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Bioactivity and Prospects of Using Ethanolic Extracts of Some Plants and Bee Glue (Propolis) to Control the Greater Wax Moth *Galleria mellonella* (L) (*Lepidoptera: Pyralidae*)

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ABSTRACT

The greater wax moth (*Galleria mellonella*) is one of the most important pests of stored or unattended combs that cause severe damage to bee broods, threatening the development of apiculture in various countries, especially Sudan. A laboratory experiment was conducted in the College of Agricultural Studies, Sudan University of Science and Technology, Shambat, Sudan, to explore the potency of ethanolic extract (EE) of different parts of three different plants and bee glue against the third larval instar of greater wax moth. The experiment was laid out in Randomized Complete Block Design and replicated five times. The percentage of mortality of GWM was recorded after 24, 48, 72, and 96 hours post-treatment. Analysis of the variance of the obtained data revealed significant differences regarding insecticidal effect between the EEs of the four test products and the control. The EE of *Eucalyptus camaldulensis*, Propolis, *Nigella sativa*, and *Carum carvi* have bioactivity action against the third instar larvae of GWM with an increased percentage of mortality according to the increase of concentration as well as elapse of time. Hence, the highest concentration (15%) caused 86.7%, 83.3%, 73.3 and 66.7% larval mortality after 96 hours for EE of leaves of *E. camaldulensis*, powder of bee glue (Propolis), seeds of *N. sativa* and seeds of *C. carvi* respectively. The results also clearly demonstrated that the EE of the powder of Propolis is significantly more toxic than *E. camaldulensis*, *N. sativa*, and *C. carvi*, where the LC50 values were 3.1% for Propolis, 5.0% for *E. camaldulensis*, 7.0 % for *N. sativa* and 7.7% for *C. carvi*. The products mentioned above at the mentioned concentration are environmental friendly, safe for honeybees, economically feasible, and affordable for small beekeepers.

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1. Introduction

The greater wax moth (*Galleria mellonella* L.) is one of the most notorious insect pests threatening apiculture worldwide. Newly hatched larvae of this pest feed on honey, nectar, and pollen, tunneling to the midrib of the comb. Chewing of worms destroy the wax cells of the combs, which become a mass of debris and dust. In addition, the feces produced by the larvae pollute the wax and become media for the growth of some contaminants [1].

According to feeding, tunneling, and pollution, the complete demolition of the bee colonies will happen and finally affect the produce and its salability [2].

The GWM is a holometabolous insect with four divergent life stages: egg, larva, pupa, and adult. According to biotic and abiotic factors, the moth life cycle is completed within weeks to months, and adult wax moth activity rises due to favorable environmental conditions [3].

Previous studies in Sudan [4] reported that the infestation percentage of bee combs with wax moths reached 86% in Gezira and Khartoum states. The control of this pest is a global problem, particularly in warm climates [5]. The assumption was that the wax moth would never be eliminated from an apiary or storage shed. It was reported that the best way to control this pest is to keep colonies strong and healthy. To reduce the population of wax moth numbers and restrict their reproduction, beekeepers have to control any damage by understanding the life cycle of the pest as well as taking measures in the field and within comb storage areas [5].

Different control measures were used in various countries to safeguard combs from infestation by the GWM.

In the past, this pest was efficiently controlled through the application of chemical fumigation, such as methyl bromide, ethylene dibromide (EDB), and paradichlorobenzene (PDB) [6]. Only paradichlorobenzene appears to have a long-term future as a registered pesticide against the wax moth [7]. Given the undesirable ecosystem outburst that succeeded following excessive and continuous reliance on conventional pesticides [8].

Several bio-agents are powerful in controlling wax moths; entomopathogenic fungus, *Beauvaria bassiana*, and *Metarrhizium anisopliae*, which attack larval and pupal stages of both wax moths *G. mellonella* and *A. grisella* in Czechoslovakia [9]. On the other hand, *Apanteles galleriae* Wilkinson, 1932, a solitary endoparasitoid of the order Hymenoptera and the family Braconidae, was found to be the most effective natural enemy to wax moths, *G. mellonella* and *A. grisella* larvae in different areas of the world [10-12].

