Egyptian Journal of Plant
Protection Research Institute
www.ejppri.eg.net

Enhancement of the efficacy of Olibanum Nanoemulsion formulation on some biological aspects of the spiny bollworm *Earias insulana* (Lepidoptera: Noctuidae)

Noha, A. M. El-Habal[1]; Mona, Sh. Abdou [1]; Mervat, H. A. Metayi[1]; Mai, M. A. Gnedy[2]; Khaled, H. M. Abdel-Rheim1[4]; Ahmed, Abdel Megeed[3]; Nariman, A. Abdel Megid[4] and Shimaa, S. I. Abd El-Naby [2].

[1] *Plant Protection Research Institute, Agricultural Research Center, El-Sabahia, Alexandria, Egypt.*
[2] *Pesticide Formulation Research Department, Central Agriculture Pesticides Laboratory, Agricultural Research Center, Egypt.*
[3] *Department of Plant Protection, Faculty of Agriculture, (Saba Basha), Alexandria University, Egypt.*
[4] *Faculty of Agriculture, Alexandria University, Egypt.*

ARTICLE INFO
Article History
Received:16 /7 /2023
Accepted:24 /9/2023
Keywords

Spiny bollworm, *Earias insulana*, *Boswellia sacra*, adjuvant, enhance and efficacy.

Abstract

The cotton crop faces direct damage from the spiny bollworm, *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae). A new, safer strategy rather than insecticide is urgently needed for its control. Nanoemulsions based on plant essential oils have a wide range of applications in pesticides and have good insecticidal activity against a wide range of agricultural pests. The purpose of this work was to enhance the efficacy of a *Boswellia sacra* essential oil-based nanoemulsion (Olibanum NE) formulation with enhanced activity by employing an adjuvant. So, olibanum NE with and without Provecta® and Polyxyethylens glycol 600 di-oleate adjuvants olibanum NE were successfully prepared by a high-energy ultrasonication process method. Then, different parameters were determined for olibanum NE with and without adjuvants. Furthermore, study their effects on the different biological aspects under laboratory conditions. Results cleared that olibanum NE droplet size of 41.30 nm, the PDI was 0.26. While olibanum NE with 0.02 % of Polyxyethylens glycol 600- di-oleate was 62.54 nm and the PDI was 0.62. Also, olibanum NE with 0.02 % of Provecta® was 72.44 nm and the PDI was 0.22, and zeta potential increased when adjuvants were added to olibanum NE. Olibanum NE, with and without adjuvants, exhibited an acidic pH value. Both adjuvant-based olibanum NE reduced the surface tension and contact angle than olibanum NE alone. The Obtained data also indicated that olibanum NE, with and without adjuvants proved to be the most effective compound against the second instar larvae of *E. insulana*, and the LC_{50} values after 72 h were 600.07 µL and 723.44 µL, respectively. While olibanum NE alone was 959.38 µL. The larvae feeding of *E. insulana* on a treated artificial diet with LC_{50} of olibanum NE with adjuvant led to a complex symptom which proved as higher prolongation for larval and pupa durations than olibanum NE alone and control. And decrease the weight of treated larvae and pupation percentage compared to untreated larvae. Also, there were significant reductions in the number of deposited eggs, hatchability percentages, and fecundity of each female, which were developed after treating the larvae with olibanum NE with Provecta® and olibanum NE with PEG 600 di-oleate, compared with olibanum NE alone and the control. As a result, olibanum NE with adjuvant may be an effective alternative to conventional pesticide formulations and may be significant in the practical application of integrated pest management (IPM) and the production of organic cotton farming.

Introduction

One of Egypt's most important commercial crops, cotton, serves as a strategic commodity by generating cash for the country. The average annual cotton production in 2022 is around 320,000 bales of 478 pounds net, with a harvested cotton area of 97,000 hectares (Omar, 2022). Bollworms are the most destructive insect pests of cotton crops, and among these spiny bollworms, *Earias insulana* ((Boisduval)) (Lepidoptera: Noctuidae) (Khan *et al*., 2007). Infestation reduces cotton quality and quantity, whereas a single larva can damage several buds and bolls throughout its life. If the incidence of spiny bollworm infection increases by 1%, production is lowered by 2.5 to 6% (Al-Juboori, 2000 and Mansour *et al*., 1990).

Chemical control plays a major role in pest management programs in both developing and industrialized countries. Cotton uses more pesticides than any other crop; it is estimated that 25% of the global use of pesticides and 10% of pesticide use are due to the cultivation of cotton (Ahmad and Hasanuzzaman, 2020). Many of today's insecticides are organic chemicals with low water solubility (Less than 1%). These include pyrethroids, neonicotinoids, polychlorinated hydrocarbons, carbamates, organophosphates, antraniliproles, benzoyl urea derivatives, and others. As a result, massive amounts of organic solvents are often needed to dissolve these products to achieve an effective and uniform application in this area. The random use of conventional pesticides has led to several problems, including resistance and pest resurgence (Bajya *et al*., 2010), and has negatively affected the population densities of natural enemies. Utilizing compounds that have an enhanced effect on insects is a novel method of controlling insect pests; as a result, pesticide usage is lowered by half while still positively impacting insect management (Dewer *et al*., 2017). Additionally, the environment is cleaner, economic profit benefits, and farmer health is enhanced (Kouser and Qaim, 2011). Considering the concern about minimizing health hazards and environmental pollution, natural plant extracts are a substantial and efficient alternative to pesticide alternatives (Sharma *et al*., 2006).

Olibanum, often known as Frankincense, is a natural resin derived from the trees of the species *Boswellia*, which is found across Arabia and India. As previously reported (Metayi *et al*., 2022), we performed essential oil analysis and oil component identification on frankincense, *Boswellia sacra*. The most prevalent chemical discovered was α-pinene **(**Subin, 2021 and Pavela *et al*., 2021**)**. In contrast, Al-Harrasi and Al-Saidi (2008) found that the most abundant compounds were *E-β-ocimene* and limonene. Recently, there has been a lot of interest in the use of self-nanoemulsifying delivery systems. Nanoemulsions are one of the most promising approaches in the field of nanotechnology. It is a collection of scattered particles. Nanoemulsion is a type of oil-in-water emulsion that contains very small, spherical oil droplets (<200 nm). As a result, they are also known as ultrafine emulsions, submicronic emulsions, or miniemulsions when compared to traditional or macroscopic emulsions, and they have a smaller polydispersity. Because of their simplicity of manufacture and a vast range of industrial applications, interest in nanoemulsion formulation research has grown.

