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Article

Comparative Study of Supplementing Two Biological Bioagents and Conventional Fungicide Against *Alternaria dauci* Pathogenicity and Monitoring Plant Growth and Yield of Coriander

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Abstract: Coriander (*Coriandrum sativum* L.) belongs to the *Apiaceae* family and is amongst the widely used medicinal plants, possessing nutritional and medicinal properties. Coriander leaf spot, caused by *Alternaria dauci* is the most devasting disease worldwide including Egypt. Therefore, the present study aimed to evaluate cumin and thyme oils nanoemulsions (EONEs) and their crude foliar application impacts in enhancing the antifungal activities against *A. dauci* and coriander growth promotion as an alternative approach for this disease management to synthetic fungicides. Cumin and thyme oils were characterized using ζ-potential, ζ-average and TEM analysis. Two field experiments were carried out during two successive winter seasons of 2021/2022 and 2022/2023 using concentrations of 0.25, 0.5, 0.75 and 1.0 ml/L for cumin and thyme oils nanoemulsions and 1.0 ml/L for their crude. The results revealed that the effects of cumin and thyme oils nanoemulsions treatments on all characteristics of coriander vegetative growth and yield were significant. The concentration of 0.5 ml/L nanoemulsion (EONEs) was the best of both cumin and thyme with the superiority of thyme helping in increasing the number of leaves, dry matter %, umbellets number, seed yield g/plant, volatile oil percentage and oil yield ml/plant compared with the control and other treatments during the two seasons. Similarly, foliar application with cumin and thyme oils (EOs) crude increased all previous characteristics compared to the control ranking third and fourth among the best treatments with the superiority of thyme oil in both seasons. The essential oil components of coriander volatile oil were also affected by the different types and concentrations of cumin and thyme oils, which increased the dominant compound percentage of L-linalool. On the other hand, the results revealed that foliar application of cumin and thyme oils nanoemulsions at 0.75 and 1.0 ml/L concentrations were the most effective treatments in reducing coriander disease leaf blight incidence (DI) and disease severity (DS) parameters during both seasons compared to control plants. Therefore, cumin and thyme oils nanoemulsions represent a promising strategy for a strong antifungal activity in controlling *A. dauc* and exhibition coriander growth and yield improvement, i.e., they provide important inferences for the development and utilization of green chemicals as antifungal delivery systems and increasing long term safety for *A. dauc* controlling and growth factors in coriander.

Key words: Coriander, *Alternaria dauci*, cumin and thyme oils, nanoemulsions, yield.

INTRODUCTION

Coriandrum sativum L. (coriander) is a member of the Apiaceae family and is the most widely used and well-liked spice because of its many applications worldwide and Mediterranean origins, Coriander is one of the most widely used and well-liked spices Coriander's distinct flavor makes it a desirable ingredient for curry powder and food processing as a preservative **(Abdou *et al.* 2015)**. According to **Kačániová *et al.* (2020)**, a chemical investigation of coriander volatile oil revealed the presence of monoterpenes, limonene, pinene, terpinene, p-cymene, citronellol, borneol, camphor, coriandrin, geraniol, dihydrocoriandrin, and coriandrons. Multiple writers have already investigated the usefulness of coriander as a medicinal plant. **Pathak Nimish *et al.* (2011)** emphasized the anthelmintic, anti-mutagenic, anti-diabetic, and antioxidant properties.

Additionally, some data about hypnotic, anticonvulsant, and diuretic effects were discovered. Furthermore, coriander's leaves, seeds, and flowers all work effectively to decrease cholesterol and guard against the toxicity of heavy metals. The anticancer, anxiolytic, hepatoprotective, post-coital, and anti-fertility activities of coriander oil can be used to sum up its health benefits **(Momin *et al.* 2012)**. The volatile oil and different extracts from coriander fruits are responsible for the antioxidant **(Kačániová *et al.*, 2020)**, anti-diabetic **(Sreelatha and Inbavalli, 2012)**, anti-cancerous, and antimutagenic activities **(Esiyok *et al.*, 2004)**.

Microbial contamination is one of the main causes of food quality loss, which in turn affects product shelf life. Major food spoilage molds are the most common opportunistic biological agents in nature. Mycotoxins affect approximately 25% of the total annual production of different crops **(FAO, 2004)**. Modern food processing methods have significantly improved food safety and quality. Certain species are phytopathogens that ruin crops during storage and after harvest, as well as causing plant illnesses in the field **(Mangwende *et al.*, 2018; Patriarca and Fernández Pinto, 2018)**.

Alternaria is one of the most destructive fungal infections that has been documented on coriander in several parts of the world **(Mangwende *et al.*, 2018)**. Numerous varieties of the common fungus *Alternaria* affect agricultural products, such as cereal grains, fruits, and vegetables, both before and after harvest. Spores from the genus *Alternaria* are commonly found in a variety of settings and are widely dispersed throughout the environment. Plant aerial portions are naturally contaminated by *Alternaria* species, which are easily separated from decomposing debris.

It was confirmed that A. alternative is the causative agent of *Alternaria* leaf spot disease when the disease manifested itself most severely (64%) on damaged coriander leaves infected with the bacterium. It has been demonstrated that Coriander seeds contaminated with *Alternaria alternata* can spread to the growing plants **(Mangwende *et al.*, 2018)**.

Essential oils (EOs), which are naturally occurring antimicrobials, are becoming more popular due to the demand for minimally processed food **(Juneja *et al.*, 2012)**. They are aromatic and volatile secondary metabolites that are primarily used for their flavor and fragrances as well as a variety of biological properties like antioxidant and antimicrobial characteristics **(Basak and Guha, 2018)**. Numerous phytochemical studies have examined the antifungal and insecticidal properties of essential oil molecules, including those conducted by **Juneja *et al.* (2012), Regnault-Roger *et al.* (2012), El-Gamal and Ghoneem (2014), and Basak and Guha (2018)**.

Ben-Jabeur *et al.* (2015) reported that the injection of thyme essential oil as a biocontrol agent enhanced the activity of peroxidase (POX), leading to a decrease in grey mold and *Fusarium* wilt caused by *Botrytis cinerea* and *Fusarium oxysporum* f. sp. *radicis lycopersici*, respectively. However, the primary component in cumin oil, cuminic aldehyde, may be responsible for its antibacterial and fungi-toxic properties **(Jacobellis *et al.*, 2005 and Donaldson *et al.*, 2005)**.

Applying excessive amounts of oil to boost its antibacterial activity may cause the meal to have a negative organoleptic effect **(Basak and Guha, 2018)**. These activities of essential oils could be increased numerous times over if the EO is applied in the form of tiny, nanoscale droplets. Their attractiveness has promoted the creation of EO nanoemulsions. According to **McClements and Rao (2011**), **Liang *et al.* (2012)**, and **Salvia-Trujillo *et al.* (2015)**, nanoemulsions are made up of an oil phase distributed in an aqueous continuous phase and a thin interfacial coating of surfactant molecules around each oil droplet with a size within the nanometer range. It is most likely possible to eliminate component interactions and maintain the highest level of efficacy for EOs in the food system by employing appropriate edible EO delivery technologies like an emulsion. When EO is nano-emulsified, it becomes more accessible in the food matrix, which may increase its bioactivity **(Basak and Guha, 2018)**.

Thus, the present study aimed to investigate the potential effects of Thyme and Cumin essential oils nanoemulsions as antifungal agents against *Alternaria dauci* pathogen and the production improvement of coriander.