Several oils and plant extracts, such as eucalyptus oil which is produced from (*Eucalyptus. spp*.) and contains eucalyptol, flavonoids, tannins, alkaloids, glycosides, terpenoids, and steroids [13], widely used to control several crop pests, including *Alphitobius diapering* and *Spodoptera frugiperda* [14].

Propolis or bee glue is a resinous product collected by honeybees from flowers leaf and buds to maintain the hive environment properly [15]. As well as seal the gaps inside the hives [16], it contains 50% resin and vegetable balsam, 30% wax and aromatic oils, 5% pollen, and 5% other substance, including minerals such as magnesium, nickel, iron, calcium, and zinc [17]. Propolis was successfully applied to prevent the beehives from ectoparasite *Varroa destructor*, the microsporidium *Nosema ceranae*, the bacterium *Paenibacillus,* and the fungus *Ascosphaera apis* [18]. On the other hand, Propolis showed high performance in controlling *Sitophilus zeamais* and *Callosobruchus maculatus* in grains [19].

Black seed, *N. sativa* is an erect annual plant that grows from 20 to 60 cm tall with one or more branching stem [20, 21].

Seeds of *N. sativa* contain fixed oil that ranges between 28 to 36% and is chiefly composed of unsaturated fatty acids that are arachidonic, eicosadienoic, linoleic and linolenic and saturated fatty acids that include palmitic, stearic and myristic [22]. In addition, the black seed is rich in bio-chemicals such as phenolic compounds, vitamins, amino acids, carbohydrates, hydrocarbons, and carboxylic acid [23, 24]. The seed oil or powder of black seeds was used to control field crop pests [23] and store pests [25] as well as insect vectors of human and animal disease [26,

27]. The Repellence of *N. sativa* seed oil to *Anopheles gambiae* is attributed to the presence of α-pinene, p -cymene, and longifolene [28].

The seed of the caraway plant is the only part that contains the essential oils Limonene and Carvone, with about a 3-6 % yield [29]. Hussein *et al.* Reported that caraway proved repellent efficacy against rice insect pests (*Sitophilus oryzae*, *Rhyzopertha dominica*, and *Cryptolestes pusillus*) [30]. In addition, the caraway significantly repelled the bean bugs [31] and affected some biotic aspects of the potato tuber moth [32]. The medicinal importance of the plant in treating some human diseases was reported by [33].

The excessive use of fumigant insecticides to control pests of honey bees caused severe health hazards to beekeepers, adversely affecting beneficial insects and generating resistance to chemical groups. Looking for an alternative to insecticides is highly required; hence this study is initiated to explore the potentiality of ethanolic extracts of a specific part of some botanicals and the powder of bee glue (Propolis) on controlling greater wax moth.

2. Materials and Methods

2.1. Study Site

The experiment was carried out at the Research Laboratory of the College of Agricultural Studies (Shambat), Sudan University of Science and Technology (SUST), Sudan, from April to June 2021. The average temperature is between 25-32°C.

2.2. Insect Collection and Rearing

Larval instars of *G. mellonella* were collected from the local honeybees' apiary in Shambat, Khartoum state, Sudan. The infested honeybee wax combs contained the developmental stages of the pest and were used to establish the stock culture for further studies. The larvae of GWM were reared on an artificial diet, according to [34].

After the emergence, emerged moths were placed in a plastic aquarium tank measuring (9.2x16x9.2 cm^3). Adult moths were allowed to reproduce in the laboratory with a temperature of 31±1°C, 66.28% RH, and 12L: 12D photoperiod (These were the average conditions for all further experiments). Then larvae were placed in a closed cm), covered with a muslin cloth, and brought to the laboratory for mass rearing. Early larval instars were reared in groups of 100 larvae in a plastic cylinder 19 cm^2in diameter covered with muslin cloth and fed on bee wax. The third larval instars were placed separately in plastic cups 5 cm in diameter and 7 cm in height to avoid cannibalism. Larvae of GWM were provided with bee wax into the bottom of each cup for pupations. Upon emergence, the adults were transferred to a glass jar (30x30x30 cm) covered with muslin cloth and fed on a 10% sugar solution [35]. The glass jar contained folded paper sheets for the deposition of eggs. The rearing process continued until a sufficient number of homogenous populations of larvae were collected for the experiments.

