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Potential of Honey Bee Propolis and Venom as Eco-friendly Control Agents in *Galleria mellonella* L.

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ABSTRACT

The study was conducted to determine the toxicity approach of four honey bee propolis and venom concentrations, 500, 1,000, 2,000 and 3,000 ppm, regarding the biological aspects of the wax moth larvae *Galleria mellonella* L. Using 2,000 and 3,000 ppm propolis concentrations gave a 100% reduction percentage on wax moth larvae after spraying the treatment for 72 and 48 hours consistently. Applying 3,000 ppm of bee venom resulted in a 100% mortality percentage for the three test times of 24, 48 and 72 hours. The lowest period of larval development was observed when propolis was used at a concentration of 3,000 ppm, with an average of 7.66±0.33 days. At the 3,000 and 2,000 ppm concentrations of propolis, the pupal stage was unable to develop for adults of the insect, as all the individuals died. For bee venom, at concentrations of 3,000 and 2,000 ppm, none of the larvae have succeeded in the development of pupa or adult insects. Bee venom was the most effective substance against wax moth larvae, followed by propolis; LC50 values were 272.62 and 3,166.42 ppm, respectively. These results clarified the need for more research to affirm their effect in the field and on honey bees.

KEYWORDS

Biological aspects, mortality rate, natural substances, suppression materials, toxicity, wax moths

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1. Introduction

Beekeeping is an important part of modern agriculture because it pollinates the country's main crops, makes honey for the food industry and produces beeswax for use in many other industries. For this reason, it is important to keep an eye on the health and productivity of honey bee colonies (Bradbear, 2004). Honey bees are often attached by *Galleria mellonella*, *Achroia grisella* and the new species of wax moths, *Galleria similes* (Ellis *et al.,* 2013). It is thought that the damage done by *G. mellonella* larvae is one reason why wild and solitary honey bee numbers are declining (Kong *et al.,* 2019). This insect breaks down beeswax, which is a valuable product that can bring in a lot of money, along with honey (Bradbear, 2004). Additionally, both adult and larval wax moth stages can spread pathogens that cause serious bee diseases, like foulbrood (Owayss and Abd-Elgayed, 2007). A variety of physical techniques have been used to control *G. mellonella* L. (James, 2011). A wide range of biological control agents (Ellis *et al.,* 2013) and the sterile insect technique have been assessed for their efficacy against this parasite (El-Kholy and Mikhaiel, 2008). Furthermore, numerous insect hormone analogues and insect growth regulators have been evaluated for their effectiveness against *G. mellonella L.* (Amany *et al.,* 2021). However, chemicals are still needed to get rid of this pest. It is easy and efficient to use these chemicals, but we should think about whether they are safe and will contaminate bee products (Naqqash *et al.,* 2016). Accordingly, a thorough assessment of viable non-chemical wax moth control strategies in dry environments might be a crucial task for bettering living circumstances, as it would help beekeepers combat this pest and boost their yield. The study objectives are to assess and validate some proposed bee products as agents for controlling wax moths and identify a possible preventive strategy for the study region.

2. Materials and Methods

2.1. Study Area and Honey Bee Colonies:

Field experiments for collecting and producing honey bee propolis and venom were evaluated in 2023 at the Research and Training Station Apiary, King Faisal University, Al-Ahsa Province, Eastern Region, Kingdom of Saudi Arabia. Local honey bee colonies (*Apis mellifera yemenitica*) with nearly equal strength in bees, brood and food were used.

2.2. Propolis Samples Collection and Extraction:

Propolis resin was obtained by scraping propolis off frame rests and edges, bottom boards and the insides of hive boxes. Propolis was refined with an ethanol solvent. Propolis was minced after being stored overnight in a deep freezer at -20°C. A propolis sample was weighed, then a 70% ethanol solvent (1:30 w:v) was added, and the mixture was left at room temperature for 24 hours. The propolis suspension was subjected to an ultrasonic bath for 20 minutes at a temperature of 20°C. The filter paper was employed to separate the resultant suspension at ambient temperature. The operation was subsequently repeated with the portion of the suspension that was retained in the filter. The residue was subsequently extracted again under identical circumstances (Popova, *et al.*, 2004). For forthcoming research, the extracted material (the stock) will undergo evaporation for drying (Netíková *et al.,* 2013).

