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Evaluation of the Insecticidal and Biochemical Impacts of Linseed Oil, KZ Oil, and Oleic Acid on *Galleria mellonella,* Larvae

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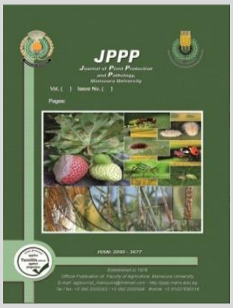

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ABSTRACT

Galleria mellonella (Lepidoptera: Pyralidae), is one of the graver pests affecting honeybee colonies, causing severe damage to stored wax combs and weakening hive productivity. The present study aimed to evaluate the insecticidal, biological, and biochemical impacts of three natural compounds, KZ oil, linseed oil, and oleic acid, on 6th instar larvae of *G. mellonella* under laboratory conditions. Results disclosed that linseed oil showed the highest mortality rates, based on LC_{50} with (8.00) µg/ml, followed by KZ oil (10.80) µg/ml, while oleic acid exhibited relatively lower toxicity with (13.02) µg/ml. Beyond direct seriousness, the tested compounds influenced several biological aspects, involving larval duration, pupation %, and adult emergence. According to the findings, all tested materials at different concentrations (2, 4, 8 and 16%) resulted in prolongation of larval and pupal duration, while caused a reduction in pupation % and adult emergence compared to the control. Notably, KZ oil at higher concentration (16%) induced a high incidence of morphological deformations in both pupae and emerged adults, further impairing successful development, whereas linseed oil produced only limited alterations. GC is a powerful analytical tool used to identify fatty acid components of linseed oil and oleic acid. In addition, all examined materials at LC_{25} and after one week, elevated the activity of chitinase enzyme and decreased that of acid phosphatase (ACP), also showing comparatively mild effects on protein content. These results highlight the potential of KZ oil, linseed oil, and oleic acid as an effective, eco-friendly alternative for greater wax moth management.

Keywords: *G. mellonella*, kz oil, linseed oil, GC, biochemical assessment.

INTRODUCTION

The greater wax moth is a major pest of honey bee (*Apis mellifera*) colonies. It causes damage to both active hives and stored combs (Kwadha *et al*., 2017). In tropical and subtropical regions, high temperatures speed up its life cycle. The moth lays their eggs in hives, their larvae feed on beeswax, pollen, honey, and brood combs, forming silk-lined tunnels that trap emerging bees. This problem is known as galleriasis (Ellis, 2013). Extensive use of synthetic pesticides against *G. mellonella* has increased resistance, contaminated hive products, and may lead to colony desertion (Mohamad, 2012). The environment and human health have suffered as a result of the extensive use of synthetic pesticides against *G. mellonella*, which has also promoted the development of resistance. On the other hand, natural plant oils are non-toxic, safe, and efficient in controlling pests (Elshafie and Camele, 2017). One of the safest ways to manage pests is using mineral oils, such as KZ oil, which has no known health risks or resistance problems (Aly *et al*., 1984). According to Messina *et al*. (2019), linseed oil is non-toxic and can lower insect populations by influencing different life phases. Unsaturated fatty acids like oleic acid are biodegradable, environmentally benign, and effective against a variety of pests. In this study, the impacts of oleic acid, linseed oil, and kz oil on *G. mellonella* larvae are examined, along with their effects on the biology and biochemical markers of the pest.

MATERIALS AND METHODS

Natural and synthetic products.

The KZ as mineral oil 95%EC, which is petroleum-derived oil produced by the Kafr El-Zayat Company for chemicals and pesticides in Egypt.

Flaxseed oil, as linseed oil, was acquired from the Agriculture Research Center's Food Technology Research Institute in Dokki, Giza Governorate, Egypt.

Oleic acid as synthetic compound, El-Gomhouria for Trading Chemicals and Medical Appliances supplied the synthesized chemical.

***G. mellonella* Larvae Rearing:**

G. mellonella eggs were taken from the wax comb of the plaguing bee and kept in jars covered with muslin fabric until they hatched. Under laboratory conditions at 30 ±2ºC and 75% relative humidity, the hatched larvae were fed an artificial diet consisting of wheat maize, glycerin, soy flour, milk powder, inactive dry yeast, and honeybee wax (Mohamed *et al*., 2014).

Preparation of KZ oil, linseed oil, and oleic acid:

KZ oil, linseed oil, and oleic acid were prepared using the dipping technique. To make the stock solution, 16 ml of oil, 83.75 ml of distilled water, and 0.25 ml of Tween-80 were combined. This stock solution was then diluted to produce three concentrations 8%, 4%, and 2% of each tested oil. The control solution included only 0.25 ml of Tween-80 and distilled water.