2.3. Test Materials and Extraction Methods

Al-Baraka Company for bee products and honey® was the provider of Propolis. The soxhlet apparatus extracted one hundred and fifty (150) grams of bee glue powder with absolute ethanol. The extraction process continued for six hours then ethanol solvent was removed from the crude extract by rotary evaporator [36].

Seeds of *C. carvi* and *N. sativa* L. were purchased from the local market of Omdurman city, while leaves of *E. camaldulensis* were collected from the Shambat area. Test samples were brought to the laboratory and dried in the shade. After complete dryness, test samples were crushed using an electronic blender, and 1600 ml of ethanol was added per sample as solvent into a council flask. A mixture of each test product was placed in a shaker operated for two days at 156 revolutions per minute. After shaking, the content of each flask was filtered using the reduced pressure filter. Then the alcoholic extract was collected, and samples were evaporated using a rotary

evaporator to remove the added solvent. After drying, the percentage of each extract was calculated, and five dilutions (5%, 7.5%, 10%, 12.5%, and 15%) were applied to run the bioassay.

2.4. Bioassay Tests

The reared third larval instars of the GWM were used to carry out this study, according to [2]. The test larvae in this study were allocated to groups of ten individuals for each. Each group of larvae was placed in a petri dish (9-cm^2 diameter) and was provided with 5 gm pure bee wax. After the preparation of different concentrations of each test material, five milliliters of each were sprayed on the wax inside each dish. The dishes were closed with ventilated lids to prevent the escape of larvae. Each treatment concentration was replicated five times, and the same number of replicates was used for the control, where larvae were provided only with bee wax. The experiment was conducted under the laboratory conditions of 31±1°C, 66.28% RH, and 12L: 12D photoperiod. The mortality percentages of larvae were recorded 24, 48, 72, and 96 hours after the application of the concentrations of different extracts.

2.5. Statistical Analysis

Analysis of variance (ANOVA) was used to analyze the obtained data, and means were separated according to Duncan's Multiple Range Test using

Genstat version 12.1 computer-based statistical software. In addition, probit analysis was conducted applying SPSS (16.0 software), and the LC50 and LC90 of each product were determined.

3. Results

Results in Table **1** and Fig. (**1**) showed that the five concentrations of the EE of all test products are significantly different from the control in their effectiveness in reducing the number of larvae of GWM. Different percentages of mortalities were obtained for different treatments according to the concentration and time of consumption. The number of dead larvae increased due to the concentration increase for each product and elapsed time, where the effect is dose and time-dependent.

3.1. Effect of Different Concentrations of Ethanolic Extracts of Some Plants and Bee Glue on the Mortality of the GWM after Different Times of Exposure

3.1.1. 24 Hours

In this study, a significant difference was recorded in the mortality percentages of the GWM between different concentrations of different treatments (Table **1**). The two higher concentrations of EE of leaves of *E. camaludolensis*, 12.5 and 15%, obtained the highest mortality percentages, 53.3 and 50.6 %, respectively. Statistically same results were documented for the middle concentration of the EE of leaves of *E. camaludolensis* (43.3%) and the highest concentrations of the EE of propolis powder, seeds of *C.carvi* as well as seeds of *N. sativa* with 46.7, 46.7 and 43.3% respectively. The rest of the percentage of mortalities of other concentrations of different test products fluctuated between 16.7% and 40%.

3.1.2. 48 Hours

The results of exposure of GWM to different concentrations of test products revealed highly significant difference among treatments for 48 hours. The same mortality percentages were obtained for the EE of *E. camalodulensis* and that of powder of Propolis at the highest concentration (15%), with 63.3% mortality. The mortality of the highest concentration (15%) of EE of seeds of *C. carvi* was found to be the same as that recorded for EEs of *E. camaludolensis* and Propolis at the concentration of (12.5%) with 56.7, 56.7, and 53.3% respectively. The remained concentrations for different treatments recorded mortality to GWM ranged from 26.7 to 50.0%.

