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Effect of Biocides and Essential Oil Nanoemulsions Against Tomato Early Blight Disease Under Greenhouse and Field Conditions

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ABSTRACT

The current research was conducted to evaluate the effectiveness of clove and thyme essential oils (emulsion and nanoemulsion) and two biocides (Bio-Arc and Bio-Zeid) as fungicidal alternatives for the management of tomato early blight disease caused by *Alternaria solani*. The images obtained by transmission electron microscopy showed the stability of the prepared clove and thyme oil nanoemulsions after 12 weeks of storage at room temperature (27°C) in spherical form and di-disperse or mildly mono. The droplet size of clove essential oil nanoemulsion ranged between 36.0 and 51.3 nm, and between 25.1 and 44.6 nm in the case of thyme. *In vitro* tests, clove oil nanoemulsion at a concentration of 6000 µg/ml was the most effective treatment, which completely inhibited the mycelial growth of *A. solani*. In greenhouse and field conditions during 2018/2019 and 2019/2020 growing seasons, Bioxan 72% was the most effective treatment for reducing disease severity, followed by clove and thyme oil nanoemulsion, clove emulsion, and Bio-Zeid. Under field conditions, clove and thyme oil nanoemulsions had superiority for increasing no. of fruits/plant, mean of fruits weight/ plant, plant height, and tomato yield, followed by clove emulsion and Bio-Zeid in both growing seasons. On the other hand, clove and thyme oil nanoemulsions had superior activity for increasing peroxidase, polyphenoloxidase, catalase, β-1, 3 glucanase, and chitinase compared to the other treatments. Therefore, it could be suggested that clove and thyme oil nanoemulsion, clove emulsion, and Bio-Zeid could be used for controlling tomato early blight disease.

Keywords: Bio-Zeid, essential oil nanoemulsion, early blight, *Alternaria solani*, Tomato.

1. Introduction

Tomato crop (*Solanum lycopersicum* L., syn., *Lycopersicon esculentum* Mill) is essential both economically and nutritionally. It is used in both food production and industry, making it one of Egypt's most essential plant crops (Abd-El-Kareem *et al*., 2006). With an average yield of 38.97 tons/hectare during the 2021 season, Egypt's tomato output has grown and now stands at 6.8 million tons (FAOSTAT, 2021). Because it is a crop with a short growing season and an elevated production, it is financially favourable, and its cultivation area is growing. One of the most virulent fungal diseases in Egypt's governorates is early tomato blight, which is caused by *Alternaria solani*. The disease requires a particular set of climate variables, including heavy rainfall, dew, and a high level of humidity. *Alternaria solani* infection on tomatoes resulted in crop losses of up to 80% (Adhikari *et al.,* 2017).

Essential oils could be extracted from herbs and spices and considered plants' secondary metabolites. As reported by Bakkali *et al*. (2008), numerous studies have mentioned the antimicrobial properties of plant essential oils, and these qualities can be related to their bioactive components**.** P-cymene, 1,8-cineole and Thymol were the major components responsible for the antifungal effect of thyme volatile oil towards certain fungi, such as *Rhizopus*, *Cladosporium*, and *Alternaria* (Šegvić Klarić *et al*., 2007). As a result of the synergy between nanotechnology and crude oil, a new substance emerged, known as nanoemulsion, which has application in the food sector, cosmetics, and medicaments within the previous ten years, but its use in agriculture is still in its infancy (Araújo *et al.*, 2019; Walker *et al.*, 2015). It is possible to enhance the activities of essential oils manyfold if they are applied as tiny droplets in nanoemulsion form, which is a colloidal system made up of an oil stage distributed in an aqueous fluid stage and a slim film of surfactant around each oil particle (McClements and Rao, 2011). In addition to enhancing plant height, leaf quality, and root length, clove oil nanoemulsion has a higher impact on reducing caraway blight disease caused by *Neoscytalidium dimidiatum* (Hashem *et al.*, 2023).

An excellent management strategy other than chemical control of fungal plant diseases is biological control (El-Rafai *et al.*, 2003). *Trichoderma* spp. has the ability to control a broad variety of plant diseases as well as enhance the growth of plants, plant defense, and production in greenhouse and field conditions (Tyśkiewicz *et al.*, 2022). High antifungal activity for *Helminthosporium sorokinana* (66.55%), *Fusarium solani* (70.82%), *Alternaria brassicicola* (70.35%), *Sclerotium rolfsii* (92.53%), and *Rhizoctonia solani* (78.58%) was demonstrated by *Trichoderma harzianum* (Poudel *et al.*, 2023).

Bacillus megaterium increased the chili crop and demonstrated highly effective control of *Sclerotium rolfsii*, the causal agent of chili southern blight disease, *in vitro* and *in vivo* (Sharf *et al.*, 2021). The most successful bio-agents for managing root rot and damping-off diseases in chickpea, as well as producing the greatest seed output in both greenhouse and field experiments, were found to be *T. viride* and *B. megaterium* (Abdel-Monaim, 2010).

The aim of the current research was to evaluate the impact of clove and thyme volatile oils (emulsion and nanoemulsion) and two biocides (Bio-Zeid and Bio-Arc) as fungicidal alternatives for the control of early blight disease of tomato incited by *A. solani* in greenhouse and field.

2. Materials and methods

2.1. The pathogenic *A. solani* isolate.

The isolate of *A. solani* used in this work was highly aggressive on tomato plants, causing a severe early blight disease and it was kindly obtained from Biological Resource Center Standard (BRCS) Unit, Plant Pathology Institute, Agricultural Research Center (ARC), Giza, Egypt (with an accession number of KY312035.1 at NCBI Genebank, El-Habbaa, G.M.D. and Ragab, A.M.E.).

2.2. Preparation of an essential oil nanoemulsion and its stability during storage

Clove and thyme volatile oils tested in the current investigation were obtained from Medicinal and Aromatic Plants dept., Horticulture Res. Inst., ARC, Giza, Egypt. For emulsion essential oil preparation, a homogenous mixture was formed by progressively adding 50 ml of the non-ionic surfactant (Tween 80) to 100 ml of essential oil while gently stirring, as described by Hassanin *et al.* (2017). A nanoemulsion of essential oil was then produced by adding 850 ml of sterilized dist. water, and the blend was then agitated using a magnetic stirrer for 40 minutes (Hotplate Magnetic Stirrer, model: JSHS – 18D, JS Research Company, Gongju-City, Korea). After that, an Elmasonic S 30 H (made in Germany by Elma) was employed to sonicate the blend for 60 min at 280 W after being further homogenised by Ultra Turrax for 40 minutes.

2.3. Droplet size measurement of essential oil nanoemulsion

The technique mentioned by Ghotbi *et al*. (2014) was conducted at Nanotechnology Laboratory, Regional Center for Food and Feed, ARC, Giza, Egypt, to measure the droplet size of the prepared essential oil nanoemulsion after being stored at room temperature (27°C) for 12 weeks. In this method, Zeta Nano ZS (Malvern Instruments, UK) was employed for a dynamic light scattering analysis (DLS) at room temperature. In order to estimate droplet size, 30 µl of nanoemulsion were added to 3 ml of dist. water, then using triplicates of nanoemulsions the mean droplet size was calculated. The polydispersity index (PDI) of the formulated nanoemulsion was also measured.

2.4. Transmission electron microscopy (TEM)

In this investigation, TEM (Tecnai G20, Super Twin, Double Tilt, FEI, The Netherlands) was employed to examine the nanoemulsion form of the prepared essential oils, as noted by Saloko *et al*. (2013). The essential oil nanoemulsion was diluted with dist. water 10 times, and 20 µl of diluted samples were put for 1 min on a film-coated 200-mesh copper grid. After that, single drop of 3% phosphotungstic acid was applied to stain the grid, followed by drying at room temperature (27 ºC). The grid was then examined by TEM, operating at 200 kV.

