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ANTIFUNGAL ACTIVITY OF SOME ESSENTIAL OIL EMULSIONS AND NANOEMULSIONS AGAINST *FUSARIUM OXYSPORUM* PATHOGEN AFFECTING CUMIN AND GERANIUM PLANTS

M.M.H. Hassanin[*], M.A. Abd-El-Sayed[**] and Mona A. Abdallah[**]

* Plant Pathology Research Institute, Agricultural Research Center, Egypt.
** Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

ABSTRACT: In the present investigation, the antifungal activity of essential oil emulsions and nanoemulsions of sweet basil (*Ocimum basilicum* L.), marjoram (*Majorana hortensis* Moench), peppermint (*Mentha piperita* L.), spearmint (*Mentha spicata* L.) and thyme (*Thymus vulgaris* L.) were evaluated against *Fusarium oxysporum* isolated from infested cumin and geranium plants. Essential oils were obtained by hydrodistillation and analyzed by gas chromatography. The nanoemulsion was formulated using the essential oils, non-ionic surfactant (Tween 80) and water by ultrasonication method for 30 min and characterized by particle size analyzer and transmission electron microscope. Essential oil emulsions were prepared as mentioned above without sonication. The transmission electron micrograph showed that the essential oil nanoemulsions were spherical in shape and moderately mono or di-dispersed. The droplet size was correlated well with the results obtained from droplet size analysis showing that droplets are present in the nanometer range, with particle size of less than 100 nm and were stable after 3 months of storage under room temperature (27 °C). Four concentrations of the emulsions and nanoemulsions were used to evaluate the anti-fusarium activity *in vitro*. The results showed that maximum inhibition against *Fusarium oxysporum.* f.sp. *cumini* was resulted by thyme essential oil nanoemulsion and emulsion at 2000 ppm and sweet basil essential oil nanoemulsion at 4000 ppm. Also, maximum inhibition against *Fusarium oxysporum* isolated from geranium plant resulted by thyme essential oil nanoemulsion and emulsion at 2000 ppm. All essential oil nanoemulsions exhibited higher activities compared to emulsions against fungal growth at all concentrations. Treating cumin seeds with each of the concentrations of essential oil emulsions did not affect germination, while seed germination percentage sharply decreased at high concentrations of nanoemulsions treatments. The results suggest the potential effects of thyme and sweet basil essential oil nanoemulsions as novel fungicide agents against *Fusarium* spp.

Key words: *Fusarium oxysporum*, essential oils, nanoemulsions, transmission electron microscopy.

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INTRODUCTION

Fungal diseases cause severe damage to a wide variety of crops. *Fusarium oxysporum* is one of the most important soil-borne pathogen that causes wilt disease in cumin and geranium plants (Arafa, 1985; Abdel-Wahed, 2011). The pathogen has the ability to persist for very long periods in soil

without a host (Larena *et al.,* 2003). Owing to the restriction of using chemical fungicides in the field of medicinal and aromatic plants, attention has been diverted towards the exploration for alternate sources of ecofriendly antifungal compounds in plants. Such compounds being biodegradable and selective in their toxicity are considered valuable for controlling some plant diseases (Reddy *et al.,* 2010).

Essential oils are volatile hydrophobic liquids extracted from different parts of the aromatic plants. The antifungal activity of essential oils against phytopathogens has previously been reported. For instance, lemongrass and thyme oils exhibited complete inhibition against *Fusarium oxysporum* (Baioumy, 1997).

Nanotechnology is a tool used to modify nano-scale material characteristics, in this case, to improve the essential oil properties (Huang *et al.,* 2010). The biggest difference between essential oil emulsion and essential oil nanoemulsion is in the size of the water particles. When the size of the oil particles becomes small, the stability of the emulsion is significantly improved. To enhance the kinetic stability of such a system, surfactants are added to oil–water mixture. A surfactant is an amphiphilic molecule that has a hydrophilic head group (polar region), which has a high affinity for water, and a lipophilic tail group (non polar region), which has a high affinity for oil (Anton and Vandamme, 2011). Essential oils incorporated in nanoemulsions seem to penetrate faster in the microbial membranes due to the increased area per weight unit. This would allow reducing the concentration to achieve an equivalent or even greater microbial effect over conventional emulsions (Odriozola-Serrano *et al.,* 2014). Therefore, there is a need for developing new fungicides using essential oil nanoemulsions as an alternative to synthetic fungicides for Fusarium control. The aim of this study was to evaluate the antifungal activity of essential oil emulsions and nanoemulsions of sweet basil, marjoram, peppermint, spearmint, and thyme against *Fusarium oxysporum* isolated from infected cumin and geranium plants under *in vitro* conditions.

MATERIALS AND METHODS

Plant materials:

Fresh herb of sweet basil (*Ocimum basilicum* L.), marjoram (*Majorana hortensis* Moench), peppermint (*Mentha piperita* L.), spearmint (*Mentha spicata* L.) and thyme (*Thymus vulgaris* L.) were collected at the beginning of the flowering stage (June 2017) from El-Kanater El-Khairia Farm, Medicinal and Aromatic Plants Research Department, Horticulture Research Institute (HRI), ARC, Egypt. Samples from each plant were then shade dried and subjected for essential oil extraction.

Essential oil extraction and analysis:

The essential oil was extracted by hydro-distillation using a Clevenger apparatus for 3 hours, then dried with anhydrous sodium sulphate and stored at 4 °C until use. Essential oils were extracted from the nanoemulsions by redistillation. The Gas chromatography analysis of the essential oil samples was carried out using Ds Chrom 6200 Gas Chromatograph apparatus, fitted with capillary column BPX-5, 5 phenyl (equiv.) polysillphenylene-siloxane 30 x 0.25 mm ID x 0.25µ film. The temperature program varied in the range of 70-200 °C, at a rate of 10 °C/min. Flow rates of gases were nitrogen at 1 ml/min, hydrogen at 30 ml/min and 330 ml/min for air. Detector and injector temperatures were 300 °C and 250 °C, respectively.