Toxicity assessment:

The competencies of the three tested materials were estimated against last instar larvae of *G. mellonella.* The tested concentrations were tested by using the dipping technique of *G. mellonella* larvae in each concentration for 30 seconds. Three replicates were prepared for each concentration, as well as the control (10 larvae / replicate). The treated insects were then transferred into 90 mm Petri dish lined with filter paper tightly closed with limy tape without food. (Hussien *et al*., 2021). The mortality averages

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were tallied after 72h, post treatment, as well as untreated control and corrected based on the Abbott formula (1925).

Analysis of linseed oil and oleic acid using gas chromatography (GC):

A cold saponification procedure was used to create fatty acid methyl esters from the oil and acid (ISO, 2011). An Agilent 6890 series gas chromatograph fitted with a DB2 capillary column (Agilent Technologies Inc., CA, USA) was used to analyze FAME. The sample size is 1µ. At a pace of 10°C per minute, the temperature program ramp rises from 150 to 220°C. At 270°C, the detector temperature (FID) is set. Nitrogen is the carrier gas, flowing at a steady 1.6 ml/min. The peaks were identified using fatty acid standards. The Horticulture Research Institute's Medicinal and Aromatic Plants Research Department Laboratory was where the GC analysis was conducted.

Biological assessment of the *G. mellonella* larvae:

For biological impacts against *G. mellonella* larvae, the compounds were used at LC_{25}. After larval mortality, surviving larvae were transferred individually to sterilized petri dishes and recorded daily. Also, different biological aspects were recorded as follows; larval duration, % pupation, pupal duration, adult emergence, adult longevity (male and female), and adult deformations.

Biochemical assessment:

Each tested compound's LC_{25} was used to combat *G. mellonella* larvae. After seven days of treatment, the larvae were chosen at random, and they were weighed before being homogenized in distilled water, while being cooled in a teflon homogenizer for three minutes. The homogenates were centrifuged for 10 minutes at 5ºC at 5000 r.p.m. In order to measure the enzyme activity, the supernatants were immediately placed in a refrigerator under chilling conditions (Abdel-Halim *et al*., 2006). Estimated levels of enzymes,

Total soluble protein (TSP):

Calorimetric measurements of total soluble protein were conducted using the Gornall *et al*. (1949) technique.

Phenoloxidase (PO):

Using catechol as the substrate, PO activity was established using Ishaaya (1971) methodology.

Transaminases:

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assessed using Reitman and Frankele (1957) methodology.

Acid phosphatase (ACP):

ACP activity was measured using disodium phenyl phosphate as the substrate in accordance with Powell and Smith (1954) methodology.

Chitinase activity:

Bade and Stinson (1981) methodology was used to determine the chitinase activity.

Statistical analysis:

The toxicity index (T.I.) and relative potency (R.P.) were calculated using the formulas of Sun (1950) and Zidan and Abdel-Maged (1988). The LC_{50} and LC_{25} values were computed using Biostat 2007 (professional Build 3200). The obtained data were then statistically analyzed using one-way ANOVA and the least significant difference (LSD) at (P>0.05) Costat statistical computer program (2005).

RESULTS AND DISCUSSION

Toxicological assessment:

The LC_{50}, LC_{25}, slope, toxicity index, and relative potency are the obtained results in Table 1. Data showed that the linseed oil as fixed oil was the most tested compound towards 6*th* instar larvae of *G. mellonella*, whereas the median concentration that kills 50% was 8 µg/ml, after 72 h. of dipping technique, while kz oil and oleic acid showed with 10.80 and 13.02 µg/ml, consecutively. Regarding toxicity indexes, the values were 74.07 and 60.00 % which netted to KZ oil and oleic acid in comparison with the most toxic compound (linseed oil), which had a 100% toxicity index 1.35 and 1.66 fold were the relative toxicity compared with the fewest compound oleic acid (Fig.1a). Sakla *et al*., (2021) reported that KZ oil proved safe for the honeybee *A. mellifera* L, an essential pollinator, to come into touch with treated leaves. Ahmed *et al*., (2020), mentioned that garlic oil was the most toxicity compound against the adult of *G.mellonella* followed with camphor, menthol oils and mixture oils and the LC_{50} values were 83%, 75%, 68% and 62% , respectively after one week under field conditions Ncibi *et al.,* (2021) looked into the effectiveness of natural compounds that were found to be safe for adult honeybees and to efficiently manage wax moth larvae in their fourth instar.Also, all tested materials caused abnormalities in shape of larvae which notice bloated, soft, and blackish brownish, that as compared to the untreated one, resulted from the oxidation of the cuticle and caused dead, (Fig.1b). This result is consistent with El-Gendy (2021), who noted some morphological alterations in *G. mellonella* larvae in their fifth instar after 24-hour dipping technique using *Lepidium sativum* seeds that were extracted with methanol.

