

# Toxicity of Some Plant Oil Nanoemulsions to Black Cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae)

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**Abstract** The insecticidal activity of some botanical oils on the black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae) was evaluated in laboratory assays. Oils of jojoba, garlic, and pumpkin plants were prepared in the form of nanoemulsion. Second and fourth instars larvae were treated with original oils and their nanoemulsions via the leaf dipping technique. The results revealed that 5% nanoemulsion oils were significantly more effective than original forms. The average size of the nanoparticles of effective nanoemulsion oils was 185.4, 362.9, and 281.5 nm for jojoba, garlic, and pumpkin, respectively. The obtained data indicated the nanoemulsion of jojoba oil was the most effective against 2nd and 4th instars larvae, causing 100% and 60% mortality after 2 days of the treatment at concentration of 5% for 2nd and 4th instars larvae, respectively. It was concluded that the formulated nanoemulsion of these botanical oils can be used as an effective alternative to commercial pesticide formulations to control *A. ipsilon* larvae.

**Keywords** black cutworm; plant oils; bioassay nanoemulsion

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## Introduction

The black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is the most destructive insect pest in Egypt. This species is a polyphagous and dangerous pest that attacks a wide range of vegetable and field crops, chewing seedling stems above soil surface, causing direct damage to the plant [1]. In the last 40 years, control of this pest has relied exclusively on synthetic conventional insecticides, especially organophosphates and pyrethroids [2]. As an outcome of such extensive use of broad-spectrum synthetic chemicals, this insect has started developing

resistance [3]. In addition, the control of agricultural pests by insecticides leads to adverse effects on beneficial insects, fish, and wildlife, as well as hazard to humans and animals by environmental pollution and residues in foods [4–7]. In previous years, research was concentrated on developing safer insecticides. Plant extracts were considered one of the richest sources which could be used as pest control agents. The natural products of plant origin are receiving considerable attention to avoid the different disadvantages of conventional insecticides, as they are mostly nonhazardous, easy to use, and specific in their action [8]. Plant extracts can be used as toxicants, repellents, synergists, growth regulators, and antifeedants to control the black cut worm [5,9].

Recent research in chemistry and material science has produced mastery in nanoparticle technology, with a wide range of different applications in the field of agriculture. Nanotechnology is the manipulation or self-assembly of individual atoms, molecules, or molecular clusters into structures to create materials and devices with new or vastly different properties. Nanotechnology can work from the top down or the bottom up. In recent years, there has been considerable interest in exploring the potential of nanotechnology in encapsulation at the nanoscale (1 nm =  $10^{-9}$  m). Nanoencapsulation is a process of coating nanoparticles such as insecticides using biodegradable polymeric matrix material [10]. Nanoparticles have a major challenge due to the extremely small size, high surface energy, and increased surface area. These characteristics could comprise possible advantages as faster dissolution, improved penetration, reduced active amount or better distribution of small amounts of active substances over a larger area [11].

Nanoemulsions are kinetically stable emulsions that contain water and oil domains separated by surfactant and sometimes using co-surfactant. They are also called submicron or ultrafine or miniemulsions [12]. Nanoemulsions serve as an attractive target of delivery systems for the pharmaceutical, food, and cosmetic industries [13,14].

The application of nanoemulsion is due to its advantages over conventional emulsions, such as its high stability and low turbidity, which makes it an attractive delivery system. Kamogawa et al. [15] and Jafari et al. [16] reported the use of mega sonic irradiation (i.e., frequency in the range of megahertz) on the formation of nanoemulsions. This technique requires less surfactant or no surfactant to produce transparent nanoemulsions. Practically, this system would be highly unstable, leading to droplet coalescence, phase separation, and possibly surfactants being needed for the formation of the nanoemulsion. The properties of the nanoemulsions are prominent because the reduced droplet size at the interface is highly significant. The unique physicochemical properties of the nanoemulsion system have led to its increasing use in different fields such as medicine, agriculture, food, and cosmetics.

