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TOXICITY AND BIOCHEMICAL EFFECTS OF MUSTARD AND NEEM OILS ON SECOND AND FOURTH LARVAL INSTARS OF COTTON LEAFWORM, *Spodoptera littoralis* (BOISD)

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ABSTRACT: In this study, mustard and neem oils trad name saif oil (0.03% EC) were tested for their insecticidal activity against 2nd and 4th instar larvae of the cotton leafworm, *Spodoptera littoralis*. Also, the biochemical changes were evaluated to compare the physiological effects between the two tested oils. Toxicity results revealed that mustard oil has low toxicity versus the neem oil with LC₅₀ values of 7.99 and 20.30 when tested against 2nd and 4th larval instars, respectively. The toxicity index were 0.0024% and 0.0014 against 2nd and 4th instar larvae, respectively. A similar trend was recorded for both neem and mustard oils in the biochemical changes of protein and transaminase enzymes activities [aspartate aminotransferase (AST), alanine aminotransferase (ALT)] which decrease in both tested 2nd and 4th instar larvae at all times intervals (1, 3, 5 and 7 days). In addition, the fluctuated effects were shown on lipids levels and amylase, invertase and trehalase activities.

Key words: *Spodoptera littoralis*, mustard oil, neem oil, insecticidal activity, biochemical effects.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis*, is one of the most destructive agricultural lepidopterous pest within its subtropical and tropical range. It is infesting over than 112 plant species belonging to 44 families due to its polyphagous feeding style (Azab *et al.*, 2001). It is known as a notoriously leaf eater accepting almost all herbaceous plants and economic crops, including cotton and many vegetable and fruit crops (Ali and Abdallah, 2018).

Becide their risk to human health, chemical pesticides have been used in cotton leafworm control for a long time (Grigoletti *et al.*, 2000) which led to the development of pest resistance and prolonging pest control process. In addition, chemical control can destroy the soil microbiota that benefit plants and causing environmental pollution (Ghorbani *et al.*, 2009). They also cause strong environmental imbalances,

distracting the natural enemies of different crop pests where they are applied.

Recently, the goal of many researchers was finding out other components from natural sources alternate to pollutant, high cost, and carcinogenic pesticides (El-Seedi *et al.*, 2017; Abd-ElAzeem *et al.*, 2019). In this regard, plant extracts showed a high potential for further development of natural pesticides. Few botanicals such as neem and mustard oils which characterized by a strong odor and plant secondary metabolites can be used as biological control agents (Bakkali *et al.*, 2008). Botanical pesticides may control the pest population through a different activity primarily through acting as an oral poison, acute toxicity, feeding deterrent, repellent, growth regulator or inhibitor reproduction.

Neem, *Azadirachta indica* Ajuss, is considered to have low toxicity for mammals, and it is efficient even with low concentrations on insects. Many studies on neem oil and derivatives had

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shown its insecticidal potential with results similar to those obtained with synthetic products (Maredia *et al.*, 1992; Martinez and Van Emden, 1999; Prates *et al.*, 2003). Like other active plant molecules, there is a lower probability of resistance development due to the complexity of its active components (Vendramim and Castiglioni, 2000). So, this work aimed to study the effect of mustard and neem oils as a potential pesticides against *S. littoralis* larval stage.

MATERIALS AND METHODS

Culture Source of the Cotton Leafworm, *S. littoralis*.

The culture of *S. littoralis* was obtained from Plant Protection Research Institute, Agriculture Research Centre (ARC), Dokki, Giza, Egypt. The culture was reared for many generations in the laboratory away of any insecticide contamination.

Used Oils

Neem oil 0.03%EC

- **Common name:** Azadirachtin
- **Neem tree:** *Azadirachta indica* Ajuss

Ministry of Agriculture, Central Agricultural Pesticide Laboratory.

Mustared oil

Extracting mustard oil

Mustard seeds (*Brassica alba* (L.) Rabenh. *Brassica hirta* Moench Family: Brassicaceae) were obtained from a local market. Seeds were dried in oven at 30°C until the weight was stabilized, then were crashed into powder. About (1 kg) of the powder was steeped at room temperature in hexane for 10 days (1L X 3). The pooled extracts were evaporated under vacuum at 50°C using rotary evaporator to yield 100g oily residue with 100% concentration.