MATERIALS AND METHODS

Essential oils extraction

Coriander and cumin seeds and thyme herb were obtained from the Medicinal and Aromatic Plants Res., Dept., HRI, ARC, Egypt and subjected to Clevenger apparatus for 3 hours (100 g /sample) to obtain their essential oils. Using a Clevenger extractor is recommended at the Lab scale for essential oil extraction according to **Guenther, (1961).** Essential oils were stored at 4 °C till use. *Alternaria dauci* (ON171224) pathogenic was isolated from coriander naturally infected cv. A local variety seed lot **(Ghoneem *et al.*, 2023).**

Cumin and thyme oils emulsion Formulation

Pinnamaneni *et al.* (2003) state the use of microfluidization to duplicate the homogenized oil-in-water emulsion formulations. The batch volume was reduced from 100 ml to 50 ml because microfluidization, as opposed to homogenization, allowed for successful formulation with a lower volume of material. Room temperature was used to handle the coarse emulsions that were produced by magnetic stirring at 30 °C for 5 minutes. The emulsion reached a temperature of 305 °C during processing after the process parameters were selected following several trial runs with emulsions kept at room temperature**.**

Measurement of Cumin and thyme oils nanoemulsion droplet size

The droplet size of the nanoemulsion was measured using dynamic light scattering analyses using the Zeta Nano sizer instrument (Malvern Instruments Ltd; zs90, Worcestershire, UK). 30 µl of the nanoemulsion was diluted with three ml of water at 25 ˚C before measurement. The particle size data was expressed as the mean of the Z-average of 3 separate batches of the oils nanoemulsion. This work was conducted at the Electron Microscopy Unit, Faculty of Agri., Mansoura Univ., Egypt. Photon correlation spectroscopy was used for the nanoemulsion characterization **(Essien *et al.*, 2011; Galindo-Rodriguez *et al.*, 2004; Rida and Harb, 2014).**

Transmission electron microscopy (TEM)

The dimension and shape of oil nanoemulsions were detected by transmission electron microscopy (TEM, JEOL, 2100). The TEM samples were prepared by dropping the solution on a 200-mesh copper specimen grid coated with film, using filter paper the excess fluid was removed after 10 minutes. Then, the grid was stained with one drop of phosphotungstic acid 3 % and was given 3 minutes for drying. The micrograph was examined by JEOLJEM 6510 at 160 kV in the RCMB, Mansoura Univ., Egypt.

Thyme, cumin and coriander essential oils Gas chromatography-mass spectrometry (GC-MS)

The volatile content of the three oils was determined to have a particular chemical composition using a Shimadzu GC-MS-QP2010 Ultra analysis system located in Tokyo, Japan. The compounds were separated using an Inc DB-5 60 m x 0.25 mm/0.25-micron column (Agilent Technologies, Santa Clara, CA, USA). The oven was set to operate at 50 °C for 3 minutes. Following that, it increased to 250 °C at a rate of 8 °C per minute, a temperature it held for ten minutes. The spectrophotometer was run in the electron impact mode. The ion source, interface, and injector were kept at 250 °C, 230 °C, and 220 °C, respectively. For the split injection, a 1 µL diluted sample was injected into n-hexane (1:1, v/v) at a split ratio of 1:20 and a column flow rate of 1.5 mL/min, and helium was utilized as the carrier gas. The components were identified using the computer controlling the GC-MS system based on a comparison of the sample's relative indices and mass spectra using computer matching with data from the WILEY and National Institute of Standards and Technology (NIST08) libraries (http://webbook.nist.gov, accessed on, 2023). Individual isolated compound identifications were also performed by comparing their mass spectra and retention durations with those of real compounds and data from the literature **(Elsherbiny *et al.*, 2017)**.

Experimental Layout

A field experiment was carried out in the winter seasons of 2021–2022 and 2022–2023, at El-Baramoon Research Farm (31°08’11.3’’N31°28’19.6’’E), Mansoura Horticulture Research Station, HRI, ARC, Egypt. The experimental field soil sample was taken from a depth of 0 to 30 cm. Its physical and chemical properties were then investigated after it was air-dried and sieved through a 2 mm sieve. The features and procedures described by **Sparks *et al.* (2020)** and **Dane and Topp (2020)** were adhered to ascertain the physicochemical characteristics of the soil which is presented in Table 1.

Table 1. Experimental field soil physicochemical properties

Mechanical analysis %				T. class	O.M%	S.P%	$CaCO_3$%	E.C $dS.m^{-1}$ 1:5	pH 1:2.5	Available ppm		
C. sand	F. sand	silt	clay							N	P	K
4.6	34.7	35.3	25.4	silt-clay-loam (S.C.L)	1.75	45.5	2.88	0.88	8.13	45.9	4.66	187.0

Every experimental plot had four ridges and measured three by six meters. The experimental field was set up with ridges placed 60 centimeters apart. Seeds were planted in hills with 30 cm between each hill in mid-October for both seasons and a month later, the plants were thinned to just one plant per hill. 200 kg/fed of phosphorus (Calcium superphosphate) was added all at once during field preparation. Each ingredient was added in two equal doses separately: nitrogen (ammonium sulfate at a rate of 100 kg/fed.) and potassium (potassium sulfate at a rate of 50 kg/fed.). The first dose was administered following thinning and the second dose at the onset of flowering.

The experimental design was a complete randomized block with four replicates. Each replicate included twelve treatments as the following: T1= Control, T2= Chemical Fungicide (Chem-cide) (Kasumin 2% SL at 25cm3 /L), T3= Cumin essential oil normal type (Crude C 1ml/L) at 1ml/L, T4= Cumin essential oil Nano type at 0.25 ml/L (C. Nano 0.25 ml/L), T5= Cumin essential oil Nano type at

0.5 ml/L (C. Nano 0.5 ml/L), T6= Cumin essential oil Nano type at 0.75 ml/L (C. Nano 0.75 ml/L), T7= Cumin essential oil Nano type at 1ml/L (C. Nano 1ml/L), T8= Thyme essential oil normal type (Crude Th 1ml/L) at 1ml/L, T9= Thyme essential oil Nano type at 0.25 ml/L (Th. Nano 0.25 ml/L), T10=Thyme essential oil Nano type at 0.5 ml/L (Th. Nano 0.5 ml/L), T11= Thyme essential oil Nano type at 0.75 ml/L (Th. Nano 0.75 ml/L), and T12= Thyme essential oil Nano type at 1ml/L (Th. Nano 1ml/L).

The used chemical fungicide was Kasumin 2% SL (Kasagamycin) and obtained from Cairo Chemical Company, Egypt.

Using a hand atomizer, plants were sprayed twice with the aforementioned treatments 8 weeks and 10 weeks after sowing, following the addition of tween 20 (0.5%) as a surfactant. After three months of sowing for each growing season, 16 plants/treatment were randomly selected to measure various vegetative growth characteristics, in terms, of the number of leaves and percentage of dry matter. At the harvest stage (when the secondary umbel color was changed to green-yellow according to **El-Gamal and Ahmed (2016a),** the number of umbellets /plant, fruit yield g/plant and 1000 fruit weight (seed index) were recorded. The volatile oil percentage was determined in air-dried fruits after oil extraction.

Antifungal assay *in vivo*

The disease incidence (DI) and disease severity (DS) of the infected leaves were measured and recorded for each of the various treatments three months after sowing. The severity of *Alternaria* leaf spot disease was evaluated using the **Boedoab *et al.* (2012)** disease rating scale. Using a 0–5 scale adapted from **Pawelec *et al.* (2006)**, the percentage of necrotic leaf area (0= no visible disease symptoms, 1= 1-4 %, 2= 5-19 %, 3= 20–39 %, 4= 40–59 %, and 5= ≥ 60% of leaf area affected) was used in this index to rate the severity on 30 plant leaves of each treatment. The values for each leaf were totaled and averaged to determine the DS for each plant. The quantity of permanently leaf-bearing infected plants

Statistical Design and Analysis

The data set was the average of a minimum of four replicates. The two field experiments and the antifungal of nanoemulsion oils assay were designed using a one-way randomized blocks design. The one-way ANOVA was carried out using CoStat software version 6.4 (CoHort Software, United States), and the difference between the averages based on the Duncan test was confirmed (CoStat, 2008). According to **Duncan (1965)**, all statistical hypothesis tests were conducted at a probability (P) value of ≤0.05.

