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Sustainable control of pomegranate carob moth with some biorationals

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Abstract
This study was carried out to investigate the effect of three types of plant extracts [Eucalyptus, *Eucalyptus camaldulensis* (Family: Myrtaceae), Oleander, *Nerium oleander* (Family: *Apocynaceae*) and Neem, *Azadirachta indica* (Family: Meliaceae)], with four concentrations as (0, 5, 10, and 15%) for each and the fungus *Metarhizium anisopliae* with three different concentrations (1×10^3, 1×10^5 and 1×10^6 spore /ml of water) against carob moth larvae. Results indicated that both plant extracts and the fungus (*Metarhizium anisopliae*) regarding the plant species, extract concentrations and exposure duration, have a high toxicity on the 3rd and 5th instar larvae of carob moth mortality. The larval mortality percentages increased significantly with Neem extract at (15%) reached (43.33and 56.70%) after 4 days of treatment for 3rd and 5th instar larvae respectively. The cumulative mortality percentage for 3rd and 5th instar larvae treated with *Metarhizium anisopliae* at (1×10^6 spore /ml) reached to (93.33%) after 6 days of treatment

Keywords: Carob moth, biorationals, plant extracts, *Metarhizium anisopliae*

Introduction
The carob moth, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) is one of the important and destructive pests of pomegranate *Punica granatum* in Iraq, which cause a big damage in pomegranate orchards and yearly causes great economic losses in (Kurdistan Region) and middle of Iraq [1]. The infestation percentage increases from 20% by early generations to more than 80% in the late generation. Furthermore, the damage rate reached 40.9% in the abandoned orchards at the fruit set stage to 94% at the harvesting stage meanwhile, 23.6% and 60.6% in the other orchards [2]. The use of conventional insecticides has caused health problems and environmental pollution. To reduce these damages, the biologically and natural based methods have been experimented. Natural compounds have been known as attractive alternatives to synthetic chemical pesticides for pest management because biorational pesticides reputedly pose much less threat to the environment and human health [3]. Recently, customer awareness has been arising with the negative impacts of chemicals on their health and environment. Accordingly, their demand has increased for non-chemical products meanwhile maintaining a high degree of control efficacy to satisfy International regulations and standards necessary for export [4].
The objective of this study is to investigate the efficacy of some treatment methods to substitute synthetic chemical pesticides with biorational pesticides that can be used as a part of insect control in IPM program.

Materials and Methods
Plant extracts preparation
Neem, Oleander and Eucalyptus leaves were collected during summer season in 2015. The harvested leaves were washed up and air-dried at room temperature for one week.
Plant leaves extracts were prepared according to Harborn (1984) [5]. Plant leaves were powdered thoroughly, and then 10 grams of powdered leaves from each plant separately was mixed with 100 ml distilled water and stirred for 15 minutes then it was left for 24 hours, followed by centrifugation at 3000r/second for 10 minutes. The supernatant was discarded, and the pellet was placed into oven at 45 °C for one hour in order to get stock solution for each plant. These extracts were used to prepare the different concentrations (5, 10, 15%) regarding the research plan.

Fungus *Metarhizium anisopliae* inoculation preparation

Metarhizium anisopliae was isolated from soil collected from alfalfa field in Duhok region in 2012. The specimen was prepared in the plant protection department laboratory at College of Agriculture in the University of Duhok by Prof. Dr. Samir Kh. Abdullah [6]. The fungus was grown on Potato Dextrose Agar (PDA) media for one week at temperature 25±2 ˚C. Three different concentrations were prepared (10^3,10^5 and 10^6 spore /ml of water). Spore concentration was determined by haemocytometer.

Insect rearing

A population of carob moth was collected from fallen pomegranate fruits at the end of October 2015 in the orchards of the Halabja Technical College of Agriculture Halabja University, Halabja city, Kurdistan Region, Northern Iraq. The larvae were classified depend on their growth stage and reared on natural diet (dates) under laboratory conditions (rearing at temperature 27–30 °C, and humidity rate 65 - 70%). Rearing is conducted in the plastic boxes (dimensions: 40x20x10 cm^3), until 3rd instar larvae stage [7].

Extract toxicity

The 3rd and 5th instar larvae were sprayed with 3ml of each Eucalyptus, Neem, and Oleander plant extracts with four different concentrations (0, 5, 10, and 15%) using perfume sprayer. The corrected mortality percentage of larvae was recorded after two and four days of treatment. Sun-Shephard's formula was used to adjust for control mortality [8].

(**Sun-Shephard's Formula**)

$$\text{Corrected\%} = \left(\frac{\text{Mortality\% in treated plot} + \text{Change\% in Control plot population}}{100 + \text{Change\% in Control plot population}}\right) * 100$$

Statistical analysis

Data were analyzed based on completely randomized design (CRD) using (SAS) software. Mean comparison was done using Duncan's multiple range tests at $p \leq 0.05$.

