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Toxicity of Essential Oils Nanoemulsion Against *Aphis craccivora* and Their Inhibitory Activity on Insect Enzymes

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Abstract: Essential oils are widely used as botanical insecticides rather than chemically synthesized pesticides which led to catastrophic effects on humans, the environment, and eutrophication. Here, encapsulation of four essential oils *Basilicum ocimum*, *Cuminum cyminum*, *Origanum marjorana*, and *Matricaria chamomilla* were utilized in the presence of 3% *v/v* ethanol, as anti-insect against *Aphis craccivora* and compared to traditional insecticides dinotefuran and pymetrozine. Different tools were used to characterize the prepared nanoemulsion such as TEM, SEM, and Zeta potential analyzer. Besides, selected *B. ocimum* and *C. cyminum* were analyzed by gas chromatography-mass GC/mass spectrometry. The results reveal that nanoemulsion exhibited considerable toxic activities against laboratory and field strains of cowpea aphid. In the toxicity bioassay test of essential oils, moderate mortality was observed at 10,000 mg/L against aphid with lethal concentration that kills 50% of insects (LC₅₀) values of basil 992 mg/L and marjoram 3162 mg/L. Else, nanoemulsion provided the highest mortality rate at 625 mg/L and the LC₅₀ values of basil nanoemulsion (NE) 45 mg/L, and marjoram NE 188 mg/L in laboratory strains. The systemic effects of the tested substances acetylcholine esterase, alkaline phosphatase, β -esterases, glutathione S-transferase (GST), and mixed-function oxidase (MFO) enzymes on insects were found to be significantly decreased and increased when compared with control groups. Overall, these results highlight that the nanoemulsion is potential tools to control cowpea aphid and could be useful in developing integrated insect management in faba bean fields.

Keywords: essential oil; nanoemulsion; *cowpea aphid*; GC/mass spectrometry; bioassay test; detoxification enzymes

1. Introduction

The cowpea aphid (*Aphis craccivora*) is one of the most harmful insects for many crops all over the world [1]. The cowpea aphid insects cause significant economic injuries by sucking sap from leaves, pods, and other organs or through the transmission of major viruses such as bean leaf roll virus (BLRV) [2]. The common method for *A. craccivora* control is chemical insecticides, but the large-scale use of synthetic insecticides resulted

in the development of resistance to insecticides and out-break of secondary pests [3,4]. Additionally, the impact of some pesticide residues and direct hazards on the users were impacted on the environment and non-target organisms like parasitoids and aphid insects [5]. However, many researchers preferred natural compounds due to their less harmful nature to non-target organisms. Plant extracts and essential oils (EOs) may be an alternative to synthetic insecticides because they are effective, eco-friendly, and easily biodegradable [6–8]. Improve metabolic detoxification by glutathione-S-transferases, esterases, P450 monooxygenases, and alkaline phosphatase are major factors in resistance to insecticides in many aphid species [9,10]. Therefore, the researchers in the insects' control field are seeking some alternative insecticides, which are more effective against the pest, have little cytotoxic effects on natural enemies, and slightly toxic to the environment [11,12]. Essential oils from plant species are mixtures of low molecular weight, produced in high amounts by an array of many plant families such as Apiaceae, Asteraceae, Lamiaceae, Lauraceae, Rutaceae, and Myrtaceae [13–15], furthermore, their monoterpenoids have attracted attention in last years as potential pest control agents. Therefore, these components had variable modes of action such as direct toxicants, antifeedants/repellants, and some effects on the enzymatic profiles [16]. Though, the efficacy of botanical insecticides may lower than synthetic insecticides, but they are prophylactic and they are not harmful on the environment and public health [17,18].

Some problems of essential oils were shown when used as pesticides such as limited physical stability, quick degradation in the environment, and poor water solubility. Therefore, there are some methods to overcome these problems with nanotechnologies such as nanoemulsion which, improves the physical stability of essential oil, water diffusion, and covering with small-sized oil droplets of the targeted surface area [19–22]. So, biological nanomaterial has taken a vast role in pest and plant disease management [23–26]. Therefore, this work was designed to study the susceptibility of *A. craccivora* to some of the new and non-traditional insecticides belongs to different groups of essential oils and their nanoemulsions. The production and properties of the fabricated nanoemulsions were verified by UV–vis spectrophotometry, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and energy-dispersive X-ray (EDX) spectroscopy techniques. Moreover, the activity of some detoxification enzymes like acetylcholinesterase (AChE), β -esterases, glutathione S-transferase (GST), alkaline phosphatase, and mixed-function oxidase (MFO) was studied in field strain.

