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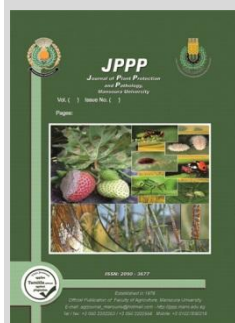
### Toxicological and Biological Effect of some Oils and their Nano-Emulsions on *Spodoptera littoralis*

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#### ABSTRACT

The cotton leafworm, *Spodoptera littoralis*, is a significant agricultural pest responsible for considerable damage to a wide range of crops. Consequently, there is a pressing need for alternative control strategies that are safer than conventional chemical insecticides. Nanoemulsions derived from certain plant oils exhibit broad-spectrum biological activities and possess low toxicity, making them promising candidates for use in integrated pest management programs. In the present study, we successfully bio-fabricated some plant extracts using their oils. This study evaluates, for the bioactivity of *Cymbopogon citratus*, *Cinnamomum zeylanicum* and *Capsicum annum* oils and their nano-emulsion against 2<sup>nd</sup> instar larvae of *S. littoralis*. We evaluated their toxicological and biological effects across various developmental stages of the insect including the increase in larval and pupal mortality when treated with nano emulsion than its oil, reduced larval and pupal weight, lower adult emergence, decreased fecundity. The three oils gave high mortality rates and low values of LC<sub>50</sub> and LC<sub>90</sub> for the larvae. Cytotoxicity evaluations revealed no harmful effects on honeybee populations, indicating their potential safety for non-target beneficial insects. These findings suggest *C. citratus*, *C. zeylanicum* and *C. annum* nano-emulsion as potential and environmentally safe alternatives to conventional chemical insecticides

**Keywords :-** nanoemulsion, *Cymbopogon citratus*, *Cinnamomum zeylanicum*, *Capsicum annum*.

#### INTRODUCTION

*Spodoptera littoralis*, a member of the order Lepidoptera, is a highly polyphagous insect capable of inflicting substantial damage to a wide range of crops, including potatoes. The larval stages are particularly voracious feeders, consuming large amounts of foliage. This feeding behavior often results in severe defoliation of potato plants, ultimately leading to reduced crop yields. Ghoneim *et al.*, 2020. The widespread use of pesticides for insect pest management has contributed to the development of resistance in many species, thereby diminishing the efficacy of conventional control measures and prompting the need for higher application rates or alternative chemical agents. Abro *et al.*, 2003. The extensive use of pesticides in insect pest management has led to the emergence of resistance in numerous pest species, thereby reducing the effectiveness of traditional control strategies and necessitating the application of higher doses or the adoption of alternative chemical compounds. Le Goff and Giraudo, 2019. Biopesticides, derived from natural sources such as plants, are environmentally friendly, exhibit lower toxicity, and are less likely to induce resistance in insect pests and pathogens. Aioub *et al.*, 2024.

Lemongrass (*Cymbopogon citratus* L.) is a globally recognized medicinal herb known for its rich content of bioactive compounds, particularly terpenes. Traditionally used in natural medicine for the treatment of various ailments, it contains a diverse array of biologically active constituents, including citral, isogeraniol, geraniol, geranyl acetate, citronellal, citronellol, germacrene-D, and elemol. (Mukarram *et al.* 2021). The essential oil of cinnamon (*Cinnamomum* spp.)

has been widely used in the food industry, medical purposes as have shown beneficial health effects, such as anti-inflammatory action, glucose control in the blood but also, used as larvicidal factors against various pest (Volpato *et al.*, 2016). The exploration of eco-friendly alternatives as chili pepper as a potential pesticide property of capsaicin and other capsaicinoids in the form of capsicum have recently been a promising area to study. Recent studies revealed that it is effective as insecticide because of its synergistic effects against numerous insects (Alarcon *et al.*, 2024).

Nanotechnology presents promising opportunities to enhance the efficacy and longevity of active compounds, reduce agricultural inputs, and overcome limitations associated with conventional pesticides (Kumar, 2019; Jasrotia *et al.*, 2022). The development of nanotechnology-based formulations can improve properties such as absorptivity, clarity, stability, and biological activity due to the increased surface area conferred by the reduced particle size (McClements, 2012). In particular, essential oil-based nanoemulsions are considered environmentally friendly and advantageous for modern agricultural practices and pest management (Mossa *et al.*, 2019). These nanoemulsions are typically water-based, thereby requiring significantly fewer organic solvents compared to traditional emulsion concentrates (Gupta *et al.*, 2016). Furthermore, nanoemulsions have been shown to enhance the stability of bioactive compounds, reduce their volatility, and mitigate potential environmental impacts (Mushtaq *et al.*, 2023). Despite these advantages, the application of nano-biopesticides in pest management remains limited, and comprehensive information regarding their synthesis,

