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Insecticidal Activity of Some Plant Extract Against Greater Wax Moth Larvae (*Galleria mellonella* L.)

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ABSTRACT

Galleria mellonella L. (Lepidoptera: Pyralidae) is a significant international pest and the most devastating pest throughout the world of beeswax. Natural pesticides must be replaced with synthetic materials since they are more ecologically friendly. This study aims to determine alternative methods of controlling the larger wax moth by assessing the efficacy of several plant-based biocides for this purpose various ethanolic plant extracts such as rosemary (Rosmarinus officinalis L), Clove (Syzygium aromaticum), Eucalyptus (Eucalyptus sp) and Cinnamon (Cinnamomum verum) where used with four extraction rates (5,10,15 and 20) against the late instar larvae of Galleria mellonella in vitro after 24,48 and 72 hr. Obtained results revealed that the pupal mortality increased when the concentration increased and the highest accumulative mortality percentage of wax moth larvae during the experiment, it was demonstrated that all studied plants were efficient at suppressing Galleria mellonella larvae. The result shows that three days after application treatment of rosemary 20% gave the maximum total mortality percentage (%100) followed by (%96.58, %92.66 and %64.86) for Eucalyptus, Cinnamon and clove after 24,48 and 72 hr. respectively. It may be suggested that spraying natural products is a good option on new wax combs to protect them against infestation by wax moth larvae.

Keywords: Plant Extracts, Galleria mellonella, Toxicity, Mortality percentage, Wax combs

1. INTRODUCTION

For many years, the honeybee has been recognized as a significant component in raising agricultural yields and producing various goods, including propels, pollen, royal jelly, bee wax, and honey. Because of its economic importance and the production of chemicals with nutritional and therapeutic advantages for various pathological disorders, such as honey, wax, royal jelly, propolis, and bee venom.

Honey bees *Apis mellifera*(L) has a variety of insect pests inside and outside their hives, the most important of which is the economically important wax worm *Galleria mellonella* L., which attacks wax frames inside bee hives and in the store, where they feed on pollen, brood, ecdysial skins, and honey, as well as causing damage to wood (Abidalla, 2017). Several pests harm honey bee colonies, including the larger wax moth (*Galleria mellonella* L.) (Lepidoptera:

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Pyralidae), which can result in considerable losses (Charriere and Imdorf, 1999) & (Chaudhary, 2017). The greater wax larvae, cover the combs with silken tunnels and feed on beeswax, are extremely destructive and can quickly destroy stored beeswax combs (Kalpana et al., 2017). Many research has been carried out in order to identify solutions to manage it. (Said, Hammam, & Kader, 2019).

For more than a century, many intervention approaches have been tested around the world to control wax moth infestation. Among them, synthetic insecticides are the most widely applied for wax moth control around the world. Synthetic pesticides, on the other hand, have significant problems, including toxicological risks to beekeepers and bees, as well as the potential of contaminating hive products, which really is a global issue (Pirali-Kheirabadi & Teixeira da Silva, 2010).

Using natural products that are readily available to the beekeeper and clear of the current problems would be an alternative and most likely the best approach to resolve the residual issues connected with chemical treatments and reduce the financial expenditures spent by physical control methods (Sanad & Mohanny, 2015). Wax moths have been found to be effectively controlled by a variety of oils and plant extracts (El-Wakeil, 2013).

Plant extracts usage is the safe way and is also being used to control the great wax worm and guard bee hives seeing as they are less polluting to bees and people (Redwane et al., 2002). Plant extracts are also utilized to combat the great wax worm and protect bee colonies since they are less polluting to both bees and humans.

Furthermore, plant material is inexpensive and effective for beekeepers, and it may also be used to attack other pests like varroa and mites. According to Redwane and colleagues (2002), using plant materials as pesticides instead of chemical pesticides decreases the risk to people and other non-target creatures. as well as the efficiency of plant pesticides against insect pests, and also their characteristics of quick decomposition, minimal pollution to the ecosystem, low toxicity to users, and the most essential attribute of the lack of insect resistance to it.

Plant Essential oils and their compounds as well as plant alcoholic extraction have been shown to be an effective source of botanical pesticides. Natural insecticides, such as essential oils, provide an alternative to synthetic pesticides. Essential oils and their constituents have been demonstrated to be a good source of botanical insecticides (Tripathi et al., 2002).

