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Effectiveness of Three Plant Oils, Argon Gas and Conjunction of them for *Galleria mellonella* Control

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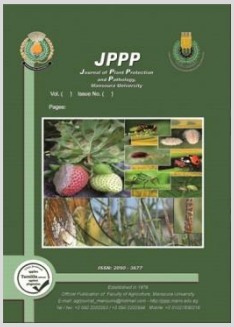

ABSTRACT

Wax moth (*Galleria mellonella*) is serious pests of beeswax worldwide. These study was conducted to estimate the effectiveness of three plant oils (Lemongrass oil, Moringa oil and Pumpkin oil), argon gas and conjunction of them to develop alternative method of controlling the 4th larval instar of *G. mellonella*. Potential activities of active ingredients on mortality percentage and duration of exposure to wax moth at four concentrations (3, 1.50, 0.75and0.37%v/w) were estimated. Data cleared that the mortality effect depends on applied material type and concentration. Lemongrass oil was recorded (100%) larval mortality 7 days post-treatment, being of highest activity comparing with the two other materials and control. Results showed that after 3 days post-treatments by Lemongrass oil, the LC_{50} value was 2.18 % (v/w). The LT_{50} values against the 4th larval instar of *G. mellonella* were 22.48, 45.09 and 68.04 hr. for Argon 100, 80 and 60%, respectively. In general, the larval mortality was higher in binary treatments (plant oils (LC_{50}) mixed with Aragon gas) than individual treatments only by the oil. It was the best treatment Lemongrass oil (LC_{50} 2.18) +Ar.(100) where the full mortality (100.0%) of larvae was achieved 24 hours after treatment, treatment with this combination Lemongrass oil (LC_{50} 2.18)+Ar.(100) increased AchE and decreased Cytochrome P-450, GST, α- amylase and invertase when compared to the control. However, these results clarified the necessity to more research in order to affirm their effect in the field and honeybee.

***Keywords*: *Galleria mellonella*,** Argon gas, plant oils, Biochemical assay**.**

INTRODUCTION

Honeybees (*Apis mellifera* L.) are known as economically insects which have a high production of honey, wax and other products and also a very good for plant pollination and environmental balance (Kebebe *et al.* 2019). Many developing countries are trying hardly to upgrade quality of honey products, but they frequently face obstacles in apiculture. Amid many interrelated agents, infestation of honeybee by pathogens and pests are eminent ones that inflict massive loss to beekeeping production. The most important pests of honeybees are small hive beetle, bee louse, Mediterranean flour moth, the lesser, varroa mite and greater wax moths (Beyene and Woldatsadik 2019).

The greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) is considered the most harmful pest of honeybee wax combs which storage and can cause major forfeiture to combs, hive material and bees in beehives all the world (Charrière and Imdorf 2004). During its developmental period, it causing silk-lined tunnels in the honey bee combs and leaves its faeces, cocoons of the bee larvae, pollen, honey and wax. That causes area damage of honey combs and sequent impairment of the weakened colonies (Zaitoun 2007). GMW exists in all beekeeping areas but is more active in warm areas at temperature higher than 27°C where it spreads rapidly (Crane 1990).

Recently, environmental issues caused by pesticides' overuse became scientists and public issue of attention. Pesticides extended by applied lead to natural enemies' destruction and also, destruction of the role of honey bees as pollinators. Natural plant products are an excellent alternative to pesticides as a tool decreasing the side effects on human health and the environment balance (Koul *et al.*, 2008 and Fawzy *et al.* 2017).

One of the main alternatives to pesticides is the botanical pesticides (plant materials) and the use of essential oils. Essential oil and their ingredients show to be a potent source of botanical pesticides. The toxicity of a many of essential oils and their ingredients has been estimates against a number of bruchid pests (Keita *et al.*, 2000, 2001 and Tripathi *et al.*, 2002).

The present study aimed to evaluate the efficacies of plant oils (Lemongrass oil, Moringa oil and Pumpkin oil) when used separately and when mixed with Argon gas against larvae of *Galleria mellonella* were evaluated.

