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Some Selected Plant Essential Oils-Based Bio-Acaricides and their Nano-Emulsions to Control, the Two Spotted Spider Mite, *Tetranychus urticae* (Koch)

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Abstract

The acaricidal potential of some eco-friendly plant-derived essential oils (EOs) against adult females of *Tetranychus urticae* Koch under laboratory-controlled conditions was assessed. Four essential oils of cumin (*Cuminum cyminum*), lemon grass (*Cymbopogon citratus*), clove (*Syzygim aromaticum*), and black pepper (*Piper nigrum*) were extracted using a hydro-distillation Clevenger-type apparatus. *In-vitro* leaf-dipping bioassay was assessed after one, two, and three days after exposure. Mortality varied according to the essential oil type, time of exposure, and the delivered dose (ppm). Clove EO was the most superior potent essential oil against adult females of *T. urticae* after 24 and 48 hours of exposure with LC_{50} values (417.68 and 247.82 ppm) but the last day cumin oil caused high mortality with LC50 value (173.62 ppm), followed by black pepper and lemon grass oils. GC-MS analysis of the most effective cumin and clove essential oils was performed and the most predominant compounds detected in clove EO were eugenol (44.66%), oleic acid (20.40 %), and eugenol acetate (15.36%), while for cumin EO were oleic acid (10.31%), *p*-Cumic aldehyde (10.24%), (-)-spathulenol (6.36%) and cumic acid (2.74%). Clove and cumin EOs-based nanoemulsions (NEs) with a high EO concentration (10%) and low Tween content were performed to minimize the volatilization and increase the acaricidal capacity. Of the nano-formulated NEs, clove NEs were the most effective after one day of exposure with an LC_{50} value of 60.55 ppm, but through the second and third days, cumin NEs were more effective than clove NEs with LC_{50} values of 39.89 and 16.36 ppm. Greenhouse experiments proved the effectiveness of the two nanoemulsions after spraying the candidate pest with their LC_{90} values on the eggplant plant. The reduction percentage for mites' population was 99.38% for clove NEs and 99.20% for cumin NEs. The effect of clove and cumin EOs on some key enzymes anticipated the toxicity mode of action. Overall, clove and cumin essential oils and their nanophase proved their ability as bio-acaricides.

Keywords: Essential oils; Nano-emulsion; *Tetranychus urticae;* Greenhouse

1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is an omnipresent and cosmopolitan phytophagous mite species exhibiting polyphagous behavior on a diverse array of horticultural crops in both enclosed and outdoor environments globally [**1**]. Mite populations can inflict considerable damage and result in substantial yield losses by feeding on the sap of various crops, including those in the Solanaceae, Fabaceae, Cucurbitaceae, and Rosaceae families, as well as fruit trees, ornamental plants, and weeds. Furthermore, they have the potential to transmit bacterial and viral pathogens to other host plants [**2**]. Chemically synthetic pesticides are the most efficient and rapid method to control *T. urticae*, however, the application of essential oils also prevents several significant risks, including potential harm to indigenous predators, adverse impacts on the environment, human health concerns, degradation of soil and water quality, and the emergence of resistant strains **[3,4]**.

Recently attention has turned to the use of natural pesticides that with a few major variations become easy to use, effective against most pests, and safe for mammals or the environment. Applying natural plant oil extracts thus appears as another good way to control mite pests [**5**] and doesn't affect natural enemies [**6]**. Essential oils are complex mixtures of volatile and semi-volatile chemical compounds, extracted from various plant parts such as buds, leaves, bark, and flowers **[7].** Essential oils possess unique odors determined by their molecular structures. Essential oils are extensively utilized across various industries such as food, agriculture, cosmetics, and pharmaceuticals [**8**], primarily due to their inherent biological properties. However, essential oils are susceptible to instability and rapid degradation when exposed to environmental factors such as heat, light, and oxidation [**9**]. Therefore, since polymeric nanocarriers act as a barrier between the oil and the environment, there is increased notice in emulsion and encapsulating essential oils [**10]**.

Nowadays, bioactive compounds are delivered using nanoemulsions such as (Rosemary and camphor), which are particularly suitable for use in crop protection [**11**, **12**]. Nanoemulsions offer major economic and safety benefits for the delivery of pesticides in agrochemicals [**13**]. A phase of oil distributed in continuous water makes up a nano-emulsion, in which each oil drop is encased in a thin layer of surface molecules that stabilizes the nanomolecular structure to a more stable formulation [**14**].

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First, unlike traditional emulsifiable concentrate (EC), oil-in-water (O/W) nanoemulsion is water-based and makes use of far less organic solvent during its production [**15, 16**]. Small droplets and particles (20–200 nm) enhance the interactions and transfer of active chemicals through biological target membranes [**17**]. Numerous studies were conducted on acaricidal activity, which included contact and fumigation toxicity against adult female *T. urticae*. Furthermore, the generated nanoemulsions' effectiveness in managing *T. urticae* populations on plants in greenhouse settings was also investigated [**18**].