3.1.3. 72 Hours

As demonstrated in Table **1**. a significant difference was revealed between different concentrations for different treatments. As shown in Table **1**, the two highest concentrations (12.5 and 15%) of Propolis were

statistically equal to the highest one of the EE of *E. camaludolensis* (15%) on the mortality of larvae of GWM with 73.3, 66.7, and 73.3% respectively. In addition, the same high mortality to larvae GWM was recorded for the highest concentration of the EX of seeds of *N. sativa* and the concentration of (12.5%) of *E. camaludolensis* with 63.3%. The mortality percentage of the other treatments fluctuated between (36.7 and 56.7%).

Table 1: Effect of *N. sativa*, *C. carvi* seeds, *E. camaldulensis* leaves and propolis EX against third larval instar of *G. mellonella*.

Treatments	Conc. (%)	Mortality (%) and Exposure Time (hrs.)			
		24	48	72	96
N. sativa	5	23.3 (4.8)fg	26.7 (5.2)fg	36.7 (6.1)fg	43.3 (6.6)g
	7.5	26.7 (5.2)ef	33.3 (6.1)e	46.7 (6.9)de	53.3 (7.3)f
	10	33.3 (5.8)de	40.0 (6.4)cde	53.3 (7.3)cd	56.7 (7.6)ef
	12.5	36.7 (6.1)cd	43.3 (6.9)bcd	56.7 (7.6)bc	66.7 (8.2)cde
	15	43.3 (6.7)ABC	50.0 (7.1)bc	63.3 (8.0)ab	73.3 (8.6)bcd
C. carvi	5	20.0 (4.5)fg	23.3 (4.8)g	33.3 (5.8)g	43.3 (6.6)g
	7.5	26.7 (5.2)ef	33.3 (5.8)ef	43.3 (6.6)ef	50.0 (7.1)fg
	10	33.3 (5.8)de	40.0 (6.4)cde	46.3 (6.9)de	53.3 (7.3)f
	12.5	40.0 (6.4)bcd	50.0 (7.1)bc	53.3 (7.3)cd	63.3 (8.0)de
	15	46.7 (6.9)abc	53.3 (7.3)ab	56.7 (7.6)bc	66.7 (8.2)cde
E. camaldulensis	5	26.7 (5.2) ef	33.3 (6.3)de	43.3 (6.6)ef	53.3 (7.3)f
	7.5	33.3 (5.8)de	40.0 (6.3)de	46.7 (6.9)de	56.7 (7.6)ef
	10	43.3 (6.6)abc	50.0 (7.1)bc	56.7 (7.6)bc	66.7 (8.2)cde
	12.5	50.6 (7.1)ab	56.7 (7.6)ab	63.3 (8.0)ab	73.3 (8.6)bcd
	15	53.3 (7.3)a	63.3 (8.0)a	73.3 (8.6)a	86.7 (9.3)a
Propolis	5	16.7 (5.1)g	26.7 (5.2)fg	40.0 (6.4) efg	56.7 (7.6)of
	7.5	23.3 (4.8)fg	36.7 (6.1)e	46.7 (6.9)de	63.3 (8.0)de
	10	33.3 (5.8)de	46.7 (6.9)bcd	56.7 (7.6)bc	70.0 (8.4)cd
	12.5	36.7 (6.1)cd	56.7 (7.6)ab	66.7 (8.2)a	76.7 (8.8)ABC
	15	46.7 (6.9)ABC	63.3 (8.0)a	73.3 (8.6)a	83.3 (9.2)ab
Control	-	0 (0.7)h	0 (0.7)h	0 (0.7)h	0 (0.7)h
C. V. %		8. 3	7. 2	5.5	5.1
SE±		0.40	0.37	0.31	0.32
L. S .D		0.82	0.74	0.63	0.64

*Means followed by the same letter (s) are not significantly different at ($p < .001$).

