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Efficacy of Carumcarvi L.(Caraway), Eculaptuscamal-dulensisdehnh(Red Gum) Andnigella Sativa(Black Seed)Against Greater Wax Mothgalleria Mellonellal.Innaeus (Lepidoptera:Pyralidae)

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Abstract

The greater wax moth considered one of the most worldwide-spread pests of the bee wax. This Study was designed to investigate the insecticidal activity of *Carumcarvi*, *Eculaptus camaldulensis* and *Nigella sativa* against 3rd larval instar of *G.mellonella* through laboratory screening. Five concentrations (5%, 7.5%, 10%, 12.5% and 15%) of each plant extract were used in this experiment. Mortality (%) was recorded after 24, 48, 72 and 96 hrs post treatment. The results showed *C. carvi*, *E. camaldulensis* and *N.sativa* have insecticidal activity against 3rd_ instar larvae of *G. mellonella*. The highest concentration (15%) caused 90% ,86.7% and 80% larval mortality after 96 hrs for *C. carvi*,*E.camaldulensis and N.sativa* respectively. The statistical analysis revealed that there is a significant difference ($p < .001$) between all treatments and control. The results also clearly demonstrate that the C. *carvi* are significantly more toxic than *E. camaldulensis* and *N. sativa,* whereas the LC50 values were 5.4% for*C. carvi*,6.0% for *E. camaldulensis* and 6.2% for *N. sativa*.

Introduction

Honey bee is attacked by many pests such as ants, termites, beetles, wasps and moths. The most serious insect pest of bee keeping in Sudan is the greater wax moth (*Galleria mellonella*) [1]. According to field observation in Khartoum and River Nile States

apiaries, there was great damage caused by the greater wax moth and also causes death to bee brood.

The greater wax moth (*Galleria mellonella* L.) is one of the most destructive insect pests that threaten apiculture. Newly hatched larvae seek out honey, nectar and pollen, and then chew their way down to the midrib of the comb. Tunneling breaks down the wax cells in the comb. It causes complete destruction of the bee colonies and affects the production quantity and quality and market value. In a survey study100% infestation of bee combs with wax moth was reported in Gezira and Khartoum States, with the mean infestation percentage in different parts of Sudan of 86% [2].

Some insecticides such as methyl bromide, ethylene dibromide (EDB) and paradichlorobenzene (PDB) have been used to control this pest. Of these only one, paradichlorobenzene, appears to have a long- term future as a registered pesticide against the wax moth [3].

Carumcarvi or caraway is the genus only annual and biennial economical one as spice, aperitif, and carminative in food and pharmaceutical industries. Caraway is widely used in food products due to its pleasant flavor and preservative properties. Caraway fruits are used as remedy to cure indigestion, pneumonia, and as carminative, appetizer, and galactagogue in different traditional systems [4].

Eucalyptus camaldulensis is a tree under the genus *Eucalyptus* which contains specific compound like essential oil in tits different parts. Also it possesses some phytochemicals which claimed to have pesticidal and also medicinal activities on various ailments [5].

*Nigella sativa*isa genus of annual plants in the Ranunculaceae family with approximately 14 species. Essential oil from dried fruits of the *Nigella sativa* L was isolated by hydrodistillation and tested for its repellent, toxic and developmental inhibitory activities against wheat flour pest *Triboliumcastaneum* [6] .

The main objectives of this study istoevaluate firstly the ethanolic and n-Hexane extracts of Caraway (*Carumcarvi*) Black seed (*Nigellasativa*) Red Gum (*Eucalyptus camaldulensis*) against the 3rd larval instars of the Greater wax moth *Galleria mellonella*and secondlyis to test the lethal effects of these extracts and determine their LC50 and LC90 and values against tested larval instars.

Materials and Methods

The experiments were conducted in the Research Laboratory, College of Agricultural Studies (Shambat), Sudan University of Science and Technology (SUST), during March - May, 2021. The average temperature is between 25-32°C.

Larval instars of *G. mellonella* were collected from local honey bee apiary located at Khartoum state, shambatarea. The infested honey bee wax combs contained all stages of insect development were used to establish the laboratory stock culture for further studies. The larvae were reared on an artificial diet [7] .The moths were placed and 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 or experiments and placed in a closed aquarium tank (9.2x16x9.2 cm), covered with muslin cloth and brought to the laboratory for mass rearing.