2.3. Bee Venom Collection and Extraction:

Bee venom was harvested using the Bee Venom Collector Device for 20 minutes every 15 days. The device was set aside the third comb from the entrance to the hive. After the collection was finished, the dried venom was scraped off with a sharp blade and placed into a dark container and kept in a cool and dry place. Bee venom extracts are made with a water solvent (Kosuge, 1969). The stock extracts,

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Ethanol Extraction of Propolis (EEP) and Water Extraction of Venom (WEV), were tested against the fifth instar of the wax moth larvae *Galleria mellonella*. Four concentrations (500, 1,000, 2,000 and 3,000 ppm) were prepared and tested from each extract by spraying over the larvae.

2.4. Preparation of *G. mellonella* L. Larvae Culture:

Local strains of *G. mellonella* L. larvae were procured from the Plant Protection Research Institute, Pest Physiology Department, ARC, Egypt. The collected larvae were placed in glass rearing jars (8 x 8 x 20 cm) and artificially raised using a medium that was created by following Wiesner's guidelines (1993). The media included bee honey (75 mL), glycerol (75 mL), rice cerelac (100 g), wheat bran (100 g), dry yeast (1 g), nipagen as an antimicrobial (0.15 g), vitamins and minerals (2 capsules; 500 mg) and water (50 mL). The collected larvae were transferred to a glass rearing jar, which was filled with 50 freshly hatched larvae per jar, 100 g of the medium and a metal cover with holes for ventilation. The jar was then sealed and kept in an incubator set at 25°C with fresh food supplied three times a week. To start a new generation, pieces of wax were put into the pupal stages' jars to facilitate their transition into moths and encourage them to deposit eggs on them (Figure 1).

Figure1. Shows adult (a), eggs (b), larva and pupa (c) and symptoms of damage (d) (the author took all photos)

2.5. Biochemical Studies:

2.5.1. Toxicity and Biological Aspects of Bee Propolis and Venom Extracts Against *G. mellonella* Larvae

Under laboratory settings, 500, 1,000, 2,000 and 3,000 ppm concentrations were prepared from the stock solutions (100%) of propolis and bee venom extracts. These tested materials were then used as liquid formulations for EEP and WEV extraction and placed onto plastic plates (10 cm) containing the required media. Ten larvae of *G. mellonella* fifth instar were put together with 10 g of tiny artificial medium fragments in each Petri plate. One millilitre of each concentration was sprayed above the wax moth larvae. Then, the larvae and medium were covered with a muslin cloth and fastened with elastic bands to keep the larvae from fleeing. The number of dead larvae was counted by daily observations. Every treatment was carried out three times. Every day, the examined larvae were inspected to count and remove any dead ones. After 24, 48 and 72 hours of treatment, mortality percentages were computed.

2.5.2. Preparation of Homogenate Samples

To homogenise the samples, all dead *G. mellonella* larvae were collected for each treatment, placed in the freezer and placed on the centrifuge device, and then a sample was taken for an analysis representative of each treatment. After homogenising the samples in distilled water, the BECKMAN GS-6R Centrifuge was used to centrifuge the mixture for 10 minutes at 5°C at 6,000 rpm. Following centrifugation, the fluid supernatant was separated into tiny aliquots (0.5 mL) and kept cold until the primary constituents were analysed. Each biochemical determination was done in three replicates (Assar *et al.*, 2016).

2.5.3. Total Carbohydrate Content Determination

Using an anthron reagent, the technique outlined by Singh and Sinha (1977) was used to calculate the total amount of carbs. (A) Anthron reagent preparation: 28 mL of H_2O, 50 mg of anthron and 72.0 mL of concentrated H_2SO_4 (98%) were combined, and the mixture was vigorously shaken. (B) Method: 100 µL of the insect homogenate sample solution was diluted to one millilitre using distilled water, and then five millilitres of anthron reagent were added. In a test tube, 1.1 mL of water and 5 mL of anthron reagent were used as a blank. After 10 minutes of being submerged in boiling water, each tube was allowed to cool for 15 minutes at room temperature. At 620 nm, the absorbance was measured. The carbohydrate content was given as milligrams per gram of body weight.