The present study attempts to evaluate the acaricidal activity of some selected plant essential oils and their nano-emulsions on the two-spotted spider mite *T. urticae.* The chemical profile of the most effective essential oils was investigated using the GC-MS technique. In addition, biochemical investigations based on studying the effect of the most potent essential oils on some enzymes were assessed to anticipate the mode of action. Essential oils' effects were believed to be strong enough to support their use in the *T. urticae* management program.

2. Materials and Methods

2.1. Equipment

A gas chromatography (Hewlett Packard 5890)/mass spectrometry (Hewlett Packard 5989B) (GC-MS) device with a flame ionization detector (FID) was used for the chemical analyses. A 30-meter HP-5MS (5 percent diphenyl) dimethyl polysiloxane capillary column with an i.d. of 0.25 mm and a film thickness of 0.25 μm served as the GC column. With an ionization energy of 70 eV, mass spectra were acquired in the electron ionization (EI) mode. The chemical constituents in each sample were identified by comparing their mass spectrum patterns with those of reliable references and the MS libraries (NIST and Wiley) database [**19 and 20**]. The percentage of each component was quantified through the peak area integration obtained from each FID chromatogram.

Transmission electron microscopy (TEM) was utilized to visualize the morphology of the nano-emulsions, revealing their size and shape by a combination of diffraction modes and bright-field imaging at increasing magnification. The nano-emulsion (50 µL) diluted with water (1/100) was added to 200-mesh form-overcoated copper TEM sample holders to perform the TEM observations. The samples of TEM were detected with JEOL JEM-2100 equipped with 200 kV, at the transmission electron microscope unit, Faculty of Agriculture, Mansoura University, Egypt.

2.2. Rearing of T. urticae

The two-spotted spider mite (TSSM) was reared on bean plants, where plastic pots (20 cm) were filled with mixed soil 1:1 v/v loam and sand. About ten seeds of beans were sown on top of the soil surface; extra soil was covered with the beans and wetted with water. After two weeks, grown seedlings were infested with *T. urticae* individuals that were collected from infested bean plants (*Phaseolus vulgaris* L.) from the greenhouse of the Agricultural Zoology Department, Mansoura University [**21**]**.**

2.3. Preparation and extraction of essential oils

Fresh aerial parts as well as seeds were collected from cloves (*Syzygim aromaticum*), cumin (*Cuminum cyminum*), and lemon grass (*Cymbopogon citratus*) from the Faculty of Agriculture, Mansoura University farm. While the seeds of black pepper (*Piper nigrum*) were purchased commercially from Aswan markets. The tested plant parts were then ground and the essential oils were extracted from them by a Clevenger hydraulic distillation device for 8 hours to obtain four essential oils [12]. The resulting oils were received on anhydrous sodium sulfate to remove water from them and the resulting oils were stored in sealed glass tubes at 4 °C until use [**18**].

2.4. Preparation and extraction of essential oils

Two steps were utilized in the preparation of the nanoemulsions. The coarse emulsions were initially formed by stirring, and a high-intensity ultrasonic method was used to further emulsify them. In a ratio of 1:2:7, the EOs were combined with surfactant (Tween 80) and water, respectively, to reach a final concentration of 10% each. Utilizing a magnetic stirrer, the organic phase, including oil, was dropped into the aqueous phase (water and surfactant) for 30 minutes at 4000 rpm to generate the emulsion. The resulting emulsions were subsequently subjected to a 15-minute ultrasonic emulsification process using a 10 kHz sonication power and pulses or cycles (9 cycles/sec) managed by the device's software (Ultrasonic Homogenizers HD 2070 with HF generator (GM 2070), probe microtip MS 73, Ø 3 mm, booster horn (SH 213 G), and ultrasonic converter UW2070). The temperature differential between the final emulsion and the original coarse emulsion was no greater than 25°C **[22]**.

2.5. Preparation of Nanoemulsions of essential oils

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device's software. Ultrasonic converter UW2070, booster horn (SH 213 G) and probe microtip MS 73, Ø 3 mm). The temperature differential between the final emulsion and the original coarse emulsion was no greater than 25°C **[22]**.

2.6. Bioassay Tests

2.6.1. Laboratory bioassay tests

Mite culture (*T. urticae*) was placed on hibiscus leaves on a sheet of cotton moistened in 12 cm Petri plates. Then the plates were placed inside a closed plastic box at 26±2°C and 75% RH. The cotton sheet is then moistened periodically by adding water droplets as needed. To prevent the escape of the mites, a barrier of wet cotton is made on the edges of leaves; later, the mites are transferred to fresh leaves every 3-4 days [**6**].

