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Toxicity and biochemical effects of citronella, mustard and sage essential oils and their nanoemulsions against Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae)

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Abstract

This study aimed to investigate the toxicity of citronella, mustard, and sage essential oils in comparison to their nanoemulsions. Laboratory bioassays were conducted against the 2nd and 4th instar larvae of *Spodoptera littoralis*. The results indicated that the LC_{50} values were 0.231, 1.898 and 2.500% for bulk oils of mustard, citronella, and sage against 2nd instar larvae of *S. littoralis*, respectively, while they were 0.253, 0.266, and 0.962% for their nanoemulsions, respectively. The LC_{50} values were 0.889, 3.358 and 0.995% for bulk oils of mustard, citronella, and sage against the fourth instar larvae, respectively, while the LC_{50} values of their nanoemulsions were 0.657, 0.572 and 0.488%, respectively. The fourth instar larvae were treated with LC_{50s} concentrations of tested essential oils and their nanoemulsions to estimate the biochemical changes. The results showed that there was a significant increase in stimulation of AChE activity, in the case of mustard and citronella bulk and nanoemulsions, while there were no significant changes in sage oil bulk treatment and its nanoemulsion compared with the control. Also, there was a significant decrease in activity of GST enzyme in mustard, citronella bulk oil and mustard nanoemulsion. In contrast, the activity of GST increased significantly in the treatments of sage bulk oil, mustard, citronella and nanoemulsion of sage oil. Alpha esterases activity decreased significantly in all treatments except nanoemulsion of mustard oil which caused a significant increase in the enzyme activities compared with the control.

Keywords: Bioassay, Essential oils, mortality, lethal concentrations, Enzymes.

1. Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is the most serious pest in Egypt and Africa, causing a significant economic loss of cotton, tomato, lettuce, strawberry, and other vegetable crops in both greenhouses and open fields **(Abd El-Razik and Mostafa, 2013; Eppo, 2014).**

Massive applications of conventional pesticides cause harmful effects on beneficial organisms, leave their residues in food that pose serious to human health and the environment, reduce the populations of natural enemies, and developed insect resistance to synthetic insecticides **(Sharma and Yadav, 2001; Saleem *et al.* 2008).**

Using natural insecticides derived from plants has become more common in recent years for the control of agricultural pests. Many authors investigated the potential toxicity of plant extracts, including **(Abdel-Raheem *et al.* 2020 (a); Abdel-Raheem *et al.* 2020 (b); Abdel-Raheem *et al.* 2019; Amr, 2001; Salem *et al.* 2016; Abdel Aziz, 2007; Colomaa *et al.* 2006)**.

To control pests, natural insecticides have been used; many of these substances are secondary plant materials (allelochemicals), such as alkaloids, quinones, and essential oils **(Appel *et al.* 2001)**. These active substances extracted from plants are effective against a wide range of insects and act as toxicants, insect growth regulators (IGR) and repellents **(Burfield and Reekie, 2005)**.

These characteristics make them appropriate bioinsecticides for organic farming and a potential replacement for those chemical insecticides. Since they have fewer detrimental effects on both human health and the environment than synthetic pesticides, various natural products like botanical pesticides have emerged as promising alternatives. Synthetic pesticides are less compatible with environmental elements than natural products. It has been demonstrated that botanical pesticides have properties that prevent a wide variety of species and stages of insects from feeding, repelling them, toxicity, and disrupting their growth.

One of the most exciting new methods for pest control is nanotechnology **(Dimetry *et al.* 2013).** Nano particles represent a new generation of environmental remediation technologies that could provide cost-effective solutions to some of the most challenging environmental clean-up problems **(Chinnamuthu and Murugesa, 2009)**.

Nanoparticles aid in the development of new pesticides, insecticides, and insect repellents. Nanotechnology is emerging as a highly attractive tool for the formulation and delivery of pesticide active ingredients as well as enhancing and offering new active ingredients, As nanocapsules based on polymers are being developed for controlled release of active ingredients as well as enhanced delivery through improving penetration through leaves **(Owolade *et al.* 2008**, **EL-Bendary and EL- Healy, 2013; Butelerb and Weaver, 2010).**

Various groups of nanomaterial function as herbicides, fungicides, and insecticides, recent developments in nanoscience with materials have unique properties to their macroscopic or bulk counterparts **(Matsumoto *et al.* 2009).** The water and oil domains in nanoemulsions are separated by a surfactant, sometimes with the help of a co-surfactant, and they are kinetically stable emulsions. They are also known as submicron, ultrafine, or mini emulsions (**Kadir, 2017)**. The pharmaceutical, food, and cosmetic industries find nanoemulsions to be an appealing target

for delivery systems **(Aubrun *et al.* 2004; Kumar *et al.* 2008).**

The main objective of the present study was to evaluate the toxic activities of three essential oils, citronella (*Cymbopogon sp*), mustard (*Brassica Nigra*) and sage (*Salvia officinalis*) in their conventional and nanoemulsions forms on the second and the fourth larvae instars of *S. littoralis* under the laboratory conditions. In addition, the effect of the same bulk and nano-formulations on some enzymatic activities, i.e. acetylcholinesterase, glutathione S-transferase and nonspecific esterases in 4th instar larvae were estimated.