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variability, efficacy, and mechanisms of action is still insufficient (Baliyarsingh & Pradhan, 2023). Therefore, optimizing the use of *Cymbopogon citratus*, *Cinnamomum zeylanicum*, and *Capsicum annuum* as botanical insecticides against *Spodoptera littoralis* through nanoemulsion-based technologies is a promising approach. This study hypothesizes that the tested plant oils—particularly *C. annuum* and their nanoemulsions—can effectively manage *S. littoralis* populations while reducing environmental risks associated with conventional insecticide use.

## MATERIALS AND METHODS

### Rearing technique of *Spodoptera littoralis*:

The *Spodoptera littoralis* laboratory strain used in this study was obtained from the Leaf Worm Research Department, Plant Protection Research Institute, Sharkia branch. The colony had been reared for multiple generations without exposure to insecticides, using castor bean leaves (*Ricinus communis* L.) as a food source, which were supplied daily until pupation. Rearing was conducted under controlled conditions in an incubator maintained at  $26 \pm 1^\circ\text{C}$  and  $70 \pm 5\%$  relative humidity (RH), following the protocol described by El-Defrawi *et al.* (1964). Pupae were individually transferred to clean tubes and incubated under the same conditions until adult emergence. Emerged adults were sexed and placed in 500 mL glass jars containing *Nerium oleander* L. (Tafla) leaves to facilitate oviposition. The adults were provided with a 10% sugar solution, which was replaced daily. Collected eggs were stored in similar jars and incubated under the same environmental conditions until hatching. Newly hatched larvae were used immediately in the subsequent experiments

### 2- Preparation of Nano-emulsions of fixed and volatile oil

The oil-in-water nano-emulsions experiments were carried out in the laboratory of the National Research Center. Tween 80, alcohol 70%, the fixed & volatile oils also, distilled water were used to create nano-emulsions. Twenty mL of *Cymbopogon citratus* oil, *Cinnamomum zeylanicum* and *Capsicum annuum* were added individually to 5mL Tween 80, 5mL alcohol 70% and 70 mL of water to reach the final 100 mL of the mixture and swirled with a magnetic stirrer for 15 minutes. After that ice bath was used while using sonicator. Finally, 25 minutes of ultrasonic emulsification at a 20 kHz sonicator with a 750 W power output according to EL-Medany, *et al.* (2022).

### 3- Toxic effect of the three oil and their nano emulsion on *S. littoralis* larvae

To assess the toxicological effects of *Cymbopogon citratus*, *Cinnamomum zeylanicum*, and *Capsicum annuum* oils and their respective nanoemulsions against second instar larvae of *Spodoptera littoralis*, 1 mL of each formulation was prepared at concentrations of 50, 100, 150, and 200 ppm. Each solution was uniformly applied to the surface of castor bean leaves (*Ricinus communis* L.). As controls, 1 mL of distilled water and 1 mL of a fungal metabolite were used separately. Twenty-five larvae were carefully transferred using a fine brush onto treated leaves, with four replicates per treatment, including the controls. All experimental units were maintained at  $27^\circ\text{C}$  for 24 hours under controlled conditions. After the exposure period, larval mortality was recorded, and lethal concentration values ( $\text{LC}_{50}$  and  $\text{LC}_{90}$ ) were subsequently calculated according to the method described by

Finney, 1971. Toxicity index (T.I) and relative toxicity were determined according to Sun, 1950. The relative potency (R.P.) values were measured according to Zidan and Abdel-Megeed, 1988.

To evaluate the biological activity of the three essential oils (*Cymbopogon citratus*, *Cinnamomum zeylanicum*, and *Capsicum annuum*) and their nanoemulsions against *Spodoptera littoralis*, second instar larvae were exposed to treated foliage. Castor bean leaves (*Ricinus communis* L.) were immersed in the nanoemulsion solutions for 10 seconds, air-dried, and subsequently placed in 500 mL glass jars. Twenty-five second instar larvae were introduced into each jar. Four replicates were conducted for each treatment and control. Leaves dipped in distilled water served as the negative control. All jars were covered with fine cloth and maintained under the previously described controlled conditions. Larvae were allowed to feed on treated leaves for 24 hours, after which they were provided with fresh, untreated castor bean leaves.