2.5. Impact of essential oil emulsions and nanoemulsions on *A. solani* mycelial growth *in vitro*

Clove and thyme oil emulsions and nanoemulsions were examined for their capability to suppress the linear growth of *A. solani*. In this regard, five concentrations (1000, 2000, 3000, 4000, and 6000 µg/ ml) of each emulsion and nanoemulsion of clove and thyme oils were prepared and added separately to sterilized PDA medium in conical flasks before its solidification. A PDA flask free of either treatment was used as an untreated control, which was poured, as well as the treated media, into Petri dishes (9 cm in diameter). The prepared Petri dishes were then inoculated with mycelial discs (5mm in diameter) of *A. solani* actively growing culture (7-day-old), followed by incubation at 28 ± 2°C. Three plates were used for each treatment and the untreated control. The average diameter of the linear growth was measured in the various applications when the control plates were completely covered with *A. solani* mycelial growth. The reduction in growth was calculated according to Ahmed (2013) as an inhibition percentage using the following formula:

$$\%\text{Inhibition} = \frac{G1-G2}{G1} \times 100$$

G1= the linear growth of the fungus in control.

G2= the linear growth of the fungus in the treatment.

2.6. Greenhouse experiments

2.6.1. Inoculum preparation

Alternaria solani growing cultures for 7 days on PDA medium at 28 ± 2°C were used to make the inoculum. The mycelial growth was repeatedly rinsed using sterilized water, followed by mixing with water for 3 minutes.

The hemocytometer was then used to adjust the fungal suspension concentration to10^5 cfu/ml (Derbalah *et al.*, 2018).

2.6.2. Impact of various treatments on the severity of early blight disease in greenhouse

In this experiment, emulsion and nanoemulsion of clove and thyme oils at three concentrations (2000, 4000, and 6000 µg/ ml), two bio-formulations (2.5 g/ l) *i.e.,* Bio-Arc (6% wettable powder, 25×10^6 cells/g of *Bacillus megaterium*) and Bio-Zeid (2.5% wettable powder, 25×10^6 spores/g of *Trichoderma album*), in addition to Bioxan fungicide 72% [Cymoxanil 8% (Cyanoacetamide Oxime) + Mancozeb 64 (Dithiocarbamate)] at 1.5 g/ l, were examined against early blight disease of tomato in greenhouse according to the designed protocol mentioned by Vloutoglou and Kalogerakis (2000). The BRCS Unit's greenhouse was used for growing tomato seedlings (cv. super strian B), which were transplanted into 30 cm-diameter pots filled with sterilized soil, 3 seedlings/ pot. All the treatments were applied to tomato plants at 4-5 weeks old (5 pots/ treatment). As an untreated control, plants were sprayed with the same amount of dist. water. After 7 days following the treatment application, the plants were inoculated with 50 ml of mycelial suspension inoculum of *A. solani* as foliar spraying, except for the healthy plant (uninoculated control). In order to increase the possibility of disease development, clear plastic bags were positioned above the inoculated plants directly after inoculation for 24 h, as well as the uninoculated control plants, which were sprayed with dist. water. At three dates; 7, 14 and 21 days after the artificial infection with *A. solani*, the numerical rates of disease symptoms were recorded using the following scale noted by Cohen *et al.* (1991);

0 = without clear symptoms;
1 = Lesions cover the plant with 1-25% /plant;
2 = Lesions cover the plant with 26-50% /plant;
3 = Lesions cover the plant with 51-75% /plant;
4 = Lesions cover the plant with 76-100% /plant.

The severity of early blight disease was then calculated using the formula described by Townsend and Heuberger (1943) as follows:

$$\text{Disease severity (\%)} = \frac{\sum nxc}{N \times C} X100$$

Where: n = Number of diseased leaves, c = Grade number, N = Total number of tested leaves and C = The maximum grade number of infection.

2.7. Enzymatic studies

2.7.1. Preparation of enzyme extract from treated tomato leaves

The impact of tested treatments on enzyme activity was evaluated through the measurement of the activity of peroxidase, polyphenol oxidase, catalase, chitinase and ß-1, 3-glucanase enzymes. In this investigation, tomato leaf samples were selected from the most efficient treatments for reducing disease severity, such as emulsion and nanoemulsion clove and thyme oils at 6000 µg/ ml concentration, biocides (Bio-Zeid and Bio-Arc), and Bioxan fungicide at the recommended concentration, in addition to uninoculated and untreated leaf plants as a control. At 1, 3, and 7 days after fungal inoculation, samples of leaves were obtained and promptly ground in liquid nitrogen. After that, one gram of ground material was added to 2 mL of 0.1 M sodium phosphate buffer (pH 7.0) followed by centrifugation at 4°C for 20 min at 4000 rpm. The filtrate was then used for enzyme activity assays according to Anand *et al.* (2007).

2.7.2. Peroxidase activity (PO)

The method of Hartee (1955) was used for peroxidase activity determination, depending on the oxidation of pyrogallol to purpyrogallin in the presence of hydrogen peroxide. The reaction mixture was prepared by adding 0.5 ml of the extracted enzyme to 1.5 ml of 0.05 M pyrogallol and 0.5 ml of 1% H_2O_2, followed by incubation at room temperature (28±1°C). Enzyme activity was determined as alteration in absorbance at 420 nm, which were measured at 30 sec intervals for 3 min as mentioned by Hammerschmidt *et al*. (1982). A

sample of boiled enzyme was used as a blank in the reaction.

2.7.3. Polyphenol oxidase activity (PPO)

Polyphenol oxidase activity determination was conducted using the method mentioned by Mayer *et al.* (1966)**.** The reaction mixture was prepared by adding 200 µl of the enzyme extract to 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of 0.01 M catechol. Enzyme activity was determined as changes in absorbance at 495 nm.

2.7.4. Catalase activity

The protocol of Kato and Shimizu (1987) was applied to measure the activity of catalase enzyme. In this protocol, 0.1 ml of the extracted enzyme was added to 0.3 ml of 0.5% hydrogen peroxide and 0.5 ml of 0.2 M sodium phosphate buffer (pH 7.6), followed by mixing and adding dist. water to reach the final volume of 3 ml. The alteration in absorbance per minute at 240 nm was used to calculate the activity of catalase enzyme.

2.7.5. Chitinase activity

The method described by Miller (1959) was followed for the determination of chitinase activity. In this method, 1 mL of 0.5% colloidal chitin in 0.1M citrate buffer (pH 7.0) was mixed with 1 mL of enzyme extract, followed by incubation for 30 min at 37˚C in water bath. After that, 2 mL of DNS (di nitro salicylic) reagent was added to stop the reaction. The activity of chitinase enzyme was determined as the optical densities of samples, which were measured at 575nm, in addition to a blank sample consisting of 1 ml sodium acetate buffer (0.1 M, pH 4.2), 2 ml DNS, and 1 ml substrate-buffer solution (0.5%). Standardization was performed using N-acetylglucosamine (GLcNAc), and enzyme activity was expressed as µmoles of GLcNAc/ml.

2.7.6. DNS reagent composition

The preparation process involved dissolving 1 g of dinitrosalicylic acid, 50 mg of sodium sulphate, and 200 mg of crystalline phenol in 1% a NaOH solution and storing the combined reagent at 4°C in a stopper bottle.

2.7.7. ß-1, 3-glucanase activity

The activity of ß-1,3-glucanase was determined using the procedure described by Marco and Felix (2007)**.** In this method, 250 µl of a laminarin solution (1%) were dissolved in 50 mM sodium acetate buffer (pH 5.0), and then 125 µl of the enzyme extract were added, followed by incubation for 30 min at 37º C. After that, 1.5 mL of DNS reagent was added to stop the reaction. Using a spectrophotometer at 550 nm, the amount of reducing sugar (µmole/min) produced by an amount of protein was expressed as enzyme units (U).