This study aimed to evaluate the use of the low dangerous products (some botanical oils) in the form of nanoparticles against the black cut worm, *A. ipsilon* larvae.

## Materials and Methods

### Tested Insect

The black cutworm, *A. ipsilon* strain used in the present study was taken from a field colony raised in the laboratory of the Bioassay Department, Central Agricultural Pesticide Laboratory, Dokki, Giza, Egypt, since 2014. This strain has been kept under constant laboratory conditions at  $25 \pm 2$  °C and  $70 \pm 5\%$  relative humidity and away from any intentional chemical pressure. Laboratory tests revealed its susceptibility. The eggs were kept separately in 400 mL jars until hatching. Newly hatched larvae were kept in new jars and fed on castor bean leaves; *Ricinus communis* for larval feeding. The larvae were reared individually once they had reached 4th instar in a separate unit of plastic cell tray (10.8 × 22.7 cm) to avoid cannibalism until pupation. The pupae were then placed in glass jars until adult emergence.

## Botanical Oils Used

Three plant oils (jojoba, garlic, and pumpkin) (Table 1) were purchased from the National Research Centre, Dokki, Giza, Egypt. Each oil sample was prepared by adding the oil to emulsifier solution which contained 1 mL of Tween 20 + 999 mL of distilled water [17–19]. Using emulsifier solution helps to prevent oil particle agglomeration. Different concentrations of 10%, 5%, 2.5%, and 1.25% of each oil solution were prepared.

**Table 1.** Family, scientific name, and common name of tested plants.

Family	Scientific Name	Common Name
Simmondsiaceae	<i>Simmondsia chinensis</i> (Link)	Jojoba
Amaryllidaceae	<i>Allium sativum</i> L.	Garlic
Cucurbitaceae	<i>Cucurbita pepo</i> L.	Pumpkin

## Synthesis and Characterization of Nanoemulsions

### Synthesis of Nanoparticles

The oil emulsions prepared as mentioned above were transformed to nanoemulsion using a high-power ultrasonicator probe (55 Watt ultrasonication) for 2 min and then stored at 4 °C for further bioassays.

### Size Characterization of Nanoparticles

The nanoparticles size and their distribution were analyzed with Zetasizer (Malven). This detection was carried out in Agriculture Research Center—Nanotechnology and Advanced Materials Central Lab (NAMCL), Giza, Egypt.

## Bioassay Tests

The leaf dipping technique was used to evaluate the efficacy of plant oils and prepared nanoemulsions of them against second and fourth instars larvae of the black cutworm *A. ipsilon*. Leaves of castor bean (*R. communist*) were dipped in the abovementioned concentrations of each plant oil for 15 s and left to dry on filter papers at room temperature (23 °C). Then, the treated leaves were placed at the bottom of glass jars (1 kg) with a filter paper and covered with muslin. The same step was repeated with the use of solvent or distilled water only as a positive and negative control. Thirty larvae were placed in the jars with three replicates; the larvae were fed on treated leaves for 24 h. Then, they were fed on fresh untreated leaves until the end of the experiment. Thereafter, the desired exposure time of mortality assessment was performed and corrected based on [20].

## Results

### Characterization of Plant Oil Nanoemulsions

The average size of the nanoparticles of the most effective nanoemulsions (5%) was 185, 362, and 281 nm for jojoba, garlic, and pumpkin plant oil, respectively. The results showed that a jojoba nanoemulsion was the smallest particle size, followed by pumpkin and garlic nanoparticles. Their size and distribution are shown in Tables 2 and 3 and Figures 1–3.