2. Materials and Methods

2.1. Chemicals, Reagents, and Essential Oils

The essential oils of *Basilicum ocimum* (basil), *Cuminum cyminum* (cumin), *Origanum marjorana* (marjoram), and *Matricaria chamomilla* (chamomile) were obtained from Hashem Brothers Company for essential oils and aromatic products, Kalyoubeya, Egypt. Pymetrozine (Chess 50% WG) belongs to pyridine azomethine derivatives and Dinotefuran (Oshin 20% SG) belongs to neonicotinoid groups were purchased from Syngenta and Mitsui Chem., Egypt. Acetylcholine iodide, 5,5-dithiobis-2-nitro benzoic acid, fast blue salt, (β -esterases), GST, alkaline phosphatase, Folin–Ciocalteu reagent were obtained from Sigma-Aldrich Chemicals (St Louis, MO, USA) and all other chemicals, as well as reagents used, were of the highest analytical grade purchased from local companies.

2.2. Preparation and Characterization of Nanoemulsions

In this study, the essential oil nanoemulsions were prepared, according to the method of [19] with some modifications by [20]. Thickened (O/W) nanoemulsions were prepared with essential oils, each representing (14%, (v/v) of the total coarse emulsion), ethanol 3% (v/v), and Tween 80 (3%, v/v), representing 20% (v/v) of the total emulsion. The components of the oil phase were vigorously mixed for 2 h. Then, they were mixed with water (80%), kept for 3 min, and finally centrifuged at 3000 rpm for 10 min. The essential oils nanoemulsion were stored in dark bottles at ambient temperature. The color of

nanoemulsion was changed and directly characterized by UV–vis. The UV spectra were recorded on (Shimadzu UV-2550 spectrophotometer, Kyoto, Japan) at 1 cm optical path quartz cuvette, in the range of 200–800 nm. Furthermore, an aliquot of nanoemulsions containing nanoparticles was used for investigation of the morphology and nanoparticle size by SEM using (JEOL-JSM IT-100, Tokyo, Japan) and operated at an accelerating voltage of 10 kV. The presence of metals was also confirmed by the energy dispersive spectroscopy (EDS) instrument equipped with the SEM. The samples were diluted to 50-folds in double deionized water and dried under vacuum at room temperature. Subsequently, the samples were coated using sputter before analysis. While the structure of synthesized nanoemulsions was observed by the TEM using a JEOL-1230, Japan transmission electron microscope operated at an accelerating voltage of 300 kV. TEM samples were prepared by placing a few drops of the emulsion-nanoparticles onto a carbon-coated copper grid to make a thin film of the sample and then it was kept in a grid box sequentially.

2.3. Droplet Size Analysis

Regarding the nanoemulsions characterization, the average size distributions of nanoemulsions were noted in the light of their intensity, number weight, and volume, respectively. Zeta potential, conductivity, viscosity, and polydispersity index (PDI) were investigated by photon correlation spectroscopy using a Zeta Plus tool (Zeta sizer, Brookhaven Instruments Corp New York, NY, USA).

2.4. Analysis of Volatiles in Essential Oils and Nanoemulsions

The GC-MS analysis was carried out using gas chromatography-mass spectrometry instrument stands with the following specifications, under a TRACE GC Ultra Gas Chromatographs instrument (THERMO Scientific Corp Carlsbad, CA, USA), coupled with a Thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer, Germering, Germany). The GC-MS system was equipped with a TR-5 MS column (30 m by 0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1 mL/min and a split ratio of 1:10 using the following temperature program: 60 °C for 1 min, rising at 40°C/min to 240 °C and held for 1min. The injector and detector were held at 210 °C. Diluted samples (1:10 hexane, *v/v*) of 1 µL of the mixtures were always injected. The spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of *m/z* 40–450. The identification of the chemical constituents of the essential oil was de-convoluted using AMDIS software (AMDIS version 2.70, www.amdis.net, accessed on 29 March 2021) and identified by its retention indices (relative to n-alkanes C8–C22), mass spectrum matching to [authentic standards (when available), Wiley spectral library collection, and NSIT library database].

