Management of strawberry gray mold disease using some essential oils and molecular identification of pathogen fungus

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Background
Evaluation of preharvest application with some essential oils (EOs) for controlling gray mold disease of strawberry plants was studied.
Objective
In vitro trails, five EOs, that is, thyme, nerol, citral, methyl anthranilate, and clove oils, were evaluated for their capability to suppress fungal growth of *Botrytis cinerea*. At the National Center for Biotechnology Information, alignment showed the percentage of identity (100%) of *B. cinerea* between our isolates and Gene bank isolate.
Materials and methods
Five EOs were evaluated for their capability to suppress fungal linear growth of *B. cinerea*. Certain volume of each oil was added to autoclaved potato dextrose agar medium flasks to obtain the proposed concentrations of 00.0, 0.25, 0.5, and 1.0% with 0.1% Tween-80. Molecular identification using the internal transcribed spacer (ITS) region of rRNA Trimmed sequences (ITS 573 bp) was conducted.
Results and conclusion
B. cinerea isolate no. 3 was identified molecular using the ITS region of rRNA Trimmed sequences (ITS 573 bp) compared with different isolates of *B. cinerea*. All tested oils significantly reduced linear growth of *B. cinerea* fungus. Complete inhibition was obtained with concentration of 1.0% with all tested EOs and at 0.5% with thyme, citral, and methyl anthranilate, whereas concentration 0.25% showed moderate effect. Moreover, in vivo trails all tested EOs treatments at concentration of 0.5% significantly reduced disease incidence under natural infection. The most effective treatments are citral, methyl anthranilate, and thyme that reduced the disease incidence by 64.3%, 67.9%, and 67.9% on average at 5°C and 75.8%, 82.3%, and77.4% on average at 20°C, respectively, whereas other treatments showed moderate effect. The same trend was observed with disease severity. Meanwhile, under artificial infection, the highest reduction was obtained with citral, methyl anthranilate, and thyme that reduced the disease incidence by 68.2%, 75.0%, and 68.0% at 5°C and 76.0%, 77.0%, and 78.0% on average at 20°C, respectively. However, other treatments showed moderate effect. The same trend was observed with disease severity. As for fruit quality, all tested treatment had no negative effect on all tested characters of fruit quality. The most effective treatments are citral, methyl anthranilate, and thyme that reduced the decay incidence by 71.0%, 71.0%, and 69.3% and weight loss percentage by 79.4%, 78.2%, and 78.8%, respectively. Also, other treatments showed moderate effect, whereas methyl anthranilate, followed by thyme and clove increased the total soluble solid by 56.9%, 41.4%, and 36.2%, respectively. As for total soluble phenol, the highest increase was obtained with all tested oils. As for, titratable acidity, there are no significant differences between all tested treatments as compared with control fruits.

Keywords:
essential oils, fruit quality, gray mold, management, strawberry

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1687-4315

Short description: Identification of *Botrytis cinerea* through morphological fungal taxonomy and molecular characterization based on their internal transcribed spacer region of ribosomal DNA. Postharvest diseases of strawberry fruit are one of most direct and indirect losses in Egypt. Chemical control (agrochemical) is inhibited to use for export fruit, because of that we are searching for active and permitted treatments control the preharvest diseases of strawberry fruits. The best treatments for controlling

gray mold are citral and methyl anthranilate. All the tested treatments did not have any negative effect on all the quality characteristics of the tested fruits, whether they were chemical or physical characteristics.

We choose the biocatalysis and agricultural biotechnology because (1) the authors published articles before and (2) published papers have higher citation and download compared with another Journal.

The goals of the paper are: (1) the results can be applied in warehouse; (2) the results showed high level against pathogen control; and (3) in Egypt, to start application the treatments on many exporter warehouses companies.

On the other hand, we hope to faster review process to be authorization report for many companies.

Introduction

Strawberry fruits contain phytochemicals that can perform different functions in the body and result in health benefits [1-3]. Gray mold is considered one of the main diseases in commercial strawberries [4] and it is caused by the necrotrophic fungus *Botrytis cinerea* Pers ex. Fr. [teleomorphic phase *Botryotinia fuckeliana* (de Bary) Whetzel] [5]. The disease can be observed in strawberries that are still in the field/greenhouse or in postharvest during storage, transport, and commercialization. In all cases, the disease can affect both qualitative and quantitative attributes [6,7].

B. cinerea initially colonizes dead or senescent tissues, which serve as a source of energy to later establish and colonize healthy tissues [4]. Favorable environmental conditions for disease occurrence are mild temperatures, high humidity, and poor ventilation; the latter usually occurs in strawberry cultivated in greenhouses [4]. Essential oils (EOs) are natural, volatile, and complex compounds known for their antimicrobial, antioxidant, and medicinal properties [8-10]. Many studies have documented the antifungal effects of plant EOs against fruit pathogens [10-12]. The potential of using EOs by spraying or dipping fruits for controlling postharvest diseases has been reported by several investigators [13,14]. The aim of this research is to evaluate preharvest application of some EOs for controlling gray mold disease of strawberry fruits under different storage conditions.