2.5.4. Total Protein Content Determination

Following centrifugation, the fluid supernatant was separated into tiny aliquots (0.5 mL) and kept cold until the primary constituents were analysed. Each biochemical determination was done in three duplicates. The Bradford (1976) technique was employed to quantify the total proteins by utilising bovine serum albumin as a reference standard. (A) Protein reagent preparation: 50 millilitres of 95% ethanol was used to dissolve 100 milligrams of Coomassie Brilliant Blue G-250. Then, 100 mL of 85% (w/v) phosphoric acid was added to this solution. One litre was the final volume at which the resultant solution was diluted. (B) Protein assay: 50 µL of pupal homogenate were pipetted into a test tube with 50 µL of phosphate buffer (pH 6.6). The test tube's contents were then vortexed after the protein reagent (5 mL) was added. After two minutes, the absorbance at 595 nm was measured in comparison to a blank made with five millilitres of protein reagent and 0.1 millilitres of phosphate buffer (pH 6.6). The protein content was calculated in milligrams per gram of body weight.

2.5.5. Total Lipid Content Determination

Phosphovanillin reagent and standard curve were used to quantify the content of total lipids in the insect homogenate following Joseph *et al.,* (1972). Following centrifugation, the fluid supernatant was separated into tiny aliquots (0.5 mL) and kept cold until the primary constituents were analysed. Each biochemical determination was done in three duplicates. (a) Preparation of the phosphovanillin reagent: 10 mL of 100% ethanol was used to dissolve 0.6 g of pure vanillin, which was then added to 100 mL of distilled water. After adding 400 mL of concentrated phosphoric acid, the mixture was kept at room temperature in a dark glass bottle. (b) Procedure: In a test tube, 250 µL of insect homogenate sample solution was combined with 5 mL of concentrated sulfuric acid, and the mixture was heated in a boiling water bath for 10 minutes. Then, 500 µl of the digest was added to the 6.0 mL phosphovanillin reagent after it cooled to room temperature. Following a 45-minute dark incubation period, the colour generated was quantified at 525 nm in comparison to a reagent blank made with 6.0 mL of phosphovanillin reagent and 500 µl of distilled water. The lipid content was given as milligrams per gram of body weight. (c) Lipid standard curve preparation: To create the standard curve, serial concentrations of a combination of palmitic and oleic acids (7:3) ranging from 0.5 to 5 mg/mL were produced in 100% ethanol and handled similarly to the unknown. Optical density was used to blot the standard curve against concentration.

2.6. Analytical Statistics:

Probit analysis was used to calculate analytical statistics and LC_{50} values, which were given in ppm units. ANOVA was used to assess the mortality data (Snedecor and Cochran, 1980). Using the Costate (version 6.204) statistical software programme, CoHort Software (CoStat Software, 2004), the least significant difference (LSD) was performed to assess the means differences at $p \leqslant 0.05$.

3. Results and Discussion

3.1. Toxicity Tests of the Bee Propolis and Venom Against Wax Moth Larvae:

Table 1 indicated that bee venom was the most potent applied compound against wax moth larvae followed by propolis. LC_{50} values were 272.62 and 3,166.42 ppm, respectively.

Table1. LC50 and LC90 values of the tested bee propolis and venom on wax moth larvae *G. mellonella*

Treatment	LC_{50} (ppm)	LC_{90} (ppm)	Upper lower value	Slope± S.E.
Propolis	3,166.42	15,419.54	2,526.15 4,518.34	1.68±0.28
Bee venom	272.62	1,569.80	128.56 403.03	1.68±0.30

S.E. = Standard Error

According to Mahgoub *et al.,* (2018), third-instar larvae were given topical applications of crude honey bee venom at doses of 0, 6.25, 12.5, 25 and 50 µg. At the lower concentration, the calculated death percentages for all treatments were 8%, and at the high concentration, 52%. The determined fatal median concentration (LC_{50}) was 38.27 µg/µl. The result of Ghoneim (2020) indicated that 3,428.9 ppm was determined to be the LC_{50}. In terms of growth and development, the larvae's somatic weight gain decreased somewhat in proportion to the concentration.

Garedew *et al.,* (2004) mentioned that propolis is toxic at high concentrations and an insect growth regulator at lower ones. In addition, using propolis on the fifth larval instar caused a very high mass-specific metabolic rate and cuticle thinner and more fragile, allowing the free transit of nonpolar toxic substances from the surroundings after being easily disrupted by propolis components.