2.5. Aphid Rearing

A laboratory strain of *A. craccivora* was obtained from the laboratory of sucking insects department, Plant Protection Research Institute, ARC, and Egypt. This strain reared on faba bean seedling (Sakh3) grown in plastic pots (20 cm diameter) under laboratory conditions of 25 ± 1 °C, 65% RH, and 12 h daily illumination by two fluorescent bulbs of 40 watts. Every week, the seedlings were replaced with new ones to keep aphid colonies alive. This laboratory strain was used as a reference strain. In the case of the field strain, faba bean leaves infested with aphids were collected from faba bean fields at Sakha Agricultural Research Station Farm, Kafr El-Sheikh governorate, Egypt, and moved to the laboratory to complete the toxicity tests.

2.6. Toxicity Tests

The rapid test was used to compare the susceptibility of the laboratory and field strains of aphids to different compounds of essential oils [21], their nanoemulsions, and two chemical insecticides (pymetrozine and dinotefuran). Where, batches of aphids were immersed and tested with serial concentrations (625, 1250, 2500, 5000, 7500, and 10,000 mg/L) of essential oils and (39.063, 78.125, 156.25, 312.5, 468.75, and 625 mg/L) of nanoemulsions for 10 s and the excess of the solution was dried on paper towels. For each strain, twenty-five

apterous adults of the same ages and sizes were placed on leaves of faba bean plants in Petri-dishes (9 cm) with the help of a soft brush, while control treatments aphids were immersed in ethanol and distilled water (20:80). Three replicates were used for each concentration of the tested compound and the control. All Petri-dishes were maintained under laboratory conditions of 25 ± 2 °C; $65 \pm 5\%$ relative humidity and 12 h daily illumination by 2 fluorescent bulbs of 40 wt. Mortality counts were observed after 24, 48, and 72 h according to the toxicant's mode of action, and the insect was considered alive if it was able to move at least one leg or antennae during probing with a soft brush. The aphid was considered dead if there's no movement or only very slight twitching observed [22]. Mortality percentages were adjusted for mortality in control according to Abbott's formula [23]. The data were analyzed according to [24] to calculate values of the lethal concentration that kills 50% of insects (LC_{50}), lethal concentration that kills 90% of insects (LC_{90}), and slope for the tested compounds. The potency level expressed as several folds were calculated by dividing the LC_{50} for the low effectiveness materials by the corresponding figure for each compound.

2.6. Biochemical Aspects

The activity of AChE was determined and the nonspecific esterases (β -esterases) was recognized by [25], GST by [26], alkaline phosphatase by [27], and MFO by [28], and all assayed in field strains of exposure insects with minor modifications. The insects were prepared as described by [29] and homogenized with cold phosphate buffer pH 7.2 (50 mg/L mL) using a glass homogenizer. The cold crude extracts were centrifuged at 8000 rpm for 15 min at 2 °C in a refrigerated centrifuge and passed through glass wool to remove the last of insoluble cell debris. The resulting supernatant of homogenate was collected as a source of the measured biochemical aspects and kept at -20 °C for further enzyme analysis.

2.7. Statistical Analysis

The percentage of mortality for aphid insects treated with essential oils, their nanoemulsions, and two chemical insecticides were subjected to probit analysis (Finney, 1971) for calculating LC_{50} , LC_{90} statistics at 95% confidence limits of lower and upper values. The differences between enzyme activities in the field strain were assessed with LSD (the least-significant-difference test) and Duncan at a 5% level of probability. Chi-square values were calculated using the SPSS software package 19.0 version (SPSS Inc., Chicago, IL, USA). The statistical analyses were performed using GenStat18 software (VSN International, 2015).

3. Results and Discussions

3.1. Characterization of the Nanoemulsion

3.1.1. Electronic Spectrum

In the present investigation, the materials of essential oils underwent reduction, which indicated the formation of nanoemulsions (Figure 1). The presence of emulsion nanoparticles was affirmed and measured by utilizing UV spectrophotometer. The formation was shown at the absorption peak of basil (Figure 1A), chamomile (Figure 1B), marjoram (Figure 1C), and cumin (Figure 1D), respectively. Such characteristics match well with those observed for a single surface plasmon resonance (SPR) and the deviation of the SPR shoulder of anisotropic particles might be possibly attributable to their shape and size [8,11,30].

Figure 1. UV spectrophotometer fingerprint peaks of prepared (A) basil, (B) chamomile, (C) marjoram, and (D) cumin.