Materials and methods

Source of essential oils

Different EOs, namely, thyme, nerol, citral, methyl anthranilate, and clove, were obtained from Oils Extract Unite, National Research Center (NRC), Giza, Egypt.

The pathogen

Botrytis spp. isolate no. 3 was isolated from naturally diseased strawberry fruits and proved to be pathogenic to healthy fruits according to pathogenicity tests conducted in previous study [15]. Preliminary identification of this isolate was done using morphological characters [16]. Molecular identification using the internal transcribed spacer (ITS) region of rRNA Trimmed sequences (ITS 573 bp) [17] was conducted as follows.

DNA extraction

DNeasy® Plant Mini Kit was used to extract DNA from fungal growth carried out according to Fan *et al.* [18].

Polymerase chain reaction amplification

Botrytis cultures were identified molecularly using the conserved ribosomal ITS region [19].

Sequencing

Using the Basic Local Alignment Search Tool for Nucleotide Sequences, the ITS nucleotide sequences for isolate no. 3 were compared with those in the public domain databases National Center for Biotechnology Information (NCBI; 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 [18].

In vitro experiments

Inhibitory effect of essential oils

Five EOs, that is, thyme, nerol, citral, methyl anthranilate, and clove were evaluated for their capability to suppress fungal linear growth of *B. cinerea*. Certain volume of each oil was added to autoclaved potato dextrose agar medium flasks to obtain the proposed concentrations of 00.0%, 0.25%, 0.5%, and 1.0% with 0.1% Tween-80. Treated or not treated (control) medium were poured into five Petri plates as replicates/treatment. After the medium had solidified, Petri plates were inoculated with 6 mm discs of a 7-day-old culture of *B. cinerea* and incubated at 20

±1°C for 7 days. Linear growth of *B. cinerea* was measured control plats are completely filled. The percentage of inhibition was calculated. The inhibition percent on mycelial growth of *B. cinerea* was calculated using the formula suggested by Fokemma [20] as follows: $\text{Reduction}\% = \left[\frac{C-T}{C}X100\right]$ where C is the maximum linear growth of B. cinerea in control and T is the maximum linear growth of B. cinerea in treatment.

In vivo experiments

Effect of some essential oils as preharvest application on gray mold disease of strawberry.

Preharvest experimental design

Field experiments were conducted in the 2018/2019 and 2019/2020 growing seasons at Bahada village, El-Qanater El-Khereia Centre, Qalyubia Governorate, Egypt, where the soil is light loamy with natural infestation. So, in a randomized complete block design with three replicates (plots) for each treatment, naturally infested plots (4×8 m) were established, each comprised of four rows (32 holes/row and one seedling was sown in each hole). There were 128 strawberry transplants in each replicate. All strawberry transplants were subjected to the same fertilizer and irrigation regime during production. Irrigation, nutrition, and other agricultural practices were implemented.

Treatments and application

Five EOs, that is, thyme, nerol, citral, methyl anthranilate, and clove at concentration of 5 ml/l were evaluated to study their effect on gray mold disease of strawberry fruits. Control plants were sprayed with sterilized water. All treatments were applied as foliar sprays at two time intervals. The first spray was carried out after 70% of strawberry plants were formed their flowers. The second spray was applied 48 h before strawberry fruits harvest.

Postharvest experiments

After harvesting, strawberry fruits were transferred to NRC laboratory to study the effect of preharvest treatments on gray mold disease developed under natural and artificial infection during storage.

Under natural infection

All fruits, whether treated or untreated (control), were packaged into 8-fruit foam tray then packed in polyethylene bags and stored at 5°C for 12 days or 20°C for 7 days with a relative humidity of 90–95%.

The fruits were regularly inspected for mold and were considered infected if a visible lesion was discovered. The findings were presented as a percentage of infected fruit. Disease incidence (%) was expressed as a percentage of infected fruit, whereas disease severity was recorded as a percentage of infected fruit [21].

