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EFFECACY OF SOME NANO-EMULSSIONS OF ESSENTIAL OILS TO CONTROL STRAWBERRY GREY MOLD POST HARVEST DISEASE

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ABSTRACT

Nano-emulsions of essential oils (NEOs) are a promising application in controlling plant pathogens, it also increases the antimicrobial properties. In this research, nano-emulsion essential oils of Clove, Rose and Bergamot were prepared and tested as potential antifungal agent at various concentrations of active ingredient against the growth of *Botrytis cinerea* isolate (BC4) and to control strawberry grey mold as post-harvest disease. Results indicated that Clove nano-emulsion was the most effective to completely inhibited the growth and sporulation of *B. cinerea* isolate, (BC4) at even the low concentration of 80 µll^{-1} for the growth and at concentration of at 50 µll^{-1} for sporulation tested fungus. Clove and Rose NEOs at both concentrations (100 µll^{-1}and 80 µll^{-}1) completely inhibited grey mold disease incidence at 7 days after fridge storage, However, Bergamot NEO consistently showed lower effect. In general, treatments with the tested nano-essential oils (NEOs) significantly maintained physical and chemical characteristics, *i.e.*, moisture content, weight loss, titrable acidity and ascorbic acid content of the treated strawberry fruits which inoculated with *B. cinerea* isolate (BC4), at the three periods of the fridge storage compared to the only inoculated fruits with fungus isolate as the control. Regarding the moisture

content, Clove, Rose and Bergamot NEOs treatments maintained fruit moisture content and also, for the ascorbic acid (AS) content, the three tested NEOs significantly maintained AS content in the inoculated treated strawberry. Meanwhile, Clove NEO showed the highest overall mean AS value being 0.64 mg/ml. based on the obtained results the use of nano-emulsion essential oils of Clove, Rose and Bergamot could be a good and environmentally safe alternative of fungicides in controlling post-harvest grey mold disease of strawberry.

Keywords: Strawberry, Grey mold, *B. cinerea,* Nano emulsion essential oils, Post harvest diseases

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INTRODUCTION

Strawberries belong to the family Rosaceae and constitute a large part of the fresh fruits worldwide. Egypt is the fourth largest producer in the world, and cultivation area of strawberry is around 20 thousand feddan in 2019 (**Shehata *et al.,* 2020**). Strawberry fruits have a limited shelf lifespan due to the highwater content, high respiration rate, and physical characteristics as well as the susceptibility to fungal rots, particularly the grey mold caused by *Botrytis cinerea*.

Grey mold or the Botrytis fruit rot, is a significant post-harvest disease of the strawberry that affect fruits at storage, long-range transport and marketing period and the most serious fruit rot of strawberry in Egypt and worldwide (**El-Korany and Mohamed, 2008; Petrasch *et al.*, 2019; Lemos Junior *et al.,* 2020**). For this reason, the use of chemical fungicides to reduce the damage caused by fungal infection after harvest is crucial to preserve the quality of strawberry fruits during storage (**Ziedan and Farrag, 2008; Bautista-Banos *et al.,* 2003**).

However, the use of chemical fungicides to control strawberry post-harvest diseases have a harmful impact on both human health and environment (**Salvia-Trujillo *et al.,* 2015**). Recently, due to the special attention to human health and the environment, efforts to find substitutes to chemical fungicides have become much more progressed. Using essential oils obtained from plants as natural antimicrobial compounds as an alternative to synthetic fungicides has been extensively reviewed in recent years (**Bhargava *et al.,* 2018**). However, the application of essential oils is usually limited regarding their minimum water solubility, volatility, physical, chemical instability and their strong taste and smell that affect the sensory properties of treated fruits (**Shang *et al.,* 2018; Hasan *et al.,* 2020; Hasheminejad and Khodaiyan, 2020**). An alternative application technique to avoid these adverse effects of using essential oils is using the nanoemulsion formulation that enhance the stability of these volatile compounds and increases antimicrobial properties by improving cellular absorption (**Hasheminejad and Khodaiyan, 2020**). Therefore, the present work was conducted to test the efficacy of Clove, Rose, and Bergamot nanoemulsions essential oils formulation against *B. cinerea* hyphal growth, sporulation *in vitro* and their inhibitory potential on grey mold disease incidence during storage period.

MATERIALS AND METHODS

Pathogenic fungus

A good growing, highly virulent isolate of the strawberry grey mold *B. cinerea* (BC4) was obtained from fungal collections of Plant Pathology Department, Faculty of Agriculture, Damanhur University. This isolate was isolated from Strawberry plants which showed grey mold symptoms in El-Behiera governorate and the identification was done at the Department of Mycology and Plant Diseases Survey, Plant Pathology Research Institute, ARC, Giza.

Preparation of essential oils nano-emulsion

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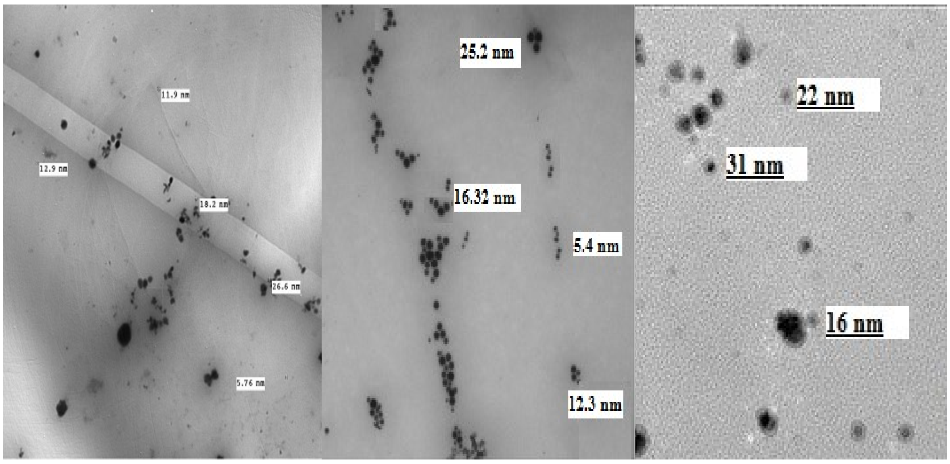
Three essential oils, namely Rose, Clove and Bergamot, were tested as formulations for their efficacy against *B. cinerea in vitro* and to control grey mold of strawberry under fridge storage conditions. All tested crude essential oils (100%) purity were of NOW Foods company, Canada.