3.1.2. Morphological Structure

Figure 2 showed the TEM images of the prepared nanoemulsion-based essential oils with polydispersity character. The TEM images indicated that the synthesized emulsion nanoparticles are mainly uniform with a spherical shape. The transparent organic layer coatings around the nanoparticles were shown in the TEM image, which was due to the phytochemicals that served as a capping agent. Therefore, the particles were polydispersed with direct contact and stable for a long period to prevent agglomeration [31].

Figure 2. TEM image of (A) basil nanoemulsions, (B) chamomile nanoemulsions, (C) marjoram nanoemulsions, and (D) cumin nanoemulsions.

The surface morphology of basil nanoemulsion as an example after lyophilization was detected by SEM analysis as in Figure 3. The nanoemulsions are mainly spherically shaped with an average range of particle size from 15.8 to 99.8 nm, and this is a good resemblance with the shape of the SPR band observed in the UV-Vis spectra [32]. The EDX pattern of nanoemulsions was shown in (Figure 3B). The presence of strong peaks of different elements after 3 keV was observed, which indicates that the Ca, Cu, Zn, K, and Mg are the major elements in nanoemulsions, thus confirming the successful biosynthesis of nanoemulsions. A similar peak after (3 keV) has been reported by other researchers [33].

Figure 3. (A) SEM morphology and (B) EDX elemental analysis of selected basil nanoemulsion.

3.1.3. Size Distribution Analysis

The zeta potential is usually utilized to know the constancy of colloidal systems. The zeta potential of nanoparticles was evaluated in water as a dispersant. In this report, zeta potentials of basil nanoemulsions, chamomile nanoemulsions, marjoram nanoemulsions, and cumin nanoemulsions were -26.2 mV, -34.9 mV, -15.8 mV, and -29.4 mV, which denoted the stability of emulsion nanoparticles suspensions (Figure 4A–D). The average size of basil emulsion nanoparticles was found to be 129.5 ± 1.3 nm, PDI = 0.28, chamomile emulsion nanoparticles 149.6 ± 1.7 nm, PDI = 0.54, marjoram emulsion nanoparticles 182.1 ± 1.8 nm, PDI = 0.22 and cumin emulsion nanoparticles 172.7 ± 1.5 nm, PDI = 0.37 (Table 1). Mostly, the suspension that displays an absolute zeta potential less than 20 mV is considered unstable and will cause precipitation of particles from solution, while the absolute zeta potential higher than 20 mV is stable [34,35]. In another scenario, the average particle size calculated using dynamic light scattering measurements (DLS) was found to be lower than 300 nm as a nanoemulsion [36]. Nanoemulsion having a particle size in the range of 100–400 nm demonstrated beneficial attributes, such as permeability, thermal stability, and solubility [34,37]. Additionally, it revealed the smallest Z-average size of 129.5 ± 1.3 nm and the lowest conductivity 0.26 mS/cm.

Figure 4. Zeta potential distributions for (A) basil nanoemulsion, (B) chamomile nanoemulsion, (C) marjoram nanoemulsion, and (D) cumin nanoemulsion, respectively.

Table 1. Mean $\pm$ (SD) particle size diameter (nm), polydispersity index (PDI), and conductivity measurements of nanoemulsion based oils.

Nanoemulsions	Particle Diameter (nm)	Polydispersity Index PDI	Conductivity (ms/cm)
<i>B. ocimum</i>	129.5 $\pm$ 1.3	0.28	0.26
<i>M. chamomilla</i>	149.6 $\pm$ 1.7	0.54	0.28
<i>O. marjorana</i>	182.1 $\pm$ 1.8	0.22	0.28
<i>C. cyminum</i>	172.7 $\pm$ 1.5	0.37	0.29

3.1.4. GC-MS Analysis and Identification of Components

A total of 14 components were determined in basil, C. cumin EO, and in their nanoemulsion by GC-MS analysis (Tables 2 and 3). Interestingly, the major components in basil oil were linalool (22.91%), eucalyptol (19.18%), eugenol (6.89%), 1,6-octadien-3-ol, 3,7-dimethyl- (6.56%), α -bergamotene (6.39%), cadinol T(2.95%), and γ -cadinene (2.55%). However, the major components in cumin oil were γ -terpinene (22.25%) L- β -pinene (21.63%), α -terpinene (19.31%), cumic aldehyde (13.02%), and β -cymene (8.78%) (Table 2). In our study, the insecticidal activity of basil and cumin essential oils may be attributed to the high level of these components (Table 2). On the other hand, the high levels of components in basil-nanoemulsions were α -bergamotene (24.73%) and linalool (10.12%). Moreover, in cumin-nanoemulsions, the high level of components was cumic aldehyde (57.28%), β -cymene (18.32%), and α -terpinene (9.91%) (Table 3). The major constituents of the basil and cumin EOs were similar to these oils which, were isolated from basil and cumin plants growing in Egypt or other countries [38–40]. Methyl chavicol, linalool, and geranial were reported as the major active components in basil oil. Linalool is characterized as the main component from basil essential oil with (22.91%), and it is an effective component as a contact insecticide and feeding deterrent [41]. Therefore, this can explain the advantage of the toxicity of basil and cumin compared to other tested essential oils.