The creation of innovative, safer, and more efficient formulations is given increased importance today. Incorporating specific additives pesticide formulations would increase their bioactivity and field performance, which would lead to reduced application rates, low-cost plant protection, and a move toward the integrated pest management (IPM) approach by reducing environmental risks (Morpeth, 1995). Numerous researchers revealed a correlation between additives and the physical properties of pesticide spray solutions because they had an impact on both retention and pesticide effectiveness. Adjuvants modify the physicochemical properties of the spray solution to make it more efficient, stable, and resistant to degrading factors. Adjuvants are used with pesticides for a variety of reasons, including improving wetting and spreading on the treated surface, increasing adhesion, decreasing drift, and improving deposit (Chapman and Mason, 1993). The goal of this research is to improve the properties of *Boswellia sacra* essential oil-based nanoemulsion (Olibanum NE) by adding adjuvants such as Polyxyethylens glycol 600 di-oleate and Provecta® against second instar larvae of the spiny bollworm, *E. insulana*, detecting a latent effect on subsequent stages, and reducing doses, which reduces the economic cost of farms and increases their income. To our knowledge, no literature exists on the insecticidal properties of olibanum nanoemulsion with different adjuvants against the second instar larvae of spiny bollworm, *E. insulana*, which stimulated our interest in investigating this further.

Materials and methods

1. Materials:

Olibanum oil (*B. sacra*) was obtained from Al Barakah Factory for Herbal Oils, L.L.C, Al Maabilah Industrial Area, Seeb, Muscat, Sultanate of Oman. Scientific distributors in Cairo, Egypt, provided Tween 80. Polyxyethylens glycol 600 di-oleate as a non-ionic surfactant produced by the Egyptian Company for Starch, yeast and Detergents, Egypt. Provecta® The organo-modified siloxanes were administered using the product (ICBPharma) which more specifically, contains polyalkyleneoxide modified heptamethyltrisiloxane. produced by ICB Pharma Tomasz Świętosławski, Paweł Świętosławski Spółka Jawna, Poland. Vitamins mixture (Grand Vit with Iron Syrup), produced by Sigma Pharmaceutical industries for Sina Pharm.

2. Insect rearing:

A lab strain of *E. insulana* was obtained from a lab colony at the Bollworms Research Department, Plant Protection Research Institute, Agricultural Research

Center, Giza, Egypt. According to Amer (2015), it had been reared on a semi-artificial diet for multiple generations without being exposed to any pesticides. The experiment was conducted in an incubator under carefully monitored conditions at 26 ± 1 °C, 65 ± 5% RH., and a photoperiod of 12: 12 hrs. (L: D).

3. Diet preparation:

To prepare the tested diets, boiling water was mixed with 250 g kidney beans and 125 g of grated wheat. It was heated for 70 minutes. After that, it was allowed for 20 minutes to cool and clear the water from them. They were mixed in an electric blender with 100 mL of milk and refrigerated for 24 hrs. Following that, further components (49 g dry active yeast, 3 g ascorbic acid, 1.75 g sorbic acid, 1.75 g methyl parahydroxy benzoate, 8 ml of vitamin combination, and 2.5 ml of formaldehyde 34-38%) were added and properly combined. Following that, the prepared diet was refrigerated for 24 hrs. before use.

4. Preparation of nanoemulsion:

Oil-in-water (O/W) nanoemulsion of olibanum oil was prepared in the laboratory of the pesticide formulation research department, central agriculture pesticides laboratory, agricultural research center, al-Sabahia, Alexandria, Egypt; the preparation process was described in a prior work by Abd El-Naby *et al*. (2020). Initially, a coarse emulsion of 5% oil, Tween 80, and water was made with a magnetic stirrer at 250 rpm for 10 min, which was then subjected to ultrasonic emulsification using a 20 kHz sonicator (BANDELIN Sonopuls, Germany). Then, the formulated olibanum NE was mixed with 0.02 % of each adjuvant, characterized, and stored at 4°C for further bioassays.

5. Characterization of prepared nanoemulsion macroscopical analysis:

Droplet size, polydispersity index (PDI) and zeta potential of nanoemulsion formulations were determined using (Zetasizer Nano ZS, Malvern Instruments, Malvern-UK, 4700 model, Germany) using a dynamic light scattering (DLS) droplet analyzer at 25 °C. The nanoemulsion sample was diluted using deionized water.

6. Physicochemical properties of olibanum nanoformulation:

6.1. pH measurement:

Utilizing a pH meter and an electrode system, the pH value of the undiluted olibanum NE alone and with two adjuvants was measured using a Mettler Toledo^{TM} Seven Easy pH meter. The pH value was measured at room temperature with the electrode submerged in the sample for 5 minutes without stirring. Before taking the measurement, the instrument must be calibrated. To eliminate all remnants of the prior sample, the electrode was carefully cleaned between samples using a stream of distilled water.

6.2. Surface tension:

The surface tension of the olibanum NE alone and with two adjuvants was determined using a du Nouy Ring, a platinum/iridium ring, and a sigma force tensiometer 700 from the United States. The instrument should be recalibrated before testing, and the material being measured should be clean, uniform, and devoid of bubbles, with a stable surface. Surface tension (dyne/cm) is measured with a tensiometer (ASTM, 2014).

6.3. Evaluation of the insecticidal activity of prepared olibanum nanoemulsion on larval mortality and different biological aspects:

6.3.1. Toxicological studies:

The toxicity of the olibanum NE alone, olibanum NE with Provecta® and olibanum NE with Polyxyethylens glycol 600 di-oleate against the second instar larvae of *E. insulana* was studied. Different volumes of tested compounds (300, 600, 900, 1200, and 1800 µL per plate) were mixed with Five grams of diet placed in Petri-dish (9 cm in diameter) while the control (Treated with water only), and then left until dry. The 15 second instar larvae were transferred to a treated diet, and the Petri dishes were covered with tissue paper below the glass cover to prevent larvae from escaping, and then left to feed. Each treatment and control were replicated four times. They were incubated under controlled conditions in an incubator at 26 ± 1°C and relative humidity of 65 ± 5%. Afterwards, Mortality percentages were recorded after 24, 48 and 72 hrs.

6.3.2. Biological studies:

The latent effects of the olibanum NE on certain biological aspects of the spiny bollworm, *E. insulana*, the calculated LC_{50} used were 959.38, 723.44, and 600.07 µL per plate for olibanum NE alone, olibanum NE with Polyxyethylens glycol 600 di-oleate and olibanum NE with Provecta®, respectively. They were mixed with three grams of diet placed in glass tubes (2 × 7.5 cm) and incubated previously under the same conditions as before. Surviving larvae from LC50 treatments were transferred individually to an untreated diet after 3 days of treatment to complete their life cycle. The tubes were inspected daily until pupation. Some parameters were estimated, such as larval mortality, larval and pupal duration, larval and pupal weight, and percentage of adult emergence. The pupae were separated in a glass jar (Half a kg) until the moth's emergence.

