EVALUATION OF THE ACTIVITY OF THYME ESSENTIAL OIL NANOEMULSION AGAINST *SCLEROTINIA* ROT OF FENNEL

HASSANIN, M. M. H.; A. E. A. HALAWA and A. A. ALI

Plant Pathology Research Institute, ARC, Giza, Egypt.

(Manuscript received 9 February 2017)

Abstract

In this work, the effect of ultrasonication time on the particle size of thyme essential oil nanoemulsion was determined. The results showed that the particle size resulting from sonication for 15 minutes was around 207.2 nm, and TEM study revealed the spherical shape. The particle size in preparation subjected to sonication for 60 min was decreased to 34.6 nm and they were spherical and shapeless. The in vitro experiments revealed that the nanoemulsion at different concentrions (0.25 – 3.0%) were capable of adversely affecting the mycelial growth of *Sclerotinia sclerotiorum.* The nanoemulsion (particle size 34.6 nm) completely prevented fungal growth at the lowest concentration. On the other hand, fungal growth was prevented by the 1% concentration of the nanoemulsion of particle size 207.2 nm. Treating fennel seeds with oil emulsion did not affect germinability, while nanoemulsion (34.6 nm) prevented feed gemination when used at 1.0 concentration and the nanoemulsion of 207.2 nm particle size was less inhibitive though a sharp decrease at 1.0% concentration occurred. Percentages of damping off were significantly reduced and plant growth was improved as well as the activities of peroxidase and polyphenol oxidase were increased as a result of treatment with namoemulsion.

INTRODUCTION

Fennel (*Foeniculum vulgare*), fam. umbelliferae, is one of the important winter medicinal and aromatic crops in Egypt. It is subjected to infection by Sclerotinia rot caused by *S. sclerotiorum* (Lib.) de Bary, resulting economic losses in plant growth and yield (Hilal *et al.,* 2007). The initial infection occurs in the late winter or early spring, and the fungal mycelium grows within and between cells. Patches of dead plant parts enlarge and coalesce through spring and cause major losses in stands (Bolton *et al.,* 2006).

Fungicides could successfully control rot diseases; however, it negatively affect the human health and the environment (Hilal *et al.*, 2007). Long-term extensive use of fungicides in open field have led to the appearance of fungicide-resistant populations of fungal pathogens (Barnet and Hollomon, 1998). Overall, intensive fungicide application results in several negative effects in the environment that cannot be ignored (Feng and Zheng, 2007). Therefore, there have been several attempts for

using natural fungicidal alternatives for controlling plant diseases. So, development of alternative eco-safe antifungal agents like bio-based nanomaterials is urgently needed. In this concern, thyme essential oil emulsion is reported to have antifungal activities against some fungi (Zengin and Baysal, 2015).

An emulsion of thyme oil is a kinetically stable system which is obtained by the dispersion of one liquid (dispersant) into another phase; where each liquid is immiscible or poorly miscible in the other, e.g. oil and water (Purwanti *et al*., 2015). The biggest difference between emulsion and nanoemulsion is in the particle size. Where, when the size of the oil particles become small, the stability of the emulsion significantly improved (Anton and Vandamme, 2011). The suitable droplet sizes for nanoemulsions are in the range of 20 to 200 nm (Shah *et al.,* 2010). Recently, nanomaterials (particle size < 100 nm) are being developed and offer the opportunity to more efficient and safer administration of pesticides, fungicides, herbicides and fertilizers by better delivery of active ingredients and less environmental drift (Gogoi *et al.,* 2009). The activity of thyme essential oil as anti-fungal agent against species of *Aspergillus, Penicillium, Alternaria, Cladosporium, Rhizopus* and *Trichoderma* was mainly attributed to p-cymene, 1,8-cineole and other thymol constituens (Šegvic Klaric *et al*., 2007). Thyme oil-in-water nanoemulsions stabilized by a nonionic surfactant (Tween 80) were prepared as potential antimicrobial delivery systems (pH = 4). The nanoemulsions were highly unstable to droplet growth and phase separation, which was attributed to Ostwald ripening due to the relatively high water solubility of thyme oil (Ziani *et al*., 2011). In this work, Thyme essential oil was selected as biocide for the preparation of nanoemulsion of oil in water was formulated using non-ionic surfactants such as Tween 80 by ultrasonic emulsification method. Effect of sonication time in droplets size of nanoemulsion was investigated. The antifungal activities of thyme essential oil nanoemulsion were evaluated *in vitro* and *in vivo*.