Larvae were monitored daily to record key biological parameters, including larval duration, larval mortality, and pupation percentage. Resulting pupae were transferred to clean jars and incubated under the same environmental conditions until adult emergence. Pupal duration and pupal mortality were also recorded. Emerged adults were sexed and grouped into pairs (three pairs per replicate), then transferred to 500 mL glass jars containing *Nerium oleander* (Tafla) leaves to facilitate oviposition. Adults were supplied with a 10% sugar solution, which was replaced daily. Four replicates were used for each treatment and control group.

### Statistical analysis

All data were statistically analyzed according to completely randomized design. The appropriate methods were used for the analysis of data according to Little & Hills (1975) and the proper “F” value was calculated as described by Fisher and Hills 1944 and Snedecor 1970

### Cytotoxic activity of the oils nanoemulsion

Honeybees (*Apis mellifera*) were selected as a model organism to evaluate the cytotoxic activity of Nano emulsions on beneficial insects. Worker bees were collected from the peripheral combs of a single colony headed by an open-mated F1 Carniolan queen, maintained at the educational and research apiary of the Plant Protection Institute, Egypt. Prior to treatment, bees were mildly anesthetized to facilitate handling. To assess the impact of nanoemulsions on bee survival, each plant oil nanoemulsion was individually mixed with a sucrose syrup solution (1:1, w/v) and offered to the bees in feeding cages (dimensions:  $9 \times 12 \times 20$  cm). Bees fed with sucrose syrup alone served as the control group. All experimental units were maintained under ambient room temperature ( $21\text{--}30^\circ\text{C}$ ) and 52–60% relative humidity. Mortality was recorded at 24- and 48 hours post-feeding. Mortality percentages were calculated using Abbott’s formula (Abbott, 1925) to account for natural mortality in the control group.

## RESULTS AND DISCUSSION

### Results

Data in Table (1) illustrated that  $\text{LC}_{50}$  recorded the highest values on 2nd larval instar of *S. littoralis* after 24 hours from treatment with *C. citratus*; at conc 13.65, but the conc  $\text{LC}_{90}$  was recorded at conc. 57.64. Also, the probability values were  $p = 0.0053$ .

**Table 1. Toxicity effect of Lemongrass oil *C. citratus* against *S. littoralis***

| Treatment                            | LC | Conc.%   | Slope $\pm$ SE       | Upper limit | Lower limit | Correlations | P-value | X <sup>2</sup> (Chi) |
|--------------------------------------|----|----------|----------------------|-------------|-------------|--------------|---------|----------------------|
| Lemongrass oil<br><i>C. citratus</i> | 25 | 6.3979   | 2.0488<br>+/- 0.1825 | 20.3087     | 9.5481      | 0.9507       | 0.0053  | 14                   |
|                                      | 50 | 13.6538  |                      | 58.6466     | 23.386      |              |         |                      |
|                                      | 75 | 29.1386  |                      | 25.71       | 16.32       |              |         |                      |
|                                      | 90 | 57.6483  |                      | 59.7533     | 28.6796     |              |         |                      |
|                                      | 95 | 86.7179  |                      | 164.5563    | 48.4824     |              |         |                      |
|                                      | 99 | 186.5054 |                      | 159.5501    | 1034.9062   |              |         |                      |

Data in Table (2) illustrated that LC50 recorded the highest values on 2nd larval instar of *S. littoralis* after 24 hours from treatment with *C. citratus* nano-emulsion; at conc 8.13, while LC90 recorded at conc. 34.9. Also, the probability values were  $p=0.0053$ .

Data in Table (3) illustrated that LC50 recorded the highest values on 2nd larval instar of *S. littoralis* after 24 hours from treatment with *C. zeylanicum*; at conc 21.75, while LC90 recorded at conc. 144.25. Also, the probability values were  $p=0.0038$ .

**Table 2. Toxicity effect of Lemongrass oil *Cymbopogon citratus* nano-emulsion against *S. littoralis***

| Treatment   | LC | Conc.%   | Slope $\pm$ SE    | Upper limit | Lower limit | Correlations | P-value | X <sup>2</sup> (Chi) |
|---|----|----------|-------------------|-------------|-------------|--------------|---------|----------------------|
| Lemongrass oil<br><i>C. citratus</i><br>nano-emulsion | 25 | 3.7784   | 2.0255 +/- 0.2208 | 4.4798      | 2.9698      | 0.9531       | 0.0604  | 9.0284               |
|   | 50 | 8.1342   |                   | 9.2102      | 7.2046      |              |         |                      |
|   | 75 | 17.5116  |                   | 22.5892     | 14.6509     |              |         |                      |
|   | 90 | 34.9187  |                   | 34.9187     | 26.2945     |              |         |                      |
|   | 95 | 52.7746  |                   | 89.9891     | 37.1213     |              |         |                      |
|   | 99 | 114.5095 |                   | 239.8315    | 70.621      |              |         |                      |