2. Materials and methods

2.1. Essential oils used:

Citronella oil (*Cymbopogon sp*), mustard oil (*Brassica Nigra*), and sage oil (*Salvia officinalis*) were obtained from Oil Extraction Unit, (National Research Center)**.**

2.2. Preparation of loaded nanoemulsion:

Oil in water (o/w), emulsifications used to create alginate nanocapsules, which were then cross-linked using polyethylene glycol (PEG) **(Lertsutthiwong *et al*. 2008)**. PEG alginate (10%, w/v) solution was made by dissolving it in distilled water at 50°C for 45 minutes. Tween 80 was used as an emulsifier to dilute the tested oils with distilled water while mechanical stirring continued for 10 min. Briefly, the PEG alginate O/W emulsion was created by continuously mechanically stirring at room temperature while dilution-wise dispersing oil into an appropriate volume of alginate solution. An ultrasonic cleaner set, model WUC-DO3H, 290 W and 60 Hz, was used to sonicate the emulsion for 30 minutes. A high-energy ultra-sonication probe, model VCX 750, 750 W, was then used to sonicate the mixture for 4 minutes. **(Yousef *et al*. 2018).**

2.3. Characterization:

Different methods of characterization were used to assess the morphological shape, size, and uniformity content of the obtained nanoformulations and/or nanoparticles to confirm that the prepared tested oils were forming nano-sized particles.

2.3.1. Transmission Electron Microscopy (TEM):

Transmission Electron Microscopy (TEM) (Jeol, JEM-2100) was used to examine the morphological shapes of the prepared nanoformulations. The nanocapsule suspensions were diluted with distilled water, applied to a copper grid with a carbon coating, stained with 1% phosphotungstic acid, and then examined and captured using a 20000X magnification.

2.4. Test insect:

The cotton leaf worm, *Spodoptera littoralis* (Boisd) was taken from Syngenta Company. The egg-masses were kept in a glass jar covered with gauze under laboratory conditions of 25- 30 ºC and 65±5% R.H. Fresh caster leaves (*Ricinus communis* L.) were added to the jars every day as a dietary medium once the egg masses had turned a deep blue color for the hatched larvae. After the first moult, groups of second-instar larvae were moved to new glass jars with a 1 kg capacity, holding 25 larvae per jar, and provided with fresh caster leaves daily. After the second moult, the number of larvae was reduced to 15 larvae per jar, and plenty of castor leaves were provided to prevent cannibalism. When the larvae reached the sixth instar, 10 of them were transferred to a clean jar with a layer of moistened sawdust that was 4 cm thick, where they were left to pupate. Both male and female full-grown pupae were moved into spherical cages covered in gauze. Moths were fed on 10% sucrose solutions. To provide suitable oviposition sites, a branch of the tafle plant *Nerium oleander* (L.) was placed inside each cage **(El-Defrawi *et al.* 1964).**

2.5. Bioassay tests:

S. littoralis larvae in their second and fourth instar were used for the bioassay tests. Different concentrations of each oil solution were created. For conventional oils were 10%, 5%, 2.5%, 1.25% and 0.625% (v/v), and nanoemulsions were 5%, 2.5%, 1.25%, 0.625% and 0.3% (v/v). The leaf dipping technique was used to evaluate the efficacy of plant oils and their prepared nanoemulsions of them against second and fourth instars larvae of the *S. litoralis*. Castor bean (*R. communist*) leaves were dipped in the aforementioned oil concentrations for 20 seconds before being allowed to air dry on filter papers at room temperature. Then, the treated leaves were placed at the bottom of glass jars (500 g) and covered with muslin. Untreated leaves used for control treatments. Ten larvae were placed in the jars with three replicates. The larvae were fed on treated leaves for 48h. Then, they were fed on fresh untreated leaves until the end of the experiment. Mortalities were recorded at 1, 2, 3, 4, 5 and 6 days and corrected based on the **Abbott formula (1925).** Concentrations mortality regression lines were corrected and plotted in form of log/probit relation and the LC_{50} values were calculated using the Ld-p line program according to **Finney (1971)**.

2.6. Determination of the effect of tested oils bulk and nanoemulsions on certain enzymatic activity:

The 4th instars larvae of *S. littoralis* were treated with LC_{50} values of tested compounds. For each concentration, 40 larvae were used, and they were transferred as described in the bioassay tests. The larvae were fed on treated leaves for 24h. Distilled water was used in control treatments.

2.6.1. Apparatus:

In a chilled glass Teflon tissue homogenizer (ST - 2 Mechanic-Preczyina, Poland), the fourth instar larvae of *S. littoralis* were homogenized for biochemical analysis. Supernatants were kept after homogenization

until they were needed for biochemical assays in a deep freezer at -20°C. The absorbance of colored substances or metabolic compounds was measured using a double-beam ultraviolet/visible spectrophotometer (Spectronic 1201, Milton Roy Co., USA).