The bioassay test of essential oils and their nanoemulsions was evaluated according to the leaf-dipping plant discs method in each of five concentrations (62.5, 125, 250, 500, and 1000 ppm) for 30 sec. The discs were left to dry, and ten fresh adult females of *T. urticae* were transported to the hibiscus disk [**23**]. This treatment was repeated five times, and the experiment was repeated twice. Also, the most effective essential oils were turned into nano-emulsion oils. The count of dead and live individual mites was calculated after 24, 48, and 72 hours with daily follow-up by stereoscopic microscopy [**23].**

2.6.2. Greenhouse experiments

The experiment was done in the Acarology greenhouse, Faculty of Agriculture, Mansoura University in the 2024 season. Plastic agricultural pots (diameter of 35 cm and depth of 30 cm) were used and filled with mixed fertilized soil sand and loam (1: 1 size/volume), then planted with 30 days eggplant seedlings, where each pot contains three plants. After that, the agricultural procedures were applied. After 40 days, the plants were infested with mite individuals. Utilizing textile barricades, the infected plants were kept apart to avoid mite contact and movement. Plants with four real leaf stages and fully expanded primary leaves were sprayed with nanoemulsions of cumin and clove EOs at LC_{90} concentration by using a hand sprayer. Twenty pots of each treatment were used, and one of these treatments was sprayed with water as a control [**24**]. The treated plants were left to dry, and the count of dead and live individual mites (immature and adult stages) was calculated by using a stereomicroscope. The *T. urticae* live individuals per leaf/replicate were recorded before and 1, 3, 7, 14, and 21 days after application. Where the reduction percentage in mite populations was calculated and corrected according to Henderson and Tilton,**[25].**

2.6.3. Biochemical investigation of T. urticae

The LC_{50} of the most potent toxicant essential oils was applied to assay the enzyme activity. Alive adult individuals from *T. urticae* were transferred and treated with each selected essential oil. The live individuals were collected, weighed, and stored at 4 ◦C after 24 h exposure. Untreated samples were employed as controls. All treated samples were sent to the Plant Protection Research Institute analysis unit, A.R.C., to assay the pest's enzyme activities of Alkaline phosphatase (ALP) **[26],** Acid phosphatase (ACP) **[27],** Aspartate transferase (AST/GOT) and Alanine transaminase (ALT/GPT) **[28]**, Acetylcholinesterase (AChE) **[29],** and Glutathione S-transferases (GST) **[30] [30]**.

2.7. Statistical analysis

Correction of the mite mortality percentages was performed using Abbott's formula [**31**] and estimation of LC_{50}, LC_{90} and slope values were established according to [**32]**. The toxicity index was generated based on Sun's equation [**33].** To compare means significance, the data was statistically examined using analysis of variance (one-way classification, ANOVA; CoHort Software, 2004) and the Duncan multiple range test at a 5% threshold **[34].**

3. Results and discussion

Discovering and commercializing natural compounds as green pesticides is an attractive and profitable pursuit that is receiving a lot of attention due to the shift toward green chemistry processes and the ongoing need to create new crop protection agents with unique modes of action **[35]**.

3.1. Toxicological effect of the selected essential oils on adult females of T. urticae under laboratory conditions

The toxic effect of the tested essential oils against the adult females of *T. urticae* using the leaf-dipping method and under controlled laboratory conditions was reported in Table (1). Data showed that all EOs were effective on *T. urticae* females and had a considerable toxic effect at the different exposure intervals. The most toxic essential oil was clove EO, with an LC_{50} of 417.68 ppm and a toxicity index of 100% after one day of application, followed by black pepper EO (1707.98ppm and 24.45%), cumin oil (2066.17ppm and 20.21%) while lemon grass EO occupied the last rank with LC_{50} 2774.79 ppm and 15.05%, respectively.

The efficiency of tested essential oils clearly increased after two days and consequently, the toxicity index increased for all tested EOs. Based on the toxicity index, clove EO still occupied the superiority, followed by cumin, black pepper, and at least lemon grass. LC_{50} values of the above-mentioned oils were 247.82, 251.45, 513.30 and 836.74 ppm, respectively.

After three days of exposure, cumin EO recorded the highest efficiency and considered to be the best essential oil for controlling *T. urticae*. Based on the toxicity index, cumin EO was the most potent followed by clove EO, black pepper and lemon grass with LC_{50} values of 173.62, 212.51, 213.66, 327.90, and 441.22 ppm, respectively.

The use of essential oils derived from *C. cyminum* and *S. aromaticum* plants to manage insect pests, certain phytophagous and ectoparasite mites has been examined by several studies. Our results were in agreement with the prior study that revealed that cumin EO and clove EO were effective for controlling *T. urticae* using the filter paper diffusion method with LC_{50} values 3.74 and 6.13 µL L^{-1}air, respectively **[36].**

Table 1: Toxicological effect of four essential oils on adult females of *T. urticae* under laboratory conditions.