**Table 3. Toxicity effect of Cinnamon oil *C. zeylanicum* against *S. littoralis***

| Treatment                            | LC | Conc.%    | Slope $\pm$ SE       | Upper limit | Lower limit | Correlations | P-value | X <sup>2</sup> (Chi) |
|--------------------------------------|----|-----------|----------------------|-------------|-------------|--------------|---------|----------------------|
| Cinnamon oil<br><i>C. zeylanicum</i> | 25 | 8.0362    | 1.5598<br>+/- 0.1360 | 10.9978     | 3.409       | 0.9543       | 0.0038  | 15.4687              |
|                                      | 50 | 21.7511   |                      | 36.8962     | 13.3341     |              |         |                      |
|                                      | 75 | 58.8722   |                      | 147.4981    | 43.7688     |              |         |                      |
|                                      | 90 | 144.2542  |                      | 567.4148    | 115.4355    |              |         |                      |
|                                      | 95 | 246.6289  |                      | 1299.3934   | 201.6887    |              |         |                      |
|                                      | 99 | 6284.7474 |                      | 561.7953    | 674.3676    |              |         |                      |

Data in Table (4) illustrated that LC50 recorded the highest values on 2nd larval instar of *S. littoralis* after 24 hours from treatment with *C. zeylanicum* nano-emulsion at conc 18.06, while LC90 recorded at conc. 80.03. Also, the probability values were  $p=0.0854$ .

LC50 recorded the highest values on 2nd larval instar of *S. littoralis* after 24 hours from treatment with *C. annum* at conc. 9.51, while LC90 recorded at conc. 33.35. Also, the probability values were  $p=0.1045$ .

Data in Table (5) illustrated that LC50 recorded the highest values on 2nd larval instar of *S. littoralis* after 24 hours from treatment with *C. zeylanicum*; at conc 21.75, while LC90 recorded at conc. 144.25. Also, the probability values were  $p=0.0038$ .

Data in Table (6) illustrated that LC50 recorded the highest values on 2nd larval instar of *S. littoralis* after 24 hours from treatment with *C. annum* nano-emulsion at conc 9.51, while LC90 recorded at conc. 32.42. Also, the probability values were  $p=0.1188$ .

**Table 4. Toxicity effect of Cinnamon oil *Cinnamomum zeylanicum* nano-emulsion against *S. littoralis***

| Treatment   | LC | Conc.%   | Slope $\pm$ SE       | Upper limit | lower limit | Correlations | P-value | X <sup>2</sup> (Chi) |
|---|----|----------|----------------------|-------------|-------------|--------------|---------|----------------------|
| Cinnamon oil<br><i>C. zeylanicum</i><br>nano-emulsion | 25 | 9.7322   | 1.9825<br>+/- 0.1910 | 8.2535      | 6.6778      | 0.9731       | 0.0854  | 6.6095               |
|   | 50 | 18.0657  |                      | 18.0657     | 15.7252     |              |         |                      |
|   | 75 | 39.5434  |                      | 39.5434     | 32.4979     |              |         |                      |
|   | 90 | 80.0379  |                      | 80.0379     | 59.9096     |              |         |                      |
|   | 95 | 122.052  |                      | 122.052     | 85.9251     |              |         |                      |
|   | 99 | 269.3047 |                      | 269.3047    | 168.2738    |              |         |                      |

**Table 5. Toxicity effect of Chili oil *C. annum* against *S. littoralis***

| Treatment                    | LC | Conc.%  | Slope $\pm$ SE    | Upper limit | Lower limit | Correlations | P-value | X <sup>2</sup> (Chi) |
|------------------------------|----|---------|-------------------|-------------|-------------|--------------|---------|----------------------|
| Chili oil<br><i>C. annum</i> | 25 | 6.0051  | 2.3523 +/- 0.3696 | 6.0051      | 3.4671      | 0.9505       | 0.1045  | 4.5178               |
|                              | 50 | 9.5124  |                   | 10.7946     | 8.2951      |              |         |                      |
|                              | 75 | 18.4088 |                   | 25.078      | 15.356      |              |         |                      |
|                              | 90 | 33.3511 |                   | 58.1658     | 24.6116     |              |         |                      |
|                              | 95 | 47.5941 |                   | 96.8013     | 32.4464     |              |         |                      |
|                              | 99 | 92.7307 |                   | 252.5857    | 54.2829     |              |         |                      |

