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Effects of *Myrtus communis* L. leaf extract on eggs and larvae of the greater wax moth *Galleria mellonella* (Lepidoptera: Pyrallidae)

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ABSTRACT

The greater wax moth was reared on its natural food consisting mainly of beeswax, and the various life stages of this insect were obtained. The results of the study showed that the aqueous and alcoholic extract of *Myrtus communis* L. leaves causes increase in mortality percentages of eggs and fourth instars larvae of the greater wax moth. In addition, there was a direct relationship between the extract concentrations used and the mortality rate of eggs and larvae. The alcoholic extract of the leaves was more effective in eggs and larval mortality percentages than the aqueous extract. Eggs at 24 h were more sensitive to the alcoholic and aqueous extracts of the leaves than eggs at 72 and 120 h. The sensitivity of eggs to extracts decreased by age. The results showed that the gastric effects of alcoholic and aqueous extracts of the *M. communis* leaves were higher on the mortality rate in the fourth instar larvae than in the contact effect.

Keywords: Alcoholic extract, Aqueous, Botanicals, Insects, Wax moth
Article type: Research Article.

INTRODUCTION

Greater Wax Moth *Gelleria mellonella* is one of the most famous and most dangerous insect pests on honeybee tires in their storage areas, causing economic losses in the production of beneficial wax in many industries, a decrease in the productivity of honey bee hives, and its negative effects on bees all over the world (Kwadhe *et al.* 2017). Beeswax contains pollen and honey, and therefore it is vulnerable to infection with various insect pests, including the greater wax moth and the smaller wax moth, which are considered among the most important insect pests that cause very great economic losses to beekeepers all over the world (Pirk *et al.* 2015). During the last fifty years, many problems have emerged as a result of the excessive use of chemical pesticides against insect pests, and this use has caused the emergence of risks and problems represented by the emergence of insecticide-resistant strains in some types of insects, in addition to their direct and residual impact polluting the environment, as well as disturbances in the life balance of the environment and its ability to create cancer, as well as genetic diseases and the problem of their high manufacturing costs (Jayaraj 2005). So, researchers have sought to find alternative, clean and effective means through the use of new means and methods for controlling insect pests (Jafari *et al.* 2010). One of these alternative methods is to employ biological control methods using pathogenic bacteria for insects, predators, parasites, fungi, pheromone traps and physical control, as well as employing plant extracts against insect pests, since they are of low toxicity to the organism and the resistance of insects has not appeared so far (Peterson *et al.* 2000; Al-Musawi 2022; Sgheer & Yassin 2022). Many studies have been conducted seeking effective ways to control this insect pest. Rahoo *et al*. (2011) studied the effectiveness of two types of entomopathogenic bacteria in killing the sixth-stage larvae of the greater wax moth, *G. mellonella* and the effect of temperature and humidity on the effectiveness of these types of bacteria. Topolska (2008) stated that Petroleum ether extract of cultivated and wild myrtle *Myrtus communis* L. led to a reduction in eggs hatching

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percentages and larva prolongation of the greater waxworm *G. mellonella* L. Abdul-Jabbar (2001) conducted a study on the tactile, infectious and respiratory effects of alcoholic and oily extracts of leaves and fruits *Eucalyptus camaldulensis* Dehnh with different concentrations in the biological performance of the greater waxworm. Saber *et al.* (2003) indicated that the diet treated with 10% alcoholic extract of *Eucalyptus* led to a significant increase in the duration of the larval stage of the larvae of the greater waxworm, which was 24 h old.

MATERIALS AND METHODS

Insects and plant samples

As for the insect mass rearing and collection, the eggs and larvae of the greater wax moth were obtained from a lab cultured colony in the insect breeding laboratory at the animal house belongs to College of Education for Girls/University of Kufa, Iraq. The insects fed on natural wax found in the wax combs and maintained in specific breeding conditions at 28 ± 2 °C and relative humidity of 50 ± 10 %, in complete darkness. In the case of plant materials, the leaves of myrtle were collected from the gardens of the College of Education for Girls in 2018. The leaf samples were taken to the laboratory, cleaned of impurities, dried in the shade, and then ground using an electric mill to obtain a fine powder. The powder of the leaves was stored in clean nylon bags in the refrigerator until use.