Rearing technique

Larvae were reared on fresh castor bean leaves, [*Ricinus communis* (L.)], which were provided daily. Towards the end of the sixth instar larvae, mist saw dust was placed at the base of the rearing jars to provide a pupation sites. The formed pupae were eventually

collected and placed in clean jars until adult's emergence. The newly emerged moths were sexed in clean jars (1 Kg in capacity). Each rearing jar was provided with 10% honeybee solution soaked in cotton wool, which was tied with wire for moths feeding. The honeybee solution was renewed daily to avoid fermentation and growth of microorganisms. Fresh green leaves of tafla, *Nerium oleander* L.) were provided for egg laying. Newly laid egg-masses were collected daily and transferred in the rearing jars (El-Defrawi *et al.*, 1964).

Toxicity experiments.

Laboratory experiments were carried out in the Laboratory of Biological Control, Plant Protection Research Institute (Sharkia Branch), Zagazig, Egypt at 26±1°C and 65±5% RH.

For studying the toxicity of neem oil and mustard oil on the 2nd and 4th instar larvae of *S. littoralis* laboratory strain, serial concentrations for each oil were prepared. The concentrations prepared were 5, 10, 15 and 20% from mustard oil and multiple of the recommended dose from neem oil (5 ml/l).

Leaf dipping technique was used to estimate the larvaicidal activity of the tested oils against newly molted 2nd and 4th instar larvae of *S. littoralis*. Fresh castor bean leaves were dipped in the tested concentrations for 10 seconds. Treated leaves were left to dry. Ten larvae were allowed to feed for 48 hr. in clean glass jar. Jars were covered with a muslin cloth and a filter paper was placed in the bottom to absorb any excess moisture. Five replicates were made (50 larvae for each concentration). Five control replicates involved using leaf disks dipped in water. This trial was carried out in the incubator at 26±1°C and 65±5% RH.

Mortality counts were recorded and mortality percentages were corrected by Abbotte's formula; (Abbott, 1925) as follow:

Corrected mortality (%) = Survival in control (%) – Survival in treatment (%) ÷ survival in control (%) × 100

Also, LC₅₀, LC₉₀, slope, were calculated. Toxicity index (TI) was determined by using (Sun, 1950) as follow:

Toxicity index = LC₅₀ or LC₉₀ of the highest efficient compound ÷ LC₅₀ or LC₉₀ of the other compound × 100

Biochemical determination

The samples of survived larvae were taken after 1, 3 and 7 days of treatment at the same time to determine the total soluble protein (TSP), total lipids (TL), activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), carbohydrate hydrolyzing enzymes and phosphatase enzymes (ALP and ACP).

Preparation of samples

Larval samples used for biochemical assays were collected at 1, 3, 5 and 7 days post treatment of the 2nd instar larvae during the biological experiments; 1, 3 and 5 days post treatment of the 4th instar larvae during the joint action trials. Untreated larvae were used as control. Samples were homogenized in distilled water (50 mg/ml) using chilled glass Teflon homogenizer. Homogenates centrifuged at 5000 rpm for 20 min. at 5°C in a refrigerated centrifuge. The deposits were discarded and the supernatants were kept in a deep freezer at -20°C till use to determine the total soluble protein, the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), carbohydrate hydrolyzing enzymes (invertase, trehalase and amylase).

Determination of Total Soluble Protein

Colorimetric determination of total soluble protein in supernatants of homogenate treated larvae of *S. littoralis* was carried out as described by **Gornall et al. (1949)**.

Determination of Total Lipids Content

The total lipids estimated according to the method of **Schmit (1964)**, using kits from Diamond Diagnostics.

Determination of Enzymes Activities

Aspartate aminotransferase activity

Aspartate aminotransferase (AST) was determined colorimetrically according to the method of **Reitman and Frankle (1957)**.

Alanine aminotransferase activity:

Alanine aminotransferase (ALT) was determined colorimetrically according to the method of **Reitman and Frankle (1957)**.

Carbohydrate hydrolyzing enzymes

The method used to determine the digestion

of trehalose, starch and sucrose by trehalase, amylase and invertase enzymes, respectively were similar to those described by **Ishaaya and Swiriski (1976)**.

Statistical Analysis

All the obtained data were statistically analyzed according to one way ANOVA (Tukey's HSD test) at least significant difference $P < 0.05$ by using a software package (**Costat® Statistical Software, 2005**).