RESULTS

GC-MS Composition results of Thyme and Cumin essential oils (EOs) identified constituents

Both Cumin and thyme essential oils were analyzed via gas chromatography-mass spectrometry (GC-MS) listed in Table (1) and shown in Figure (1). The GC-MS analysis results of cumin EO were presented in Table and Figure 1 showing eighteen identified compounds. The major constituents of cumin oil were Cumin aldehyde (20.41%), O-Cymene (17.73%), β-Pinene (15.80%), γ-Terpinene (15.29), 1-Phenylethanol (11.62%) and bicyclic monoterpenoid (9.00%). Meanwhile, the GC-MS analysis results of thyme EO were presented at the same Table 1 and Figure 1, showing bioactive twenty-seven identified compounds. Particularly, the major constituents were O-Cymene (30.83%), Thymol (24.02%), and Carvone (5.30%).

Cumin oil **Thyme oil**

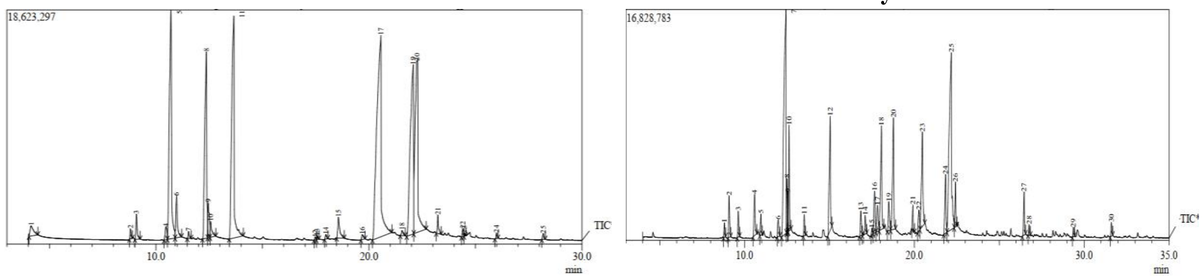


Fig. (1). GC-Ms chromatogram of bioactive metabolites of Thyme and Cumin essential oils Nanoemulsions

*= Total ion chromatogram of the tentatively identified compounds by GC-Ms. Arrows represent the beginning and end of the base of the compound peak.

Table (2). Identified active constituents of cumin and thyme essential oils (EOs) using GC-MS analysis

ID	Cumin		Thyme	
	constituent	percentage	constituent	percentage
1	Glycyl-D-asparagine	2.05	(3-Thujene	0.65
2	Thujene	0.51	α.-Pinene	1.58
3	α.-Pinene	1.02	Camphene	0.72
4	β-Phellandrene	0.71	1-Octen-3-ol	2.03
5	β-Pinene	15.80	β-Myrcene	0.83
6	β-Myrcene	1.28	-Carene	0.42
7	α.-Phellandrene	0.35	O-Cymene	30.83
8	O-Cymene	17.73	D-Limonene	0.53
9	Cineole	0.19	β-Phellandrene	1.49
10	γ-Terpinene	15.29	Eucalyptol	2.01
11	Phellandral	0.51	γ-Terpinene	0.55
12	1-(1-Ethyl-2,3-dimethyl-cyclopent-2-enyl)-ethanone	0.39	β- linalool	3.85
	Cumin aldehyde	20.41	Camphor	0.71
14	bicyclic monoterpenoid	9.00	endo-Borneol	3.13
15	1-Phenylethanol	11.62	Menthol	0.60
16	Benzene, (1-methoxypropyl)-	0.59	Terpinenol	4.07
17	beta.-Citral	0.23	α.-Terpineol)	1.29
18	Phthalic acid, 2-isopropylphenyl methyl ester	0.11	Estragole	4.19
19	-----	-----	Thymol methyl ether	1.33
20			Benzenemethanol, 4-(1,1-dimethylethyl)-	1.11
21			(+)-Carvone	5.30
22			β-methylstyrene	1.25
23			Thymol	24.02
25			Carvacrol	3.31
26			Caryophyllene	0.51
27			Caryophyllene oxide	0.19
Total identified constituents %		97.79	Total identified constituents %	96.50

TEM Characterization of cumin and thyme oils nanoemulsions

Essential oils (EOs) offer a wealth of potential benefits due to their unique bioactive compounds **(Saber *et al.*, 2009).** However, their limited water solubility and extreme sensitivity to light, heat, and oxygen hinder their practical applications. Fortunately, nanotechnology provides intriguing solutions through the development of Essential oils nanoemulsions (EONEs). The structural characteristics of EONEs prepared from cumin and thyme oils were investigated using TEM (Figure 2).

The TEM analysis of the size and morphology of cumin EONEs revealed spherical particles with an average size of 29.82 nm and a range of 15.27 to 39.7 nm. This agrees well with **Abdelaal *et al.* (2021),** who reported a similar size range (15.8 to 99.8 nm) for cumin EONEs. Thyme EONEs displayed almost spherical droplets with an average size of 0.033 µm and a range of 0.03 to 0.04 µm. This finding aligns with previous research by **Hassanin *et al.* (2017)** and **El-Sayed and El-Sayed (2021),** who observed spherical thyme EONEs in the range of 20-52 nm and 26.6-45.3 nm, respectively.

Both cumin and thyme EONEs showcased exceptional dispersion and uniformity as evidenced by the TEM images. This finding aligns with **Sundararajan *et al.* (2018)**, who highlighted the spherical shape and uniform distribution of EONEs as crucial characteristics for enhanced stability and release of bioactive compounds. Accordingly, the TEM study confirms the successful formation of EONEs with desirable characteristics, including small size, spherical morphology, and uniform dispersion. These features contribute to improved solubility, stability, and potential delivery of EOs for various applications.