2.8. Field experiment

During the 2018/2019 and 2019/2020 successive cultivation seasons, the field trial was performed at the Giza Experimental Station, ARC, Giza, Egypt. The aim of this trial was to evaluate the efficacy of clove and thyme essential oils (emulsions and nanoemulsions) at 6000 µg/ ml, two biofungicides (Bio-Zeid and Bio-Arc) at 2.5 g/l, and one fungicide (Bioxan 72%) at 1.5 g/l, for controlling early blight disease on tomato (cv. super strain B) under natural field conditions. All treatments, according to El-Mougy (2009), were used twice, separated by 15 days. Using a completely randomized block design, five replicates of each treatment and an untreated control were used in the field experiment, including plots of 3 x 7 m each with seven rows and 15 holes per row. The disease severity symptoms were noted after 10 days from the second treatment application, and the percentages of disease severity were estimated according to the formula mentioned before in the greenhouse experiment. Additionally, the fruits number / plant, the fruits weight average/ plant, plant height (cm), and the mean harvested fruit yield (g /plot) were noted at the end of each growing season.

2.9. Statistical analysis

Analysis of Variance (ANOVA) between treatments was used to analyze all obtained data, and averages were compared with least significant differences (L.S.D.) at $p \leq 0.05$ as reported by Song and Keane (2006).

3. Results

3.1. Measurement of essential oil nanoemulsion droplets size

The droplet size of the thyme and clove essential oil nanoemulsion produced by the ultra-sonication at 280 W for 60 min was measured by a dynamic light scattering analysis (DLS) after being stored at room temperature (27°C) for 12 weeks. The droplet size ranged between (96.0 nm and 0.445) in the case of clove (Fig. 1) and (77.5 nm and 0.276) in the case of thyme (Fig. 2) (droplet size and PDI, respectively).

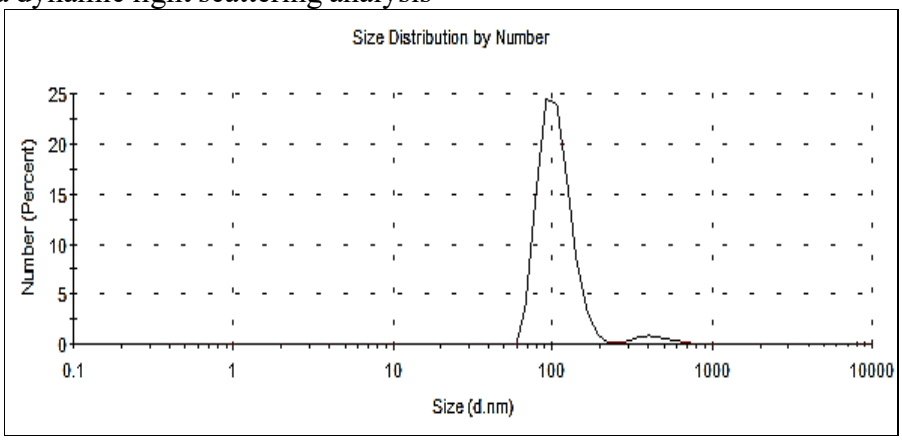

Fig. 1. Particle size of clove essential oil nanoemulsion after 12 weeks of using the ultrasonication method (droplet size = 96 nm and PDI=0.445)

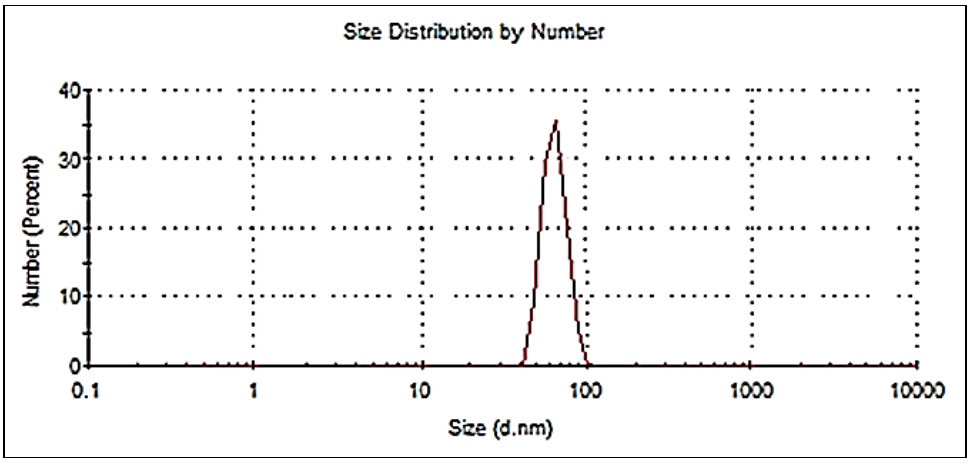

Fig. 2. Particle size of thyme essential oil nanoemulsion after 12 weeks of using the ultrasonication method (droplet size = 77.5 nm and PDI=0.276)

3.2. TEM of essential oil nanoemulsion

Clove and thyme essential oil nanoemulsions were described using transmission electron microscopy (TEM), which reveals the exact size and form of the droplets. The nanoemulsions of the tested oils appeared spherical in form and di-disperse or mildly mono, based on TEM images. Fig. 3 shows the droplet size of clove essential oil nanoemulsion, which ranged between 36.0 and 51.3 nm, while it ranged between 25.1 and 44.6 nm in the case of thyme (Fig. 4).

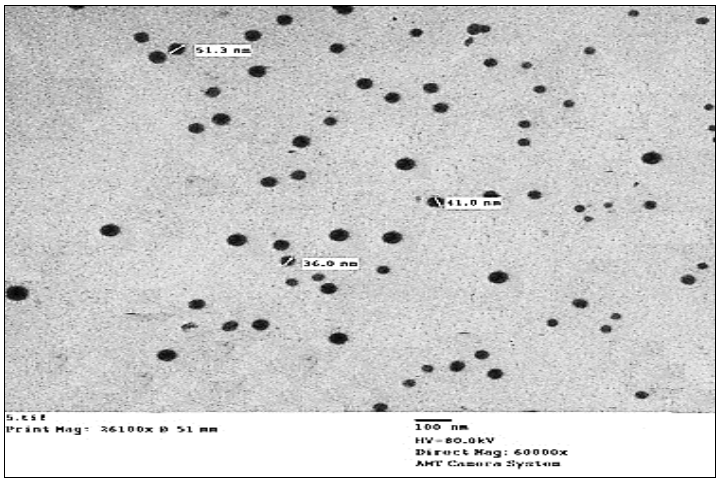

Fig. 3. Transmission electron microscopic image of clove essential oil nanoemulsion

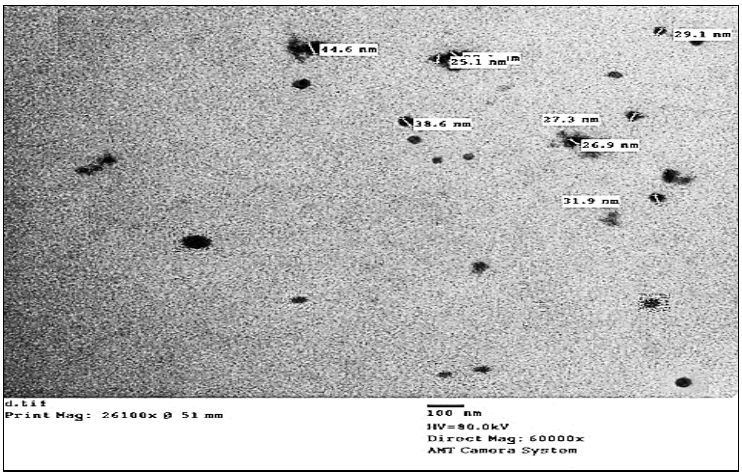

Fig. 4. Transmission electron microscopic image of thyme essential oil nanoemulsion