Early larval instars were reared in groups of 100 larvae in plastic cages 19 cm in diameter covered with muslin cloth and fed on bee wax. The 3rd_ larval instars were reared separately in plastic cups 5 cm in diameter and 7 cm. in height to avoid cannibalism. The bottom of each cup was filled with bee wax for pupations. Upon emergence, the adults were transferred to glass cages30*30*30 cm. covered with muslin cloth and fed on 10% sugar solution [8]. The glass cages contained a comprised folded paper sheets for the deposition of eggs. The rearing process continued until a sufficient number of homogenous populations of larvae was collected for the experiments.

For preparing plant materials and extraction methods, seeds of *C. carvi* and *N. sativa* L. were collected from Omdurman market. Leaves of *E. camaldulensis* were collected from Shambat area in the Khartoum State and

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brought to the laboratory for shade-drying. After complete dryness the plant samples were crushed into powder by an electronic blender. The sample was weighed and then solvents were added sequentially from the lowest polarity N-hexane to the highest polarity ethanol in council flask, 1600 liters of ethanol were added to each sample and placed in a shaker at 156 rpm for two days using the reduce pressure filter and then the alcoholic extract was collected then the sample was evaporated using rotary evaporator to get rid of the added solvent down to the drying stage. Then, the sample was weighed and the percentage of the extract was calculated, then five dilutions were taken from each extract.

The third larval instar of the Greater wax moth *G. mellonella* was used in this study [9]**.** However, ten (10) newly hatched instar larvae of the greater wax moth were placed in each dish contained 5g. of small pieces of pure wax, and then 5ml of each prepared concentration of each tested material was sprayed to the Petri dish above the pieces of wax and covered to prevent larvae from escaping.

The experiments were conducted under the laboratory conditions of 31±1°C, 66.28% RH and 12L: 12D photoperiod. Treated larvae were provided with fresh wax pieces till the end of the experiment. The mortality percentage was recorded 24, 48, 72 and 96 hrs. after application. The collected data were statistically analyzed using analysis of variance (ANOVA); Duncan's Multiple Range Test and Statistix 8 for means separation. Also the data were subjected to probability analysis using SPSS 16.0 software to get LC50 and LC90.

Results and Discussion

Results

All results showed that the mortality % increases with the increase of both concentration and exposure period.

The results in table (1) showed the *E. camaldulensis* leaves gave highest mortality % (86.7%) at the highest concentration (15%) after 96 hours (the longest exposure period), followed by *N. sativa* seeds under the same condition and gave mortality of 73.3%, while the *C. carvi* seeds records gave the lowest mortality % (66.7%) under the same condition.

Considering the result in table (2), it revealed the higher concentration (15%) of *C.carvi* seeds gave 90 % mortality after 96 hours, while under the same concentration, *E. camaldulensis* leaves gave 83.3 % mortality after 96 hours and *N.sativa* seeds gave 80 % mortality after 96 hours. The least concentrations (5%, 7.5%and 10%) of n, hexane extract of *N.sativa* seeds after 24 hours gave mortality % of 23.3 %, 26.7% and 33.3% respectively compared with the control which records the worst percentage result throughout the experimental period.

The data presented in table (3) and Figure (1) Provided clear evidence that the oily extracts of all tested plants have a lethal effect against the 3rd larval instars of the Greater wax moth. Probability analysis of the mortality data showed that the lethal concentrations of the extracts vary from one plant to another. The lowest LC_{50} value for n-hexane extract was recorded by *C.carvi (*5.4), followed by *E.camaldulensis* (6.0) and *N. sativa (6.2)*.

Regarding the results in table (4) and Figure (2), they showed ethanolic extracts of all tested plant scored a significantly lethal effect against the 3rd larval instars of the Greater wax moth. The probability analysis of the mortality data showed that the lethal concentrations of the extracts differ from one plant to another. The lowest LC_{50} value for ethanolic extract was recorded by *E.camaldulensis(5.0)*, followed by *N. sativa(7.0) and C. carvi (7.7).*

Discussion

Plants have long been proposed as smart alternatives to synthetic insecticides for pest management because they are safe to the environment and human health. More than thousands species of plants have been reported to have chemicals in its various parts which have insecticidal properties. However, a few of them were used for insect control on a commercial scale [10]. The study findings clearly proved the efficacy of *C. carvi* against

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Table 1. Lethal effects of *N.sativa*seeds,*C.carvi* seeds and *E. camaldulensis*leavesethanolic extract against 3rd larval instar*G.mellonella*.Shambat-Sudan (01/05/2021)

