Research Article

Antagonistic Effect of Nanoemulsions of Some Essential Oils against *Fusarium oxysporum* and Root-Knot Nematode *Meloidogyne javanica* on Coleus Plants

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Abstract | Efficacy of nanoemulsions and emulsions of thyme and spearmint essential oils were examined as an alternative to chemical control for suppressing *Meloidogyne javanica* and *Fusarium oxysporum* on coleus plants, *Coleus forskohlii in vitro* and greenhouse conditions. Nanoemulsions of thyme (droplets size was in the range of 25.4–32.9 nm) and spearmint (droplets size was in the range of 5.91–9.77 nm) at concentrations 4000 and 5000ppm separately, recorded the best results *in vitro* investigations, completely prevented *F. oxysporum* growth at all concentrations, and increased *M. javanica* mortality by 100%, in comparison to the non-treated control. In the greenhouse, thyme and spearmint essential oils nanoemulsions and emulsions (at 5000 ppm) affected significantly on the final population (Pf) of *M. javanica* (671.8 and 1072.4), while emulsions of spearmint and thyme nanoemulsion completely infection prevented after 50 days from planting in infested soil with *F. oxysporum*, but thyme and spearmint essential oils nanoemulsions and emulsions recorded effect on Pf about 1193.6 and 1465.6, respectively, compared to Fenamiphose (328.4) and fungicide, Occidor 50% SC was completely infection prevented. A similar pattern was discovered in a greenhouse with a positive effect on increased shoot dry weight for coleus plants, where thyme and spearmint essential oil nanoemulsions at (5000ppm) achieved 3.3 and 3.9 g/plants for root-knot nematode infected plants, respectively, compared to 2.7 and 4.4 g/plants for *F. oxysporum* infected plants.

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Introduction

Coleus, *Coleus forskohlii* (Wild) Briq. Belonging to the family Lamiaceae is one of the commercial ornamental plants grown extensively in Egypt and cultivated primarily in India, Sri Lanka, Nepal, and Yunnan Province in China. It is used as a bedding plant for public gardens in Egypt. *Coleus forskohlii*

is susceptible to many diseases, of which root rot and wilt disease caused by *Fusarium oxysporum* is a major constraint throughout the world (Miao *et al*., 2021; Halawa *et al*., 2018). Symptoms of the disease, which included wilting of the leaves, discolouration of the stem to brown and root rot (Zheng *et al*., 2012), However, root tubers are especially susceptible to the root-knot nematode *Meloidogyne incognita* Chitwood, which can reduce yields by up to 86 percent (Senthamarai *et al*., 2006a). Root-knot nematode also makes the root system vulnerable to infections by disease-causing fungi and bacteria. *Meloidogyne incognita* and *M. arenaria* have been shown to induce root-knot disease in *C. forskohlii* (Seenivasan and Devrajan, 2008) showed that *M. incognit*a infestations cause yield reductions of up to 86 percent, whereas *M. arenaria* infestations produce severe loses in *C. forskohli* (Bhandari *et al*., 2007; Kulkarni *et al*., 2007) identified a collar rot the complex including *C. forskohlii, F. chlaydosporum,* and *Rhizoctonia bataticola (Macrphomonia phaseolina)*. *C. forskohlii* has also been linked to a complex illness involving both fungal and nematode infections (Senthmarai *et al*., 2006b). Essential oils are compounds extracted from plants. The oils capture the plant's scent and flavor. Essential oils are also known as volatile oils, ethereal oils. The antifungal activity of essential oils against phytopathogens have previously been reported. For instance, lemongrass and thyme oils exhibited complete inhibition against *Fusarium oxysporum* (Baioumy, 1997). Also, soil treating with essential oils of *Eucalyptus citriodora* Hook., *E. globulus* Labill., *Pelargonium asperum* Ehrh. ex. Spreng. and *Ruta graveolens* L. reduced *Meloidogyne* sp. (Kofoid and White) Chitwood multiplication and gall formation on tomato roots (Laquale *et al*., 2015). Since *Meloidogyne* sp. is an endoparasite, a translatable compound that could affect the nematode inside root plants is desirable. This compound could be found in the essential oils, once it contains several antimicrobial ingredients that work through various modes of action. Eugenol, a constituent of *Ocimum sanctum* L. and others, have shown effect on the viability of nematodes, in addition to a systemic the effect (Bala and Sukul, 1987; Li *et al*., 2013; Moreira *et al*., 2013). Using Nanotechnology is a tool to modify nano-scale material characteristics, to improve the properties of the essential oils (Huang *et al*., 2010). The difference between essential oil emulsion and essential oil nanoemulsion is in the size of the oil particles. The stability of the emulsion is significantly improved when the size of the oil particles becomes small. Surfactants are added to oil-water mixture to enhance the kinetic stability of such a system. 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, the objective of this study was to assess the effects of emulsion and nanoemulsions of essential oils against *Fusarium oxysporum* and *Meloidogyne javanica* root-knot nematodes pathogenic infecting coleus plants to develop a management strategy for these pathogens.