Results and Discussion

All three types of plant extract (Neem, Olender and Eucalyptus) and *Metarhizium anisopliae* fungus tests had an effect on the correct mortality of pomegranates' carob moth, however there was a difference in their efficacy against the insect larvae. In almost all cases, the mean numbers of correct mortality were significantly higher than that of controls (See Table 1& 2).

The effectiveness of the tested extracts and the fungus rising correct mortality was: *Metarhizium anisopliae* fungus > Neem > Eucalyptus > Olender, compared to the controls.

None of the plant extracts spray treatments performed better than *Metarhizium anisopliae* fungus treatment, in rising correct mortality of carob moth.

As shown in table 1, the mortality percentage of 3rd instar larvae after 2 and 4 days treated with Neem, Olender and Eucalyptus at 0, 5, 10 and 15% extracts concentration and *Metarhizium anisopliae* fungus at (10^3, 10^5, 10^6 spore/ml) concentrations, there were differences in the correct mortality percentages for each treatments.

The highest mortality percentage achieved for the larvae treated with Eucalyptus extract (30.0%) at (15%) concentration, followed by Neem extract (23.33%) at the same (15%) concentration compare to control treatment (0.0%) after 2 days, whereas the lowest rate of the correct mortality recorded (0.0%) for Olender extract at 5% concentration after 2 days.

As illustrated in table 1, the highest rate of correct mortality was recorded for Neem extract (43.3%) at 15% concentration after 4 days, which is not significantly differ from the mortality percentage recorded for Eucalyptus extract (40.0%) at the same concentration. The mortality percentages of larvae treated with (Neem, Eucalyptus, and Oleander) extracts after 4 days at 10% concentration were (26.66, 23.33 and 20.0%) chronologically. Meanwhile, the lowest mortality percentage was recorded for larvae treated with Oleander extract (0.00%) at 5% concentration followed by Eucalyptus extract (3.33%) at the same concentration.

Significant differences were achieved for the *Metarhizium anisopliae* fungus (30.0 and 46.6%) at 10^6 spores/ml concentration after 2 and 4 days respectively (Fig.1), as well as, Eualyptus after 2 days and neem after 3 days among plant extracts, performing an effective rise in correct mortality of the treated larvae (See Table 1).

Table 1: Effect of different concentrations of different plant extracts on correct mortality percentages of carob moth 3rd instar larvae on after 2 and 4 days of treatment

Concentration plants	Correct mortality 3rd instar after 2 days				Correct mortality 3rd instar after 4 days			
	5%	10%	15%	Control	5%	10%	15%	Control
Neem	6.7 ± 3.3 fg	16.7 ± 3.3 def	23.3 ± 3.3 bcd	0.0 ± 0.0 g	16.7±6.7 de	26.7± 3.3 cd	43.3±3.3 a	0± 0 f
Oleander	0.0 ± 0.0 g	10.0 ± 0.0 efd	16.7 ± 0.0 def	0.0 ± 0.0 g	10.0 ± 3.3 ef	20.0 ± 5.57 cde	30.0 ± 5.8 bc	0± 0 f
Eucalyptus	3.3 ± 3.3 g	20.0 ± 0.0 cde	30.0 ± 0.0 bc	0.0 ± 0.0 g	16.7 ±3.3 de	23.33 ± 3.33 cd	40.0 ± 0.0 ab	0± 0 f
Metarhizium anisopliae	10^3	10^5	10^6	0.0 ± 0.0 g	10^3	10^5	10^6	0± 0 f
	6.7 ± 3.3 fg	3.3 ± 3.3 b	46.7 ± 6.7 a		26.7 ± 3.3 cd	43.3 ± 6.7 a	46.6 ± 3.3 a	

*Means followed by the same letter are not significantly different at the 0.05 level

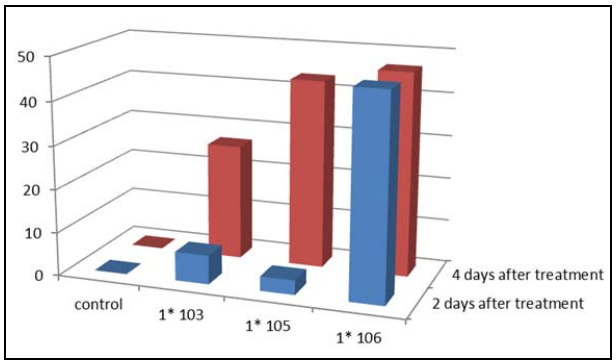

Fig 1: Mortality percentage of carob moth 3rd instar larvae treated with different concentrations of *Metarhizium anisopliae* fungus.

The results in the table 2 presents the correct mortality percentages of 5th instar larvae treated with plant extracts (Neem, Eucalyptus and Olender) and *Metarhizium anisopliae* fungus after 2, 4, and 6 day.