Treatments	**Conc. (%)**	**Means mortality (%)**			
		Exposure time (hrs.)			
		24	**48**	**72**	**96**
N. sativa+Ethanol	5	23.3 (4.8)f	26.7 (5.2)fg	36.7 (6.1)fg	43.3 (6.6)f
	7.5	26.7 (5.2)ef	33.3 (6.1)e	46.7 (6.9)de	53.3 (7.3)de
	10	33.3 (5.8)de	40.0 (6.4)cde	53.3 (7.3)cd	56.7 (7.6)cde
	12.5	36.7 (6.1)cd	43.3 (6.9)bcd	56.7 (7.6)bc	66.7 (8.2)bc
	15	43.3 (6.6)abc	50.0 (7.1)bc	63.3 (8.0)ab	73.3 (8.6)b
C. carvi+Ethanol	5	20.0 (4.5)f	23.3 (4.8)g	33.3 (5.8)g	43.3(6.6)f
	7.5	26.7 (5.2)ef	33.3 (5.8)ef	43.3 (6.6)ef	50.0 (7.1)ef
	10	33.3 (5.8)de	40.0 (6.4)cde	46.3 (6.9)de	53.3 (7.3)de
	12.5	40.0 (6.4)bcd	50.0 (7.1)bc	53.3 (7.3)cd	63.3 (8.0)bcd
	15	46.7 (6.9)abc	53.3 (7.3)ab	56.7 (7.6)bc	66.7 (8.2)bc
E. camaldulen-sis+Ethanol	**5**	**26.7 (5.2)ef**	**33.3 (5.8)ef**	**43.3 (6.6)ef**	**53.3 (7.3)de**
	7.5	33.3 (5.8)de	40.0 (6.3)de	46.7 (6.9)de	56.7 (7.6)cde
	10	43.3 (6.6)abc	50.0 (7.1)bc	56.7 (7.6)bc	66.7 (8.2)bc
	12.5	50.6 (7.1)ab	56.7 (7.6)ab	63.3 (8.0)ab	73.3(8.6)b
	15	53.3 (7.3)a	63.3 (8.0)a	73.3 (8.6)a	86.7 (9.3)a
Control	-	(0.7000)g	(0.7000)h	(0.7000)h	(0.7000)g
C. V. %		**8.9**	**7.3**	**5.9**	**5.6**

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

* Means between brackets are transformed according to √ (x+0.5)

*C. V. = Coefficient of Variation.

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Table 2. Lethal effects of *N.sativa*seeds,*C.carvi* seeds and *E. camaldulensis*leavesN-hexane extract against 3rd larval instar*G.mellonella*.Shambat-Sudan (06/05/2021)

Treatments	Conc. (%)	Means mortality (%)			
		Exposure time (hrs.)			
		24	48	72	96
N. sativa +N-hexane	5	23.3 (4.8)f	26.7 (5.2)g	33.3 (5.8)d	46.7 (6.9)h
	7.5	26.7 (5.2)ef	33.3 (5.8)fg	43.3 (6.6)cd	53. (7.3)fgh
	10	33.3 (5.8)de	40.0 (6.4)def	50.0 (7.1)de	63.3 (8.0)de
	12.5	43.3(6.6)bc	46.7 (6.9)cd	60.0 (7.8)bcd	73.3 (8.6)bcd
	15	46.7 (6.9)abc	53.3 (7.6)b	70.0 (8.4)abc	80.0 (8.9)ab
C. carvi +N-hexane	5	20.0 (4.5)f	23.3 (5.8)fg	43.3 (6.6)ef	50.0(7.1)gh
	7.5	33.3 (5.8)de	40.4 (6.6)de	50.0 (7.1)de	60.0 (7.8)ef
	10	40.0 (6.3)cd	53. (7.3)bc	63.3 (7.9)bcd	66.7 (8.2)cde
	12.5	46. (6.9)abc	63.3 (8.0)ab	70.0 (8.4)abc	76.7 (8.8)bc
	15	56.7 (7.6)a	73.3 (8.6)a	80.0 (8.9)a	90. (9.5)a
E. camaldulen-sis +N-hexane	5	23.3 (4.8)f	33.3(5.8)fg	40.0(6.3)ef	46.7 (6.9)h
	7.5	33.3 (5.8)de	36.7(6.1)ef	50.0(7.1)de	56.7 (7.6)efg
	10	40.0(6.4)bcd	46.7 (6.9)cd	56.7(7.6)cd	63.3(8.0)de
	12.5	46.7 (6.9)abc	56.7 (7.6)b	66.7(8.2)abc	73.3 (8.6)bcd
	15	50.0(7.1)ab	63.3 (8.0)ab	73.3(8.6)ab	83.3 (9.2)ab
Control	-	(0.7000)g	(0.7000)h	(0.7000)g	(0.7000)i
C. V. %		7.9	6.4	7.6	5.1