Preparation of essential oil emulsions and nanoemulsions:

A volume of 10 ml of each essential oil and 5 ml of non-ionic surfactant Tween 80 were added slowly under gentle stirring until a homogeneous mixture formed. Water (85 ml) was then added to reach the final mixture of each oil to 100%, to help distribute and completely incorporate the essential oils and then stirred using a magnetic stirrer for 30 min. The mixture was sonicated using an

Ultrasonicator (Bande-lin SONOPULS HD 2200, Germany) for 30 min. at 700 W. The particle size of 10% essential oils nanoemulsion for each amount was detected by Hydrodynamic light scattering analyzer (DLS) after 90 days of storage under room temperature (27 °C). Essential oil emulsions were prepared as mentioned above without sonication.

Droplet size analysis:

Measurement of droplet size of nanoemulsions was performed by a dynamic light scattering analyses using Zeta Nano ZS (Malvern Instruments, UK) at room temperature. Prior to measurement, 30 µl of each nanoemulsion was diluted with 3ml of water at 25 °C. Particle size data were expressed as the mean of the Z-average of 3 independent batches of the nanoemulsions. The droplet size and the poly dispersityindex (PDI) of the formulated nanoemulsion were measured. This work was performed by Nanotechnology Laboratory, Regional Center for Food & Feed, ARC, Giza, Egypt.

Transmission electron microscopy (TEM):

Twenty microliters of diluted samples were placed on a film-coated 200-mesh copper specimen grid for 10 min and the excess fluid was eliminated using filter paper. The grid was then stained with one drop of 3% phosphotungstic acid and allowed to dry for 3 min. The coated grid was dried and examined under the TEM microscope (Tecnai G20, Super twin, double tilt, FEI, The Netherlands). The samples were observed by operating at 200 kV.

Antifungal activity assay:

The efficacy of the emulsions and nanoemulsions in reducing the fungal growth was tested. Essential oil emulsions and nanoemulsions of sweet basil, marjoram, peppermint, spearmint and thyme were added to sterilized potato dextrose agar (PDA)flasks before solidifying to obtain the proposed concentrations of 1000, 2000, 4000 and 8000 ppm (v/v). The bactericide chloramphenicol (0.1mg/l) was added to the medium to avoid bacterial contamination. Three plates containing PDA medium were inoculated with discs (5-mm-diam.) of *Fusarium oxysporum* f.sp. *cumini* (isolated from infected cumin plants) and *Fusarium oxysporum* (isolated from infected geranium plants). Petri dishes were incubated at 25 °C. Percentages of fungal growth inhibition were calculated when the fungal growth of the control plates (without treatments) completely filled the plates according to formula suggested by Topps and Wain (1957) as follows:

$$\textbf{Inhibition \%} = \frac{A - B}{A} \times 100$$

A= the linear growth in control treatment.

B= the linear growth of treated fungus.

Evaluation of sporulation:

Number of spores was counted in both treated and non-treated plates (7 days after incubation at 27 °C) with essential oil emulsions and nanoemulsions at a concentration of 4000 ppm. Spores were harvested from margins of the colonies using sterile needle and transferred into the test tubes, each contained 10 ml of sterile distilled water. The tubes were centrifuged at 1000 rpm for 3 minutes to allow for even dispersal of spores. A drop of spore suspension was placed on a haemocytometer slide, then spores formed were counted in 5 microscopic fields.

Germination test:

Sets of 300 cumin seeds were soaked in each preparation of sweet basil, marjoram, peppermint, spearmint and thyme essential oil emulsions and nanoemulsions at the rates of 0.0, 1000, 2000, 4000 and 8000 ppm for 10 min. Seeds treated with each concentration were placed in Petri-dishes on layers of moistened blotters at the rate of 100 seeds/dish. Dishes were then incubated in controlled environment (27 °C) under alternating cycles of 12 hrs. light and 12 hrs dark for 15 days. Percentage of germination was finally measured for each treatment as follows:

$$\textbf{Germination \%} = \frac{\text{No. of germinated seeds}}{\text{Total number of experimented seeds}} \times 100$$

Statistical analysis:

The layout of this experiment as designed factorial experiment in a complete randomized design with three replicates (Snedecor and Cochran, 1980). This statistical analysis was done by using the computer program MS-TATEC software version (4) using the L.S.D. test at 0.05.

RESULTS AND DISCUSSION

Chemical composition of essential oils and essential oil nanoemulsions:

Chemical composition of investigated essential oils and essential oil nanoemulsions analyzed by gas chromatography are presented in Table (1). Linalool (40.72%), methyl chavicol (9.41%), 1,8-cineole (5.31%), α-pinene (3.11%) and eugenol (2.73%) were identified as major constituents of sweet basil essential oil. Terpinene-4-ol (30.28%), γ-terpinene (13.32%), α-terpinene (12.46%) and sabinene (10.49%) were identified as the major constituents of marjoram essential oil. Menthol (30.51%), menthyl acetate (23.06%) and menthone (14.45%) were identified as the major constituents of peppermint essential oil. Carvone (60.03%), limonene (16.63%) and 1,8-cineole (6.54%) were identified as the major constituents of spearmint essential oil. ρ-cymene (52.36%), thymol (23.62%) and borneol (5.80%) were identified as the major constituents of thyme essential oil. Changes in the essential oil components were observed in the nanoemulsions compared with the corresponding essential oils. Data presented in Table (1) show that main component content of peppermint and thyme oil nanoemulsions were decreased. On the contrary, higher content of major components were observed in basil, marjoram and spearmint essential oil nanoemulsions. Decreases in the main component content in essential oil nanoemulsions could be due to that volatile compounds might be able to migrate from the oil droplets through the water phase and further be volatilized or they might be oxidized to unstable forms (Mirhosseini *et al.,* 2008).