MATERIALS AND METHODS

Preparation of nano- and non-nanoemulsion of thyme essential oil:

Essential oil of air dried herb thyme plants was extracted by using steam distillation method according to Al-Shahrani *et al.* (2017). Ten ml of thyme essential oil and 5 ml of non-ionic surfactant Tween 80 (5 ml) were added slowly under gentle stirring until a homogeneous mixture formed. Then, water (85 ml) was added to reach the final mixture to 100 ml, to help distribute and completely incorporate the thyme oil was then stirred using a magnetic stirrer for 30 min. The mixture was divided into two parts and were sonicated using an Ultrasonicator (Bande-lin SONOPULS HD 2200, Germany) for 15 and 60 min. at 700 W. The particle size of 10% thyme essential oil

nanoemulsion for each amount was detected by Hydrodynamic light scattering analyzer (DLS) after 90 days of storage under room temperature (27 ˚C). Thyme essential oil non-nanoemulsion was prepared as mentioned above before, without sonication.

Measurement of nanoemulsion droplet size:

Measurement of droplet size of nanoemulsion was performed by a dynamic light scattering analyses using Zeta Nano ZS (Malvern Instruments, UK) at room temperature. Prior to measurement, 30 µl of the 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. This work was performed by Nanotechnology Laboratory, Regional Center for Food & Feed, ARC, Giza, Egypt.

Transmission electron microscopy (TEM):

Twenty microliters of diluted samples was 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.

Effect of nano- and non-nanoemulsion of thyme volatile oil on growth of *S. sclerotiorum*:

The efficacy of volatile substances in reducing the fungal growth was also tested using the paper disc method as described by Šegvic Klaric *et al.*, (2007). Three plates containing PDA medium were inoculated with discs (5-mm-diam.) of *S. sclerotiorum*. The inoculated Petri dishes were inverted, then filter paper discs (5 mm) were saturated with different concentrations (0.0, 0.25, 0.50, 0.75, 1.0, 2.0, 3.0 %) of the different preparations of the volatile oil and placed at the center of the plate lids and incubated at 20 ˚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:

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

A= The linear growth in control treatment.

B= The linear growth of treated fungus.

Effect of nano- and non-nanoemulsion of thyme essential oil on seed germination:

Sets of 300 fennel seeds were soaked in each preparation of thyme essential oil (emulsion and nanoemulsion) at the rates (0.0, 0.25, 0.50, 0.75, 1.0, 2.0 and 3.0 %) for 15 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:

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

Greenhouse experiments:

In all greenhouse experiments, pots (15-cm-diam.) filled with soil (1 sand : 1 clay ,w/w) were used for planting. Formalin-disinfected soils were infested with *S. sclerotiorum* at a rate (3%) of soil weight. A set of three replicates were used for each treatment.

Suspension of the systemic fungicide, *i.e.* Switch 62.5% WG (a-Cyprodinil b-Fludioxonil) (2 g/L water) was used to compare the inhibitory effect of the fungicide. Seeds were soaked for 15 minutes in the previously prepared emulsions and the fungicide or in water only as control and planted in the infested soil. A set of 3 pots were used for each treatment and control. Sixty fennel seeds were planted for each treatment (20 seeds / pot). Percentages of pre- and post-emergence damping-off were recorded after 15 and 45 days. Healthy survival plants and their root length and plant height were determined 90 days after sowing.

Effect of nano- and non-nanoemulsion of thyme essential oil on some defense-related enzymes:

Fennel plants samples (about 10 gm/treatment) were obtained from each greenhouse treatments and control to determine peroxidase and polyphenoloxidase enzymes in plants at the same time of measuring the diseased stem parts. The samples were homogenized by a mortar and pestle with 10 ml of sodium phosphate buffer pH 6.8 (0.1 M). These triturated tissues were strained through four layers of cheese-cloth and the filtrates were centrifuged at 3000 rpm for 20 min. at 6 ^{o}C. The supernatant fluids were used for enzymes assay. The clear supernatant was taken as the enzyme source.

a. Peroxidase :