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

* Means between brackets are transformed according to √ (x+0.5)

*C. V. = Coefficient of Variation

Table 3. LC values for N-hexane extracts of tested plants against 3rd larval instar of *G. mellonella*after 96 hrs of exposure. Shambat-Sudan (11/05/2021)

Plant extract	LC* values and 95% Confidence limits (Lower – Upper)		
	LC_{50}	LC_{90}	Chi- square $\chi 2$
Carum carvi	5.4(-0.4 - 19.2)	16.4(13.5 - 25.3)	0.6
Eucalyptus camaldulensis	6.0(-0.3 – 8.2)	18.7 (14.9 – 32.9)	0.03
Nigella sativa	6.2 (-0.6– 8.5)	19.6(15.4 – 37.1)	0.14

* LC = Lethal Concentration

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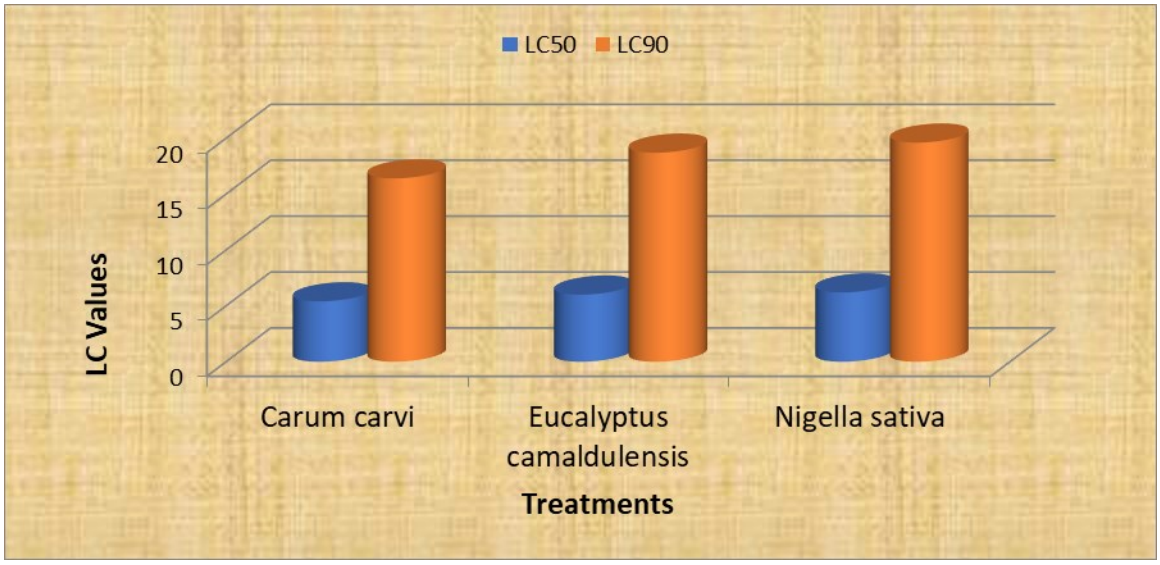

Figure 1. LC values for n-Hexane extracts of *C.carvi*seeds,*E. camaldulensis*leaves, and *N.sativa*seeds against 3rd larval instar of *G.mellonella* after 96 hrs of exposure.

Table 4. LC values for ethanolic extracts of tested plants against 3rd larval instar of *G. mellonella*after 96 hrs of exposure. Shambat-Sudan (16/05/2021)

Plant extract	**LC* values and 95% Confidence limits (Lower – Upper)**		
	LC_{50}	**LC_{90}**	**Chi- square $\chi 2$**
Eucalyptus camaldulensis	5.0(-3.6 – 7.6)	18.3 (14.4 – 34.1)	0.1
Nigella sativa	7.0 (-3.3– 9.7)	23.6(17.3 – 68.2)	0.6
Carum carvi	7.7(-27.8 -11.3)	28.6(19.2 - 315.7)	0.4