MATERIALS AND METHODS

1.Experiment place

The experiment was preceed in the laboratory of the Plant Protection Dept. Fac. of Agriculture, Benha University. The aim of this study was to evaluate the effectiveness of 3 plant oils. Separately and when mixed with Aragon gas against 4th instar larvae of greater wax moth (GWM), *Galleria mellonella.*

2.Rearing of GWM

The conceptuality by PDBC 2007 is the artificial trophic system which was used for rearing GWM larvae by mixing the whole wheat bran (100 g), hominy or corn flour (100 ml), powder of skimmed milk (100g), dehydrated grains of yeast (100g), glycerin (175 ml) and honey (175ml).

Wax combs samples which are suffering of the infection of great wax moth were collected from the apiary of faculty of agriculture, Moshtohor, Benha University. To prevent the insect from escaping; samples were kept in glass

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jars with plastic lids purveyed with wiry nets. Larvae were maintained under laboratory conditions (30 ± 2°C and 65 ± 5% R.H.) in the dark (controlled by incubator). GWM moth that emerged daily were collected and kept in separate glass jars. Jars used for mating adults were provided with soft tissues, a piece of cotton soaked in honey, and small pieces of artificial feed for the larvae, all covered with plastic lids fitted with wire mesh. Neonate larvae dig tunnels and are immediately fed on an artificial diet to pupate outside and inside the tunnels. Shortly after emergence, the butterflies were collected and the rearing units were supplied with fresh artificial food for the larvae.

3.Plant oils

Lemongrass oil (*Cymbopogaon Citratus*, Fam.: Poaceae), Moringa oil (*Moringa oleifera*, Fam.: Moringaceae) and Pumpkin oil (*Cucurbita pepo* Fam.: Cucurbitaceae) were bought from the National Research Center.

The concentrations of plant oils used in this study were 3, 1.50, 0.75 and 0.37% (v/w%).

Bioassay experiment

The fourth larval instar of *G. mellonella* was used in all experiments. Three replicates were conducted in the various treatments. Thirty larvae were placed in each replicate.

4. Argon gas

Steel cylinders contain pressurized pure Argon gas were prepared. A pressure regulator should be connected to cylinder. Dilution method was used to obtain required argon concentration. To obtain an almost pure Ar. atmosphere (100%), the valve of each cylinder was operated for three minutes in order to fill a sealed Drechel exposure flask with gas. Modified atmosphere (MA) of 60 and 80% argon concentration was prepared in air using a gas diffuser. Determination of Ar. gas concentrations were monitored using a Model 2 gas analyzer (10-600 Gow-Mac Instruments Company USA).

Preparing larvae of GWM for bioassay tests of modified atmosphere

Number of 30 4th larval instar *G. mellonella* placed in small cloth bags (4×8 cm) filled with about 25 gm. artificial diet and closed with rubber bands. Cloth bags were placed into the gastight Drechel-flasks of 0.55L volume. The larvae in the gastight flasks treated with the MA at mentioned concentrations for different exposure periods ranged from 12 to 120 hrs. at 30 ± 2°C. After the exposure periods, the flasks were aerated for 24 hrs. and larvae were transferred into Petri-dishes and kept at (30 ± 2°C and 65 ± 5% R.H.), larvae were examined to record mortality %., daily

5. Mixing plant oils with Argon gas

Efficacies of binary combinations of LC_{50}s in 3 days of the plant oils mixed with Argon 100, 80 and 60%, were assayed, against the 4th larval instar of GWM.

6. Biochemical assay

The 4th instar larvae of GWM survival after 6 hours of feeding on artificial diet treated with Argon gas (100%) with plant oils LC_{50} (2.18%) used for different biochemical tests. 5 replicates of 10 larvae each were maintained for treatment. Untreated diet served as a control by adding distilled water according to the method of Sorour (2021). Insects were prepared as explained by Amin (1998). The whole body larval weight of was homogenized in appropriate amount of homogenizer and centrifuged at 10000g for 5 min at 4°C. The deposits were removed away, and the supernatants kept in deep freezer until using it.

- **Acetylcholine esterase activity determination:**

A substrate acetylcholine bromide (nchBr) was used to measure the acetylcholine esterase activity according to the method described by Simpson *et al.* (1964).

- **Cytochrome P-450 Mono oxygenase assay:**

P-nitroanisole (PNOD) was used to measure cytochrome P-450 activity as described by Hansen and Hodgson (1971) and Moustafa *et al.* (2021).

