Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Evaluation of toxicity and repellent effect of some oils with qualitative analysis of spray droplets against spiny bollworm *Earias insulana* (Lepidoptera: Nolidae) and fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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ARTICLE INFO
Article History
Received: 8/7 /2023
Accepted:20 /9/2023
Keywords

Essential oil, Captiva Prime oil and eucalyptus oil, toxicity, repellence, survived, some biological parameters, and Hand –Held laboratory sprayer.

In the perspective of studying new pesticide alternatives for evaluation, two repellent oils (Captiva Prime oil and Eucalyptus oil) were tested against neonate larvae of *Earias insulana* (Boisduval) (Lepidoptera: Nolidae) and *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae. Transaction completed using the method of spray drops technology by Hand –handheld laboratory sprayer at 0.5m spray height at a spray height of 0.5 cm and water-sensitive paper (Novartis cards). The toxic as well as repellent effects of tested compounds on the survivor and duration of larval and pupal stages of *E. insulana* or *S. frugiperda* were tested. Results showed that the Captiva Prime oil exhibited higher toxicity on direct spray where, The LC_{50} values were 1.747 and 1.134%, for *S. frugiperda* and *E. insulana* respectively, followed by Eucalyptus oil. Captiva Prime and Eucalyptus oils produced a repellent activity estimated by 25.3 and 27.0 in Captiva Prime oil and 7.0 and 5.3 in Eucalyptus oil against *S. frugiperda* and *E. insulana*, respectively. The biological studies cleared that treating *E. insulana* and *S. frugiperda*, with the two tested oils greatly affected the survival, mortality, and malformed percentages of treated larvae as well as resulted pupae with significant effects on their durations at ($P<0.05$).The toxic and repellent activities of Captiva Prime and Eucalyptus oils allow us to conclude that both presented significant values and potentials as bio-pesticides for the two economically important crop pests; *E. insulana* and *S. frugiperda*. In addition, the directed homogeneity of spray spectrum ratio ($VMD/N/cm^2$) of oils improved the mortality percentages of treated pests.

Introduction

Earias insulana (Boisduval) (Lepidoptera: Nolidae) the Egyptian stem borer, Egyptian bollworm, spiny bollworm or cotton spotted bollworm. The species was first described by Jean Baptiste Boisduval in 1833. The larvae feed on okra, cotton and hibiscus, the larva tunnels into the buds of their host plant. Later, it feeds on the bolls, which become brown and fall off. Secondary invasion by fungi and bacteria sometimes occurs (Poole, 1989 and Zahiri *et* *al*., 2013). The fallarmy worm (FAW) *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is one of the important invasive polyphagous pests, Larvae cause different damage by consuming foliage, it usually reduced to one to two per plant when larvae feed near one another, due to cannibalistic behavior and causing high economic losses in crops (Montezano *et al*., 2018).

Chemical control became dominant in the late 1940s and is still an important part of integrated pest management systems currently used. But pest problems have increased over the years and change has occurred in the methods and materials used to control them. The experiment of some compounds in research and extension programs has been very important in developing for use in control.The majority of the ecosystem services include some simple products as oils. Such as eucalyptus oil which is a complex mixture of a variety of monoterpenes and sesquiterpenes, with a great source of antioxidants, particularly flavonoids and aromatic phenols, esters, alcohols, oxides, ethers, aldehydes and ketones; however, the exact composition and proportion of which varies with species (Bakkali *et al*. 2008). Eucalyptus oil is a natural insect repellent, mainly due to its eucalyptol content, which is considerable a type of evergreen tree.

Already, essential oils have been studied for control of *S. frugiperda*. Niculau *et al.* (2013) studied the oils of plants *Pelargonium graveolens* and isolated some compounds geraniol, citral, carvone, and linalool observing high toxicity in the *S. frugiperda* pest. The essential oil of candeeiro (*Vanillosmopsis arborea*) has considerable action insecticidal on the insect vector of *Aedes aegypti* and has proven efficiency by Silva *et al.* (2017). Less attention was given to the application methods. Hindy (1992) and Dar (2020) discovered a significant variation in the spray deposit due to nozzle arrangement, spray technique, and the rate of application.

The current paper aims to evaluate oil pesticides and oil plant extract, number of droplets, and droplet sizes produced by Hand-Held laboratory sprayer, essays against two major pests *E. insulana* and *S. frugiperda*.