The resulting moths were sexed and divided into four groups based on the combination: group one consisted of treated males with untreated females (T♂xU♀), group two included treated males with treated females (T♂ x T ♀), group three included untreated males with treated females (U ♂ x T ♀) and group four, which served as the control group, was untreated males with untreated females (U ♂ xU♀), each group was replicated four times. The moths were fed a 10% sugar solution. The number of deposited eggs as well as the hatchability percentage were both recorded. The hatchability percentages of the eggs were calculated using the following equation by El-Shennawy *et al*. (2019):

$$\% \textbf{ Egg hatchability } = \frac{\textbf{no.of hatched eggs}}{\textbf{no. of deposited eggs}} \textbf{ X 100}$$

The Fecundity percentage was calculated according to Crystal and Lachance, (1963) as follows:

$$\% \textbf{ Fecundity } = \frac{\textbf{no. of eggs/ treated female}}{\textbf{no. of eggs/ untreated female}} \textbf{ X 100}$$

7. Statistical analysis:

All data were subjected to a one-way ANOVA, then a Duncan multiple ranges test (Duncan, 1955) to identify significant differences in treatment mean values at 0.05 probabilities. Furthermore, using the Ldp-line software (www.Ehabbakr software/Ldp line), the LC_{50} of the tested compounds was determined in accordance with Finney (1971).

Results and discussion

1. Characterization of fresh olibanum nanoemulsion:

1.1. Droplet size and Poly dispersity index (PDI):

The mean size (z-average diameter) can be obtained by using various algorithms from the correlation function, which can be taken by fitting a single exponential to the correlation function and an estimate of the width of the distribution (PDI). According to Figure (1), olibanum NE formulation is considered a good quality nanoemulsion with an average droplet size of 41.30 nm, characterizes the uniform distribution of droplets in the nanoemulsion the PDI was 0.26. While, as shown in Figure (2) libanum NE formulation with 0.02 % of Polyxyethylens glycol 600 di-oleate adjuvant was 62.54 nm and the PDI was 0.62. Also, as shown in Figure (3) olibanum NE formulation with 0.02 % of Provecta®

adjuvant was 72.44 nm and the PDI was 0.22. When the adjuvant was mixed with olibanum NE, the droplet size increased slightly, but it remained in the nano scale. As demonstrated by this finding, the nano metric size preparation for olibanum NE was successful. Our findings corroborate those that good nanoemulsion had droplet sizes of 20–200 nm in accordance with those reported by other researchers (Massoud *et al*., 2018; Al-Mansoori, 2018; Molai *et al*., 2022 and Abd El-Naby *et al*., 2022).

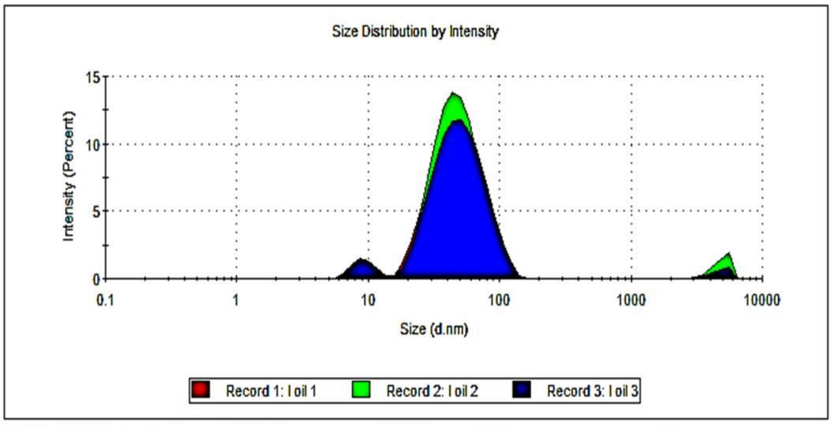

Figure (1): Olibanum NE alone with droplet size distribution measured by dynamic light scattering (DLS).

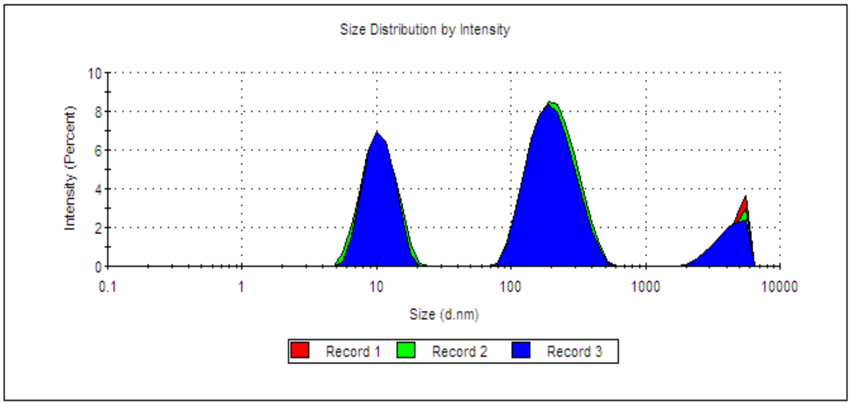

Figure (2): Olibanum NE with Polyxyethylens glycol 600 di-oleate adjuvant with droplet size distribution measured by dynamic light scattering (DLS).

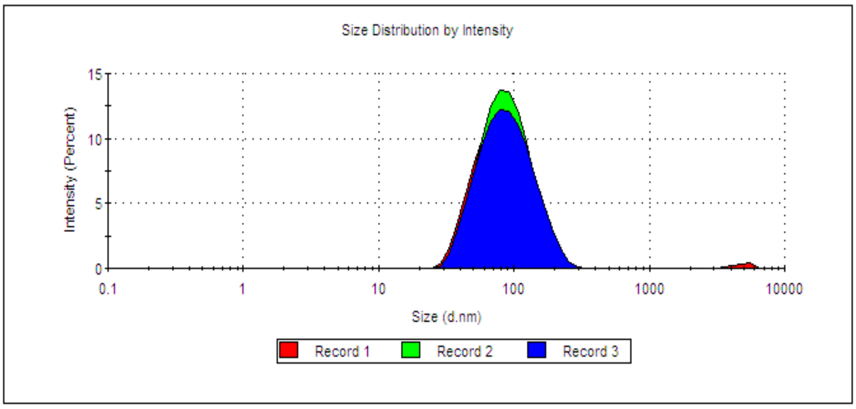

Figure (3): Olibanum NE with Provecta® adjuvant with droplet size distribution measured by dynamic light scattering (DLS).