- **Glutathione S- transferase (GST) determination:**

(GST) catalyzed the conjugation of reduced glutathione (GSH) with 1- chloro 2, 4dinitrobenzene (CDNB) viathe-SH group of glutathione. The conjugate, S (2, 4-dinitro-phenyl) 1- glutathione could be described according to the method of Habig *et al.* (1974).

- **α- amylase and invertase determination of:**

Carbohydrate digestive enzymes activity (α-amylase and invertase) were measured by the method of Ishaaya and Swirski (1976).

Statistical Analysis

Abbott's formula (1925) used for correcting mortality %. Dosage mortality response estimated by Probit analysis (Finney, 1971) with using a computer program of Noack and Reichmuth (1978). Data presented as the mean ± standard error (SE) and were analyzed using Student's t-test between treatments and control.

RESULTS AND DISCUSSION

1. Plant oils toxicity on the 4th instar larvae of *Gallaria mellonella*:

Data in Table (1) indicates effect of plant oils toxicity (Lemongrass oil; *Cymbopogaon citratus*, Fam.: Poaceae), Moringa oil (*Moringa oleifera*, Fam.: Moringaceae) and Pumpkin oil (*Cucurbita pepo*; Fam. Cucurbitaceae)) on 4th instar larvae of *G. mellonella* at 30 ± 1°C and 65 ± 5% R.H. Results showed that by increasing the plant oils concentration and the prolongation of exposure period mortality % increased. Efficacies of assayed plant oils on the 4th larval instar of *G. mellonella* were as followed:

Lemongrass oil:

Data in Table (1) shows that, after one day of *G. mellonella* 4th instar larvae treatments by Lemongrass oil at 3, 1.50, 0.75 and 0.37% concentrations, mortalities among larvae reached 20.21±1.53, 11.11±1..15, 9.87±0.58 and 2.22±1.53%, respectively. These percentages increased, successively, reached 69±0.58, 36.66±2.08, 30±0.58 and18.67±0.58 %, respectively, 3 days post-treatment. Also, exposure period was prolonged to seven days, total accumulative larval mortality reached their higher rates, being100±0.00, 83.33±2.08, 70±0.00 and 36.67±1.15 %, respectively (Table 1).

Moringa oil

At 3, 1.50, 0.75 and 0.37% concentrations of Moringa oil, larval mortality percentages were 20.11±0.58, 8.67±1.00, 5.45±1.53 and1.11±0.58 %, respectively, after one day exposure. These mortality percentages very much increased to 81.33±1.53, 66.67±1.00, 36.67±0.58 and 25.55±1.00 %, respectively, after 7 days of treatments. (Table 1).

Pumpkin oil

As shown in Table (1) larval mortality percentages after treatments by Pumpkin oil indicated lower efficiency

against 4th instar larvae of *G. mellonella* larvae than Lemongrass and Moringa oils.

After one day of larvae treatment by Pumpkin oil at concentrations; 3, 1.50, 0.75 and 0.37%, larval mortality percentages recorded 13.33±1.15, 8.87±0.58, 0.0 and 0.0%, respectively, (Table 1). Mortality % increased, successively, during the subsequent days after treatment until reaching the maximum of 60±0.00, 48.89±1.00, 28.67±0.58 and 13.33±1.15 %, respectively, by the same concentrations, respectively, after 7 days of treatments.

Table 1. Toxicity of plant oils treatments on *G. mellonella* 4th instar larval mortality.