Droplet size and polydispersity index (PDI):

The droplet size of prepared essential oil nanoemulsions with polydispersity nature was examined using particle size analyzer after 3 months of storage under room temperature. Tween-80 was used as surfactant for its high hydrophile-lipophile balance(HLB) value that favor's formulation of oil-in-water emulsion. Also, small molecule surfactant like Tween-80 gets rapidly adsorbed onto emulsion droplet surface and hence they are more effective on reducing droplet diameter than polymeric surfactants (Ghosh *et al.,* 2014). The nanoemulsions showed different mean diameters as shown in Table (2) & Figure (1). The mean droplet diameter for nanoemulsions containing sweet basil, marjoram, peppermint, spearmint and thyme oils was 23.09, 46.39, 73.76, 58.34 and 64.49 nm, respectively. The type of essential oil had an impact on the droplet size of nanoemulsion. Nanoemulsions containing sweet basil oil presented the smallest average droplet diameter compared to the other essential oil nanoemulsions. The differences among the droplet size obtained in nanoemulsions containing several essential oils has been attributed to different polarities of the volatile compounds forming each oil phase (Salvia-Trujillo *et al.,* 2015; Guerra-Rosas *et al.,* 2017). The values of polydispersity index (PDI) are also presented in Table (2). The polydispersity index (PDI) is a measure of homogeneity and stability of the droplet size in the nanoemulsion system. PDI value below 0.2 indicates a narrow size distribution and thus provides long-term stability to the formulated nanoemulsion (Sampathi *et al.,* 2015).

Table 1. Chemical composition of essential oils (EO) and essential oil nanoemulsions (EONE).

Components (%)	Sweet basil		Marjoram		Peppermint		Spearmint		Thyme	
	EO	EONE	EO	EONE	EO	EONE	EO	EONE	EO	EONE
α-thujene	-	-	1.38	9.09	-	-	-	-	-	-
α-pinene	3.11	0.58	-	-	0.29	0.75	1.07	0.72	1.76	1.63
Camphene	-	-	-	-	-	-	-	-	1.83	1.76
Sabinene	-	-	10.49	1.37	-	-	-	-		
β-pinene	1.18	1.51	-	-	1.80	2.35	-	-	1.87	1.76
β-myrcene	1.00	0.35	10.67	6.51	-	-	2.49	1.18	1.75	7.25
α-terpinene	-	-	12.46	5.54	-	-	-	-	-	-
ρ-cymene	-	-	5.60	3.75	-	-	1.27	7.36	52.36	29.18
Limonene	-	-	4.81	5.62	0.66	7.39	16.63	4.96	0.96	6.74
1,8-cineol	5.31	3.23	-	-	0.71	4.44	6.54	2.04	-	-
β-ocimene	-	-	-	-	-	-	1.48	1.58	1.10	9.45
γ-terpinene	-	-	13.32	-	-	-	-	-	-	-
α –terpinolene	-	-	-	-	-	-	-	-	2.09	3.98
Linalool	40.72	54.24	-	-	-	-	-	-	-	-
Menthone	-	-	-	-	14.45	16.57	-	-	-	-
Iso menthone	-	-	-	-	5.44	9.43	-	-	-	-
Menthofuran	-	-	-	-	6.26	5.17	-	-	-	-
Borneol	-	-	-	-	-	-	-	-	5.80	9.03
Menthol	-	-	-	-	30.51	20.05	-	-	-	-
Terpinene-4-ol	-	-	30.28	45.69	-	-	-	-	-	-
γ-terpineol	-	-	-	-	-	-	1.12	1.47	-	-
Dihydrocarveol	-	-	-	-	-	-	3.32	3.83	-	-
Dihydrocarveon	-	-	-	-	-	-	0.29	2.41	-	-
Methyl chavicol	9.41	4.83	-	-	-	-	-	-	-	-
Carvone	-	-	-	-	-	-	60.03	66.34	-	-
Menthyl acetate	-	-	-	-	23.06	13.39	-	-	-	-
Thymol	-	-	-	-	-	-	-	-	23.62	14.40
Eugenol	2.73	-	-	-	-	-	-	-	-	-
β-caryophyllene	0.76	0.97	-	-	-	-	-	0.95	2.65	0.79
Caryophyllene oxide	-	-	-	-	-	-	-	2.59	-	-
Essential oil (%)	0.6		1.00		1.5		1.00		2.00	

Table 2. Mean droplet and PDI of investigated essential oil nanoemulsions.