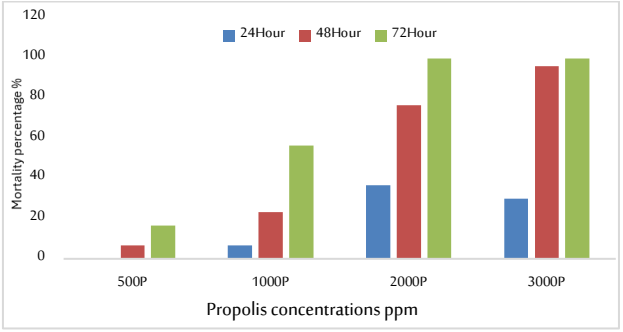
3.2. Effect of Bee Propolis and Venom on the Larval Stage of *G. mellonella*:

Table 2 and Figure 2 present the mean mortality and reduction percentage of wax moth larvae when applied to four concentrations of propolis 500, 1,000, 2,000 and 3,000 ppm. Propolis recorded the highest mortality number for concentrations of 2,000 and 3,000 ppm after treatment for 72 hours. The mean mortality values were 10 for both concentrations, followed by propolis concentrations of 2,000 and 3,000 ppm after 48 hours with mean mortality values of 7.66±0.33 and 9.6±0.33, respectively. The lowest percentages of the mortality rate for using a propolis concentration was observed for 500 ppm after 24 hours of treatment: 0.0% and 6.6%, respectively. Using 2,000 and 3,000 ppm of propolis concentrations gave a 100% reduction percentage after treatment for 72 hours, followed by using the propolis after 48 hours, which led to the percentage of mortality being 76.6% and 96%, respectively. According to Shimanuki and Knox (1997), honey, pollen, wax combs and the skins of bee larvae are the main foods for wax moth larvae. A statistical analysis indicated that significant differences in the mortality rate of the wax moth among all treatments were observed. According to Hussein *et al.,* (2022), depending on the concentration and length of exposure, there are substantial changes in the percentage of killing between propolis extract concentrations at a probability threshold of 5%. During the initial therapeutic period, the mortality percentage observed at a concentration of 2% was 53.3%. For one week, the interaction resulted in the highest death rate of 76.6% when the concentration was 3%. In contrast, the comparator medication did not cause any mortality. The experiment also revealed the initial larval stage's susceptibility to compounds extracted from propolis and cinnamon plants. The results showed that there were considerable variations in the proportion of organisms killed based on the concentration and duration of exposure to the extracts. As an illustration, the maximum mortality rate observed at the given concentration was 3%, which increased to 76.6% during one week of treatment. Conversely, the minimum mortality rate at the same dose was 2%, reaching 53.3% on the first day of the experiment. Sanad and Mohany (2015) indicated that mortality percentages went up by increasing the time after application. The highest percentages of late instar larvae were obtained after 72 hours of treatment of all tested materials, except for the mint treatment. Mint oil recorded the highest accumulative mortality percentage after the three days at 4%, giving 70%, pursued by 4% Chinese propolis and 4% cinnamon, which gave 60%, while 4% clove gave 50%, and 4% of Egyptian propolis gave only 40%. These results agree with Izhar-ul-Haq *et al.,* 2008.

Table 2. Mean mortality and percentages of the late wax moth stage treated with propolis

Propolis concentrations(ppm)	24 hours	%	48 hours	%	72 hours	%
500	0.0b	-	0.66d±0.33	6.6	1.66c±0.881	16.6
1,000	0.66b±0.33	6.6	2.33c±0.66	23.3	5.66b±0.33	56.6
2,000	3.66a±0.33	36.6	7.66b±0.33	76.6	10.0a	100
3,000	3.0a±0.57	30	9.6a±0.33	96.0	10.0a	100
Control	0.0b	-	0.0d	-	0.0d	-
$LSD_{5\%}$	1.050		1.242		1.328	
F	7.3		121.214		10.187	
P	.0000***		.0000***		.0000***	

Figure 2. Mortality percentages of the wax moth larvae stage treated with propolis