Concentrations	Accumulative adult mortality (%) after treatment (days)				
% (v/w)	1	2	3	5	7
			Lemongrass oil		
3	20.21±1.53	53.33±0.58	69±0.58	83.33±0.58	100±0.00
1.50	11.11±1..15	22.22±1.00	36.66±2.08	60±0.00	83.33±2.08
0.75	9.87±0.58	18.67±1.53	30±0.58	46.67±1.00	70±0.00
0.37	2.22±1.53	8.87±0.00	18.67±0.58	26.67±1.15	36.67±1.15
			Moringa oil		
3	20.11±0.58	31.11±1.53	49.67±0.58	69.67±0.58	81.33±1.53
1.50	8.67±1.00	19.11±1.15	26.67±1.53	58.33±2.08	66.67±1.00
0.75	5.45±1.53	11.11±0.58	18.89±0.58	24.44±1.15	36.67±0.58
0.37	1.11±0.58	8.67±1.53	13.33±0.58	16.67±0.58	25.55±1.00
			Pumpkin oil		
3	13.33±1.15	22.22±1.53	30±0.00	42.22±1.00	60±0.00
1.50	8.87±0.58	14.44±0.58	21.11±0.58	31.11±2.08	48.89±1.00
0.75	0±0.00	6.67±1.00	12.22±1..15	17.78±2.08	28.67±0.58
0.37	0±0.00	0±0.00	3.33±1.53	8.89±1..15	13.33±1.15
Control	0	0	0	0	0

2. Toxicity of different concentrations of plant oils on the 4th larval instar of *G. mellonella*:

Table (2) indicates results of lethal concentrations (LC_{50}, LC_{90} and LC_{95}) of Lemongrass oil, Moringa oil and Pumpkin oil to 4th instar larvae of GWM after 3,5 and 7 days post-treatments.

After 3 days post-treatments, LC_{50} value was 2.18 % (v/w). The corresponding value at 7 days post-treatment was significantly lower being (0.50 % (v/w)).

As for Moringa oil treatment, the LC_{50} value was recorded 3.78 % (v/w) 3 days post-treatments. While after 7 days post-treatment was significantly lower being 1.04 % (v/w).

In case of Pumpkin oil three days post-treatments, LC_{50} value was higher, being 6.12 % (v/w). The corresponding value at 7 days post-treatment was significantly lower being 1.82 % (v/w).

Obtained results confirmed that Lemongrass oil was the highest toxic to 4th instar larvae of *G. mellonella* (lowest LC_{50}), followed by Moringa oil while Pumpkin oil demonstrated the lowest toxicity (highest LC_{50}). These results matched with other studies which indicated that the plant oil extracts were very efficient in control larval stage of greater wax moth. Also, *A. indica* oil extract was very active oil in controlling larvae of *G. mellonella* (Beyene and Woldatsadik 2019). In last years, authors were looking for environmentally and safe methods to control insect pests, which led to using plant derivatives with significant insecticidal effects, which have been considered as new source of pesticides without side effects on the environment and human (Balandrin *et al.*, 1985). These natural plant products considered more economical comparing with other chemicals. Plant oils from some medicinal and aromatic plants are known to possess bioactive compounds which have either toxic effect to a number of insect pests at various stages of life or elicit antifeedant properties to these pests (Huang *et al.*, 2000). According to Asawalam *et al.* (2007), controlling activity of plant extracts depends on their active constituents in the plant extract. So, in general, use of natural plant products as insecticides may help to minimize the problem of environmental pollutions which resulting of other synthetic insecticide applications.

Table 2. Toxicities of plant oils against 4th instar larvae of *G. mellonella* at (30±1° C, 65±5% R.H.) and various exposure periods:

Exposure period	LC (%v/w) and their 95% confidence limits.			Slope ±	R
(Days).	LC_{50}	LC_{90}	LC_{95}	SD	
			Lemongrass oil		
3 days	2.18 (0.99-4.77)	25.45 (11.61-55.81)	51.10 (23.30-112.04)	1.20±0.83	0.96
5 days	0.99 (0.50-1.96)	8.75 (4.41-17.37)	16.24 (8.18-32.25)	1.35±0.74	0.99
7 days	0.50 (0.31-0.81)	1.95 (1.21-3.16)	2.87 (1.77-4.65)	2.17±0.46	0.97
			Moringa oil		
3 days	3.78 (1.64-8.71)	45.81 (19.89-105.5)	92.90 (40.34-213.95)	1.18±0.84	0.94
5 days	1.43 (0.83-2.46)	7.59 (4.42-13.03)	12.17 (7.09-20.9)	1.77±0.56	0.95
7 days	1.04 (0.65-1.67)	4.41 (2.74-7.09)	6.64 (4.13-10.68)	2.04±0.49	0.96
			Pumpkin oil		
3 days	6.12 (2.78-13.43)	49.21 (22.37-108.22)	88.90 (40.42-195.53)	1.41±0.70	0.96
5 days	3.97 (1.81-8.69)	39.07 (17.84-85.567)	74.71 (34.12-163.62)	1.29±0.77	0.99
7 days	1.82 (0.98-3.40)	12.48 (6.7-23.26)	21.52 (11.55-40.11)	1.53±0.65	0.98