Under artificial infection

Strawberry fruits were stored under artificial infection at 5±1°C for 12 days or 20±1°C for 7 days and 90–95% relative humidity. Spore suspension of *B. cinerea* was obtained by flooding 10-day-old cultures sterile distilled water containing 0.1% (v/v) Tween 80. Spores were counted with a hemacytometer slid, and the pathogen's spore concentration was adjusted with sterile distilled water to achieve (10^6 spores/ml). Under field conditions, treated strawberry fruits were inoculated with a spore suspension of *B. cinerea* (10^6 spores/ml) and air dried. All treated or untreated (control) fruits were packed into carton boxes at a rate of 20 per box and stored at 5±1°C for 12 days or 20±1°C for 7 days, with a relative humidity of 90–95%. The fruits were regularly inspected for mold and were considered infected if a visible lesion was discovered. The findings were presented as a percentage of infected fruit. The disease incidence (%) was expressed as percentage of fruit infected, whereas disease severity was recorded according to Romanazzi *et al.*'s [21] empirical scale with six degrees: 0, healthy strawberry; 1, 1–20% fruit surface infected; 2, 21–40% fruit surface infected; 3, 41–60% fruit surface infected; 4, 61–80% fruit surface infected; 5, more than 81% of the strawberry surface infected and showing sporulation.

Strawberry characterization

A random sample of 20 fruit from each treatment or untreated (control) under natural infection and stored at 5±1°C for 12 days was chosen to determine the following properties.

Incidence of decay

When a visible surface lesion was observed on a strawberry, it was considered infected. The disease incidence percentage of coated and uncoated fruits was calculated by dividing the number of spreading lesions by the total number of lesions and multiplying by 100 [21].

Loss of weight

The weight loss percentage was calculated using the standard method using the weights at days 0 and 7 [22]. The experiment was carried out three times.

Total soluble solids (TSS)

At 20°C, the strawberry purees were placed on the prism glass of the refractometer (Atago Co., Tokyo,

Japan) and a direct reading as a percentage was taken [23]. Six replicates of the measurements were taken.

Total soluble phenolics (TSP)

Fruit samples (0.5 g) were extracted with 10 ml of 95% ethanol before being frozen for 48–72 h. The samples were homogenized using Tissue Tearor before being centrifuged at 10 000 rpm for 10 min. TSP content was determined at 725 nm using a spectrophotometer and 50% Folin-Ciocalteu phenol reagent, and absorbencies were converted to µg gallic acid equivalents/g fresh fruit [24,25]. Each reported value is the average of three replicate assays performed on three different samples for each replicate.

Total titratable acidity (TA)

Total TA was determined by titrating fruit juice against 0.1 N NaOH to pH 8.1 and was expressed as a percentage of citric [26].

Statistical analysis

The Tukey test was used for multiple comparisons of means [27].

Results

Identification of *Botrytis cinerea* using molecular biology

Botrytis spp. ITS genes, including the 5.8S ribosomal rRNA, were amplified and DNA sequences were determined. A new polymerase chain reaction 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% of the time.

Results indicate that the NCBI alignment showed the percentage of identity (100%) of *B. cinerea* between studied isolates and Gene bank isolate, whereas results in Figure 1 indicated that the phylogenic tree showed convergence between our isolates (yellow color) and Gene bank isolate. Our isolates are shown in separated cluster that means it had diversity.