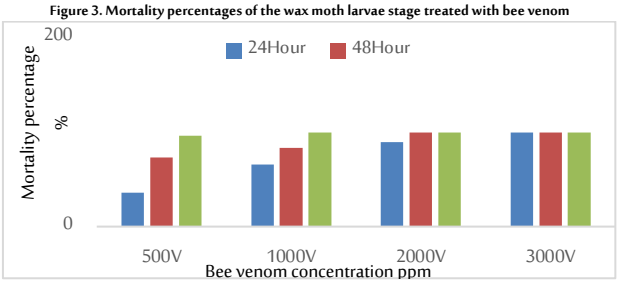


From Table 3 and Figure 3, it could be concluded that applying 3,000 ppm of bee venom concentration on wax moth larvae gave a 100% mortality percentage for the three tested times of 24, 48 and 72 hours. The mean mortality value was 9±0.57 of 2,000 ppm bee venom concentration with a 90% mortality percentage after 24 hours of applying. Then, the mean mortality value reached 10 with a 100% mortality percentage after 48 and 72 hours of using 2,000 ppm concentration. The mean mortality values of wax moths were 6.66±0.33 and 8.33±0.33 and 10 with mortality percentages of 66, 83.3 and 100% after 24, 48 and 72 hours with 1,000 ppm concentration, respectively. Data showed that applying 500 ppm of bee venom on wax moths gave the lowest mortality values after 24, 48 and 72 hours, giving 3.66±0.33, 7.33±0.33 and 9,66±0.33, with the lowest mortality percentages of 36, 73.3 and 96.6%, respectively.

Table 3. Mean mortality and percentages of the late wax moth stage treated with bee venom

Bee venom concentration(ppm)	24 hours	%	48 hours	%	72 hours	%
500	3.66b±0.33	36	7.33c±0.33	73.3	9.66a±0.33	96.6
1,000	6.66b±0.33	66	8.33b±0.33	83.3	10.0a	100
2,000	9.0a±0.57	90	10.0a	100	10.0a	100
3,000	10.0a	100	10.0a	100	10.0a	100
Control	0.0d	-	0.0d	-	0.0b	-
$LSD_{5\%}$	1.050		0.664		0.469	
F	150.3		387		886	
P	.0000***		.0000***		.0000***	

Figure 3. Mortality percentages of the wax moth larvae stage treated with bee venom



3.3. Effect of Bee Propolis and Venom Extracts During Post-embryonic Development of *G. mellonella* Larvae:

Table 4 shows the impact of four propolis concentrations of 500, 1,000, 2,000 and 3,000 ppm on the number of days experienced by larvae, pupae and adults after treatment. The shorter period of the larval stage with propolis at a 3,000-ppm concentration was followed by a concentration of 2,000 ppm and then 1,000 ppm with averages of 7.66, 11.33 and 18 days, respectively. The highest period of larval development for control was, on average, 24 days. Sanad and Mohany (2015) mentioned that the larval stage duration decreased by increasing the Egyptian propolis concentration, where it was 14.9±2.6 days at a 4% concentration, compared with 24.2±4.1 days at the control. The mean duration of the larval stage was decreased by increasing the Chinese propolis by a 4% concentration compared with 25.2±5.1 days at control.

A significant difference between the control and each of the tested four concentrations was observed. The lowest periods of pupa were 3,000 and 2,000, followed by 1,000 ppm. The average pupa stages were 6.66±0.33, 8.66±0.88 and 12.33±0.33 days. Sanad and Mohany (2015) described that as the Egyptian propolis concentration increased, the mean duration of the pupa stage decreased. It was 15.0±1.3 at 4% concentration, compared with 17.1±1.7 days for the control. The mean duration period of the pupal stage decreased by increasing the Chinese propolis concentration, giving 15.0±1.3 days at 4% concentration, compared with 16.1±1.8 days at control. At 3,000 and 2,000 ppm concentrations of propolis, the pupae were unable to develop for adults, where all the individuals died. On the other hand, with a 1,000-ppm concentration of propolis, all pupae were developed for adults. The average was 8.33±0.33 days, and the control was the highest average of 13±0.57 days. Sanad and Mohany (2015) mentioned that adult longevity was 10.1±1.7 days at 4% of the Egyptian propolis concentration compared with 14.2±2.3 days at the control.

Wax moth larvae are at the harmful stage that destroys wax combs with food and brood. Therefore, the longer the duration of the larvae stage, the greater the damage, and vice versa: the shorter the duration of the larvae stage, the lower the damage. Any substance that causes the wax moth eggs to not hatch or leads to a shorter duration of the larvae phase is important and can be included in the integrated control programmes of the greater wax moths (Ellis *et al.,* 2013). According to Garedew *et al.,* (2004), the wet weight of the larval instars increased drastically, from a mean value of 23±2.5 mg at L5 to 65.7±5.8 mg at L6 more than twice, achieving its maximum mean value of instars used in the investigation. Furthermore, the weight change during the entire larval developmental stage ranges from <1 mg at L1 to nearly 400 mg at L7.