3. Effect of MA enriched with Argon 100, 80 and 60 % on 4th instar larvae of *G. mellonella*:

After evaluation of efficacy of MA concentration with Argon 100, 80 and 60% on fourth larval instar of GWM are cleared in Table 3. Results indicated that decreasing the numbers of larvae increases gradually with increasing the exposure period. Complete reduction (100%) recorded in larvae after 60 hrs. exposure to pure Argon, whereas, in the case of 80% Ar. complete reduction (100% mortality) occurred after 108 hrs. exposure time. Whereas, by exposure of larvae to atmosphere of 60% Argon, reduction percentage reached (100% mortality) occurred after 120 hrs. (Table 3).

Table 3. Toxicity of MA enriched with Argon 100, 80 and 60 % on 4th instar larvae of *G. mellonella*:

Concentrations % (v/w)	Reduction% Exposure time (hr.)									
	12	24	36	48	60	72	84	96	108	120
100	15.56±0.58	55.56±1.15	76.39±1.53	88.89±2.08	100±0.00	-	-	-	-	-
80	3.36±1.15	14.81±0.58	28.89±0.58	44.44±1.53	65.51±0.58	77.78±2.08	89.90±1..15	98.89±1.15	100±0.00	
60	-	2.12±0.58	12.26±1.15	24.13±0.58	38.49±1.53	45.56±1.15	65.51±0.58	72.22±0.58	88.89±2.08	100±0.00
Control	0	0	0	0	0	0	0	0	0	0

4. Lethal times and parameters of probit regression line of the 4th larval instar of *G. mellonella* exposed to Argon MA at 60, 80 and 100%:

Results in Table 4 indicates LT_{50}, LT_{90} and LT_{95} of 4th larval instar of *G. mellonella* being: 2.48, 50.15 and 62.97 hrs., respectively, Argon 100%. In the same line of results, the LT_{50}, LT_{90} and LT_{95} values were 45.09, 105.44 and 134.15 hrs., respectively, for Argon 80%. While, for exposure to MA 60% Ar., the LT values were recorded 68.04, 130.4 and 156.81 hrs. for Argon 60%, respectively. It is clear that the LT was prolonged when the applied concentration of Argon gas was decreased.

Table 4. LTs and parameters of probit regression line estimated for 4th instar larvae of *G. mellonella* exposed to Argon at 60, 80 and 100%:

Exposure period	Lethal times and their 95% confidence limits			Slope ± SD	R
	LT_{50} (hr.)	LT_{90} (hr.)	LT_{95} (hr.)		
100%	22.48 (17.03-29.67)	50.15 (38-66.2)	67.17 (49.62-90.94)	3.677±0.27	0.99
80%	45.09 (36.66-55.45)	105.44 (85.73-129.68)	134.15 (109.07-164.99)	3.56±0.28	0.95
60%	68.04 (58.04-79.76)	130.4 (111.23-152.87)	156.81 (133.76-183.83)	4.57±0.22	0.98

5. Effect of plant oils (LC_{50}) mixed with Aragon gas on 4th instar larvae of *G. mellonella*:

Effect of binary combinations of plant oils (LC_{50}) and Aragon on 4th instar larvae of *G. mellonella* is presented in Tables 5. The larval mortality of *G. mellonella* larvae were increased in all treatments with increasing both application % and exposure perid. In general, binary treatments induced higher toxicity to *G. mellonella* larvae than individual treatments. The accumulative mortality percentage (100.0%) of larvae was achieved in treatment of Lemongrass oil (LC_{50} 2.18) + Ar. (100) after 24 (hrs.). While, treatment of Lemongrass oil (LC_{50} 2.18) + Ar. (80 and 60) caused 100% mortality of *G. mellonella* larvae after 72 and 96 hrs., of exposure, respectively.

After 6 hrs. of larval treatment by Moringa oil (LC_{50} 3.78) + Ar. (100, 80 and 60), mortality percentages were 33.33±1.15, 11.11±0.00 and 2.11±1.00 %, respectively, (Table 5). The total mortality (100.0%) of larvae achieved after 48, 84 and 108 hrs., of exposure, respectively.