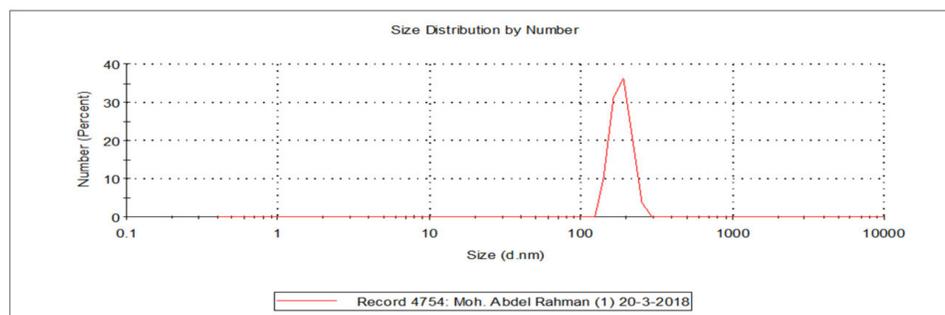


Figure 1. Particle size and zeta potential of jojoba oil nanoemulsion.

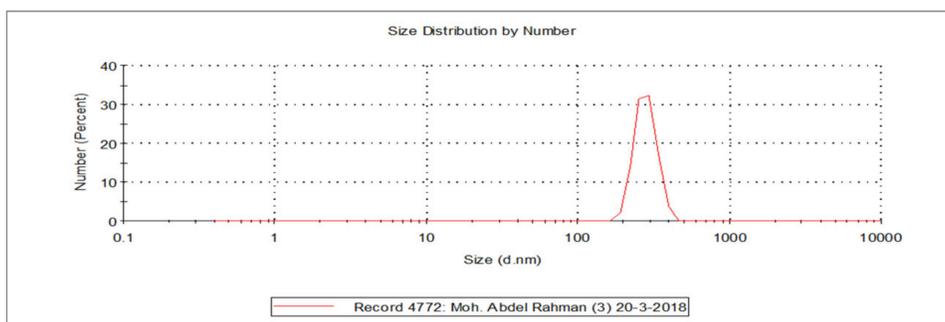


Figure 2. Particle size and zeta potential of garlic oil nanoemulsion.

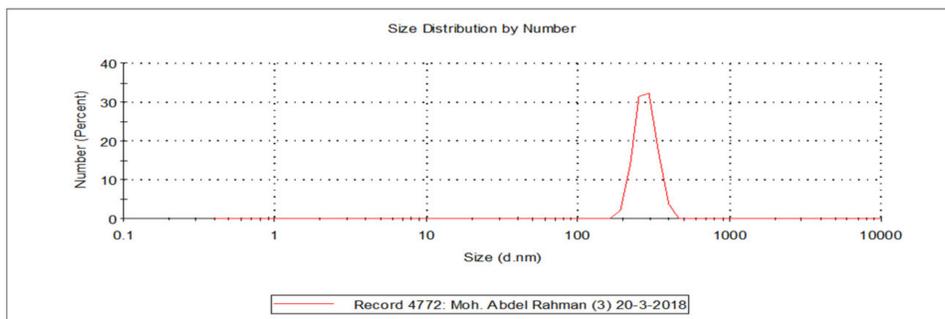


Figure 3. Particle size and zeta potential of pumpkin oil nanoemulsion.

**Table 2.** Physicochemical characterization of plant oils.

Parameters	Plant Oils		
	Jojoba	Garlic	Pumpkin
Droplet diameter (nm)	00	00	00
pH	00	00	00
Temperature	25.5	25.3	25.4
Viscosity	2.25	4.13	2.31
Absorbance	00	00	00

**Table 3.** Physicochemical characterization of oil nanoemulsions.

Parameters	Plant Oils		
	Nanojojoba	Nanogarlic	Nanopumpkin
Droplet diameter (nm)	185	362	281
pH	6.08	5.67	6.90
Temperature	22.7	21.8	21.7
Viscosity	14.74	14.23	16.26
Absorbance	2.99	3.031	3.031
PDI	0.991	0.836	1.00
Zeta Potential	40.1	34.4	35.1