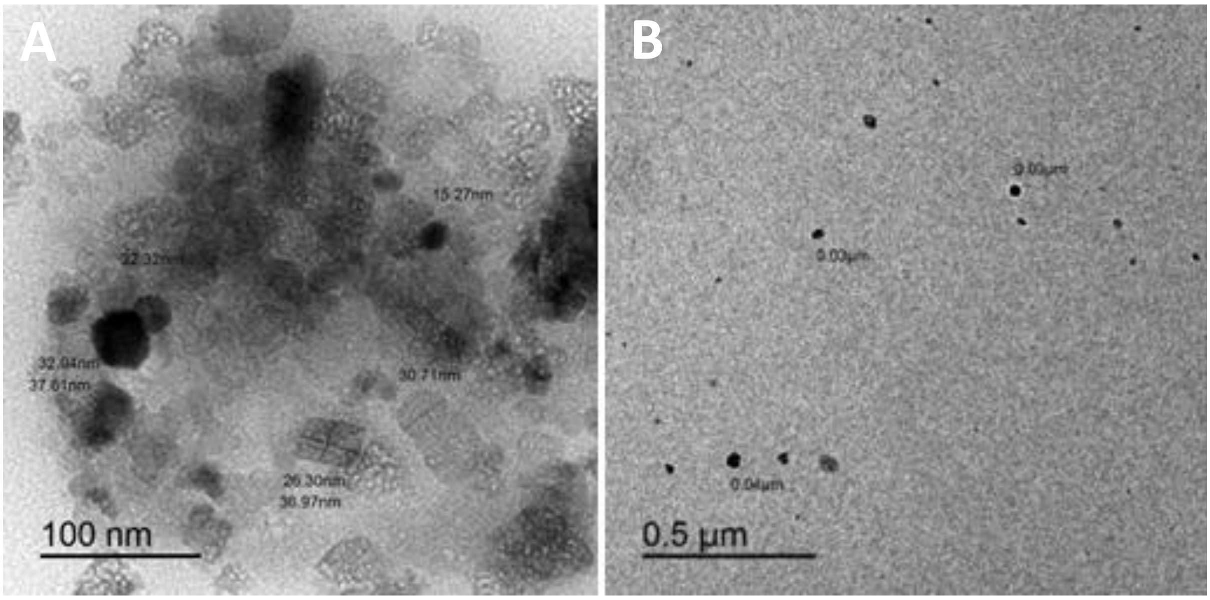
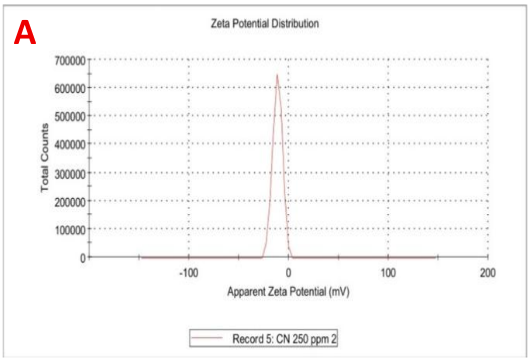


Fig. (2.) TEM image showing the morphological characterization of cumin and thyme EONEs at 160 kV; (a) cumin nanoemulsion, scale bar 100 nm, and (b) thyme nanoemulsion, 0.5 µm magnifications

Zeta potential of EOs Nanoemulsion

The zeta potential reveals the electrical charge surrounding particles in dispersion, essentially acting as a shield against aggregation **(Hanaor *et al.*, 2012).** The current study found that both cumin and thyme EONEs had zeta potential values in water exceeding -10 mV (cumin: -11.4 mV, thyme: -18.3 mV). This negative charge indicates strong electrostatic repulsion between neighboring droplets, effectively preventing unwanted clustering and sedimentation (Figure 3). This translates to enhanced stability of the EONEs, ensuring their longevity and functionality. By combining high zeta potential values with appropriate particle sizes, these EONEs hold promise for improved stability, enhanced bioavailability, and ultimately, greater effectiveness in various delivery and therapeutic applications **(Benelli *et al.*, 2017; Kumar and Dixit, 2017)**.



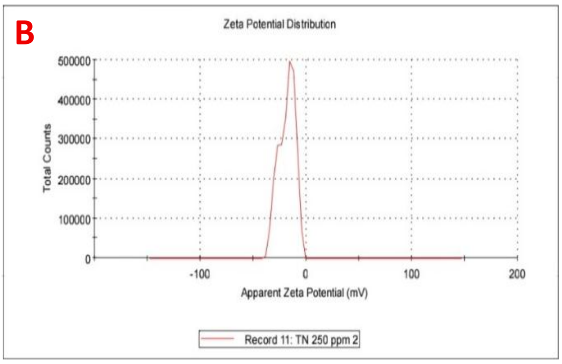


Figure 3. Zeta potential of cumin (A) and thyme (B) essential oils nanoemulsions (EONEs)

In vivo Antifungal Assay

In the in vivo antifungal assay conducted to determine the optimal concentration of cumin and thyme for controlling coriander leaf spot disease, various concentrations were tested (Table 3). Notably, both cumin and thyme EONEs, particularly at higher concentrations, exhibited significant efficacy in reducing symptoms of leaf spot disease compared to the fungicide and control treatments. A positive correlation between EONE concentrations and effectiveness was evident. In terms of treatment outcomes, thyme essential oil nanoemulsion (EONE) at 0.5 and 0.75 ml/L (Th. Nano 0.5 and 0.75 ml/L), demonstrated the most substantial reduction in DI and DS parameters during the first season (58.19 & 60.71% and 45.73 & 64.85%, respectively) and the second season (56.56 & 61.47% and 46.59 & 49.55%, respectively). Following closely, Cumin EONE at concentrations of 0.5, 0.75, and 1 ml/L exhibited notable reductions in DI and DS parameters during both the first (35.44, 40.13, & 46.28% and 33.45, 38.91, & 47.44%, respectively) and second seasons (38.30, 45.87, & 49.52% and 36.20, 40.06, & 47.18%, respectively) when compared to the untreated control.

Table (3). The leaf spot disease parameters of coriander plants, showcasing the impact of the applied treatments using cumin and thyme essential oils nanoemulsions under field conditions

Treatments	1st year		2nd year	
	DI	DS	DI	DS
T1	47.60 a	2.93 a	56.40 a	3.37 a
T2	30.33 b-d	2.12 b	37.00 bc	2.00 bc
T3	22.70 cd	2.00 bc	37.97 bc	2.23 bc
T4	24.03 cd	1.63 cd	30.83 de	1.90 c
T5	19.90 d	1.59 cd	24.50 fg	1.80 c
T6	18.70 d	1.03 e	21.73 g	1.70 c
T7	37.48 a-c	2.07 b	37.03 bc	2.26 bc
T8	32.87 a-d	2.10 b	36.13 c	2.63 b
T9	30.73 b-d	1.95 b-d	34.80 cd	2.15 bc
T10	28.50 b-d	1.79 b-d	30.53 de	2.02 bc
T11	25.57 cd	1.54 d	28.47 ef	1.78 c
T12	41.77 ab	2.57 a	41.67 b	2.56 b

Statistical significance was determined using the Duncan test at a significance level of $P \leq 0.05$, with a sample size of n = 16 plants. In each column, averages denoted by a different letter(s) are considered significantly different.

Where, T1= Cont., T2= Chem-cide,T3= Crude C 1ml/L,T4= C. Nano 0.25 ml/L, T5= C. Nano 0.5 ml/L, T6= C. Nano 0.75 ml/L, T7= C. Nano 1ml/L, T8= Crude Th 1ml/L, T9= Th. Nano 0.25 ml/L, T10= Th. Nano 0.5 ml/L, T11= Th. Nano 0.75 ml/L, T12= Th. Nano 1ml/L

Our findings align with the research conducted by **Attia *et al.* (2023)**. They reported that thyme oil nanoemulsion and emulsion effectively reduced the disease index of fennel plants infected by *Fusarium oxysporum* by up to 17.5, and 42.5%, respectively, compared to the untreated infected control with a disease index of 85%. Additionally, the percentage of protection increased to 79.4, and 50%, respectively.

In this context, it is suggested that thyme EONE can impede the growth of the pathogen either directly through antibiosis, as indicated by **Sharma *et al.*, 2023**, or indirectly by enhancing plant resistance. The enhancement of plant resistance involves mechanisms such as increasing phenol, proline, and antioxidant enzymes like peroxidase and polyphenol oxidase activities. Moreover, it contributes to the reduction of oxidative stress by minimizing the formation of malondialdehyde and hydrogen peroxide, as highlighted in the study by **Attia *et al.* (2023)**.

Growth Characterization

As shown in Figure (4), the number of leaves, dry matter %, and the number of umbellets/plant were increased in treated plants with cumin and thyme essential oils nanoemulsion (EONEs) in both seasons. In the first year, the highest values of leaves number per plant and dry matter % were recorded in coriander plants treated with thyme EONE (0.5 ml/L) with values of 53 leaves/plant and 10.31%, respectively followed by coriander treated with cumin EONE (0.5 ml/L) with values of leaves/plant and 10.16%, respectively. The second season had the same trend of the first one. In the same Figure (4), The highest number of umbellets/plant was of the treatment thyme EONE (0.5 ml/L) 456 and 468 umbellets/plant followed by the treatment cumin EONE (0.5 ml/L) which gave 438 and 444 umbellets/plant in the first and second seasons, respectively. Meanwhile, the lowest values were recorded in untreated plants (15 and 16 leaves/plant & 8.21 and 8.25% dry matter & 100 and 108 umbellets/plant) in the first and second growing seasons, respectively.