1.2. Zeta potentials (Electrophoretic properties):

The zeta potential is a crucial variable that has a direct bearing on the colloidal system's physical stability (Pawar and Vavia, 2016). Figures 4, 5 and 6 showed that the zeta potential of the olibanum nanoformulation with both adjuvants was in the negative zeta potential region. The zeta potential of olibanum NE alone was in the negative zeta potential region, has a zeta potential of -29.8 mV. While olibanum NE with adjuvant Polyxyethylens glycol 600 di-oleate was -40.67 mV. Also, olibanum NE with Provecta® adjuvant has a zeta potential of-50.3 mV. When adjuvants were mixed with olibanum NE, the zeta potential increased when adjuvants added to olibanum NE, based on the findings, we can conclude that stable nanoemulsion have zeta potential values of more than 30 mV, independent of the prefix being positive or negative.

Analogous results were reported by Wahba (2020) reported comparable results, demonstrating that the high stability of grape seed oil and pomegranate fruit peel oil could be attributed to their high zeta potentials of -36.7 and -31.0 mV, respectively. Colloidal stability for a range of zeta potentials displays quick flocculation or coagulation from 0 to 5, incipient instability from 10 to 30, moderate stability from 30 to 40, and good stability from 40 to 60 (Kumar and Dixit, 2017). When the zeta potential increases, the attractive force between the droplets decreases and the repulsive force rises.

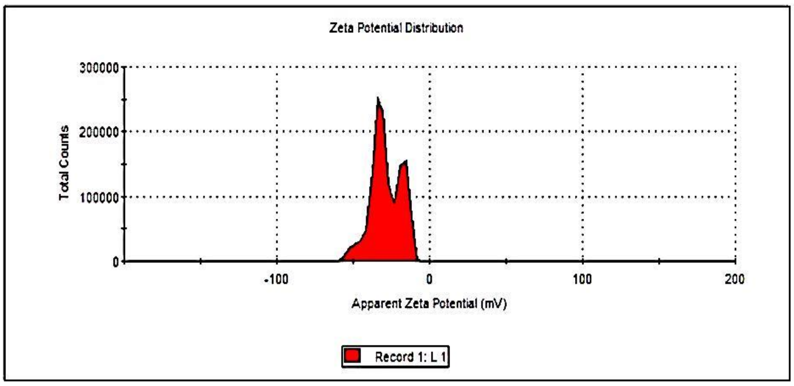

Figure (4): Zeta potential measurement of olibanum NE alone using Malvern Zetasizer Nano ZS.

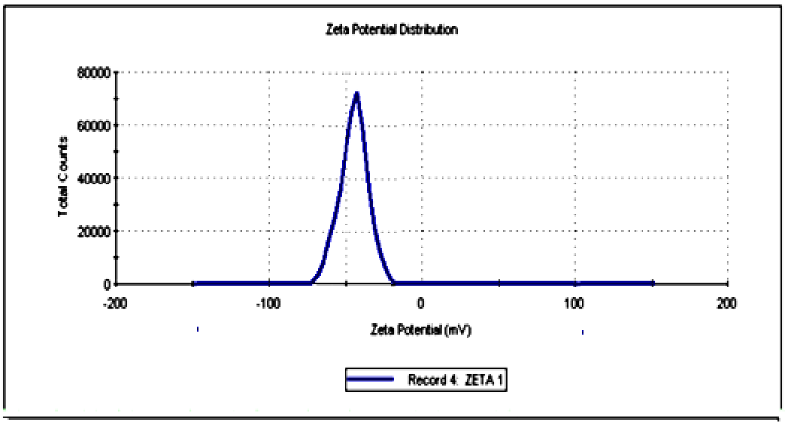

Figure (5): Zeta potential measurement of olibanum NE mixed with Polyxyethylens glycol 600 di-oleate adjuvant using Malvern Zetasizer Nano ZS.

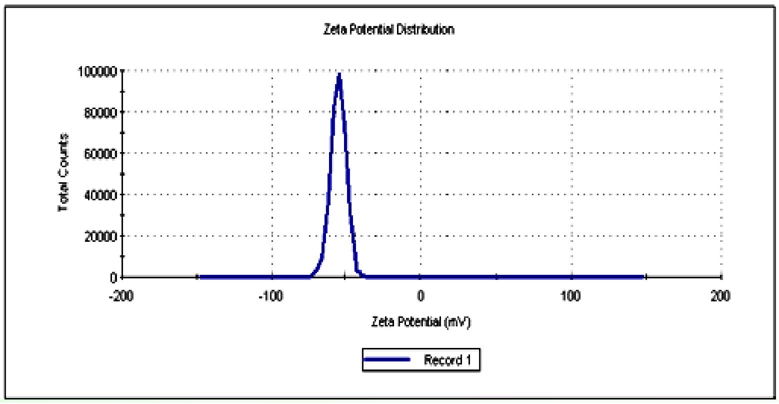

Figure (6): Zeta potential measurement of olibanum NE mixed with Provecta® adjuvant using Malvern Zetasizer Nano ZS.

1.2. Physicochemical properties of the nanoformulation:

1.2.1. The pH value:

The most important aspects of chemical stability are accelerated testing results and pH profile kinetics (Issa *et al*., 2000). The surface properties around the droplet determine the pH value as an indicator of nanoformulation stability. It was observed from the results that the olibanum NE with adjuvants exhibited an acidic pH value. Also, the olibanum NE formulation alone was 6.45. It is noticeable that when olibanum NE was mixed with the Provecta® adjuvant, the pH value slightly decreased to 6.20 compared to olibanum NE alone was 6.45, and the lowest pH value was given when olibanum NE was mixed with PEG 600 di-oleate adjuvant was 5.03. Results showed that they had an acidic character, which is in agreement with Abd El-Naby *et al.* (2022) reported that the prepared castor oil nanoemulsion had an acidic pH value. Also, Mesbah *et al.* (2022) showed that the prepared geranium nanoemulsion exhibited an acidic pH value.

We deduce from that that they will have high biological activity (Issa *et al*., 2000). Lowering the pH of the spray solution may improve the attractiveness of the sprayed solution to the treated plants, hence enhancing the insecticidal efficacy (Tawfic and El-Sisi, 1987). Previous research has found that excessive alkalinity or acidity of nano-formulations causes neem component breakdown, lowering the formulation's bio-efficacy (Gianeti *et al*., 2011).