Table 1. Median and sub-lethal concentrations of KZ oil, linseed oil, and oleic acid on *G. mellonella* 6*th* instar larvae.

	Median and sub-lethal concentrations and their 95% confidence				
Tested Materials(µg/ml)	**LC_{50}**	**LC_{25}**	**Slope**	**Toxicity index**	**Relative potency**
KZ oil	10.80(7.29-25.79)	4.13(0.39-1.73)	1.43±0.13	74.07	1.35
Linseed oil	8.00(3.88-16.46)	2.70(0.18-1.01)	1.61±0.15	100	1.66
Oleic acid	13.02(5.94-16.46)	5.19(0.14-6.83)	1.34 ±0.56	60.00	1.00

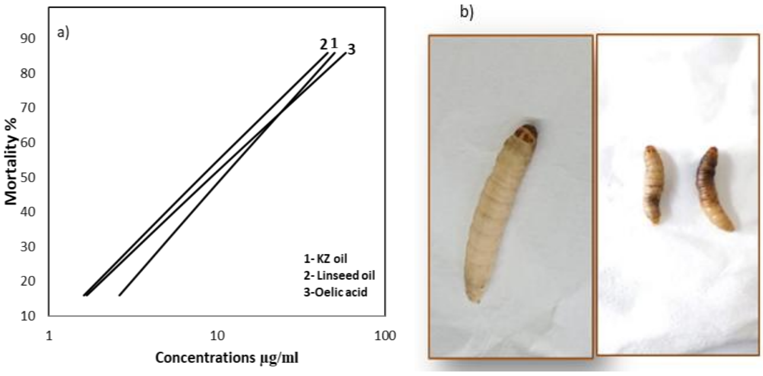

Fig.1. a. Toxicity regression line of KZ oil, linseed oil, and oleic acid, b. comparison of dead larvae, which are bloated, soft, and blackish brownish after 72 hours of the dipping method, with untreated larvae, which are creamy in color.

Linseed oil and synthetic oleic acid fatty acid composition:

Table (2) and Figures (2 & 3) noticed the analysis of the fatty acid components of linseed oil and synthetic oleic acid, showed the presence of unsaturated fatty acids, including palmitoleic acid (0.057, 0.0%), myristoleic acid (0.034, & 0.0%), oleic acid (16.818, & 82.156%), linoleic acid (15.034, 27.033, 7.950 & 7.456%), linolenic acid (15.034 & 8.950%), and eicosenic acid (0.158 & 0.0%), respectively. Additionally, there were substantial amounts of saturated fatty acids such as stearic acid (5.130 & 2.984%) and palmitic acid (4.671 & 7.410%). Abdel-Haleem (2021) found similar results when analyzing the fatty acid components of synthetic oleic acid and linseed oil whether it fresh or expired. Marinova *et al.,* (2012) detected that linoleic acid content is abounds used as a cursors of rater to oil degradation, wherein the polyunsaturated linoleyl chain is profoundly sensitive to oxidation.

Table 2. Fatty acids composition percentage of linseed oil and synthetic oleic acid.

Fatty acid	Structural Formula	Linseed oil	Synthetic oleic acid
Lauric acid C12:0	CH3(CH2)10COOH	____	1.710
Myristic acid C14:0	CH3(CH2)12COOH	____	1.893
Pentadecylic acid C15:0	CH3(CH2)13COOH	____	____
Palmitic acid C16:0	CH3(CH2)14COOH	4.671	7.410
Palmitoleic acid C16:1	CH3(CH2)5CH=CH(CH2)7COOH	0.057	____
Margaric acid C17:0	CH3(CH2)15COOH	0.064	____
Myristoleic acid C17:1	CH3(CH2)10CH=CH-COOH	0.034	____
Stearic acid C18:0	CH3(CH2)16COOH	5.130	2.984
Oleic acid C18:1	CH3(CH2)7CH=CH(CH2)7COOH	16.818	82.156
Linoleic acid C18:2	CH3(CH2)4CH=CHCH2CH=CH(CH2)7COOH	15.034	8.950
Linolenic acid C18:3	CH3(CH2)4CH=CHCH2CH=CHCH2CH=CH- (CH2)4COOH	58.957	___
Arachidic acid C20:0	CH3(CH2)18COOH	0.123	____
Eicosenic acid C20:1	CH3(CH2)7CH=CH(CH2)9COOH	0.158	____
Behenic acid C22:0	CH3(CH2)20COOH	0.082	____