RESULTS AND DISCUSSION

Toxicity Effects

The toxic effects were recorded after 48 hr. for both oils (mustard and neem) according to their mode of action. The results in Table 1 clear the acute toxicity of mustard and neem oils on the 2nd instar larvae. The efficiency of the tested oils were observed at LC₅₀ level. Neem oil was the most toxic recorded ($1.94 \times 10^{-4}\%$) at LC₅₀ whereas, mustard oil recorded lower effect with LC₅₀, 7.99%. Same results can be obtained from Table 2 that reveal the acute toxicity on 4th instar larvae where neem oil was the most toxic with LC₅₀, ($2.86 \times 10^{-4}\%$) and mustard oil recorded lower effect with LC₅₀ (20.30%).

The toxicity index was obtained by comparing the efficiency of the mustard oil at LC₅₀ with neem oil. The strength of insecticidal activity of mustard oil was 0.0024% and 0.0014% against 2nd and 4th instar larvae, respectively compared with the remarkable efficiency of neem.

Despite, the low toxicity of mustard oil on cotton leafworm, it provided a promising botanical safe control agent as no toxicity recorded against mammals and human versus neem oil which have low toxicity for mammals (**Naumann and Isman, 1996**). Regarding to recent researches that revealed the insecticidal activities of mustard oil; **Sabbour (2010)** recorded that mustard oil is one of natural preparation active against insects, where mustard oil revealed a strong repellent activity after 7 days (89%) against *Bruchidius incarnatus* beetles. Moreover, **Paes et al (2012)** evaluated the fumigant toxicity of synthetic mustard

Table 1. Acute toxicity of mustard and neem oils on 2nd instar larvae of *S. littoralis*

Treatment	LC ₅₀ (%)	Confidence limit		LC ₉₀ (%)	Confidence limit		Slope	Toxicity index LC ₅₀
		Lower	Upper		Lower	Upper		
Mustard oil	7.99	3.25	19.62	31.19	3.39	286.40	2.16	0.0024
Neem oil	1.94×10 ⁻⁴	0.98×10 ⁻⁴	3.84×10 ⁻⁴	10.04×10 ⁻⁴	1.27×10 ⁻⁴	78.90×10 ⁻⁴	1.80	100.0

Table 2. Acute toxicity of mustard and neem oils on 4th instar larvae of *S. littoralis*

Treatment	LC ₅₀ (%)	Confidence limit		LC ₉₀ (%)	Confidence limit		Slope	Toxicity index LC ₅₀
		Lower	Upper		Lower	Upper		
Mustard oil	20.30	18.78	22.03	42.37	36.02	53.83	4.009	0.0014
Neem oil	2.86×10 ⁻⁴	1.58×10 ⁻⁴	5.14×10 ⁻⁴	11.40×10 ⁻⁴	1.80×10 ⁻⁴	71.94×10 ⁻⁴	2.13	100.0

essential oil (SMEO) (90% allyl isothiocyanate) on the developmental stages of the maize weevil (*Sitophilus zeamais*). The vapors of SMEO caused mortality of all stages of *S. zeamais*. (Konecka et al., 2018) reported novel information concerning the insecticidal activity of mustard oil (*Brassica alba*) when applied the intestinal tract via insects' diet against pests from the order Lepidoptera. Finally, Aly and Ali (2017) evaluated the LC₅₀ and latent effects; LC₂₅ of three essential plant oils; coriander, basil and mustard oils against fourth larval instar of *S. littoralis* under laboratory conditions. The LC₅₀ of mustard oil recorded 851.14 ppm.

Effects of Mustard and Neem Oils on Lipids and Proteins

Lipids contents increased significantly with treatments of neem as well as mustard oil at all-time intervals. More precisely, neem oil was more effective recording the highest lipid level as 40.09 mg/g in the 4th instar after 5 days and 28.98 mg/g in 2nd instar after 7days versus mustard oil that revealed nearly the same increase effect as 12.97 and 35.43 mg/g in 2nd instar after 1day and 4th instar after 5 days, respective (Tables 3 and 4).

On the contrary of lipids, total soluble protein showed significant decreases in both 2nd and 4th instars at all-time intervals with mustard and neem oils. Mustard was more effective on

protein levels recording the lowest concentration (2.32 mg/g) in 2nd instar after 3days. It is worth to mention that, the 2nd instar larvae were more sensitive to all treatments (Tables 3 and 4).