2.6.2. Preparation of insects for analysis:

As stated by **Amin (1998)**, the insects were prepared. They were homogenized (50 mg/ml) in distilled water. In a refrigerated centrifuge, homogenates were centrifuged at 2 ºC for 15 min at 8000 rpm. The deposits were discarded, and the supernatants, also known as enzyme extract, can be kept for at least a week without significantly losing their activity when kept at temperatures below 0 °C.

2.6.3. Determination of acetylcholinesterase activity:

Acetylcholine bromide (AchBr) was used as the substrate as **Simpson *et al.* (1964)** described to measure AChE (acetylcholinesterase) activity. 200 µl of enzyme solution, 0.5 ml of pH-7.0 phosphate buffer, and 0.5 ml of AchBr (3 mM) made up the reaction mixture. For precisely 30 minutes, the test tubes were incubated at 37 ^{o}C. The test tubes received 1 ml of alkaline hydroxylamine, which was made up of an equal volume of 3.5 M NaOH and 2 M hydroxylamine chloride. Then 0.5 ml of HCl was added (1 part of concentrated HCl and 2 parts of ΔH_2O). After giving the mixture a vigorous shake, the mixture was left to stand for two minutes. Then, 0.5 ml of the ferric chloride solution (0.9 M Fecl3 in 0.1 M HCl) was added and thoroughly mixed. At 515 nm, the reduction in AChBr brought on by AChE hydrolysis was measured.

2.6.4. Determination of glutathione S-transferase (GST) activity:

By using the -SH group of glutathione, glutathione S- transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2, 4-dinitrobenzene (CDNB). The method of **Habig *et al.* (I974)** could be used to detect the conjugate, S-(2,4-dinitro-phenyl)-L-glutathione. The reaction mixture contained 200 µl of larval homogenate, 100 µl of GSH, and 1 ml of the potassium salt of phosphate buffer (pH 6.5). With the addition of 25 µl of the substrate CDNB solution, the reaction began. GSH and CDNB concentrations were changed to 5mM and 1mM, respectively. For five minutes, enzymes and reagents were incubated at 30 °C. With the exception of the enzyme, the increase in absorbance at 340 nm was measured against a blank. The nanomole substrate conjugated/min/larva was then determined using a molar extinction coefficient of 9.6 mM/cm.

2.6.5. Determination of nonspecific esterases activity:

Using α-naphthyl acetate or β-naphthyl acetate as the substrate in either case, beta esterases (-esterases) and alpha esterases (-esterases) were identified in accordance with **Van Asperen (I962)**. The reaction mixture contained 20 ml of larval homogenate and 5 ml of the substrate solution (3 10 4 M or-naphthyl acetate, 1% acetone, and 0.1 M phosphate buffer, pH 7). 1 ml of diazo blue color reagent (prepared by combining 2 parts of 1% diazo blue B and 5 parts of 5% sodium lauryl sulfate) was added after the mixture had been incubated at 27 °C for exactly 15 minutes. The developed color was read at 600 or 555 nm for α- and β-naphthol produced from the hydrolysis of the substrate, respectively. Standard curves for α-and β-naphthol were made by dissolving 20 mg of α- or β-naphthol in 100ml of phosphate buffer, pH7 (stock solution). Ten milliliters of stock solution were diluted up to 100ml by the buffer. Aliquots of diluted solution in the amounts of 0.1, 0.2, 0.4, 0.8, and 1.6 ml (equal to 2, 4,8,16, and 32 µg naphthol) were pipetted into test tubes and phosphate buffer was used to complete them to 5 ml. Following the addition of one milliliter of diazo blue reagent, the developed color was assessed as before.

2.7. Statistical analysis:

Probit analysis was used to determine the dosage mortality response by **Finney (1952)**. Using SAS (Statistic Analysis Software) **SAS (1999)**, all data were statistically analyzed as means and standard errors. One-way analysis of variance (Danken) ($P \leq 0.05$) was used to determine the statistical significance of differences between the various study groups. Duncan's multiple range tests were used to differentiate between means (to determine differences between means of treatments at significance rates of ($P \leq 0.05$). According to **Finney (1971)**, probit analysis was used to calculate the LC_{25}, LC_{50}, and LC_{90}.