After 6 hrs. of larval treatment by Pumpkin oil (LC_{50} 6.12) + Ar. (100, 80 and 60) mortality percentages were 7.79±0.58, 0±0.00 and 0±0.00%, respectively. The mortality (100.0%) of larvae were achieved in treatment of Pumpkin oil (LC_{50} 6.12) + Ar. (100 and 80) after 60 and 96 (hrs.).

In the present study, the combined treatments of three plant oils (LC_{50}) with Argon recorded higher toxicty than individual treatments on larvae of *G. mellonella*. The obtained results agreed with Gad *et al.* (2022) who reported that binary treatments of inert dusts (ZE and KA), with CA are considered a promising and alternative to different chemical insecticides for the control of bruchid insects infesting different stored legumes.

Table 5. Effect of plant oils (LC_{50}) with Argon 100, 80 and 60 % on fourth instar of *G. mellonella* larvae:

Concentrations % (v/w)	Reduction% Exposure time (hr.)								
	6	12	24	36	48	60	72	84	96	108
Lemongrass oil										
Ar. (100)+LC_{50} (2.18)	48.81± 1.53	73.36± 0.58	100± 0.00							
Ar. (80)+LC_{50} (2.18)	12.26± 1.53	26.67± 2.08	35.51± 1.15	52.22± 1.53	63.36± 1.15	88.89± 2.08	100± 0.00			
Ar. (60)+LC_{50} (2.18)	8.81± 0.58	16.66± 1.00	31.32± 1.15	46.67± 1.53	58.89± 0.58	71.11± 0.58	78.87± 1.53	85.56± 0.58	100± 0.00	
Moringa oil										
Ar. (100)+LC_{50} (3.78)	33.33± 1.15	56.66± 0.58	78.89± 2.08	93.31± 1.15	100± 0.00					
Ar. (80)+LC_{50} (3.78)	11.11± 0.00	21.11± 0.58	32.22± 1.00	76.67± 1.15	55.5± 1.53	73.31± 0.58	81.11± 2.08	100± 0.00		
Ar. (60)+LC_{50} (3.78)	2.11± 1.00	11.11± 1.15	26.67± 1.53	36.67± 0.58	48.89± 1.00	63.31± 1.15	72.22±1.00	80.01± 0.58	95.51± 1.00	100± 0.00
Pumpkin oil										
Ar. (100)+LC_{50} (6.12)	7.79± 0.58	16.66± 0.58	58.13± 2.08	80.01± 0.58	91.11± 1.53	100± 0.00				
Ar. (80)+LC_{50} (6.12)	0± 0.00	5.51± 0.58	18.89± 1.15	32.22± 1.00	51.11± 0.58	68.89± 0.58	83.31± 2.08	96.67± 1.53	100± 0.00	
Ar. (60)+LC_{50} (6.12)	0± 0.00	0± 0.00	6.66± 1.00	20.01± 1.53	26.67± 0.58	40.01± 2.08	51.11± 1.53	68.89± 1.00	77.71± 0.58	95.51± 0.58

6. Biochemical assay

Treatment by Argon (100%) with Lemongrass oil (LC_{50}) (2.18%) affected some enzymes activity in larvae of *G. mellonella* as shown in Table (6). There was insignificant increase in the activity of AchE (118.2 ug. AchBr/min/g.b. wt) in treated larvae opposed to 112.8 ug. AchBr/min/g.b.wt in larvae of control. While, a significant decreasin in the activity of P-4540 after treated with Argon and Lemongrass oil treatment, being 0.209 for P-4540 and 0.469 (nmol/ mg) for the control. According to the present results, it was clean that treatment had influence the respiration phase in insects. Also, treatment caused a significant decrease in GST, α-amylase and invertase enzymes of *G. mellonella* larvae compared to the control. Regarding α- amylase and invertase, results in Table (6) showed that the averages were 27.13, 18.4 and 90.4, 20.6 (mg glucose min /g. protein) for α- amylase, invertase activities and their control, respectively.