*Means between brackets are transformed according to $\sqrt{X + 0.5}$

*C. V. = Coefficient of Variation.

*S .E. = Stander Error

*L. S. D. = Least Significant Difference

3.1.4. 96 Hours

High significant differences were obtained between different treatments after 96 hours of exposure. The highest concentration of the EE of leaves of *E. camalodolensis gave* the highest mortality percentages of GWM (86.7%), followed by EE of powder of Propolis (83.3%) of the same concentration.

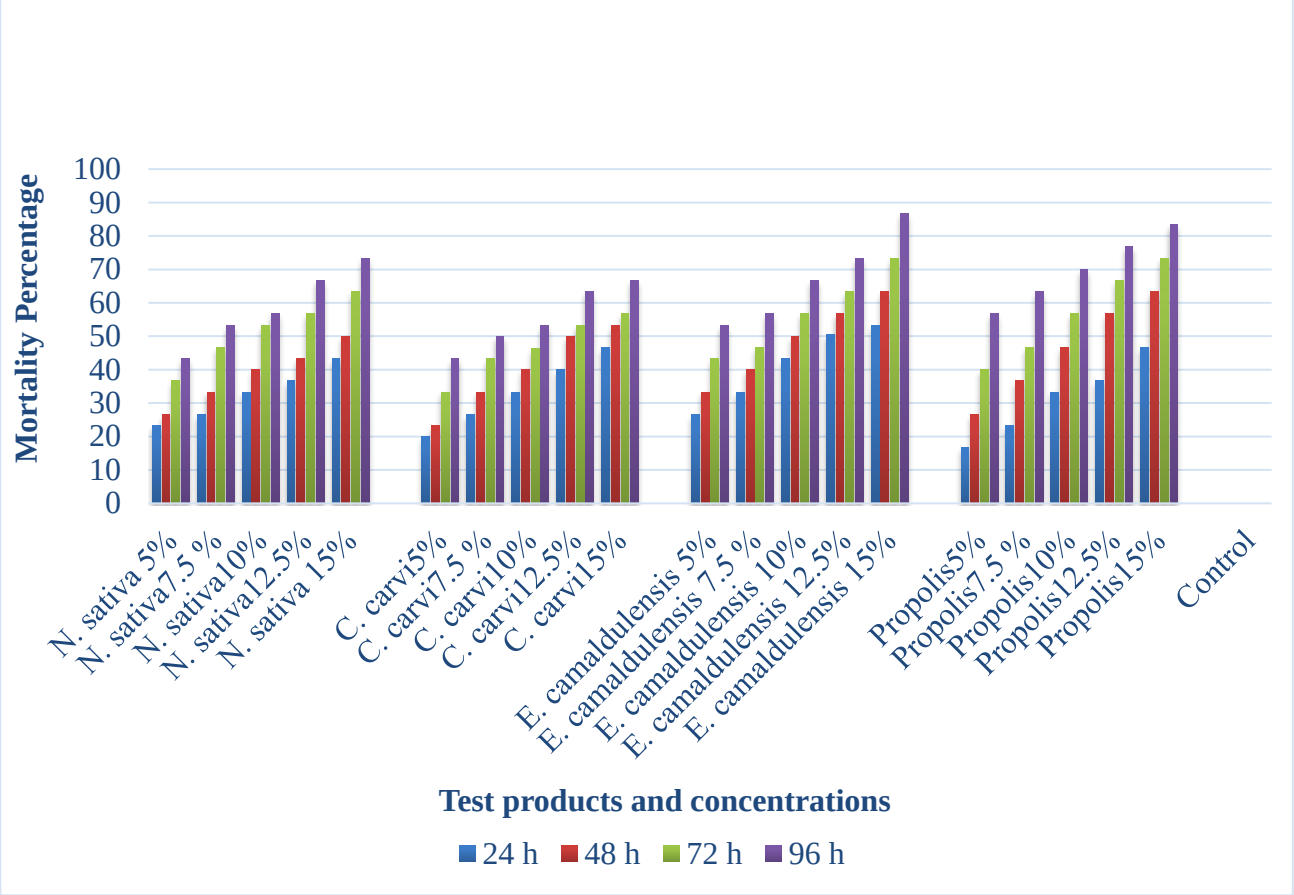

Figure 1: Concentration and time dependent of different ethanolic extracts of some plants and Propolis and their effect on the mortality of the third instar of greater wax moth.