Essential oil nanoemulsion	Mean droplet size (nm)	Poydispersity index (PDI)
Sweet basil	23.09	0.244
Marjoram	46.39	0.125
Peppermint	73.76	0.436
Spearmint	58.34	0.181
Thyme	64.49	0.165

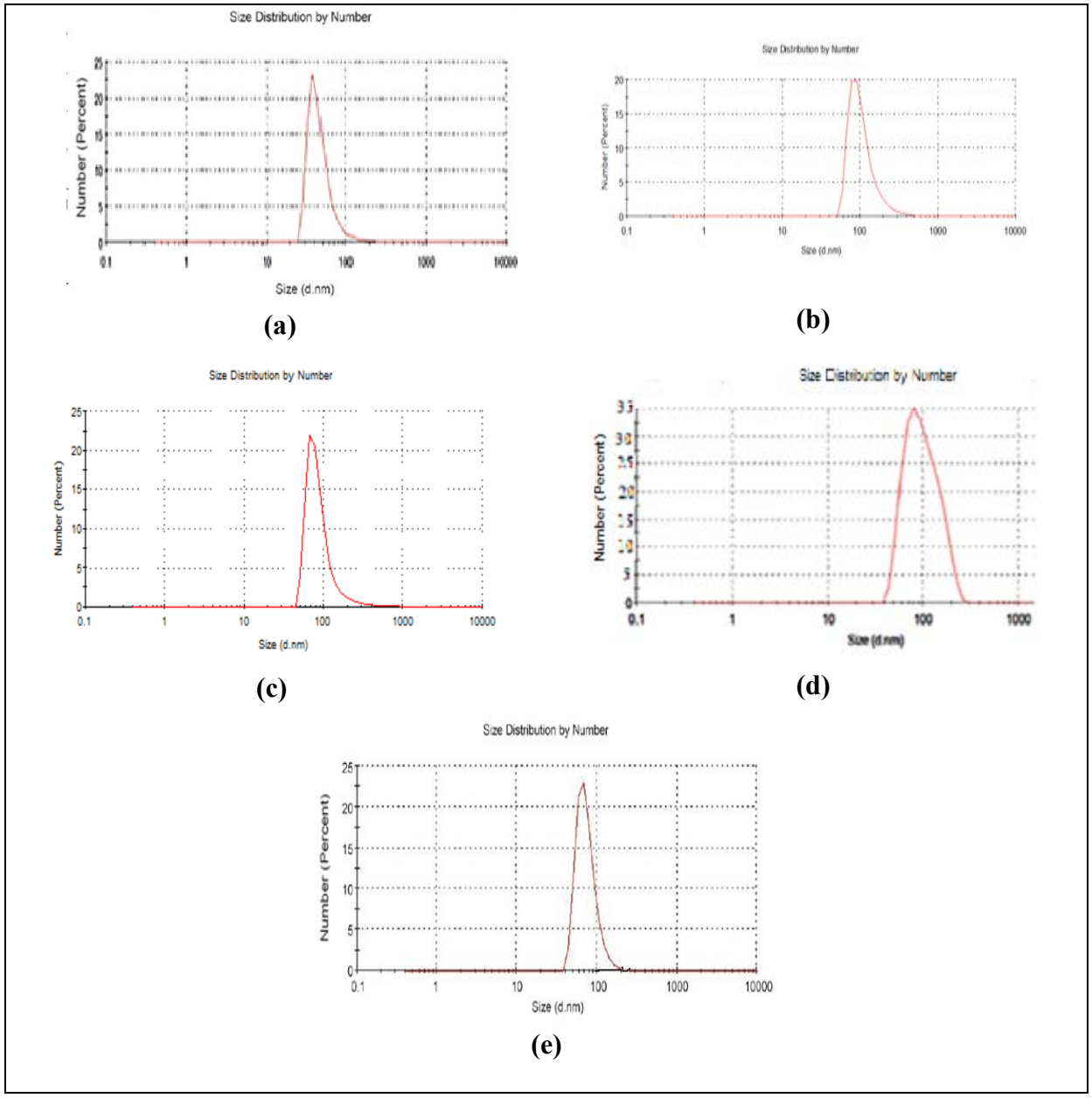

Fig.1. Mean droplet size (nm) of investigated essential oils nanoemulsion.
(a) Sweet basil oil nanoemulsion, (b) Marjoram oil nanoemulsion (c) Peppermint oil nanoemulsion, (d) Spearmint oil nanoemulsion, (e) Thyme oil nanoemulsion.

Transmission electron microscopy (TEM):

Transmission electron microscopy characterization of essential oils nanoemulsion gives the actual size and shape; the droplets in the nanoemulsion appear dark as shown in Figure (2). The TEM micrograph showed that the essential oil nanoemulsions were spherical in shape and moderately mono or di-dispersed. Sweet basil nanoemulsion droplets were in the range of 24.4 - 54.2 nm. Marjoram nanoemulsion droplets were in the range of 96.8 – 158 nm. Peppermint nanoemulsion droplets were in the range of 36.5 – 95.3 nm. Spearmint nanoemulsion droplets were in the range of 58.6 – 95.1 nm. Thyme nanoemulsion droplets were in the range of 34 – 80.8 nm. The droplet size was well correlated with the results obtained from