Table 2. Volatile components obtained from basil and cumin oils.

Compounds	Other Names	RT ^a (min)	Molecular Formula	Concentrations %	
				Basil Oil	Cumin Oil
Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-, (1s)-	L-β-Pinene	6.55	C ₁₀ H ₁₆	-	21.63
Benzene, methyl(1-methylethyl)-	β-Cymene	7.46	C ₁₀ H ₁₄	-	8.78
Eucalyptol	-	7.68	C ₁₀ H ₁₈ O	19.18	-
2-oxabicyclo[2.2.2]octane, 1,3,3-trimethyl-	furan	7.81	C ₁₀ H ₁₈ O	5.58	-
1,4-cyclohexadiene, 1-methyl-4-(1-methylethyl)-	γ-Terpinen	8.38	C ₁₀ H ₁₆	-	22.25
Linalool	-	9.93	C ₁₀ H ₁₈ O	22.91	-
1,6-octadien-3-ol,3,7-dimethyl-	Linalool	10.27	C ₁₀ H ₁₈ O	6.56	-
Benzaldehyde, 4-(1-methylethyl)-	Cumic aldehyde	12.17	C ₁₀ H ₁₂ O	-	13.02
4-Isopropyl cyclohexa-1,3-dienecarbaldehyde	α-Terpinene	13.63	C ₁₀ H ₁₄ O	-	19.31
Eugenol	-	14.75	C ₁₀ H ₁₂ O ₂	6.89	-
Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	α-Bergamotene	15.89	C ₁₅ H ₂₄	6.39	-
(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.0.2,7]decane-rel-	β-Copaen-4α-ol	16.66	C ₁₅ H ₂₄	1.58	-
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-	γ-Cadinene	17.25	C ₁₅ H ₂₄	2.55	-
tau.-Cadinol	Cadinol T	19.25	C ₁₅ H ₂₆ O	2.95	-

^a RT, Retention time (minutes).**Table 3.** Volatile components obtained from basil and cumin nanoemulsions.

Compound	Other Names	RTa (min)	Molecular formula	Concentrations %	
				Basil NE	Cumin NE
Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-	β-Pinene	6.28	C ₁₀ H ₁₆	2.65	5.53
Benzene, methyl(1-methylethyl)-	β-Cymene	7.25	C ₁₀ H ₁₄	1.12	18.23
Eucalyptol	Eucalyptol	7.38	C ₁₀ H ₁₈ O	2.65	-
1,4-cyclohexadiene, 1-methyl-4-(1-methylethyl)	γ-Terpinen	7.88	C ₁₀ H ₁₆	-	2.20
ς-Terpinene	ς-Terpinene	7.91	C ₁₀ H ₁₆	1.29	-
Linalool	Linalool	9.03	C ₁₀ H ₁₈ O	10.12	-
Benzaldehyde,4-(1-methylethyl)-	Cumic aldehyde	11.81	C ₁₀ H ₁₂ O	-	57.28
Bornyl acetate	Bornyl acetate	12.46	C ₁₂ H ₂₀ O ₂	1.06	-
4-Isopropyl cyclohexa-1,3-dienecarbaldehyde	α-Terpinene	12.57	C ₁₀ H ₁₄ O	-	9.91
p-Cymen-7-ol	p-Cymen-7-ol	12.67	C ₁₀ H ₁₄ O	-	6.11
Eugenol	Eugenol	13.90	C ₁₀ H ₁₂ O ₂	2.57	-

Table 3. Cont.

Compound	Other Names	RTa (min)	Molecular formula	Concentrations %	
				Basil NE	Cumin NE
Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	α -Bergamotene	15.53	C ₁₅ H ₂₄	24.73	-
Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1 α ,4 α ,8 α)-	γ -Cadinene	16.93	C ₁₅ H ₂₄	9.67	-
tau.-Cadinol	Cadinol T	18.91	C ₁₅ H ₂₆ O	5.64	-

RTa—retention time (minutes).