Materials and Methods

Extraction, preparation and analysis of essential oils

Fresh herb of spearmint (*Mentha spicata* L.) and thyme (*Thymus vulgaris* L.) were collected from El-Kanater El- Khairia Farm, Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Center, Egypt. Samples from each plant were then shade dried and subjected to essential oil extraction. The essential oils were extracted by hydrodistillation using a Clevenger apparatus for 4 hours. Ten ml of each essential oil and 5 ml of non-ionic surfactant Tween 80 was added slowly with gentle stirring until a homogeneous mixture formed. Then, water (85 ml) was added to reach the final mixture of each oil to 100 ml, then stirred using a magnetic stirrer for 30 min. The mixture was sonicated using an Ultrasonicator (Bandelin Sonopuls HD 2200, Germany) for 30 min. at 700 W, all the treated essential oil was placed in an ice bath during the time of work. The particle size of 10% essential oils nanoemulsion for each amount was detected by Hydrodynamic light scattering analyzer (DLS) after 90 days of storage at room temperature (27°C). Essential oils emulsions were prepared as mentioned above before without sonication. 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 phenyls (equiv.) polysillphenylene-

siloxane 30 x 0.25 mm ID x 0.25 µ film. The temperature the 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. This work was performed by Laboratories of Medicinal and Aromatic Plants Research Department, Horticulture Research Institute and Research Department of Ornamental, Medicinal and Aromatic Plants Diseases, Plant Pathology Research Institute, Agricultural Research Center, Egypt.

Measurement of nanoemulsion droplet size

Measurement of droplet size of nanoemulsions was performed by dynamic light scattering analyses using Zeta Nano ZS (Malvern Instruments, UK) at room temperature. Before 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 disparity index (PDI) of the formulated nanoemulsion were measured. This work was performed by Nanotechnology Laboratory, Regional Center for Food and Feed, ARC, Giza, Egypt according to (Ghotbi *et al.*, 2014).

Transmission electron microscopy (TEM)

Twenty micro liters of diluted samples were placed on a film-coated 200-mesh copper specimen grid for 10 min and the excess fluid was eliminated using a 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), operating at 200 kV (Saloko *et al.*, 2013).

Extraction of Meloidogyne javanica

Root-knot nematode eggs were isolated from the egg masses on the roots of the *Coleus blumei* plant using the Hussey and Barker (1973) method. The eggs were transferred to flasks and incubated at 28°C for three days, after which the eggs hatched in water and active juveniles (J2s) of *M. javanica* were collected, according to Coyne *et al.*, (2007). A calculated suspension comprising viable juveniles was employed as an inoculum.

Source of F. oxysporum

The isolate of *F. oxysporum* used in this study was obtained from the fungal collection of Ornamental, Medicinal and Aromatic Plant Dis. Dept., Plant Pathol. Res. Inst. ARC, Giza, Egypt (Halawa *et al.*, 2018).

Effect of emulsions and nanoemulsions of spearmint and thyme essential oils on inhibition of F. oxysporum and M. javanica in vitro

Nematode, *M. javanica*: The inhibitory impact of the usual particle size of spearmint and thyme essential oil nanoemulsion against second-stage juveniles (J2s) of *M. javanica* was determined using five rates (1000, 2000, 3000, 4000, and 5000ppm). Second stage juveniles were extracted, counted, and concentrated in suspension until they reached contented 1 ml of distilled water roughly 100 J2s, according to the methodology reported by Demeure *et al.* (1981). 1 ml of juvenile suspension was placed into screw-capped test tubes containing 5 ml of various rates of tested materials and incubated at 26±2°C for 3 days, with the number of active and inactive juveniles counted at 24, 48, and 72 hours using a nematode counting slide (Hussey, 1973). Each treatment was repeated five times. The distilled water serve as a control. The Schneider and Orelli's formula was used to compute the revised mortality percentages (Schneider and Orelli, 1947).

$$\text{Mortality inhibition \%} = \left(\frac{\text{Number of dead juveniles in treatmentl} - \text{Number of jdead juveniles in control}}{100 - \text{mortality percentage in the negative control)] } \times \text{ 100Number ofdead juveniles in control}}\right) x100$$

***Fusarium oxysporum*:** The efficacy of volatile substances in reducing fungal growth was tested. Essential oils of spearmint and thyme (emulsions and nanoemulsions) were added to sterilized PDA flasks before solidifying to obtain the proposed concentrations of 1000, 2000, 3000, 4000 and 5000 ppm (v/v). The bactericide (Chloramphenicol, 0.1mg/l) was added to the medium to avoid bacterial contamination. Three plates for each treatment were inoculated with discs (5-mm-diam.) of *F. oxysporum*. Petri dishes were incubated at 27±1°C is randomized complete design. Percentages of fungal growth inhibition was calculated when the fungal growth of the control plates filled the plates according to the formula as follows:

$$\text{Inhibition \%} = \left(\frac{\text{The linear growth in control treatment} - \text{The linear growth of treated fungus}}{\text{The linear growth in control treatment}}\right) x100$$

Greenhouse experiments

Essential oils emulsions and nanoemulsions of spearmint and thyme at the concentration (5 ml/L water) were tested for controlling *C. forskohlii* wilt and root rot diseases caused by a root-knot nematode, *M.*

javanica and *F. oxysporum* fungus in pot experiments compared with treatments of nematicide, Fenamiphose 40 % EC. (Common name: Nemacur, Chemical composition: Ethyl-3methyl-4 (methyl thiophenyl)-methyl-ethyl) phposphoramidate. (6 ml/100 L water) and fungicide, Occidor 50% SC [Common name: Carbendazim, Chemical composition: Methyl 2-benzimidazole carbamate, Manufacture: Agriphar S.A., Belgium.] (2 g/L water) and untreated plants under greenhouse conditions during spring season 2021.