Nano-emulsions of essential oils (Rose, Clove and Bergamot) were prepared according to **Shafiq and Shakeel (2010)**. The coarse-emulsion was then subjected to ultrasonic emulsification using a 20 kHz Sonicator (Ultrasonics, USA), with a power output of 750 W, dipped into essential oil coarse- emulsion. Sonicator probe generates disruptive forces that reduce the droplet diameter converting coarse emulsion to nano-emulsion. The morphological features and size particles of the nano-emulsion were investigated by using the Transmission Electron Microscope (TEM, EM Sciences, Hatfield, PA, USA) at Faculty of Science, Alexandria University (Figure 1). The formulated Nano emulsion stability was also investigated according to **Ghosh *et al.,* (2018)** and the particle size of nano-emulsion was determined to be 12± 1 nm 14.8 ±0.8 nm and 22 ± 0.95 nm for Clove, Rose and Bergamot essential oils, respectively.



Clove NEO **Rose NEO** **Bergamot NEO**

Fig.1: The morphological features and particle size of the tested Clove, Rose, and Bergamot nano-emulsion essential oils (NEOs) under the Transmission Electron Microscope.

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***In vitro* effects of nanoemulsion essential oils on mycelial radial growth and sporulation of *B. cinerea* isolate (BC4)**

Rose, Clove and Bergamot nanoemulsion essential oils (NEOs) tested were applied at concentrations of 20, 50, 80 and100 µl/l (oil-in-water nano-emulsions). The nano-emulsion essential oils were tested for their inhibition efficacy against isolate of *B. cinerea*. The tested concentrations of the nanoemulsion essential oils mentioned were added directly to a molten Potato dextrose agar (PDA) medium before pouring the plates. These plates were inoculated with *B. cinerea* isolate using 5mm-diameter fungal discs taken from active margins of 7-day-old cultures. Four replicates were used for each tested concentration. Inoculated plates were incubated at 20±2 ºC. Sterilized distilled water was applied in control treatment. When mycelial growth covered the entire surface in control plates, *B. cinerea* growth (mycelial radial growth) was measured and inhibition percentage was calculated using the formula described by **Pandey *et al.*, (1982)** as follows:

Where: $$I = \frac{C - T}{C} \times 100$$

I= Inhibition of fungal growth.
C= Colony diameter of control.
T= Colony diameter of treatment.

After determining mycelial radial growth and inhibition percentage of the pathogen, conidia spores were collected by washing the plates with 20 ml/ treatment. Spores were then counted by a hemocytometer and spore concentration per milliliter was determined according to the following formula of **Kamaruzzaman *et al.*, (2016)**:

Spore conc. = Total spores count in four squares x 2500x dilution factor.

In order to reveal the effect of the tested nano-emulsions on hyphal morphology, plates with the 100 µl/l were investigated using the Scanning Electron Microscope (SEM) technique at Faculty of Science, Alexandria University. The obtained images for both the treated and the untreated mycelia were investigated.

Effect of nano-emulsion essential oils on grey mold disease incidence under fridge storage

Inoculum of the *B. cinerea* isolate was prepared by harvesting spores from plates of two-week-old culture on PDA medium. Conidia spore suspension was collected by washing culture plate with 20 ml sterile distilled water, mixed with 1%, v/v tween-80 and filtered through three layers of cheese cloth. Spores were then counted by a hemocytometer and spore concentration per milliliter was adjusted to 10^4 spore/ml.

The tested strawberry fruits of cv. Florida were obtained from EI- Tahreer city. Fruits were fresh, mature, and of healthy appearance. Fruits were washed with distilled water and surface sterilized by 70% ethyl alcohol for 2 minutes. Sterilized fruits were left to dry for one hour, and then singly treated by dipping for one minute in the most effective concentrations, (80 µl/l and100 µl/l) of the tested nanoemulsion essential oils. Spore suspensions at the rate of 10^4 spore/ml in sterilized distilled water were applied by spraying strawberry fruits surface previously dipped in the tested nano-emulsion essential oils. Inoculated fruits were then placed in sterilized plastic box and kept incubated at fridge temperature of 5°C. Grey mold disease incidence (Percentages of infection) was calculated at 7, 14, and 21 days after treatment. Four replicates were used for each treatment of each of four fruits. Control treatment was sprayed with sterilized distilled water only. The percentage of infection was calculated as follows:

$$\text{Infection (\%)} = \frac{Number\ of\ rotted\ fruits}{Total\ number\ of\ inoculated\ fruits}$$

Effect of nano-emulsion essential oils on physical characteristics of strawberry fruits.

Measurement of moisture content

Moisture content was determined according to **Mrad *et al.,* (2012)** by drying fruit samples in the oven for 5 hours at temperature of 105ºC. Mass weight was determined before and after drying. The moisture content percentage was determined from the difference in weight before and after drying.

Measurement of weight loss:

Weight loss was determined according to **Ali *et al.* (2011)** as follows:

Weight of individual fruits was recorded at the beginning before treatments as reference weight as well as after treatments and storage and weight loss (%) was calculated by using following formula (**Ali *et al.,* 2011**):

Weight loss (%) = initial weight – final weight / initial weight × 100.

Effect of nano-emulsion essential oils on chemical characteristics of strawberry fruits.