Peroxidase enzyme activity was determined colormetrically by measuring the oxidation of pyrogallol to purpurogallin in the presence of H_2O_2 at 425 nm. The reaction mixture contained 0.5 ml of 0.1 M sodium phosphate buffer solution at pH=7.0, 0.3 ml sample extract, 0.3 ml 0.05 M pyrogallol, and 0.1 ml of 1.0 % H_2O_2 , then completed with distilled water up to 3 ml. The activity was expressed as absorbance change per minute (Abs/min.) .

b. Polyphenoloxidase:

The activity of polyphenoloxidase was measured by the colorimetric method of (Quiles *et al.,* 2005). The reaction mixture contained 1.0 ml enzyme extract, 1.0 ml of 0.2 M sodium phosphate buffer at pH 7.0 and 1.0 ml of 10^3 N catechol brought to a final volume of 6.0 ml with distilled water. The activity of polyphenol oxidase was expressed as the change in absorbance 1.0 ml of extract per min. at 495 nm.

RESULTS AND DISCUSSION

Effect of ultrasonication time on nanoemulsion droplet size:

The effect of ultrasonication time on the droplet size of thyme essential oil nanoemulsion was determind. (Fig. 1,2). It showed the stable thyme essential oil nanoemulsions prepared by ultrasonication method with different ultrasonication times 15 and 60 min. at 700 W after 3 months of storage under room temperature. Sonication time had considerable effect on droplet size. The relative increase in sonication time could be due to an increase in shear forces applied on the droplets which cause more deformation and more fragmentation of the droplets. It is suggested to have small droplets size with increasing sonication time. Figure (1) show a particle size prepared by ultrasonication method for 15 min. It was tiny (around 207.2 nm), while particle size of nanoemulsion prepared by ultrasonication method for 60 min was decreased to around 34.6 nm as illustrated in Fig. (2). The decrease in the droplets size, may depend on the ability and performance of surfactants. Stirring is known to reduce droplet size in an oil – in – water emulsion (Sajjadi *et al*., 2002). The fabrication of nanoemulsion with lesser droplet size in the presence of double bonds in the nonpolar chain of non – ionic surfactants was evaluated by Dai *et al.* (1997). The obtained results were in agreement with different results (Anton and Vandamme, 2011 and Shahavi *et al.,* 2015).

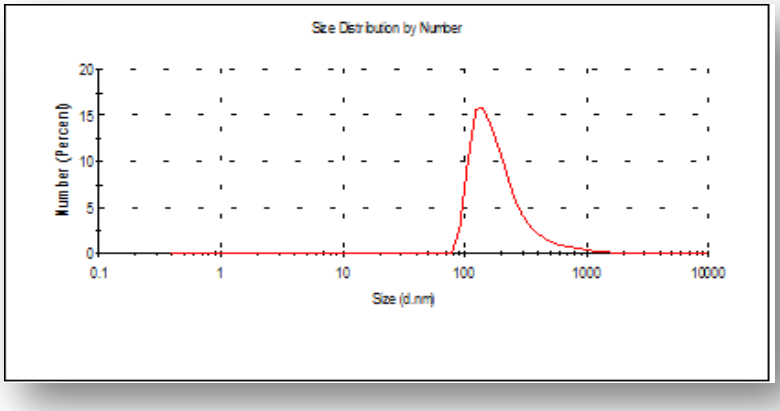

Fig. 1. Effect of ultrasonication on particle size of thyme essential oil nanoemultion prepared by ultrasonication method for 15 min. (ranging between 164 nm and 252 nm ; peak at 207.2 nm).

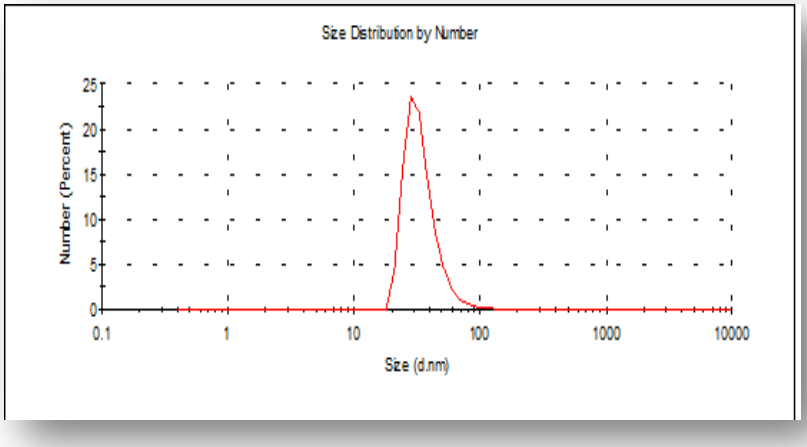

Fig. 2. Effect of ultrasonication on particle size of thyme essential oil nanoemultion prepared by ultrasonication method for 60 min. (ranging between 26.6 nm to 45.3 nm and peak at 34.6 nm)