## Toxic Effect of Plant Oils and Their Nanoemulsion against the 2nd Instar Larvae of *A. ipsilon*

The results showed that larval mortality was increased by increasing the plant oil concentration and period of exposure. The mortality percentages were 60%, 33.3%, and 3.33% after 2 days of exposure and increased after 7 days of treatment to 61.0%, 43.3%, and 20% for jojoba, garlic, and pumpkin oils (at the highest concentration of 10%), respectively (Table 4). Furthermore, the data also indicated that jojoba oil had the highest effect among the oils, followed by garlic and pumpkin oils.

**Table 4.** Toxicity of the plant oils against the 2nd instar larvae of *Agrotis ipsilon*.

Botanical Oil	Conc. % (v/v)	Accumulative Mortality % after Indicated Days			
		2	3	5	7
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Jojoba	10	60.0 ± 3.00	60.0 ± 3.00	60.0 ± 3.00	60.0 ± 3.00
	5	43.3 ± 0.33	43.3 ± 0.33	50.0 ± 1.53	50.0 ± 1.53
	2.5	36.67 ± 1.20	40.0 ± 0.58	43.3 ± 0.33	43.3 ± 0.33
	1.25	33.3 ± 1.67	36.67 ± 1.20	43.3 ± 0.33	43.3 ± 0.33
Garlic	10	33.3 ± 1.67	40.0 ± 0.58	40.0 ± 0.58	43.3 ± 0.33
	5	20.0 ± 0.58	23.3 ± 1.45	23.3 ± 1.45	23.3 ± 1.45
	2.5	13.3 ± 0.88	20.0 ± 0.58	20.0 ± 0.58	20.0 ± 0.58
	1.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 0.33
Pumpkin	10	3.33 ± 0.33	13.3 ± 0.88	16.67 ± 0.33	20.0 ± 0.58
	5	3.33 ± 0.33	10.0 ± 0.58	10.0 ± 0.58	10.0 ± 0.58
	2.5	0.00 ± 0.00	6.67 ± 0.67	6.67 ± 0.67	10.0 ± 0.58
	1.25	0.00 ± 0.00	3.33 ± 0.33	6.67 ± 0.67	6.67 ± 0.67
Control	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Jojoba oil in the form of nanoparticles proved that it came in the first category, causing 100% mortality at 5% and 2.5% concentration after 7 days of treatment. The obtained results also showed that nanoparticles of garlic oil came in the second position, producing 96.6%–16.6% mortality at its different concentrations. Pumpkin nanoemulsion was the lowest one, producing 33.3% of mortality at the higher concentration after 7 days of treatment (Table 5).

**Table 5.** Toxicity of nanoemulsion of the plant oil against the 2nd instar larvae of *A. ipsilon*.

Botanical Oil	Conc. % (v/v)	Accumulative Mortality % after Indicated Days			
		2	3	5	7
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Jojoba	5	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
	2.5	90.0 ± 0.58	90.0 ± 0.58	100 ± 0.00	100 ± 0.00
	1.25	46.6 ± 2.40	53.3 ± 2.67	56.6 ± 2.85	60.0 ± 3.00
	0.625	13.3 ± 0.88	16.6 ± 0.88	16.6 ± 0.88	16.6 ± 0.88
Garlic	5	93.3 ± 0.33	96.6 ± 0.33	96.6 ± 0.33	96.6 ± 0.33
	2.5	50.0 ± 1.53	56.6 ± 2.85	56.6 ± 2.85	60.0 ± 1.00
	1.25	40.0 ± 0.58	43.3 ± 0.33	50.0 ± 1.53	50.0 ± 1.53
	0.625	6.60 ± 0.67	6.60 ± 0.67	10.0 ± 0.58	16.6 ± 0.33
Pumpkin	5	23.3 ± 1.45	23.3 ± 1.45	26.6 ± 1.20	33.3 ± 1.67
	2.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.60 ± 0.66
	1.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.30 ± 0.33
	0.625	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Control	0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

### Toxic Effect of Plant Oils and Their Nanoemulsions against the 4th Instar Larvae of *A. ipsilon*

The result revealed that the mortality was consistent with an increase of the plant oil concentration and time period of exposure. At the highest concentration of 10%, mortality was 20%, 30%, and 3.33% after 2 days of exposure and increased to 46.6%, 31%, and 33.3% for jojoba, garlic, and pumpkin oils after 7 days of treatment, respectively.