**Table 6. Toxicity effect of Chilli oil *C. annum* nano-emulsion against *S. littoralis***

| Treatment                                      | LC | Conc.%  | Slope $\pm$ SE    | Upper limit | Lower limit | Correlations | P-value | X <sup>2</sup> (Chi) |
|--|----|---------|-------------------|-------------|-------------|--------------|---------|----------------------|
| Chilli oil<br><i>C. annum</i><br>nano-emulsion | 25 | 5.0519  | 2.4228 +/- 0.3714 | 6.1169      | 3.6423      | 0.9554       | 0.1188  | 4.2611               |
|  | 50 | 9.5909  |                   | 10.848      | 8.4061      |              |         |                      |
|  | 75 | 18.2079 |                   | 24.4229     | 15.2823     |              |         |                      |
|  | 90 | 32.4221 |                   | 54.7771     | 24.2253     |              |         |                      |
|  | 95 | 45.7924 |                   | 89.3357     | 31.7323     |              |         |                      |
|  | 99 | 87.5069 |                   | 224.4125    | 52.4544     |              |         |                      |

At all, the previous data showed that, nano-emulsions have the greatest toxic effect on 2<sup>nd</sup> larval instar of *S. littoralis* than the oil in crude form. Also, *C. annum* has the highest toxic effect on larvae than the others

Data in table (7) show the effect of tested oils and their nano-emulsion on percentage of larval duration, mortality and weight. Analysis of variance revealed highly significant effects on larval mortality percentage for all treatments than the

control. *C. annuum* showed the highest larval mortality (72.47%). The larval durations have also highly significant effect between all oils and the control, *C. annuum* NE elongate the larval duration. On the other hand, there are non-significant effect on larval weight between the treatments and control.

**Table 7. Effect of some oils and their nano-emulsions on larval stage of *S. littoralis*.**

| Treatments              | Larval duration (days)       | Larval mortality %           | Larval weight (g)            |
|-------------------------|------------------------------|------------------------------|------------------------------|
| <i>C. citratus</i>      | 9.0033 <sup>b</sup> ±0.4211  | 61.62 <sup>c</sup> ±0.6123   | 0.1062 <sup>c</sup> ±0.0029  |
| <i>C. citratus</i> NE   | 9.0533 <sup>b</sup> ±0.0504  | 67.2566 <sup>b</sup> ±0.4378 | 0.1092 <sup>ab</sup> ±0.0040 |
| <i>C. zeylanicum</i>    | 8.6066 <sup>bc</sup> ±0.2653 | 47.0666 <sup>c</sup> ±0.8301 | 0.1081 <sup>ab</sup> ±0.0035 |
| <i>C. zeylanicum</i> NE | 8.5233 <sup>bc</sup> ±0.1934 | 51.25 <sup>d</sup> ±0.7158   | 0.1169 <sup>ab</sup> ±0.0077 |
| <i>C. annuum</i>        | 9.7833 <sup>a</sup> ±0.0868  | 60.7066 <sup>c</sup> ±0.2321 | 0.0982 <sup>c</sup> ±0.0136  |
| <i>C. annuum</i> NE     | 9.8533 <sup>a</sup> ±0.0938  | 72.4733 <sup>a</sup> ±0.5159 | 0.0995 <sup>c</sup> ±0.0013  |
| Control                 | 8.2233 <sup>c</sup> ±0.0896  | 0.00 <sup>f</sup> ±0.00      | 0.1265 <sup>a</sup> ±0.0040  |
| p-value                 | 0.0005                       | 0.0000                       | 0.0994                       |
| F test                  | ***                          | ***                          | ns                           |
| LSD <sub>0.05</sub>     | 0.6405                       | 1.6567                       | 0.0199                       |

Data in table (8) illustrated the highest pupal mortality by the effect of indicated that effects of *C. annuum* in nano-emulsion form recorded 18.85% showing highly significant effect between the treatments and control. The pupation

percent showed highly effect between control and other treatments. *C. annuum* showed the least pupation percent followed by *C. citratus* NE while the highest pupation recorded by *C. zeylanicum* (52.9333) compared to control 100%. There are also, highly significant effect on the pupal duration and weight between all treatments and control.

Data presented in Table 9 reveal that all treatments had a highly significant effect on adult emergence percentage compared to the control. The nanoemulsion of *Cymbopogon citratus* (*C. citratus* NE) resulted in the lowest adult emergence (73.41%) relative to the control (100%). In addition, significant differences were observed among all treatments in terms of deformed adult percentages, with all treatments causing notable deformities compared to the control group, which exhibited no malformations (0%).

Furthermore, the longevity of emerged females was significantly affected by all tested oils, both in their crude and nanoemulsion forms. These treatments also had a significant impact on the pre-ovipositional, ovipositional, and post-ovipositional periods. Regarding male longevity, all treatments also produced significant reductions when compared to the control group.