Plant extracts

The aqueous extract of the leaves of the myrtle plant was prepared according to the method of Harborn (1984), where 20 g of plant leaf powder was placed in a 1000 mL glass beaker containing 400 mL distilled water. The contents were mixed using a magnetic mixer for 15 min, then the solution was left for 24 h in lab temperature. The solution was filtered by tamping cloth and the filtrate was centrifuged at 3000 rpm for 10 min. Then the filtrate was transferred to an electric oven to dry it at a temperature of 45 °C. The remaining dry matter was weighed and the following concentrations (35, 25, 15 and 5 mg mL^{-1}) were prepared. In the case of alcoholic extract, we prepared ethanol extract of the myrtle leaves according to AL- Manhel & Niamah (2015). A 20 g of leaf powder was placed in an extraction tube (thimble), placed in the Soxhelt extractor and then 400 mL ethyl alcohol was added to the extraction flask and left for 24 h. Thereafter, the filtrate was transferred to an electric oven for drying at a temperature of 45 °C. The remaining dry matter was weighed, from which the following concentrations (5, 15, 25, and 35 mg mL^{-1}) were prepared

Effect of myrtle extracts treatment application

On eggs

From adult mating cages, egg masses on folded cardboard tapes were collected at age 24, 72, and 120 h. From each egg age group, 100 eggs were counted to serve as one replicate of three replications for each plant extract concentration. The alcoholic and aqueous extracts at different concentrations were sprayed on insect's eggs using hand sprayer, while the control eggs were sprayed with distilled water. The treated eggs were transferred to incubate at 28 °C and 50% relative humidity (RH). It is followed up daily for 8 days and number of hatched eggs was recorded and hatching rate (%) was calculated.

On larvae

In the case of contact effect, the fourth instar larvae were collected from the laboratory colony. A hand sprayer was used to spray every 10 larvae (one replicate) of three replicates for each concentration (5, 15, 25, and 35 mg mL^{-1}) of alcoholic or aqueous extracts of myrtle leaves, the control larvae were distilled water treated. The treated and control larvae were transferred to Petri dishes containing wax food feed, incubated at 28°C and 50% RH. Mortality rate (%) was calculated after five days of treatment. As for the digestive effect, the food (wax) was sprayed in Petri dishes at each concentration (5, 15, 25, and 35 mg mL^{-1}) of alcoholic and aqueous extracts of myrtle leaf with the same number of larvae and replications. The control larvae fed on wax treated with distilled water only, then the treated and control larvae dishes were transferred to an incubator at 28 °C and 50% RH.

Experiment design and statistical analysis

The experiments in this study were factorial according the completely randomized design (CRD) with three replications for each treatment. The data were analysed and ANOVA tables were performed using GenStat 2012, computing program. The least significant difference (L.S.D) was used at the significant level of 0.05 to test the significance of differences between the treatments means (Al-Rawi & Khalaf Allah 2000).

RESULTS AND DISCUSSION

The results (Table1) indicated that the alcoholic extract of the myrtle leaves was more effective than the aqueous one in the egg mortality rate of the greater wax moth. It was also found that there was significant difference between the two types of extract. In addition, a direct relationship was found between the concentrations used for the alcoholic and aqueous extracts. The highest mortality rate (94%) was recorded in eggs treated at the age of one day (24 h) with a 5% alcoholic extract on the myrtle leaves, while the lowest mortality rate (4.33%) was recorded in eggs treated with aqueous extract at age 120 h. This suggests that eggs at the age of 24 h are more sensitive to alcoholic and aqueous extracts of the myrtle leaves and in all concentrations used in the experiment than eggs at age 72 and 120 h. The findings showed the existence of a direct relationship between the plant extract concentration and mortality rates of eggs treated with different extracts. This came in agreement with the results of Awmack & Leather (2002) and Basker & Ignacimuthu (2012), revealing an increase in the mortality of eggs treated with the elevated concentrations of the various plant extracts they used.

Table1. Effect of different concentrations of myrtle leaf extract (alcoholic and aqueous) on the greater wax moth eggs mortality rate at 8 days post treatment.

Myrtle leaf extract Conc. (mg mL^{-1})	Egg mortality rate (%)						
	Eggs at age 24 h		Eggs at age 72 h		Eggs at age 120 h		LSD at ($P \leq 0.05$)
	Alco.	**Aq.**	**Alco.**	**Aq.**	**Alco.**	**Aq.**	
Control	0.00	0.00	0.00	0.00	0.00	0.00	
5	33.67	13.33	15.33	8.00	6.33	4.33	4.2
15	69.00	44.67	35.33	21.67	21.00	19.33	3.6
25	77.67	68.67	56.33	43.67	41.00	27.67	2.2
35	94.67	84.00	77.67	57.33	50.67	39.00	3.2
LSD at $p \leq 0.05$	4.3	5.2	6.2	5.8	4.7	6.0	