3.3. Impact of essential oil emulsions and nanoemulsions on *A. solani* mycelial growth *in vitro*

According to the presented data in Table (1), clove and thyme essential oils (emulsions and nanoemulsions) had an inhibiting impact on the linear growth of *A. solani* at all tested concentrations. There was a noticeable decrease in mycelial growth with increasing concentrations, which reached its minimum level at the maximum concentration (6000 µg/ ml). The highest concentration of clove and thyme nanoemulsion oil (6000 µg/ ml) resulted in 100% and 86.6% inhibition of fungal growth, respectively. The following effective treatment for mycelial growth was the highest concentration of clove and thyme emulsion oil (6000 µg/ ml), which caused 80.0% and 67.0% inhibition of fungal growth, respectively. It was clear that nanoemulsion essential oils (clove and thyme) at all concentrations have a higher antifungal impact on mycelial growth compared to emulsion essential oils.

Table 1. Antagonistic effect of clove and thyme oil emulsion and nanoemulsion against *A. solani* isolate on PDA medium.

Concentrations of the tested oils (µg/ ml)	Growth reduction %			
	Clove		Thyme	
	Emulsion	Nanoemulsion	Emulsion	Nanoemulsion
1000	25.5	64.4	38.8	61.1
2000	44.4	74.4	53.6	67.7
3000	51.1	88.5	50.0	76.6
4000	72.2	90.0	58.8	81.1
6000	80.0	100	67.0	86.6
Control	0	0	0	0
L.S.D at 5%	4.320	5.513	6.924	4.506

3.4. Effect of various treatments on the severity of early blight disease of tomato in greenhouse

Results in Table (2) show that all applications resulted in a significant reduction in disease severity (%) compared to the untreated control; however, compared with the other applications, Bioxan was the best one, which reduced disease severity to 15.71, 20.22 and 20.55% at 7, 14 and 21 days from the fungal inoculation date, respectively. Following that, clove and thyme nanoemulsion at a concentration of 6000 µg/ ml reduced disease severity to 25.21, 30.91 and 40.1% (with clove nanoemulsion), and 30.90, 36.55 and 48.33% (with thyme nanoemulsion) at 7, 14 and 21 days from the fungal inoculation date, respectively. Clove emulsion at a concentration of 6000 µg/ ml recorded the next order, followed by Bio-Zeid, since they reduced disease severity to 33.21, 40.18 and 48.33% (with clove emulsion) and 34.81, 42.81 and 49.11% (with Bio-Zeid) at 7, 14 and 21 days from the fungal inoculation date, respectively. On the other hand, a negative correlation was observed between the concentration of oil and the severity of the disease. In contrast, the emulsion of thyme volatile oil was the least efficient application regarding disease severity reduction (%), especially using the lower concentration at 2000 µg/ ml, which reduced disease severity to 45.22, 50.92 and 62.31% at 7, 14 and 21 days from the fungal inoculation date, respectively.

Table 2. Impact of different treatments on the severity of early blight disease of tomato caused by *A. solani* under greenhouse conditions.

Treatments	Concentration (µg/ ml)	Disease severity % at days		
		7	14	21
Thyme Nanoemulsion	2000	40.65	45.73	51.22
	4000	37.11	41.54	50.71
	6000	30.90	36.55	48.33
Thyme emulsion	2000	45.22	50.92	62.31
	4000	43.21	48.34	59.73
	6000	40.55	46.87	53.01
Clove Nanoemulsion	2000	30.81	35.67	48.32
	4000	29.72	33.43	45.32
	6000	25.21	30.91	40.1
Clove emulsion	2000	40.90	45.51	52.96
	4000	38.67	44.01	49.28
	6000	33.21	40.18	48.33
Bio-Arc		35.1	43.75	51.84
Bio-Zeid		34.81	42.81	49.11
Bioxan 72%		15.71	20.22	20.55
Infected control		69.33	80.71	91.53
Control		0	0	0
L.S.D at 5%		4.640	4.954	4.309

3.5. Effect of various treatments on oxidative and hydrolytic enzyme activities

3.5.1. Peroxidase, polyphenoloxidase, and catalase activities

According to the data in Table (3), treated tomato leaves had higher levels of peroxidase, polyphenoloxidase, and catalase activity than untreated ones. However, plants treated with clove and thyme oil nanoemulsion showed the highest activity of the peroxidase, polyphenol oxidase, and catalase enzymes, recording 4.495, 1.280, and 1.820 (units/ml enzyme), respectively (with clove oil nanoemulsion), and 4.385, 0.874, and 1.740 (units/ml enzyme), respectively (with thyme oil nanoemulsion) compared with the other treatments at 7 days post-infection. Following that, treatment with clove oil emulsion increased the activity of peroxidase and polyphenol oxidase at 7 days post-infection, recording 4.374 and 0.730, respectively, and Bio-Zeid increased catalase activity to 1.152 (units/ml enzyme). In contrast, the least activity of peroxidase, polyphenol oxidase and catalase enzymes at 7 days post-infection was recorded with the treatment of thyme oil emulsion recording 4.159, 0.500 and 1.083 (units/ml enzyme), respectively, compared with the other fungicidal alternatives tested.

Table 3. Impact of different applications on the activity of peroxidase, polyphenol oxidase and catalase at different incubation periods after fungal inoculation.

Treatments	Incubation period (days)	Enzyme activity (units/ml enzyme)		
		Peroxidase	Polyphenoloxidase	Catalase
Thyme oil nanoemulsion	1	3.951	0.361	0.636
	3	4.260	0.389	0.940
	7	4.385	0.874	1.740
Thyme oil emulsion	1	3.526	0.445	0.154
	3	4.110	0.455	0.761
	7	4.159	0.500	1.083
Clove oil nanoemulsion	1	3.604	0.191	0.459
	3	4.324	0.514	0.614
	7	4.495	1.280	1.820
Clove oil emulsion	1	3.677	0.484	0.640
	3	4.038	0.518	0.814
	7	4.374	0.730	1.103
Bio-Arc	1	3.946	0.368	0.640
	3	4.111	0.434	0.730
	7	4.245	0.487	1.148
Bio-Zeid	1	3.896	0.446	0.780
	3	4.022	0.448	0.813
	7	4.350	0.567	1.152
Bioxan 72%	1	3.764	0.410	0.772
	3	3.876	0.484	0.780
	7	4.076	0.485	0.850
Control	1	3.941	0.373	0.680
	3	4.000	0.433	0.784
	7	4.016	0.498	0.899
L.S.D at 5%		0.459	0.251	0.260

3.5.2. The activity of β- 1, 3 glucanase and chitinase

Data in Table (4) show that the application of the examined treatments resulted in an increase in β-1, 3 glucanase and chitinase activities in treated plants compared with the untreated ones. However, the treatment of clove and thyme oil nanoemulsion, recorded the highest increase in β-1, 3glucanase and chitinase activities at 7 days post-infection compared with other treatments, recording 1.641 and 1.977 (units/ml enzyme), respectively (with

clove oil nanoemulsion) and 1.450 and 1.882 (units/ml enzyme), respectively (with thyme oil nanoemulsion). The following order was the treatment with Bio-Zeid which increased β-1, 3glucanase and chitinase activities at 7 days post-infection to 1.414 and 1.505 (units/ml enzyme), respectively.