**Table 8. Effect of some oils and their nano-emulsions on pupal stage of *S. littoralis***

| Treatments              | Pupation %                   | Pupal duration (days)        | Pupal weight (g)             | Pupal mortality %            |
|-------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| <i>C. citratus</i>      | 38.38 <sup>d</sup> ±0.6123   | 7.55 <sup>ab</sup> ±0.11     | 0.265 <sup>cd</sup> ±0.0025  | 9.3 <sup>c</sup> ±0.0964     |
| <i>C. citratus</i> NE   | 32.7433 <sup>c</sup> ±0.4378 | 7.12 <sup>bc</sup> ±0.1053   | 0.2542 <sup>d</sup> ±0.0032  | 15.64 <sup>b</sup> ±0.6702   |
| <i>C. zeylanicum</i>    | 52.9333 <sup>b</sup> ±0.8301 | 7.5233 <sup>ab</sup> ±0.1245 | 0.2900 <sup>b</sup> ±0.0003  | 6.9266 <sup>e</sup> ±0.3819  |
| <i>C. zeylanicum</i> NE | 48.75 <sup>c</sup> ±0.7158   | 7.06 <sup>bc</sup> ±0.0321   | 0.2759 <sup>c</sup> ±0.0018  | 13.8066 <sup>c</sup> ±0.7940 |
| <i>C. annuum</i>        | 39.2933 <sup>d</sup> ±0.2321 | 7.1966 <sup>a</sup> ±0.3722  | 0.2156 <sup>e</sup> ±0.0028  | 10.97 <sup>d</sup> ±0.3153   |
| <i>C. annuum</i> NE     | 27.5266 <sup>e</sup> ±0.5159 | 7.95 <sup>a</sup> ±0.4623    | 0.20563 <sup>c</sup> ±0.0027 | 18.85 <sup>a</sup> ±0.3467   |
| Control                 | 100 <sup>a</sup> ±0          | 6.43 <sup>c</sup> ±0.3102    | 0.3301 <sup>a</sup> ±0.0087  | 0 <sup>e</sup> ±0            |
| p-value                 | 0.0000                       | 0.0056                       | 0.0000                       | 0.0000                       |
| F test                  | ***                          | **                           | ***                          | ***                          |
| LSD <sub>0.05</sub>     | 1.6567                       | 0.7661                       | 0.0121                       | 1.3827                       |

**Table 9. Effect of some oils and their nano-emulsions on mature stage of *S. littoralis***

| Treatments                      | Adult emergency %            | Deformed adult %             | Male longevity                | Female longevity             |                              |                              |                              |
|---------------------------------|------------------------------|------------------------------|-------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
|                                 |                              |                              |                               | Pre oviposition              | oviposition                  | post oviposition             | Total                        |
| <i>Cymbopogon citratus</i>      | 86.59 <sup>c</sup> ±0.1946   | 4.11 <sup>cd</sup> ±0.11     | 7.4366 <sup>bc</sup> ±0.0578  | 1.3033 <sup>ab</sup> ±0.0266 | 4.29 <sup>bcd</sup> ±0.0305  | 2.2866 <sup>ab</sup> ±0.0384 | 7.88 <sup>b</sup> ±0.0493    |
| <i>Cymbopogon citratus</i> NE   | 78.6433 <sup>c</sup> ±0.2127 | 5.7166 <sup>bc</sup> ±0.5569 | 7.2033 <sup>bcd</sup> ±0.1169 | 1.1633 <sup>bc</sup> ±0.0902 | 4.1966 <sup>cd</sup> ±0.0284 | 2.12 <sup>c</sup> ±0.0057    | 7.48 <sup>cd</sup> ±0.0901   |
| <i>Cinnamomum zeylanicum</i>    | 91.2966 <sup>b</sup> ±1.3714 | 1.7766 <sup>c</sup> ±1.1760  | 7.4433 <sup>b</sup> ±0.0578   | 1.38 <sup>a</sup> ±0.05      | 4.3333 <sup>ab</sup> ±0.1066 | 2.3133 <sup>a</sup> ±0.0088  | 8.0266 <sup>ab</sup> ±0.1297 |
| <i>Cinnamomum zeylanicum</i> NE | 83.3666 <sup>d</sup> ±1.244  | 2.8266 <sup>bc</sup> ±0.5889 | 7.09 <sup>d</sup> ±0.0251     | 1.3633 <sup>a</sup> ±0.0333  | 4.4733 <sup>ab</sup> ±0.0938 | 2.2933 <sup>ab</sup> ±0.0371 | 8.13 <sup>ab</sup> ±0.0907   |
| <i>Capsicum annuum</i>          | 82.8166 <sup>d</sup> ±0.2677 | 6.2133 <sup>ab</sup> ±0.1974 | 7.2266 <sup>bcd</sup> ±0.0548 | 1.1 <sup>c</sup> ±0.0472     | 4.25 <sup>cd</sup> ±0.0945   | 2.2 <sup>bc</sup> ±0.0404    | 7.55 <sup>c</sup> ±0.1274    |
| <i>Capsicum annuum</i> NE       | 73.4066 <sup>e</sup> ±0.5983 | 7.74333 <sup>a</sup> ±0.2566 | 7.1233 <sup>cd</sup> ±0.0611  | 1.0533 <sup>c</sup> ±0.0233  | 4.1066 <sup>d</sup> ±0.0392  | 2.1 <sup>c</sup> ±0.0208     | 7.26 <sup>d</sup> ±0.0435    |
| Control                         | 100 <sup>a</sup> ±0          | 0 <sup>f</sup> ±0            | 8.4 <sup>a</sup> ±0.2227      | 1.3066 <sup>ab</sup> ±0.0392 | 4.55 <sup>a</sup> ±0.0723    | 2.39 <sup>a</sup> ±0.0599    | 8.2466 <sup>a</sup> ±0.0491  |
| p-value                         | 0.0000                       | 0.0000                       | 0.0000                        | 0.0012                       | 0.0103                       | 0.0003                       | 0.0000                       |
| F test                          | ***                          | ***                          | ***                           | **                           | *                            | ***                          | ***                          |
| LSD <sub>0.05</sub>             | 2.2760                       | 1.6838                       | 0.3189                        | 0.1485                       | 0.2224                       | 0.1065                       | 0.2717                       |