Essential oils	LC_{50} (ppm)	Confidence limit at 95%		LC_{90} (ppm)	Confidence limit at 95%		Slope±SE	Toxicity index
		Lower limit	Upper limit		Lower limit	Upper limit		
24h of leaf-dipping method								
P. nigrum	1707.98	824.60	18085.03	127920.00	14019.84	8.94 E+8	0.684±0.211	24.45
C. cyminum	2066.17	928.96	29017.50	36063.33	6140.44	2.41 E+7	1.032±0.305	20.21
S. aromaticum	417.68	254.74	729.78	13411.10	3338.96	4.83 E+6	0.851±0.271	100.0
C. citratus	2774.79	1565.26	23259.70	602410.00	10238.11	3.49 E+7	0.631±0.463	15.05
48h of leaf-dipping method								
P. nigrum	513.30	369.49	696.986	2796.36	1538.32	15329.42	1.741±0.440	48.27
C. cyminum	251.45	134.54	469.24	6466.82	1897.13	366091.49	0.909±0.255	98.55
S. aromaticum	247.82	115.43	344.59	1310.55	869.48	3816.23	1.772±0.449	100.0
C. citratus	836.74	593.85	2028.79	6791.16	2486.70	478366.58	1.409±0.451	29.61
72h of leaf-dipping method								
P. nigrum	327.90	233.33	407.42	1082.46	813.99	1865.28	2.471±0.468	52.94
C. cyminum	173.62	101.55	260.23	1984.94	962.23	10548.23	1.211±0.263	100.0
S. aromaticum	212.51	111.37	289.61	811.94	610.59	1424.42	2.202±0.485	81.69
C. citratus	441.22	318.50	564.03	1941.49	1235.53	5763.44	1.992±0.448	39.34

Toxicity index = LC_{50} of the most effective compound/ LC_{50} of the tested compound ×100.

3.2. Chemical Compositions of the Potent Essential Oils (EOs)

The chemical composition of clove EO and the relative concentration of bioactive components were identified with the aid of GC/MS technique. The GC chromatogram of clove EO represented fourteen peaks corresponding to fourteen compounds that were characterized by comparing their mass spectra with the help of NIST and WILEY libraries (table 2, Fig. 1The main detected bioactive compounds were eugenol (44.66 %), oleic acid (20.40 %), and eugenol acetate (15.36 %). While, cumin EO represented twenty- three peaks corresponding to twenty-three compounds (Table 2~~, Fig. 2~~). The most predominant constituents were Oleic acid (10.31%), p-Cumic aldehyde (10.24%), n-Hexadecanoic acid, (9.37%), (-)-Spathulenol (6.36%), Dimethylbicyclo[2.2.1]hept-2-yl acetate (3.36%), 4-(2-Methyl-3-oxocyclohexyl)butanal (3.28%),Octadecanoic acid(2.91%), Linoleic acid (2.80%), and Cumic acid (2.74%). The acaricidal activities of eugenol, and acetyleugenol, the main constituents in clove EO, have been reported in contact bioassays against scabies mite populations and there was no significant difference between the activity observed compared with the positive control acaricide (benzyl benzoate) ($p > 0.11$) **[37]**. The larvicidal activity was recorded for cuminaldehyde against *Musca domestica* with LC_{50} value of 1.90 mg/kg 72 h post-exposure **[38],** and also showed high acaricidal potency against *Dermanyssus gallinae* with LC_{50} value 11.474 mg/ml **[39]**. Strong repellent activity, fumigation toxicity, and AChE inhibition were previously recorded for cuminaldehyde **[40].**

Table 2: GC-MS chemical profiling of *C. cyminum* and *S. aromaticum* essential oils

No.	Compound name	*Rt	Clove Area %	Cumin Area %	Molecular weight	Molecular formula	Class
1	α-Pinene	4.82	0.23	0.53	136	$C_{10}H_{16}$	Monoterpene hydrocarbon
2	*β*-Pinene	5.66	0.99	1.93	136	$C_{10}H_{16}$	Monoterpene hydrocarbon
3	*p*-Cymene	6.69	0.90	1.99	134	$C_{10}H_{14}$	Monoterpene hydrocarbon
4	cis-4-Thujanol	6.84	0.30	-----	154	$C_{10}H_{18}O$	Oxygenated monoterpene
5	*β*-Pinone	9.09	-----	0.52	138	$C_9H_{14}O$	nor Oxygenated monoterpene
6	2(10)-Pinen-3-ol	9.57	-----	0.78	152	$C_{10}H_{16}O$	Oxygenated monoterpene