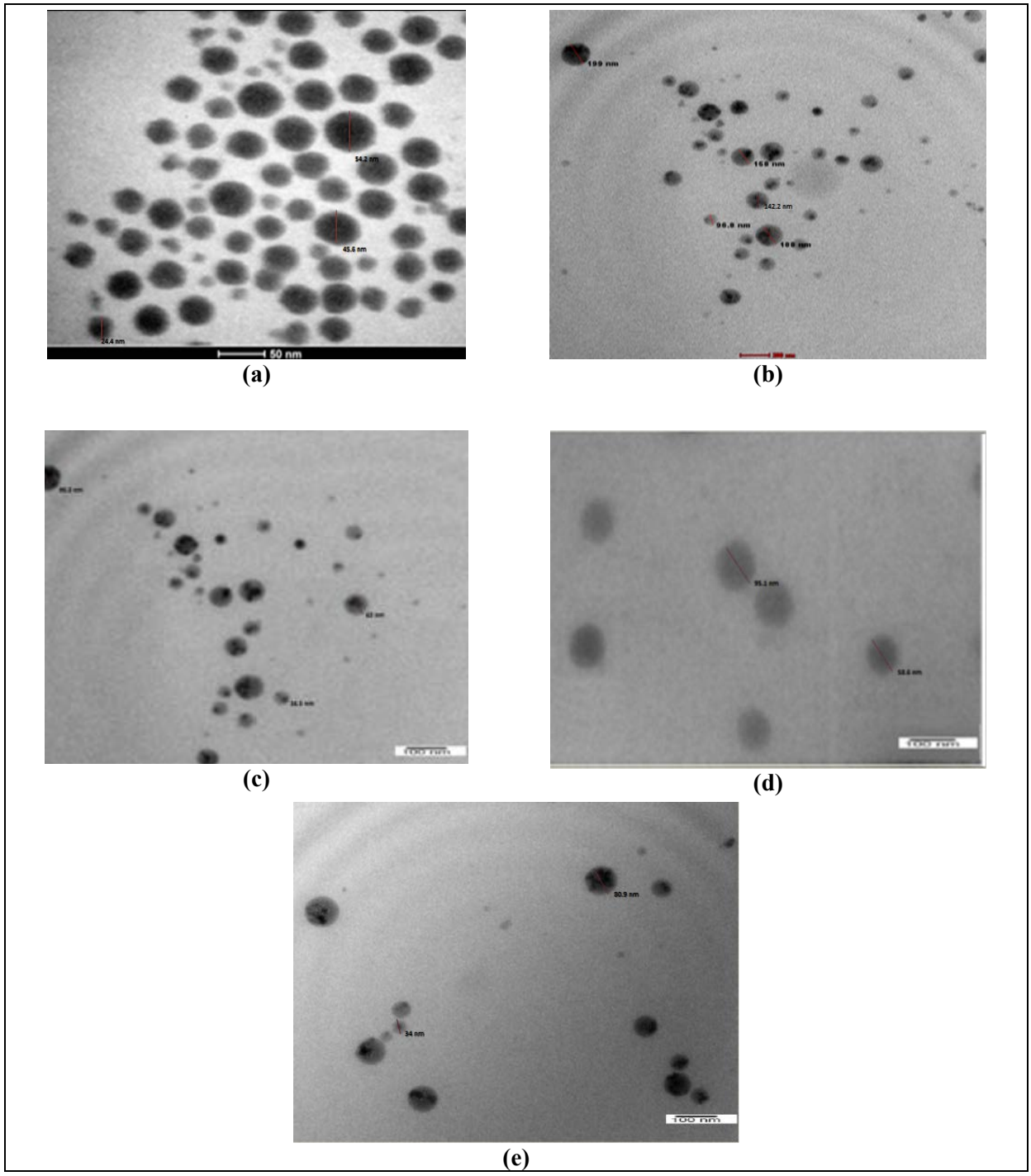

Fig. 2. Transmission electron micrographs of essential oil nanoemulsions.
(a) Sweet basil oil nanoemulsion, (b) Marjoram oil nanoemulsion (c) Peppermint oil nanoemulsion, (d) Spearmint oil nanoemulsion, (e) Thyme oil nanoemulsion.

droplet size analysis using the dynamic light scattering (Abd-Elsalam and Khokhlov, 2015) showing that droplets are present in the nanometer range, with particle size of less than 100 nm.

Antifungal activity:

The antifungal activity of 5 essential oil emulsions and nanoemulsions at 1000, 2000, 4000 and 8000 ppm was evaluated against *F. oxysporum* f.sp. *cumini* and *F. oxysporum*

isolated from infected cumin and geranium plants, respectively under *in vitro* conditions. After seven days of incubation the fungal isolates exhibited growth inhibition in a dose dependent manner (Tables 3 & 4). The statistical analysis showed that essential oil emulsions and nanoemulsions at different concentrations significantly affected radial growth. The growth inhibition of *F. oxysporum* was linearly increased with an increase in concentration of the essential oil emulsions and nanoemulsions. Data revealed that all essential oil emulsions and nanoemulsions at 8000 ppm concentration completely inhibited the mycelial growth.

The treatment with thyme essential oil emulsion and nanoemulsion at 2000 ppm and 4000 ppm completely inhibited the radial growth of both isolates of *F. oxysporum.* At 4000 ppm concentration, sweet basil nanoemulsion completely inhibited the radial growth of both isolates of *F. oxysporum.*

The treatments evaluated at 4000 ppm were significantly superior to those of 2000 ppm, however, thyme oil emulsion and nanoemulsion were highly effective (100%) even at 2000 ppm concentration, whereas, spearmint oil was the least effective (34.44%; 43.33%) against *F. oxysporum* f. sp. *cumini* and *F. oxysporum* isolated from geranium plant, respectively as shown in Tables (3 & 4).

The inhibitory effect of the essential oils might be attributed to the presence of antifungal compounds. The differences might be due to the difference in nature, quality and quantity of the inhibitory substances present in the essential oils. The essential oil of thyme contains thymol (23.62%) and borneol (5.80%) as main constituents as shown in Table (1). These compounds possess an antimicrobial activity against a number of fungi as reported by ŠegvicKlaric *et al.* (2007). Greater antimicrobial potential could be ascribed to the oxygenated terpenes, especially phenolic compounds such as thymol (Soković *et al*., 2002). However, thyme oil activity might be due to its chitin penetration of cell wall which damages the lipoprotein cytoplasmic membrane, leading to escape of cytoplasm (Zambonelli*et al*., 1996).