The results obtained are consistent with the findings of **Kumari *et al.*, (2018)** for soybeans. They came to the conclusion that spraying soybean plants with thymol nanoemulsion greatly reduced the severity of the disease, increased the percent efficacy of disease control, and significantly improved plant growth characteristics. Thymol nanoemulsion may have applications in agriculture as an antimicrobial and plant growth promoter. Furthermore, thymol nanoemulsion's direct antibacterial activity and ability to induce plant defensive responses by stimulating the synthesis of phenolic compounds and peroxidase activity may account for the much-decreased disease incidence **(Ben-Jabeur *et al.*, 2015)**. The growth-stimulating properties of thyme and cumin oils nanoemulsion on plants, to determine an ideal dosage for disease prevention that doesn't interfere with long-term plant growth. Additional studies revealed that, when tested against the four test fungi *Fusarium oxysporum*, *Penicillium digitatum*, *Rhizoctonia solani*, and *Asperigallus niger*, thymol was the most effective monoterpene antifungal drug **(Marei *et al.*, 2012)**.

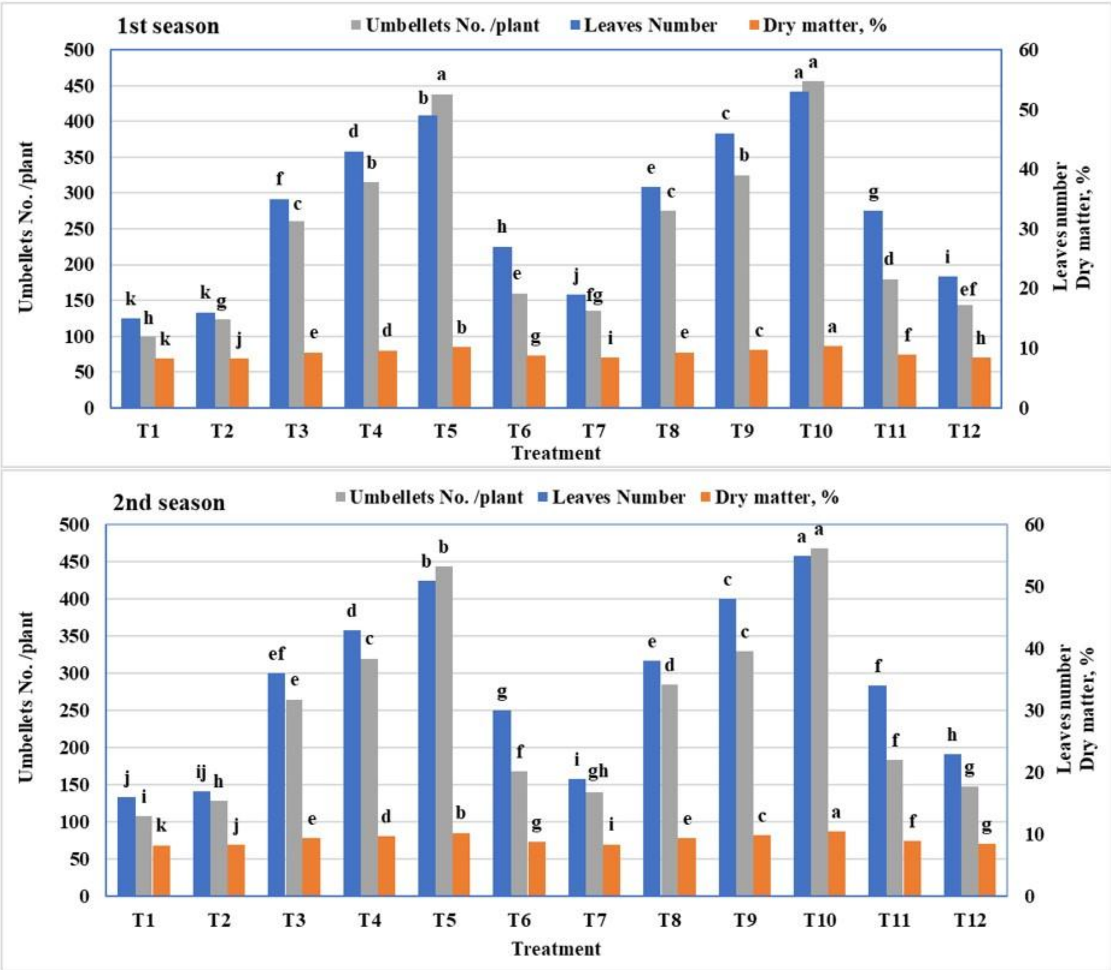


Fig. (4). Cumin and thyme essential oils nanoemulsions (EONEs) foliar application effects on coriander growth characterization during seasons of 2021/2022 and 2022/2023

Statistical significance was determined using the Duncan test at a significance level of $P \leq 0.05$. For each season, and each criterion, the averages denoted by a different letter(s) are considered significantly different.
T1= Cont., T2= Chem-cide,T3= Crude C 1ml/L,T4= C. Nano 0.25 ml/L, T5= C. Nano 0.5 ml/L, T6= C. Nano 0.75 ml/L, T7= C. Nano 1ml/L, T8= Crude Th 1ml/L, T9= Th. Nano 0.25 ml/L, T10= Th. Nano 0.5 ml/L, T11= Th. Nano 0.75 ml/L, T12= Th. Nano 1ml/L

Seed and volatile oil yield characterization

Data presented in Figure (5) indicate that cumin and thyme essential oils nanoemulsion (EONEs) foliar application significantly raised coriander seed index (g/1000 seeds), seed yield (g/plant), volatile oil % and oil yield (ml/plant) in both seasons relative to control plants (water spraying). The results of seed yield per plant and seed index showed that cumin and thyme EONPs spraying had a significant impact on seed yield per plant and seed index in the first and second seasons. In the first season, the height values of seed yield per plant and seed index were of plants sprayed with nanoemulsions of cumin and thyme oils at the rate of 0.5 ml/L with the superiority of thyme EONE (16.07 g/1000 seeds and 52.48 g/plant for thyme EONE and 15.76 g/1000 seeds and 48.29 g/plant for cumin EONE meanwhile, the lowest values were detected in untreated plants (11.82 g/1000 seeds and 29.95 g/plant). The second season had the same trend as the first one.

Regarding the volatile oil% and oil yield per plant, the highest oil %, and oil yield (ml/ plant), were recorded by spraying thyme EONE at the rate of 0.5 ml/L (1.49 and 1.52 % & 0.780 and 0.823 ml/plant) followed by spraying cumin EONE at the rate of 0.5 ml/L (1.45 and 1.49 % & 0.698 and 0.727 ml/plant) in the first and second seasons, respectively. Meanwhile, the lowest values were recorded in control plants (Figure 5).

The most useful instrument in the seed trade today is the seed health report, which allows for the execution of necessary preventative measures to lower the danger of spreading illnesses into areas that are not yet afflicted. Thus, the pathogenicity, seed transmissibility, and effects on seed germination of an *Alternaria l*eaf spot pathogen linked to coriander seed lots generated in South Africa are reported in this work. Referring to the **International Seed Federation (2017)** regulations, it was discovered that every coriander seed lot had more germination than was necessary. Both China and India have long employed thymol as a biocontrol agent for a variety of biological applications **(Li *et al.*, 2006)**. Moreover, **El-Gamal and Ghoneem (2014)** conducted a study on anise and disapproved of the effects of caraway, lemongrass, and thymes volatile oils as antifungal agents. These oils might be widely employed as natural fungicides to guard against *Alternaria radicina* disease and enhance the growth and productivity of anise plants.