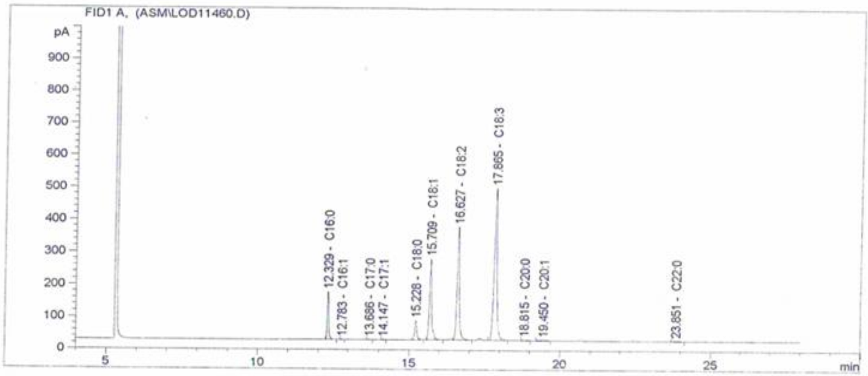

Fig. 2. Fatty acid composition of linseed oil.

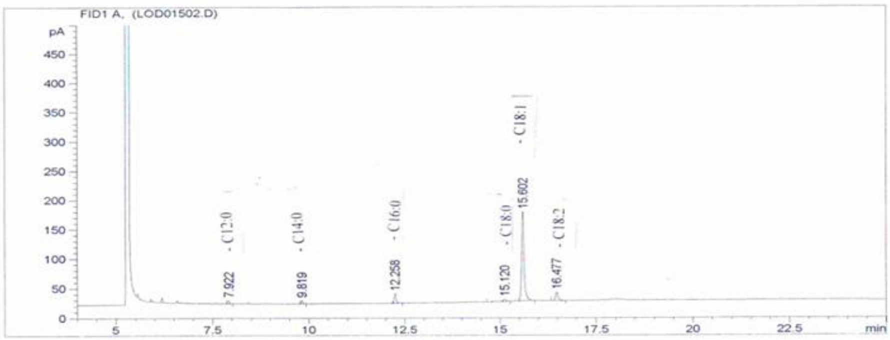

Fig. 3. Fatty acid compositions of synthetic oleic.

Biological assessment:

In laboratory conditions, the data in Table 3 demonstrated the effects of varying KZ oil concentrations on a number of biological parameters, such as larval duration, pupation percentage, adult emergence, and longevity of both male and female of G. *mellonella*. The results showed that the duration of larval stage and longevity recorded with non-significant impact compared with the control, at the same time the pupation percentage declined by increasing KZ oil concentrations (30.00 ±3.33) at 16%, (56.67±6.93) at 8%,(74.00±3.84) at 4% and (87.00± 5.09) % in comparison with the control (100%). For the pupal duration, the following concentrations, 4, 8, 16% caused noticeable prolongation in the days, compared to concentration 2% and the control. Elbarky *et al.*, (2015) demonstrated that essential oils prolonged the larval and pupal durations while reducing the rates of pupation and adult emergence. Significant differences between adults emergence were demonstrated by statistical analysis. Additionally, the highest rates of malformation in pupae and adults were caused by KZ oil at 16%, with 28.00±8.48 % and 18.00±2.06%, consecutively. Kaur *et al.*, (2023) elucidated that the Pongamia oil, Peppermint oil, Basil oil, and Thyme oil as botanical plants at 0.5, 1, 2, and 4% caused a reduction in larval duration, while led to increase in time of pupation and adult longevity of 3rd and 7th instar *G.mellonella* with dipping method.

Table 3. Impact of variation concentrations of KZ oil on some biological parameters of *G. mellonella under* laboratory conditions.