Materials and methods

1. Rearing culture of *Earias insulana*:

The susceptible strain neonate larvae of spiny bollworm (SBW), *E. insulana* used in the experiment, it was obtained from laboratory strain culture, which rearing in Bollworms Research Department, Plant Protection Research Institute reared for several generations away from any contamination with insecticides on an artificial diet that previously described by Amer (2015).

2. Collection and rearing culture of *Spodoptera frugiperda*:

The different instar larvae from (1st to 6th instar larvae) and pupal stage of *S. frugiperda* were collected from corn field in El-Fayum Governorate, during the Summer session (May to September 2022). All field population pests were placed in bottles and transferred to the laboratory. Different larval instars were separated in glass tubes and fed on corn, this population was reared at 26 ± 1 C, 60 ± 5% RH. The larvae were provided with a natural diet (Corn) until pupation. The formed pupae were shifted in tubes (3.5x 12 cm) with tissue paper until the emergence of adults. The eggs laid by female were collected and incubated until larvae hatched for using the first instar larvae in experiments. All cultures were reared under controlled conditions 26 ± 1 °C and 60 ± 5% RH.

3. Oils used:

3.1. Captiva Prime oil; as a synthesized oil: combination of plant extracts.

Active ingredients:

Capsicum oleoresin extract, 7.6%,

Garlic oil-3.4%

Canola oil*55.0%

Another ingredients*14%

3.2. Essential Oil

Eucalyptus oil (5%)

Eucalyptus (Family: Myrtaceae).

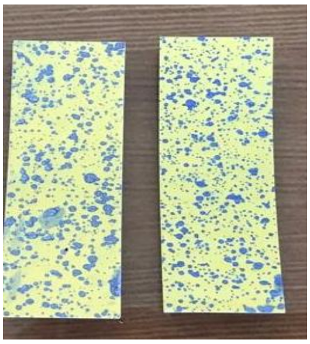
4. Prepared the oil:

To estimate the toxicity of the Captiva Prime oil and eucalyptus oil (5%) against first instar larvae of *E. insulana* and *S. frugiperda*, serial concentrations of (1.211, 0.515, 0.257, 0. 123, and 0.061 ppm) for Captiva Prime oil and (20, 10, 5 and 2.5 ml-100ml water) for eucalyptus oil **(**5%) were prepared**.**

5. Toxicity assessment:

By Spray technic method used by Hand –Held Laboratory sprayer at 0.5m spray height, Water sensitive paper (Novartis cards) was fixed in each treatment to determine both number of droplets per square centimeter and volume mean diameter (VMD) from the resultant droplets at 28^{0}C, and 42% RH. to evaluate the spray coverage of this sprayer (Figures 1-3).


(1)


(2)

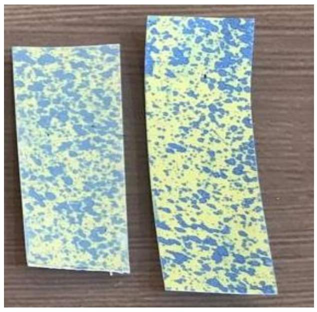

(3)

Figure (1): Water sensitive paper collecting spray droplets of water.
Figure (2): Water sensitive paper collecting spray droplets of synthesized oil (EOs **Oil) of Captiva Prime.**
Figure (3): Water sensitive paper collecting spray droplets of eucalyptus oil.

The toxicity assessment for each Captiva Prime oil and eucalyptus oil (5%) oil under the laboratory is important for determining the potential of synthesized or natural oil, it is called an *in-vitro* assay. For estimated the toxicity of two tested oils, against first instar larvae of *E. insulana* or *S. frugiperda* larvae:

For each **c**oncentration/treatment, sixty 1^{st} instar larvae (0-24hrs). were used for each pest; the numbers were divided into three replicates, each replicate containing twenty ***E.*** *insulana* or *S. frugiperda* larvae. *S. frugiperda* larvae were transferred for feeding on parts of plant corn spray by each concentration/ oil. At the same time *E. insulana* were transferred for fed on diet sparing by each concentration/oil treatment (Figure 1) and the same number of larvae fed on corn without treatment as a control. It was kept under constant conditions of 25 ± 1°C and 60-65 % RH.

