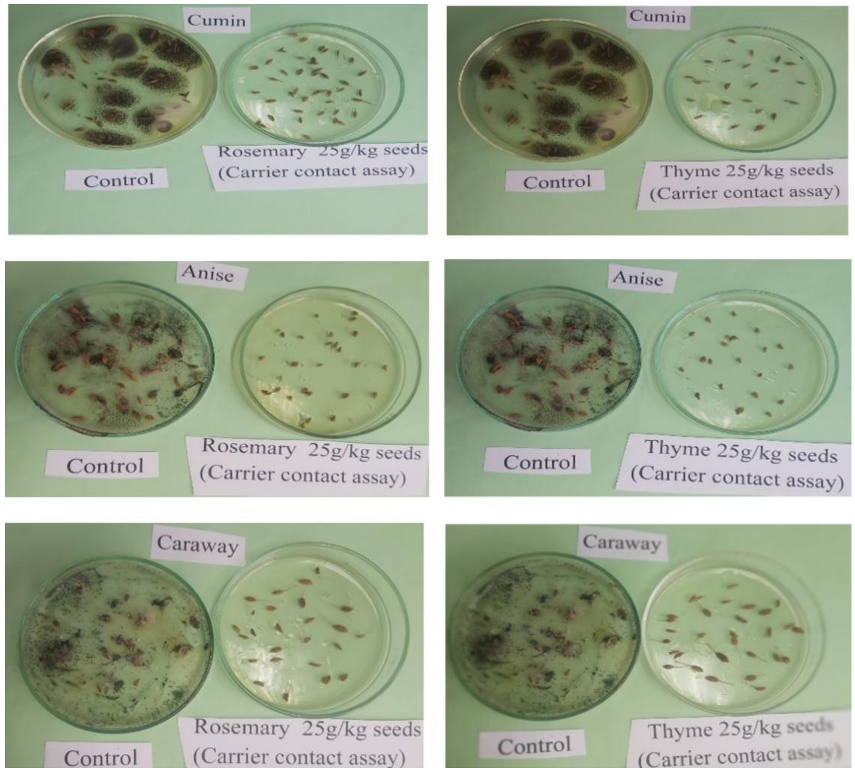

Fig. 1: Impact of essential oils applied as Carrier contact assay on natural infection of some medicinal and aromatic seeds.

Impact of essential oils treatment applied as carrier contact assay on germination percent of some medicinal and aromatic seeds

Results in Table (4) indicate that all tried concentrations of essential oil carrier decreased the germination tested seeds. The highest inhibited was achieved with Thyme and Rosemary carrier at 25.0 g /kg seeds which recorded the germination seeds as 19.0, 24.0 &25.0 and 22.0, 28.0 &30.0 % for cumin, anise and caraway respectively. Followed by both essential oils carrier at 20.0g/kg seeds which recorded germination seeds more than 28.0 % for all tested seeds. Other concentrations showed moderate effect.

Table 4: Impact of essential oils treatment applied as Carrier contact assay on germination percent of some medicinal and aromatic seeds

Essential oil Carrier (g/kg seeds)	**Germination %**		
	Cumin	**Anise**	**Caraway**
Thyme treatment			
5.0	74.0a	62.0b	60.0b
10.0	61.0c	55.c	52.0c
15.0	42.0d	44.0d	45.0d
20.0	28.0e	36.0e	34.0e
25.0	19.0f	24.0f	25.0f
Rosemary treatments			
5.0	70.0b	64.0b	61.0b
10.0	58.0c	56.0c	50.0c
15.0	44.0d	45.0d	41.0d
20.0	35.0e	38.0e	38.0e
25.0	22.0f	28.0f	30.0f
Control	100.0a	100.0 a	100.0 a

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

Protective effect of essential oils on artificial infection with *A. flavus* during 90 days of storage

Thyme and rosemary essential oils was mixed individually with wheat barn and seeds were treated with mixture at rate of 0.00, 20.0 and 25.0 g/kg seeds. The protective impact of Thyme and rosemary essential oils carrier against *A. flavus* infection was assessed after 90 days of storage. Results in Table (5) show that all tried concentrations of essential oil carrier decreased the artificial infection with *A. flavus* of tested seeds. The highest inhibited was achieved with Thyme and Rosemary carrier at 25.0 g /kg seeds which decreased the infection more than 88.0, 89.0 and 91.0 %, for cumin, anise and caraway respectively. Followed by both essential oils carrier at 20.0g/kg seeds which reduced the infection more than 76.0 % for all tested seeds. Other concentrations showed moderate effect.