1.2.2. Surface tension:

Surface tension values were lower with using adjuvants than without, where the olibanum NE formulation alone was 53.69 dyne/cm and when olibanum NE was mixed with PEG 600 di-oleate adjuvant was 43.81 dyne/cm and when mixed with the Provecta® adjuvant, the surface tension value was 45.16 dyne/cm. Based on the findings, we can conclude that both adjuvant based olibanum NE can reduce surface tension and contact angle. These results are in harmony with those obtained by Iqbal *et al*. (2022) who tested and proved that neem colloidal nanoemulsion with botanical adjuvant based nanoemulsion improved adhesiveness and wetting with reduced surface tension and contact angle. This decrease in surface tension predicts an increase in wettability and spreading on the treated plant surface (Jiang *et al*., 2008 and Hussein *et al*., 2010). Wetting is a crucial factor that is closely related to surface tension and contact angle (Hussein *et al*., 2010).

2. Toxicological effect of the examined compounds:

The susceptibility of *E. insulana* larvae to olibanum NE alone, olibanum NE with PEG 600 di-oleate adjuvant, and olibanum NE with Provecta® adjuvant was shown in Table (1). The obtained results revealed that olibanum NE with the Provecta® adjuvant was the most toxic insecticide against the second instar larvae of *E. insulana*, whereas olibanum NE alone was the least toxic one. Among the compounds, olibanum NE with PEG 600 dioleate adjuvant occupied the middle position. The corresponding LC_{50} values were 600.07, 723.44, and 959.38 µL. In the same trend, Metayi *et al.* (2022) revealed that the accumulated mortality percentages of *E. insulana* increased significantly more than untreated larvae with a formulated frankincense nanoemulsion. With regard to the fourth instar larvae of the cotton leaf worm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) , Abd El-Naby *et al.* (2022) discovered that castor oil nanoemulsion was more effective than castor bulk oil under laboratory conditions. Moustafa *et al.* (2015) assessed the effectiveness of the eucalyptus nanoemulsion against the larvae of *E. insulana* and *Pectinophora gossypiella* and discovered excellent effectiveness in reducing cotton bollworms.

Table (1): Toxicity of tested insecticides on second instar larvae of *Earias insulana*.

Compound	LC_{50} µL	Confidence limits		Slope	X^2
		Lower	Upper		
O NE	959.38	733.2	1025	2.86	22.21
O NE + Provecta®	600.07	489.8	749.1	2.39	22.91
O NE + PEG 600 di-oleate	723.44	562	976.2	2.31	17.38

3. Biological aspects:

3.1. Insecticidal activity of the three formulations of olibanum essential oil against the second instar of *Earias insulana* larvae.

The data in Table (2) showed that the addition of both Provecta® and PEG 600 di-oleate adjuvants to olibanum NE improved its bioefficacy against second-instar larvae of *E. insulana* compared to olibanum NE alone. The larval mortality percentages were 51.94, 58.03, and 63.93% for olibanum NE alone, olibanum NE with PEG 600 di-oleate adjuvant, and olibanum NE with Provecta® adjuvant, respectively. These results were attributed to the adjuvants' ability to either lower the surface tension or boost the surface activity of a pesticide solution. The result is in agreement with Saad *et al.* (2013), who discovered that adding PEG 600 di-oleate adjuvants to pesticides such as lambda-cyhalothrin, abamectin, emamectin benzoate, imidaclopride, and oxamyl improved both their effectiveness and their residual effects.

Also, all treatments caused a significant reduction in pupation percentage, especially treatment olibanum NE with Provecta® and treatment olibanum NE with PEG 600 di-oleate compared to treatment with olibanum NE alone, as shown in Table (2). The highest pupal percentage in treatments was 35.96% with olibanum NE alone, and the lowest was 11.98% with olibanum NE with Provecta®; olibanum NE with PEG 600 di-oleate adjuvant treatment recorded 18.62%, while the pupal percentage in control was 96.60%.

These results agree with Metayi *et al.* (2022), who reported that the treatment with frankincense nanoemulsion caused a significant decrease in pupal percentages of *E. insulana*. Also, Ammar and Abd-ElAzeem (2021), observed that application of gelatin-copper nanoparticles to the spiny bollworm induced the highest levels of pupal mortality and pupation while releasing the lowest LC_{50} levels. The failure in pupation as a result of olibanum NE with adjuvant and olibanum NE alone treatment which caused incomplete larval molting and failure to pupate this explained by (Adel *et al*., 2018 and Abd El-Naby *et al*., 2022) who recorded that the harmful effects of nano-emulsion to insects may be explained by their small size and large surface area, which increases biological activity and penetration in the living cells.

In addition, all tested compounds caused a significant reduction in *E. insulana* moth emergence. The average percentage of adult emergence was 93.30% in the control. This average decreased significantly to 49.23, 33.33, and 10.63% for olibanum NE alone, olibanum NE with PEG 600 di-oleate adjuvant, and olibanum NE with Provecta® adjuvant, respectively. These results corresponded with the findings of Metayi *et al.* (2022), who reported that the frankincense nanoemulsion caused a significant reduction in *E. insulana* moth emergence. Also, El-Din *et al.* (2020) found that jojoba oil significantly reduced the percentage of adult emergence.

Table (2): Effect of Olibanum NE with and without adjuvant on larval mortality, pupation percentage and adult emergence percentages of *Earias insulana.*

Tested Compound	Conc. μL	Accumulated mortality of larval stages %	Pupation %	Adult emergence %
Olibanum NE	959.38	51.94	35.96	49.23
Olibanum NE **+ Provecta®**	600.07	63.93	11.98	10.63
Olibanum NE **+ PEG 600 di-oleate**	723.44	58.03	18.62	33.33
Control	00.00	00.00	96.60	93.30
LSD 0.05		9.01*	4.59*	7.20*

Data is the means ±SD of the three replicates of immature stages. LSD mean not significantly (P>0.05).

3.2. Larval duration and weight:

Data in **Table (3)** illustrates the latent effect of tested compounds on the larval duration and weight of *E. insulana*. The three tested compounds prolonged the duration of the larval stage significantly. While olibanum NE treatment with adjuvant prolonged the duration more than olibanum NE alone. These periods were estimated at 17.14, 19.31and 21.29 days for olibanum NE alone, olibanum NE with Provecta® adjuvant and olibanum NE with PEG 600 di-oleate adjuvant, respectively, compared with 14.20days in control. In addition, the average larval weight decreased significantly in 0.044, 0.038, and 0.035g/ larva for olibanum NE alone, olibanum NE with Provecta® adjuvant, and olibanum NE with PEG 600 di-oleate adjuvant, respectively, while it was 0.080g/ larva in control. Also, statistically, there was a non-significant difference found between the larval duration of the three tested compounds and the control. While there was a significant difference found between the larval weight of the three tested compounds and control.

