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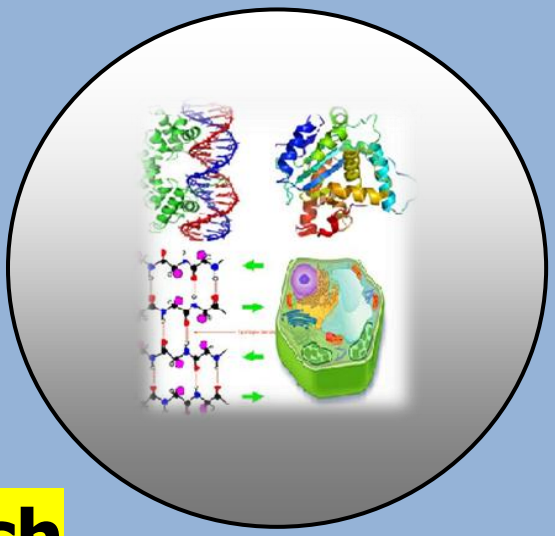

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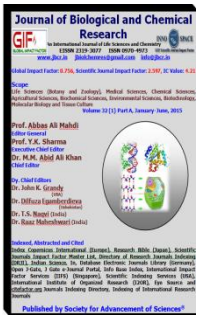

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Effect of Formulated Nanoemulsion of Eucalyptus Oil on the Cotton Bollworms

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ABSTRACT

Nanotechnology has emerged as a promising area for innovative products, including insecticides. Cotton bollworms are the most destructive insect pest infested cotton plants, due to negative impacts to the environment caused by synthetic chemicals used for cotton bollworms control. Thus, developing of natural products based insecticidal are considered very promising. On this context, the aim of the present study deals with the formulation and characterization of bio-based oil in water nanoemulsion and its potential insecticidal activity. Eucalyptus oil was prepared by ultrasonication method. The prepared nanoemulsion presented low polydispersity 0.106 and mean droplet size 8.003 nm. Further, the nanoemulsion had insecticidal and biological activity against newly-hatched larvae of Pectinophora gossypiella and Earias insulana. The LC_{50} values after 72h were 195.44 ppm and 470.18 ppm, respectively. Also, the rate of larval duration of P.gossypiella was increased than E. insulana. These values were 23.18±6.81and 21.82±4.62 days, respectively as compared to the control. Meanwhile, the rate of pupal duration was increased in case of E. insulana than P.gossypiella 9.45±3.059 and 7.55±0.577 days, respectively. This study contributes to nanobiotechnology of natural products, presenting a potential insecticidal nanoemulsion prepared with eucalyptus essential oil.
Keywords: Eucalyptus Oil, Nanoemulsion, Formulation and Cotton Bollworms.

INTRODUCTION

Cotton bollworms are the most destructive insect pest infested cotton plants. The pink bollworm (PBW) *Pectinophora gossypiella* and spiny bollworm *Earias insulana* (Boisd.)

larvae damage squares, flower buds, flowers and bolls that cause decreasing in the quality and quantity in the lint and the oil of the obtained yield. To control the cotton bollworms many chemical insecticides are used. The indiscriminate use and the excessive reliance on chemical pesticides in crop protection have resulted in serious problems linked to water and environmental contamination, phytotoxicity, and toxic hazards to human and non-target organisms. (Boyer et al., 2012; Kohler and Triebskorn, 2013).

In recent years, many research studies focused on the use of essential oils from higher plants. They have the advantages of low mammalian toxicity and selectivity with varying sites of action, alternative as pest control including repellent, insecticidal and larvicidal properties (Koul et al., 2008; Conti et al., 2010; Regnault-Roger et al., 2012).The problem that arises with the use of essential oils as pesticides is that their effect is short lasting (Barnard and Xue, 2004). An additional important problem in practical application of essential oil in agriculture is the lake of persistent efficacy in field. Chen et al., (2013).To overcome this problem; essential oils are formulated into nanoemulsions. Nanoemulsions are colloidal dispersion of oil and water having droplet size in the range of 10–100 nm. (European Food Safety Authority, 2009; McClements, 2011; Caroline et al., 2015). potential advantages of nanoemulsion is more environmentally from the conventional emulsifiable concentrates (EC), nanoemulsions are large surface area of the emulsion system allows rapid penetration of activities (Tadros, 2004).So, the aim of the present study was to prepare nanoemulsion formulation of eucalyptus oil and study the insecticidal activity of the stable prepared formulation against newly hatched larvae of *Pectinophora gossypiella* and *Earias insulana*

MATERIAL AND METHODS

Chemicals

Eucalyptus oil was purchased from Kato Aromatic Company for Flavors, Fragrances, Coloring Extracts and Essential Oils, Giza, Egypt, non-ionic surfactant; Tween 20 (polyoxyethylene (20) sorbitan monolaurate; hydrophile-lipophile balance, HLB=16.7) was purchased from Sigma-Aldrich Chemie GmbH, Riedstr, Steinheim, Germany. Deionized water was used for all the experiments throughout the study; obtained from Water distiller LABCONCO water PROT.M PS LABCONCO Corporation, KANSAS City, Missouri 64132-USA.