Table 4. Effect of different treatments on β- 1, 3 glucanase and chitinase activities at different incubation periods after fungal inoculation.

Treatments	Incubation period (days)	Enzyme activity (units/ml enzyme)	
		β-1,3 glucanase	Chitinase
Thyme oil nanoemulsion	1	0.628	0.875
	3	0.984	1.137
	7	1.450	1.882
Thyme oil emulsion	1	0.685	0.895
	3	1.069	1.118
	7	1.173	1.176
Clove oil nanoemulsion	1	1.002	0.860
	3	1.156	1.034
	7	1.641	1.977
Clove oil emulsion	1	1.066	1.126
	3	1.250	1.406
	7	1.358	1.366
Bio-Arc	1	0.726	0.878
	3	1.027	1.042
	7	1.049	1.332
Bio-Zeid	1	1.024	1.066
	3	1.176	1.492
	7	1.414	1.505
Bioxan 72%	1	0.765	0.585
	3	1.133	1.112
	7	1.171	1.126
Control	1	1.023	0.485
	3	1.082	1.013
	7	1.111	1.106
L.S.D at 5%		0.266	0.266

3.6. Effect of various treatments on the severity of early blight disease of tomato and some growth parameters in the field

The data presented in Table (5 and 6) show that all the tested applications resulted in a significant reduction in disease severity (%) in the two cultivation seasons compared to the untreated control. However, Bioxan 72% was the most efficient application, which decreased disease severity to 39.7 and 42.4% in 2018/2019 and 2019/2020 growing seasons, respectively. Following that, the application of clove oil nanoemulsion significantly decreased the disease severity to 41.6 and 45.2%, as well as its superiority for improving the crop parameters: the fruits number/ plant, the fruits weight average/ plant, plant height (cm), and tomato yield (g/ plot) by (94.6, 1025.3, 109.0 and 5945) and (97.3, 1031.0, 112.6 and 5952) in 2018/2019 and 2019/2020 growing seasons, respectively. The biocide Bio-Zeid followed clove oil emulsion, and it was better than Bio-Arc for reducing disease severity to 54.4 and 55.7% in 2018/2019 and 2019/2020 growing seasons, respectively, as well as for improving plant crop parameters: the fruits number / plant, the fruits weight average/ plant, plant height (cm), and tomato yield (g/ plot) by (83.1, 888.3, 87.5 and 4632) and (88.5, 893.6, 92.1 and 4646) in 2018/2019 and 2019/2020 growing seasons,

respectively. In contrast, emulsion of thyme volatile oil was the least efficient treatment regarding disease severity reduction to 59.9 and 64.6%, as well as for improving the crop parameters: the fruits number / plant, the fruits weight average/ plant, plant height (cm), and tomato yield (g/ plot) by (69.2, 792.1, 80.25 and 3911) and (74.5, 800.0, 84.4 and 3919) in 2018/2019 and 2019/2020 growing seasons, respectively.

Table 5. Effect of various treatments on the severity of early blight disease of tomato caused by *A. solani* and certain growth parameters under field conditions during season 2018/2019.

Treatments	Disease severity (%)	Fruits no. / plant	Fruits weight average (g)/ plant	Plant height (cm)	Fruit yield [g /plot]
Thyme oil nanoemulsion	48.4	90.7	970.3	90.8	5320
Thyme oil emulsion	59.9	69.2	792.1	80.25	3911
Clove oil nanoemulsion	41.6	94.6	1025.3	109.0	5945
Clove oil emulsion	52.4	88.3	948.5	89.7	4841
Bio-Arc	59.1	79.3	821.1	82.3	4346
Bio-Zeid	54.4	83.1	888.3	87.5	4632
Bioxan 72%	39.7	91.8	991.3	93.8	5630
Control	80.2	23.3	590.2	78.6	2065
L.S.D. 5%	12.142	14.953	18.186	13.509	18.859

Table 6. Effect of various treatments on the severity of early blight disease of tomato caused by *A. solani* and certain growth parameters under field conditions during season 2019/2020.

Treatments	Disease severity (%)	Fruits no./ plant	Fruits weight average (g)/ plant	Plant height (cm)	Fruit yield [g /plot]
Thyme oil nanoemulsion	50.5	93.8	976.5	95.2	5331
Thyme oil emulsion	64.6	74.5	800.0	84.4	3919
Clove oil nanoemulsion	45.2	97.3	1031.0	112.6	5952
Clove oil emulsion	58.3	91.2	955.4	91.4	4854
Bio-Arc	62.6	82.6	830.1	85.7	4355
Bio-Zeid	55.7	88.5	893.6	92.1	4646
Bioxan 72%	42.4	96.2	1001.5	97.7	5645
Control	86.4	30.7	600.0	82.8	2070
L.S.D. 5%	12.913	10.981	12.135	14.604	16.649

4. Discussion

In the current research, thyme and clove volatile oil nanoemulsions were produced by the ultra-sonication method at 280 W for 60 min. Due to Tween 80's high HLB value, which encourages the production of oil emulsion in water, it was employed as a surfactant in the current investigation. The use of Tween 80 in this respect of our current study is supported by Ghosh *et al.* (2014), who reported that Tween 80 is a small particle surfactant that adheres quickly to the emulsion surface, thereby reducing the diameter of droplets more effectively than polymeric surfactants. The level of non-uniformity in a particle size spreading is expressed by the poly dispersity index (PDI) (Bera, 2015). Danaei *et al.* (2018) reported that when PDI values exceed 0.7, the sample most likely will not be suitable for dynamic light scattering (DLS) analysis. According to these reports, the prepared essential oil nanoemulsions in the current study have a good PDI, with tiny droplets that measured (96.0 nm and 0.445) in the case of

clove and (77.5 nm and 0.276) in the case of thyme (droplet size and PDI, respectively). Additionally, the results of Abd-Elsalam and Khokhlov (2015) of droplet size investigation utilizing DLS, which demonstrated the presence of droplets with particle sizes smaller than 100 nm, were strongly in accordance with the droplet size of nanoemulsion essential oils in the current study. The effectiveness and capability of surfactants may be a factor in the reduction of particle size, as reported by Dai *et al.* (1997). Also, Sajjadi *et al.* (2002) mentioned that in the oil-in-water emulsion, stirring decreases the size of the droplets. Generally, our findings were in accordance with Hammad and Hassanin (2022), who found that the nanoemulsion droplets of thyme produced ultrasonically at 700 W for 30 min were very small (around 48.1 nm) after storing them at room temperature for 3 months.

The images obtained by TEM showed the prepared clove and thyme oil nanoemulsions in spherical form and di-disperse or mildly mono, and correlated with the results obtained by DLS technique. The droplets size of clove nanoemulsion ranged between 36.0 and 51.3 nm, while it ranged between 25.1 and 44.6 nm in the case of thyme. Other investigations on some nanoemulsion essential oils, including basil oil nanoemulsion (Ghosh *et al.*, 2014) and neem oil nanoemulsion (Anjali *et al.*, 2012), are in agreement with our findings. Also, Sugumar *et al.* (2014) reported that the size of essential oil nanoemulsion droplets ranges between 20 and 200 nm. Furthermore, the results of Hammad and Hassanin (2022) revealed the spherical form of thyme essential oil nanoemulsion with droplet sizes varying between 25.4 and 32.9 nm, which support our obtained results.