Mainly, the highest mortality percentage for 5th instar larvae has reached to (56.7%) treated with Neems extract followed by Eucalyptus extract (53.3%) then Olender (50.0%) at 15% concentration after 6 days of treatment, whereas the lowest rate of the correct mortality was recorded for Olender (0.0%) following by Neem (3.3%) then Eucalyptus (6.7%) at 5% concentration after 2 days among plant extracts (See Table 2).

Significant differences were achieved for *Metarhizium anisopliae* fungus (93.3%) at the concentration 10^6 spores/ml after 6 days achieving an effective increase in the mortality rate of the treated larvae (See Fig. 2 and Table 2).

The effects of biorationals have increased on correct mortality in accordance to (type, concentration of biorational and number of days after treatment). In other words, the results indicated that the mortality percentage is rising up within increasing the concentration and the number of days after treatment. For example the efficacy of Neem extract was increased from (23.3%) after 2 days to (36.7%) after 4 days then to (56.7%) after 6 days at the same concentration (15%). In the meantime, the correct mortality has increased from 46.7% at concentration (10^3 spore/ml) to (63.3%) at concentration (10^5 spore/ml) then to (93.3%) at concentration (10^6 spore/ml) (See Fig. 2).

The results showed that effect of the plant extracts varies between instars and the period after treatments. The previous studies showed that the seed and leaf extract of eucalyptus and neems contain compounds that are toxic and time dependence insect larvae [9], [10], and [11]

Table 2: Effect of different concentrations of different plant extracts on correct mortality percentages of carob moth 5rd instar larvae on after 2 and 4 days of treatment.

Concentration plants	Correct mortality 5th instar after 2 days				Correct mortality 5th instar after 4 days				Correct mortality 5th instar after 6 days			
	5%	10%	15%	Control	5%	10%	15%	Control	5%	10%	15%	Control
Neem	3.3 ± 3.3 fg	16.7 ± 3.3 cd	23.3 ± 3.3 c	0± 0 g	13.3± 3.3 gh	26.7 ± 3.33 de	36.66± 3.3 c	0 ± 0 j	23.3 ± 3.3 g	40 ± 5.6 de	56.7 ± 3.3 bc	3.3 ± 3.3 i
Oleander	0± 0 g	10.0 ± 0.0 def	16.7 ± 3.3 cd	0± 0 g	10 ± 0 hi	20.0 ± 0.0 efg	33.3± 3.3 cd	3.3± 3.3hi	16.7 ± 3.3 gh	36.7 ± 6.7 ef	50 ± 0 cd	6.7 ± 6.7 hi
Eucalyptus	6.7 ± 3.3 efg	13.3 ± 3.3 de	16.7 ± 3.3 cd	0± 0 g	16.7± 3.3 fgh	20.0 ± 0.0 efg	23.3 ± 3.3 ef	0 ± 0 j	26.7 ± 3.3 fg	36.7 ± 3.3 ef	53.3 ± 3.3 bc	6.7 ± 6.7 hi
Metarhizium anisopliae	10^3	10^5	10^6	0± 0 g	10^3	10^5	10^6	3.3± 3.3hi	10^3	10^5	10^6	6.7 ± 6.7 hi
	16.7 ± 3.3 cd	46.7 ± 3.3 b	73.3 ± 3.3 a		40 ± 0 c	50 ± 0 b	80 ± 0 a		46.67 ± 3.33 cde	63.33 ± 3.33 b	93.33 ± 3.33 a	

*Means followed by the same letter are not significantly different at the 0.05 level

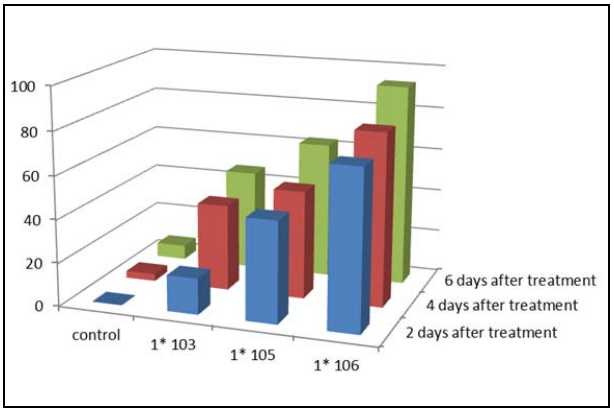

Fig 2: Mortality percentage of carob moth 5th instar larvae treated with *Metarhizium anisopliae* in different concentrations

Our study revealed that Neem extract has higher toxicity to carob moth larvae compared to others. Moreover, bioassay results showed that 5th instar larvae of the carob moth were more sensitive to the biorationals used in this study than were earlier instars larvae (Table 1 and 2).

In sum, these natural extracts and *Metarhizium anisopliae* fungus has been used in this study can be considered as potential tools for controlling this pest as a part of IPM control, as has been previously shown by different authors for other insect pests.

Recommendation

Further studies need to be carried out on the nature of plant extracts and their phytotoxicity potential to fruit and foliage for better understanding of their mode of action and limitation of the chemical pesticides use.

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