Determination of titrable acidity (TA)

Titrable acidity was determined using titration method of **Geransayeh *et al.,* (2015)**. Fruits of each replicate were blended and strained to get clear fresh fruit juice. A 5 ml fruit juice was added to 25 ml distilled water plus two drops of phenolphthalein and titrated with 0.1N NaOH up to pH 8.1. The results were converted to percent citric acid and expressed as g/100 ml fresh juice.

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Determination of Ascorbic acid (Vitamin C)

Ascorbic acid concentration was determined according to **Todeschini *et al.* (2018)**. Fruits of each replicate were homogenized using tissue homogenizer. Then a 5 g fruit homogenate was diluted with 50ml of deionized water. The solution was shaken at 200 rpm for 10 min, filtered with filter paper and decolorized twice with polyvinyl pyrrolidone (PVP-0.1 g per 10 ml) and pH was adjusted between 3.5 and 4.0.The Ascorbic acid content was measured at 578 nm using resolution range spectrophotometer and expressed as mg/g fresh weight.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) and the least significant difference test to estimate statistical differences between means at p = 0.05 using the SAS program version 9.2 (**SAS, 2009**).

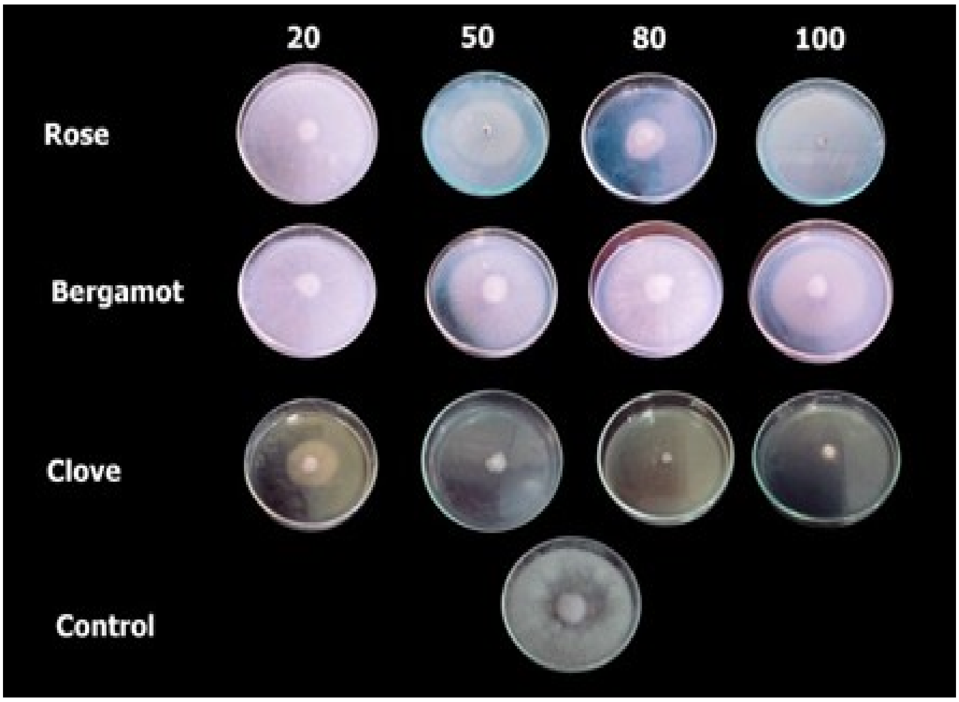
EXPERIMENTAL RESULTS

***In vitro* effects of nano-emulsion essential oils on mycelial radial growth and sporulation of *B. cinerea* isolate (BC4)**

It was obvious from Fig. (2) That all the three tested nano-emulsion of essential oils (NEOs) significantly decreased both growth (colony diameter) and sporulation of *B. cinerea* isolate (BC4) compared with the control treatment. However, Clove nano-emulsion was the most effective and completely (100%) inhibited the growth and sporulation of *B. cinerea* isolate at even the low concentration of 80 µl/l for colony diameter inhibition and at concentration of 50 µl/l for sporulation inhibition. Also, Rose NEO showed complete colony growth inhibition at the highest concentration of 100 µl/l with (100%) sporulation inhibition at 80 µl/l while Bergamot showed 30% the growth inhibition at the highest tested concentration (100µl/l) and 72.5% sporulation inhibition at the same concentration Table (1).

Effect of nano-emulsion essential oils on hyphal morphology of *B. cinerea*

In order to reveal the effect of the tested NEOs on hyphal morphology of *B. cinerea*, mycelia in the above treated *in vitro* plates (at the 100 µl/l) were investigated using the Scanning Electron Microscope (SEM). The NEOs caused severe hyphal distortion, lyses and unusual bulges on the surface of fungal hyphae, while, the control



showed typical net structure and smooth surface (Fig.3).

Fig. 2: Inhibition the growth of *Botrytis cinerea* fungus on potato dextrose agar medium with application of Cloe, Rose and Bergamot nano-emulsion essential oils, *in vitro*.

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Table 1: Inhibition (%) of the growth and sporulation of *B. cinerea* (BC4) with application of Clove, Rose, and Bergamot nano-emulsion essential oils, at different concentrations, *in vitro*.

Conc. µl/l	Colony diameter inhibition (%)				Sporulation inhibition (%)			
	Clove	**Rose**	**Bergamot**	**Mean**	**Clove**	**Rose**	**Bergamot**	**Mean**
20	69.57	0	0	23.19^{D}	64.67	45.33	12.33	40.78^{D}
50	97.33	62.43	11.2	56.99^{C}	100	57	34	63.67^{C}
80	100	95.0	23.07	72.69^{B}	100	100	44.83	81.61^{B}
100	100	100	30.80	76.93^{A}	100	100	72.50	90.83^{A}
Mean	**73.38^{A}**	**51.49^{B}**	**13.01^{C}**		**91.17^{A}**	**75.58^{B}**	**40.92^{C}**	

Means followed by different letter(s) for each single parameter are significantly different at p= 0.05.