7	*p*-Menth-3-en-7-al	10.90	-----	0.55	152	$C_{10}H_{16}O$	Oxygenated monoterpene
8	(-)-α-Terpineol	11.01	-----	0.61	154	$C_{10}H_{18}O$	Oxygenated monoterpene
9	Myrtenol	11.16	-----	0.54	152	$C_{10}H_{16}O$	Oxygenated monoterpene
10	6-Hydroxy-3-bornanone	11.80	-----	0.50	170	$C_{10}H_{16}O_2$	Oxygenated monoterpene
11	*p*-Cumic aldehyde	12.06	-----	10.24	148	$C_{10}H_{12}O$	Oxygenated monoterpene
12	α-Terpinen-7-al	13.26	-----	1.00	150	$C_{10}H_{14}O$	Oxygenated monoterpene
13	2-Methyl-5-(propan-2-ylidene)cyclo hexane-1,4-diol	14.19	-----	1.4	170	$C_{10}H_{18}O_2$	Oxygenated monoterpene
14	7,7-Dimethylbicyclo[2.2.1]hept-2-yl acetate	16.33	-----	3.36	182	$C_{11}H_{18}O_2$	nor Oxygenated monoterpene
15	Cumic acid	17.73	-----	2.74	164	$C_{10}H_{12}O_2$	Oxygenated monoterpene
16	4-(2,6,6-Trimethyl-cyclohex-1-enyl)-butyric acid	22.98	0.79	-----	210	$C_{13}H_{22}O_2$	Oxygenated monoterpene
17	Eugenol	15.39	44.66	-----	164	$C_{10}H_{12}O_2$	Shikimate
18	Eugenol acetate	19.26	15.36	-----	206	$C_{12}H_{14}O_3$	Shikimate
19	Caryophyllene	17.31	0.69	-----	204	$C_{15}H_{24}$	Sesquiterpene hydrocarbon
20	Caryophyllene oxide	20.95	1.44	-----	220	$C_{15}H_{24}O$	Oxygenated sesquiterpene
21	Humulene-1,2-epoxide	21.52	0.22	-----	220	$C_{15}H_{24}O$	Oxygenated sesquiterpene
22	(-)-Spathulenol	20.87	-----	6.36	220	$C_{15}H_{24}O$	Oxygenated sesquiterpene
23	Caryophylla-4(12),8(13)-dien-5α-ol	31.27	-----	0.41	220	$C_{15}H_{24}O$	Oxygenated sesquiterpene
24	4-(2-Methyl-3-oxocyclohexyl)butanal	13.93	-----	3.28	182	$C_{11}H_{18}O_2$	Acetogenin
25	n-Hexadecanoic acid	29.34	8.58	9.37	256	$C_{16}H_{32}O_2$	Acetogenin
26	Ethyl(9Z,12Z)-9,12-octadecadienoate	32.33	-----	0.58	308	$C_{20}H_{36}O_2$	Acetogenin
27	Linoleic acid	32.34	2.73	2.80	280	$C_{18}H_{32}O_2$	Acetogenin
28	Oleic acid	32.48	20.40	10.31	282	$C_{18}H_{34}O_2$	Acetogenin
29	Octadecanoic acid	34.47	2.70	2.91	284	$C_{18}H_{36}O_2$	Acetogenin
30	7,10,13-Eicosatrienoic acid, methyl ester	37.60	-----	0.36	320	$C_{21}H_{36}O_2$	Acetogenin
	Monoterpenes		**3.21**	**26.69**			
	Sesquiterpenes		**2.35**	**6.77**			
	Shikimate		**60.02**	-----			
	Acetogenin		**34.41**	**29.61**			

*Rt, retention time (min)

3.3. Characterization of the prepared nano-emulsions

All the prepared nano-emulsions (Clove and cumin EOs) were characterized through Zetasizer and transmission electron microscopy (TEM) analyses (Fig. 1 and 2). The droplet size of the prepared nano-emulsions was around 21 to 38 nm for clove NE and around 25-80 nm for cumin NE and showed a single peak droplet size distribution. Droplet size is a significant indicator of emulsion stability and is one of the most crucial elements in stabilizing emulsions **[41]**. The values of Z-average size, polydispersity index (PDI), and zeta potential (ZP) of clove oil NE were found to be 243 nm, 0.48, and -16.8mV, respectively and for cumin oil NE were found to be 144 nm, 0.64, and -4.9mV, respectively.

The effective surface potential of a droplet suspended in a medium is the zeta potential, which offers a strong energy barrier and good electrostatic repulsion between droplets.

The produced nano-emulsions EOs have a spherical shape, they likely have a low surface energy and strong thermodynamic stability. The idea that the produced nano-emulsions have a significant zeta potential is further supported by this observation **[42]**.

The micromorphological studies, which display the droplet sizes of the emulsions under intuitive settings, demonstrated that the nano-emulsions were nearly uniformly dispersed and spherical in accordance with the observation of Bagher and Dornoush's **[43].**