Three seedlings of coleus (20 days old) was planted in 25 cm plastic pot. Pots containing a mixture of clay and sandy soil (1:1 w/w). The coleus seedlings were infected with *F. oxysporum* at the rate of 1% (w/w), and 1000 newly hatched juveniles (J2s) of *M. javanica* for each one kg soil, so each pot contained 3000 hatched juveniles (J2s) of *M. javanica* plant after one week from seedlings. Each application was repeated five times, with the application of each spearmint and thyme essential oil nanoemulsion. As well as the same applications with nematicides Fenamephose (6 ml/100 L water) and fungicides Occidor 50% SC (2 g/L water) were replicated five times. All treatments were applied as a soil drench. Five inoculated by *M. javanica* and another separated inoculated by *F. oxysporum* pots were left without adding any materials as a negative control, in addition to another five replicated healthy seedlings without inoculation with nematodes and fungus as positive control. All pots were arranged in the greenhouse at 27±5°C in randomized block design. After fifty days of inoculation with *M. javanica* and *F. oxysporum,* the plants were harvested. Percent dead plants were recorded (Booth, 1971) and juveniles of *M. javanica* were extracted from one kg. soil/pots by sieving and modified Baeman technique (Seinhorst, 1962). Roots were stained by acid fuchsin (Bybd *et al.*, 1983) and examined under a stereomicroscope for counting developmental stages, females, galls, and egg masses. Root galling or egg masses were rated on a scale of 0-5 where 0= no galls or egg masses, 1= 1-2 galls or egg masses, 2= 3-10 galls or egg masses, 3= 11-30 galls or egg masses, 4= 31-100 galls or egg masses, 5= more than 100 galls or egg masses per root system (Taylor and Sasser, 1978). At the end of the experiment, shoot length, root length, shoot weight, root weight and shoot dry weight per plant were also recorded.

$$\text{Dead plants \%} = \left(\frac{\text{No. of dead plants}}{\text{Total No. of plants.}}\right) x100$$

Data analysis

The data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) and Duncan's multiple range tests in the form of probability ($P \leq 0.05$) (Duncan, 1955) using Costat software was used to separate means.

Results and Discussion

Chemical composition of essential oils emulsions and nanoemulsions

Chemical composition of investigated essential oils emulsions and nanoemulsions analyzed by gas chromatography is presented in Table 1. Carvone (60.03%) and Limonene (16.63%) were identified as major constituents of spearmint essential oil emulsion. Thymole (23.62%) and ρ-cymene (52.36%) were identified as the major constituents of thyme essential oil emulsion. Changes in the essential oil components were observed in the nanoemulsions compared with the corresponding original essential oils emulsions. Data presented in Table 1 show the main component content of spearmint oil nanoemulsions Carvone and ρ-cymene (66.34% and 7.36%, respectively) were increased, also, in thyme oil nanoemulsions was Borneol increased (9.03%), while, ρ-cymene and Thymol (29.18% and 14.40%, respectively) were decreased compared with essential oil emulsion.

Effect of ultrasonication on nanoemulsion droplet size

The effect of ultrasonication on the droplet size of essential oils of spearmint and thyme nanoemulsions was determined. Figure 1A shows the stable essential oil nanoemulsions prepared by ultrasonication method for 30 min. at 700 W after 3 months of storage under room temperature. Tween 80 was used as the surfactant for its high HLB value that favors the formulation of oil in water emulsion. Also, small molecule surfactants like Tween 80 gets rapidly adsorbed onto emulsion droplet surface and hence they are more effective in decreasing droplet diameter than polymeric surfactants (Ghosh *et al.*, 2014). Spearmint nanoemulsion droplets were tiny (around 6.4 nm) (Figure 1). Thyme nanoemulsion droplets were tiny (around 48.1 nm) (Figure 2A) (Sampathi *et al.*, 2015; Hassanin *et al.*, 2017).

Transmission electron microscopy (TEM)

Transmission electron microscopy characterization of spearmint and thyme essential oil nanoemulsions gives the actual size and shape, the droplets in the

nanoemulsion appears dark. The TEM micrograph showed that the essential oils nanoemulsions were spherical and moderately mono or di-dispersed. Spearmint nanoemulsion droplets were in the range of 5.91–9.77 nm (Figure 1B). Thyme nanoemulsion droplets were in the range of 25.4 – 32.9 nm (Figure 2B). The droplet size was correlated well with the results obtained from droplet size analysis using the dynamic light scattering (Abd-Elsalam and Khokhlov, 2015; Hassanin *et al.*, 2017).

Table 1: *Chemical composition of essential oils emulsions and nanoemulsions.*

Components (%)	Essential oils of			
	Spearmint (*Mentha spicata* L.)		Thyme (*Thymus vulgaris* L.)	
	Emulsion	Nano-emulsion	Emul-sion	Nano-emulsion
α-thujene	-	-	-	-
α-pinene	1.07	0.72	1.76	1.63
Camphene	-	-	1.83	1.76
Sabinene	-	-		-
β-pinene	-	-	1.87	1.76
β-myrcene	2.49	1.18	1.75	7.25
α-terpinene	-	-	-	-
ρ-cymene	1.27	7.36	52.36	29.18
Limonene	16.63	4.96	0.96	6.74
1,8-cineol	6.54	2.04	-	-
β-ocimene	1.48	1.58	1.10	9.45
γ-terpinene	-	-	-	-
α –terpinolene	-	-	2.09	3.98
Linalool	-	-	-	-
Menthone	-	-	-	-
Iso menthone	-	-	-	-
Menthofuran	-	-	-	-
Borneol	-	-	5.80	9.03
Menthol	-	-	-	-
Terpinene-4-ol	-	-	-	-
γ-terpineol	1.12	1.47	-	-
Dihydrocarveol	3.32	3.83	-	-
Dihydrocarveon	0.29	2.41	-	-
Methyl chavicol	-	-	-	-
Carvone	60.03	66.34	-	-
Menthyl acetate	-	-	-	-
Thymol	-	-	23.62	14.40
Eugenol	-	-	-	-
β-caryophyllene	-	0.95	2.65	0.79
Caryophyllene oxide	-	2.59	-	-