Regarding to the effect of time, time intervals had shown notable fluctuated levels of total lipids and protein. Same findings were reported by Khosravi, and Sendi, (2013), as they recorded same results with neem pesticide (Achook) on lipids and protein in the hemolymph of lesser mulberry pyralid, *Glyphodes pyloalis* Walker also, Huang et al. (2004) found that azadirachtin significantly influenced the protein level in *S. litura*. Another research showed that neem oil interfered with protein synthesis, in the desert locust (Annandurai and Rembold, 1993).

Contrarily, the amount of lipid was decreased by different concentrations of neem in the hemolymph of fifth instar larvae of *G. pyloalis* after feeding on treated mulberry leaves with *Artemisia annua* extract (Khosravi et al., 2010). While Zibae et al. (2011) demonstrated that with the highest pyriproxifen concentration, the amount of lipid significantly decreased in the hemolymph of *Eurygaster integriceps*. Dos Santos Silva et al. (2016) mentioned that citronella oil (*Cymbopogon winterianus* Jowittes) was effective repellent and insecticide on *S. frugiperda*, a reduction in proteins and carbohydrates were determined.

Table 3. Changes in total lipids and total soluble proteins of 2nd instar larvae treated with LC₅₀ of mustard and neem oils

Treatment	Total lipid (mg/g)				Total soluble protein (mg/g)			
	Time interval (day)							
	1	3	5	7	1	3	5	7
Mustard oil	12.97 ^a	9.95 ^b	6.92 ^b	11.04 ^c	3.55 ^d	2.32 ^c	2.66 ^d	4.09 ^c
Neem oil	10.12 ^b	9.76 ^b	6.78 ^c	28.98 ^a	4.54 ^c	3.4 ^b	4 ^b	4.59 ^c
Control	8.70 ^c	8.71 ^c	6.57 ^c	12.2 ^b	4.88 ^b	2.66 ^{bc}	2.96 ^c	6.02 ^b
Control hexane	10.37 ^b	10.30 ^a	12.53 ^a	11.00 ^c	7.06 ^a	6.46 ^a	5.57 ^a	15.55 ^a
LSD	0.49	0.56	0.64	0.44	0.33	0.78	0.16	0.85
P	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

LSD: The lowest significance differences at P < 0.05 P: Porpability

Table 4. Changes in total lipids and total proteins of 4th instar larvae treated with LC₅₀ of mustard and neem oils

Treatment	Total lipid (mg/g)			Total soluble protein (mg/g)		
	Time interval (day)					
	1	3	5	1	3	5
Mustard oil	28.50 ^b	30.13	35.43 ^c	4.68 ^c	5.62 ^c	7.84 ^c
Neem oil	29.8 ^b	32.32	40.09 ^b	6.81 ^b	10.07 ^b	6.17 ^d
Control	25.43 ^c	21.67	23.67 ^d	5.48 ^c	10.91 ^a	18.76 ^a
Control hexane	46.05 ^a	47.21	50.18 ^a	8.24 ^a	5.12 ^c	15.5 ^b
LSD	0.73	0.53	0.41	1.13	0.63	1.15
P	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000

LSD: The lowest significance differences at P < 0.05 P: Porpability

Effects of Mustard and Neem Oils on Carbohydrate Hydrolyzing Enzymes

Metabolism of carbohydrates controlled mainly by amylase, trehalase and invertase enzymes. They play a principal role in the digestion and utilization of carbohydrates by insect (Wyatt, 1967; Wigglesworth, 1972). Indeed, little are known about the relation between carbohydrates hydrolyzing enzymes with both of plant extracts and botanical insecticides. We shall light on work done in this respect as follows:

Generally, fluctuated effects were recorded with all treatments on carbohydrate hydrolyzing enzymes activity through time intervals of treatment. Neem showed a fluctuated increase in

amylase and invertase compared to the control group while mustard oil attained contrary effect as they significantly decreased where the activity reduction of amylase reached 49.12 µg/g bodyweight after 3 days and invertase reached to 503.58 µg/g bodyweight after 7 days, respectively in 2nd larval instar compared to its control (Table 5). Trehalase activity was different whereas increased in general by treatments with neem oil in 2nd instar and valued 999.18, 574.28, 373.91 and 764.02 µg glucose/g while started with an increase followed by drop-down 442.41, 559.63 and 402.48 (µg glucose/g) in 4th instar. The same trend was followed by mustard oil which showed the same effects compered to control as shown at Table 6.