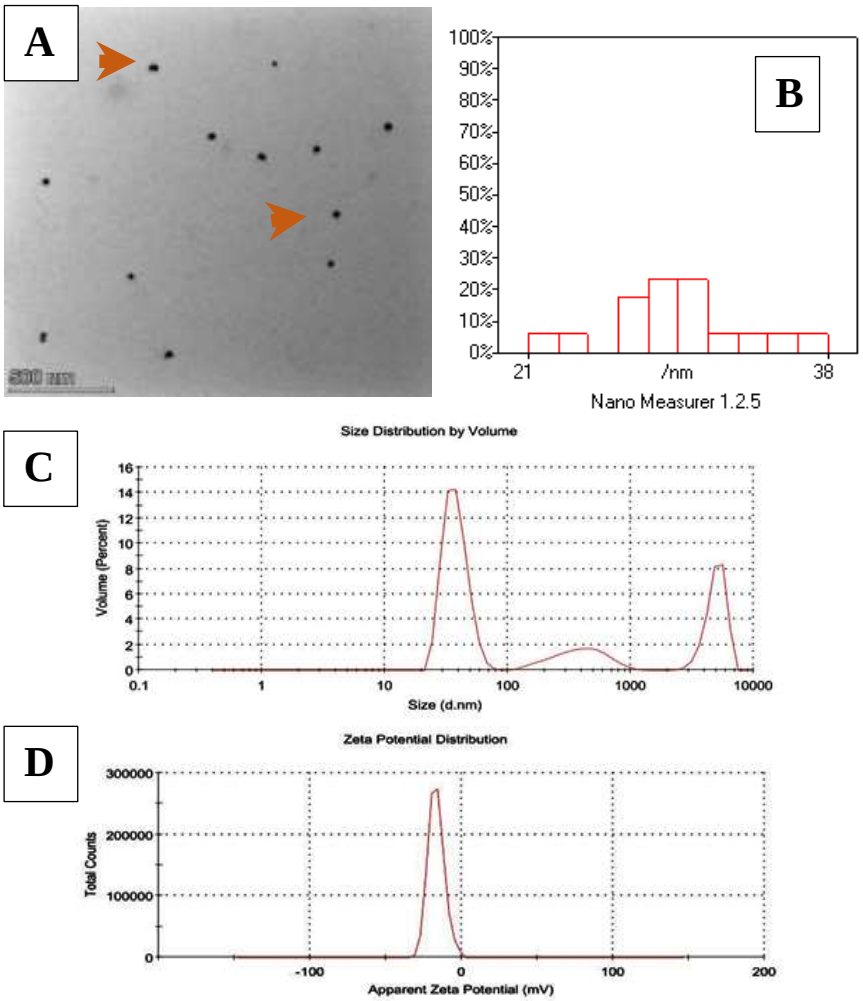

Fig. 1. (A)The morphology of clove oil Nano emulsion by TEM showing nearly spherical particles (arrowheads), (B) histogram shows that most particles cluster around 21 to 38 nm, with a good distribution, C) Zeata size distribution by volume. D) Zeta potential distribution. The values of Z-average size, PDI, and ZP of clove oil Nano emulsion were found to be 243 nm, 0.48, and -16.8mV respectively.

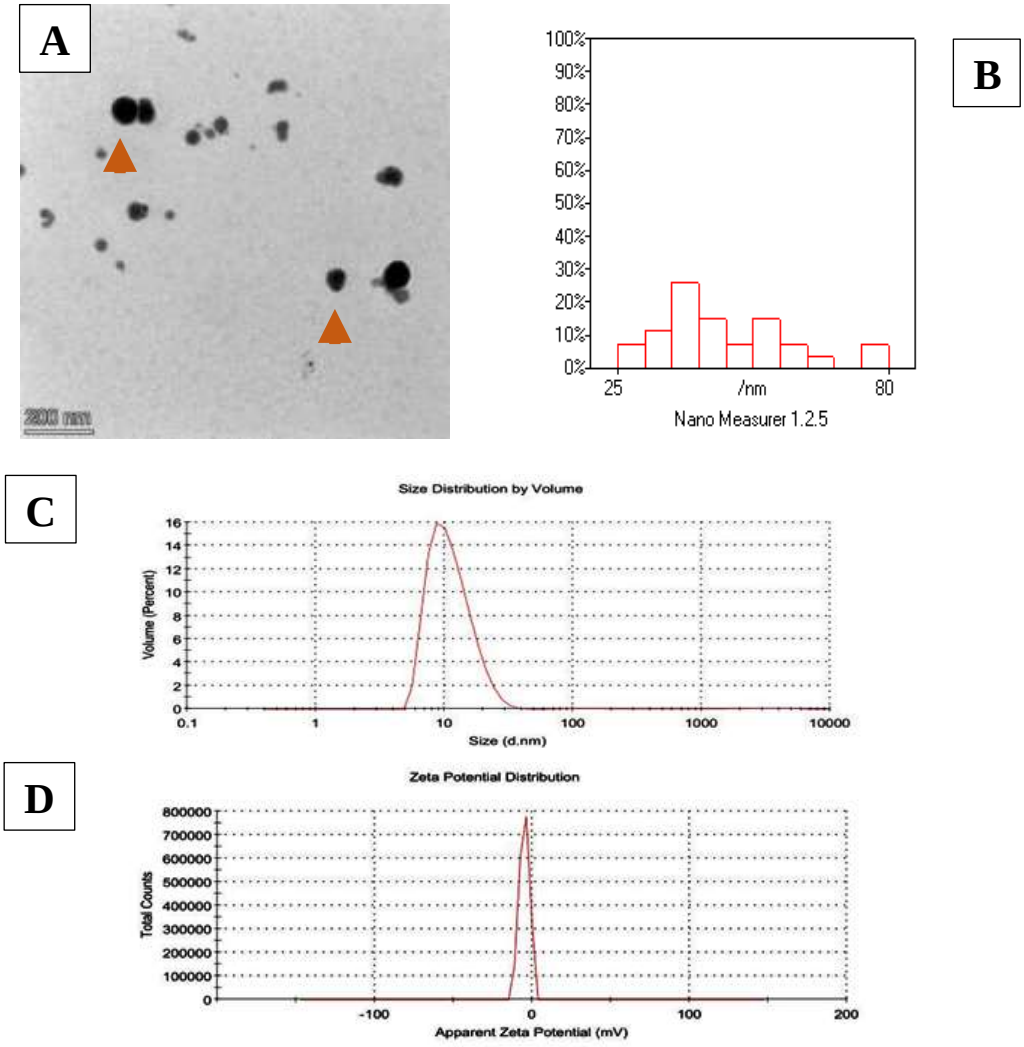

Fig. 2. (A) The morphology of cumin oil Nano emulsion by TEM shows nearly spherical particles (arrowheads), (B) histogram shows that most particles cluster around 25 to 80 nm, with a good distribution, C) Zeta size distribution by volume. D) Zeta potential distribution. The values of Z-average size, PDI, and ZP of cumin oil Nano emulsion were found to be 144 nm, 0.64, and -4.9 mV respectively.