6. Effect of oil compounds used on some parameters assay:

After estimating the LC_{25}, LC_{50} and LC_{90} values for each Captiva Prime oil and eucalyptus oil (5%) tested oil; LC_{50} values for both oils were prepared and the newly hatched larvae of *E. insulana* alive results from each treatment were transferred individually to the diet tubes (2.5 x7.0 cm) each containing about 3 gm artificial diet (Without any treatment) by camel hairbrush and another group of larvae used as a control. All groups were kept under the constant conditions of 27±1°C and 60-65 %RH. and observed daily until pupation.

The following biological parameters were investigated as follows:

6.1. Larval stage, Larval duration in days, Percent of larval mortality.

% Larval mortality = No. dead larvae/ Total tested larvae X 100

6.2. Pupal stage: Pupal duration in days, Percent of pupal mortality.

% pupation = No. produced pupal/ Total tested larval X 100

Bioassay data, including estimate toxicity at mean lethal concentration ($LC_{25,}$ LC_{50} and LC_{90}) values (Finney, 1971). Results were correct for control mortality by using Abbott's (1925), and their 95% confidence limits were calculated from probit regressions using the computer program. LC_{50} values of specific two oils against two pests *E. insulana* and *S. frugiperda* treated were considered significantly different ($P < 0.05$).

Results and discussion

1. Evaluation of tested Captiva Prime and eucalyptus oils compounds against *Earias insulana* and *Spodoptera frugiperda* treated as 1st instar larvae:

1.Toxicity assessment:

Data as shown in Table (1) show that the LC_{25}, $LC_{50,}$ and LC_{90} values of Captiva Prime oil treating against both newly hatched larvae of *E. insulana* and *S. frugiperda* under laboratory conditions. The LC_{50} values compound is considered the highest potency and is more toxic against *E. insulana* than *S. frugiperda*. The LC_{25}, $LC_{50,}$ and LC_{90} values were estimated at (0.529, 1.134 and 7.263) respectively for *E. insulana* and, (0.681, 1.747 and 9.805), respectively, for *S. frugiperda*. While LC_{50} values for natural eucalyptus oil were estimated at 8.7% and 10.5% against both newly hatched larvae of *E. insulana* and *S. frugiperda* sprayed by technic method used; Hand-Held Laboratory sprayer at 0.5m spray height, Water-sensitive paper (Novartis cards) was fixed in each treatment.

Table (1): Toxicological evaluation of Captiva Prime and eucalyptus oils compounds against the two pests *Earias insulana* and *Spodoptera frugiperda* under laboratory conditions of (26.0 ± 1.0 °C and 75.0 ± 5.0% RH.).

Compound used	Kind of insects	LC (ppm)			
		LC_{25}	LC_{50}	LC_{95}	Slope ± SE
Captiva Prime oil	*Spodoptera frugiperda*	0.681	1.747	9.805	2.1±0.198
	Earias insulana	0.529	1.134	7.263	2.039 ±0.216
Eucalyptus oil	*Spodoptera frugiperda*	7.3	10.5	53.0	1.55±0.2
	Earias insulana	5.0	8.7	40.0	2.08 ±0.3

2. Repellents and biological immature stages:

The initial number used from spiny bollworm and fall armyworm did not successfully complete their development to the adult stage with Captiva Prime oil or eucalyptus oil treated, the larval greatly affecting with more specifically, significant differences various were recorded in developmental parameters (Table 2).

3. Repellents *Spodoptera frugiperda and Earias insulana* with two oils:

The repellence with Captiva Prime oil or eucalyptus oil treated was presented in (Table 2). Results show that after contact with each pest with the surface, highly significant differences were observed in the percentage of repellence individuals for both insects' exposure to Lc_{50} values. Both pests left the diet treated on corn or in the Petri dish for both treatments. It was recorded 25.3 and 27.0 with Captiva Prime oil and 7.0 and 5.3 with eucalyptus oil-treated larval stages of *S. frugiperda and E. insulana*, respectively (Table 2).