3.2. Susceptibility of *A. craccivora* to Tested Insecticides

In this study, we compared the toxicity of two insecticides: Pymetrozine (Chess 50%WG), Dinotefuran (Oshin 20%SG), and four essential oils basil, cumin, marjoram, and chamomile as well as their nanoemulsions against apterous adult stage of laboratory and field strains of aphid *A. craccivora*. Where, data in (Table 4) cleared that pymetrozine and dinotefuran were the most effective against laboratory strains of *A. craccivora* with LC₅₀ (9.4 and 4.6 mg/L), respectively. Furthermore, (Table 4) showed that the toxicity of essential oils and nanoemulsion of four essential oils, where basil was the most effective oil followed by cumin, whereas chamomile and marjoram oils were the least effective after different exposure times against the laboratory strains of *A. craccivora* with LC₅₀ (992, 1710, 2327, and 3162 mg/L), respectively. Furthermore, the toxicity activity of nanoemulsions for four essential oils against the laboratory strains of *A. craccivora* demonstrated the same results of essential oils but with low concentrations and LC₅₀ were (45, 79, 134, and 188mg/L), respectively (Table 4). Moreover, the data in Table 4 showed that the highest degree of homogeneity for the laboratory population of *A. craccivora* was observed to basil with a slope value of 2.1 and 1.9 for EOs and nanoemulsion (NE), respectively, while the other tested compounds exhibited low slope values, and this indicates heterogeneity in the aphid response to these essential oils. On the other hand, in field strain, basil oil, and their nanoemulsions were the most effective on *A. craccivora* where recorded LC₅₀ values 1376 and 54 mg/L, followed by cumin with LC₅₀ values 2263 and 83 mg/L after 72 h of exposure.

Table 4. Toxicity of four essential oils, nanoemulsions, and chemical insecticides against *A. craccivora* laboratory strains.

Treatments	LC ₅₀ mg/L (95% LCL–UCL)		LC ₉₀ mg/L (95% LCL–UCL)		Slope $\pm$ SE		χ^2		PL	
	EO	NE	EO	NE	EO	NE	EO	NE	EO	NE
<i>O. basilicum</i>	992 (820–1163)	45 (35–55)	3998 (3358–4969)	209 (176–256)	2.1 $\pm$ 0.17	1.9 $\pm$ 0.17	2.9	1.8	3.2	69.5
<i>C. cyminum</i>	1710 (1462–1967)	79 (65–94)	7600.32 (6282–9650)	425 (349–545)	1.9 $\pm$ 0.15	1.8 $\pm$ 0.14	3.5	2.8	1.9	39.7
<i>M. chamomilla</i>	2327 (2024–2653)	134 (114–155)	9962.66 (8160–12821)	726 (572–990)	2.0 $\pm$ 0.15	1.9 $\pm$ 0.14	1.9	1.8	1.4	23.5
<i>O. marjorana</i>	3162 (2753–3624)	188 (163–216)	14862 (11736–20206)	938 (734–1293)	1.9 $\pm$ 0.15	1.8 $\pm$ 0.14	2.4	2.6	1.0	16.8
Pymetrozine	9.4 (8.1–10.9)		44.0 (34.1–61.8)		1.9 $\pm$ 0.16		1.9		335	
Dinotefuran	4.6 (3.8–5.6)		34.0 (23.7–57.0)		1.5 $\pm$ 0.15		3.3		679	

LC₅₀—lethal concentration that kills 50% of insects, LC₉₀—lethal concentration that kills 90% of insects, LCL—lower confidence limit, UCL—upper confidence limit, χ^2 = Chi-square value, PL—potency level, SE—standard error, EO—essential oil, and NE—nanoemulsion.