The obtained results indicated that jojoba oil had the higher mortality, and it was more effective than other oils (Table 6). The toxicity effect of nanoemulsion particles of plant oils against the 4th instar larvae of *Agroitis ipsilon* is shown in Table 7. Jojoba and garlic nanoparticles recording 76.7% mortality at concentration of 5% after 7 days of treatment, but pumpkin nano oil produced 73.3% of mortality at the same concentration and after the same period of treatment.

**Table 6.** Toxicity of some plant oils against 4th instar larvae of *A. ipsilon*.

Botanical Oil	Conc. % (v/v)	Accumulative Mortality % after Indicated Days			
		2	3	5	7
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Jojoba	10	43.33 ± 0.33	46.67 ± 2.40	46.67 ± 2.40	46.67 ± 2.40
	5	30.0 ± 1.67	33.33 ± 1.67	43.33 ± 0.33	43.33 ± 0.33
	2.5	20.0 ± 0.58	23.33 ± 1.45	36.67 ± 1.20	43.33 ± 0.33
	1.25	3.33 ± 0.33	16.67 ± 0.88	36.67 ± 1.20	36.67 ± 1.20
Garlic	10	30.0 ± 1.67	30.0 ± 1.67	30.0 ± 1.67	30.0 ± 1.67
	5	26.67 ± 1.20	26.67 ± 1.20	26.67 ± 1.20	30.0 ± 1.67
	2.5	6.67 ± 0.67	16.67 ± 0.88	23.33 ± 1.45	26.67 ± 1.20
	1.25	6.67 ± 0.67	16.67 ± 0.88	20.0 ± 0.58	20.0 ± 0.58
Pumpkin	10	3.33 ± 0.33	33.3 ± 1.67	33.3 ± 1.67	33.3 ± 1.67
	5	3.33 ± 0.33	20.0 ± 0.58	20.0 ± 0.58	20.0 ± 0.58
	2.5	3.33 ± 0.33	13.33 ± 0.88	13.33 ± 0.88	13.33 ± 0.88
	1.25	0.00 ± 0.00	3.33 ± 0.33	3.33 ± 0.33	3.33 ± 0.33
Control	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

**Table 7.** Toxicity of nanoemulsion particles of the plant oils against 4th instar larvae of *A. ipsilon*.

Botanical Oil	Conc. % (v/v)	Accumulative Mortality % after Indicated Days			
		2	3	5	7
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Jojoba	5	60.0 ± 3.00	70.0 ± 1.00	76.67 ± 0.67	76.67 ± 0.67
	2.5	36.67 ± 1.20	43.33 ± 0.33	46.67 ± 2.40	60.0 ± 0.58
	1.25	26.67 ± 1.20	26.67 ± 1.20	33.33 ± 1.67	43.3 ± 0.33
	0.625	20.0 ± 0.58	20.0 ± 0.58	23.33 ± 1.45	36.67 ± 1.20
Garlic	5	66.67 ± 1.45	73.33 ± 2.19	76.67 ± 0.67	76.67 ± 0.67
	2.5	20.0 ± 0.58	30.0 ± 1.67	30.0 ± 1.67	40.0 ± 0.58
	1.25	23.33 ± 1.45	23.33 ± 1.45	33.33 ± 1.67	36.67 ± 1.20
	0.625	13.33 ± 0.88	16.67 ± 0.88	16.67 ± 0.88	33.3 ± 1.67
Pumpkin	5	66.67 ± 1.45	70.0 ± 1.00	73.33 ± 2.19	73.33 ± 2.19
	2.5	30.0 ± 1.67	33.33 ± 1.67	36.67 ± 1.20	36.67 ± 1.20
	1.25	6.67 ± 0.67	6.67 ± 0.67	10.0 ± 0.58	10.0 ± 0.58
	0.625	3.33 ± 0.33	3.33 ± 0.33	6.67 ± 0.67	6.67 ± 0.67
Control	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