The same mortality to GWM was reported with the EE for the powder of Propolis and EE of leaves of *E. camaludolensis at* the concentration of (12.5%), EE of seeds of *N. sativa* at (15%) concentration and EE of the powder of Propolis at (10%) concentration with (76.7%), (73.3%), (73.3%) and (70%) mortalities respectively**.** Other treatments in this study obtained mortalities ranging between 43.3 to 66.7% after 96 hours of exposure.

3.2. Determination of the Lethal Dose of Each Product against the Third Larval Instar of GWM

Results presented in (Table **2** and Fig. **2**) provided clear evidence that all concentrations of EEs of all test plants and that of the bee glue (Propolis) have a lethal effect against the third larval instars of the GWM. Probit analysis of the mortality data demonstrated that the lethal concentrations of the extracts vary from one plant to another and to bee glue (Propolis). The lowest LC50 value for EE was recorded by Propolis (bee glue), followed by *E. camaldulensis*, *N. sativa*, and *C. carvi with* 3.1, 5.0, 7.0, and 7.7, respectively.

4. Discussion

Results of this study revealed the potentiality of the five concentrations of the EEs of the test plants and the bee glue to control the GWM, where all treatments recorded a high percentage of mortalities than the control.

The findings in this study are the triumphant story of using botanical insecticides in bee colonies to manage parasites and pathogens for long history [37]. Thyme oil, blueberry oil, eucalyptus oil, walnut leaf oil, bay leaf oil, pine oil, guar gum, iroba oil, citronella oil, garlic extract, and other vegetable oils are among the historically used botanicals [38, 39].

Table 2: LC50 and LC90 values for EXs of tested plants and bee glue against 3rd larval instar of *G. mellonella* after 96 hrs of exposure.

Plant Extract	LC* Values and 95% Confidence Limits (Lower – Upper)		
	LC_{50}	LC_{90}	Chi- Square χ2
Propolis	3.1(-20.7 – 6.7)	19.3(14.6 -54.3)	0.03
E. camaldulensis	5.0(-3.6 – 7.6)	18.3 (14.4 – 34.1)	0.1
N. sativa	7.0 (-3.3– 9.7)	23.6(17.3 – 68.2)	0.6
C. carvi	7.7(-27.8 -11.3)	28.6(19.2 - 315.7)	0.4

*LC = Lethal concentration.