3. Results and Discussion

3.1. Characterization of the prepared nano-formulations:

3.1.1. Electron microscopy examination:

Nano-samples of the tested oils were examined by TEM to study the morphological shapes and sizes of the prepared nano-formulations. Fig. (1)

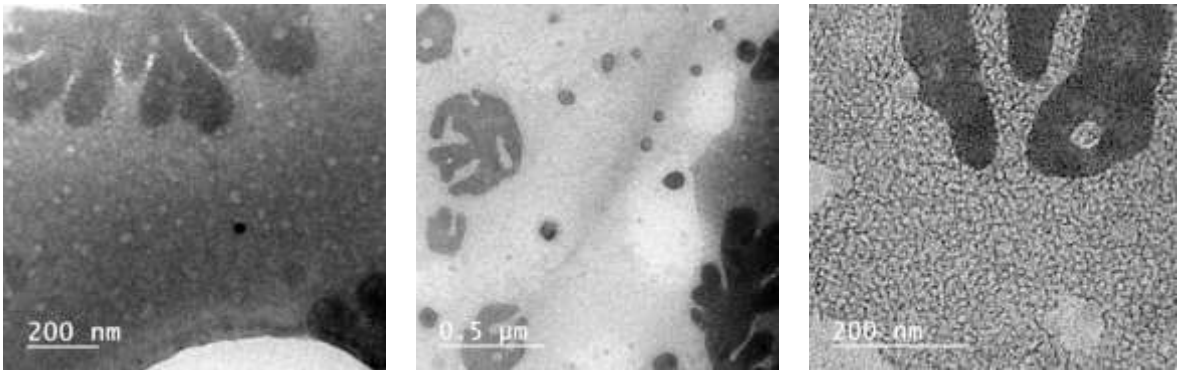

Fig. (1) (A) Images of the prepared mustard nanoemulsion from transmission electron microscopy.

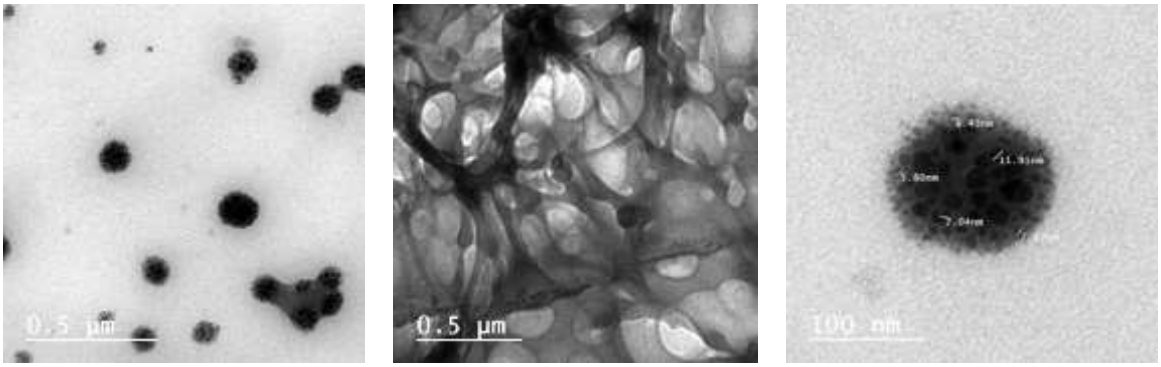

Fig. (1) (B) Images of the prepared citronella nanoemulsion from transmission electron microscopy.

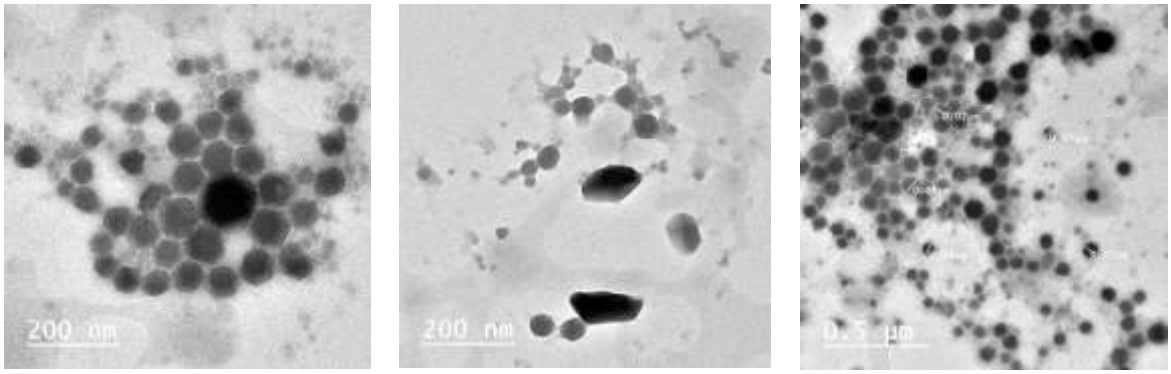

Fig. (1) (C) Images of the prepared sage nanoemulsion from transmission electron microscopy.

TEM was used to determine the particle size and shape **(Sanghi and Verma, 2009).** The average particle size for the mustard nanoemulsion was 40 nm, with the mean particle sizes ranging from 30 to 60 nm **Fig. 1. (A)**, the mean particle sizes for citronella nanoemulsion ranged between 50 and 100 nm with an average size of 50 nm **Fig. 1. (B)**. While the mean particle sizes for sage nanoemulsion ranged between 100 and 200 nm with an average size of 100 nm **Fig. 1. (C)**.