Table 6. Biochemical activities of Argon (100%) with plant oil (LC_{50}) at 6 hours post- treatment on 4th instar larvae of *G. mellonella*:

Enzymes treatment	AchE (ug.AchBr/min/g.b.wt)	Cytochrome P-450 (nmol/ mg)	GST (nmol sub. Coj. /min/g.b.wt)	α- amylase (mg glucose min /g.protein)	invertase (mg glucose min /g.protein)
Control	112.8±1.2	0.469±0.02	47.0±4.36	90.4±2.4	20.6±2.2
Ar. (100)+LC_{50} (2.18)	118.2±0.9	0.209±0.01	28.33±6.03	27.13±0.8	18.4±1.4

These results coincide with that reported by Said *et al.*, (2019) who assessed the biochemical parameters of total protein and acetylcholine esterase in *G. mellonella* the 3rd instar larvae fed by treated artificial diets which containing LC_{50} of lavender, camphor, mint, clover and rosemary oils for 48 hrs. Data revealed that tested essential oils caused an increasing on the total protein and AchE.

Studies showed that resistant populations to toxic compounds, in principle, are susceptible to plant essential oils and their components, so that the activity of cytochrome P-450 is usually decreased (Norris *et al.* 2018; O'Neal *et al.* 2019; Gaire *et al.* 2021).

Enzymes of Cytochrome P-450 are playing a critical role in metabolism of toxic substances of insects (Prasannakumar *et al.* 2023). Glutathione-s-transferases used in detoxification of compounds remaining from some metabolic reactions or toxins entering the body exogenously (Dasari 2018).

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تقييم فعالية ثلاثة زيوت نباتية وغاز الأرجون وخلطهم معا في مكافحة دودة الشمع الكبري

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الملخص

دودة الشمع الكبري *Galleria mellonella* تعتبر من أخطر الآفات التى تصيب أقراص شمع العسل في جميع أنحاء العالم. أجريت هذه الدراسة لتقييم وتحديد فعالية ثلاثة زيوت نباتية (زيت عشبة الليمون، زيت المورينجا وزيت اليقطين) وخلطها مع غاز الأرجون لتطوير طريقة بديلة وآمنة لمكافحة العمر اليرقي الرابع لدودة الشمع. تم تقييم كفاءة المواد المستخدمة على نسب الموت وفترة التعرض لدودة الشمع الكبرى لأربعة تركيزات مختلفة من كل زيت (3 ، 50.1، 0.75 و0.37% حجم/وزن). أوضحت البيانات أن نسب الموت تعتمد على نوع المادة والتركيز المعامل بهما. سجل زيت عشبة الليمون (100 ٪) موت لليرقات بعد 7 أيام من المعاملة، وهو أعلى تأثير مقارنة بالمادتين الأخرين والكنترول. كما أظهرت النتائج أنه بعد 3 أيام من المعاملة بزيت عشبة الليمون، كانت قيمة LC_{50} 2.18% (حجم/وزن). وكانت قيم LT_{50} كالأتي (22.48، 45.09 و68.04 ساعة) لمكافحة العمر اليرقي الرابع لدودة الشمع بإستخدام غاز الأرجون بالتركيزات الآتية 100، 80 و 60٪ على التوالي. بشكل عام، كان معدل موت اليرقات أعلى في المعاملات الثنائية (الزيوت النباتية (LC_{50}) الممزوجة بغاز الأرجون) مقارنة بالمعاملات الفردية بالزيت فقط. حيث أن أفضل معاملة كانت زيت عشبة الليمون LC_{50} 2.18 % + تركيز 100% من غاز الأرجون حيث سجلت نسبة الموت التراكمية (100%) لليرقات بعد 24 ساعة من المعاملة بهذا التركيز. كما أظهرت النتائج أن معاملة يرقات العمر الرابع لدودة الشمع الكبيرة بزيت عشبة الليمون LC_{50} 2.18 % + تركيز 100% من غاز الأرجون أدى لإرتفاع انزيم الأسيتيل كولين إستيريز AchE وإنخفاض في السيتوكروم P-450، GST، α- الأميليز والإنفيرتاز مقارنة بالكنترول. إلا أن هذه النتائج أوضحت ضرورة إجراء المزيد من الأبحاث للتأكد من فعاليتها في الحقل وعلى نحل العسل.