Linseed oil	Larval duration(days)	Pupation (%)	Pupal duration (days)	Deformed pupae (%)	Adult emergency (%)	Longevity of adult (days) ♂	Longevity of adult (days) ♀	Malformation (%)
2%	$8.00^{ab}\pm0.58$	$83.63^{ab}\pm2.51$	$13.66^{a}\pm0.68$	$11.11^{b}\pm6.41$	$87.50^{a}\pm4.16$	8.06a±0.59	$7.00^{a}\pm2.33$	0.00^{b}
4%	$8.76^{ab}\pm0.51$	$81.07^{ab}\pm2.08$	$17.00^{a}\pm1.00$	$23.61^{ab}\pm3.49$	$76.42^{ab}\pm3.48$	$7.83^{a}\pm2.29$	$6.16^{a}\pm0.35$	0.00^{b}
8%	$11.00^{ab}\pm1.33$	$70.55^{b}\pm1.39$	$17.43^{a}\pm1.28$	$31.94^{a}\pm6.26$	$52.22^{ab}\pm9.04$	$6.08^{a}\pm1.83$	$6.00^{a}\pm0.76$	$4.16^{b}\pm2.40$
16%	$15.33^{a}\pm1.67$	$42.00^{c}\pm5.50$	$19.16^{a}\pm3.12$	$83.63^{ab}\pm2.51$	$35.00^{b}\pm7.26$	$5.40^{a}\pm1.61$	$5.76^{a}\pm2.45$	$24.54^{a}\pm2.56$
Control	$6.33^{b}\pm0.38$	100.00^{a}	$9.67^{a}\pm0.94$	0.00^{b}	$97.76^{a}\pm1.92$	$9.83^{a\pm}0.75$	$7.33^{a}\pm0.31$	0.00^{b}
P	*	***	NS	**	***	NS	NS	***
L.S.D.	8.01	22.75	12.53	49.87	44.97	8.51	5.17	12.20

Newly moulted 6*th* instar larvae of a laboratory colony of*G. mellonella* were treated using dipping technique, P. = Probability.

L.S.D: The least significant difference ns, not significant at p>0.05, *: significant at p<0.05, **:highly significant at p<0.01, and *:very high significant at p<0.001.**

At all concentrations, the data in Table 4 proved that the linseed oil increased the larval duration. The values were (8.00±0.58, 8.76±0.51, 11.00±1.33, and 15.33±1.67) days, for 2, 4, 8, and 16% of concentrations, consecutively, compared with the control (6.33±0.38) days. Likewise, the effect of linseed oil on *G. mellonella* pupation percentage showed a significant decline between the tested material and control; the lowest average pupation percentage was 42.00±5.50% at concentration 16%, while the highest was observed at concentration 2% with 83.63±2.51%% compared to 100% for the control. All concentrations resulted in a prolongation of pupal duration Pastagia and Patel (2007) indicated that the pupal stage lasts between 12 and 19 days. On the other hand, the linseed oil could cause deformed pupae at all tested concentrations, whereas at concentration 16% led to the highest deformed pupa with (83.63±2.51) %, while at concentration 2% noticed with (11.11±6.41) %. According to the statistical study, adult emergence was significantly impacted by depression, with an average adult emergence range of (35.00±7.26, 52.22±9.04, 76.42±3.48, and 87.50±4.16) % at concentrations 16, 8, 4, and 2%, constructively. Else data mentioned that the linseed oil at a concentration 16% triggered the utmost percentage of malformation with (24.54±2.56) %. Moawad *et al*., (2015) mentioned that the clove oil and geranial at high concentrations (0.5µl/50ml) caused obvious effect at all stage of *G.mellonella* when treated 1st and 6th instar. Else, Photo (1) showed various deformations in the pupae and adult stage.

Table 4. Impact of variation concentrations of linseed oil on some biological parameters of *G. mellonella under* laboratory conditions.

KZ oil	Larval duration (days)	Pupation (%)	Pupal duration (days)	Deformed pupae (%)	Adult emergency (%)	Longevity of adult (days) ♂	Longevity of adult (days) ♀	Malformation (%)
2%	$5.60^{a}\pm0.076$	$87.00^{ab}\pm5.09$	$8.47^{c}\pm0.48$	0.00^{c}	$84.72^{ab}\pm2.01$	$8.87^{a}\pm1.53$	$7.06^{a}\pm1.38$	0.00^{b}
4%	$5.51^{a}\pm0.086$	$74.00^{c}\pm3.84$	$12.83^{bc}\pm0.98$	$4.67^{ab}\pm2.74$	$72.33^{ab}\pm0.69$	7.00a±3.15	$6.05^{a}\pm0.86$	$11.11^{a}\pm6.41$
8%	$5.33^{a}\pm0.016$	$56.67^{bc}\pm6.93$	$15.40^{ab}\pm1.03$	$8.33^{ab}\pm4.81$	$71.66^{ab}\pm1.34$	$6.61^{a}\pm3.00$	$6.76^{a}\pm2.03$	$15.87^{a}\pm5.57$
16%	$5.31^{a}\pm0.012$	$30.00^{d}\pm3.33$	$17.58^{a}\pm1.18$	$28.00^{a}\pm8.48$	$59.85^{b\pm}3.01$	$4.76^{a}\pm0.25$	$4.33^{a}\pm1.71$	$18.00^{a}\pm2.06$
Control	$6.33^{a}\pm0.38$	100.00^{a}	$9.67^{c}\pm0.94$	0.00^{c}	$97.76^{a\pm}1.92$	$9.83^{a\pm}0.75$	$7.33^{a}\pm0.31$	0.00^{b}
P	Ns	***	**	**	*	Ns	Ns	.*
L.S.D.	7.33	24.04	4.41	24.73	34.62	7.06	4.80	22.63

Newly moulted 6*th* instar larvae of a laboratory colony of *G. mellonella* were treated using dipping technique, P. = Probability.