Tested insect

Newly-hatched larvae of *Pectinophora gossypiella* (Saund.) and *Earias insulana* (Boisd.) were obtained from the Bollworm Research Department, Plant Protection Research Institute, Dokki, Giza. Rearing conditions were controlled at 27°C ± 2 and 70-85% RH. Feeding the newly hatched larvae on semi-artificial diet according to Rashad and Ammar (1985).

Preparation of nanoemulsion (Ultrasonication method)

Nanoemulsion was prepared by the procedure previously reported by Sugumar et al., (2014). Initially coarse emulsion (16.66% oil, 16.66% Tween 20, 66.68% water in v/v proportion) was made with magnetic stirrer at 250 rpm for 10 min. which was then subjected to ultrasonic emulsification using a 20 kHz Sonicator (Ultrasonics, USA). Sonicator probe diameter was13 mm dipped into coarse emulsion. The temperature difference between initial coarse emulsions to final nanoemulsion should be less than 10°C. Heat is generated during the process of high energy ultrasonic emulsification. This heat is diminished by keeping the emulsion sample beaker in a comparatively bigger beaker containing ice. Then, the formulated nanoemulsion was characterized and also stability of the emulsion was investigated.

Characterization of prepared nanoemulsion

Macroscopical analysis

Stability of the prepared nanoemulsion was evaluated immediately and after 1 and 30 days of manipulation by macroscopic analysis, such as color, visual aspect, phase separation and sedimentation. During this period the nanoemulsions was maintained under room temperature (25 °C ± 2) in screw capped glass test tube. (Fernandes et al., 2014).

Centrifugation test

Laboratory Centrifuge REMI Centrifuge REMI Equipments Bombay-India- R32A.4000002 was used to determine the stability of the prepared nanoemulsion against gravity. Formulation was centrifuged for 10 min at 5000 rpm in 10 ml graduated plastic test tube, to monitor whether phase separation occurred. The formulation was centrifuged at 25°C.

Mean droplet size and polydispersity index

The mean droplet size and polydispersity index (PDI) of eucalyptus oil nanoemulsion formulation was performed by a dynamic light scattering method using Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature. Formulated emulsion was diluted with milli-Q (Millipore Corporation) deionized water to avoid multiple scattering effects.

pH Measurement

pH value of the prepared nanoemulsion was measured by using a pH Meter (Jenway model pH 3510). It was recalibrated before testing.

Refractive Index

Refractive index of the prepared nanoemulsion was measured by using ABBE Refractometer, ATAGO, Co., LTD, Japan. ASTM (2002).

Surface Tension

Surface tension of the prepared nanoemulsion was measured using “Sigma 700” by du Noüy Ring, a platinum/iridium ring. The instrument recalibrated before testing, the sample measured should be clean, homogenous and free from any bubbles and has a stable surface. Recording the surface tension of the prepared nanoemulsion.

Flash point

Measurement of flash point of the prepared nanoemulsion was carried out by tag open-cup method by Koehler instrument company, INC, USA. The flash point was recorded as the temperature at the thermometer when a flash appeared. CIPAC (MT12).

Evaluation of the insecticidal activity of prepared nanoemulsion against newly hatched larvae of *P. gossypiella* and *E. insulana*

The activity of the prepared nanoemulsion of eucalyptus oil was tested against the newly hatched larvae of *P. gossypiella* and *E. insulana* by applied gradual concentrations; 207.5, 415, 830, 1660 and 3320 ppm of the prepared formulation on artificial diet in glass tubes of (2x7.5cm), each tube was infested by neonatal larvae and capped with cotton piece. Percentage mortality was calculated after 24, 48 and 72 hours of exposure and corrected by Abbott's formula (1952). The LC_{50} value for each insect was calculated according to Finney (1971).

RESULTS AND DISCUSSION

Formulation characteristics

Nanoemulsions have been considered advantageous for optimizing delivery of water-insoluble compounds or active ingredients (Wang et al., 2007; Megha et al., 2014).

The small size of the droplets allows them to deposit or spray uniformly on target areas (Solans et al., 2003). The mean droplet size of the prepared nanoemulsion formulation was 8.003 nm Fig. (1). The polydispersity index (PDI) is a measure of homogeneity and stability of the droplet size in the nanoemulsion system. PDI value below 0.2 indicates a narrow size distribution and thus provides long-term stability to the formulated nanoemulsion (Sampathi et al., 2015). PDI was found to be 0.106; this confers more uniformity in size distribution and their stability.

Nanoemulsion is kinetically stable systems and is formed from a particular concentration of oil phase, surfactant and water, with no phase separation during macroscopical analysis and centrifugation. The prepared nanoemulsion formulation exhibited acidic pH value. The pH value of the prepared nanoemulsion was 4.23, indicating that the formulation having acidic character implying that it will have good biological activity. Issa et al., (2000). The prepared nanoemulsion having the surface tension (29.31mN/m). Wetting, spreading, and penetration may also be enhanced as a result of the low surface tension of the whole system and the low interfacial tension of oil in water droplets due to nano-range particle size (Tadros et al., 2004). The refractive index of the prepared formulation was 1.3673. Finally, the flash point of the prepared formulation was more than 70°C and this is quite safe.