Al-Ghamdi (2013) mentioned that plant extracts work to kill insect eggs in many ways with a physiological effect, including that they contain a percentage of oils that cover the surface of the eggs with a thin layer that prevents gas exchange between the egg embryo and its external surroundings or works to harden. The shell of the egg prevents its hatching or the penetration of the extracts into the egg, which affects the protoplasm, which leads to killing the embryos. Abdul-Jabbar (2001) attributed the cause of eggs mortality to the inhibitory effect of the turbine compounds in the oil extract of eucalyptus on embryos development of these eggs. Kumar *et al.* (2009) reported that the alcoholic and aqueous extracts of four different plants, including the rosemary plant, had a significant effect on the egg hatching rates of the diamond-back moth *Plutella xylostella,* where the alcoholic extract was superior to the aqueous extract of the four studied plants. As for the effect of myrtle leaf extract on the fourth instar larvae of the greater wax moth, in a similar way to the effect on eggs, the results showed that the alcoholic extract had a higher effect on the elevated insect mortality rate than the aqueous extract (Table 2 and Fig. 1). In general, the insecticidal efficacy of the extract upraised by the elevation in the concentration used, regardless of extract type. On the other hand, the digestive effect was higher in elevating insect mortality compared to the contact effect. The lowest mortality rate was recorded in the aqueous extract treatment at a concentration of 5 mg mL^{-1}, leading to 15.7% and 20.3% mortality in the contact and digestive treatment, respectively. Once using alcoholic extract at a concentration of 35 mg $mL^{-}1$, the highest mortality rate in the contact and digestive treatments were 72.3% and 82.3% respectively (Table 2 and Fig. 1). Prophiro *et al.* (2008) explained that the alcoholic extract is more effective than the aqueous extract, due to the fact that many active substances, especially limonoids, are lipophilic substances that dissolve easily in alcohol and acetone and are difficult to dissolve in water. The cause of larvae mortality resulting from contacting them with plant extracts may be due to the penetration of toxic substances into the insect's body through the membranous areas between the body rings.

Table 2. Contact and digestive effect of alcoholic and aqueous leaves myrtle extracts on larval mortality of fourth instar of the greater wax moth.

Concentrations of myrtle leaf extracts (mg mL^{-1})	Mortality rate (%) by contact effect		Mortality rate (%) by digestive effect	
	Alcoholic extracts	Aqueous extracts	Alcoholic extracts	Aqueous extracts
Control (DW)	0.0	0.0	0.0	0.0
5	32.3	15.7	34.3	20.3
15	43.3	22.3	52.3	36.0
25	52.3	56.7	71.3	54.7
35	72.3	62.7	82.3	68.3
L.S.D. ($P \leq 0.05$)	Between extracts	Among concentrations	Between extracts	Among concentrations
	5.6	8.9	8.0	10.1

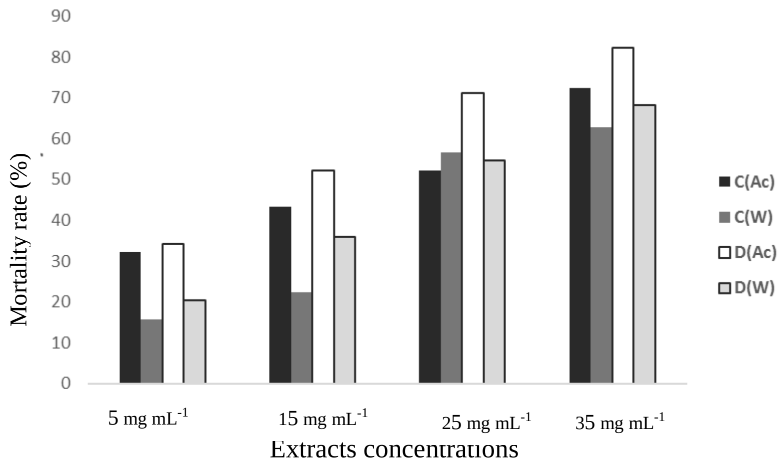

Fig. 1. Contact (C) and digestive (D) effect of alcoholic (Ac) and aqueous (W) leaf extracts of myrtle plants on mortality of fourth instar larvae of the greater wax moth.

As for the digestive effect, it is through the absorption of toxic substances by the epithelial membranes lining the central digestive tract. Schmuterer & Singh (1995) indicated that the cause of the contact toxicity and the digestive effect of the active substance, i.e., Azadiraachtin, which is found in the fruits neem plant, is due to its effectiveness in being a toxic substance and an anti-feeding substance, and its anti-feedant activity is stronger than its action as a poisonous substance through contact. Moreover, Arivoli & Tennyson, (2012) found contact and infectious mortality in the third instar larvae of *Spodoptera litura* that were contact-treated on one side and fed with the diet mixed with the methanolic extract of a number of plants they used in their experiment.

CONCLUSION

The findings of this study showed that the myrtle leaf extract had insecticidal effect on the greater wax moth eggs. The results of the current study also showed that the alcoholic extract of the myrtle leaves was more effective by contact or digestion on the fourth instar larvae than the aqueous extract.

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