3.4. Toxicological effect of the potent essential oils nanoemulsions *on adult females of T. urticae under laboratory conditions*

The susceptibility of *T. urticae* females to clove and cumin essential oil nanoemulsions (NEs) using the spraying method at different exposure intervals is mentioned in Table 3. Clove NEs was the most toxic, with an LC_{50} 60.55 ppm and a toxicity index 100% one day after application, followed by cumin NEs (163.86 ppm and 36.95%). After 48h of spraying, cumin NEs became more toxic to *T. urticae* females with LC_{50} 39.89 ppm and a toxicity index 100% than clove nano oil (45.84 ppm and 87.02%). Cumin NEs still occupied the superiority after 3 days of exposure followed by clove NEs with LC_{50} values 16.36 and 18.91 ppm, respectively.

The higher toxicity of essential oil nanoemulsions over the ordinary EOs appeared as result of the increasing of the area contact through the spread of nano droplets **[44].** The efficiency of all NEs due to the toxic action on nerves that leads to the breakdown of insects and mites' functions **[45].** Some of nanomaterials can produce reactive oxygen (ROS) and its species that causing damage in the mechanism of mitochondria defense, which, leads to deactivation or malfunction in protein functions till cells death **[46].**

Clove and cumin NEs are considered repellent agents for spider mites it's due to their components as described before [**47].** Clove and cumin affect the cholinergic nerve system, as it is an important target for the development of acaricides **[48]**.

Similar results were confirmed by [**49]** who recorded that clove nano-emulsion oil at a concentration 10% caused 93.3% mortality for adult females and also, have a strong reduction in egg hatching for phytophagous mites' species and ticks **[50]**.

Table 3: Toxicological effect of nano-emulsion essential oils on adult females of *T. urticae* using spraying method under laboratory conditions.

NEs	LC_{50} (ppm)	Confidence limit at 95%		LC_{90} (ppm)	Confidence limit at 95%		Slope±SE	Toxicity index
		Lower limit	Upper limit		Lower limit	Upper limit		
24h of spraying technique								
C. cyminum	163.86	79.74	1662.69	12577.73	1381.49	89687628.0	0.680± 0.211	36.95
S. aromaticum	60.55	33.77	258.58	1988.22	377.81	1083566.39	0.845±0.845	100
48h of spraying technique								
C. cyminum	39.89	21.49	19911.81	1759.44	322.47	2373210.79	0.779±0.249	100
S. aromaticum	45.84	26.73	129.83	1279.67	297.43	194779.12	0.886±0.254	87.02
72h of spraying technique								
C. cyminum	16.36	7.98	26.35	300.82	118.71	4004.93	1.013±0.256	100
S. aromaticum	18.91	8.80	8.80	494.81	156.93	20492.29	0.904±0.252	86.51

3.5. Effect of LC_{50} of most effective essential oils on enzyme activities of T. urticae adult female.

The effect of the median lethal concentration of clove and cumin EOs on some key enzyme activity of *T. urticae* adult female (GST) and (AChE), (AST/GOT), (ALT/GPT), (ACP) was biochemically investigated and reported in Table 4.

Data represented in Table 4 showed that a significant inhibition in the activity of GST enzyme, detoxifying enzyme level, was recorded for cumin EO cased high (-43.55%) than clove EO (-4.84%) compared with control. Also, a significant inhibition in the activity of AChE enzyme level for cumin EO (-64.67%) while a remarkable activation was noticed for clove EO (91.77%) compared with the untreated one.

Both clove EO and cumin EO showed non-significant activation in the level of the transaminase GOT enzyme while a significant reduction for the transaminase GPT enzyme was noticed (-18.74%) and (-72.00%), respectively compared with control. The change in the level of ACP enzyme showed a notable reduction by clove EO (-13.00%) and a significant activation by cumin EO (15.65%) compared with control.

Regardless, the introduction of any toxicant into the insect's body has a direct impact on its metabolic, physiological, and reproductive processes, causing a significant alteration compared to the control, accomplished by a change in the ALT and AST enzyme levels **[51].**

The insect nervous system is a primary site of action, several essential oils constituents have been reported to inhibit acetylcholine esterase *in vitro* **[52,53]** and this clearly observed in cumin EO which targeted acetylcholinesterase activity and acts as a neurotoxic compound.

These results agree with [**54**] who reported that Glutathione enzyme was inhibited by EOs in the 5th instar of *M. separata* which, cased high inhibition for metabolism. The inhibition of all enzymes such as glutathione (GST) leads to cell damage, prevents cells from growth and accumulation of toxins inside mites bodies [**54**].