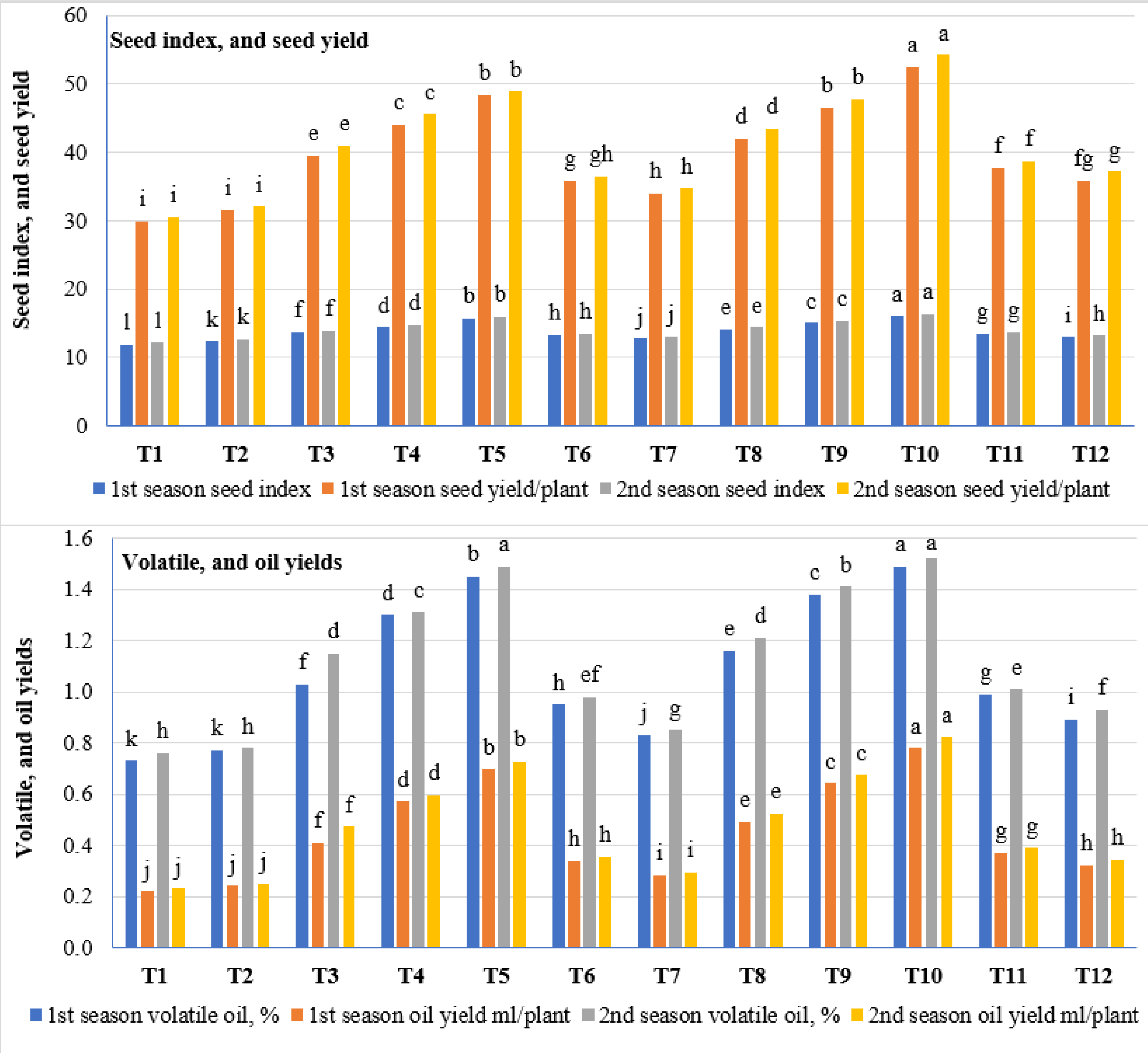


Fig. (5). Effect of cumin and thyme essential oils nanoemulsions (EONEs) foliar application on coriander seed and volatile oil yield characterization during 2021/2022 and 2022/2023 seasons.

Statistical significance was determined using the Duncan test at a significance level of $P \leq 0.05$. For each season, and each criterion, the averages denoted by a different letter(s) are considered significantly different.

T1= Cont., T2= Chem-cide,T3= Crude C 1ml/L,T4= C. Nano 0.25 ml/L, T5= C. Nano 0.5 ml/L, T6= C. Nano 0.75 ml/L, T7= C. Nano 1ml/L, T8= Crude Th 1ml/L, T9= Th. Nano 0.25 ml/L, T10= Th. Nano 0.5 ml/L, T11= Th. Nano 0.75 ml/L, T12= Th. Nano 1ml/L

Volatile oil components of coriander

The coriander oil of the seeds was extracted and analyzed via GC-MS. Twenty-one components were identified in 2023 coriander seeds oil samples, representing a total of 95.32 – 99.94 % of all volatiles as affected by thyme and cumin oils types and concentrations of the foliar application (Table 4) and (Figure 6)**.**

The dominant compound in all investigated treatments was linalool, which was influenced by the different treatments and ranged from 36.09 to 59.63 % from volatiles of the examined volatile oil samples of coriander. Moreover, it was noticed that all foliar application treatments with crude cumin and thyme crude EOs (1 ml/L) and the low concentrations (0.25 and 0.5 ml/L) of EONEs increased the percentage Linalool to record 48.29 % and 48.39 % for cumin and thyme EOs crude (1 ml/L), respectively and 55.42 & 59.63 % and 53.67 & 55.11 % for (0.25 and 0.5 ml/L) concentrations of cumin and thyme EONEs, respectively. In contrast, Linalool showed the lowest percentages in control and high concentration of (0.75 and 1 ml/L) EONEs treatments, which were 39.17 % in the control sample and (47.89 & 36.09 %) and (46.29 & 37.13 %) for 0.75 and 1 ml/L concentrations of cumin and thyme EONEs treatment samples, respectively.

The predominant compounds varied depending on the type and concentration of cumin and thyme EOs, for instance, the predominant compound was 1,22-Docosanediol of control (10.37 %) and thyme EONE (11.99 %) at the rate of 0.75 ml/L treatments. On the other hand, the predominant compound of chemical fungicide (Chem-cide) and the rate of 1 ml/L of cumin EONE treatments was Oxirane, hexadecyl (12.63 & 14.46 %, respectively). Meanwhile, the predominant compound of 1 ml/L thyme EONE treatment was 1,37-Octatriacontadiene (14.48 %).

The predominant compound of cumin and thyme EONEs at the rate of 0.5 ml/L treatment samples was alpha-Pinene (8.05 & 5.99 %, respectively). It was noticed that the infected plants (control), and plants that were sprayed with chemical fungicide or the high concentrations (0.75 and 1 ml/L) of cumin and thyme EONEs had 1,37-Octatriacontadiene, 1,22-Docosanediol, and Oxirane, hexadecyl as predominant compounds, vise verse, of the low concentrations (0.25 and 0.5 ml/L) of cumin and thyme EONEs in which these compounds did not find. The α.-Pinene (ranged from 2.28 to 8.05 %), p-Cymene (ranged from 1.60 to 7.37%), γ-Terpinene (ranged from 2.49 to 4.91 %), Camphor (ranged from 0.93 to 3.93 %), Geraniol (ranged from 1.45 to 3.09 %) and Geranyl acetate (ranged from 3.52 to 6.66 %) took the same trend of Linalool trend. Generally, treatments of cumin and thyme EOs crude gave a close result with the results of optimum doses of these oils' nanoemulsions.