L.S.D: The least significant difference ns, not significant at p>0.05, *: significant at p<0.05, **:highly significant at p<0.01, and *:very high significant at p<0.001.**

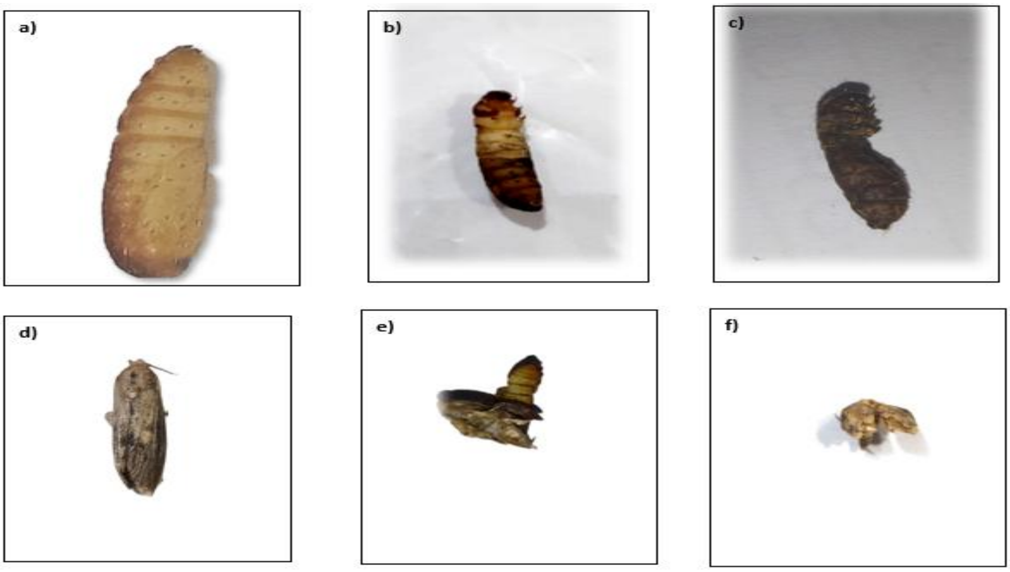

Photo 1. Deformation types which detected in pupae and adult stages which describe as following: a); normal pupae, b); pupal larval-intermediate, c); pupae with larval head and thoracic, d); normal adult, e); partial emergence of adults with head, thorax and wings and f); complete emerged moths with wrinkled wings.

Data in Table 5 demonstrated that the oleic acid at concentrations 4, 8, and 16% caused a slight extension in larval duration, and the values were (9.00±0.5, 9.84±0.59, and 11.23±0.75) days compared with the control (6.33±0.38) days. Moreover, all concentrations contributed to a decrease in pupation percentage. The Statistical study revealed no significant differences in pupal duration, adult emergence, and longevity, whilst oleic acid fulfilled noticeable abnormalities in pupae and adults, and the values were (15.00±8.66, 15.87±5.57, 20.11±6.72, and 37.5±4.16) % and (4.17±2.41, 8.33±4.81, 13.92±4.24, and 25.00±11.43) % at concentrations 2, 4, 8, and 16%, consecutively. The higher rate of metamorphosis may result in malformed and immature individuals (Assegid *et al*., 2004). Crude extracts and essential oils have been investigated for repellent, fumigant, larvicidal, and adulticidal activities (Beyene and Woldatsadik, 2019).

Table 5. Impact of variation concentrations of oleic acid on some biological parameters of *G. mellonella* under laboratory conditions.