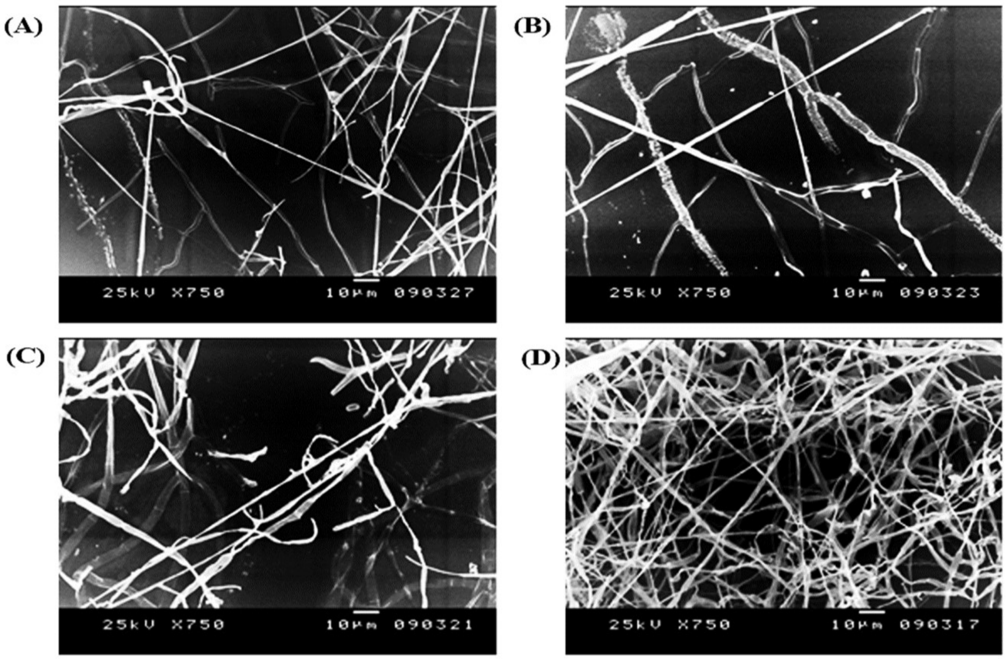

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Fig. 3: The deformation effect, under the Scanning Electron Microscope (SEM), of (A): Clove, (B): Rose, and (C): Bergamot nano-emulsion essential oils on hyphal morphology of *B. cinerea* (BC4) where (D): *B. cinerea* (BC4) as control.

Effect of nano-emulsion essential oils on grey mold disease incidence under fridge storage

Data in Table (2) showed that all tested nano-emulsion essential oils (NEOs) significantly decreased severity of grey mold disease on the treated fruits compared to the untreated inoculated control over the three intervals of the fridge storage. Also, at 7 days after fridge storage, Clove NEO at both concentrations (100 µl/land 80 µl/l) completely inhibited grey mold disease and showed zero as percentage of infection. This effect was not significantly different with Rose NEO suppression at both NEO concentrations or Bergamot NEO at the higher concentration of 100 µl/l. However, at 14 and 21 days of the fridge storage, both Clove and Rose NEOs at the high concentration of 100 µl/l were the most effective to suppress grey mold disease incidence. Besides, Bergamot NEO consistently showed lower effect but exhibited overall mean infection of 5.91% over the three periods of storage which was not significantly different from Rose NEO.

Table 2: Grey mold disease incidence (percentage of infection, %) on strawberry fruits (cv. Florida) inoculated with *B. cinerea* isolate (BC4), treated with Clove, Rose, and Bergamot nano-emulsion essential oils, stored at 5°C for 7, 14 and 21 days under fridge conditions.

Days Post inoculation	**Inoculated Untreated Control**	**Clove (µl/l)**		**Rose (µl/l)**		**Bergamot (µl/l)**	
		80	**100**	**80**	**100**	**80**	**100**
7	15.7^{a}	0.5^{c}	0.0^{c}	0.6^{c}	0.5^{c}	2.5^{b}	1.5^{bc}
14	18.0^{a}	6.3^{bc}	1.7^{e}	7.3^{bc}	3.6^{de}	9.0^{b}	5.0^{cd}
21	26.9^{a}	7.6^{c}	4.2^{e}	8.5^{bc}	5.2^{de}	10.4^{b}	7.1^{c}
Mean	20^{A}	4.8^{C}	1.9^{E}	5.5^{C}	3.1^{DE}	7.3^{B}	4.5^{CD}
Overall Mean	20^{A}	3.39^{C}		4.28^{BC}		5.91^{B}	

Values followed by different letter(s) for each single parameter are significantly different at p= 0.05.

Effect of nano-emulsion essential oils on physical characteristics of strawberry fruits inoculated with *B. cenerea*.

Data in Table (3) showed that strawberry fruits treatments with the tested nano-essential oils (NEOs) significantly maintained physical characteristics, *i.e.* moisture content, and weight loss of the treated fruits with *B.cinerea*, at the three periods of the fridge storage compared to the untreated inoculated fruits and this effect increased with increasing concentration.

Regarding the moisture content, the different treatments with the tested three NEOs maintained fruit moisture content over the three periods of storage and exhibited mean moisture contents of 82.8%, 76.9%, and 71.8% with Clove, Rose, and Bergamot NEOs treatments, respectively, compared with 63.6% for the untreated inoculated control. Meanwhile, at the 7th days of storage, there was no significant difference between Clove and Rose NEOs at both tested concentrations (80µl/l & 100µl/l) and also were not significantly different from the healthy untreated control. However, after 14 and 21 of storage, Clove treatments showed significantly higher effect than Rose while Bergamot treatments exhibited the lowest effect over the three periods of storage (Table 3).

Concerning the weight loss (%), data shown in Table (3), exhibited that fruit treatments with the three tested NEOs, and previously inoculated with *B. cinerea*, and significantly controlled fruit weight loss (%) in the inoculated fruits. However, Clove NEO consistently controlled weight loss with overall mean as low as 12.5% over the three intervals of the fridge storage which was not significantly different from 8.7% for the healthy untreated control. This was followed by Rose and Bergamot NEOs with overall means being 21.7% and 24.9%, respectively, which still significantly lower than weight loss (%) in the untreated inoculated control which exhibited 60.6 % mean weight loss (Table 3).