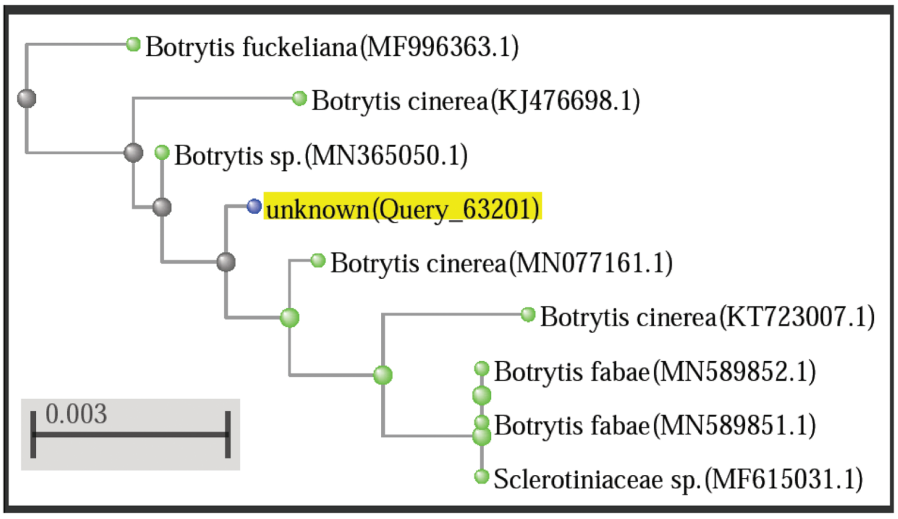
NCBI Ref. / GenBank: ON059698.1

>CGTAGGTACTGCGGAGATCATTACAGA
GTTCATGCCCGAAAGGGTAGACCTCCCA
CCCTTGTGTATTATTACTTTGTTGCTTTG
GCGAGCTGCCTCCGGGCCTTGTATGCTC
GCCAGAGAATACCAAAACTCTTTTTATT
AATGTCGTCTGAGTACTATATAATAGTTA
AAACTTTCAACAACGGATCTCTTGGTTCT
GGCATCGATGAAGAACGCAGCGAAATGC
GATAAGTAATGTGAATTGCAGAATTCAG
TGAATCATCGAATCTTTGAACGCACATTG
CGCCCCTTGGTATTCCGGGGGGCATGC
CTGTTCGAGCGTCATTTCAACCCTCAAGC
TTAGCTTGGTATTGAGTCTATGTCAGTAA
TGGCAGGCTCTAAAATCAGTGGCGGCGC
CGCTGGGTCCTGAACGTAGTAATATCTCT
CGTTACAGGTTCTCGGTGTGCTTCTGCCA
AAACCCAAATTTTTCTATGGTTGACCTCG
GATCAGGTAGGGATACCCGCTGAACTTA
AGCATATCATAAAGAGGCGGAGGA

Effect of some essential oils on linear growth of *Botrytis cinerea*, in vitro

Results in Table 1 indicate that all tested oils at different concentrations significantly reduced linear growth of *B. cinerea* fungus. Complete inhibition was obtained with concentration of 1.0% with all

Figure 1



The phylogenic tree showed convergence between our isolated shaded area and Gene bank isolate. Our isolate are shown in separated cluster that means it had diversity.

tested oils and at 0.5% with thyme, citral, and methyl anthranilate, whereas concentration of 0.25% showed moderate effect.

Effect of some essential oils as preharvest application on gray mold disease of strawberry plants under natural infection and stored at 5 or 20±1°C

The five tested EOs essential (as mentioned before) at concentration 0.5% were applied on two seasons 2018–2019 and 2019–2020 to study their efficiency of controlling gray mold disease. Results in Tables 2 and 3 indicate that all tested treatment significantly reduced disease incidence and severity. The most effective treatments are citral, methyl anthranilate, and thyme that reduced the disease incidence by 64.3%, 67.9%, and 67.9% when strawberry fruit stored at 5°C for 12 days on average and 75.8%, 82.3%, and 77.4% when strawberry fruit stored at 20°C for 7 days on average, whereas other treatments showed moderate effect. The same trend was observed with disease severity.

Table 1 Linear growth and reduction of *Botrytis cinerea* incubated at 20±1°C for 7 days in response to different concentrations of some essential oils

Tested oil	Con. (%)	*Botrytis cinerea* Linear growth (mm)	Reduction (%)
	0.25	22.3[(1)] c	75.2
Nerol	0.5	12.3 d	86.3
	1.0	0.0 e	100.0
	0.25	12.3 d	86.3
Citral	0.5	0.0 e	100.0
	1.0	0.0 e	100.0
Methyl anthranilate	0.25	27.3 b	69.6
	0.5	0.0 e	100.0
	1.0	0.0 e	100.0
	0.25	27.3 b	69.6
Thyme	0.5	0.0 e	100.0
	1.0	0.0 e	100.0
	0.25	27.3 b	69.6
Clove	0.5	12.7 d	85.9
	1.0	0.0 e	100.0
Control	90.0 a	0.0	

[1]Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.

Effect of some essential oils as preharvest application on gray mold disease of strawberry under artificial infection and stored at 5 or 20±1°C

Five EOs, that is, thyme, nerol, citral, methyl anthranilate, and clove at concentration of 0.5% were applied to study their effect on gray mold disease. Results in Tables 4 and 5 indicate that all tested treatment significantly reduced disease incidence and severity. The most effective treatments are citral, methyl anthranilate, and thyme that reduced the disease incidence by 68.2%, 75.0%, and 68.2% at 5°C and 76.0%, 77.0%, and 78.0% at 20°C, respectively, whereas other treatments showed moderate effect. The same trend was observed with disease severity.

Effect of some essential oils as preharvest application on fruit quality of strawberry fruits stored at 20±1°C for 7 days

Five EOs, that is, thyme, nerol, citral, methyl anthranilate, and clove at concentration of 0.5% were applied to study their effect on fruit quality of strawberry fruits.

Effect on decay incidence and weight loss percentage

Results in Table 6 indicate that all tested treatment had no negative effect on all tested characters of fruit quality. The most effective treatments are citral, methyl anthranilate, and thyme that reduced the decay incidence by 71.0%, 71.0%, and 69.3% and weight loss percentage by 79.4%, 78.2%, and 78.8%, respectively, whereas other treatments showed moderate effect.