4. Duration of immature stages:

Concerning the effects of the LC_{50} values for two tested oils on the larval duration of *E. insulana* and *S. frugiperda*, it was noticed generally that the larval duration period increased with LC_{50} of two oils, where the average durations were 24.33±1.7 and 18.0±1.5 days with Captiva Prime oil and 21.3±1.4 and 17.3±1.6 days, respectively, when treated larval stages of *S. frugiperda* and *E. insulana* with eucalyptus oil, compared with 17.3±0.8 days/ larvae of S. *frugiperda* and14.3±0.5 *days E. insulana* (Table 2). Statistically, there are significant differences between the values of larval durations at the two treatments. Table (2) recorded the effects of two oils Captiva Prime and eucalyptus compounds on larval mortality and malformed of *S. frugiperda and E. insulana*. The two oils Captiva Prime oil treated increased the % larval mortality, it recorded by 61.0 and 73.3 % larvae of S. *frugiperda* and *E. insulana*, respectively, and 59.0 and 63.3 with eucalyptus, compared with untreated check (4.6 and 6.6 %). Moreover, larvae malformation (%) significantly increased to 6.3, and 7.0% with Captiva Prime oil and 3.3 and 1.3, respectively, different from the untreated check (0.00).

Table (2) Impact of two oils Captiva Prime and eucalyptus compounds on biological aspects of larval stage *Spodoptera frugiperda* and *Earias insulana.*

		Impact of Captiva Prime							
	Variables	***Spodoptera frugiperda***				***Earias insulana***			
		Untreated	Treated		**LSD**	**Untreated**	**Treated**		**LSD**
		Mean± SE (Rang)	**Rang**	**Mean± SE**		**Mean± SE(Rang)**	**Rang in days**	**Mean± SE**	
Captiva Prime oil	**Larval duration in (days)**	17.3±0.8 (15-18)	21-27	24.33±1.7	1.336	14.3±0.5 (13-16)	16 - 20	18.0±1.5	1.106
	% Larval mortality	4.6±0.3 (2-5)	57-71	61.0±7.1	7.181	6.6±0.5 (5-8)	68 - 83	73.3±3.4	10.215
	Larval survival	95.4±4.8 (85-98)	43-20	33.3±2.8	9.715	93.4±1.5 (92 – 95)	9 - 12	10.3±0.8	6.054
	% Larval malformed	0.0	5-8	6.3±0.8	0.118	2.0±0.1 (0-4)	5 - 9	7.0±1.2	0.351
repellence		0.0	21-30	25.3	6.191	0.0	19 - 39	27.0±4.1	3.780
Impact of eucalyptus oil									
	Variables	***Spodoptera frugiperda***				***Earias insulana***			**LSD**
		Untreated	**Treated**		**LSD**	**Untreated**	Treated		
		Mean± SE(Rang)	**Rang**	**Mean± SE**		**Mean± SE(Rang)**	**Rang in days**	**Mean± SE**	
Eucalyptus oil	**Larval duration in (days)**	17.3±0.8	19-24	21.3±1.4	0.667	14.3±0.5a (13-16)	16-21	19.3±1.6b	0.869
	% Larval mortality	4.6±0.3	54-63	59.0±2.6	9.271	6.6±0.5 a (5-8)	57-66	63.3±1.2b	11.210
	Larval survival	95.4	35- 42	39.0±1.3	13.31	93.4±1.5b (92 – 95)	43-39	39.6±1.7a	7.581
	% Larval malformed	0.0	1- 5	3.3±0.6	0.88	2.0±0.1b (0-4)	0 - 3	1.3±0.1a	0.032
Repellence		**0.0**	0.0	7.0±0.3	1.44	0.0a	0.0	5.3±0.2b	0.311

5. Pupal duration and mortality %:

The biological studies clearly demonstrated the great effect of both tested oils on the survival and mortality percentages of *E. insulana* and *S. frugiperda* resulting in pupae stage with significant effects on their duration (Table, 3). Captiva Prime and Eucalyptus compounds decreased the pupation times duration to 14.0±0.9 and 10.3±0.6 days respectively, With Captiva Prime oil and 12.6±1.1and 8.6±03days with Eucalyptus oil for *S. frugiperda and E. insulana* compared with the untreated (9±0.31and 7.0±0.4 days). Moreover, Pupa mortality (%) significantly increased to 15.0 and 16.2 % for *S. frugiperda and E. insulana* respectively, in Captiva Prime oil treatment and to 7.4 and 7.7% respectively in Eucalyptus oil treatment, differently compared to3.3 and 2.3 % in the untreated for both insects, respectively.