Several essential plant oils and plant ethanolic extraction have been shown to have repelling qualities in recent years. Citronella, cedar, verbena, pennyroyal, geranium, lavender, pine,

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cinnamon, rosemary, basil, thyme, allspice, garlic, and peppermint were some of the plants used (Jarial, 2001)

Cinnamon alcohol extract can lower a 3% weight first instar and third larger wax moth concentration. Studies has shown that spraying wax frames with alcohol extract and oil from eucalyptus leaves can prevent damage for three months (Kwadha, Ong'amo, Ndegwa, Raina, & Fombong, 2017), Moreover, it should be mentioned that frames treated with 2% extract concentration provided 10 months of protection. Such non-harmful treatment approaches might help manage this pest and decrease the possibility of beehive product contamination, as well as provide a potential solution to this of the problem.

Eucalyptus oil (*Eucalyptus* spp.) contains eucaliptol, which can successfully manage a vari ety of agricultural pests, including *Alphitobius diaperinus* (Pinto Junior et al., 2010). Insecticidal components of chemicals derived from plants have recently been shown to be effective against particular target species, biodegradable, non-toxic products, and potentially appropriate for use in integrated management programs. (Markouk et al., 2000) & (Tare, Deshpande, & Sharma, 2004)

2. RESEARCH METHOD

Study location:

An experiment was conducted at the laboratory of Zanko private sector for agricultural research in Allai-Sulaimani during the period 2020-2021

Sources of tested plant:

Clove and cinnamon were obtained from the local market, the leaf of rosemary and eucalyptus were collected from public parks during April 2020 the leaves are cleaned of dust and brushed on newsprint at appropriate temperatures with ventilation after being collected, continuous stirring to prevent the occurrence of rot until they dry completed up to ten days.

The dried leaves, bark and clove were ground with a pestle and mortar sieved to obtain the fine powder particles (Mukhtar & Tukur, 2000) separately and the powder was placed in 300g nylon bags, with the sample information recorded and kept in the freezer until extraction.

Table 1. Source of the tested plants

English name	Scientific name	Family	Used part
Clove	*Syzygium aromaticum*	Myrtaceae	flower
Eucalyptus	*Eucalyptus spp*	Myrtaceae	leaf
Rosemary	*Rosmarinus officinalis*	Lamiaceae	Leaf

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Cinnamon	*Cinnamomum verum*	Lauraceae	Bark

Rearing of the greater wax moth:

Old wax frames were collected which were heavily infested with grater wax moth. Newly emerging wax moth females and males were obtained from naturally infected wax combs in order to achieve pure wax moth culture from the apiary of technical institute of bakrajo in sulaimani,Iraq. then placed in 1kg clean glass jars sterilized with alcohol at a concentration of 96%. supplied cotton moistened with a 10% sugar solution in a glass tube placed in the box and replaced every 2-3 days when they've dried and therefore are not contaminated by fungus, the cans were covered with a boring cloth and secured with a rubber band to avoid escaping.

Females were left to deposit eggs on black ribbon cloth was hanged inside each cage to serve as ovipositional site after they had mated. The eggs were kept in a sterilized dishes the depth 1.5 cm and a diameter of 9 cm and in a warm (27 ± 2 °C) with a relative humidity of around 65± 5 % and 24 hours of darkness until the grater wax moth emerged (Owayss and Abd-Elgayed, 2007). Hatching larvae were transferred from the egg dish collection to new dishes containing applied natural bee wax.

Preparation of Tested plants extracts:

300 hundred grams of grounded powder were mixed with 700 mL of 96 percent ethanol at room temperature for 48 hours. The suspensions were filtered through Whitman no.1 filter paper. The solvents were evaporated from the filtrate by leaving it at the room temperature to obtain crude extracts and the extract was kept in a fridge at 4°C. Different concentrations (5, 10, 15, and 20 percent) of the solution were obtained by diluting it in distilled water (Al-Ghannoum et al., 2015).

Toxicity of tested plant extracts:

To determine the effect of clove, eucalyptus and rosemary against 5^{th} instar of *Galleria mellonella* larvae at different time intervals (24h,48h and 72hours) under laboratory conditions. Different concentrations (5,10,15 and %20) of each extract were prepared from the stock solutions (30%), 3ml of the tested materials were sprayed on (2x2x1cm) pieces of pure wax in Petri dishes (9 cm) and only distilled water was used in the controlled treatment. For each concentration of all treatments, three replications were conducted and each treatment contain 10 of 5^{th} instar *G mellonella* larvae (Ellis et al., 2013). After mentioned period of the treatment, percentage mortality was recorded, and mortalities were calculated and adjusted using Abbott's formula (1925).