The essential oil of sweet basil contains linalool (40.72%), 1,8- cineol (5.31%) and eugenol (2.73%) as main constituents as shown in Table (1). The antifungal activity of *Ocimum basilicum* might be attributed to the presence of these compounds as reported by Oxenham *et al.* (2005). The antifungal activity of eugenol depends on cell proliferation. Eugenol treatment altered cell membrane and cell wall structures of proliferating *Saccharomyces cerevisiae* cells resulting in the release of cellular content as reported by Bennis *et al.* (2004). The obtained results showed that all essential oil nanoemulsions had increased antifungal activity compared to essential oil emulsions. This could be attributed to larger surface area of nano material (as a result of their very small size), which probably enable a greater interaction between the active compounds and cell surfaces of the microorganisms (Lboutounne *et al.*, 2002).

Data presented in Table (5) indicated that the tested essential oil emulsions and nanoemulsions significantly inhibited sporulation of *Fusarium* spp. Data showed also that the sporulation of *F. oxysporum* f. sp. *cumini* was completely inhibited with thyme and sweet basil essential oils. Also, the sporulation of *F. oxysporum* (isolated from geranium) was completely inhibited with the thyme essential oil.

It was observed that all essential oil nanoemulsions exhibited higher activities against sporulation than the respective essential oil emulsions. The mode of action of the active substances in essential oils was reported by many authors. Zambonelli *et al*. (1996) mentioned that these antifungal substances have high capabilities to damage structure and function of the enzymatic bioactivity. The fungal growth damages caused by essential oils might be due to their capabilities to penetrate into the fungal cells. They added that the antifungal activities might be due to increase in the permeability

Table 3. Effect of essential oil emulsions and nanoemulsion at different concentrations on mycelial growth of *Fusarium oxysporum* f. sp. *cumini*.

Type of essential oil (A)	Type of treatment (B)	Linear growth (cm) and % Inhibition of mycelial growth at different concentrations (ppm) (C)										Mean
		Control		1000		2000		4000		8000		
		Linear growth (cm)	Inhibition (%)	Linear growth (cm)	Inhibition (%)	Linear growth (cm)	Inhibition (%)	Linear growth (cm)	Inhibition (%)	Linear growth (cm)	Inhibition (%)	
Sweet basil	Emulsion	9.00	0.000	5.80	35.55	3.20	64.44	1.70	81.11	0.00	100.00	3.94
	Nanoemulsion	9.00	0.000	5.70	36.66	2.80	68.88	0.00	100.00	0.00	100.00	3.50
Mean		**9.00**	0.000	**5.75**	36.11	**3.00**	66.67	**0.86**	**90.56**	**0.00**	100.00	**3.72**
Marjoram	Emulsion	9.00	0.000	9.00	0.0	7.60	15.55	5.00	44.44	0.00	100.00	6.12
	Nanoemulsion	9.00	0.000	8.10	10.0	7.30	18.88	4.60	48.88	0.00	100.00	5.80
Mean		**9.00**	0.000	**8.60**	5.00	**7.45**	17.22	**4.80**	**46.66**	**0.00**	100.00	**5.96**
Peppermint	Emulsion	9.00	0.000	7.00	22.22	4.60	15.55	3.00	66.66	0.00	100.00	4.72
	Nanoemulsion	9.00	0.000	6.20	31.11	4.10	18.88	2.10	76.66	0.00	100.00	4.28
Mean		**9.00**	0.000	**6.60**	26.67	**4.35**	17.22	**2.55**	**71.66**	**0.00**	100.00	**4.50**
Spearmint	Emulsion	9.00	0.000	8.60	4.44	7.90	12.22	5.90	34.44	0.00	100.00	6.28
	Nanoemulsion	9.00	0.000	8.10	10.00	7.40	17.77	5.70	36.66	0.00	100.00	6.04
Mean		**9.00**	0.000	**8.35**	7.22	**7.65**	15.00	**5.80**	**35.55**	**0.00**	100.00	**6.16**
Thyme	Emulsion	9.00	0.000	2.80	68.88	0.00	100.00	0.00	100.00	0.00	100.00	2.36
	Nanoemulsion	9.00	0.000	2.50	72.22	0.00	100.00	0.00	100.00	0.00	100.00	2.30
Mean		**9.00**	0.000	**2.65**	70.55	**0.00**	100.00	**0.00**	**100.00**	**0.00**	100.00	**2.33**
Mean of emulsions		**9.00**	0.000	**6.64**	26.22	**4.66**	**48.22**	**3.12**	**65.33**	**0.00**	100.00	**4.68**
Mean of nanoemulsion		**9.00**	0.000	**6.12**	32.00	**4.32**	**52.00**	**2.48**	**72.44**	**0.00**	100.00	**4.38**
Means		**9.00**	0.000	**6.38**	29.11	**4.49**	**50.11**	**2.80**	**68.89**	**0.00**	100.00	**4.53**

L.S.D. at 5% : A = 0.14, B =0.06 , C = 0.13, A X B = 0.24, A X C = 0.30, B X C = 0.17 and A X B X C = 0.41

Table 4. Effect of essential oil emulsions and nanoemulsions at different concentrations on mycelial growth of *Fusarium oxysporum* isolated from geranium plants.