In vitro, the tested essential oil nanoemulsions showed high antifungal effect towards the growth of *A. solani*, and the most effective treatment was clove oil nanoemulsion at a concentration of 6000 µg/ml, which completely inhibited the mycelial growth of *A. solani*. Many studies have documented the antagonistic impact of plant essential oils towards a number of plant fungi. Oliva *et al.* (2015) reported that plant disease can be controlled more successfully with the use of essential oils from plants. Also, Akthar *et al.* (2014) mentioned that the antibacterial and antifungal impacts of essential oils are a result of phenolic compounds found in plant essential oils. Furthermore, Tanovic *et al.* (2007) reported that the fungicidal action of thyme essential oil against plant pathogenic fungi, *Rhizoctonia* sp., *penicillium*, *pythium* sp., *Cladosporium, Verticilium albo-atrum*, *Alternaria*, *Aspergillus*, and *Fusarium oxysporum f. sp. lycopersici*, extends a broad spectrum. Additionally, the results obtained by Thabet and Khalifa (2018) indicated that the linear growth of *F. oxysporum* and *Rhizoctonia solani*, which were isolated from tomato plants, was inhibited by the use of clove essential oil. Generally, our obtained results are in accordance with those obtained by Hassanin *et al*. (2017), who mentioned that essential oil nanoemulsions have a higher antifungal impact on the mycelial growth of *Fusarium oxysporum*, isolated from cumin plant, compared to essential oil emulsions. Anton and Vandamme (2011) reported a negative correlation between oil emulsion stability and oil particle size.

As a result of greenhouse and field experiments conducted during the 2018/2019 and 2019/2020 growing seasons, Bioxan 72% was the most efficient treatment regarding disease severity reduction, followed by clove and thyme volatile oil nanoemulsion, clove emulsion, and Bio-Zeid. It is clear from the obtained results that essential oil nanoemulsions were better than essential oil emulsions for reducing disease severity, which may be investigated by the reduction in particle size of the nanoemulsion. This interpretation may be based on the results obtained by Anton and Vandamme (2011) who revealed that the reduction in oil particle size led to a significant increase in oil emulsion stability. Our obtained results are also somewhat in agreement with

those obtained by Thabet and Khalifa (2018), who mentioned that 4% of clove oil nanoemulsion significantly decreased disease incidence and severity in tomato wilt and root rot diseases. Moreover, the results conducted by Sharma *et al*. (2017) may support our obtained results, which revealed that increasing the clove oil concentration led to a reduction in the severity of tomato *Fusarium* wilt in greenhouse compared to control. *Trichoderma* spp. has the ability to inhibit a broad variety of plant pathogens, as reported by Tyśkiewicz *et al.* (2022). Also, the results of Poudel *et al.* (2023) revealed the high antifungal activity of *Trichoderma harzianum* against *Helminthosporium sorokinana*, *Fusarium solani*, *Alternaria brassicicola*, *Sclerotium rolfsii* and *Rhizoctonia solani*. Bolar *et al.* (2000) mentioned that *T. harzianum* secretes a number of lysis enzymes, including chitinase, which lyses the cell wall of the fungal pathogen.

The clove and thyme oil nanoemulsions had superior results under field conditions in both cultivation seasons in terms of plant height, mean of fruits weight/ plant, no. of fruits/plant, and tomato yield, followed by the clove emulsion and Bio-Zeid. Our obtained results are in accordance with Thabet and Khalifa (2018), who noted that clove oil nanoemulsion treatment resulted in a significant increase in plant height, plant fresh weight, and number of branches per plant in treated tomato plants compared to the untreated ones. Additionally, Hashem *et al.* (2023) revealed that clove oil nanoemulsion showed high efficacy for reducing the blight disease of caraway plants, which is caused by *Neoscytalidium dimidiatum*, in addition to enhancing plant height, leaf quality, and root length. The authors also mentioned that clove nanoemulsion oil stimulates plant resistance through the modification of proline, antioxidant enzymes, phenol, and hydrogen peroxide. As reported by Hou *et al.* (2022), the development of plant growth can be attributed to the capability of clove oil nanoemulsion to promote plant growth through its bioactive compounds, in addition to its antifungal activity. Sattary *et al.* (2020) indicated that the helpful effect of clove oil nanoemulsion may result from the induction of the plant's defense mechanisms, which increased its resistance to the fungal infection, fixed the physiological failing, and subsequently improved the plant's growth. Tyśkiewicz *et al.* (2022) reported that *Trichoderma* spp. has the ability to inhibit a broad variety of plant pathogens as well as improve plant development, plant defense, and plant growth in greenhouse and field conditions. Moreover, Poudel *et al.* (2023) mentioned that a high antifungal activity for *Helminthosporium sorokinana* (66.55%), *Fusarium solani* (70.82%), *Alternaria brassicicola* (70.35%), *Sclerotium rolfsii* (92.53%) and *Rhizoctonia solani* (78.58%), was demonstrated by *T. harzianum*. Also, Rahman *et al.* (2023) noted that tomato bacterial wilt caused by *Pseudomonace solanacearum* was significantly suppressed by the two isolates, *T. harzianum* NBG and *T. harzianum* MC2, in addition to enhancing the yield output.

Furthermore, clove and thyme oil nanoemulsions enhanced peroxidase, polyphenoloxidase, catalase, β-1, 3 glucanase, and chitinase activities more effectively than the other treatments. According to our findings, the time extension following inoculation with the tested fungus from 1 to 7 days gradually raised the activity of the peroxidase, polyphenoloxidase, and catalase enzymes. The obtained results could be supported by those recorded by Fahiem (2010), who mentioned that all the mould fungi studied, including *Fusarium*, *Rhizoctonia*, *Alternaria*, *Pythium*, *Botrytis*, *Mucor*, and *Sclerotinia*, were capable of producing peroxidase and polyphenoloxidase enzymes in their culture filtrates. Additionally, the author noted that the activity of the peroxidase and polyphenoloxidase enzymes steadily rose when the incubation times of the investigated fungi were extended from 4 to 13 days. According to Hassanin (2013), the application of fungicide-alternatives resulted in a valuable rise in the

activity of peroxidase and polyphenol oxidase in black cumin plants as a type of induced defense. Increasing the activity of the polyphenol oxidase will protect the plant from the fungal infection, as reported by Newman *et al.* (2011), since it stimulates the phenol oxidation to quinones in addition to the lignification process. Moreover, Sekiguchi *et al*. (1994) noted that peroxidase and polyphenol oxidase are oxidative enzymes that promote phenol oxidation into more toxic quinones, lignin synthesis and are involved in forming defense barriers for cell protection, which are also confirmed by EL-Tanany *et al.* (2018).

Also, our obtained data are in accordance with those noted by Al-Sohaibani *et al*. (2011) and Sagitov *et al.* (2011), who reported that enzymatic responses, such as the accumulation of β-1, 3glucanases and chitinases, the induction of lignification, and the production of phytoalexins, are closely associated with induced defense reactions in plants.

Conclusions

The current research evaluated the efficacy of clove and thyme essential oils (emulsion and nanoemulsion) and two biocides (Bio-Zeid and Bio-Arc) as fungicidal alternatives for the management of early blight disease of tomato incited by *Alternaria solani*. Our obtained results revealed the high antagonistic effect of clove and thyme oil nanoemulsion, clove emulsion, and Bio-Zeid against early blight disease of tomato in greenhouse and in the field, in addition to enhancing plant growth parameters and crop yield. These findings support the possibility of using the tested treatments as fungicidal alternatives for management of tomato early blight disease.

List of Abbreviations

ANOVA	Analysis of Variance
ARC	Agricultural Research Center
BRCS	Biological Resource Center Standard
DLS	Dynamic light scattering
DNS	Di nitro salicylic
L.S.D.	Least significant differences
PDI	Poly dispersity index
TEM	Transmission electron microscopy

Conflict of interest

The authors declare non-existence of any conflict of interests.

Ethical approval

Not applicable.

5. References

Abd-El-Kareem, F., El-Mougy, N. S., El-Gamal, N. G., & Fotouh, Y. O. (2006). Use of chitin and chitosan against tomato root rot disease under greenhouse conditions. *Research Journal of Agriculture and Biological Sciences*, *2*(4), 147-152.