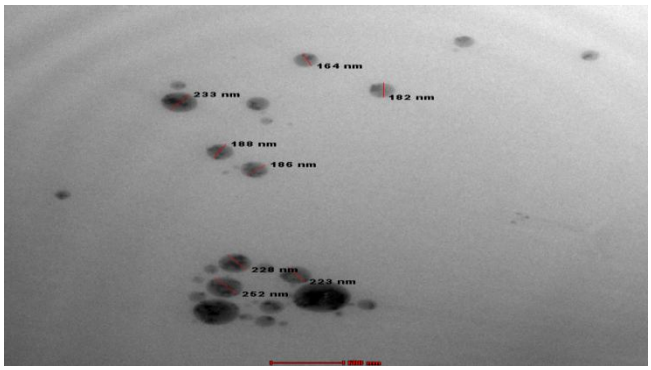

Fig.3. Transmission electron microscopic image of thyme essential oil nanoemultion prepared by ultrasonication method for 15 min. (size ranging from 164 nm to 252 nm).

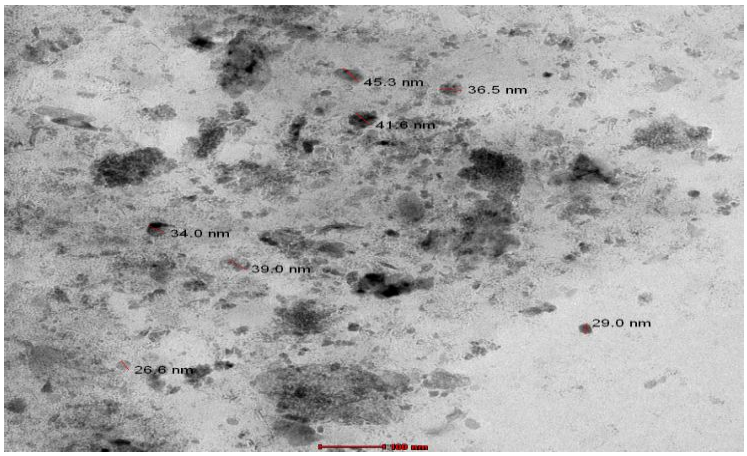

Fig.4. Transmission electron microscopic image of thyme essential oil nanoemultion prepared by ultrasonication method for 60 min. (size ranging from 26.6 nm to 45.3 nm).

Transmission electron microscopy (TEM):

Transmission electron microscopy characterization of thyme essential oil nanoemulsion gives the actual size and shape; the droplets in the nanoemulsion appear dark. The TEM micrograph showed that the thyme essential oil nanoemulsion was shapeless and spherical in shape and moderately mono or di-dispersed and was in the range of 164 – 252 nm (Fig. 3). While, TEM micrograph showed that the thyme essential oil nanoemulsion was spherical in shape and moderately mono or di-dispersed and was in the range of 26.6 – 45.3 nm (Fig.4). The droplet size was correlated well with the results obtained from droplet size analysis using the dynamic light scattering (Abd-Elsalam and Khokhlov, 2015).

Effect of nano- and non-nanoemulsion of thyme essential oil on fungal growth of *S. sclerotiorum*:

Data in Table (1) indicated that increasing concentration of thyme essential oil emulsion (TEOE) was always correlated with a decrease in linear fungal growth. The mycelial growth diameters, however, were 9.0 (control), 5.6, 3.5, 1.0 and 0.0 cm at 0.0(control), 0.25, 0.50, 0.75 and 1.0%, respectively. Whereas, the fungal growth was equal to the control (9 cm) with thyme essential oil nanoemulsion (TEON) (particle size >200 nm: around 207.2 nm) at the concentrations of 0.25, 0.50 and 0.75%. In addition, increased concentration of TEON (207.2 nm) to 1, 2 and 3% caused reductions in the fungal growths since they were 7.3, 3.7 and 0.0 cm, respectively. Using TEON (particle size <100 nm : around 34.6 nm) gave complte inhibition (100%) of the growth at the lowest concentration (0.25%). The biggest difference between emulsion and nanoemulsion is in the size of the nanoemulsion particles. When the size of the oil particles become small, the stability of the emulsion significantly improved (Anton and Vandamme, 2011). Nanomaterials (particle size <100 nm) are being developed and offer the opportunity to more efficiently and safely administration of pesticides, fungicides, herbicides and fertilizers by better delivery of active ingredients and less environmental drift (Gogoi *et al*., 2009). The antifungal toxic effects of different essential oils including thyme one under study were reported by Zedan *et al*. (1994). Thymol. p-cymene and 1,8-cineole present in thyme oil were found to be responsible for its antifungal activity as reported by Šegvic Klaric *et al.* (2007). However, thyme oil activity might due its chitin penetration of cell wall which damages the lipoprotein cytoplasmic membrane, leading to escape of cytoplasm (Zambonelli *et al*., 1996).

Table 1. The *in vitro* effect of six concentrations of nano- or non-nanoessential thyme oil on linear growth of *S. sclerotiorum*.

	Linear growth (cm) at concentration of:						
Treatments	0.0%	0.25%	0.50%	0.75%	1%	2%	3%
A*	9.0	5.6	3.4	1.0	0.0	0.0	0.0
B**	9.0	0.0	0.0	0.0	0.0	0.0	0.0
C***	9.0	9.0	9.0	9.0	7.3	3.7	0.0
L.S.D. at 5% for: Treatment (A)= 1.02 Concentrations (B)= 1.2							

*Thyme essential oil emulsion.

** Thyme essential oil nanoemulsion; particle size <100 nm : around 34.6 nm.

*** Thyme essential oil nanoemulsion; particle size >200 nm : around 207.2 nm.

Effect nano- and non-nanoemulsion of thyme essential oil on seed germination:

The results showed the effect of the nano- and non-nanoemulsions on germination of fennel seeds when used as soaking treatment to seeds for a certain period (Table, 2). Using nanoemulsion (particle size, around 207.2 nm) yielded 100% germination for treated seeds at the concentrations of 0.25%, 0.50% and 0.75%, while % of germination were sharply decreased to 26%, 22% and 20% at the concentrations of 1%, 2% and 3%, respectively. While, thyme essential oil nanoemulsion (particle size <100 nm: around 34.6 nm) gave 100% germination for seed at only the concentrations of 0.25% and 0.50%, then the germination% were sharply decreased to 20% and 0.0% at 0.75% and (1, 2 & 3%), respectively. Germination (%) were gradually increased by increasing concentration in case of thyme essential oil emulsion to reach 36.7%, 40.0%, 64.0%, 66.7% and 100% at the concentrations of 0.25%, 0.50%, 0.75%, 1% and (2% & 3%), respectively. Our 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) revealed that germination percentage of two thyme species attended a value of about 100% at control level (without treatment). In addition, germination percentages of *Lepidium sativum* values of 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 activity such nano compounds. The effect was both concentration and preparation dependent.

Table 2. Effect of nano- and non-nanoemulsion of thyme essential oil on percentage of seeds.germination

	% Concentrations						
Treatment	0.0%	0.25%	0.50%	0.75%	1%	2%	3%
A*	100	36.7	40.0	64.0	66.7	100	100
B**	100	100	100	20	0.0	0.0	0.0
C***	100	100	100	100	26	22	20
L.S.D. at 5% for: Treatment (A)= 13.4 Concentrations (B)= 9.8							

*Thyme essential oil emulsion.

** Thyme essential oil nanoemulsion; particle size <100 nm : around 34.6 nm.

*** Thyme essential oil nanoemulsion; particle size >200 nm : around 207.2 nm.

Greenhouse experiments :

Data presented in Table (3) showed that all the tested treatments significantly decreased the percentages of pre- and post- emergence damping-off caused by *S. sclerotiorum* and significantly increased survival plants compared with the controls. However, nanoemulsion (particle size around 34.6 nm) was the most effective treatment for decreasing percentages of pre- and post- emergence damping-off (13.3% and 16.7%, respectively). Subsequently, it recorded the highest percentage of increase in survival seedlings (319.2%) over the control.