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Table 3: Effect of Clove, Rose, and Bergamot nano-emulsion essential oils at two concentrations on physical characteristics of strawberry fruits (cv. Florida) inoculated with *B. cinerea* (isolate, *B.c.*4), treated and stored at 5° C for7, 14 and 21 days under fridge conditions.

Days Post inoculation	**Healthy untreated control**	**Inoculated untreated control**	**Clove (µl/l)**		**Rose (µl/l)**		**Bergamot (µl/l)**	
			80	**100**	**80**	**100**	**80**	**100**
Moisture Content (%)								
7	92.6^{a}	72.0^{c}	89.6^{a}	90.1^{a}	89.1^{a}	89.5^{a}	79.6^{b}	81.5^{b}
14	89.2^{a}	68.0^{c}	86.2^{a}	87.6^{a}	75.3^{b}	76.5^{b}	73.0^{b}	74.1^{b}
21	83.6^{a}	51.0^{f}	71.4^{bc}	72.1^{b}	64.3^{de}	66.5^{cd}	60.1^{e}	62.2^{de}
Mean	88.5^{A}	63.6^{E}	82.4^{AB}	83.3^{AB}	76.2^{C}	77.5^{BC}	70.9^{D}	72.6^{CD}
Overall mean	88.5^{A}	63.6^{D}	82.8^{AB}		76.9^{BC}		71.8^{C}	
Weight Loss (%)								
7	5.0e	24.1^{a}	6.3^{d}	0.0^{e}	10.3^{bc}	8.4^{cd}	12.0^{b}	11.3^{b}
14	6.2e	61.8^{a}	11.4^{d}	10.4^{d}	21.8^{c}	21.5^{c}	26.0^{b}	24.2^{bc}
21	15.0e	95.8^{a}	24.5^{e}	22.7^{e}	35.8^{cd}	32.2^{d}	39.4^{b}	36.5^{bc}
Mean	8.7^{C}	60.6^{A}	14.0^{C}	11.0^{C}	22.6^{B}	20.7^{B}	25.8^{B}	24.0^{B}
Overall mean	8.7^{C}	60.6^{A}	12.5^{C}		21.7^{B}		24.9^{B}	

Values in each single period of storage, or in each single parameter, followed by different letter(s) are significantly different at p= 0.05.

Effect of nano-emulsion essential oils on chemical characteristics of strawberry fruits inoculated with *B. cinerea*

It is evident from data in Table (4) that treatments with the three tested nano-emulsions (NEOs) significantly improved chemical characteristics of the strawberry fruits, inoculated with *B. cinerea*, at the different three periods of the fridge storage compared to the untreated inoculated fruits.

Concerning titrable acidity (TA), Treatments with Clove NEO were the most effective and showed overall mean of 0.63g/100ml fresh juice which was even higher than TA in the healthy un-inoculated control which exhibited overall mean being 0.58g/100ml fresh juice and followed by Rose NEO which exhibited 0.56g/100ml that was not significantly different from the healthy un-inoculated fruits. Bergamot EO treatments, however, showed the lowest effect with overall TA being 0.50 g/100 ml which was lower than TA overall mean value of the healthy control but still significantly higher than TA value of the inoculated untreated control fruits which exhibited 0.42 g/100 ml. Meanwhile, no significant differences were revealed for Clove NEO between the two tested concentrations (800 and 1000 µl/l) over the three periods of storage (Table 4).

Table 4: Effect of Clove, Rose, and Bergamot nano-emulsion essential oils at two concentrations on chemical characteristics of strawberry fruits (cv. Florida) inoculated with *B. cinerea* (isolate, BC4), treated, and stored at 5°C for7, 14 and 21 days under fridge conditions.

Days Post inoculation	**Healthy Untreated Control**	**Inoculated Untreated Control**	**Clove E.O (µl/l)**		**Rose E.O (µl/l)**		**Bergamot E.O (µl/l)**	
			80	**100**	**80**	**100**	**80**	**100**
Titrable acidity (g/100ml Fresh juice)								
7	0.69^{b}	0.53^{d}	0.73^{a}	0.73^{a}	0.68^{b}	0.68^{b}	0.60^{d}	0.64^{c}
14	0.56^{c}	0.40^{d}	0.69^{a}	0.71^{a}	0.56^{c}	0.62^{b}	0.52^{c}	0.56^{c}
21	0.49^{a}	0.33^{d}	0.46^{ab}	0.47^{ab}	0.42^{bc}	0.45^{ab}	0.33^{d}	0.39^{c}
Mean	9.4^{A}	0.42^{D}	0.62^{A}	0.64^{A}	0.55^{B}	0.58^{B}	0.48^{D}	0.53^{C}
Overall mean	0.58^{B}	0.42^{D}	0.63^{A}		0.56^{B}		0.50^{C}	
Ascorbic acid (mg/ml Fresh juice)								
7	0.52c	0.08^{f}	0.70^{a}	0.73^{a}	0.57^{b}	0.60^{b}	0.43^{d}	0.48^{e}
14	0.43c	0.05^{e}	0.65^{a}	0.69^{a}	0.52^{b}	0.55^{b}	0.35^{d}	0.40^{cd}
21	0.36c	0.02^{e}	0.52^{a}	0.56^{a}	0.42^{b}	0.45^{b}	0.25^{d}	0.33^{c}
Mean	0.43^{C}	0.05^{E}	0.63^{A}	0.66^{A}	0.51^{B}	0.53^{B}	0.35^{D}	0.40^{CD}
Overall mean	0.43^{C}	0.05^{E}	0.64^{A}		0.52^{B}		0.37^{D}	

Values in each single period of storage, or in each single parameter, followed by different letter(s) are significantly different at p= 0.05.