2.3. Pupal duration and weight:

Analysis of the variance of the results in Table (3), showed that olibanum NE alone gave a slight prolongation in pupal duration, while olibanum NE with adjuvant showed a significant prolongation in pupal duration compared with the control. These periods were estimated at 7.11, 7.51 and 7.90 days for olibanum NE alone, olibanum NE with Provecta® adjuvant and olibanum NE with PEG 600 di-oleate adjuvant, respectively, compared with 7.00 days in control. From the data presented in Table (3), showed that all tested compounds led to a significant decrease in pupal weight, but only olibanum NE with PEG 600 di-oleate adjuvant was the highest one that caused a significant decrease in pupal weight. These weights were estimated at 0.041, 0.034 and 0.030 grams for olibanum NE alone, olibanum NE with Provecta® adjuvant and olibanum NE with PEG 600 di-oleate adjuvant, respectively, compared with 0.052 grams in control. Statistically, there was a non-significant difference found between the pupal duration of the three tested compounds and control. While there was a significant difference found between the pupal weight of the three tested compounds and control.

Table (3): Latent effects of Olibanum NE with and without adjuvant on immature stages of *Earias insulana*.

Tested Compound	Conc. *μL*	Larval		Pupal	
		duration (days) Mean± SE	Weight (g) Mean± SE	duration (days) Mean± SE	Weight (g) Mean± SE
Olibanum NE;	959.38	17.14	0.044	7.11	0.041
Olibanum NE + Provecta®	600.07	19.31	0.038	7.51	0.034
Olibanum NE + PEG 600 di-oleate	723.44	21.29	0.035	7.90	0.030
Control	0	14.20	0.080	7.00	0.052
LSD 0.05	-	5.36	0.02	3.23	0.02

Data is the means ±SD of the three replicates of immature stages. LSD mean not significantly (P>0.05).

Results on the latent effect of olibanum NE on the rate of egg production are presented in Table (4). The results indicated that when adult males that came from treated larvae mated with untreated females, the fecundity of these females decreased with treatment with olibanum NE alone, olibanum NE with Provecta® adjuvant, and olibanum NE with PEG 600 di-oleate adjuvant which gave 37.8, 27.2 and 0.00 % respectively. The reduction in fecundity was increased when olibanum NE treatment with adjuvant was applied to adult males. A significant reduction of 0.00% occurred when adult males were treated with olibanum NE with PEG 600 di-oleate adjuvant. Also, from the results, when adult males and females that came from treated larvae mated, the fecundity of these females decreased with olibanum NE treatment with adjuvant which gave 18.3 and 13.9% with olibanum NE with Provecta®, and olibanum NE with PEG 600 di-oleate adjuvant respectively. The reduction in fecundity was increased when olibanum NE treatment with adjuvant was applied to adult males than treatment with olibanum NE alone. No hatched egg occurred when adult males were treated with olibanum NE + PEG 600 di-oleate. On the other hand, when females that emerged from treated larvae were mated with untreated males, the fecundity of such females decreased significantly with treatment of olibanum NE with adjuvant.

It is noticeable from Table (4), that when the treated females were mated with untreated males, the fecundity was much lower than when the treated males were mated with untreated females. The results from the present study highlighted the inhibitory effect of olibanum NE with and without adjuvant on fecundity and fertility of *E. insulana* with types of mating combination have a significant effect.

Table (4:) Latent effect of Olibanum NE with and without adjuvant and combination between male and female adults on total eggs laid and hatchability percentages of *Earias insulana* under laboratory conditions.

Tested Compound	Conc. *µL*	Mating possibilities		Average no. of eggs/♀ (Mean ± SD)	Hatched Eggs (Mean ± SD)	Hatchability %	Fecundity %
		♀	♂				
Olibanum NE	959.38	T	T	50.0± 3.35	26.0± 2.35	52.0	27.8
		T	U	54.0± 3.35	32.0± 2.00	59.3	30.0
		U	T	68.0± 5.00	46.0± 3.35	67.7	37.8
Olibanum NE + Provecta®	600.07	T	T	33.0± 2.00	10.0± 4.16	30.3	18.3
		T	U	42.0± 1.35	19.0± 2.00	45.2	23.3
		U	T	49.0± 3.35	28.0± 2.35	57.1	27.2
Olibanum NE + PEG 600 di-oleate	723.44	T	T	25.0± 1.65	10.0± 4.16	40.0	13.9
		T	U	33.0± 2.00	6.00± 1.15	18.2	18.3
		U	T	40.0± 1.35	0.00± 0.00	0.0	0.00
Control	0	U	U	180±10.35	173± 8.35	96.11	100
LSD 0.05	-	-	-	14.9	15.5	-	-

Data are the means ±SD of the three replicates of immature stages. LSD mean not significantly (P>0.05).

These results concurred with Metayi *et al.* (2022) confirmed that frankincense nanoemulsion caused a significantly lower hatchability percentage compared to the untreated control and the types of mating combinations have a significant effect on the fecundity of *E. insulana*. In addition, using a bio-inspired adjuvant of *Cymbopogon citratus* and *Prosopis juliflora* **(**Iqbal *et al*., 2022), they demonstrated significant insecticidal activity against *Bemisia tabaci* in eggplant (91.24%) by lowering the surface tension and contact angle. *Ocimum basilicum* oil was tested in a bioassay against late third-instar *Anopheles Stephensi* larvae with and without the synergist PBO **(**Yadav *et al*., 2009**),** and the LC_{50} value for *O. basilicum* was shown to have been decreased from 44.19 ppm to 23.87 ppm.

When geranium essential oil-loaded nanoparticles were used to treat *Phthorimaea operculens*, Adel *et al*. (2014) found that both the percentage of hatchability relative to untreated females and the percentage of fecundity with regard to control decreased significantly. Hategekimana and Erler (2020) reported that EOs had an inhibitory influence on fecundity, with eucalyptus oil treatment T♀ × U♂ combination recording 47.55% greater than U ♀× T ♂ recorded 37.68% and anise oil treatment T♀× U♂ combination recording 74.24% higher than U ♀ × T ♂ recorded 49.33%. In this line, Ammar and Abd-ElAzeem (2021) also showed that gelatin-copper nanoparticles (G-CuNPs) treatment significantly reduced the percentage of hatchability and egg counts of *E. insulana* as compared to the untreated group.