Such a profile is similar to the reports of other authors with linalool as the main component of volatile oil in coriander seeds, like **Mandel and Mandel, 2015 and Khater *et al.*, 2022.** The terpene alcohol compound linalol possesses sedative, antimicrobial, and anti-inflammatory qualities (**Nurzyńska-Wierdak, 2013**). Coriander oil's oleic acid, an omega-9 fatty acid, has been demonstrated to increase the generation of antioxidants and delay the onset of heart disease **(Nurzyńska-Wierdak, 2013).** Although there is little data on how thyme EONEs interact with plants, it supports the theory that the thymol component of the nanoemulsion modifies the biochemical responses in plants by activating stored food and raising the activity of antioxidant enzymes (**Kumari *et al.*, 2018**).

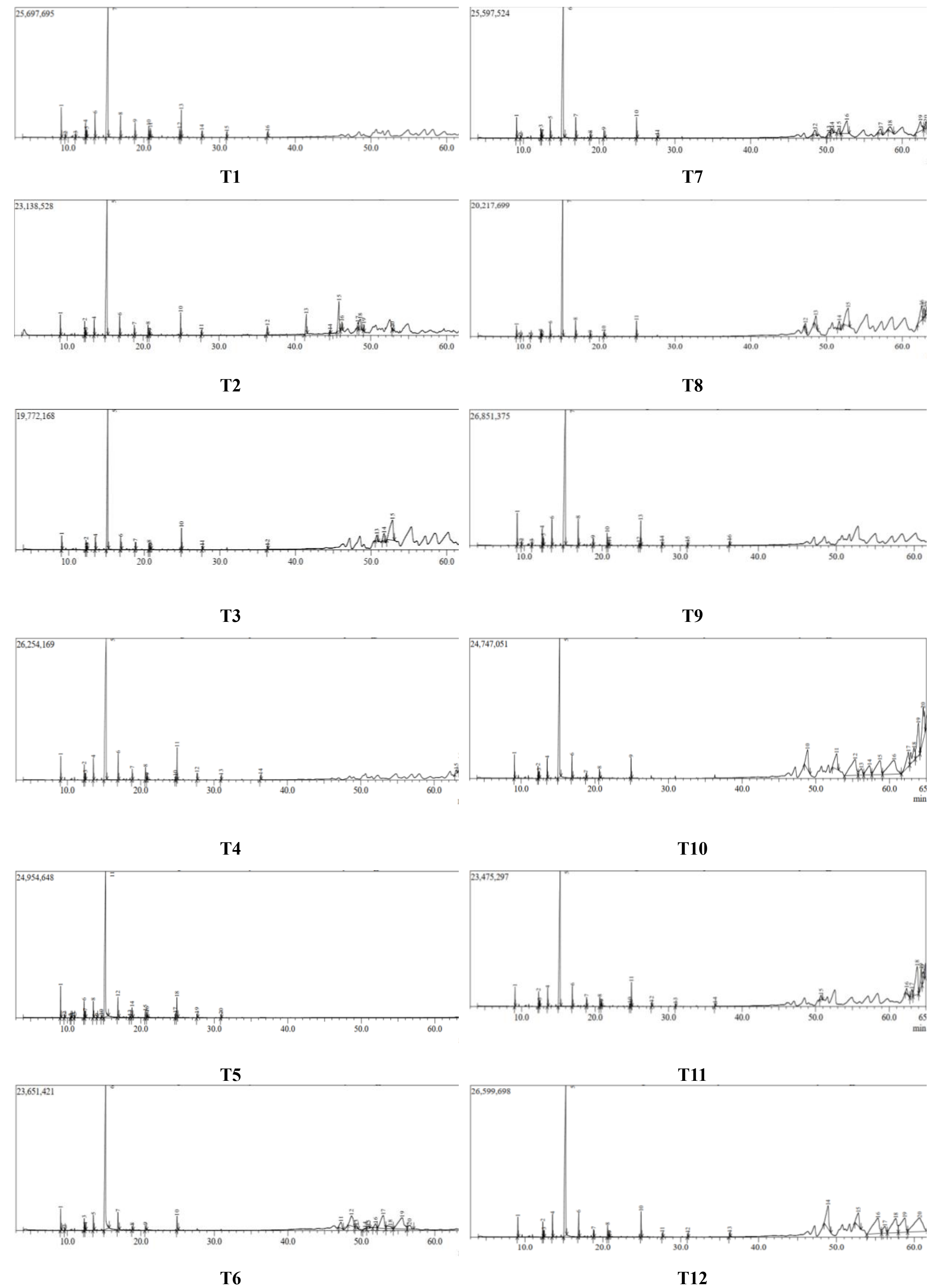


Fig. (6). GC-Ms chromatogram of bioactive metabolites of Coriander volatile oil as influenced by cumin and thyme oils Nanoemulsions treatments

*= Total ion chromatogram of the tentatively identified compounds by GC-Ms. Arrows represent the beginning and end of the base of the compound peak. where, T1= Cont., T2= Chem-cide,T3= Crude C 1ml/L,T4= C. Nano 0.25 ml/L, T5= C. Nano 0.5 ml/L, T6= C. Nano 0.75 ml/L, T7= C. Nano 1ml/L, T8= Crude Th 1ml/L, T9= Th. Nano 0.25 ml/L, T10= Th. Nano 0.5 ml/L, T11= Th. Nano 0.75 ml/L, T12= Th. Nano 1ml/L

Table (4). Coriander volatile oil constituent percentages in samples of the 2023 season as affected by treatments of either crude EOs or EONEs