Table 5: Evaluation of essential oils as protective treatment against artificial infection of *Aspergillus flavus* after 90 days of storage

Essential oil carrier (g/kg seeds)	Seeds infection with *Aspergillus flavus*					
	Cumin		Anise		Caraway	
	Infection %	Efficacy %	Infection %	Efficacy %	Infection %	Efficacy %
			Thyme treatment			
20.0	22.0b	78.0	18.0a	82.0	17.0a	83.0
25.0	12.0c	88.0	11.0b	89.0	9.0b	91.0
			Rosemary treatments			
20.0	24.0b	76.0	20.0a	80.0	18.0a	82.0
25.0	11.0c	89.0	10.0b	90.0	8.0b	92.0
Control	100.0a	0.0	100.0 a	0.0	100.0 a	0.0

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

Impact of essential oils treatment applied as carrier contact assay on germination percent of some medicinal and aromatic seeds

Results in Table (6) indicate that all tried concentrations of essential oil carrier decreased the germination tested seeds. The highest inhibited was achieved with Thyme and Rosemary carrier at 25.0 g /kg seeds which recorded the germination seeds as 40.0, 35.0.0 &36.0 and 36.0, 31.0 &32.0 % for cumin, anise and caraway respectively. Followed by both essential oils carrier at 20.0g/kg seeds recorded germination seeds more than 37 % for all tested seeds. Other concentrations showed moderate effect.

Table 6: Impact of essential oils on germination percent of some medicinal and aromatic seeds after 90 days of storage

Essential oil Carrier (g/kg seeds)	Germination seeds%		
	Cumin	Anise	Caraway
	Thyme treatments		
20.0	44.0	40.0	50.0
25.0	40.0	35.0	36.0
	Rosemary treatments		
20.0	42.0	38.0	37.0
25.0	36.0	32.0	31.0
Control	100.0	100.0	100.0

4. Discussion

The most significant medicinal and aromatic plants in Egypt and the world are caraway (*Carum carvi*), anise (*Pimpinella anisum*) and cumin (*Cuminum cyminum*). Aspergillus spp., Penicillium spp., Rhizopus spp. and Fusarium spp. are among the many fungus that attack and harm them after harvest and while they are being stored (Moharram *et al.* 1989; Regina & Roman 1992 and Said & El-Hady Goder 2014).

Essential oils (EOs) are aromatic, volatile, sweet-smelling liquid oils that are obtained from flavors and plants by hydrodistillation or supercritical liquid extraction. They are widely available in bioactive combinations that have antioxidant and antibacterial properties (Taghavi *et al.* 2018; Mari *et al.,* 2016; Prakash *et al.,* 2015 Danh *et al.,* 2021).

Results in current study revealed that *in vitro* trails, inhibited completely of mycelial growth and spore germination was achieved with Thyme and Rosemary at 0.75 % for *A. flavus* and *F. verticillioides* while at 1.0 % for *A. niger.* While in vivo, trails, thyme and rosemary essential oils was mixed individually with wheat barn and seeds were treated with mixture at rate of 0.00, 20.0 and 25.0 g/kg seeds. The protective impact of Thyme and rosemary essential oils carrier against *A. flavus* infection was assessed after 90 days of storage. Results in showed that all tried concentrations of essential oil carrier decreased the artificial infection with *A. flavus* of tested seeds. The highest inhibited was achieved with Thyme and Rosemary carrier at 25.0 g /kg seeds which decreased the infection more than 88.0, 89.0 and 91.0 %, for cumin, anise and caraway respectively. Followed by both essential oils carrier at 20.0g/kg seeds which reduced the infection more than 76.0 % for all tested seeds. Thyme and Rosemary carrier at 25.0 g /kg seeds recorded lower percent of the germination seeds for all tested seeds.