The decrease in hatchability percentage might be caused by sterilizing the eggs or the sperms, or it can be caused by the sperm's failure to reach the female during copulation (**Ismail, 1980**). The sterility of treated larvae may be due to the malnutrition caused by the antifeedant effect, which requires food before they can deposit fertile eggs, as in the case of many lepidopteron species. Malnutrition may also inhibit the development of sex organs, resulting in infertile eggs.

Our findings have shown satisfactory results in improving the bioinsecticidal properties of olibanum nanoemulsion and biological activity by adding an adjuvant; the results revealed enhanced insecticidal activity and decreased lethal concentration values (LC_{50}). Alsocaused long-term inhibition effects on larvae, pupae and the adult longevity and reproductive capacity of *E.insulana*.

On the other hand, the physical properties of the prepared olibanum nanoemulsion with and without adjuvant, such as pH, surface tension, mean droplet size, and polydispersity index, were studied. Thus, the olibanum nanoemulsion with adjuvant might be a viable alternative to traditional pesticide formulations and could play an important role in Integrated Pest Management (IPM) and organic cotton agriculture. However, more research is required before these compounds can be used in the field, particularly to evaluate their non-target toxicity on aquatic and soil invertebrates and to confirm the bioactivity that has been observed over time.

References

Abd El-Naby, S. S. I.; Mahmoud, F. H. E.; El-Habal, N. A. M.; Abdou, M. Sh. and Abdel-Rheim, K. H. M. (2020): Efficacy of prepared castor oil nanoemulsion formulation against rice weevil *Sitophilus Oryzae* on stored wheat grain and its acute toxic effect on albino rats. Egy. Sci. J. Pestic. 6 (4): 33–44.

Abd El-Naby, S. S.; Abdou, M. S.; Metayi, M. H.; Mahmoud, F. H.; El-Habal, N. A.; Abdel-Megeed, A. and Cipriano-Salazar, M. (2022): Development and evaluation of castor oil nanoemulsion on the cotton leaf worm *Spodoptera littoralis* (Boisd.) and its adverse effects on the reproductive system of albino rats. *Biomass Convers. biorefin.*, 1–13.

Adel, M. M.; Massoud, M.; Mohamed,M. I.E.; , Abdel-Rheim K. H. and Abd El-Naby, S. S. (2018): New nano-biopesticide formulation of *Mentha piperita* L.(Lamiaceae) essential oil against stored product red flour beetle *Tribolium castaneum* Herbs and its effect on storage. Adv. Crop.Sci. Techno., 6(6): 409. doi: 10.4172/2329-8863.1000409.

Adel, M.M; Atwa, W. A.; Hassan, M. L.; Salem, N. Y.; Farghaly, D. S. and Ibrahim, S. S. (2014): Biological activity and field persistence of *Pelargonium graveolens* (Geraniales:Geraniaceae) loaded solid lipid nanoparticles (SLNs) on *Phthorimaea operculenlla* (Zeller) (PTM) (Lepidoptera: Gelechiidae). Int. l J. Sci. Res., 4 (11): 514–520.

Ahmad, S. and Hasanuzzaman, M. (2020): Cotton Production and Uses: Agronomy, Crop Protection, and Postharvest Technologies. Berlin: Springer, 1–641. doi: 10.1007/978-981-15-1472-2.

Al-Harrasi, A. and Al-Saidi, S. (2008): Phytochemical analysis of the essential oil from botanically certified oleogum resin of *Boswellia sacra* (Omani luban). Molecules, 13: 2181–2189.

Al-Juboori, I. J. (2000): The importance of natural enemies in IPM of cotton and its pests. Proceedings of the I^st^ Workshop in International Iraqi Atomic Center, Iraq, November 25-26, pp.18–27.

Al-Mansoori, D. H. (2018): Efficacy of essential oil nanoemulsion delivery system for strong antimicrobial action against Pathogen Listeria Monocytogenes. Ph.D. Thesis. United States: University of New Jersey.

Amer, A. E. (2015): Economic artificial diets for rearing spiny bollworm, *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae). J. Plant Prot. Pathology 6 (3): 527–534.

Ammar, H. A. and Abd-ElAzeem, E. M. (2021): Novel treatment of gelatin copper bio-nanoparticles as a management method against the spiny bollworm, *Earias insulana*, (Boisd) (Lepidoptera: Noctuidae) in comparison studies with the uncoated nanoparticles. Inorg. Nano Met. Chem., 51 (3): 309–321.

ASTM (2014): American society of testing and materials standard test methods for surface and interfacial tension of solution of surface-active agents, 1-5.

Bajya, D. R.; Monga, D.; Meena, B. L. and Tyagi, M. P. (2010): Insecticide resistance management strategies for managing cotton pest complex. Ann. Plant Prot. Sci., **18**(1): 1-5.

Chapman, P.J. and Mason, R. D. (1993): British and European Community regulations and registration requirements for non-pesticidal co formulants in pesticides and adjuvants. Pest. Sci., 37: 167-171.

Crystal, M. M. and Lachance, L. E. (1963): The modification of reproduction in insects treated with alkylating agents. Inhibition of ovarian growth and egg reproduction and hatchability. Biol. Bull., 25: 270–279.

Dewer, Y.; Elbassoiuny, H. M. and El-Shahaat M. S. (2017): Improving the properties of emulsifiable formulation of pesticides in relation to efficacy against insect pests. Egypt Sci. J. Pestic., **3**(3): 1– 9 .

Duncan, D. B. (1955): Multiple range and multiple F tests. Biometrics, 11: 1–42.

El-Din, H. S.; Rashad, A. M.; El–Khayat, E. F.; Shams El–Din, A. M. and Abd–Elzaher, T. R. (2020): Toxicological and biological effects of jojoba and flaxseed oils against Pink Bollworm *Pectinophora gossypiella* (Saund.) and spiny bollworm *Earias insulana* (Boisd.). J. Plant Prot. Pathol., 11 (9): 461–466.

El-Shennawy, R. M.; Husseen, A. M. and Kandil, M. A. (2019): Latent effects on adults' emergence of *Pectinophora gossypiella* (Saund.) and *Earias insulana* (Boisd.), resulted from magnetized pupae. J. Plant Prot. Pathology 10 (1): 77–80.

Finney, D.J. (1971): Probit analysis. 3rd Edn. Cambridge University press, Cambridge, UK. , 318.

Gianeti, M. D.; Wagemaker, T. A. L.; Seixas, V. C. and Maia, Campos P. M. B. G. (2011): The use of nanotechnology in cosmetic formulations: the influence of vehicle in the vitamin a skin penetration. Nano, Sci., 8: 526–534.