It should also be noted that marjoram oil and nanoemulsions with LC₅₀ values of 3624 and 221 mg/L were the least effective after 72 h (Table 5). In addition, the field strain was less sensitive to the insecticides tested than the laboratory strain. The results cleared that pymetrozine and dinotefuran were the most effective against field strain of *A. craccivora* with LC₅₀ 12.5 and 27.4 mg/L with significant differences between treatments (Table 5). At all treatments, the toxicity increased dramatically with increasing exposure time. Moreover, the population of *A. craccivora* field strain reflected the different degrees of homogeneity in response to tested with slope values ranged varied from 1.6 to 2.4 (Table 5). Concerning of resistance ratio of the tested essential oils and their nanoemulsions revealed that the field strain of *A. craccivora* had a low level of resistance to all the tested essential oils and their nanoemulsions. Where, these results suggested that basil, cumin, and their nanoemulsions can be used to control *A. craccivora* as a safer and effective insecticide with varying modes of action targeting aphid. The obtained results are in agreement with [42], they showed that the field population of *A. craccivora* exhibited low resistance to acetamiprid, imidacloprid, primiphose-methyl, Pymetrozine, and primicarb ranged from 1.29- to 2.78-fold. In general, the laboratory strain was more susceptible to the tested oils than that the field strain of *A. craccivora*. Essential oils were promising against many insects, but it had registered some problems i.e., the rate of essential oils, water solubility, and oxidation play a pivotal role in its application and efficiency, therefore, nano-formulation can resolve many problems in essential oils application, where nanoparticles lead to keep the essential oils from degradation, improve their residue half-life by decreasing evaporation, high surface area, solubility, and mobility [43]. The positive effect of these compounds (neemix and basil oil) may be due to their effects on the prolongation of nymph duration and decreasing the number of adults of *A. craccivora*; also the changes in biochemical biomarkers (aspartate transaminase, AST; alanine transaminase, ALT; and alkaline phosphatase ALP) in *A. craccivora* [44]. Basil showed insecticidal activity in controlling *A. Craccivora* in faba bean plants systematically or by contact, caused toxicity to the adult stage of *A. craccivora* and the accumulative mortality reached 100% after 7 days of treatment [44].

Table 5. Toxicity of four essential oils, nanoemulsions, and chemical insecticides against *A. craccivora* field strains.

Treatments	LC ₅₀ mg/L (95% LCL–UCL)		LC ₉₀ mg/L (95% LCL–UCL)		Slope ± SE		χ ²		PL	
	EO	NE	EO	NE	EO	NE	EO	NE	EO	NE
<i>O. basilicum</i>	1376 (1148–1607)	54 (43–64)	6778 (5628–8541)	225 (191–273)	1.9 ± 0.14	2.1 ± 0.16	2.1	1.5	2.6	67.6
<i>C. cyminum</i>	2263 (1963–2584)	83 (67–99)	9873 (8071–12744)	535 (419–738)	2.0 ± 0.15	1.6 ± 0.14	4.6	1.9	1.6	43.6
<i>M. chamomilla</i>	2857 (2455–3310)	163 (140–188)	15,727 (12,104–22,244)	856 (670–1179)	1.7 ± 0.14	1.8 ± 0.14	3.8	2.5	1.3	22.2
<i>O. marjorana</i>	3624 (3143–4191)	221 (194–253)	18,541 (14,202–26,419)	962 (765–1293)	1.8 ± 0.15	2.0 ± 0.15	2.4	1.9	1.0	16.3
Pymetrozine	12.5 (10.7–14.3)		49.1 (40.0–64.0)		2.2 ± 0.18		2.2			291.1
Dinotefuran	27.4 (23.9–31.1)		94.1 (78.1–119.4)		2.4 ± 0.19		2.5			132.4

LC₅₀—lethal concentration that kills 50% of insects, LC₉₀—lethal concentration that kills 90% of insects, LCL—lower confidence limit, UCL—upper confidence limit, χ² = Chi-square value, PL—potency level, SE—standard error, EO—essential oil, and NE—nanoemulsion.

3.3. Inhibitory Activity Evaluation of Essential Oils and Synthesized Nanoemulsions on Insect Enzymes

The activities of essential oils and synthesized nanoemulsions on the biochemical ingredients (acetylcholine esterase, alkaline phosphatase, beta esterase, glutathione S-transferase, and mixed-function oxidase) of a field strain of aphid *A. craccivora* were tested and the results are showed in (Figure 5). The treated samples with essential oils showed decreasing in the activity of acetylcholine esterase compared to the control group, where the acetylcholine esterase activity values of control and basil oil were 20.1 and 12.6 ug/min/mg

protein, respectively. However, the level of acetylcholine esterase significantly reduced after the treatment with all four nanoemulsions as compared with the control group (Figure 5A). The toxicity of tested compounds inhibited AChE involved in the process of catalyzing the hydrolysis of the neurotransmitter AChE at nerve synapses and neuromuscular junctions and their activity. The inhibition of acetylcholine esterase activity was confirmed after treated against *A. Aegypti* by α -chitin nanoparticles (CNP) [30]. On the other hand, as shown in (Figure 5B,C), the exposure of aphid insects to basil and cumin essential oils and their nanoemulsions resulted in the reduction of the level of alkaline phosphatase and beta esterase activities when compared with the control groups. In the alkaline phosphatase assay, the level of alkaline phosphatase activity decreased from the absorbance value of control 18.7 to values of cumin EO and cumin NE 11.3 and 11.6 mU/mg protein, respectively. Whereas in the beta esterase assay, the control, basil NE, and cumin NE against aphid insects showed absorbance values of 51.4, 20.4, and 24.5 $\mu\text{g}\beta\text{-naphthol/min/mg protein}$, respectively.