3.2. Efficiency of tested oils bulk and their nano-emulsions against 2nd and 4th instar larvae of *S. littoralis*:

The findings regarding the impact of mustard oil in various forms bulk and nano-emulsions against *S. littoralis* 2nd instar larvae are listed in **(Table 1)**. This comparison was intended to see the effectiveness of mustard oil compared to citronella oil and sage oil against 2nd instar *S. littoralis* larvae mortality. The mortality rate of *S. littoralis* was 96.67, 86.67, 80, 70 and 63.33% at concentrations of 10%, 5%, 2.5%, and 0.625%, respectively, after 6 days of exposure time for mustard oil, proving that oil was more effective in controlling *S. littoralis*. Whereas the mortality values for citronella oil were 80, 73033, 60, 50 and 40%, followed by sage oil caused mortality percentages of 66.67, 63.33, 56.67, 50 and 46.67% at concentrations of 10%, 5%, 2.5% and 0.625%, respectively, after 6 days of exposure. **Al-Keridis (2020)** reported that purslane oil (bulk and nano) has been shown to have the highest effectiveness of the oils tested against *Spodoptera littoralis* larvae, followed by mustard oil. At

concentrations of 3, 1.5, 0.5 and 0.05% in purslane oil (bulk), the mortality rates of larvae were 75.3, 55.5, 37.11 and 10.7%, respectively. However, mortality rates of the larvae treated with nano-purslane at various concentrations were recorded as 97.2, 78.9, 67.13 and 25.2%, respectively, for concentrations of 1.0, 0.5, 0.05 and 0.005%. In bulk mustard oil, the mortality rates were 45.77, 33.26, 27.8, and 16.33% at concentrations 3, 1.5, 0.5 and 0.05 %, respectively. However, at concentrations of 1.0, 0.5, 0.05 and 0.005%, the mortality rates of the larvae treated with nano mustard were 82.70, 70.1, 34.9, and 19.30, respectively.

Table (1) Efficiency of tested bulk oils against 2nd instar larvae S. littoralis

Bulk oils	Conc.% (v/v)	Accumulative Mortality % after Indicated Days					
		1	2	3	4	5	6
	10	36. 67	43. 33	50	60	70	96. 67
	5	30	36. 67	46. 67	53. 33	66. 67	86. 67
Mustard	2.5	23. 33	30	40	50	60	80
	1.25	20	26. 67	36. 67	46. 67	53. 33	70
	0.625	10	23. 33	30	43. 33	46. 67	63. 33
	10	6. 67	13. 33	20	40	60	80
	5	3. 33	6.67	13. 33	26. 67	50	73.33
Citronella	2.5	3. 33	3.33	10	16. 67	36.67	60
	1.25	0	3.33	6. 67	13. 33	23.33	50
	0.625	0	0	3. 33	6. 67	16.67	40
	10	13. 33	20	33. 33	43. 33	50	66.67
	5	10	16.67	23. 33	33. 33	46.67	63.33
Sage	2.5	6.667	13.33	20	30	43.33	56.67
	1.25	3.33	6.67	16. 67	26. 67	36.67	50
	0.625	0	3.33	13. 33	20	33.33	46.67

Table (2) Efficiency of nanoemulsions of tested oils against 2nd instar larvae of S. littoralis

Nanoemulsion oils	Conc.% (v/v)	Accumulative Mortality % after Indicated Days					
		1	2	3	4	5	6
	5	43. 33	50	73. 33	96. 67	100	100
	2.5	36. 67	40	66. 67	83. 33	93.33	100
Mustard	1.25	33. 33	40	60	70	76.67	93.33
	0.625	30	36.67	56. 67	66. 67	73.33	83.33
	0. 3	20	30	46. 67	63. 33	66.67	80
	5	10	20	26. 67	66. 67	80	90
	2.5	6. 67	16.67	23. 33	60	63.33	86.67
Citronella	1.25	3. 33	13. 33	20	56. 67	60	76.67
	0.625	0	6.67	13. 33	36. 67	43.33	70
	0. 3	0	3.33	10	26. 67	40	56.67
	5	16. 67	23.33	43. 33	53. 33	76.67	100
	2.5	13. 33	20	26. 67	43. 33	66.67	93.33
Sage	1.25	10	16.67	23. 33	40	56.67	73.33
	0.625	6.67	13.33	20	36. 67	50	70
	0. 3	3. 33	10	16. 67	33. 33	46.67	63.33

The obtained results in **Table (2)** summarized the toxicity of nanoemulsion forms of mustard, citronella and sage at different concentrations against the 2nd larval instar of *S. littorals*. The percentage of larval mortality correlated positively with the tested materials' concentrations and the length of time after exposure, according to the data. The mortalities increased as concentrations and time after treatment increased. The maximum concentration of 5% resulted in the highest cumulative larval mortalities of mustard nanoemulsion produced 100% larval mortalities after 5 days of exposure, followed by 100% of sage nanoemulsion, and 90% of citronella nanoemulsion after 6 days of treatment. These findings concur with those of **Abd El-Zaher (2017)** who claimed that jojoba oil in the form of Nano-proved that it comes in the first category, recording 100% mortality at 5% and 2.5% concentration, and the minimum mortality was 86.6% at 0.625% concentration after 7 days of treatment. Approximately Flax oil in Nano form caused the same mortality. The data consistently showed that the nano form of garlic oil came in third position and finally ginger oil came in last and that it caused between 81.1 and 33.3% mortality at various concentrations.