Table 2 Effect of preharvest application with some essential oils on gray mold disease of strawberry fruits under natural infection and stored at 5±1°C for 12 days

	Gray mold disease Grown seasons							
	2018/2019		2019/2020		Mean			
Treatment	Disease incidence	Disease severity	Disease incidence	Disease severity	Disease incidence	Efficacy %	Disease severity	Efficacy %
Nerol	20.0[(1)] b	1.5 c	22.0 b	1.7 c	21.0 b	25.0	1.6 c	48.4
Citral	10.0 c	1.2 d	10.0 c	1.0 d	10.0 c	64.3	1.1 d	64.5
Methyl anthranilate	8.0 c	1.0 d	10.0 c	1.0 d	9.0 c	67.9	1.0 d	67.7
Thyme	10.0 c	1.2 d	8.0 c	0.8 d	9.0 c	67.9	1.0 d	67.7
Clove	22.0 b	2.4 b	20.0 b	2.6 b	21.0 b	25.0	2.5 b	19.4
Control	26.0 a	3.0 a	30.0 a	3.2 a	28.0 a	0.0	3.1 a	0.0

[1]Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.

Table 3 Effect of preharvest application of some essential oils on gray mold disease of strawberry fruits under natural infection and stored at 20±1°C for 7 days

Treatment	Gray mold disease Grown seasons 2018/2019 Disease incidence	2018/2019 Disease severity	2019/2020 Disease incidence	2019/2020 Disease severity	Mean Disease incidence	Mean Efficacy %	Mean Disease severity	Mean Efficacy %
Nerol	22.0[(1)] c	2.5 c	26.0 c	2.4 c	24.0 c	61.3	2.5 c	37.5
Citral	14.0 d	2.0 d	16.0 d	1.8 d	15.0 d	75.8	1.9 d	52.5
Methyl anthranilate	12.0 d	2.0 d	10.0 e	1.8 d	11.0 d	82.3	1.9 d	52.5
Thyme	16.0 d	2.5 c	12.0 e	2.0 d	14.0 d	77.4	2.3 d	42.5
Clove	48.0 b	2.9 b	40.0 b	3.1 b	44.0 b	29.0	3.0 b	25.0
Control	58.0 a	4.0 a	66.0 a	4.0 a	62.0 a	0.0	4.0 a	0.0

[1]Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.

Table 4 Effect of preharvest application of some essential oils on gray mold disease of strawberry fruits under artificial infection and stored at 5±1°C for 12 days

Treatment	Gray mold disease grown seasons 2018/2019 Disease incidence	2018/2019 Disease severity	2019/2020 Disease incidence	2019/2020 Disease severity	Mean Disease incidence	Mean Efficacy %	Mean Disease severity	Mean Efficacy %
Nerol	26.0[(1)] c	2.5 c	24.0 c	2.4 c	25.0 c	43.2	2.5 c	34.2
Citral	16.0 d	2.0 d	12.0 d	2.0 e	14.0 d	68.2	2.0 d	47.4
Methyl anthranilate	10.0 d	2.2 d	12.0 d	2.0 e	11.0 d	75.0	2.1 d	44.7
Thyme	14.0 d	2.5 c	14.0 d	2.2 d	14.0 d	68.2	2.4 c	36.8
Clove	32.0 b	2.9 b	34.0 b	3.2 b	33.0 b	25.0	3.1 b	18.4
Control	40.0 a	3.5 a	48.0 a	4.0 a	44.0 a	0.0	3.8 a	0.0

[1]Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.

Table 5 Effect of preharvest application of some essential oils on gray mold disease of strawberry fruits under artificial infection and stored at 20±1°C for 7 days

Treatment	Gray mold disease grown seasons 2018/2019 Disease incidence	2018/2019 Disease severity	2019/2020 Disease incidence	2019/2020 Disease severity	Mean Disease incidence	Mean Efficacy %	Mean Disease severity	Mean Efficacy %
Nerol	42.0[(1)] c	2.5 c	40.0 c	2.4 c	41.0 c	59.0	2.5 c	50.0
Citral	26.0 d	2.0 d	22.0 d	2.1 d	24.0 d	76.0	2.1 d	58.0
Methyl anthranilate	26.0 d	2.0 d	20.0 d	2.0 d	23.0 d	77.0	2.0 d	60.0
Thyme	24.0 d	2.2 d	20.0 d	2.0 d	22.0 d	78.0	2.1 d	58.0
Clove	58.0 b	3.0 b	56.0 b	3.2 b	57.0 b	43.0	3.1 b	38.0
Control	100.0 a	5.0 a	100.0 a	5.0 a	100.0 a	0.0	5.0 a	0.0

[1]Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.

Effect on total soluble solid (TSS), total soluble phenol (TSP), and titratable acidity (TA)

Results in Table 7 indicate that all tested treatment had no negative effect on all tested characters of fruit quality. The most effective treatment was methyl anthranilate, followed by thyme and clove that increased the TSS by 56.9%, 41.4%, and 36.2%, respectively. As for TSP, the highest increase was obtained with all tested oils. As for TA, there are no significant differences between all tested treatments as compared with control fruits.