Data (Table, 4) showed that all treatments tested increased plant growth parameters, *i.e.* plant height (cm) and root length (cm) than the control (without treatment). Differences between these treatments and the controls, were significant

Nanoemulsion (particle size around 34.6 nm) was generally the best seed soaking treatment which led to increase in plant growth parameters (plant height: 43.2% & root length: 103.7%) than the other treatments, followed by nanoemulsion (particle size around 207.2 nm) and Switch fungicide, except with Switch in case of root length. In contrast, Thyme essential oil emulsion showed the least effect on plant height (34.2%) and root length (92.6%) comparing with the control.

Efficiency of nanoemulsion may be due to the small size of its particles (Anton and Vandamme, 2011). The suitable droplet sizes for nanoemulsions are in the range of 20 to 200 nm (Shah *et al.,* 2010). Recently, nanomaterials (particle size < 100 nm) are being developed and offer the opportunity to more efficiently and safely administration of pesticides, fungicides, herbicides and fertilizers by better delivery of active ingredients and less environmental drift (Gogoi *et al.,* 2009).

Table 3. Effect of nano- and non-nanoemulsion of thyme essential oil and the fungicide Switch 62.5%WG on damping-off incidence % of fennel seedlings, grown in artificially infested soil, Under greenhouse conditions.

Treatments	Pre- emergence (%)	Post- emergence (%)	Survivals (%)	Increase # (%)
A*	23.3	26.7	50.0	199.4
B**	13.3	16.7	70.0	319.2
C***	20.0	30.0	50.0	199.4
Switch	23.3	26.7	50.0	199.4
Control	40.0	43.3	16.7	-
L.S.D. at 5%	14.3	9.7	21.4	-

Increase relative to the control.

*Thyme essential oil emulsion.

** Thyme essential oil nanoemulsion (particle size <100 nm : around 34.6 nm).

*** Thyme essential oil nanoemulsion (particle size >200 nm : around 207.2 nm).

Table 4. Effect of nano- and non-nanoemulsion of thyme essential oil on growth of fennel Plants grown in infested soil, under greenhouse conditions.

Treatments	Plant height (cm)	Increase # (%)	Root length (cm)	Increase # (%)
A*	25.5	34.2	15.6	92.6
B**	27.2	43.2	16.5	103.7
C***	27.0	42.1	16.4	102.5
Switch	27.2	42.1	13.7	69.1
Control	19.0	-	8.1	-
L.S.D. at 5%	5.6	-	5.2	-

Increases relative to the control .

*Thyme essential oil emulsion.

** Thyme essential oil nanoemulsion; particle size <100 nm : around 34.6 nm.

*** Thyme essential oil nanoemulsion; particle size >200 nm : around 207.2 nm.

Effect of nano- and non-nanoemulsion of thyme essential oil and the fungicide Switch on defense-related enzymes:

Data in Table (5) showed increases in the oxidative enzyme activities (Peroxidase and Polyphenoloxidase) due to treating fennel seeds with nano- and non-nanoemulsion of thyme essential oil and the fungicide Switch. Thyme essential oil nanoemulsion (particle size around 34.6 nm) was the treatment that led to the highest increase in peroxidase and polyphenoloxidase activities. In contrast, thyme essential oil emulsion exhibited lower Peroxidase and Polyphenoloxidase activities. The present results, in general, are in agreement with Hassanin (2013) who found that garlic and clove oils affected the presence of *F. oxysporum* in the soil when black cumin seeds were treated with oils before planting. Clove extract had the same effect in the presence of *F. semitectum* and *R. solani*. These plant extracts increased Peroxidase and Polyphenoloxidase activities in plants. Also, the obtained results are partially similar to those reported by Zambonelli *et al*. (1996) and Quiles *et al.* (2005). It was obvious that nano oil emulsions had an inhibitory effect on fungal growth, which was reflected in reduced with of pre- and post- emergence damping- off and an incease in peroxidase and polyphenoloxidase activities, respectively and increase in plant disease defense.

Table 5. Activity of Peroxidase and Polyphenoloxidase enzymes in fennel plants, resulting from seeds treated with nano- and non-nanoemulsion of thyme essential oil and the fungicide Switch.

Treatment	Peroxidase activity	Polyphenoloxidase activity
A*	0.59	0.17
B**	1.21	0.49
C***	1.19	0.43
Switch	1.12	0.33
Control	0.33	0.09

*Thyme essential oil emulsion.