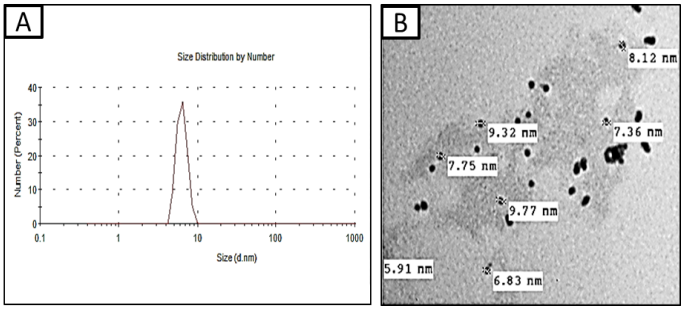

Figure 1: *Effect of ultrasonication on particle size (A) and TEM (B) of spearmint essential oil nanoemulsion.*

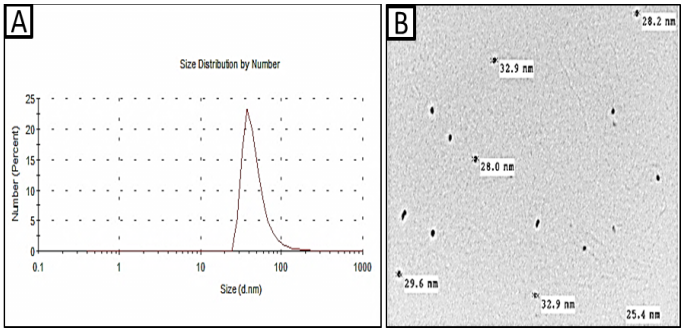

Figure 2: *Effect of ultrasonication on particle size (A) and TEM (B) of thyme essential oil nanoemulsion.*

In vitro efficacy of essential oils emulsions and nanoemulsions on two bioagantis

Mycelial growth of *F. oxysporum*: Data in Tables 2 and 3, represented the effect of spearmint and thyme essential oil emulsions and essential oil nanoemulsion solutions at five concentrations of 1000, 2000, 3000, 4000 and 5000 ppm (v/v) on juveniles mortality of *M. javanica* and mycelial growth of *F. oxysporum*. Data in Table 2, indicated that increasing concentration of essential oil emulsions and nanoemulsions can be correlated with a decrease in linear fungal growth. Nanoemulsion of thyme oil completely inhibited growth of *F. oxysporum* at the 2000 ppm concentration. While, spearmint essential oil nanoemulsion completely inhibited the growth of *F. oxysporum* at the 4000 ppm concentration. Nanoemulsions were always the most active against fungal growth at all concentrations. The biggest difference between emulsion and nanoemulsion is in the size of the nanoemulsion particles.

The mortality percentage of *M. javanica*

The effect of spearmint and thyme essential oil emulsions and essential oil nanoemulsion solutions at five concentrations of 1000, 2000, 3000, 4000, and 5000 ppm (v/v) on *M. javanica* juvenile mortality was reflected in Table 3 and Figure 1. When compared to the non-inoculated control, all of the tested therapies

were found to generate a significant increase in juvenile *M. javanica* death %. In general, the effect of the essential oil emulsions and nanoemulsions investigated varies depending on the concentrations and exposure time. Whereas, in terms of the effect of the relationship between concentration and exposure time as a steady relationship, all treatments were defined by a stable line, as this offered a stable perception of the effective value, especially at higher concentrations. According to the findings, the higher rates (5000 ppm) of thyme and spearmint essential oil nanoemulsions resulted in the highest mortality increase percentages (100%) at the end of the 24-hour test. When evaluating the middle effect of the lowest rates (2000 and 3000ppm) of thyme and spearmint essential oil emulsions (1000 ppm), which was the polar opposite of what was expected as a biomaterial, a reduced percentage of the morality of *juvenile* to *M. javanica* juvenile was reached, respectively, of 61.8 and 37.5%.

Table 2: *Effect of essential oils emulsions and nanoemulsions at different concentrations on mycelial growth of F. oxysporum*

Mean	Linear growth (cm) of mycelial growth at different concentrations (ppm)						Treatments
	5000	4000	3000	2000	1000	Control	
5.5	0.0 i	3.0fg	5.1d	7.5 b	8.3 ab	9.0 a	Spearmint emulsion
4.6	0.0 i	0.0 i	4.6 e	6.0 c	7.9 b	9.0 a	Spearmint nanoemulsion
2.4	0.0 i	0.0 i	0.0 i	1.5 h	3.9 f	9.0 a	Thyme emulsion
2.0	0.0 i	0.0 i	0.0 i	0.0 i	2.7 g	9.0 a	Thyme nanoemulsion
-	Treatments (T)= 0.5 Concentrations (C) = 0.1 T X C = 0.2						L.S.D. at 5% :

Each value is the mean of five replicates. Means in each column followed by the same letter(s) are not significantly different (P≤0.05) by Duncan's multiple range test.

Greenhouse experiments

Evaluation of spearmint and thyme essential oil emulsions and essential oil nanoemulsion solutions against *Fusarium oxysporum* wilts of coleus plants and root-knot nematodes in a greenhouse setting *(M. javanica*). This investigation looked at the effects of two essential oil emulsions and two essential oil nanoemulsion solutions on wilt disease and root-knot incidence and severity % on coleus plants inoculated with *F. oxysporum* and *M. javanica* in greenhouse settings.