Hategekimana, A. and Erler, F. (2020): Fecundity and fertility inhibition effects of some plant essential oils and their major components against *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). J. Plant Dis. Prot. 127 (5): 615–623.

Hussein, I.H.; Hashim, E.F. ; Toson, M.G. and Gnedy, M.M. (2010): Physico-chemical studies and aphicidal effect of some locally formulated plant oils. J. Egyp. Biolo. Pest Control., 20(2):115-120.

Iqbal, N.; Hazra, D. K.; Purkait, A.; Agrawal, A. and Kumar, J. (2022): Bioengineering of neem nano-formulation with adjuvant for better adhesion over applied surface to give long term insect control. Colloids and Surfaces B: Biointerfaces, 209, 112176. doi.org/10.21203/rs.3.rs-116370/v1.

Ismail, I. E. (1980): Physiological studies on the effect of juvenile hormone analogues upon the cotton leafworm, *Spodoptera littoralis*. Ph. D. Thesis, Cairo University, Egypt.

Issa, T. S.; Philippe, B.; Raymond, H.; Michel, H. and Jacques, D. (2000): Improved kinetic parameter estimation in pH-profile data treatment. Int. J. Pharm., 198: 39–49.

Jiang, J.; Oberdörster, G. and Biswas, P. (2008): Characterization of Size, Surface Charge, and Agglomeration State of Nanoparticle Dispersions for Toxicological Studies. J. Nanopart. Res., 11: 77-89.

Khan, R. R.; Ahmed, S.; Saleem, M. W. and Nadeem, M. (2007): .Field evaluation of different insecticides against spotted bollworms *Earias* spp. at district Sahiwal. Pak. Entomol., 29(2):129-134.

Kouser, S. and Qaim, M. (2011): Impact of Bt cotton on pesticide poisoning in smallholder agriculture: a panel data analysis. Ecol. Econ., **70**(11): 2105–2113.

Kumar, A., and Dixit, C. (2017): Methods for characterization of nanoparticles," in Advances in nanomedicine for the delivery of therapeutic nucleic acids Sawston, UK: Woodhead Publishing,43–58.

Mansour, M. H.; Aboul Nasr, A. E. and Amr, E. M. (1990): Dietary influence of two allelochemical on larval growth of the spiny bollworm *Earias insulana* Boisd. Z. - fuer, pflanzcnkr – ankheiten – pflanzenschutz, 97 (6): 580–587.

Massoud, M. A; Adel, M. M.; Zaghloul, O. A.; Mohamed, M. I. and Abdel-Rheim, K. H. (2018): Eco-friendly nano-emulsion formulation of *Mentha piperita* against stored product pest *Sitophilus oryzae*. Adv. Crop. Sc.i Techno., 6(6):404–409.

Mesbah, H. A.; Massoud, M. A.; Aajel, M. S.; Abdelsalam, N. R. and Adel, M. M. (2022): Nano-formulation of *pelargonium graveolens* essential oil: Physicochemical characterization and its bioactivity against rice weevil *sitophilus oryzae* on stored wheat grain. Egypt. J. Chem. 0, 0. Article in Press. doi:10.21608/EJCHEM.2022.119611.5375.

Metayi, M.H.; Abd El-Naby, S.S.; El-Habal, N. A.; Fahmy, H.H.; Abdou, M.S.; Ali, B.; Abdel-Rheim, K.H. and Abdel-Megeed, A. (2022): Omani Frankincense nanoemulsion formulation efficacy and its latent effects on biological aspects of the spiny bollworm *Earias insulana* (Boisd.). Front. Physiol. 13:1001136. doi: 10.3389/fphys.2022.1001136.

Molai, S.; MemarMaher, B.; Anarjan, N. and Hamishehkar, H. (2022): The effects of main formulation and process parameters on characteristics of frankincense essential oil microemulsions. Iranian Journal of Chemistry and Chemical Engineering. Doi:10.30492/IJCCE.2022.530681.4742.

Morpeth, F. F. (1995): "Preservations of surfactants formulations", London, Weinheim, New York, pp: 134.

Moustafa, H. Z.; Mohamad, T. G. M. and Torkey, H. (2015): Effect of Formulated nanoemulsion of eucalyptus oil on cotton bollworms. J. Biolo. Chem., 32(2): 478-484.

Omar, S. (2022): Cotton and Products Annual. Attaché Report (GAIN), Report No: EG2022-0011. https://apps.fas.usda.gov/newgainapi/api/Report/DownloadReportByFileName?fileName=Cotton%20and%20Products%20Annual_Cairo_Egypt_EG2022-0011.pdf

Pavela, R.; Maggi, F.; Giordani, C.; Cappellacci, L.; Petrelli, R. and Canale, A. (2021): Insecticidal activity of two essential oils used in perfumery (Ylang ylang and frankincense). Nat. Prod. Res., 35 (22): 4746–4752.

Pawar, S. and Vavia, P. (2016): Glucosamine anchored cancer targeted nano-vesicular drug delivery system of doxorubicin. J. Drug. Target., 24(1): 68-79.

Saad, A. S. A.; Tayeb, E. H.; El-Shahaat, M. S.; Zaghloul, O. A. and Abdel-Naby, S. S. (2013): Evaluation of certain adjuvants to enhance pesticides efficacy against the cotton leafworm, *Spodoptera littoralis*, 18(3): 624-635.

Sharma, A.; Kaushal, P.; Sharma, K. and Kumar, R. (2006): Bio efficacy of some plant products against Diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). J. Entomol. Res. Soc., 30: 213–217.

Subin, K. (2021): Synergistic mechanism of insecticidal activity in basil (*Ocimum basilicum*) and Mandarin (*Citrus reticulata*) essential oils against the tobacco cutworm. MSc Thesis. South Korea: Seoul National University.

Tawfic, M. H. and El-Sisi, A. G. (1987): The effect of mixing some foliar fertilizers on the physico-chemical properties and insecticidal activity of some locally spray oils against the scale insect *Parlatoria ziziphus* (Lucas). 2nd Nat. Conf. Ismailia, 367-376.

Wahba, T. F. (2020): Antifeedant activity of three essential oils and their nanoemulsions against antifeedant activity of three essential oilsand their nanoemulsions against the rice weevil *Sitophilus oryzae* (L.). Egypt. Sci. J. Pestic., 2: 19–31.

Yadav, S.; Mittal, P. K.; Saxena, P. N. and Singh, R. K. (2009): Effect of Synergist Piperonyl Butoxide (PBO) on the Toxicity of Some Essential Oils Against Mosquito Larvae. J. Commun. Dis., 41 (1): 33-38.