ID	Component		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
1	α.-Pinene	RT	9.09	9.11	9.1	9.11	9.09	9.11	9.09	9.09	9.11	9.09	9.1	9.09
		RI	948	948	948	948	948	948	948	948	948	948	948	948
		Area%	3.87	4.18	4.33	4.45	8.05	5.9	2.28	3.51	6.34	5.99	4.08	2.86
2	Camphene	RT	----	----	9.64	9.93	9.62	9.64	9.63	----	9.65	9.62	----	----
		RI	----	----	943	943	943	943	943	----	943	943	----	----
		Area%	----	----	0.39	3.65	0.6	0.54	0.22	0.95	0.52	0.49	----	----
3	β.-Myrcene	RT	----	----	----	----	10.96	10.98	10.97	----	10.99	----	----	----
		RI	----	----	----	----	958	958	958	----	958	----	----	----
		Area%	----	----	0.55	0.72	0.72	0.65	0.32	0.59	0.7	0.44	----	----
4	p-Cymene	RT	12.29	12.32	12.31	12.32	12.29	12.31	12.29	12.29	12.32	12.29	12.31	12.29
		RI	1042	1042	1042	1042	1042	1042	1042	1042	1042	1042	1042	1042
		Area%	3.48	4.65	3.41	4.8	7.37	4.81	1.6	4.52	4.96	5.82	5.3	3.31
5	Limonene	RT	12.41	12.46	12.45	12.46	12.43	12.46	12.44	12.44	12.46	12.43	12.46	12.44
		RI	1018	1018	1018	1018	1018	1018	1018	1018	1018	1018	1018	1018
		Area%	0.79	0.63	0.78	0.84	1.05	1.01	0.46	0.77	1.05	0.92	0.77	0.51
6	γ-Terpinene	RT	13.54	13.56	13.56	13.57	13.53	13.56	13.54	13.54	13.57	13.53	13.56	13.54
		RI	998	998	998	998	998	998	998	998	998	998	998	998
		Area%	3.28	3.07	3.76	4.11	4.34	4.54	2.49	3.94	4.91	4.31	3.69	2.55
7	L -Linalool	RT	15.22	15.26	15.26	15.3	15.21	15.28	15.18	15.24	15.28	15.19	15.24	15.17
		RI	1082	1082	1082	1082	1082	1082	1082	1082	1082	1082	1082	1082
		Area%	39.17	45.58	48.29	55.42	59.63	47.89	36.09	48.39	53.67	55.11	46.29	37.13
8	(-)-Camphor	RT	16.89	16.92	16.91	----	16.88	16.92	16.88	16.89	16.93	16.88	16.91	16.88
		RI	1121	1121	1121	----	1121	1121	1121	1121	1121	1121	1121	1121
		Area%	2.79	2.88	3.18	0.93	3.93	3.63	2.4	3.14	3.44	3.62	3.11	1.96
9	Decanal	RT	18.82	18.85	18.84	18.85	18.82	18.84	18.82	18.82	18.86	18.81	18.84	18.82
		RI	1204	1204	1204	1204	1204	1204	1204	1204	1204	1204	1204	1204
		Area%	0.41	2.44	0.43	1.02	1.33	0.59	0.33	0.63	1.53	0.58	0.94	0.76
10	Geraniol	RT	20.62	20.66	20.64	20.66	20.61	20.64	20.62	20.62	20.66	20.61	20.64	20.62
		RI	1228	1228	1228	1228	1228	1228	1228	1228	1228	1228	1228	1228
		Area%	2.01	2.65	2.19	3.09	2.31	2.75	1.45	2.7	2.96	2.07	2.48	1.86
11	(Z)-2-Decenal	RT	----	20.88	----	20.88	20.84	----	----	20.85	20.88	----	20.87	20.85
		RI	----	1212	----	1212	1212	----	----	1212	1212	----	1212	1212
		Area%	----	0.46	----	0.47	0.94	----	----	0.39	1.07	0.61	0.56	0.43

RT: retention time (min), RI: retention index, Area%: component percentage, Where: T1= Cont., T2= Chem-cide,T3= Crude C 1ml/L,T4= C. Nano 0.25 ml/L, T5= C. Nano 0.5 ml/L, T6= C. Nano 0.75 ml/L, T7= C. Nano 1ml/L, T8= Crude Th 1ml/L, T9= Th. Nano 0.25 ml/L, T10= Th. Nano 0.5 ml/L, T11= Th. Nano 0.75 ml/L, T12= Th. Nano 1ml/L

cont. Table (4). Coriander volatile oil constituent percentages in samples of the 2023 season as affected by treatments of either crude EOs or EONEs

ID	Component		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
12	Geranyl acetate	RT	24.92	24.95	24.94	24.96	24.92	24.94	24.92	24.92	24.96	24.91	24.95	24.93
		RI	1352	1352	1352	1352	1352	1352	1352	1352	1352	1352	1352	1352
		Area%	3.73	4.68	4.77	6.66	6.02	4.65	3.52	4.79	6.27	4.57	5.3	4.94
13	2-Dodecenal, (E)-	RT	----	27.69	27.68	27.69	27.65	27.67	----	27.65	27.69	----	27.68	27.67
		RI	----	1410	1410	1410	1410	1410	----	1410	1410	----	1410	1410
		Area%	----	0.58	----	0.7	0.63	----	----	0.43	0.74	0.33	0.49	0.44
14	4,4-Dimethoxy-2-pentyl-cyclohexanone	RT	----	----	----	30.96	30.91	30.94	----	30.92	30.96	----	30.95	----
		RI	----	----	----	1636	1636	1636	----	1636	1636	----	1636	----
		Area%	----	----	----	0.59	0.56	0.35	----	0.46	0.8	0.36	0.39	----
15	Oleyl oleate	RT	48.87	48.64	48.62	48.62	----	---	47.23	48.94	----	47.22	----	----
		RI	3783	3783	3783	3783	----	---	3783	3783	----	3783	----	----
		Area%	7.25	5.13	2.54	4.12	----	---	5.86	8.58	----	4.77	----	----
16	Oleic acid	RT	52.84	63.64	51.65	63.01	----	63.87	48.6	52.83	63.68	51.94	----	63.19
		RI	3783	3783	3783	3783	----	3783	3783	3783	3783	3783	----	3783
		Area%	5.21	2.51	3.54	1.11	----	8.85	2.67	3.47	5.96	5.03	0.46	2.2
17	Oleyl alcohol, heptafluorobutyrate	RT	57.28	62.47	50.73	64.31	----	63.85	63.24	60.68	----	53	63.09	62.57
		RI	1940	1940	1940	1940	----	1940	1940	1940	----	1940	1940	1940
		Area%	2.49	2.45	1.37	3.12	----	6.03	2.33	1.24	----	2.86	1.93	2.76
18	Oleyl alcohol, trifluoroacetate	RT	58.73	64.27	52.71	63.9	----	64.47	62.57	58.77	64.34	55.61	62.38	62.83
		RI	2019	2019	2019	2019	----	2019	2019	2019	2019	2019	2019	2019
		Area%	3.93	2.39	1.7	0.69	----	5.21	5.2	5.11	3.08	0.65	5.44	4.76
19	1,37-Octatriacontadiene	RT	60.48	---	62.43	----	----	---	51.7	62.57	---	---	----	63.89
		RI	3779	---	3779	----	----	---	3779	3779	---	---	----	3779
		Area%	1.54	---	4.08	----	----	---	12.56	1.21	---	---	----	14.48
20	1,22-Docosanediol	RT	62.54	---	63.16	64.89	----	---	63.91	63.58	---	---	63.81	62.57
		RI	---	---	---	----	----	---	----	----	---	---	----	----
		Area%	10.37	---	7.84	1.07	----	---	1.08	1.2	---	---	11.99	1.11
21	Oxirane, hexadecyl-	RT	64.59	----	64.42	----	----	64.56	----	63.98	64.94	---	64.72	64.54
		RI	---	----	---	----	----	---	---	----	---	---	----	---
		Area%	9.62	12.63	4.93	----	----	1.02	14.46	3.49	---	---	6.24	14.11
Total		99.94		96.91	98.08	97.56	97.48	98.42	95.32	99.51	98	98.53	99.46	96.17

RT: retention time (min), RI: retention index, Area%: component percentage, Where: T1= Cont., T2= Chem-cide,T3= Crude C 1ml/L,T4= C. Nano 0.25 ml/L, T5= C. Nano 0.5 ml/L, T6= C. Nano 0.75 ml/L, T7= C. Nano 1ml/L, T8= Crude Th 1ml/L, T9= Th. Nano 0.25 ml/L, T10= Th. Nano 0.5 ml/L, T11= Th. Nano 0.75 ml/L, T12= Th. Nano 1ml/L

CONCLUSION & RECOMMENDATION

Based on our study's findings, it can be concluded that *Alternaria dauci*-induced disease significantly diminished various vegetative growth and yield parameters in coriander. Application of thyme or cumin EONE topically at a rate of 0.5 ml/L (with thyme proving more effective) exhibited robust antifungal efficacy against *Alternaria dauci* pathogens, concurrently promoting coriander growth and yield. These results hold substantial implications for developing and employing environmentally friendly biocontrol agents as antifungal delivery systems, contributing to the long-term safety of growth factors and disease management in coriander. Consequently, we recommend the application of thyme or cumin (EONEs) at a rate of 0.5 ml/L to coriander, serving as an effective strategy to prevent and control *Alternaria dauci* pathogens while enhancing coriander production.

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