Table 3: *Evaluation of thyme and spearmint essential oil nanoemulsion solutions on the mortality percentage of second stage juveniles of M. javanica at different exposure periods in vitro (25±2 ˚C).*

Treatments	concentrations (ppm) V/V	Mortality %		
		24h.	48h.	72h.
Spearmint emulsion	1000	35.7	52.6	70.1
	2000	49.7	68.9	84.3
	3000	66.5	81.2	97.2
	4000	78.9	96.6	100
	5000	100	100	100
Spearmint nanoemulsion	1000	62.1	76.2f	82.3
	2000	73.6	82.0	89.7
	3000	85.1	90.1	99.9
	4000	96.3	100	100
	5000	100	100	100
Thyme emaulsion	1000	47.8	63.	86.7
	2000	61.8	79.8.6	96.0
	3000	77.8	88.9	98.7
	4000	87.2	96.7	100
	5000	100	100	100
Thyme nanoemaulsion	1000	61.8	75.3	91.2
	2000	75.1	90.2	99.1
	3000	82.6	91.3	100
	4000	94.3	100	100
	5000	100	100	100
Control	-	1.4	2.9	3.8

Each value presented the mean of five replicates.

Evaluation of spearmint and thyme essential oil emulsions and essential oil nanoemulsion solutions against *F. oxysporum*: Drenching soil with various control treatments resulted in an increase in coleus resistance against infection with the tested fungi and nematode (Table 4). Data in Table 4 indicate that all treatments reduce disease incidence. Spearmint emulsion, thyme emulsion, thyme nanoemulsion and fungicide, Occidor 50% SC completely infection prevented after 50 days from planting in infected soil with *F. oxysporum* compared with the control. Whereas spearmint emulsion and spearmint nanoemulsion completely infection prevented after 50 days from planting in infected soil with nematodes compared with the control. The contrast, spearmint nanoemulsion was the least effective treatment in decreasing disease incidence (%) in infected soil with *F. oxysporum*, while thyme emulsion was the least effective treatments in decreasing disease incidence (%) in infected soil with the nematode.

Table 4: *Effect of various control treatments as soil drenching on incidence (%) of root rot and wilt disease of coleus plants 50 days after planting in soil infected with pathogenic root-knot nematode, M. javanica and F. oxysporum fungus, under greenhouse conditions (27±3 °C).*

Treatment	% Dead plants after 50 days		% Plant survival	
	Fungi	Fungi+ Nematode	Fungi	Fungi+ Nematode
Spearmint emulsion	0.0 c	0.0 d	100 a	100 a
Spearmint nanoemulsion	13.3 b	0.0 d	86.7 b	100 a
Thyme emaulsion	0.0 c	20.0 b	100 a	80 b
Thyme nanoemaulsion	0.0 c	6.7 c	100 a	93.3 a
Occidor 50% SC	0.0 c	0.0 d	100 a	100 a
Untreated plant(inoculated)	40.0 a	53.3 a	60 c	46.7 c
Untreated plant(uninoculated)	0.0 c	0.0 d	100 a	100 a
LSD at 0.05	1.5	2.1	12.4	8.6

Each value is the mean of five replicates. Means in each column followed by the same letter(s) are not significantly different (P≤0.05) by Duncan's multiple range test.

Table 5: *Effect of spearmint and thyme essential oils emulsions and nanoemulsions solutions on plant length of coleus infected with, M. javanica and F. oxysporum under greenhouse conditions (27±3°C).*

Treatment	Shoot length			Root length		
	Nematode	Fungi	Nematode + Fungi	Nematode	Fungi	Nematode + Fungi
Spearmint emulsion	30.7_{d}	36.7_{d}	32.4_{d}	6.0_{b}	7.1_{cd}	6.4_{d}
Spearmint nanoemulsion	37.7_{c}	43.5_{c}	40.0_{c}	$9.0a_{b}$	9.7_{bc}	7.4_{bc}
Thyme emaulsion	49.4_{ab}	52.7_{b}	$41.4b_{c}$	12.4_{a}	6.4_{de}	9.7_{b}
Thyme nanoemaulsion	52.7_{a}	61.7_{a}	$43.4b_{c}$	6.7_{b}	16.7^{a}	9.4_{b}
Fenamephose and/or Occidor 50% SC	47.1_{b}	45.2_{c}	45.9_{b}	8.4_{ab}	5.4_{de}	9.5_{b}
Untreated plant (inoculated)	27.8_{d}	30.3_{e}	27.2_{e}	6.7_{b}	4.4_{e}	3.2_{c}
Untreated plant (uninoculated)	52.7_{a}	52.7_{b}	52.7_{a}	12.4_{a}	12.4_{b}	12.4_{a}
L.S.D. at 5%:	4.49	4.26	4.91	4.27	3.02	2.36

Each value is the mean of five replicates. Means in each column followed by the same letter(s) are not significantly different (P≤0.05) by Duncan's multiple range test.

Effect of experimental spearmint and thyme essential oil emulsions and nanoemulsion solutions on growth parameters of coleus plants which afflicted with pathogenic *M. javanica* and *F. oxysporum* under greenhouse conditions: Data presented in Table 5 show that all treatments tested gave significant increases in shoot length compared with the controls (without treatment) in soil infected with each of pathogenic root-knot nematode, *M. javanica* and *F. oxysporum* fungus, except spearmint emulsion with soil infested with the nematode, however, thyme nanoemulsion was the superior treatments in increasing shoot length compared with the other treatments in cases of nematode and fungi followed by thyme emulsion treatment. Whereas (Fenamephose + Occidor 50% SC) was the superior treatment in increasing shoot length compared with the other treatments in cases of (nematode + fungi). In this respect, spearmint emulsion was the least effective treatment in increasing shoot length with the nematode, fungi and (nematode + fungi) tested. On the other hand, thyme emulsion was the superior treatments in increasing root length compared with the other treatments in cases of nematode and (nematode + fungi), while thyme nanoemulsion was the superior treatments in increasing root length compared with the other treatments in cases of fungi.