Table 10 presents data on the number of eggs laid, hatchability percentage, and sex ratio. The results indicate that all treatments had a highly significant effect on these reproductive parameters relative to the control. In particular, the

percentage of female offspring (sex ratio), egg production, and hatchability were all significantly reduced. Overall, the findings suggest that *Acremonium* sp. was the most effective treatment across all evaluated biological and reproductive parameters.

**Table 10. Effect of some oils and their nano-emulsions on sex ratio, fecundity and fertility of *S. littoralis***

| Treatments              | Sex ratio %                    |                               | No. of eggs                     | Hatchability %                |
|-------------------------|--------------------------------|-------------------------------|---------------------------------|-------------------------------|
|                         | Female                         | Male                          |                                 |                               |
| <i>C. citratus</i>      | 46.3 <sup>d</sup> ±0.4425      | 53.7 <sup>ab</sup> ±1.3839    | 2105.25 <sup>b</sup> ±42.4347   | 85.8133 <sup>bc</sup> ±0.2391 |
| <i>C. citratus</i> NE   | 47.14 <sup>cde</sup> ±0.6423   | 52.86 <sup>abc</sup> ±0.6423  | 2031.9333 <sup>b</sup> ±26.0795 | 81.2066 <sup>de</sup> ±0.4787 |
| <i>C. zeylanicum</i>    | 52.1466 <sup>a</sup> ±1.4497   | 47.8533 <sup>c</sup> ±1.4497  | 1857.69 <sup>c</sup> ±36.1937   | 87.3666 <sup>b</sup> ±0.8849  |
| <i>C. zeylanicum</i> NE | 50.67 <sup>ab</sup> ±0.13      | 49.33 <sup>d</sup> ±1.4497    | 1746.69 <sup>c</sup> ±0.6042    | 82.8233 <sup>cd</sup> ±1.7723 |
| <i>C. annuum</i>        | 48.73 <sup>bcd</sup> ±0.8888   | 51.27 <sup>bcd</sup> ±0.8888  | 1545.2466 <sup>d</sup> ±54.6759 | 79.49 <sup>c</sup> ±0.6187    |
| <i>C. annuum</i> NE     | 44.62 <sup>c</sup> ±0.5488     | 55.38 <sup>a</sup> ±0.5488    | 1333.9133 <sup>c</sup> ±54.8070 | 75.4833 <sup>d</sup> ±1.2488  |
| Control                 | 49.7733 <sup>abc</sup> ±0.4425 | 50.2266 <sup>de</sup> ±0.4425 | 2327.2166 <sup>a</sup> ±31.9971 | 97.1533 <sup>a</sup> ±1.0198  |
| p-value                 | .0005                          | .0005                         | .0000                           | .0000                         |
| F test                  | ***                            | ***                           | ***                             | ***                           |
| LSD <sub>0.05</sub>     | 2.74510                        | 2.7451                        | 119.1876                        | 3.0748                        |

## Discussion

The use of synthetic pesticides for controlling *Spodoptera littoralis* has not only contributed to the development of resistance in this pest species (Aioub *et al.*, 2024), but also poses significant risks to ecosystems and non-target organisms (Elhamalawy *et al.*, 2024). Consequently, there has been a growing shift toward the use of plant-based insecticides as promising alternatives to synthetic chemicals. This shift is supported by the identification of naturally occurring phytochemicals as active compounds in botanical formulations, offering a more sustainable approach to insect pest management (Majeed, 2021; Ngegba *et al.*, 2022).