Type of essential oil (A)	Type of treatment (B)	Linear growth (cm) and % Inhibition of mycelial growth at different concentrations (ppm) (C)										Mean
		Control		1000		2000		4000		8000		
		Linear growth (cm)	Inhibition (%)	Linear growth (cm)	Inhibition (%)	Linear growth (cm)	Inhibition (%)	Linear growth (cm)	Inhibition (%)	Linear growth (cm)	Inhibition (%)	
	Emulsion	9.00	0.00	8.10	10.00	5.00	44.44	2.40	73.33	0.00	100.00	4.90
Sweet basil	Nanoemulsion	9.00	0.00	7.00	22.22	4.60	48.88	2.00	77.77	0.00	100.00	4.52
Mean		**9.00**	0.00	**7.55**	**16.11**	**4.8**	**46.66**	**2.20**	**75.55**	**0.00**	100.00	**4.71**
	Emulsion	9.00	0.00	8.30	7.77	6.80	24.44	4.20	53.33	0.00	100.00	5.66
Marjoram	Nanoemulsion	9.00	0.00	7.80	13.33	6.10	32.22	3.10	65.55	0.00	100.00	5.20
Mean		**9.00**	0.00	**8.05**	**10.55**	**6.45**	**28.33**	**3.65**	**59.44**	**0.00**	100.00	**5.43**
	Emulsion	9.00	0.00	8.30	7.77	5.80	35.55	2.70	70.00	0.00	100.00	5.16
Peppermint	Nanoemulsion	9.00	0.00	7.70	14.44	5.10	43.33	2.20	75.55	0.00	100.00	4.80
Mean		**9.00**	0.00	**8.00**	**11.11**	**5.45**	**39.44**	**2.45**	**72.78**	**0.00**	100.00	**4.98**
	Emulsion	9.00	0.00	8.30	7.77	7.50	16.66	5.10	43.33	0.00	100.00	5.98
Spearmint	Nanoemulsion	9.00	0.00	7.90	12.22	6.00	33.33	4.60	48.88	0.00	100.00	5.50
Mean		**9.00**	0.00	**8.10**	**10.00**	**6.75**	**25.00**	**4.85**	**46.11**	**0.00**	100.00	**5.74**
	Emulsion	9.00	0.00	2.90	67.77	0.00	100.00	0.00	100.00	0.00	100.00	2.38
Thyme	Nanoemulsion	9.00	0.00	2.70	70.00	0.00	100.00	0.00	100.00	0.00	100.00	2.34
Mean		**9.00**	0.00	**2.80**	**68.89**	**0.00**	**100.00**	**0.00**	**100.00**	**0.00**	100.00	**2.36**
Mean of emulsions		**9.00**	0.00	**7.18**	**20.22**	**5.02**	**44.22**	**2.88**	**68.00**	**0.00**	100.00	**4.82**
Mean of nanoemulsion		**9.00**	0.00	**6.62**	**26.44**	**4.36**	**51.56**	**2.38**	**73.56**	**0.00**	100.00	**4.47**
Means		**9.00**	0.00	**6.90**	**23.33**	**4.69**	**47.89**	**2.74**	**69.56**	**0.00**	100.00	**4.67**

L.S.D. at 5% : A = 0.19, B =0.41 , C = 0.34, A X B = 0.45, A X C = 0.71, B X C = 0.55 and A X B X C = 1.05

Table 5. Effect of essential oil emulsions and nanoemulsions at the concentration of 4000 ppm on sporulation of *Fusarium* spp. after 7 days incubation at 27 °C.

Type of essential oil (A)	Mean sporulation (x10^3)					
	F. oxysporum f. sp. *cumini* isolated from cumin plants			*F. oxysporum* isolated from geranium plants		
	Type of treatment (B)		Mean	Type of treatment (B)		Mean
	Emulsion	Nanoemulsion		Emulsion	Nanoemulsion	
Control	290.00	290.00	290.00	295.00	295.00	295.00
Sweet basil	0.00	0.00	0.00	30.00	25.00	27.50
Marjoram	205.00	175.00	190.00	100.00	85.00	92.50
Peppermint	50.00	25.00	37.50	55.00	30.00	42.50
Spearmint	235.00	200.00	217.50	225.00	205.00	215.00
Thyme	0.00	0.00	0.00	0.00	0.00	0.00
Mean	130.00	115.00		117.50	106.67	
L.S.D. at 5%	**A=** 2.85			**A=** 3.04		
	B= 4.85			**B=** 5.27		
	AX B = 11.92			**AX B=** 12.89		

of the fungal cell as well as inhibition in the fungal detoxificating enzymes of the antifungal oil substances. The essential oils are also capable to affect respiration of the fungal cell (oxygen uptake) and having toxic substances acting as antisporulation compounds (Inouye *et al.,* 1988).

Effect of essential oil emulsions and nanoemulsions at different concentrations on seed germination:

The effect of essential oil emulsions and nanoemulsions at different concentrations on cumin seed germination is presented in Table (6). Generally, there was a significant increase in germination percentage as essential oil emulsions increased up to 4000 ppm then was declined at 8000 ppm. In the case of essential oil nanoemulsions, maximum germination percentage (100%) was recorded with all essential oil nanoemulsions at 1000 ppm and minimum germination percentage (52.7%) was recorded with thyme oil nanoemulsion at 8000 ppm.

The obtained results may be interpreted in terms of different kinds of terpenoids present in plant essential oils and their probable effects on seed germination.

It is reported that the terpenoid, particularly the sesquiterpene, is a group of compounds with variable biological activities. At low concentrations, these compounds exhibited specific structure-activity relationship (Beekman *et al.*, 1997). Hemada and El-Darier (2011) showed that germination percentage of *Lepidium sativum* seeds obtained a value of about 100% at control level (without treatment) and when soaked in two thyme species, germination percentage values were about 33% and 60% for *Thymus capitatus* and *T. vulgaris*, respectively at the maximum oil concentration.

This showed that the use of nanoemulsions above a certain concentration had an inhibitory effect on seed germination, which may be attributed to higher levels of nano compounds. The effect was both concentration and preparation dependant.

Conclusion:

Nanoemulsions represent a promising strategy for overcoming essential oil limitations, lowering their dose and increasing long term safety of these constituents. Thus, the results suggest the potential effects of thyme and sweet basil essential oil nanoemulsions as novel fungicide agents against *Fusarium* spp.

Table 6. Effect of essential oil emulsions and nanoemulsions at different concentrations on seed germination percentage of cumin seeds.