Table 4. Effect of different propolis concentrations on the post-embryonic development period of wax moth larvae

Propolis concentration (ppm)	Period of stages (day)		Adult longevity
	Larval	Pupal	
500	21.33a±0.88	15.33a±0.66	11.66b±0.33
1,000	18.0b±1.15	12.33b±0.33	8.33c±0.33
2,000	11.33c±1.45	8.66c±0.88	0.0d
3,000	7.66d±0.33	6.66d±0.33	0.0d
Control	24.0a±0.57	16.0a±0.57	13a±0.57
$LSD_{5\%}$	3.044	1.878	1.050
F	49.976	46.812	352.7
P	0.0000***	0.0000***	0.0000***

In Table 5, the lowest period of larvae development was at the concentration of 3,000 followed by 2,000, and the least was 500 ppm of bee venom with averages of 3.0±0.57, 3.3±0.88 and 12.33±1.45 days, respectively. For the concentrations of 3,000 and 2,000 ppm, none of the larvae have succeeded in the development of pupa or adult insects. The concentrations of 500 and 1,000 ppm larvae evolved into pupa and did not succeed in reaching the adult stage. The study conducted by Ghoneim (2020) evaluated the effects of treating newly molted third-instar larvae of *G. mellonella* with scorpion L. *quinquestriatus* venom. The inability of *G. mellonella* larvae during ecdysis to shed their exocuticle (Linton *et al.*, 1997) or the suppression of chitin production (Adel, 2012) could be the reason for the larvae's death when exposed to the studied arthropod products.

Ghoneim *et al.*, (2000) mentioned that larval death may be attributed to the antifeedant impact of the venom and secretion, leading to persistent starvation in the larvae. The poison had a lethal effect on the pupae, as the severity effect was directly proportional to the dosage. In addition, the tested venom can induce pupal deaths in *G. mellonella* by interfering with important processes like suffocation, bleeding and desiccation (Possani *et al.,* 1999). In contrast, the venom did not have any impact on the survival of adult individuals. These deaths occur because the venom hinders proper exuviation and disrupts essential homeostatic mechanisms (Ghoneim *et al.*, 2000).

Table 5. Effect of different bee venom concentrations on the post-embryonic development period of wax moth larvae

Propolis concentration (ppm)	Period of stages (day)		Adult longevity
	Larval	Pupal	
500	12.33b±1.45	8.33b1.76	0.0 d
1,000	10.33b±2.60	7.66b±1.45	0.0 d
2,000	3.33c±0.88	0.0 c	0.0 d
3,000	3.0 c±0.57	0.0 c	0.0 d
Control	25.0a±1.15	17.0a±1.15	13.0a±1.20
$LSD_{5\%}$	4.744	3.608	1.627
F	35.38	38.025	126.75
P	0.0000***	0.0000***	0.0000***

3.4. Effect of Bee Propolis and Venom on Total Lipid, Protein and Carbohydrates of *G. mellonella* Larvae:

The present investigation indicates that all propolis concentrations impact lipids, protein and carbohydrates compared with control on wax moths. The data in Table 6a and Figure 4, the highest average of lipids, was achieved by propolis concentrations 2,000 and 1,000 ppm with mean values of 10.93±0.67 and 10.55±0.34, respectively. The lowest average of lipids was achieved by the control, giving a 3.16±0.26 value. Lipids are a vital energy source, precursors to hormones and structural components for insects. They are carried from their place of production of storage to the user organs by the hemolymph (Zhou and Miesfeld, 2009). However, the current findings partially align with previous studies that indicate an increase in lipids in different insect species following treatment with certain insect growth regulators during the larval stage (Bouaziz *et al.,* 2011). Protein had the highest average when a concentration of 3,000 ppm of propolis was used, with a mean value of 31.94±4.01. Control had the second-highest average, giving a mean value of 27.94±0.44. Protein metabolism is vital for generating energy and facilitating insect reproduction (Taskín and Aksoylar, 2011). It also plays a mandatory role in the development of adult structures in insects during their transformation from larvae and pupae to adults (Resmitha *et al.,* 2014). Through hormones, enzymes and nucleoproteins, proteins integrate and regulate a number of physiological and metabolic processes in the body of an insect (Chapman, 2012). For the carbohydrate analysis, the highest average rate was achieved by the control with a mean value of 2.59±0.006. A significant analysis was observed for all treatments compared with the control. During an insect's metamorphosis, carbohydrates are crucial to the composition and operation of every tissue. Furthermore, carbohydrates are necessary metabolites for the development of the embryo and the proper operation of the reproductive systems in both sexes. Carbohydrates are generally important for the physiology of insects exposed to external poisons (Kaufmann and Brown, 2008).