** Thyme essential oil nanoemulsion; particle size <100 nm : around 34.6 nm.

*** Thyme essential oil nanoemulsion; particle size >200 nm : around 207.2 nm.

ACKNOWLEDGMENT

The researchers are deeply grateful to the late Dr. Arafa A. Hilal, Prof. of Plant Pathology, Ornamental, Medicinal and Aromatic Plant Dis. Res. Dept., Plant. Pathol. Res. Inst., ARC, Giza, Egypt, for useful suggestion and providing all the needed facilities.

REFERENCES

1. Abd-Elsalam, K. A. and A. R. Khokhlov. 2015. Eugenol oil nanoemulsion: antifungal activity against *Fusarium oxysporum f.* sp. *vasinfectum* and phytotoxicity on cotton seeds. Appl. Nanosci., 5: 255–265.
2. Al-Shahrani, M. H., M. Mahfoud, R. Anvarbatcha , Md T. Athar , A. Al Asmari. 2017. Evaluation of antifungal activity and cytotoxicity of *Thymus vulgaris* essential oil. Pharmacogn. Commn., 7(1) : 34-40.
3. Anton, N. and T. F. Vandamme. 2011. Nano-emulsions and micro-emulsions:clarifications of the critical differences. Pharm. Res., 28 : 978–985.
4. Barnet, K.J. and D.W. Hollomon. 1998. Fungicide resistance: The assessment of risk, pp. 48. FRAC Monograph No. 2, Global Protection Federation.
5. Beekman, A.C.; H.J. Woerdenbag; W.V. Uden; N. Pras; Wikstroemtiv and T.J. Schmidt. 1997. Structure-cytotoxicity relationship of some phelenanolide-type sesquiterpene lactones. J. of Nat. Products, 60:252-257.
6. Bolton, N.D.; P.H.J. Thoma and B.D. Nelson. 2006. *Sclerotinia sclerotiorum*(Lib) de Bary: Biology and molecular traits of cosmopolitan pathogen. Mol. Plant Pathol.,7: 1-16.
7. Dai, L.; W. Li and X. Hou. 1997. Effect of the molecular structure of mixed nonionic surfactants on the temperature of miniemulsion formation. Colloids and Surf. A-physicochemical and Eng. Aspects, 125: 27-32.
8. Feng, W. and X. Zheng. 2007. Essential oils to control *Alternaria alternate in vitro* and *in vivo*. Food Control 18:1126–1130.
9. Gogoi, R.; P.S. Dureja and K. Pradeep. 2009. Nanoformulations-A safer and effective option for agrochemicals. Indian Farming, 59:7-12.
10. Hassanin, M.M.H. 2013. Pathological studies on root rot and wilt of black cumin (*Nigella sativa*)and their management in Egypt. Ph. D. Thesis, Fac. Agric., Al-Azhar Univ. (Egypt), 137pp.
11. Hemada, M. and S. El Darier. 2011. Comparative study on composition and biological activity of essential oils of two *Thymus* species grown in Egypt. American-Eurasian J. of Agric. and Environ. Sci., 11(5): 647-654.