Also, data in Table 6 show that spearmint nanoemulsion was the superior treatment in increasing shoot weight, root weight and shoot dry weight compared with the other treatments in cases of nematode and/or fungi, except root weight and shoot dry weight in case of (nematode + fungi), while thyme nanoemulsion treatment was the superior treatments in increasing root weight compared with the other treatments in cases of (nematode + fungi), whereas (Fenamephose + Occidor 50% SC) was the superior

treatments in increasing shoot dry weight compared with the other treatments in cases of (nematode + fungi).

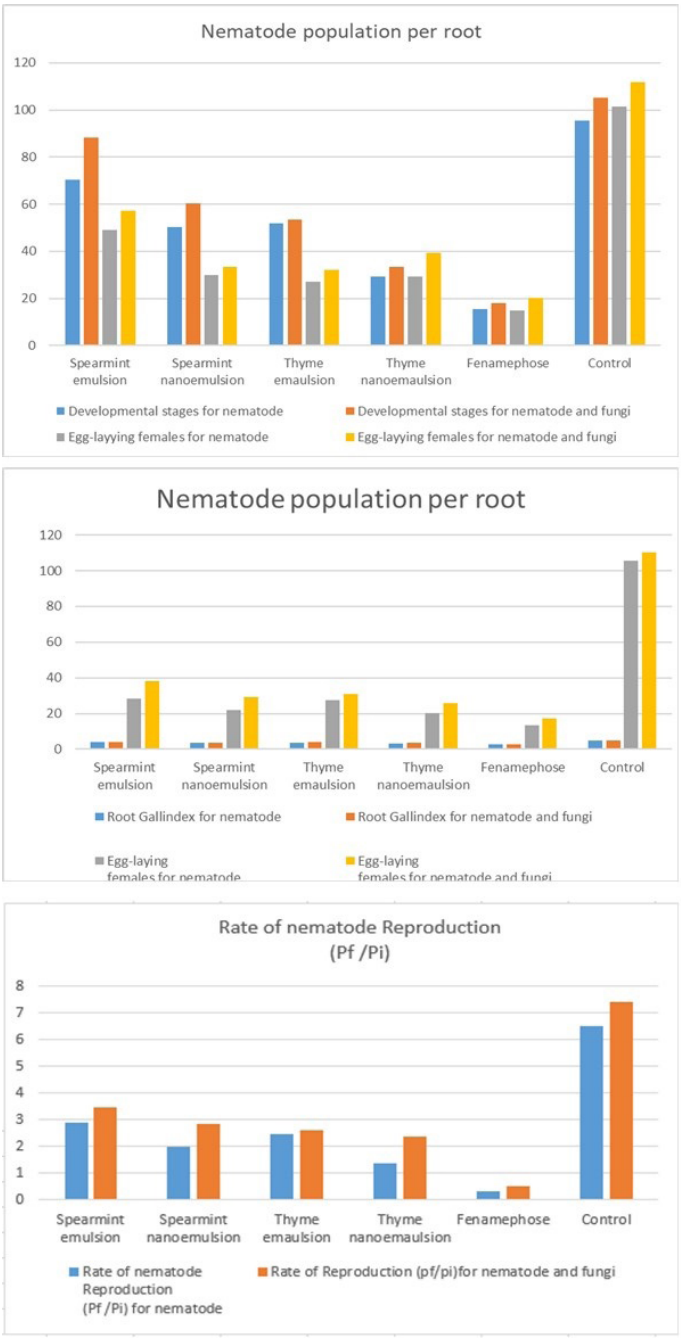

Figure 3: *Nematode population per root of plants as second stage J_2 in soil, developmental stages in root Egg-laying females, root gall index and rate of reproduction (pf/pi) for root-knot nematode M. javanica.*
Pf= (no. Egg-masses per root × no. Eggs per Egg – masses) + (Development stage/ root) + (Juveniles in soil) + (Adult females/ root). Nematode build –up = Pf/ Pi

Efficacy of tested spearmint and thyme essential oils emulsions and nanoemulsions solutions against *M. javanica*: The purpose of this study was to see how they tested materials (spearmint and thyme essential oil emulsions and essential oil nanoemulsion solutions 5000 ppm. and Fenamiphose) affected the population of *M. javanica* infected coleus plants during the regular growing season (2021). In general, the current study found that all tested treatments reduced root-knot nematode parameters, such as the number of root galls, build-up, and nematode reduction, to varying degrees, as compared to control and chemical nematicide. All treatments resulted in a significant reduction in the total number of root-knot juvenile nematodes in the soil and on the roots of coleus plants, as shown in Figure 3 and Tables 7, with nematode build-up rates ranging from 0.35 percent for infected only with *M. javanica* to 0.5 percent for infected only with *M. javanica* and *F. oxysporum* for Fenemiphose to 3.46 and 2.89 percent Fenemiphose provided the greatest reduction in the nematode population, root galls index, and egg masses for *M. javanica* witch infested coleus plants only (328.4, 13.9, and 9.2), while in complex infection by *M. javanica* and *F. oxysporum*, Fenemiphose provided the the greatest reduction in the nematode population, root galls index, and egg masses for *M. javanica* witch infested coleus plants only (328).

Following that, Thyme nanoemulsion solutions 5000 ppm resulted in significant reductions in nematode population, root galls index, and egg masses (671.8, 26.7, and 17.7) for *M. javanica* alone infested coleus plants, while mixed infection yielded (695.9, 29.5, and 19.6).

In both cases, infection to coleus plants, Spearmint nanoemulsion, and Thyme emulsion solutions 5000 ppm caused a medium reduction in nematode population, root galls index, and egg masses for root-knot nematode. Infected only by *M. javanica* 1465.6 and 1582.3 for infected with root-knot nematode and *F. oxysporum*, respectively, Spearmint emulsion, solutions 5000 ppm, had the lowest nematode population levels, with infected only by *M. javanica* 1465.6 and 1582.3 for infected with root-knot nematode and *F. oxysporum*, respectively. Root gall index and egg masses of *M. javanica* infested coleus plants or *F. oxysporum* revealed a similar pattern of treatments (Table 7).