Table (3) Efficiency of tested bulk oils against 4th instar larvae of S. littoralis

Bulk oils	Conc.% (v/v)	Accumulative Mortality % after Indicated Days					
		1	2	3	4	5	6
	10	16. 67	33. 33	56. 67	63. 33	83. 33	96. 67
	5	13. 33	23. 33	36. 67	46. 67	63. 33	76. 67
Mustard	2.5	10	16. 67	23. 33	36. 67	50	70
	1.25	6. 67	13. 33	20	26. 67	46. 67	60
	0.625	3. 33	10	13. 33	20	40	56. 67
	10	10	20	30	36. 67	46.67	76.67
	5	6.67	13.33	16. 67	20	26.67	60
Citronella	2.5	3.33	10	13. 33	16. 67	20	53.33
	1.25	0	6.67	10	16. 67	16.67	46.67
	0.625	0	3.33	6. 67	13. 33	13.33	36.67
	10	13. 33	16.67	20	36. 67	46.67	76.67
	5	10	13.33	13. 33	23. 33	36.67	70
Sage	2.5	3. 33	6.67	10	16. 67	33. 33	66.67
	1.25	0	6.67	10	16. 67	30	56.67
	0.625	0	3.33	6.667	10	26.67	53.33

Data in **Table (3**) revealed that after 6 days of exposure against *S. littoralis* 4nd instar larvae, citronella and sage oils caused a mortality percentage of 76.67%, while mustard oil at its highest tested concentration of 10% caused the highest mortality rate of 96.67%. The other concentrations, 5, 2.5, 1.25 and 0.625%, had respective mustard oil readings of 76.67, 70, 60 and 53.33%. Citronella oil was tested at 60, 53.33, 46.67, and 36.67%, and sage oil at 70, 66.67, 56.67, and 53.33%, respectively. According to **Marouf *et al.* (2021)**, who assessed the toxic effects of compher essential oil and its nanoemulsion against *Spodoptera littoralis*, the camphor oil nanoemulsion is the most effective substance compared to the essential oil, with LC_{50} values of 88.67 ppm and 1699.85 ppm for the camphor nanoemulsion and camphor oil, respectively. This outcome was consistent with the findings.

According to information in **Table (4)**, mortality rates for fourth instar larvae of *S. littoralis* were studied for 6 days after the application. The percentages of larvae that died after being exposed to mustard nanoemulsion were 100, 86.67, 80, 70 and 63.33%, respectively, at concentrations of 5, 2.5, 1, 25, 0.625, and 0.3%. On the other hand citronella nano emulsion caused larvae mortality recorded at 83.33, 73.33, 70, 63.33 and 43.33%, followed by sage nanoemulsion which recorded 76.67, 73.33, 66.67, 56.67 and 53.33% at concentrations 5, 2.5, 1.25, 0.625 and 0.3%, respectively. **Kasmara *et al* (2018)** compared the acute toxicity test of *Lantana. camara* crude extract with *L. camara* nano extract with a concentration of 0 ppm (control), 2,500 ppm, 5,000 ppm, 10,000 ppm, and 20,000 ppm. The purpose of this comparison was to evaluate how well *L. camara* nano extract and crude extract performed in terms of reducing third-instar *S. litura* larvae mortality. Nano extracts from L. camara were found to be more effective at controlling *S. litura*; the mortality rate of *S. litura* was found to be 3–10% higher in 24 hours and 13-26% higher in 48 hours. Nano extracts had a mortality rate of 20% in a 24 h treatment at the lowest concentration of 2,500 ppm (0.4%), which was higher than the result of 16.7% for crude extracts. As a result, at 48 hours, nano extract performs better than crude extract in terms of causing 80% mortality and effectively controlling 66.7% mortality in *S. litura* 3rd instar larvae.

Table (4) Efficiency of nanoemulsions of tested oils against 4th instar larvae of *S. littoralis*

Oil's nanoemulsions	Conc.% (v/v)	Accumulative Mortality % after Indicated Days					
		1	2	3	4	5	6
	5	20	36.67	60	70	86.67	100
	2.5	16. 67	26.67	46. 67	56. 67	73.33	86.67
Mustard	1.25	13. 33	23.33	33. 33	43. 33	63.33	80
	0.625	10	16.67	23. 33	33. 33	56.67	70
	0. 3	6. 67	13.33	20	30	46.67	63.33
	5	6. 67	13.33	33. 33	46. 67	63.33	83.33
	2.5	3. 33	6.67	26. 67	40	46.67	73.33
Citronella	1.25	0	3.33	23. 33	36. 67	43.33	70
	0.625	0	3.33	16. 67	33. 33	40	63.33
	0. 3	0	0	6. 67	23. 33	30	43.33
	5	20	40	50	63. 33	70	76.67
	2.5	10	30	43. 33	60	63.33	73.33
Sage	1.25	10	26.67	40	50	56.67	66.67
	0.625	6. 67	23.33	33. 33	46. 67	50	56.67
	0. 3	3. 33	20	30	40	46.67	53.33