Discussion

Gray mold is one of the most common diseases in commercial strawberries [4] and it is caused by the necrotrophic fungus *B. cinerea* Pers ex. Fr. [teleomorphic phase *Botryotinia fuckeliana* (de Bary) Whetzel] [5]. The disease can be seen in strawberries that are still in the field or greenhouse, as well as postharvest during storage, transport, and commercialization. In all cases, the disease can have an impact on both qualitative and quantitative

Table 6 Effect of preharvest application of some essential oils on decay incidence and weight loss % of strawberry fruit stored at 20°C for 7 days

Treatments	Strawberry fruit quality			
	Decay incidence	Efficacy %	Weight loss	Efficacy %
Nerol	18.0[(1)] b	60.0	6.2 b	63.5
Citral	13.3 c	71.0	3.5 c	79.4
Methyl anthranilate	13.3 c	71.0	3.7 c	78.2
Thyme	13.8 c	69.3	3.6 c	78.8
Clove	18.3 b	59.3	6.8 b	60.0
Control	45.0 a	0.00	17.0 a	0.0

[1]Means designated with the same letter in the same column are not significantly different at 0.05 level of probability

Table 7 Effect of preharvest treatments on total soluble solid (TSS), total soluble phenol (TSP), and titratable acidity (TA) of strawberry fruit stored at 20±1°C for 7 days

Treatment	Strawberry fruit quality					
	TSS	Increase %	TSP	Increase %	TA	Increase %
Nerol	7.0[(1)] c	20.7	68.1 a	19.5	0.8 a	14.3
Citral	7.2 c	24.1	69.0 a	21.1	0.7 a	0.0
Methyl anthranilate	9.1 a	56.9	68.2 a	19.6	0.8 a	14.3
Thyme	8.2 b	41.4	69.0 a	21.1	0.8 a	14.3
Clove	7.9 b	36.2	68.2 a	19.6	0.7 a	0.0
Control	5.8 d	0.0	57.0 b	0.0	0.7 a	0.0

[1]Means designated with the same letter in the same column are not significantly different at 0.05 level of probability.

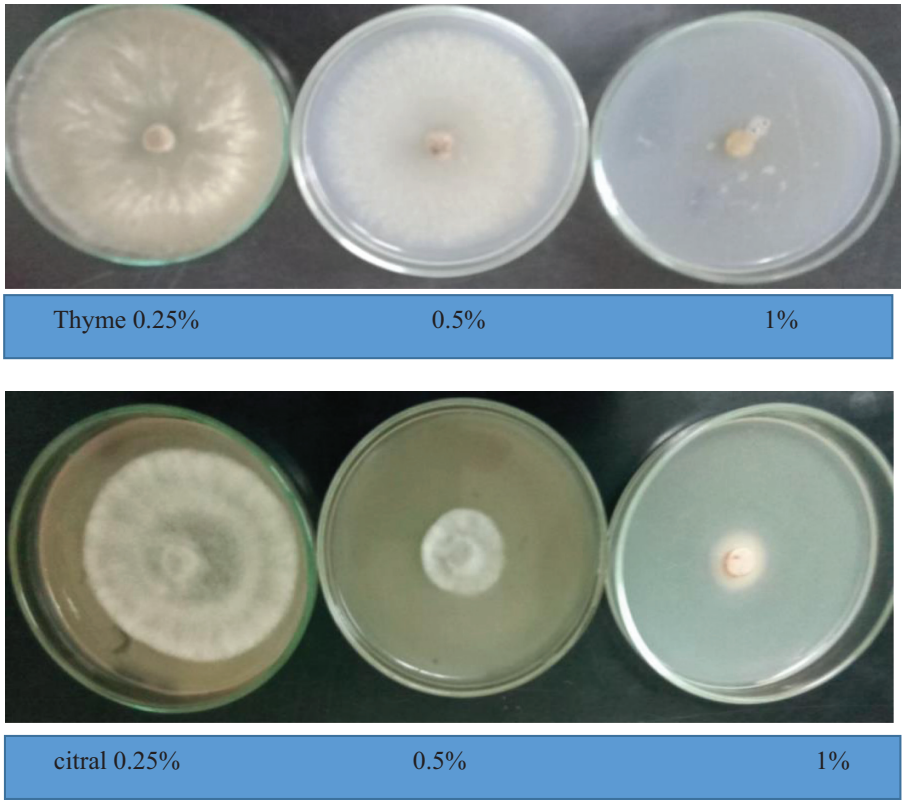
attributes [6,7]. EOs are natural, volatile, and complex compounds with antimicrobial, antioxidant, and medicinal properties [8-10].