In the present study, we assessed the toxicological effects of three plant-derived oils—*Cymbopogon citratus*, *Cinnamomum zeylanicum*, and *Capsicum annuum*—and their nanoemulsions against various developmental stages of *S. littoralis*. The LC<sub>50</sub> values for *C. citratus* oil and its nanoemulsion against second instar larvae were 9.51% and 9.59%, respectively. These findings align with those of Eldesouky *et al.* (2019), who demonstrated that oleic and linoleic acids possess insecticidal properties against *S. littoralis* larvae. Similarly, Mishra *et al.* (2020) reported that  $\beta$ -sitosterol induced systemic toxicity and growth inhibition in *Helicoverpa armigera*, while Kannan *et al.* (2020) highlighted the potential of nanomaterials as effective, eco-friendly bioinsecticides.

Our findings indicate that the tested oils and their nanoemulsions significantly increased mortality rates across larval, pupal, and adult stages of *S. littoralis*. They also prolonged developmental periods, reduced larval and pupal weights, and decreased egg hatchability. In addition, both crude and nanoemulsified oils affected the longevity of adult moths, altered the sex ratio, and negatively influenced reproductive parameters. These results are consistent with prior studies, including that of Eldesouky *et al.* (2019), which reported significant reductions in pupal weight, pupation rate, adult emergence, fecundity, and longevity in *S. littoralis* treated with oleic and linoleic acids. Likewise, Mishra *et al.* (2020) noted higher prepupal and pupal mortality in *H. armigera* and a marked decrease in adult emergence and weight gain in later instars, underscoring the cumulative effects of  $\beta$ -sitosterol.

Furthermore, El-Medany *et al.* (2022) demonstrated that *Mentha pulegium* nanoemulsion increased larval and pupal mortality and deformities, as well as prolonged larval and pupal durations in *Earias insulana*. Collectively, these findings support the efficacy of the three tested oils and their nanoemulsions in controlling multiple developmental stages of *S. littoralis* and highlight their potential as environmentally friendly alternatives for integrated pest management.

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## التأثيرات السمية والبيولوجية لبعض الزيوت وصورتها النانوية على دودة ورق القطن

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### المخلص

تسبب دودة ورق القطن *Spodoptera littoralis* أضرارًا كبيرة للعديد من المحاصيل، مما يستدعي ضرورة إيجاد وسيلة جديدة وأمنة أكثر من المبيدات الحشرية لمكافحةها. تمتلك المستحلبات النانوية لبعض الزيوت النباتية ذات الأنشطة البيولوجية الواسعة، كما أن لها سمية منخفضة ويمكن استخدامها بأمان في إدارة الآفات. في هذه الدراسة، تم تصنيع مستحلبات نانوية لبعض المستخلصات النباتية باستخدام زيوتها بنجاح. تهدف هذه الدراسة إلى تقييم النشاط الحيوي لزيوت (*Cymbopogon citratus* عشبة الليمون)، (*Cinnamomum zeylanicum*) (القرفة)، و (*Capsicum annuum*) (الفلفل الحلو) ومستحلباتها النانوية ضد يرقات الطور الثاني من دودة ورق القطن. تم تقييم تأثيراتها السمية والحيوية عبر مراحل النمو المختلفة، وشملت النتائج زيادة في معدلات موت اليرقات والعذارى عند المعالجة بالمستحلب النانوي مقارنة بالزيت الخام، وانخفاضًا في أوزان اليرقات والعذارى، وتراجعًا في نسبة خروج الحشرات الكاملة، وانخفاضًا في الخصوبة. أظهرت الزيوت الثلاثة معدلات موت عالية وقيم منخفضة لـ LC50 و LC90 لليرقات. كما أشارت تجارب السمية الخلوية إلى أمان استخدامها على نحل العسل. تشير هذه النتائج إلى أن مستحلبات *C. annuum* و *C. zeylanicum* و *C. citratus* النانوية تُعد بديلًا آمنًا وفعالًا للمبيدات الحشرية الكيميائية.