Type of essential oil (A)	Type of treatment (B)	Seed germination (%) at different concentrations (ppm)(C)					Mean
		Control	1000	2000	4000	8000	
Sweet basil	Emulsion	100.00	90.00	100.00	100.00	64.00	90.80
	Nanoemulsion	100.00	100.00	95.00	81.70	91.60	93.66
Mean		**100.00**	**95.00**	**97.50**	**90.85**	**77.80**	**92.23**
Marjoram	Emulsion	100.00	95.00	100.00	100.00	90.00	97.00
	Nanoemulsion	100.00	100.00	100.00	90.00	62.70	90.54
Mean		**100.00**	**97.50**	**100.00**	**95.00**	**76.35**	**93.77**
Peppermint	Emulsion	100.00	67.00	95.00	100.00	85.00	89.40
	Nanoemulsion	100.00	100.00	100.00	100.00	82.30	96.46
Mean		**100.00**	**83.50**	**97.50**	**100.00**	**83.65**	**92.393**
Spearmint	Emulsion	100.00	65.00	100.00	100.00	89.30	90.86
	Nanoemulsion	100.00	100.00	100.00	100.00	92.30	98.46
Mean		**100.00**	**82.50**	**100.00**	**100.00**	**90.80**	**94.66**
Thyme	Emulsion	100.00	66.70	100.00	100.00	92.30	91.80
	Nanoemulsion	100.00	100.00	100.00	100.00	52.70	90.54
Mean		**100.00**	**83.35**	**100.00**	**100.00**	**72.50**	**91.17**
Mean of emulsions		**100.00**	**76.74**	**99.00**	**100.00**	**84.12**	**91.97**
Mean of nanoemulsion		**100.00**	**100.00**	**99.00**	**94.34**	**76.32**	**93.93**
Means		**100.00**	**88.37**	**99.00**	**97.17**	**80.22**	**92.95**

L.S.D. at 5%: A= 1.66, B =0.40, C= 1.73, A X B= 2.24, A X C= 2.21, B X C= 3.83 and A X B X C= 5.33

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النشاط المضاد للفطريات لمستحلبات بعض الزيوت العطرية العادية والنانومترية لمقاومة فطر الفيوزاريوم أوكسيسبورم الممرض لنباتات الكمون والعتر

محمود محمد حسانين حسانين*، محمد عبدالقوي عبدالسيد** و منى عبدالمنعم عبد الله**
* معهد بحوث أمراض النباتات، مركز البحوث الزراعية، مصر.
** قسم بحوث النباتات الطبية والعطرية، معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر.

في هذا البحث، تم تقييم النشاط المضاد للفطريات لكل من مستحلبات الزيوت العطرية العادية والنانومترية من الريحان، البردقوش، النعناع البلدي، النعناع الفلفلي والزعتر ضد فطر الفيوزاريوم أوكسيسبورم المعزول من نباتات الكمون والعتر. تم الحصول على الزيوت العطرية من العشب بواسطة التقطير باستخدام الماء وتحليلها بواسطة الكروماتوجرافي الغازي. تم تحضير المستحلبات النانومترية باستخدام الزيوت العطرية، مادة خافضة للتوتر السطحي توين ٨٠ والماء بواسطة طريقة الموجات فوق الصوتية لمدة ٣٠ دقيقة، وتوصيفها بجهاز تحليل حجم الجزيء والميكروسكوب الإلكتروني، تم تحضير مستحلبات الزيوت العادية كما ذكر أعلاه دون إستخدام موجات فوق صوتية. وأظهرت الصور المجهرية للميكروسكوب الإلكتروني النافذ أن الزيوت العطرية كانت كروية الشكل ومعتدلة أحادية أو عديدة في شكل متناثر. وقد أكدت نتائج حجم القطرات المأخوذة بالمجهر الالكتروني نتائج حجم القطرات باستخدام جهاز تحليل حجم القطرات والذي تبين أن القطرات موجودة في النطاق النانومتري مع حجم قطرات أقل من ١٠٠نانومتر والتي ظلت مستقرة طبيعيا لمدة ثلاثة أشهر عند الاحتفاظ بها في درجة حرارة الغرفة (٢٧ °م). تم استخدام أربعة تركيزات من المستحلبات العادية والمستحلبات النانومترية لتقييم النشاط المضاد للفيوزاريوم في المعمل. أظهرت النتائج أن أقصى نشاط ضد فطر فيوزاريوم أوكسيسبورم والذي يصيب نبات الكمون بالذبول نتج من إستخدام مستحلب الزعتر العادي والنانومتري حيث أعطى تثبيطا كاملا عند تركيز ٢٠٠٠جزء في المليون ومستحلب زيت الريحان النانومتري عند تركيز ٤٠٠٠ جزء في المليون. أيضا أعطى مستحلب زيت الزعتر النانومتري أقصى نشاط ضد فطر الفيوزاريوم أوكسيسبورم والمعزول من محصول العتر والذي ثبط تثبيطا كاملا بتركيز ٢٠٠٠جزء في المليون. وأظهرت النتائج أن جميع مستحلبات الزيوت النانومترية كانت أكثر نشاطا ضد نوعي الفيوزاريوم مقارنة بمستحلبات الزيوت العادية. لم يتأثر إنبات بذور الكمون باستخدام أي من تركيزات مستحلبات الزيوت العطرية، في حين انخفضت نسبة إنبات البذور بحدة عند المعاملة بالتركيزات العليا من المستحلبات النانومترية. وتشير هذه النتائج إلى أن المستحلبات النانومترية لزيوت الزعتر والريحان يمكن أن تشكل مصدرا جديدا كمبيدات فطرية لاستخدامها في مقاومة فطر الفيوزاريوم.