In vitro trails, thyme, nerol, citral, methyl anthranilate, and clove were evaluated for their capability to suppress fungal growth of *B. cinerea*. All tested concentrations of all tested oils significantly reduced linear growth of the *B. cinerea* fungus. Complete inhibition was obtained with concentration of 1.0% with all tested oils and at 0.5% with thyme, citral, and methyl anthranilate, whereas concentration of 0.25% showed moderate effect. In this respect, many studies have shown that plant EOs have antifungal properties against fruit pathogens [10-12]. Mohammadi and Aminifard [28] tested in vitro conditions the antifungal properties of EOs against the fungal pathogen *B. cinerea*, the causal agent of peach grey mold disease (*Prunus persica* L.). Tao *et al.* [29] also showed that Transmission electron microscopes (TEM) and A scanning electron microscope (SEM) images of *P. italicum* treated with the highest concentration of citral revealed structurally disorganized plasma and cytoplasm with distorted and collapsed filaments.

EOs are an effective tool for reducing the environmental impact of fruit production [30]. In vitro research on the efficacy of plant EOs has been extensive [8], but a few studies have been performed in vivo [31,32]. The chemical composition of EOs determines their antifungal activity. Pathogen growth is significantly inhibited by aldehydes, phenols, and ketones in particular. Thymol, carvacrol, and p-anisaldehyde have been shown to have fungicidal activity, and EOs rich in these components had the highest inhibitory activity against many postharvest pathogens, including *Penicillium digitatum* [33], *Colletotrichum gloeosporioides* [34], and *Rhizopus stolonifer* [35]. EOs of thyme (*Thymus vulgaris*) and savory (*Satureja montana*) are largely made up of thymol and carvacrol, which are particularly effective in management fungal infections [31]. In general, the effectiveness of EOs is assessed by contacting the fruit directly, spraying, or dipping it [36]. Most studies have shown that EOs and their components can breach the cell wall and cell membrane, coagulate the cytoplasm, and therefore damage cellular organelles and escape macromolecules [30,37]. EOs can permeate the cell wall and affect the cytoplasmic membrane, disturbing different layers of polysaccharides, fatty acids, and phospholipids and finally making them permeable due to their lipophilic nature [38,39]. EOs have a significant impact on fruit quality and postharvest degradation in several fruits [14,40].

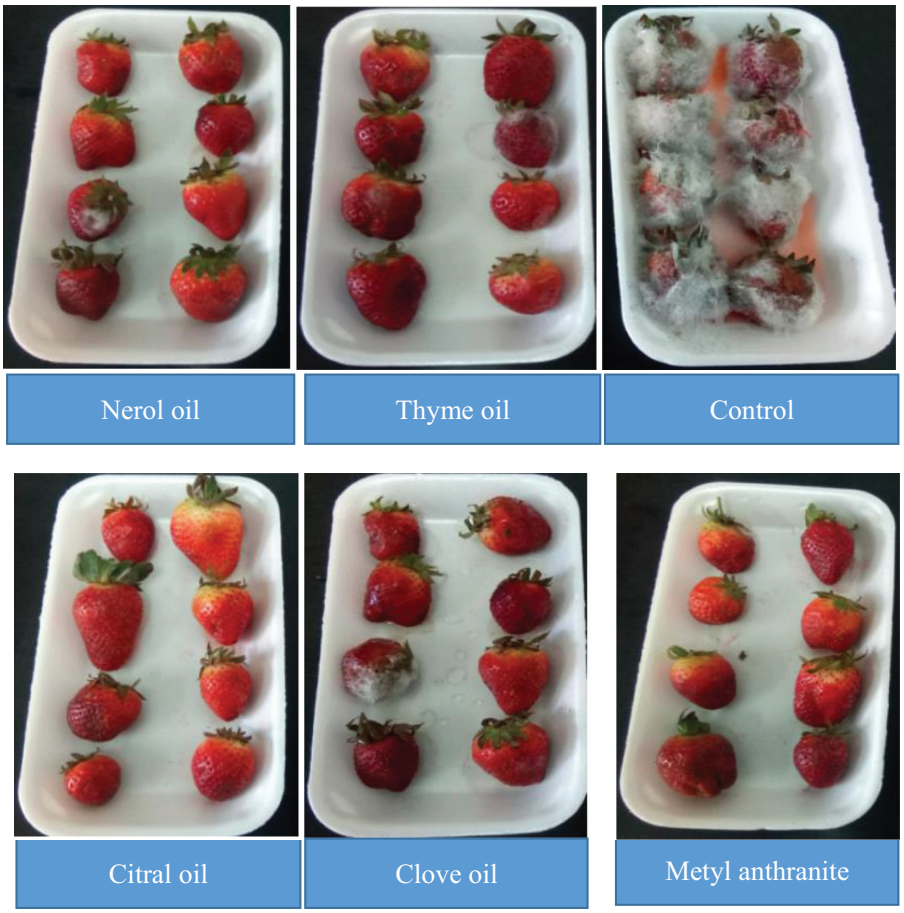
In vivo trails, five EOs, that is, thyme, nerol, citral, methyl anthranilate, and clove at concentration of 0.5% were applied to study their effect on gray mold disease. Results indicated that all tested treatment significantly reduced disease incidence and severity under natural infection. The most effective treatments are citral,

Figure 2



Effect of preharvest application with some essential oils on gray mold disease of strawberry plants under natural infection.