Abdel-Monaim, M. F. (2010). Integrated management of damping-off, root and/or stem rot diseases of chickpea with sowing date, host resistance and bioagents. *Egyptian Journal of Phytopathology, 38*(1), 45-61.

Abd-Elsalam, K. A., & Khokhlov, A. R. (2015). Eugenol oil nanoemulsion: antifungal activity against Fusarium oxysporum f. sp. vasinfectum and phytotoxicity on cottonseeds. *Applied Nanoscience*, *5*, 255-265. https://doi.org/10.1007/s13204-014-0398-y

Adhikari, P., Oh, Y., & Panthee, D. R. (2017). Current status of early blight resistance in tomato: an update. *International journal of molecular sciences*, *18*(10), p.2019. https://doi.org/10.3390/ijms18102019

Ahmed, M.F.A. (2013). Studies on non-chemical methods to control some soil-borne fungal diseases of bean plants *Phaseolus vulgaris* L. Ph.D. Thesis. Faculty of Agriculture, Cairo University, pp: 137.

Akthar, M. S., Degaga, B., & Azam, T. (2014). Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: A review. *Issues in Biological Sciences and Pharmaceutical Research, 2*(1): 001-007.

Al-Sohaibani, S. A., Mahmoud, M. A., Al-Othman, M. R., Ragab, M. M., Saber, M. M., & Abd El-Aziz, A. R. (2011). Influence of some biotic and abiotic inducers on root rot disease incidence of sweet basil. *African Journal of Microbiology Research, 5*(22): 3628-3639.

Anand, T., Chandrasekaran, A., Kuttalam, S., Raguchander, T., Prakasam, V., & Samiyappan, R. (2007). Association of some plant defense enzyme activities with systemic resistance to early leaf blight and leaf spot induced in tomato plants by azoxystrobin and *Pseudomonas fluorescens*. *Journal of Plant Interactions, 2*(4): 233-244. https://doi.org/10.1080/17429140701708985

Anjali, C. H., Sharma, Y., Mukherjee, A., & Chandrasekaran, N. (2012). Neem oil (*Azadirachta indica*) nanoemulsion—a potent larvicidal agent against *Culex quinquefasciatus*. *Pest management science, 68*(2): 158-163.

Anton, N., & Vandamme, T. F. (2011). Nano-emulsions and micro-emulsions: clarifications of the critical differences. *Pharmaceutical research, 28*: 978–985. https://doi.org/10.1007/s11095-010-0309-1

Araújo, G. M. F., Barros, A. R., Oshiro-Junior, J. A., Soares, L. F., da Rocha, L. G., de Lima, Á. A. N., da Silva, J.A., Converti, A., & Damasceno, B. P. G. D. L. (2019). Nanoemulsions loaded with amphotericin B: development, characterization and leishmanicidal Activity. *Current pharmaceutical design, 25*(14): 1616–1622. https://doi.org/10.2174/1381612825666190705202030

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and chemical toxicology, 46*(2): 446-475. https://doi.org/10.1016/j.fct.2007.09.106

Bera, B. (2015). Nanoporous silicon prepared by vapour phase strain etch and sacrificial technique; Proceedings of the International Conference on Microelectronic Circuit and System (Micro); Kolkata, India. *International Journal of Computer Applications,* 42–45.

Bolar, J. P., Norelli, J. L., Wong, K. W., Hayes, C. K., Harman, G. E., & Aldwinckle, H. S. (2000). Expression of endochitinase from *Trichoderma harzianum* in transgenic apple increases resistance to apple scab and reduces vigor. *Phytopathology, 90*(1): 72-77. https://doi.org/10.1094/PHYTO.2000.90.1.72

Cohen, Y., Gisi, U., & Mosinger, E. (1991). Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acids. *Physiological and Molecular Plant Pathology, 38*(4): 255 – 263. https://doi.org/10.1016/S0885-5765(05)80117-1

Dai, L., Li, W., & Hou, X. (1997). Effect of the molecular structure of mixed nonionic surfactants on the temperature of mini emulsion formation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 125*(1): 27-32. https://doi.org/10.1016/S0927-7757(96)03859-9

Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., & Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics, 10*(2): p.57. https://doi.org/10.3390/pharmaceutics10020057

Derbalah, A., Shenashen, M., Hamza, A., Mohamed, A., & El Safty, S. (2018). Antifungal activity of fabricated mesoporous silica nanoparticles against early blight of tomato. *Egyptian journal of basic and applied sciences, 5*(2):145-150. https://doi.org/10.1016/j.ejbas.2018.05.002

El-Mougy, N.S. (2009). Effect of some essential oils for limiting early blight (*Alternaria solani*) development in potato field. *Journal of Plant Protection Research, 49*(1): 57-62. https://doi.org/10.2478/v10045-009-0008-2

El-Rafai, I. M., Asswah, S. M., & Awdalla, O. A. (2003). Biocontrol of some

tomato disease using some antagonistic microorganisms. *Pakistan Journal of Biological Sciences, 6*(4): 399- 406. https://doi.org/10.3923/pjbs.2003.399.406

El-Tanany, M. M., Hafez, M. A., Ahmed, G. A., & El-Mageed, M. A. (2018). Efficiency of biotic and abiotic inducers for controlling tomato early blight disease. *Middle East Journal of Agriculture Research, 7*(2): 650-670.

Fahiem, M.A. (2010). Studies on bean fruit in Egypt. M.Sc. Thesis, Faculty of Agriculture, Benha University, pp: 121.

FAOSTAT. (2021). Global tomato production in 2019. Rome, FAO.

Ghosh, V., Mukherjee, A., & Chandrasekaran, N. (2014). Eugenol-loaded antimicrobial nanoemulsion preserves fruit juice against, microbial spoilage. *Colloids and Surfaces B: Biointerfaces,* 114:392–397. https://doi.org/10.1016/j.colsurfb.2013.10.034

Ghotbi, R. S., Khatibzadeh, M., & Kordbacheh, S. (2014). Preparation of neem seed oil nanoemulsion. In *Proceedings of the 5th International Conference on nanotechnology: Fundamentals and Applications* (pp. 11-13).

Hammad, E. A., & Hasanin, M. M. H. (2022). Antagonistic effect of nanoemulsions of some essential oils against *Fusarium oxysporum* and root-knot nematode *Meloidogyne javanica* on coleus plants. *Pakistan Journal of Nematology, 40*(1): 35-48. https://dx.doi.org/10.17582/journal.pjn/2022/40.1.35.48

Hammerschmidt, R., Nuckles, E. M., & Kuć, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology, 20*(1):73-82. https://doi.org/10.1016/0048-4059(82)90025-X

Hartree, E.F. (1955). Haematin Compounds. In: Paech, K., Tracey, M.V. (eds) Modern Methods of Plant Analysis / Moderne Methoden der Pflanzenanalyse, Springer Verlag, Berlin, Heidelberg, 4 : 197- 245. https://doi.org/10.1007/978-3-642-64961-5_7

Hashem, A. H., Abdelaziz, A. M., Hassanin, M. M., Al-Askar, A. A., Abd-Elgawad, H., & Attia, M. S. (2023). Potential impacts of clove essential oil nanoemulsion as bio fungicides against Neoscytalidium Blight Disease of *Carum carvi* L. *Agronomy, 13*(4): p.1114. https://doi.org/10.3390/agronomy13041114

Hassanin, M. M. H., Abd-El-Sayed, M. A., & Abdallah, M. A. (2017). Antifungal activity of some essential oil emulsions and nanoemulsions against *Fusarium oxysporum* pathogen affecting cumin and geranium plants. *Scientific Journal of Flowers and Ornamental Plants, 4*(3): 245-258. https://doi.org/10.21608/SJFOP.2017.11326

Hassanin, M.M.H. (2013). Pathological studies on root rot and wilt of black cumin (*Nigella sativa*) and their management in Egypt. Ph. D. Faculty of Agriculture Al-Azhar University, (Egypt), pp: 137.