Changes in the essential oil components were observed in the nanoemulsions compared with the corresponding original essential oils emulsions. Carvone and Limonene were identified as major

Table 6: *Impact of spearmint and thyme essential oils emulsions and nanoemulsions solutions on fresh and dry weight of coleus plant infected with M. javanica and F. oxysporum under greenhouse conditions(27±3˚C).*

Treatments	Shoot weight			Root weight			Shoot dry weight		
	Nematode	Fungi	Nematode + Fungi	Nematode	Fungi	Nematode + Fungi	Nematode	Fungi	Nematode + Fungi
Spearmint emulsion	20.7^{cd}	20.8^{c}	17.7^{c}	5.83^{d}	4.7^{b}	2.8^{d}	3.4^{a}	3.1^{bc}	2.6^{b}
Spearmint nanoemulsion	26.8^{a}	27.0^{a}	20.1^{ab}	7.47^{a}	5.63^{a}	5.7^{b}	3.9^{a}	4.4^{a}	2.6^{b}
Thyme emaulsion	23.2^{bc}	26.3^{a}	19.5^{b}	3.73^{g}	5.57^{a}	5.8^{b}	1.8^{bc}	3.7^{ab}	2.7^{b}
Thyme nanoemaulsion	24.9^{ab}	23.3^{b}	17.1^{cd}	6.46^{b}	3.37^{d}	6.2^{a}	3.3^{a}	2.7^{c}	1.4^{c}
Fenamephose and/or Occidor 50% SC	24.1^{ab}	20.8^{c}	16.2^{e}	4.13^{f}	4.03^{c}	2.9^{d}	2.9^{ab}	4.1^{a}	4.1^{a}
Untreated plant (inoculated)	18.1^{d}	20.8^{c}	16.2^{de}	6.1^{c}	3.53^{d}	5.6^{b}	1.5^{c}	1.2^{d}	0.9^{c}
Untreated plant (uninoculated)	20.1^{cd}	20.8^{c}	21.4^{a}	4.83^{e}	4.83^{b}	4.8^{c}	3.8^{a}	3.8^{ab}	3.8^{a}
L.S.D. at 5%:	2.9	2.5	0.99	0.175	0.23	0.27	1.26	0.96	0.95

Each value is the mean of five replicates. Means in each column followed by the same letter(s) are not significantly different (P≤0.05) by Duncan's multiple range test.

Table 7: *Efficiency of essential oil emulsions and essential oil nanoemulsion solutions on population density of M. javanica alone or interacted with Fusarium wilt disease under greenhouse condition(27±3˚C).*

Treatment	Nematode final population		Galls (RGI)		Egg masses (GI)	
	Nematode	Nematode+ Fungi	Nematode	Nematode+ Fungi	Nematode	Nematode+ Fungi
Spearmint emulsion	1465.6b	1582.3b	60.8b	67.1b	40.2b	44.6b
Spearmint nanoemulsion	1072.4d	1171.8c	44.9c	49.7cd	29.1c	32.9b
Thyme emaulsion	1193.6c	1221.6c	46.8c	51.8c	31.1c	36.5b
Thyme nanoemaulsion	671.8e	695.9d	26.7d	29.5d	17.7d	19.6c
Fenamiphose	328.4f	361.9e	13.9e	15.4e	9.2d	12c
Control	3490.6a	3645.8a	139.7a	154.7a	92.7a	102.8a
L.S.D. at 5%	50.9	233.4	11.41	13.5	10.2	12.3

Each value is the mean of five replicates. Means in each column followed by the same letter(s) are not significantly different (P≤0.05) by Duncan's multiple range test. Nematode final population= No. of nematode in one Kg. soil + one root for plant.

constituents of spearmint essential oil emulsion. Thymole and ρ-cymene were identified as the major constituents of thyme essential oil emulsion.

Data presented show that main component content of spearmint oil nanoemulsions Carvone and ρ-cymene were increased, also, in thyme oil nanoemulsions was Borneol increased, while, ρ-cymene and Thymol were decreased compared with essential oil emulsion.

The components of essential oils include different groups of distinct biosynthetically origin. The main group is composed of terpenoids, phenylpropanoids, and short-chain aliphatic hydrocarbon derivatives, which are all characterized by low molecular weight. Terpenes are made from combinations of several 5- carbon-base (C5) units called isoprene and form structurally and functionally different classes. The biosynthesis of the terpenes consists of synthesis of the isopentenyl diphosphate (IPP) precursor, repetitive addition of IPPs to form the prenyldiphosphate precursor of the various classes of terpenes, modification of the allylic prenyldiphosphate by terpene specific synthetases to form the terpene skeleton, and, finally, secondary enzymatic modification (redox reaction) of the skeleton to attribute functional properties to the different terpenes (Bilia *et al.*,2014). It is suggested that, maybe there was a breakdown of ultrasonic chemical bonds during the manufacture of the nanoemulsion. Once again the atoms are redistributed through the donor atom, which carries a positive charges such as the hydrogen atom and the recipient the atom with a negative charge such as the oxygen atom.

The compounds are then formed within aromatic oils again but at different concentrations. *In vitro* and *in vivo* investigations, the results showed that all treatments, including thyme and spearmint essential oil nanoemulsions, solutions, have a positive effect on *F. oxysporum*, and *M. javanica*. At 4000 and 5000 ppm

concentrations from two essential oil nanoemulsion were used to completely stop *F. oxysporum* growth at all concentrations. Nanoemulsions have been working efficiently for six months, compared to ordinary oil emulsions. The most notable difference between emulsion and nanoemulsion is the size of the nanoemulsion particles.