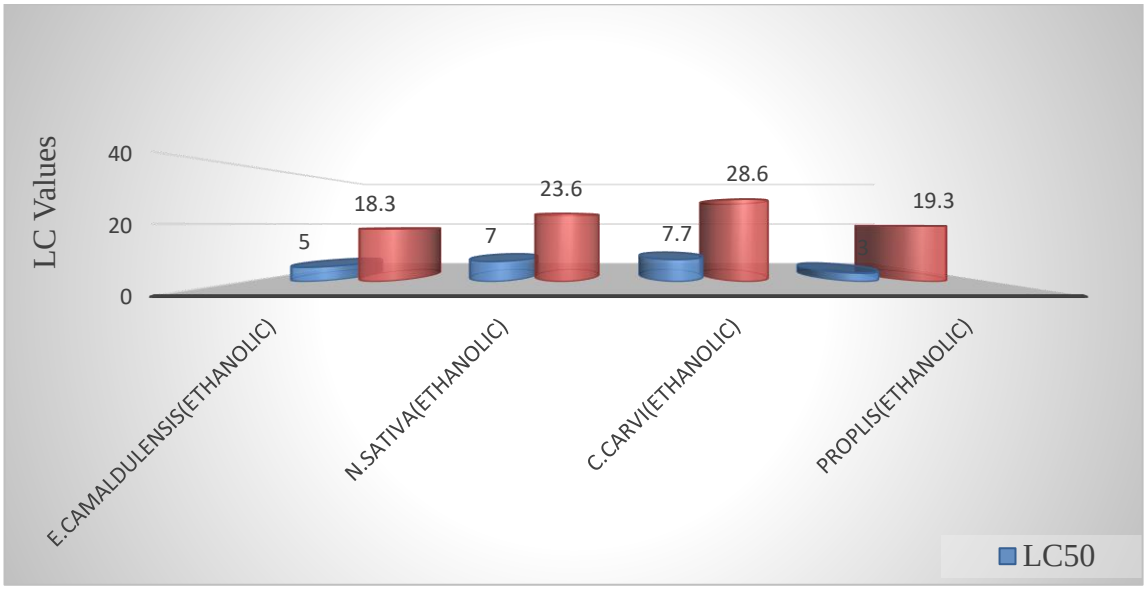

Figure 2: LC values for EXs of *E. camaldulensis* leaves, *N.sativa*, *C.carvi* seeds and bee glue against 3rd larval instar of *G. mellonella* after 96 hrs of exposure.

EEs of leaves of *E. camaludolensis* in this study showed excellent mortality to GWM through all test concentrations compared to EE of other plants and the control for the four assessment times. The findings mentioned above are the same as that reported by [40], who stated that the leaves of *E. camaludolensis* affect the duration of larval, pupal, and adult stages as well as the incubation period. In addition, the results of this study agreed with the findings of [41], who reported the larvicidal effect of the essential oils extracted from five *Eculayptus* species against *Tribolium castaneum* and *Tribolium confusum*.

As demonstrated in this study, EE of powder of Propolis or (bee glue) was found to be very effective in controlling the GWM at different concentrations compared to EE of seeds of *N. sativa* and *C. carvi.* The highest concentration15%, gave mortality percentages of 46.7, 63.3, 73.3, and 83.3%, increased according to the elapse of time of exposure.

The above findings regarding the efficacy of EE of Propolis against the GWM are by [42], who stated that the solvent extract of propolis samples from Brazil and Bulgaria are potent to control the larger grain borer *Prostephanus truncates* (Horn) in maize grains.

In addition, the results are the same as the findings of [43], who proved that the extract of phenolic Propolis gave high mortality percentage to the last instars of the greater wax moth. On the other hand, the EE of Propolis

gave high mortality to red spider mites *Tetranychus sp* [44] and to the Asian fruit fly *Bacterocera dorsalis* in food bait application [45].

The results revealed the EE of seeds of *N. Sativa*gave (73.3%) mortality percentage at the concentration of (15%) after 96 hours. This result is identical to results achieved by [46, 47] for *N. sativa* against to control tropical stored pests and by findings of [48], who highlighted similar effects for the same product to manage *Tuta absoluta.*

The good performance of the EX of Caraway (*C. carvi* against the GW in this recent study is in harmony with the results obtained by the same product in the reduction of several eggs, percentage of hatching, and incubation period of GWM *G. mellonella* [49]. Also, the obtained result of using Caraway in controlling GWM in this study is in agreement with [50], who found that the oil extracts of the same product have high repellent activities (96.7%) against *Sitophilus oryzae.*

5. Conclusion

The obtained results in this study verified that all test products had insecticidal activity against the 3rd instar larvae of *G. mellonella.* The EE of *E. camaldulensis* leaves was the best, followed by the EE of powder of Propolis, EE of seeds of *N. Sativa*, and eventually, the EE *of C. carvi*. For all test products, the increase in mortality is attributed to the increase in concentration and time elapsed.

The authors recommend using EEs of leaves of *E. camaludolensis* and powder of bee glue at 12.5% and 15% and EE s of seeds of *N. sativa* and *C. carvi* at the concentrations of 15% to control GWM in hives of honeybees. The use of the products mentioned above at the mentioned concentrations is environmentally safe, economically feasible, and affordable and participated positively in increasing the productivity of beehives.

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