Table (5) Lethal concentrations of tested oils bulk and nanoemulsions against 2nd instar larvae of Spodoptera littoralis

Oils		Day*	Lethal concentrations % (v/v) and their95% confidence limits			Slope ± SE
			LC_{25}	LC_{50}	LC_{90}	
Bulk	Mustard	6	0.040 (0.015 - 0.109)	0.231 (0.086 - 0.621)	6.358 (2.372 - 17.044)	0.894±0.218
	Citronella	6	0.337 (0.135 - 0.842)	1.898 (0.760 - 4.740)	50.633 (20.272 - 126.466)	0.899±0.203
	Sage	6	0.078 (0.013 - 0.471)	2.500 (0.415 - 15.073)	1811.321 (300.429 - 10920.674)	0.448±0.398
Nano-emulsion	Mustard	4	0.050 (0.020 - 0.124)	0.253 (0.102 - 0.627)	5.516 (2.225 - 13.674)	0.980±0.201
	Citronella	6	0.038 (0.013 - 0.111)	0.266 (0.091 - 0.777)	10.678 (3.656 - 31.189)	0.801±0.237
	Sage	5	0.087 (0.025 - 0.305)	0.962 (0.274 - 3.370)	92.416 (26.376 - 323.806)	0.648±0.278

***: days after treatment**

The LC_{25}, LC_{50} and LC_{90} values for mustard, citronella, and sage oil bulk and nanoemulsions against the second instar larvae of *S. littoalis* were shown in **Table (5)**. The results showed that the LC_{5o} values for mustard, citronella, and sage were 0.231, 1.898 and 2.500%, respectively. In contrast, for mustard citronella and sage nanoemulsions, they were 0.253, 0.266, and 0.962%, respectively. Based on the LC_{50} values in Table (5) showed that oils nanoemulsions were superior to their bulk formulations when used against *S. littoalis* second larvae. According to **Yousef *et al.* (2018)**, who estimated the effect of peppermint oil in different formulation forms, i.e. bulk, nanoemulsions, and loaded nano-emulsion against 2nd and 4th instar larvae of *S. littoralis*, it was found that bulk and loaded nano-emulsions performed best in nano-emulsion form. For 2nd instar larvae, the LC_{50} values for bulk oil were 70.59 ppm, 12.23 ppm for nanoemulsion and 21.72 ppm for loaded nanoemulsion. The LC_{90} values for loaded nanoemulsion, nanoemulsion, and bulk oil were 30.14 ppm, 33.97 ppm and 104.75 ppm, respectively.

Table (6) Lethal concentrations of tested oils bulk and nanoemulsions against 4th instar larvae of *Spodoptera littoralis*

Oils		Day*	Lethal concentrations % (v/v) and their95% confidence limits			Slope ± SE
			LC_{25}	LC_{50}	LC_{90}	
Bulk	Mustard	6	0.180 (0.077 - 0.423)	0.889 (0.378 - 2.091)	18.483 (7.858 - 43.475)	0.998±0.190
	Citronella	6	0.720 (0.317- 1.637)	3.358 (1.478- 7.630)	62.557 (27.528-142.161)	0.809±0.224
	Sage	6	0.049 (0.010 - 0.238)	0.995 (0.207 - 4.794)	299.294 (62.127 - 1441.850)	0.517±0.348
Nano-emulsion	Mustard	5	0.107 (0.041 - 0.281)	0.657 (0.251 - 1.720)	20.519 (7.835 - 53.739)	0.860±0.213
	Citronella	6	0.080 (0.028 - 0.227)	0.572 (0.201 - 1.628)	24.099 (8.467 - 68.589)	0.790±0.232
	Sage	6	0.023 (0.005 - 0.114)	0.488 (0.099 - 2.397)	157.742 (32.097 - 775.240)	0.511±0.353

***: days after treatment**

The toxicity values of the tested oils based on LC_{50} are given in **Table (6)** against the 4th instar larvae of *S. littoralis*. According to the data, mustard oil caused the highest level of current mortality, whereas citronella oil had the lowest level of effectiveness. For bulk mustard, citronella, and sage, the LC_{50} values were 0.889, 3.358, and 0.995%, respectively. However, the LC_{50} values for the nanoemulsions of mustard, citronella, and sage were 0.657, 0.572, and 0.488, respectively. These findings concur with those of **Dimetry *et al.* (2019)**, who examined the toxicity of four essential oils, peppermint, thyme, camphor, and sage oil against *Agrotis ipsilon* larvae in their fourth instar. The outcomes revealed that peppermint essential oil was the most efficient oil and thymus oil was the least efficient oil. After 96 hours post-treatment, the LC_{50} values for peppermint, camphor, sage, and thyme were 0.45%, 0.60%, 0.73%, and 0.86%, respectively. **Elhadek *et al.* (2015)** investigated the toxicity of four natural source essential oils, including *Trigonella foenum graecum*, *Sesamum indicum*, *Eucalyptus camaldulensis*, and *Nigella sativa*, on *Spodoptera littoralis* 4th larval instars and calculated the LC_{25}, LC_{50}, and LC_{90} values of the tested plant essential oils. The results showed that the LC_{50} values for *T. foenum graecum*, *S. indicum*, *E. camaldulensis*, and *N. sativa* were 8.69, 10.24, 11.79, and 24.46 ppm, respectively.