Concerning the ascorbic acid (AS) content, data shown in Table (4), exhibited that fruit treatments with the three tested NEOs significantly maintained AS content in the inoculated treated strawberry over the three intervals of the fridge storage compared to the untreated inoculated control fruits. Meanwhile, Clove NEO showed the highest overall mean AS value being 0.64 mg/ml which followed by Rose NEO with 0.52 mg/ml. Bergamot NEO, however, showed the lowest AS content being 0.37 mg/ml which was significantly lower than SA overall mean value (0.43 mg/ml) in the healthy untreated control but still significantly higher SA mean value in the inoculated untreated control which exhibited 0.05 mg/ml (Table 4).

DISCUSSIONS

El-Beheira Governorate is a leading governorate for strawberry production and exportation (**Abd-Elgawad, 2019**). However, strawberry fruits are characterized by their thin and tinder skin, which is easily broken and makes fruits vulnerable to spoilage and fungal infection. Various environmental and human health concerns over the use of fungicides led to the search for safer alternative methods to control *B. cinerea,* incitant of the grey mold of strawberry, especially during storage. Use of resistant cultivars is considered a reliable way in this concern (**Petrasch *et al.,* 2019**). In the present study supported cv. Festival was the most tolerant strawberry tested cultivar to the infection with *B. cinerea* particularly 7 days and 14 after storage at 5^{o} C as exhibited the lowest percentage of infection being 15.15%, 31.46%, respectively. This is compared to 21.04%, 43.34% for cv. Fortuna and 29.25%, 53.01% for cv. Florida, for the previous two storage periods, respectively. These results supported results obtained by other investigators (**Mertely and Peres, 2006; Feliziani and Romanazz, 2016; Petrasch *et al.,* 2019**). The integration of the use of such tolerant strawberry cultivars with other ecofriendly control measures could be an effective safer way for post harvest control of the grey mold of strawberry.

The present study, showed that nano-emulsions of tested essential oils (nano-essential oils, NEOs) were effective, All tested NEOs significantly decreased the growth of *B. cinerea* (isolate, BC4) grown on amended PDA and decreased the *in vitro* sporulation. Clove NEO was most effective and completely (100%) inhibited the growth of the *B. cinerea* isolate at even the low concentration of 80 µl/l and totally (100%) inhibited sporulation at even the low concentration of 50 µl/l, *in vitro*. Meanwhile, under fridge storage, all tested NEOs significantly decreased grey mold disease incidence. Clove NEO completely (100%) inhibited grey mold disease incidence with concentration as low as 100 µl/l, seven days after storage. Also, at 14 and 21 days after fridge storage at 5° C, grey mold disease incidence with Clove NEO at100 µl/l was as low as 1.7%, 4.2%. It was also notable that Clove NEO suppression effects, at 100 µl/l, were not significantly different from Rose NEO, at 100 µl/l, over the three periods of the fridge storage. Bergamot NEO, however, consistently showed lower effects. These findings are in harmony with **Aoudou *et al.,* (2010), Pedro *et al.,* (2013), Anwar *et al*., (2014), Aguilar-Gonzalez *et al.,* (2015), and Robledo *et al.,* (2018)**.

The obtained effect of the tested essential oils (EOs) against *B. cinerea* and grey mold of strawberry can be explained in view that several EOs have antifungal activities **(Bouchra *et al.,* 2003; Daferera *et al*., 2003; Plotto *et al*., 2003; Simic *et al*., 2004; Tzortzakis and Economakis, 2007; El-Tahan, 2017; El-Naqa, 2020**). Also, the nano-formulations of the different materials even were more effective at much lower concentrations to control plant diseases due to their capability of improving the solubility, stability and penetration (**Pedro *et al.,* 2013; Worrall *et al*., 2018; Amjadi *et al*., 2019; Shang *et al*., 2018**). Nanomaterials are of quick dissolution, improved penetration through membranes lower doses and lower dose-dependent toxicity (**Bhushan, 2004; Rai *et al*., 2015**).

Meanwhile, efficacy of the tested NEOs could be explained in view that the present study revealed that treatments with the tested NEOs significantly maintained and controlled physical characteristics of the treated strawberry fruits, *i.e.* moisture content, weight loss, and also the chemical characteristics, *i.e.* titrable acidity and ascorbic acid content, of strawberry fruits inoculated with *B.cinerea* (isolate, BC4) at the different three periods of the fridge storage compared to the inoculated untreated fruit control. This could make strawberry fruits more tolerant to fungal infection. Also, the present study showed that the Scanning Electron Microscopy (SEM) investigation revealed that the tested NEOs caused severe hyphal distortion, lyese and unusual bulges on surface of *B. cinerea* fungal hyphae, *in vitro*, while the untreated control showed typical net structure and smooth surface of mycelia. These findings are in agreement with **El-Tahan (2017) and El-Naka (2020)**.

Regarding to the results of this research, the use of nano-emulsion essential oils of Clove, Rose and Bergamot could be a good and environmentally safe alternative of fungicides in controlling post-harvest grey mold disease of strawberry, enhanced control activity and management of strawberry quality by nano-emulsions proposed that such a formulation is too promising to use it commercially as an alternative for chemical fungicides in order to reduce the postharvest grey mold for strawberry.

CONCLUSION

Application of nano-emulsion essential oils (NEOs) is a promising application in controlling plant pathogens. Findings of this research indicated that nano-emulsion essential oils of Clove, Rose and Bergamot had good potential as antifungal agents at various concentrations of active ingredient against the growth of *Botrytis cinerea* isolate (BC4) and to control strawberry grey mold as post-harvest disease. Also, treatments with the above-mentioned nano-essential oils (NEOs) significantly maintained physical and chemical characteristics, i.e., moisture content, weight loss, titrable acidity and ascorbic acid content of the treated strawberry fruits which inoculated with *B. cinerea* isolate (BC4), under fridge storage. In conclusion the use of nano-emulsion essential oils of Clove, Rose and Bergamot could be a good and environmentally safe alternative of fungicides in controlling post-harvest grey mold disease of strawberry.