Table 5. Changes in carbohydrate hydrolyzing enzymes activities of 2nd instar larvae treated with LC₅₀ of mustard and neem oils

Treatment	Amylase (µg glucose /g)				Trehalase (µg glucose /g)				Invertase (µg glucose /g)			
	Time interval (day)											
	1	3	5	7	1	3	5	7	1	3	5	7
Mustard	64.02 ^b	49.12 ^c	57.18 ^d	110.18	576.48 ^c	685.63	683.80 ^b	898.46 ^a	506.51 ^b	587.43 ^c	554.5 ^c	503.58 ^d
Neem	44.73 ^c	80.63 ^a	102.85 ^b	148.03	999.18 ^a	574.28	373.91 ^d	764.02 ^b	839.846 ^a	926.29 ^a	937.65 ^a	870.62 ^a
Control	38.87 ^d	52.3 ^b	69.15 ^c	123.36	538.75 ^d	537.28	717.87 ^a	644.24 ^c	496.26 ^b	499.19 ^d	655.6 ^b	612.74 ^c
Control hexane	95.52 ^a	79.89 ^a	136.30 ^a	133.62	939.84 ^b	644.61	422.26 ^c	588.57 ^d	874.28 ^a	601.02 ^b	517.50 ^d	673.18 ^b
LSD	1.91	1.82	3.62	6.18	21.72	22.37	19.33	34.30	48.18	26.85	31.33	37.73
P	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

LSD: The lowest significance differences at P < 0.05 P: Porpability

Table 6. Changes in carbohydrate hydrolyzing enzymes activities of 4th instar larvae treated with LC₅₀ of mustard and neem oils

Treatment	Amylase (µg glucose /g)			Trehalase (µg glucose /g)			Invertase (µg glucose /g)		
	Time interval (day)								
	1	3	5	1	3	5	1	3	5
Mustard	36.91 ^d	29.34 ^d	58.9 ^c	455.6 ^b	544.61 ^b	572.08 ^c	895.52 ^c	871.35 ^c	965.61 ^b
Neem	117.25 ^a	80.14 ^a	220.8 ^a	442.41 ^b	559.63 ^b	402.48 ^d	733.25 ^d	788.55 ^d	984.54 ^a
Control	57.18 ^c	57.43 ^b	29.59 ^d	385.27 ^c	672.08 ^a	971.71 ^a	992.71 ^b	1103.33 ^a	875.74 ^c
Control hexane	88.69 ^b	52.06 ^c	194.43 ^b	471.35 ^a	399.19 ^c	879.04 ^b	1019.09 ^a	1053.76 ^b	870.62 ^c
LSD	3.96	2.89	8.58	14.64	30.23	20.22	3.44	4.16	14.10
P	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

LSD: The lowest significance differences at P < 0.05 P: Porpability

These results clearly that disagree with all finding of **Amin *et al.* (2019)** who recorded decrease in digestive enzymes including amylase and invertase by neem oil while, mustared oil results agreed with **Rao *et al.* (1999)** who pointed out that botanical oils have great effect on digestive enzymes Also, **Hill and Orchard (2005)** referred the reduction of enzymatic activities of digestive enzymes by the lack of food intake. **Bezzar-Bendjazia *et al.* (2017)** and **Kilani-Morakchi *et al.* (2017)** cleared that azadirachtin reduced significantly the activity of α -amylase and protease in *Dorsophila melanogaster* larvae. **Tatun *et al.* (2014)** tested the inhibitory effect of plant extracts (*Ricinus communis* and papaya) on α -amylase activity in the red flour beetle and decided that α -amylase activity was inhibited and glucose content was reduced in the larvae and adults of this insect.