3.1.4. Effect of tested oil bulk and nanoemulsions on biochemical aspects of *Spodoptera littoralis*:

Based on the LC_{50} values of the bulk oil and its nanoemulsion, the data in **Table (7)** demonstrated the impact of mustard, citronella, and sage oils on the activities of AChE, GST and alpha esterases in the total homogenate of 4th instar *S. littoralis* larvae after the treated larvae were exposed for 24 hours. The findings showed that the bulk and nanoemulsions of mustard and citronella significantly increased the stimulation of AChE activity, whereas the bulk and nanoemulsions of sage oil did not significantly differ from the control. For bulk mustard, citronella, and sage, the values of AChE were 71.67, 102.33 and 46.33 ug AChBr/min/g.b.wt, respectively. The AChE values were 116.0, 110.33 and 40.33 ug AChBr/min/g.b.wt for mustard, citronella, and sage nanoemulsions, respectively. However, the GST enzyme levels significantly decreased for mustard, citronella, and mustard nanoemulsions (5.97, 9.37 and 11.80 mmol sub. Conj./min/g.b.wt, respectively), while they significantly increased for sage bulk oil (15.47), mustard, citronella and sage nanoemulsions (11.80, 26.50 and 16.0 mmol sub. Conj./min/g.b.wt, respectively). With the exception of mustard nanoemulsion, all treatments resulted in a significant decline in alpha esterase enzyme activities when compared to the control. **Abdel-Aziz *et al.* (2013)** demonstrated the effect of thyme, bitter, and neem oils on chitinase and protease activities in the total homogenate of 6th instar larvae of *S. littoralis* resulted from the treated when treated as 4th instar for 48 hrs with LC_{50} values of the three tested oils. The results showed that all treatments significantly increased α-esterase activity, which was significantly higher in the bitter and neem treatments (61.16% and 60.75%, respectively), and lower in the thyme treatment (26.86%, compared to the control). Thyme, bitter, and neem oils, when compared to controls, increased the activity ratio of -esterase by 1.27, 1.61, and 1.61 times, respectively. Similarly, all treatments showed highly significant stimulation of α-esterase activity, with bitter being the most effective (120.7%), followed by neem (97.27%), and thyme showing the least stimulation (52.14%). In a similar pattern, when treated with thyme, bitter, or neem oils instead of control, the activity ratio of esterase was increased by 1.52, 2.21, and 1.97 times, respectively. **Fergani *et al.* (2020)** said that when exposed to LC_{50} concentrations of crane's-bill, basil, dill, citronella, and clove oils, the AChE activity in 3rd instar larvae of *S. littoralis* significantly increased ($P \leq 0.05$) in comparison to control larvae. Crane's-bill oil treatment led to the greatest (1.79 fold) change in enzyme activity. In contrast, exposure to cinnamon oil significantly ($P \leq 0.05$) reduced the activity of AChE. Contrarily, basil oil exposure resulted in a significant increase (1.37 fold) in AChE activity in the 5th instar larvae, while treatment with clove, citronella, crane's-bill, cinnamon, and dill oils resulted in a significant decrease in the enzyme's activity when compared to the control larvae.

Table (7) Effect of tested plant essential oils on the certain enzymatic activity of *Spodoptera littoralis*

Tested oils		Enzymatic activity (Mean ± SD) AChE (ug AchBr/min/g.b.wt)	GST (mmol sub. Conj./min/g.b.wt)	Alpha esterases (ug α-naphthol/min/g.b.wt)
Bulk	Mustard	71.67^{b} ± 7.37	5.97^{e} ± 1.19	920.0^{bc} ±20.0
	Citronella	102.33^{a} ± 2.52	9.37^{d} ± 1.58	620.67^{f} ± 26.10
	Sage	46.33^{c} ±8.14	15.47^{b} ± 2.24	904.67^{c} ±16.04
Nanoemulsions	Mustard	116.0^{a} ± 11.53	11.80^{cd} ± 0.72	1005.0^{a} ±13.23
	Citronella	110.33^{a} ± 8.96	26.50^{a} ± 3.50	721.0^{e} ±26.66
	Sage	40.33^{c} ± 6.81	16.0^{b} ± 1.0	838.67^{d} ±26.76
Control		43.67^{c} ± 3.21	13.10^{bc} ± 0.90	954.0^{b} ±44.14

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