Hou, T., Sana, S. S., Li, H., Xing, Y., Nanda, A., Netala, V. R., & Zhang, Z. (2022). Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Bioscience, 47*: p.101716. https://doi.org/10.1016/j.fbio.2022.101716

Kato, M., & Shimizu, S. (1987). Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves; phenolic-dependent peroxidative degradation. *Canadian Journal of Botany, 65*(4): 729-735. https://doi.org/10.1139/b87-097

Marco, J. L. D., & Felix, C. R. (2007). Purification and characterization of a beta-Glucanase produced by *Trichoderma harzianum* showing biocontrol potential. *Brazilian Archives of Biology and Technology, 50*: 21-29. https://doi.org/10.1590/S1516-89132007000100003

Mayer, A. M., Harel, E., & Ben-Shaul, R. (1966). Assay of catechol oxidase—a critical comparison of methods. *Phytochemistry,*

5(4):783-789. https://doi.org/10.1016/S0031-9422(00)83660-2

McClements, D. J., & Rao, J. (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Critical reviews in food science and nutrition, 51*(4): 285-330. https://doi.org/10.1080/10408398.2011.559558

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry, 31*(3): 426-428. https://doi.org/10.1021/ac60147a030

Newman, S. M., Tantasawat, P., & Steffens, J. C. (2011). Tomato polyphenol oxidase B is spatially and temporally regulated during development and in response to ethylene. *Molecules, 16*(1): 493-517. https://doi.org/10.3390/molecules16010493

Oliva, M. D. L. M., Carezzano, M. E., Giuliano, M., Daghero, J., Zygadlo, J., Bogino, P., Giordano, W., & Demo, M. (2015). Antimicrobial activity of essential oils of *Thymus vulgaris* and *Origanum vulgare* on phytopathogenic strains isolated from soybean. *Plant Biology, 17*(3): 758–765. https://doi.org/10.1111/plb.12282

Poudel, S., Khanal, P., Bigyan, K. C., Pokharel, S., & Gauli, S. (2023). Biological control of fungal phytopathogens with *Trichoderma harzianum* and its fungicidal compatibility. *International Journal of Applied Biology, 7*(1): 47-58. https://doi.org/10.20956/ijab.v7i1.26502

Rahman, M., Borah, S. M., Borah, P. K., Bora, P., Sarmah, B. K., Lal, M. K., Tiwari, R.K., & Kumar, R. (2023). Deciphering the antimicrobial activity of multifaceted rhizospheric biocontrol agents of solanaceous crops viz., *Trichoderma harzianum* MC2, and *Trichoderma harzianum* NBG. *Frontiers in Plant Science, 14*: p.1141506. https://doi.org/10.3389/fpls.2023.1141506

Sagitov, A. O., El-Habbaa, G. M., & El-Fiki, I. A. (2011). Studies on tomato wilt caused by *Fusarium oxysporum f.sp. lycopersici* in Kazakhstan. 2: Effect of exogenous application of plant extracts and safe chemicals as resistance inducer treatments on the activity of the oxidative enzymes. *Physiologiae Plantarum, 1*(49):107-113.

Sajjadi, S., Zerfa, M., & Brooks, B. W. (2002). Dynamic behaviour of drops in oil/water/oil dispersions. *Chemical Engineering Science, 57*(4): 663-675. https://doi.org/10.1016/S0009-2509(01)00415-8

Saloko, S., Darmadji, P., Setiaji, B., Pranoto, Y., & Anal, A. K. (2013). Encapsulation of coconut shell liquid smoke in chitosan-maltodextrin based nanoparticles. *International Food Research Journal, 20*(3): 1269-1276.

Sattary, M., Amini, J., & Hallaj, R. (2020). Antifungal activity of the lemongrass and clove oil encapsulated in mesoporous silica nanoparticles against wheat's take-all disease. *Pesticide Biochemistry and Physiology, 170*: p.104696. https://doi.org/10.1016/j.pestbp.2020.104696

Šegvić Klarić, M., Kosalec, I., Mastelić, J., Piecková, E., & Pepeljnak, S. (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. https://doi.org/10.1111/j.1472-765X.2006.02032.x

Sekiguchi, S., Miura, Y., Kaneko, H., Nishimura, S. I., Nishi, N., Iwase, M., & Tokura, S. (1993). Molecular weight dependency of antimicrobial activity by chitosan oligomers. In: Nishinari K., Doi E., editors. *Food Hydrocolloids: Structures, Properties, and Functions*. Springer; Boston, MA, USA pp. 71–76. https://doi.org/10.1007/978-1-4615-2486-1_6

Sharf, W., Javaid, A., Shoaib, A., & Khan, I. H. (2021). Induction of resistance in chili against *Sclerotium rolfsii* by plant-growth-promoting rhizobacteria and *Anagallis arvensis*. *Egyptian Journal of Biological Pest Control, 31*, 1-11.

Sharma, A., Rajendran, S., Srivastava, A., Sharma, S., & Kundu, B. (2017). Antifungal activities of selected essential oils against *Fusarium oxysporum* f. sp. *lycopersici* 1322, with emphasis on *Syzygium aromaticum* essential oil. *Journal of Bioscience and Bioengineering, 123*(3): 308 -313. https://doi.org/10.1016/j.jbiosc.2016.09.011

Song, W., & Keane, A. (2006). Parameter screening using impact factors and surrogate-based ANOVA techniques. In *11th AIAA/ISSMO Multidisciplinary Analysis and Optimization Conference* (p. 7088). https://doi.org/10.2514/6.2006-7088

Sugumar, S., Clarke, S. K., Nirmala, M. J., Tyagi, B. K., Mukherjee, A., & Chandrasekaran, N. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against *Culex quinquefasciatus*. *Bulletin of Entomological Research, 104*(3): 393-402. https://doi.org/10.1017/S0007485313000710

Tanovic, B., Milijasevic, S., & Obradovic, A. (2007). In vitro effect of plant essential oils on growth of some soil-borne pathogens. *Acta Horticulturae, 729*: 467–471. https://doi.org/10.17660/ActaHortic.2007.729.79

Thabet, M., & Khalifa, W. (2018). Antifungal activities of clove oil against root rot and wilt pathogens of tomato plants. *American-Eurasian Journal of Agricultural and Environmental Sciences, 18*(3): 105-114. https://doi.org/10.5829/idosi.aejaes.2018.105.114

Townsend, G.R., & Heuberger, J.W. (1943). Method for estimating losses caused by diseases in fungicide experiments. *Plant Disease Reporter, 27*: 340-343.

Tyśkiewicz, R., Nowak, A., Ozimek, E., & Jaroszuk-Ściseł, J. (2022). *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *International Journal of Molecular Sciences, 23*(4): p.2329. https://doi.org/10.3390/ijms23042329

Vloutoglou, I., & Kalogerakis, S. N. (2000). Effects of inoculum concentration, wetness duration and plant age on development of early blight (*Alternaria solani*) and on shedding of leaves in tomato plants. *Plant pathology, 49*(3): 339–345. https://doi.org/10.1046/j.1365-3059.2000.00462.x

Walker, R., Decker, E. A., & McClements, D. J. (2015). Development of food-grade nanoemulsions and emulsions for delivery of omega-3 fatty acids: opportunities and obstacles in the food industry. *Food and fuood and function, 6*(1): 41–54. https://doi.org/10.1039/C4FO00723A