Alkaline phosphatase and beta esterase play an important role in the hydrolytic cleavage of phosphoric acid esters and they regulated the alkaline balance [12]. Moreover, these enzymes are important for many remarkable physiological processes such as metabolism and cellular signaling processes [16]. Esterases are the primary enzymes involved in the development of resistance mechanisms to chemical insecticides by splitting the carboxyl ester and phosphodiester bonds. The detoxifying activity of β -carboxylesterase was used as biomarkers in studies with different insects [45,46]. According to the presented data in Figure 5D,E, the exposure of essential oils and synthesized nanoemulsions increased GST and MFO activity in the laboratory and field strains of aphid *A. craccivora* when compared with control groups. The activity of GST increased in chamomile EO, chamomile NE, marjoram EO, and marjoram NE with values 5.8, 5.4, 6.2, and 5.3 mmole sub. conjugated/min/mg protein, respectively, but in basil EO, basil NE, and cumin NE the values decreased where were 3.8, 2.8, and 4.0 mmole sub. conjugated/min/mg protein, when compared to control groups 4.1 mmole sub. conjugated/min/mg protein, respectively. Furthermore, the effectiveness of mixed-function oxidase increased in all essential oils and their nanoemulsions except chamomile EO 999.3, marjoram EO 931 $\mu\text{mole sub. oxidized/min/mg protein}$, when compared with control groups 1021 $\mu\text{mole sub. oxidized/min/mg protein}$. The MFO activity values of aphid *A. craccivora* were 1724.7, 1525.7, 1344.3, 1213.3, and 1053.3 $\mu\text{mole sub. oxidized/min/mg protein}$ for basil NE, cumin NE, basil EO, chamomile NE, and marjoram NE, respectively. In previous studies, the detoxification enzyme system, known as the mixed-function oxidase system, protect insects from poisons. Moreover, lepidopterous larvae show that the MFO system is an effective biochemical defense, mainly because this system plays an important role in the primary degradation and inactivation of a wide variety of exogenous lipophilic substances, the high concentration of these enzymes occurs in plant-eating insects, in which they detoxify the natural toxins in the plants [47,48].

Figure 5. Impact of four essential oils and their nanoemulsions on enzyme activities. (A) acetylcholine esterase, (B) alkaline phosphatase, (C) β -esterase, (D) glutathione S-transferase (GST), and (E) mixed-function oxidase (MFO) enzymes. Each bar represents the mean $\pm$ SE of three replicates using different preparations of insect homogenates (a, ab, and bc).

4. Conclusions

In the current study, we synthesized nanoemulsion as environmentally friendly organic natural compounds and compared them with two selective insecticides dinotefuran, pymetrozine, and essential oils. The TEM image of basil NE, chamomile NE, marjoram NE, and cumin NE, indicated that the synthesized emulsion nanoparticles are mainly uniform with a spherical shape. Furthermore, the EDX pattern of nanoemulsion was indicated, where the presence of strong peaks of different elements that Ca, Cu, Zn, K, and Mg were the major elements in nanoemulsion, thus confirming the successful of nanoemulsion. Furthermore, the obtained oils and their nanoemulsions showed considerable toxicity against laboratory and field strains of cowpea aphid *A. craccivora* with the highest mortality rate of nanoemulsions when compared with their essential oils and also at the same time with synthetic insecticides. All this is due to the high efficiency of nanoemulsions, their small size, and their ability to penetrate the body of the pest. Moreover, the inhibitory activity evaluation of essential oils and synthesized nanoemulsion on insect enzymes showed that the treated samples showed decreased activity of acetylcholine esterase when compared to the control groups. Further studies are required to characterize the interaction pathway between nanoemulsion and the cell organelles including mitochondria and the nucleus. Thus, the synthesized nanoemulsion may be employed as an eco-friendly insecticide and at low dosages to control cowpea aphid in faba bean fields and protect the environment.

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