% Corrected mortality = % Test mortality – % Control mortality / % Control mortality X 100

% Mortality larvae = No. of dead larvae / No. of introduced larvae X 100

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Experimental design and statistical analysis:

The experiments were arranged in a completely randomized design (CRD). The obtained data were statistically analyzed according to the analysis of variance (ANOVA); Duncan's Multiple Range Test was used for mean separation using xlstat. All cultures and experiments were conducted in an incubator that was set to 30 ± 2 °C and 65 ± 5 % R.H. in darkness.

3. RESULTS AND DISCUSSION

The Impact of tested materials against the larvae of greater wax moth, *Galleria mellonella* : The potential activities of active materials on the mortality percent of greater wax larvae moth at four concentrations after 24,48 and 72hrs were reported in (Table 2) according to data the mortality percentage increased with longer exposure times reaching its highest level when the concentration increased.

The highest mortality percentage of late instar larvae was obtained after 72hr of treatment of all tested material, especially in the highest concentration (20%); where it recorded (60.00,66.66 and 96.58), (83.35,100 and 100), (65.57,66.66 and 92.66) and (53.33,58.33 and 64.86) after 24.48 and 72hr. for Eucalyptus, Rosemary, Cinnamon and Clove, respectively. The mortality rate climbed with longer exposure times, according to the data in (Table 2).

Similar results were reported by (Surendra et al., 2010) It was discovered that the larval mortality of wax moth changed dramatically with three different plant product concentrations when natural plant products were used in the management of the greater wax moth, *G. mellonella*, under experimental conditions on the other hand (Abdelrahman et al., 2012) demonstrated that ginger and peppermint were more harmful than lemon and camphor against middle and late larval instars of *Galleria mellonella*.

Abdelsalam (2009), when treated with the higher doses of either peppermint oil or lemon oil, higher reduction percentages of *G. mellonella* population (90%) were obtained. The tested dosages of lemon oil or the higher doses of peppermint oil maintained a mortality rate of 100%. When applied at larger concentrations, only the oils of peppermint and lemon significantly reduced the number of emerging adults.

Table 2. Effect of different concentrations of botanical alcoholic extract (Eucalyptus, Rosemary, Cinnamon and Clove) on mortality percentage of 5th instar larvae of *Galleria mellonella*.

Conc (%)	Corrected Mortality (%)											
	24h				48h				72h			
	Eu	Ro	Cn	Cl	Eu	Ro	Cn	Cl	Eu	Ro	Cn	Cl

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0	0.00	0.00	3.33	0.00	0.00	0.00	0.00	0.00	2.18	0.00	1.72	1.72
5	66.67	20.00	13.22	10.00	21.11	29.16	27.77	22.22	32.87	46.66	25.94	35.47
10	33.33	56.66	23.22	23.33	25.39	70.00	36.31	26.19	41.56	83.33	42.62	33.57
15	43.33	73.33	59.90	46.66	53.33	83.33	63.88	31.11	57.68	100.00	75.98	48.48
20	60.00	83.35	65.57	53.33	66.66	100.00	66.66	58.33	96.58	100.00	92.66	64.86

According to an ANOVA was represented in (Table 3) the major impacts of plant variety, exposure period, and concentration significantly influenced the larger wax moth, *Galleria mellonella*, larval mortality. The highest larvae mortality was obtained from the main effect of *Rosmarinus officinalis* was dramatically the mortality rate increased and recorded the highest value after 24,48 and 72hr respectively, on the other hand *Cinnamomum verum* and *Eucalyptus spp* become the second plants as a natural insecticide for the number of larval mortality especially in 72hr, while the least larvae mortality was recorded from *Syzygium aromaticum* . The larger wax moth larvae *Galleria mellonella* might be effectively controlled by these plant materials' plant extracts.

According to (Asawalam et al., 2009) the insecticidal activity of any plant extract depends on the active ingredient of the plant extract. This could be explained that due to its insecticidal, growth-regulating, and antifeedent capabilities against insects, Azadirachata indica (Neem) extracts may have resulted in high larval mortality (Larry, 2004). In their work on the *Spodoptera littoralis*. (El- Shall et al., 2005) demonstrated that using ethanol, petroleum, or chloroform extracts of *Eucalyptus camaldulensis* alone or in combination with gamma radiation had substantial chronic effects on larvae, pupae, and adult emergence.