12. Hilal, A.A., M.G.A. Nada and Wafaa H. Zaky. 2007. Induced resistance against *Sclerotinia sclerotiorum* disease in some umbelliferous medicinal plants as a possible and effective control mean. Egypt. J. Phytopathol., 34(2):85-101.
13. Purwanti, N., M.A. Neves, K. Uemura, M. Nakajima, and I. Kobayashi. 2015. Stability of monodisperse clove oil droplets prepared by microchannel emulsification. Colloids and Surf. A: Physicochemical and Eng. Aspects, 466: 66–74.
14. Quiles, A., I. Hernando, I. Perez-Munuera, V. Larrea, E. Llorca and M. A. Lluch. 2005. Polyphenoloxidase (PPO) activity and osmotic dehydration in Granny Smith apple. J. Sci. Food Agric., 85:1017–1020.
15. Sajjadi, S.; M. Zerfa and W.B. Brooks. 2002. Dynamic behavior of drops in oil/water/oil dispersions. Chem. Eng. Sci., 57:663-675.
16. Šegvic Klaric, M., I. Kosalec, J. Mastelic, E. Pieckova and S. Pepeljnak. 2007. Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. Letters in applied microbiology, 44(1):36-42.
17. Shahavi, M. H. ; M. Hosseini; M. Jahanshahi; R. L. Meyer and G. N. Darzi 2015. Clove oil nanoemulsion as an effective antibacterial agent: Taguchi optimization method. Desalination and Water Treatment, 1-12.
18. Topps, J.H. and R.L.Wain. 1957. Investigations on fungicides: III.The fungitoxicity of 3- and 5-alkyl-salicylanilides and parachloroanilides. Ann. Appl. Biol. 45(3):506-511.
19. Zambonelli, A.; A. Bianchi and A. Albasini. 1996. Effect of essential oils on phytopathogenic fungi *in vitro* . Phytopathology, 86: 491-494.
20. Zedan, A. M.; A. M. E1- Toony and N. G. Awad. 1994. Comparative study on antifungal activity of certain plant extracts ,essential oils and fungicides on tomato wilt pathogens. A1-Azhar J.Agric. Res. (Egypt.), 20:217-236.
21. Zengin, H. and A. H. Baysal. 2015. Antioxidant and antimicrobial activities of thyme and clove essential oils and application in minced beef. J. of Food Processing and Preservation, 39 : 1261–1271.
22. Ziani,K.; Y. Chang; L. McLandsborough and D. J. McClements. 2011. Influence of surfactant charge on antimicrobial efficacy of surfactant-stabilized thyme oil nanoemulsions. J. Agric. Food Chem., 59 (11): 6247–6255.

تقييم فعالية المستحلب النانومترى لزيت الزعتر الطيار فى مقاومة مرض عفن الاسكليروتينيا على نبات الشمر

محمود محمد حسانين حسانين، أشرف عز الدين على حلاوة ، عبدالله عبدالمجيد على

معهد بحوث أمراض النباتات- مركز البحوث الزراعية- الجيزة- مصر

في هذا العمل، تم تحديد تأثير الزمن عند المعاملة بالموجات فوق الصوتية على حجم قطرات المستحلب النانومترى لزيت الزعتر الطيار. وأشارت النتائج إلى انخفاض حجم جزيئات المستحلب النانوى المعد بطريقة الموجات فوق الصوتية لمدة 15 دقيقة إلى حجم (حوالي 207.2 نانومتر) وأظهر الميكروسكوب الإلكترونى الشكل الكروى للجزيئات النانومترية، في حين انخفض حجم جزيئات المستحلب النانومترى المعد بطريقة الموجات فوق الصوتية لمدة 60 دقيقة إلى أصغر حجم (حوالي 34.6 نانومتر) وأظهر الميكروسكوب الإلكترونى أشكالا مشوهة وأخرى كروية للجزيئات النانومترية. علاوة على ذلك عند استخدام مستحلب زيت الزعتر الطيار بستة تركيزات (0.25% – 3%) ضد فطر *Sclerotinia sclerotiorum* كان قادرا على منع النمو الميسليومى للمسبب المرضى بدرجات مختلفة فى المعمل. وعند دعم بيئة آجار البطاطس بتركيزات من المستحلب النانومترى (حجم الجزيئات حوالي 34.6 نانومتر) ثبط النمو الفطرى تماما، بينما عند المعاملة بالمستحلب(حجم الجزيئات حوالي 207.2 نانومتر) توقف النمو الفطرى تماما عند التركيز 1٪. وعلى ذلك كانت المعاملة الأكثر فعالية في هذا الصدد المستحلب النانومترى(حجم الجزيئات حوالي 34.6 نانومتر). لم يتأثر إنبات بذور الشمر باستخدام أي من تركيزات مستحلب زيت الزعتر، في حين انخفضت نسبة إنبات البذور بحدة عند المعاملة بالتركيزات العليا من المستحلب النانومترى ، وقد كان المستحلب النانومترى (حجم الجزيئات حوالي 34.6 نانومتر) الأفضل في هذا المجال، حيث كانت المعاملة الأكثر فعالية فى خفض نسبة مرض موت البادرات قبل وبعد ظهورها فوق سطح التربة وتحسين نمو النبات في الصوبة. ولقد كانت المعاملة بالمستحلب النانومترى من أفضل المعاملات فى زيادة نشاط إنزيمى البيروكسيديز والبولى فينول أوكسيديز مقارنة بالمعاملات الأخرى.