REFRENCES

Abd-Elgawad, M. M. (2019) Plant-parasitic nematodes of strawberry in Egypt: a review. Bulletin of the National Research Centre.43:7.

Aguilar-González, A. E., Palou, E. and López-Malo, A. (2015) Antifungal activity of essential oils of clove (*Syzygium aromaticum*) and/or mustard (*Brassica nigra*) in vapor phase against gray mold (*Botrytis cinerea*) in strawberries. Innov. Food Sci. and Emerging Technol. 32:181–185.

Ali, A., Muhammad, M.T., Sijam, K. and Siddiqui, Y. (2011) Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (*Carica papaya* L.) fruits during cold storage, Food Chemistry124:620-626.

Amjadi, S., Almasi, H., Ghorbani, M. and Ramazani, S. (2019) Reinforced ZnONPs/ rosemary essential oil-incorporated inelectrospun nano fibers by κ-carrageenan. Carbohydr Polym. 2020 Mar 15; 232:115800.

Anwar, K. M., Jamil, S., Ibnouf, O. E., Shakeel, F. (2014) Enhanced antibacterial effects of clove essential oil by nano-emulsion. J. Oleo Sci.63: 347-354.

Aoudou, Y., Léopold, T.N., Michel, J.D., Xavier, E.F. and Moses, M.C. (2010) Antifungal properties of essential oils and some constituents to reduce foodborne pathogen. Trends Food Sci. and Technol.101:89-105.

Bhargava P., Kumar A., Kumar S., Azad C.S. (2018) Impact of fungicides and nanoparticles on *Ustilaginoidea virens* causing false smut disease of rice. J Pharmacogn Phytochem 7:1541–1544.

Bhushan, B. (2004) Handbook of Nanotechnology. Berlin-Heidelberg, Springer- Verlag, Germany.

Bautista-Banos, S., Garcia-Dominguez, E., Barrera-Necha, L.L., Reyes-Chilpa, R. and Wilson, C.L. (2003) Seasonal evaluation of the postharvest fungicidal activity of powders and extracts of huamuchil (Pithecellobiom dulce): Action against *Botrytis cinerea, Penicillium digitatum* and *Rhizopus stolonifer* of strawberry fruit. Postharvest Biology and Technology. 29:1.81–92.

Bouchra, C., Achouri, M.,Hassani, M. L., Hmamouchi, M. (2003) Chemical composition and antifungal activity of essential oils of seven Moroccan labiates against *Botrytis cinerea.* J. Ethno Pharmacology 89:165-169.

Daferera, D.J., Ziogas, B.N. and Polissiou, M.G. (2003) The effectiveness of plant essential oils on the growth of *Botrytiscinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Eur. PMC. 22:39-44.

El-Korany. A. E., and Mohamed, R. A. (2008) The use of antioxidants to control grey mold and to enhance yield and quality of strawberry. J. Agric. & Env. Sci. Alex. Univ., 7: 1-30.

El-Tahan, R. M. (2017) Studies on some root rot diseases of sugar beet. Ph. D. Thesis, Fac. Agric., Damanhour Univ., Egypt.

El-Naqa, S. A. (2020) New approaches to control charcoal rot of sesame caused by *Macrophomina phaseolina*. Ph. D. Thesis, Fac. Agric., Damanhour Univ., Egypt.

Feliziani, E. and Romanazz, G. (2016) Postharvest decay of strawberry fruit: Etiology, epidemiology, and disease Management. Journal of Berry Research 6: 47–63.

Geransayeh, M., Sepahv, M., Abdossi, V., Nezhad, R. (2015) Effect of thymol treatment on decay, postharvest life and quality of strawberry (*Fragaria ananassa*) fruit cv. Gaviota. Int. J. Agron. & Agric. Res.6: 151-162.

Ghosh, V., Saranya, S., Mukherjee, A., Chandrasekaran, N. (2018) Cinnamon oil nanoemulsion formulation by ultrasonic emulsification: investigation of its bactericidal activity. J Nanosci Nanotechnol.13:114-22.

Hasan S. K., Ferrentino G., Scampicchio M. (2020) Nanoemulsion as advanced edible coatings to preserve the quality of fresh-cut fruits and vegetables: a review. Int. J. Food Sci. Technol 55 1–10.

Hasheminejad, N. and Khodaiyan, F. (2020) The effect of clove essential oil loaded chitosan nanoparticles on the shelf life and quality of pomegranate arils. Food Chemistry 309:125520.

Kamaruzzaman, M., Rahman, M.M., Islam, M.S., andAhmad, M. U. (2016) Efficacy of four selective Trichoderma isolates as plant growth promoters in two peanut varieties. International J. Biol. Res. 2:409-413.

Lemos Junior, W. J. F., Binati, R. L., Felis, G. E., Slaghenaufi, D., Ugliano, M., and Torriani, S. (2020) Volatile organic compounds from Starmerella bacillaris to control grey mold on apples and modulate cider aroma profile. Food Microbiology. 89:103446.

Mrad, N. D., Boudhrioua, N., Kechaou, N., Courtois, F., and Bonazzi, C. (2012) Influence of air-drying temperature on kinetics, physicochemical properties, total phenolic content and ascorbic acid of pears. Food and Bioproducts Processing. 90: 433-441.

Mertely, J.C. and Peres, N.A. (2006) Botrytis fruit rot or grey mold of strawberry. University of Florida Publications, USA.

Pandey, D.K., Tripathi, N.N., Tripathi, R.D. and Dixit, S.N. (1982) Fungitoxic and phytotoxic properties of the essential oil of the Hyptissuaveolens. Z. Pflakkkrankh PflSchutz. 89:344-349.

Petrasch S., Knapp, S. J.; Vankan, J. A. L. and Blanco-Ulate, B. (2019) Grey mold of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen *Botrytis cinerea.* Molecular Plant Pathology. 20(6), 877–892.