Plant extracts are currently being studied more and more due of their utility in urban entomology and plant protection (Din et al., 2001). Plant extracts are less dangerous to non-target creatures including humans, plant-based formulations are more environmentally friendly than synthetic larvicides. Comparing these organic plant products to other chemicals, they are more affordable. Using natural products as an insecticide may help us reduce the environmental pollution caused by synthetic insecticides. Aromatic plants and their essential oils are significant sources of synthetic substances utilized in different contexts (Amer & Mehlhorn, 2006).

Table 3. The interaction effects of plant type and exposure time on the larval mortality of greater wax moth, *Galleria mellonella*.

Category	Mean Value after 24hr	Groups		
Rosmarinus officinalis	0.47	A		
Cinnamomum verum	0.33		B	

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Eucalyptus spp	0.29			C
Syzygium aromaticum	0.27			C
Category	Mean Value after 48hr	Groups		
Rosmarinus officinalis	0.57	A		
Cinnamomum verum	0.39		B	
Eucalyptus spp	0.33		B	C
Syzygium aromaticum	0.28			C
Category	Mean Value after 72hr	Groups		
Rosmarinus officinalis	0.66	A		
Cinnamomum verum	0.48		B	
Eucalyptus spp	0.49		B	
Syzygium aromaticum	0.37			C

Remarks: Mean values within a column followed by the same letters are not significantly different at $p \leq 0.01$ according to Duncan's Multiple Range Test.

Control not add plant extracts. An ANOVA showed that the increased wax moth larval mortality was significantly influenced by the interaction of plant type and concentration. Therefore, the application of higher level of plant extracts increased the percentage of *Galleria mellonella* larval death. 24hrs after treatment lowest mortality rate was achieved at a concentration of 5% while the maximum death rate was seen at concentration 20% of plant extract.

After 48hrs the larval mortality was different significantly according to the different rate of and highest mortality was recorded for the second time at the concentration of 20%. In the final day (72hr) for another time the highest concentration level of the plant extract (%20) recorded the maximum rate at larval mortality of *Galleria mellonella*. by increasing concentrations from 5% to 20% and exposure times from 24 to 72 hrs, the percentage of larval mortality was substantially increased both vertically and horizontally. These observations demonstrate that higher concentrations of plant extracts significantly increased the mortality of larger wax moth larvae compared to lower concentrations and untreated controls. The current finding agrees with the results of (Mohamed, 2012) who noted that increasing both the horizontal duration and the vertical extract concentration had an increase in the effect of plant extract on the percentage of larval mortality of the Greater Wax Moth, *Galleria mellonella*. In the same year in another finding (Mohamad, 2012) investigated the impact of plant extract (Neem) at concentrations 0, 10, 15, 20, 25, 50, 75, and 100 ppm on the percentage of pupation, emergence, and adult survival.

(Elbehery et al., 2016) observed the results demonstrated that the tested 2nd instar larvae were significantly affected by the various doses. Even at the lowest measured concentration, there was a remarkable elongation of the second larval instar in compared to the control.

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Table 4. The interaction effects of concentration and exposure time on the 5th larval mortality of greater wax moth, *Galleria mellonella*

Concentration	Mean Value after 24hr	Groups				
20	0.66	A				
15	0.56		B			
10	0.34			C		
5	0.12				D	
0	0.01					E
Concentration	Mean Value after 48hr	Groups				
20	0.73	A				
15	0.58		B			
10	0.39			C		
5	0.25				D	
0	0.00					E
Concentration	Mean Value after 72hr	Groups				
20	0.89	A				
15	0.71		B			
10	0.51			C		
5	0.36				D	
0	0.02					E

Remarks: Mean values within a column followed by the same letters are not significantly different at $p \leq 0.01$ according to Duncan's Multiple Range Test. Control not add plant extracts.

4. CONCLUSIONS

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According to the results of the laboratory experiment, the larger wax moth larvae *Galleria mellonella* responded differently to different plant extracts. *Rosmarinus officinalis*, *Eucalyptus spp* and *Cinnamomum verum* extracts triggered the greatest larval mortality after application %15 and %20 after 48,72hr respectively. Therefore, as a result, plant extracts from rosemary, Eucalyptus and cinnamon can be developed as sources of bio-pesticide for the control of larger wax moth larvae.

Additionally, it can provide the beekeepers with satisfactory results for protecting periodically kept wax combs in the apiary. To determine its usefulness on the field and with honeybees, additional investigation is required on these findings. Also essential for wax moth control is the identification of bioactive substances from beneficial plant material.

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