Because essential oils are generally thought to be safe, they are used practically as mould growth inhibitors (carrier contact assay). They could be used to stop fungus from growing on seeds that have been kept. Chang *et al.* (2022) reported that EOs have previously demonstrated a strong effect has previously been obtained with EOs towards seed infection. Although, the EO in present study, in the carrier contact assay showed higher inhibition against storage fungi and natural infection.

Our result showed that seeds exposed to tested essential oils as carrier contact significantly reduced the percent of germinated seeds.

As an alternative control method for seed protection, the EO carrier coating disinfects the artificially inoculated seeds and the therapy shown protective effects against bacterial infections (Yilmaz *et al.,* 2021).

Results in current study, the protective impact of Thyme and rosemary essential oils carrier against *A. flavus* infection was assessed after 90 days of storage. All tried concentrations of essential oil carrier contact decreased the artificial infection with *A. flavus* of tested seeds. The highest inhibited was achieved with Thyme and Rosemary carrier at 25.0 g /kg seeds which decreased the infection more than 88.0, 89.0 and 91.0 %, for cumin, anise and caraway respectively. In this respect, a film coating pesticide is an alternative and economically available option **(**Jacob *et al.,* 2009 and Keawkham *et al.,* 2014).

A 6% concentration of C. citratus was found to be efficient in reducing the seed-borne infection of F. moniliforme and other pathogens in sorghum seed by Somda *et al.* (2007). According to studies conducted by other researchers, essential oils can considerably slow the growth of Fusarium species pathogens (Perczak *et al.,* 2019). The development of the pathogens under study was greatly inhibited by clove essential oil.

Moreover, essential oils (EOs) have been shown to be an effective control measure for mitigating the environmental impact of fruit production (Bakkali *et al.,* 2008); however, the majority of these research were conducted under greenhouse conditions (Lopez-Reyes *et al.,* 2010 and 2013).

The way that essential oils (EOs) like aldehydes, phenols, and ketones interact with pathogens is determined by their molecular makeup. These substances efficiently inhibit the growth of pathogens. Furthermore, environmental elements rich in fungicidal chemicals, such as thymol, carvacrol, and b-anisaldehyde, have the strongest inhibitory impact against *Penicillium digitatum* (Daferera *et al.,* 2000) and *Colletotrichum gloeosporioides* (Barrera-Nacha *et al.,* 2008). According to several researchers, EO has antifungal activity in vitro against a variety of postharvest fungi, including *Aspergillus* spp. (Tang *et al.,* 2018), *Penicillium* spp. (Xing *et al.,* 2016), *Alternaria* spp. (Chen *et al.,* 2016 a, b), *Colletotrichum* spp. (Bill *et al.,* 2016), and *Botrytis cinerea* (Banani *et al.,* 2018).

Generally, direct contact with the fruit, spraying, or dipping are the methods used to verify the effectiveness of EOs (Elshafie *et al.,* 2016). Moreover, the most malleable biopolymer, chitosan exhibits antibacterial activity against a range of foodborne pathogens, sparking curiosity as a potential preservative (Ganan *et al.,* 2009).

According to Abd-El-Kareem *et al.* (2022), reported that using thyme and nerol at 1.5% as well as 8.0 g/L of chitosan completely suppressed the mycelial growth and spore germination of all fungi. They added that in order to investigate their potential protection against green and blue molds as well as sour rot diseases of Washington navel orange fruits, thyme or nerol at a concentration of 1.5% and chitosan at 8.0 g/L were administered separately or in combination. The greatest inhibition, according to the results, was achieved when thyme or nerol at 1.5% was mixed with 8.0g/L of chitosan. This combination reduced the incidence and severity of green, blue mold, and sour rot diseases by more than 88.0 and92.0%, respectively. Fruit quality was unaffected negatively by any of the studied procedures.

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