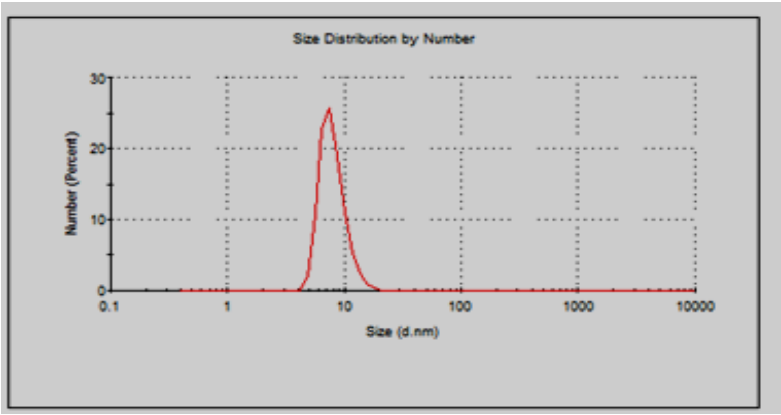

Fig. 1. Mean droplet size of eucalyptus oil nanoemulsion

Table.1. Physicochemical characterization of eucalyptus oil nanoemulsion.

Physical Properties	Values
pH value	4.23
Refractive index	1.3673
Surface tension(mN/m)	29.31
Flash Point (°C)	Over 70°C
Mean droplet size (nm)	8.003
Polydispersity index	0.106

Formulation activity

Insecticidal activity of the nanoemulsion formulation against newly hatched larvae of *E. insulana* and *P. gossypiella*

The insecticidal activity of the prepared eucalyptus oil nanoemulsion formulation on the newly-hatched larvae of *P. gossypiella* and *E. insulana* after 24, 48 and 72 hours as shown in Table (2).

The obtained LC_{50} values revealed that the higher mortality of *P. gossypiella* larvae than *E. insulana*, the LC_{50} values after 72 h were 195.44 and 470.18 ppm, respectively.

Table 2. LC_{50} values from the toxicity of nanoemulsion formulation on *E . insulana* and *P. gossypiella*.

Insect	Time	LC_{50}	Slope
E. insulana	24h	694.91(388.8-1131.99)	0.25±1.04
	48h	561.77(290.87-889.77)	0.26±1.06
	72h	470.18(244.87-716.09)	0.26±1.16
P. gossypiella	24h	412.99(136.03-708.33)	0.25±0.89
	48h	311.73(11.75-522.19)	0.26±1.01
	72h	195.44(21.68-389.30)	0.26±0.86

Biological effects of the nanoemulsion formulation against *E. insulana* and *P. gossypiella*:
Survived larvae from treated with the LC_{50} values were inspect for latent effects on some biological aspects compared to control. Data presented in Table (3) showed that, the prepared nanoemulsion formulation has been affected against newly-hatched larvae of *P. gossypiella* and *E. insulana*. It was appeared that, the rate of larval duration of *P.gossypiella* was increased than *E. insulana*. These values were 23.18**±**6.81and 21.82**±**4.62 days, respectively as compared to the control. While the rate of pupal duration was increased in case of *E. insulana* than *P.gossypiella*. The values were 9.45**±**3.059 and 7.55**±**0.57 days, respectively. The larval and pupal weight was decreased as compared to the control due to the effect of nanoemulsion formulation on the newly-hatched larvae of *P. gossypiella* and *E. insulana*. These results are in accordance with those obtained by Sabbour and Abd El-Aziz (2002) mentioned that eucalyptus oil caused retarded larval development.

Table 3. Latent effects of the nanoemulsion formulation on some biological aspects of *E. insulana* and *P. gossypiella*.

Tested Compound	Test insect	Larval duration		Pupal duration	
		Larval period	Weight	Pupal period	Weight
		Mean± SE	Mean± SE	Mean± SE	Mean± SE
Formulation	***E. insulana***	21.82**±**4.62	0.058**±**0.0124	9.45**±**3.059	0. 041**±**0.009
Control		15.44**±**8.830	0.186**±**0.271	7.17**±**6.51	0.05**±**0.006
Formulation	***p. gossypiella***	23.18**±**6.81	0.019**±**0.010	7.55**±**0.577	0.018**±**0.005
Control		14.05**±**11.93	0.048**±**0.077	4.68**±**14.85	0.035**±**0.006

CONCLUSION

Eucalyptus oil could be successfully formulated in the form of nanoemulsion. Physical properties of the prepared nanoemulsion formulation such as pH, surface tension, refractive index, flash point, mean droplet size and polydispersity index were studied. The prepared nanoemulsion presented low polydispersity 0.106 and mean droplet size 8.003 nm. Also, the results demonstrate the prepared nanoemulsion formulation exhibited insecticidal activity against newly hatched larvae of *P. gossypiella* and *E.insulana*.

The findings of the present study suggested that eucalyptus oil nanoemulsion can be used as a safe and effective alternative in the control of *P. gossypiella* and *E.insulana* larvae.

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