Figure 3



Effect of preharvest application with some essential oils on gray mold disease of strawberry plants under artificial infection.

methyl anthranilate, and thyme that reduced the disease incidence by 64.3%, 67.9%, and 67.9% at 5°C and 75.8%, 82.3%, and 77.4% at 20°C, respectively, whereas other treatments showed moderate effect. The same trend was observed with disease severity. Meanwhile, under artificial infection, the most effective treatments are citral, methyl anthranilate, and thyme that reduced the disease incidence by 68.0%, 75.0%, and 68.0% at 5°C and 76.0%, 77.0%, and 78.0% at 20°C, respectively, whereas other treatments showed moderate effect. The same trend was observed with disease severity.

However, the treatments that reduced decay incidence or severity would also be expected to delay decay onset and thereby prolong the postharvest life of the fruit in commercial handling. As expected, disease incidence and severity increased with inoculation and with storage at a warmer temperature (20±1°C). Gray mold develops more rapidly at warmer storage temperatures [41-43] particularly if the fruit have been inoculated [44]. In this study, storage at 5±1°C instead of 20±1°C delayed the onset of decay even when fruit were inoculated. In fact, the lower storage temperature delayed the development of disease by about 1 week, which is consistent with the report of Sommer *et al.* [41] who reported that after 7 days at 5 ±1°C, fruit wound-inoculated with 103 spores per wound (the same inoculum concentration used in this study) had a trace of rot development, whereas large lesions were found on inoculated fruit held at 20 ±1°C for 7 days.

As for fruit quality, all tested treatment had no negative effect on all tested characters of fruit quality. The most effective treatments are citral, methyl anthranilate, and thyme that reduced the decay incidence by 71.0%, 71.0%, and 69.3% and weight loss percentage by 79.4%, 78.2%, and 78.8%, respectively, whereas other treatments showed moderate effect. Methyl anthranilate, followed by thyme and clove increased the TSS by 56.9%, 41.4%, and 36.2%, respectively. As for TSP, the highest increase was obtained with all tested oils. As for TA, there are no significant differences between all tested treatments as compared with control fruits. Several studies have looked into the possibility of spraying or dipping fruits in EOs to control postharvest diseases [13,14] (Figs. 2–4).

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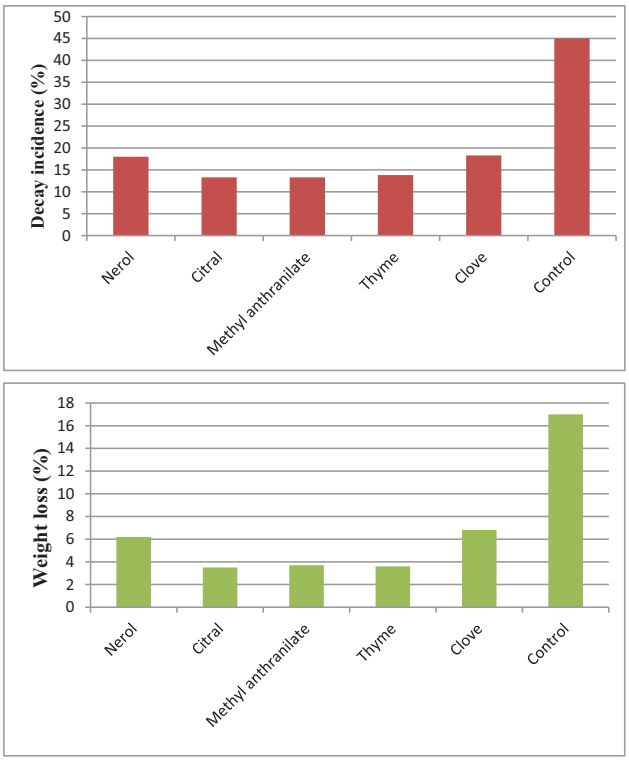
Figure 4



Effect of some essential oils as pretreatment on percentage of decay incidence (A) and percentage of weight loss of strawberry fruit (B) with gray mold disease after stored at 20°C for 7 days.

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Conflicts of interest

There are no conflicts of interest.

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