A wax moth larvicidal effect was observed when treated larvae with propolis and increased with the concentrations. In addition, higher concentrations of propolis extract accelerated the larvae and pupae

development in adults, which might lead to malformed and immature individuals. This higher rate of development requires more energy and feeds, which can be obtained by lipids and carbohydrate metabolic (Ararso and Legesse, 2016).

Table 6a. Total lipids, protein and carbohydrate values (mg/gram) for wax moths in different concentrations of propolis

Propolis concentration (ppm)	Lipids	Protein	Carbohydrates
500	7.88b±0.90	18.55b±0.28	1.11d±0.066
1,000	10.55a±0.34	19.82b±0.43	1.60c±0.04
2,000	10.93a±0.67	21.82b±0.30	1.74b±0.043
3,000	6.19b±0.58	31.94a±4.01	0.57e±0.01
Control	3.16c±0.267	27.94a±0.44	2.59a±0.006
$LSD_{5\%}$	1.89	5.758	0.127
F	28.598	9.770	342.67
P	0.0000***	0.0017***	0.0000***

Figure 4. Content of lipids, protein and carbohydrates of the wax moth larvae treated with propolis

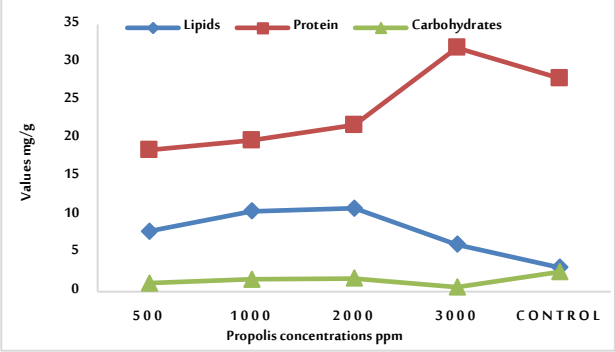


Table 6b and Figure 5 show the analysis of wax moths' lipids, protein and carbohydrates when extracting four concentrations of bee venom. The results are as follows: Wax moths' lipids were higher when treated with bee venom at a concentration of 1,000 ppm, followed by concentrations of 2,000 ppm and 500 ppm. The value of the lipids was lower in the control treatment.

According to Tanani *et al.,* (2021), in the fifth and seventh instars of normal larvae, respectively, the lipid content dropped steadily with instar (265.77±18.0 and 247.75 mg/g). This data demonstrates that all arthropod venoms have a shared impact of inducing larvae to amass a greater amount of lipids compared to control counterparts. Apitoxin had the lowest level of larval-boosting activity during their fifth instar. The scorpions' venom had the smallest enhancing impact on lipids in the seventh stage of larval development.

Lipids are a vital source of energy, precursors to hormones and structural components for insects. They are carried from their place of production of storage to the user organs by the haemolymph (Zhou and Miesfeld, 2009). According to Canavoso *et al.,* (2001), the reduction of lipids in wax moth larvae bodies treated with bee venom might be due to the venom's toxic compounds, which influence the synthesis of lipids in *G. mellonella* larvae and pupae bodies. In addition, bee venom disruptively affected physiology and subsequently deranged vital growth and reproduction functions (Bouaziz *et al.,* 2011).

The treatment with 2,000 ppm of bee venom had the highest protein content of the wax moth body, followed by the control and 3,000 ppm with negligible variations among them (27.39±0.48, 27±0.44 and 25.38±1.58). The treatment with 500 ppm of bee venom produced the lowest protein value (20.03±0.10, $\boldsymbol{P}$<0.0003). Tanani *et al.,* (2021) indicated that the treated larvae had significantly less total protein, irrespective of the venom amount or larval stage development. The effectiveness of the bee venom, observed in the larvae during their fifth instar with an inhibition rate of 19.80%, and wasp venom, observed in their seventh instar with a reduction rate of 34.77%, showed the most significant decline. Scorpion venom showed the least amount of strength loss (17.45% and 15.7% decreases in the fifth and seventh instar larvae, respectively).