Table (3): Impact of Captiva Prime and eucalyptus oil compounds on pupal stage *Spodoptera frugiperda* and *Earias insulana.*

Variables		Impact of Captiva Prime							
		Spodoptera frugiperda				*Earias insulana*			LSD
		Untreated	Treated			Untreated	Treated		
		Mean± SE (rang)	Rang	Mean± SE	LSD	Mean± SE (rang)	Mean± SE (rang)	Mean± SE	
Captiva Prime oil	Pupal duration in (days)	9±0.3a (7-11)	11-16	14.0±0.9b	2.53	7±0.4a (6-9)	10-13	10.3±0.6	0.361
	% Pupal mortality	3.3±0.1a (0.4)	13-17	15.1±1.1b	4.31	2.3±0.1 (1.5)	16-20	17.6±1.2	5.17
	Pupal survival	96.7b (96-100)	83-90	86. ±2.1a	6.01	95-99	87-90	87.6±2.6	3.614
Impact of eucalyptus oil									
eucalyptus oil	Variables	*Spodoptera frugiperda*				*Earias insulana*			
		Untreated	Treated		LSD	Untreated	Treated		LSD
		Mean± SE (rang)	Rang	Mean± SE		Mean± SE (rang)	(rang)	Mean± SE	
	Pupal duration in (days)	9±0.3b (7-11)	9-17	12.6±1.1b	0.721	7.0±0.4a (6-9)	8-10	8.6±03a	1.31
	% Pupal mortality	3.3±0.1a (0.4)	7-9	7.6±0.3b	1.530	2.3±0.1a (1.5)	6-11	9.0±0.6b	3.011
	Pupal survival	96.7b (95-97)	91-93	91.6±0.6a	2.411	95-99	89 – 94	92.3±1.3	1.041

6. Effect of oil droplet size on toxicity:

Results presented in Table, (4) and Figures (1-3) indicate the relationship between laboratory spray quality and toxicity values and larval mortality by the Hand –Held Laboratory sprayer. According to Table (4), there is a difference in the size of the spray drops for each tested oil. A satisfactory coverage was obtained under laboratory experiments on Corn plants or on diet (Figures 4-7), and the optimum droplet sizes were agreed with the droplet spectrum for controlling insects of *S. frugiperda* and *E. insulana.* It should be sized between 135 and 161 µm (VMD) with a number not less than 131 droplets/cm^2.

Table (4): Qualitative analysis of spray droplets.

Conc. %	LC_{25}			LC_{50}		
Insecticides used	N/cm^2	VMD	SQ	N/cm^2	VMD	SQ
Eucalyptus oil (5%)	140^{b}	135^{a}	0.96	131^{a}	137^{a}	1.05
Captiva Prime oil	137^{a}	137^{b}	1	133^{b}	137^{a}	1.03
LSD	2.26	8.63	--	0.055	6.79	--

For water only; N/cm^2 =179, VMD =161
The spray height is constant ~ 0.5 meter in all treatments.
VMD= Volume mean diameter, N/cm^2= Number of droplets/cm^2
VMD/N/cm^2= Spray quality (degree of homogeneity)
Numbers followed by the same letter at the same column are not significantly different at P= 0.05.

S. frugiperda

Figure (4): Untreated immature stages.

Figure (5): Treated immature stages.

E. insulana

Figure (6): Untreated immature stages.

Figure (7): Treated immature stages.

The present result shows the bioassay; toxicity or bioassay was dependent on the type of oil used in the experiment. The mortality of *E. insulana* or *S. frugiperda* was significantly different among the tested LC_{50} for both oils and sizes of spray. However, the mortality was significantly different between *E. insulana* and *S. frugiperda* when treated with Captiva Prime oil as A major chemical compound oil and eucalyptus as a natural oil used. The high or % mortality, survival, and reduction with prolonged the duration of the *E. insulana* and *S. frugiperda* is presented in Table (2). In two treatment oils, a noticeable variation of mortality was observed with prolonged duration, due to the different active ingredients in both oils. Kandil and El- Shennawy (2018) recorded that the α-pinene had the most potent compound against neonate larvae of PBW (LC_{50} = 1.43%), followed by sesame oil (LC_{50} = 3.46%), *Thuja orintalis (*LC_{50} = 4.88%), and bug oil (LC_{50}= 6.29%). A Similar, trend appeared where LC_{95} values were 8.84, 33.5, 14.08 and 52.76 %, respectively. The geographic origin and plant organ can be the cause of such differences in LC_{50}. Rozman *et al.* (2006) indicated that pure camphor compound shows contact activity against *Sitophilus oryzae* and *Rhyzopertha dominica* but does not affect *Tr. castaneum* after 24 hrs. of exposure at 0.1 ml/720 ml. Bekele and Hassanali (2001) demonstrated the insecticidal activity of camphor EO against *R. dominica* and *Si. zeamais* is attributed to camphor and the combined effects of different components, but camphor alone does not affect rice weevil at an LC50> 100 ml/liter.