Oleic acid	Larval duration (days)	Pupation (%)	Pupal duration (days)	Deformed pupae (%)	Adult emergency (%)	Longevity of adult (days)		Malformation (%)
						♂	♀	
2%	6.33^{b}± 0.96	90.00^{ab}±1.92	8.49^{b}±0.64	15.00^{ab}±8.66	75.00^{ab}±11.98	9.63^{a}±2.71	7.33^{a}±0.77	4.17^{ab}±2.41
4%	9.00^{ab}±0.5	74.00^{b}±1.76	10.76^{ab}±1.02	15.87^{ab}±5.57	69.04^{ab}±3.43	6.66^{a}±1.95	6.01^{a}±1.66	8.33^{a}±4.81
8%	9.84^{ab}±0.59	53.00^{b}±4.25	12.83^{ab}±1.43	20.11^{ab}±6.72	56.53^{ab}±4.11	7.61^{a}±0.65	5.73^{a}±0.84	13.92^{a}±4.24
16%	11.23^{a}±0.75	37^{c}±3.84	15.73^{a}±0.73	37.5^{a}±4.16	50.00^{b}±3.33	5.83^{a}±0.67	5.16^{a}±0.54	25.00^{a}±11.43
Control	6.33^{b}±0.38	100.00^{a}	9.67^{ab}±0.94	0.00b	96.76$^{a±}$1.92	9.83$^{a±}$0.75	7.33^{a}±0.31	0.00ab
P	*	***	NS	*	NS	NS	NS	*
L.S.D.	3.53	20.84	7.11	31.69	33.39	8.66	5.17	38.99

Newly moulted 6th instar larvae of a laboratory colony of*G. mellonella* were treated using dipping technique, P. = Probability.

L.S.D: The least significant difference ns, not significant at p>0.05, *: significant at p<0.05, **:highly significant at p<0.01, and *:very high significant at p<0.001.**

Biochemical assessment:

Data in table 6 observed the biochemical markers of the sixth instar of *G. mellonella* to the LC_{25} of KZ oil, linseed oil, and oleic acid after one week of treatment. The findings showed that KZ oil reduced the protein level and gave (10.89) mg/g with (-48.02%) protein content. Protein synthesis, which produces lisle silk, a protective tube utilized in larval feeding during the pupa stage, may be impacted by the drop in protein levels due to insufficient demand for necessary amino acids (Shaik *et al*., 2017). In the country, linseed oil achieved a reduction in phenol oxidase (PO) and gave (13.09O.D.unit/min/g.b.wt) and percentage (-61.67), followed by oleic acid, which showed (18.84 O.D. unit/min/g.b.wt) with (-44.83%) compared with untreated larvae (Fig., 4). (González-Santoyo, and Córdoba-Aguilar, 2012) who assessed phenol oxidase, which is thought to be an important part of insects' immune systems and whose primary function in melanogenesis is to convert phenols to quinones. KZ oil efficacy in ALT enzyme through exposure gave (-17.41%), whereas results highlighted that linseed oil induced a rise in the ALT level with (34.69%), and led to a diminution in AST level with (-33.64%).

The examined chemical's binding to proteins that inhibit amino-transferase activity, which is known to be tightly related to protein synthesis, may be the reason for the decrease in AST and ALT.

Table 6. The biochemical indicators on the 6th instar larvae of *G. mellonella* exposure to LC_{25} of KZ oil, linseed oil, and oleic acid after one week.

Tested materials (LC25)	TSP mg/g	C %	PO O. D. unit/ min/g. b. wt	C %	ALT µg pyruvate / g. b. wt.	C %	AST µg oxaloacetate/ g.b.wt.	C %	ACP µg phenol/ g.b.wt	C %	Chitinase µg/NAGA/ min/g.b.wt	C %
KZ oil	10.89b	-48.02	49.01b	17.15	59.49b	-17.41	30.13ab	13.91	226.77b	-58.43	53.11a	22.14
Linseed oil	22.95a	42.36	13.09a	-61.67	97.02a	34.69	17.55c	-33.64	343.75ab	36.98	74.03a	77.65
Oleic acid	17.38a	7.81	18.84c	-44.83	81.11b	12.61	23.33a	11.79	152.54bc	-72.03	81.88a	96.49
Control	16.12b	-	34.15ab	-	72.03b	-	26.45c	-	545.54a	-	41.67ab	-
P	*		***		*		***		***		*	
L.S.D.	0.034		21.45		43.05		0.371		26.57		3.72	

C% = Change in activity, Increase or decrease than control = (treated – control) ÷ control x 100, g. b. w.t = gram body weight, P. = Probability.
L.S.D: The least significant difference, *: significant at p<0.05, **:highly significant at p<0.01 and *:very high significant at p<0.001.**

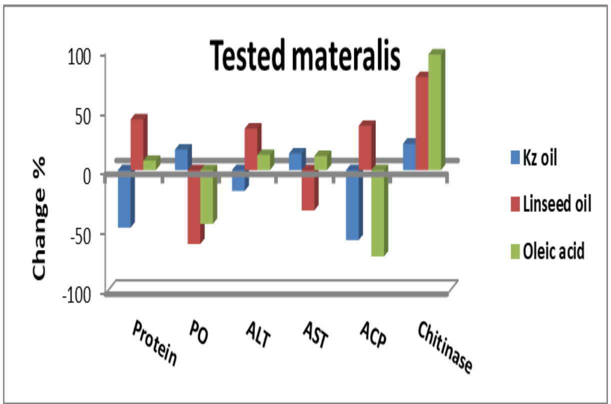

Fig. 4. Change percentages of protein content, PO, ALT, AST, ACP, and Chitinase enzymes through one week of total body homogenate for *G.mellonela*, larvae treated with LC_{25} of Kz oil, linseed oil, and oleic acid by dipping technique.