According to the data, the highest concentrations (5000 ppm) of thyme and spearmint essential oil nanoemulsions resulted in the highest mortality reduction percentages (100%) for *M. javanica* at the end of the 24-hour test. *In vivo* experiments, the results observed in decreasing diseases incidence (%) in infested soil with *F. oxysporum*, while and a greater reduction in the final population *M. javanica* juveniles in the soil. The most notable difference between emulsion and nanoemulsion is the size of the nanoemulsion particles. According to the data, the highest concentrations (5000 ppm) of thyme and spearmint essential oil nanoemulsions resulted in the highest mortality percentages (100%) at the end of the 24-hour test.

The main finding was that essential oil nanoemulsion solutions were more effective as a natural nematicide on *M. javanica* and a fungicide on *F. oxysporum*. The size of the nanoemulsion particles is the difference between emulsion and nanoemulsion.

The emulsion's stability improves considerably when the size of the oil particles is lowered (Anton and Vandamme, 2011). Nanomaterials (tiny particles) are a type of material (Zedan *et al*., 1994). Because essential oils (which have low water solubility) are larger than nanoemulsion particles, they cannot easily interact with cell membranes. However, nanoemulsion particles can deliver essential oils to the surface of nematode cell membranes, which could be related to the ability of smaller particles to kill or hinder the nematode at any stage of its life cycle (Pérez *et al*., 2003; Barbosa *et al*., 2010).

Nanoemulsions are generated from a specific the concentration of oil phase, surfactant, and water, with no phase separation, and are kinetically stable for more than six months at room temperature (Abd-Elsalam and Khokhlov, 2015; Hassanin *et al*., 2018). The ability and performance of surfactants may influence the size reduction of droplets. In oil in water emulsion, stirring is known to diminish droplet size (Sajjadi *et al*., 2002). Dai *et al*. (1997) investigated the synthesis of nanoemulsions with smaller droplet sizes in the presence of double bonds in the nonpolar chain of non-ionic surfactants. The findings were consistent with previous findings (Shahavi *et al*., 2015).

Essential oils as nanoemulsions or natural nematicides, on the other hand, have a variety of efficiency mechanisms (Park *et al*., 2005; Laquale *et al*., 2015). The chitin penetration of the cell wall destroys the lipoprotein cytoplasmic membrane, enabling cytoplasm to escape, resulting in antifungal action. Or, as Mendes *et al*. (2018) suggested, the nanoemulsion's antipictide activity was boosted while cytotoxicity was lowered. In this study, a nanoemulsion containing mint essential oil extract and chitosan has nematicidal activity against root-knot nematodes with low cytotoxicity in a human cell line (Kumar *et al*., 2019).

Because the optimal sample contained chitosan-containing mint essential oil nanoemulsion extract, it is reasonable to speculate that the presence of chitosan can govern the nanoemulsion's size, nucleation, and nematicidal activity. Essential oil emulsion and nanoemulsion components may adversely affect nematodes nervous system. Another possibility is that essential oils disrupt the cell membrane of the nematode and change its permeability.

This mechanism has also been suggested to explain the fungicidal activity of essential oils. Aldehydes of essential oil components may cause irreversible changes to protein structures, especially those located on the nematode surface, like formaldehyde and other aldehydes. Interestingly, benzaldehyde and furfural (D2-furaldehyde) have been found to attract *C. elegans* at low concentrations (Bargmann *et al*., 1993). Also, the antifungal activity of essential oils emulsion and nanoemulsion against *F. oxysporum* was reported by (Hassanin *et al*., 2017). The essential oil can penetrate and disrupt the fungal cell wall and cytoplasmic membranes, permeabilize them and finally damage mitochondrial membranes.

The changes in electron flow through the electron transport system inside the mitochondria damage the lipids, proteins, and nucleic acid contents of the fungal cells (Arnal-Schnebelen *et al*., 2004). The nematicidal action of benzaldehyde in conjunction with thymol on *M. javanica* J2 has been extensively studied

(Serratosa *et al*., 1995). In soil infested with root-knot nematodes, a combination of chitin and benzaldehyde increased tomato plant growth and health (Kokalis-Burelle *et al*., 1999). Citral, an aliphatic aldehyde found in essential oils like Cymbopogon grasses, and perillaldehyde have also been reported to exhibit nematicidal properties (Sangwan *et al*., 1985; Tsao and Yu, 2000). In addition, sabinene, myrcene, and trans-caryophyllene concentrations in Thyme essential oil nanoemulsions, which is a group of terpenoid compounds, sabinene plays a role in nematicidal activity (Santana *et al*., 2014; Bahmani *et al*., 2020; Sarkar, 2020).

According to the chemical makeup of thyme essential oil nanoemulsion, the primary components are sabinene, myrcene, and trans-caryophyllene, which may be responsible for its anti-nematode capabilities (Sangwan *et al*., 1990; Oka *et al*., 2000). The gains in plant growth metrics might be attributable to biochemical changes in the stem base tissues, or they could be owing to their effectiveness in partially or preventing disease infection and development. Peroxidase enzyme activity, growth hormones, and phenol chemicals all increase as a result of this shift. Zedan *et al*. (2011) and Hassanin (2013) observed somewhat comparable results on several crops in naturally or artificially contaminated soil.

Conclusions and Recommendations

The efficacy of treatments with thyme and Spearmint essential oil nanoemulsions compared to Nematicide and fungicide resulted in higher coleus development, indicating a viable use as an eco-friendly nematode and fungal control technique.

Novelty Statement

Developing effective methods for using natural extracts in the soil to reduce nematode populations and boost plant productivity.

Author's Contribution

Both Authors contributed equally.

Conflict of interest
The authors have declared no conflict of interest.

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