## Discussion

The obtained results of oil Nanoemulsion showed that the particulation of plant oils in the form of nanoparticle emulsions was more effective against treated insects and minimized the concentration to its half. Nanoparticles can cover more and larger surface areas with particles of insecticides. Further, nanoparticles have a higher ability to penetrate the target protected plants. These characters make these products more effective against insect pests attacking the plants [21,22]. The efficacy of essential oils varies according to the phytochemical profile of their plants. The mortality of target insects may be due to the effect of fatty alcohols as well as hydrocarbons and sterols on the cuticle and/or may be due to the disturbance of the hormonal regulation caused by sterols. The mortality was increased with the existence of the fatty acids. Al-Sharook et al. [23] mentioned that the death of treated

insects caused by plant extracts may be due to their inability to swallow sufficient volumes of air to split the old cuticle and expand the new one during ecdysis, or to a metamorphosis-inhibiting effect of the plant extract, which is possibly based on the disturbance of the hormonal regulation. Essential oils are very complex natural mixtures and can contain about 20 to 60 compounds at different concentrations, characterized by two or three major components at fairly high concentrations compared to other components present in trace amounts. Generally, these major constituents determine the biological properties of the essential oils. Terpenes and terpenoids are the most representative molecules, constituting 90% of the essential oils, and allow a great variety of structures with diverse functions [24]. Among components of essential oils, terpenes, especially monoterpenoids, have been shown to be toxic to a variety of insects.

Abd El-Rahman [25] mentioned that jojoba oil caused 83.8% and 90.8% mortality against *Liriomyza trifolii* larvae at 0.5% and 1%, respectively. In the same subject, the authors of [26] revealed that jojoba oil formulation was a potent agent against both white fly and leafhopper species, where the LC<sub>50</sub> was 5.4% for *Bemisia tabaci* and 6.4% for *Empoasca discipiens*, respectively. The potential of garlic oil and its main compounds to control the *Tenebrio molitor* in starches and stored products was great. In order to prevent or retard the development of insecticide resistance, the toxicity and repellency effects of garlic oil and its toxic compounds on *T. molitor* show that it can be used individually or as part of a mixture to manage populations. The lethality of diallyl sulfide and diallyl disulfide on *T. molitor* may have advantages through their mode of action on this insect. These findings show that the compounds of garlic essential oil are a potential source of insecticidal compounds and warrant further exploration [27].

On the tested target species, nanomodified emulsion has a greater toxic effect at a lower concentration compared to their conventional compounds. Thus, it can be concluded that formulated oil nanoemulsions (5%) can be used as an effective alternative to commercially available formulation of oils and pesticides to control *A. ipsilon* and may be safer after more studies to evaluate their mammalian toxicity. Jojoba nanoemulsion achieved 100% mortality at 5% and 2.5% concentrations, whereas garlic oil caused 96.6% mortality at 5% concentration against the 2nd instar larvae of cut worm after 7 days of treatment.

## Conclusion

This study has provided evidence that plant oils in the form of nanoemulsion were more effective than in their traditional form. Thus, it can be concluded that jojoba and garlic oil nanoemulsion can be used as an alternative form of commercially available formulation of oils and pesticides using on *A. ipsilon* control and has a promising future in the field of plant protection and IPM programs.

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