Also, results clarify the activity of acid phosphates (ACP) after one week of exposure, both KZ oil & oleic acid led to a decline and gave (226.77 & 152.54µg phenol/g. b. wt) with (-58.43&%-72.03), consecutively in comparison with the control. This is expected to compound may cause destabilization of the lysosomal membrane and the consequent release of the enzyme into the hemolymph or can trigger the hyper synthesis of acid phosphatase, which is subsequently released into the hemolymph (Beltagi *et al*., 2011). All compounds attained elevation in chitinase activity, ranging between 22.14, 77.65, and 96.49%, consecutively in comparison with untreated larvae. There are two possible explanations for the increase in chitinase activity: either the chitin synthesis inhibitor had a secondary effect, or the β-ecdysone metabolizing enzymes' decreased activity was followed by β-ecdysone buildup, which led to hyperchitinase activity (Yu and Terriere, 1977).

CONCLUSION

Overall, these finding demonstrated that, linseed oil, KZ oil, and oleic acid are safe substitutes for traditional pesticides and show promise as environmentally benign methods of managing *G. mellonella*, larvae and diminish the pest population by interfering with essential enzyme functions, disrupting normal development, and decreasing survival. In order to create sustainable integrated pest management strategies.

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تقييم التأثيرات الحشرية والبيوكيميائية لزيت بذرة الكتان، زيت كزد وحمض الأوليك على يرقات دودة الشمع الكبرى جاليريا ميلونيلا

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الملخص

تُعتبر دودة الشمع الكبرى من أخطر الآفات التي تصيب مستعمرات نحل العسل ، مسببة أضرارًا بالغة في الأقراص الشمعية حيث تؤدي إلى إضعاف إنتاجية الخلايا، لذلك استهدفت الدراسة الحالية تقييم التأثيرات ا،البيولوجية ،والبيوكيميائية لثلاثة مركبات طبيعية : كزد أويل ،زيت بذرة الكتان وحمض الأوليك على يرقات العمر السادس لدودة الشمع الكبرى تحت الظروف المعملية. أظهرت النتائج أن زيت بذرة الكتان حقق أعلى تأثير قاتل حيث كانت قيم التركيز النصف مميت المتحصل عليها ، ٨,٠٠ ميكروجرام/مل، يليه كزد اويل بقيمة ١٠,٨٠.ميكروجرام/مل ، بينما أظهر حمض الأوليك أقل تأثير قاتل بقيمة ١٣,٠٢ ميكروجرام/مل. كما أثرت المركبات المختبرة على عدة جوانب بيولوجية تضمنت، مدة الطور اليرقي، نسبة التعذر، ونسبة خروج الحشرات الكاملة. وأوضحت النتائج أن جميع المواد المختبرة عند التركيزات المختلفة ٢٪ ،٤٪ ،٨٪ و ١٦٪ أدت إلى إطالة الطور اليرقي و طور العذراء، بينما أدت الى انخفاض في نسب التعذر وخروج الحشرات الكاملة مقارنة بالغير معاملة. ومن الجدير بالذكر، أن كزد اويل عند تركيز ١٦٪ أحدث أعلى نسب من التشوهات المورفولوجية فى العذارى والحشرات الكاملة حديثة الخروج أدى الى أعاقة نموها الطبيعى. كما أثبت التحليل الكروماتوجرافي الغازى فعاليته في تحديد مكونات الأحماض الدهنية لكلا من زيت بذرة الكتان وحمض الأوليك. وأيضاً ، تبين أن جميع المواد المختبرة عند التركيز تحت المميت وبعد أسبوع من المعاملة أدت الى زيادة نشاط إنزيم الكيتيناز، بينما تسببت فى انخفاض نشاط إنزيم الفوسفاتاز الحامضي، كما أدت الى حدوث تأثيرات طفيفة نسبيًا على محتوى البروتين الكلى. لذا توصلت الدراسة انه يمكن استخدام كزد أويل، زيت بذرة الكتان،وحمض الأوليك كبدائل فعالة وصديقة للبيئية لمكافحة دودة الشمع الكبرى تحت الظروف المعملية.