Protein metabolism is critical for insect energy production and reproduction (Kong *et al.*, 2019), as well as for putting together adult structures when insects change from larvae or pupae to adults (Resmitha *et al.*, 2014). Proteins are essential for controlling and organising many of the body's natural and metabolic processes. This is achieved through the action of hormones, enzymes and nucleoproteins (Chapman, 2012). When it comes to analysing carbohydrates, the control group outperformed the others with an impressive average rate of 2.58±0.006. There was a noticeable analysis conducted for all treatments in comparison to the control. When *G. mellonella* larvae in their third instar were exposed to the LC_{50} value of each venom by Tanani *et al.,* (2021), there was a significant decrease in the amount of carbohydrates found in their bodily tissues during both their fifth and seventh instars. For regular larvae, there was a decrease in the amount of carbohydrates as they progressed through each instar. Specifically, in the fifth and seventh instars, the amount dropped to 0.0366±0.007 mg/g. When it comes to the impact on carbohydrates, it is interesting to note that the wasp venom had a minimal effect on the fifth larval instar. On the other hand, the scorpion venom had a similar effect on the seventh larval instar, resulting in reductions of 11.78% and 21.04%, respectively.

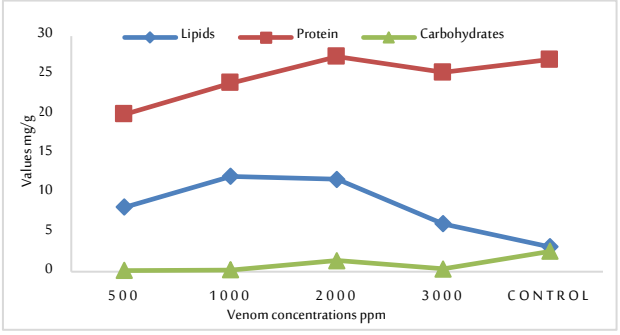
Carbohydrates are crucial for the development and functioning of various tissues during the process of metamorphosis in insects. In addition, carbohydrates initially support the proper functioning of the reproductive organs in both males and females, as well as in the development of embryos (Kaufmann and Brown, 2008). Carbohydrates significantly impact the physiology of insects exposed to foreign toxins, as highlighted by Kaufmann and Brown (2008).

The toxicity effect of bee venom on corn earworms, tobacco hornworms and lesser and greater wax moths was recorded (Ghoneim *et al.*, 2019a). In addition, Ghoneim *et al.,* (2019b) mentioned that bee venom causes reduced larval growth rate and weight and prevents adult emergence and fecundity for *G. mellonella* L.

Table 6b. Total lipids, protein and carbohydrate values (mg/gram) for wax moths in different concentrations of bee venom

Bee venom concentration(ppm)	Lipids	Protein	Carbohydrates
500	8.23ab±0.61	20.03c±0.10	0.14d±0.020
1,000	12.13a±0.285	24.04b±0.65	0.22d±0.003
2,000	11.75a±4.85	27.39a±0.48	1.40b±0.07
3,000	6.11ab±0.46	25.38ab±1.58	0.34c±0.003
Control	3.16b±0.26	27a±0.44	2.58a±0.006
$LSD_{5\%}$	6.948	2.586	0.103
F	2.969	14.886	105.177
P	0.0741(ns)	0.0003***	0.0000***

Figure 5. Content of lipids, protein and carbohydrates of wax moth larvae treated with bee venom



4. Conclusion

Based on the current findings, the applications of high concentrations of bee venom and propolis result in a complete reduction (100% reduction percentage) of wax moth larvae. At concentrations of 3,000 and 2,000 ppm of propolis, the pupal stage of the insect was unable to develop into the adult stage, resulting in the death of all individuals. At

concentrations of 3,000 and 2,000 ppm of bee venom, none of the larvae were able to reach the pupae or adult stage. At concentrations of 500 and 1,000 ppm, the bee larvae underwent metamorphosis into pupae but were unable to complete their development into adults. Accordingly, we recommend using bee venom with a concentration of 2,000 ppm and propolis with a concentration of 3,000 ppm to control the greater wax moth *Galleria mellonella* under laboratory conditions. However, bee venom and propolis can find their way into integrated pest management programmes to manage the greater wax moths in the foreseeable future.

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