Effects of Tested Oils on Transaminase Enzymes Activities, AST and ALT

It is a fact that the maintenance of the balanced amino acid pool in insects is the result of various biochemical reactions carried out by-aminotransferases enzymes (**Meister, 1957**). The activities of AST and ALT enzymes were decreased with neem oil as a result of treatment comparing to control. In contrast, mustard oil resulted also in reduction of activity with the exception of increasing the activity of AST, in 2nd instar samples at all-time intervals (164.64, 158.02, 166.44 and 107.54 µg pyruvate/g) compared to its control 144.44, 175.81, 153.46 and 67.16 µg pyruvate/g (Table 7). Generally, neem and mustard oils caused decrease in the activities of AST and ALT enzymes in 4th instar larva, the maximum reduction induced with LC₅₀ mustard oil after 3 day 19.24 and 11.23 µg pyruvate/g for AST and ALT activity, respectively (Table 8).

Table 7. Changes in transaminase enzymes activities of 2nd instar larvae treated with LC₅₀ of mustard and neem oils

Extract	AST (µg pyruvate /g)				ALT(µg pyruvate /g)			
	1	3	5	7	1	3	5	7
Mustard	164.64a	158.02b	166.44a	107.54a	91.32a	44.00b	65.51b	66.05d
Neem	30.62d	35.19c	33.63c	87.47b	19.81c	31.64	25.72c	82.18c
Control	78.94c	159.35b	118.36b	110.43a	68.2b	118.73a	126.8a	125.19b
Control hexan	144.44b	175.81a	153.46a	67.16c	68.19b	112.28a	116.04a	154.22a
LSD	3.74	6.07	14.31	4.51	10.55	12.71	12.76	14.84
P	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

LSD: The lowest significance differences at P< 0.05 P: Porpability

Table 8. Changes in transaminase enzymes activities of 4th instar larvae treated with LC₅₀ of mustard and neem oils

Extract	AST (µg pyruvate /g)			ALT (µg pyruvate /g)		
	1	3	5	1	3	5
Mustard	20.69c	19.24c	21.85b	16.58a	11.23b	43.47c
Neem	24.49b	34.83b	12.47c	15.51a	26.26a	34.86d
Control	30.06a	39.04a	26.54a	7.44b	39.16a	66.05a
Control hexan	8.91d	15.12d	6.82d	3.13b	33.25a	54.22b
LSD	1.26	1.10	1.68	6.67	12.50	4.84
P	0.0000	0.0000	0.0000	0.0044	0.0005	0.0000

LSD: The lowest significance differences at P< 0.05 P: Porpability

Similar observation was recorded by **Bakr et al. (2002)** investigated the effects of botanical extracts on some biochemical parameters in the *S. littoralis*. Further **Hasheminia et al. (2011)** stated that plant extracts exhibited an endocrine disruption by way of progressive or retrogressive larval duration; this explanation could be pointed out for reduced alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Conclusion

Mustard oil exhibited an insecticidal activity against *Spodopetra littoralis* comparing with neem oil. The botanical recommended insecticides seems to be an effective botanical pesticide with biochemical effects as well as neem effects. So, it is great importance due to the fast development

of insect resistance against synthetic insecticides. Mustard and neem oil could be used in IPM programs.

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السمية والتأثيرات البيوكيميائية لزيت الخردل وزيت النيم على العمر اليرقي الثاني والرابع لدودة ورق القطن

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في هذه الدراسة، تم تقييم كل من زيت الخردل وزيت النيم من حيث الفعل الأبادي على يرقات العمر الثاني والرابع لدودة ورق القطن معمليا وكانت النتائج المتحصل عليها كما يلي: أظهرت دراسات السمية أن لزيت الخردل سمية منخفضة مقابل زيت النيم بقيمة LC_{50} بلغت 7.99% و 20.30% حيث كان مؤشر السمية 0.0024% و 0.0014% ضد يرقات العمر الثاني والرابع على التوالي مقارنة بالكفاءة الملحوظة للنيم، كما تم تقييم التغيرات البيوكيميائية لمقارنة الفعل الحيوي بين الزيتين قيد الدراسة، وتم تسجيل نفس الإتجاه لكل من زيت النيم والخردل في التغيرات البيوكيميائية لنشاط الإنزيمات الناقلة للأمين (AST and ALT) والبروتين الكلي والتي انخفضت في كل من العمرين الثاني والرابع لدودة ورق القطن في جميع الفترات الزمنية (1-3-5-7 أيام) بالإضافة إلى إظهار تأثيرات متذبذبة على مستويات الدهون ونشاط انزيمات الأميليز والانفرتيز والتريهايز.

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