Our results show widely recognized that, when two insect pests *were exposed and* fed with Captiva Prime and eucalyptus oils compounds treated *during the entire larval stage*, they had different responses or susceptibility to the kind of oil used. It is believed that these changes are related to the inhibition of *S. frugiperda* and *E. insulana* pests, because it may be cussed the repellents, resting slowly in fed for both insects. Although the studies point clear that synthetic chemical oil is still more frequently used as repellents, followed by natural products (Essential oils) eucalyptus oil against *E. insulins* or *S. frugiperda*. Usually, insect repellents work by providing a vapor barrier deterring the arthropod from coming into contact with the surface (Brown, 1997).

Also, our results show that they significantly affected the *S. frugiperda and E. insulana* larval growth, causing a prolonged larval stage in approximately six to seven days compared to the control. In addition, elongated the pupal stage than the control (Tables 2 and 3). As previously verified *eucalyptus compounds* were those that highly affected S. frugiperda, replant, survival, and growth, justifying the increased mortality observed in this assay compared to *eucalyptus as a natural oil used* to the untreated. Batish_*et al.* (2008) recorded that eucalyptus oil possesses a wide spectrum in biological activity as insecticidal/insect repellent. Also, eucalyptus oil has also been used as an anti-feeding, particularly against biting insects (Thorsell *et al.*, 1998). Trigg and Hill (1996) reported that eucalyptus-based products are used for insect repellent. Mumcuoglu *et al.* (1996) recorded that the insect-repellent activity could be extended up to 8-days, when eucalyptus essential oils are applied. Seyoum *et al.* (2003) reported that burning leaves of *E. citriodora* provides a cost-effective method of household protection against mosquitoes in Africa. Ceferino *et al. (*2020) demonstrated the fumigant toxicity/repellent activity of essential oil from *E. cinerea*, *E. viminalis*, and *E. saligna*, against permethrin-resistant human head lice with KT 50 (Time for 50% knockdown) values of 12.0, 14.9 and 17.4 min, respectively. Homogeneous spray spectrum on the treated target in the optimum droplet size to control the cotton leaf worm in the cotton fields by ground equipment (Himel,1969 and Himel *et al*., 1969). The usage of Hand-Held Laboratory sprayer to resemble the spray in field with a number, and volume of droplets can be identified. The ratio of [VMD/N/cm^2] was 0.96, 1.05 for eucalyptus oil (5%) and 1,1.03 for Captiva Prime oil. These phenomena resulted from the physical properties of the used liquid certainly the ratio between the viscosity and the surface tension into the different concentrations used and the effected on the homogeneity, this result agreed with both (Fraser and Eisenklam, 1956 and Nordby and Skuterud, 1975). The ratio (VMD/N/cm^2) was developed toward a tendency to homogeneity of spray spectrum with increasing mortality produced by the insecticide. These results agreed with Bakr *et al*. (2004 and 2013), Dar (2020) and Alakhdar *et al*. (2021). This is the first study demonstrating the repellency and toxicity activity of EOs Captiva Prime oil or eucalyptus oil on *E. insulana* or *S. frugiperda* the results obtained in this study demonstrated that highly significant effects on some parameters such as survivor, repellent, and duration larvae and pupal stages for both insects. Positive results indicated that oil could potentially be used to develop eco-friendly control products. In additional the ratio (VMD/N/cm^2) by Hand-Held Laboratory sprayer was developed toward a tendency to homogeneity of spray spectrum with increasing mortality produced by compounds.

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