3.6. Effect of LC_{90} of the essential oil nano-emulsions on T. urticae under greenhouse conditions

The effectiveness of the tested NEs at LC_{90} on *T. urticae* population under greenhouse conditions was conducted and one day before, 1-, 3-, 7-, and 14-days observation of *T. urticae* population was recorded and tabulated in Table 5.

Data represented in Table 5 showed a significant reduction in the spider mite individuals after one-day post-treatment for clove NEs (98.56%) and cumin NEs (98.15%). Over varying time intervals, the tested nano-oils achieved considerable mortality, particularly after 7 and 14 days. Treatment of mites with clove NEs at LC_{90} concentration, showed a high reduction in mite population after 3 and 7 days reached to 98.96% and 100%, respectively and the overall reduction percentage was 99.38%. Consequently, cumin NEs showed a reduction in the mite population reached 98.98%, 99.76% and 99.93% after 3, 7 and 14 days of application and with an overall reduction percentage 99.20% as shown in Table (5).

Clearly investigating the greenhouse results, it can be concluded that both nano oils caused a significant reduction in the spider mites population reached to 100% after spraying clove NEs in the seventh day after the application and these results were on par with those reported previously **[55,56].**

Tunc and Şahinkaya [57] also confirmed that the two tested nano essential oils instigated 100% mortality in *T. urticae* individuals after 21 days of application. The current findings also have been confirmed by **[58, 59, 47** and **60]** who reviewed that the EOs used for control and kill mites under greenhouse conditions with mortality reached to 88%.

Plant oil extracts are considered to be natural pesticides against both mites and insects, especially against both *T. urticae* and *Bemisia tabaci*. Also, [**61]** indicated that ESo have a high potential for application in integrated management programs against *T. urticae*, which is a major pest in greenhouse.

The potential effect of NEs in greenhouses is due to its ability to cover the plant surface, especially the shelters (domatia) that hide the egg and immature stages, which are considered a way to escape from external influences. NEs are more capable

of penetrating the plant surface due to the small size of their molecules, which affects individual mites during feeding, disrupting the metabolic processes in the digestive system **[62].**

Table 4: Effect of LC_{50} of two essential oils on enzyme activities of *T. urticae* adult female

EOs	GST activity (mmol sub. conjugated/ min/mg protein	Change %	AChE activity (ug AchBr /min/gm b wt.)	Change %	GOT (U/L) ±SE	Change %	GPT (U/L) ±SE	Change %	ACP (U/L) ±SE	Change %
S. aromaticum	0.59^{b}±0.00	-4.84	271.09^{a}±8.33	91.77	34.67^{a}±0.38	6.12	9.80^{b}±0.26	-18.74	33.00^{c}±0.57	-13
C. cyminum	0.35^{c}±0.00	-43.55	49.94^{c}±3.00	-64.67	35.10^{a}±0.78	7.44	3.30^{d}±0.20	-72.00	44.33^{a}±0.33	15.65
Control	0.62^{a}±0.001	0.00	141.36^{b}±7.99	0.00	32.67^{b}±0.23	0.00	12.06^{a}±0.32	0.00	38.33^{b}±0.88	0.00
LSD $_{0.05}$	0.0029	0.00	23.82	0.00	1.80	0.00	0.925	0.00	2.208	0.00

Change % = (sample-control/control) x100.

Table 5: Effectiveness of two nano-emulsion essential oils at lC_{90} level against *T. urticae* under greenhouse conditions

NEs	Pre-spray	Mean number per plant and percent reduction o of *T. urticae*									
		Time after treatment (days)								Overall Mean	Overall Reduction
		1		3		7		14			
		Mean No.	Reduc . %	Mean No.	Reduc . %	Mean No.	Reduc . %	Mean No.	Reduc . %		
S. aromaticum	94.50^{b}± 2.43	1.40^{b}± 0.42	98.56	1.30^{b}±0.56	98.96	0.00^{b}±0.00	100	0.00^{b}±0.00	100	0.68^{b}±0.39	99.38
C. cyminum	86.4^{b}±3.40	2.60^{b}±0.58	98.15	1.5^{b}± 0.45	98.98	1.40^{b}± 0.45	99.76	0 1.9 b± 0.38	99.93	1.85^{b}± 0.27	99.20
Control	149.4^{a}±9.55	159.50^{a}±9.32	------	172.00^{a}±10.45	----	230.20^{a}±14.11	-----	429.5^{a}±20.55	----	247.75^{a}±62.50	----
F	32.39	283.64		265.21		263.91		434.57		15.55	
P	0.0000 ***	0.0000 ***		0.0000 ***		0.0000 ***		.0000 ***		.0012 **	
LSD $_{(P=0.05)}$	17.474	15.667		17.550		23.667		34.440		115.442	

Mean ±SE followed by different letters in each column have significantly differences

4. Conclusions

The acaricidal potential of some selected plant essential oils and their developed EO nano-emulsions-formulations oils against adult females of *Tetranychus urticae* Koch under both laboratory and greenhouse-controlled conditions were assessed. The use of essential oils and/or nano-formulated essential oils could be considered as an integrative eco-friendly tool to reduce the damages caused by *T. urticae* and support the reduction of synthetic chemical pesticides use.

5. Conflicts of interest

There are no conflicts to declare.

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