Pedro, S. A., Santo, E. I., Silva, V. C., Detoni, C. and Albuquerque, E. (2013) The use of nanotechnology as an approach for essential oil-based formulation with antimicrobial activity. Salvador, Bahia, Brazil. In: Mendez-Vilas, A., Ed., Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education, Formatex Research Center, Badajoz, 251-257.

Plotto, A., Bai, J. and Baldwin, E. A. (2003) Effect of pretreatment of intact ‘Kent’ and ‘Tommy atkins’ Mangoes with ethanol vapour, heator 1-methyl cyclo propene on quality and shelf life of fresh-cut slices. Proc. Fla. State Hort. Soc. 116:394-400.

Rai, M., Ribeiro, C., Mattoso, L. and Duran, N. (2015) Nanotechnologies in food and agriculture. Springer Professional, Heidelberg, Germany.

Robledo, N., Vera, P., López, L., Yazdani-Pedram, M., Tapia, C. and Abugoch, L. (2018) Thymol nanoemulsions incorporated in quinoa protein/chitosan edible films, antifungal effect in cherry tomatoes. Food Chem. 246, 211–219.

SAS (2009) SAS/STAT 9.2 Users Guide. SAS Institute, Cary.

Salvia-Trujillo L., Rojas-Graü A., Soliva-Fortuny R., and Martín-Belloso O. (2015) Use of antimicrobial nanoemulsions as edible coatings: Impact on safety and quality attributes of fresh-cut Fuji apples. Postharvest Biology and Technology. 105:8–16.

Shafiq, S. and Shakeel, F. (2010) Stability and self-nanoemulsification efficiency of ramiprilnanoemulsion containing labrasol and plurololeique. Clinical Research and Regulatory Affairs 27:7-12.

Shang, Y., Hasan, M.K., Ahammed, G.J., Li, M., Hand, Y., Zhou, J. (2018) Applications of nanotechnology in plant growth and crop protection: A Review. Molecules 24: 2558-2569.

Shehata, H. M. A., Soilman, M. A., and Ewis, D. M. (2020) An analytical study of strawberry crop in Egypt. Assiut Journal of Agricultural Sciences. 51:9–10.

Simic, A., Socovic, M.D., Ristic, M., Grujic-jovanovic, V.J. and Marin, P.D. (2004) The chemical composition of some Lauraceae essential oils and their antifungal activities. Phytotherapey Res.18: 713-717.

Todeschini, V., Lahmidi, N., Mazzucco, E., Marsano, F., Gosetti, F., Robotti, E., Bona, E., Massa, N., Bonneau, L., Marengo, E., Wipf, D., Berta, G. and Lingua, G., (2018) Impact of beneficial microorganisms on strawberry growth, fruit production, nutritional quality, and volatiles, Front. Plant Sci. 9: 1611-1619.

Tzortzakis, N.G. and Economakis, C.D. (2007) Antifungal activity of lemongrass (Cympopogoncitratus L.) essential oil against key postharvest pathogens. Innov. Food Sci. & Emerging Technol.8: 253-258.

Worrall, E. A., Hamid A., Mody, K.T, Mitter, N. and Pappu, H. (2018) Nanotechnology for Plant Disease Management. Agronomy 8:285-290.

Ziedan, E.H.E. and Farrag, E.S. (2008) Fumigation of peach fruits with essential oils to control postharvest decay.2008. Research Journal of Agriculture and Biological Sciences, 4:5,512–519.

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الملخص العربى

فاعلية بعض مستخلصات الزيوت العطرية النانوية فى مقاومة مرض العفن الرمادى في الفراولة مابعد الحصاد

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تعتبر المستخلصات النانويه للزيوت العطرية طريقة واعدة فعاله لمقاومة المسببات المرضية. وفي هذه الدراسة تم تحضيرمستحلبات الزيوت العطرية النانويه لكلا من القرنفل – الورد – البرجموت وإختبارها كمضاد فطري محتمل بتركيزات مختلفة من المادة الفعالة لتثبيط نمو الفطر بوترايتس سيناريا (ب س4) المسبب لمرض العفن الرمادي في الفراولة معمليا ومكافحة المرض تحت ظروف التخزين. أشارت النتائج إلي أن مستخلص القرنفل النانوي كان الأكثر تأثيرا بنسبة ١٠٠% في تثبيط النمو القطري والتجرثم للعزله (ب س4) المختبره من فطرالبوترايتس سيناريا وذلك حتي بتركيز أقل 80 ميكروليترمل -1 للنمو القطري و 50 ميكروليترمل -1 لتثبيط التجرثم. مستخلصات القرنفل و الورد النانويه بتركيزات (100 ميكروليترمل -1 و 80 ميكروليترمل -1) ثبطت تماما تكشف مرض العفن الرمادي بعد 7 أيام من التحضين بينما مستخلص البرجموت النانوي أعطى تأثيرا أقل في تثبيط مرض العفن الرمادي. عموما فإن المعاملة بالمستخلصات النانويه للزيوت العطرية حافظت علي الخصائص الفيزيائية والكيميائية (المحتوي الرطوبي – فقدان الوزن – الحموضه المعايره – محتوي حمض الأسكوربيك) في الثمار المعاملة والمعداه بالعزله الفطرية المختبره وذلك علي ثلاث فترات من التخزين المبرد مقارنة بالثمار المعداه والغير معاملة. بناء علي النتائج المتحصل عليها فأن إستخدام

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المستخلصات النانويه للزيوت العطرية يمكن إعتبارها طريقه جيده وصديقه للبيئه وأمنه كبديل لإستخدام المبيدات الفطرية في مقاومة مرض العفن الرمادي في الفراولة مابعد الحصاد.

الكلمات الدالة: العفن الرمادي – *البوترايستس سيناريا* - المستخلصات النانويه للزيوت العطرية – أمراض مابعد الحصاد