LC = Lethal Concentration

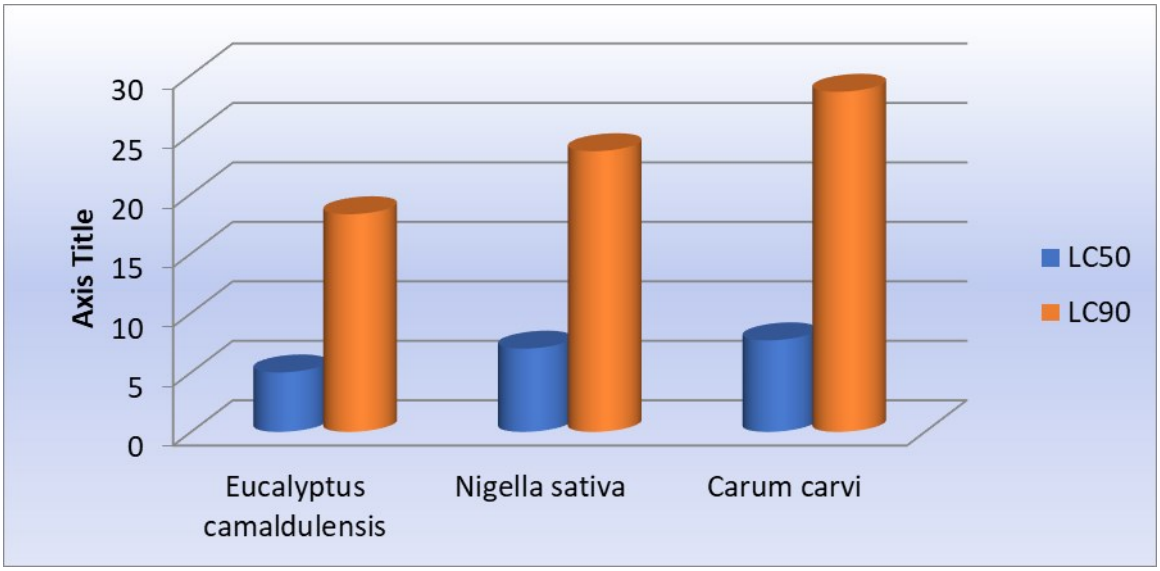

Figure 2. LC values for ethanolic extracts of *E. camaldulensis*leaves,*N.sativa* and*C.carvi* seeds against 3rd_ larval instar of *G.mellonella* after 96 hrs of exposure

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3rd_ larval instar of *G. mellonella*. In fact its highest concentration (15%) gave 90% mortality of tested larvae after 96 hrs of application.

This result agree with results obtained a researcher [11] who analyzed the repellent effects of six essential oils extracted from caraway, grapefruit, clary sage, strawberry and thyme white on *Sitophilus oryzae*. They found that the highest repellent activities (96.7%) were obtained with caraway oil. Also these results agreed with the results of other researchers [12] who found that the volatile oil carvone against the larvae and adult of greater wax moth has insecticidal activity.

The mortality % recorded after72 and 96 hours of exposure15% and12.5% respectively o f N-hexane extract of *E. camaldulensis* leaves does not change. The present results are also in line with research the finding that a species of *Eculayptus* successfully controlled the larval stages of the stored wax greater wax moth [13]. This maybe due to an acute action of this plant extract. Similar results were obtained by researchers [14]; who found that the leaves of *E. camaldulensis* duration of larval, pupal and adult stages and incubation period. The effect is dose dependent. Also these results agreed with the results of researchers [15]; who studied the effect of essential oils extracted from five *Eculayptus*n species and found that all tested essential oils have larvicidal effect against *Tribolium castaneum* and *Tribolium confusum*.

The results revealed that the *N. Sativa* N, hexane extract at the concentration 15% gave mortality percentage of 80% after 96 hours. The present results are also in line with results of other researchers [16,17] who found that *N. Sativa* at higher concentration was better than conventional insecticides in tropical stored pest. Similar results were demonstrated by [18] who emphasis that the highest concentration of N. *Sativa* gave a 100% mortality against *Tuta absoluta*.

Conclusion and Recommendations

The obtained results clearly proved that the C. carvi, E. camaldulensis and N. Sativa have insecticidal activity against 3rd_ instar larvae of *G. Mellonell*. Through this study, it was found that the N, hexane extract of *C. carvi* seeds was the best, followed by the ethanolic *E. Camaldulensis* leaves extract and then the n, hexane *E. Camaldulensis* leaves extract and then *N. Sativa*n, hexane extract. Thiscleary means that the above mention plants can be used in an integrated control program against greater wax moth.

Caraway (*C. carvi*) N, hexane extract can be used as a bio pesticides to safe environment; the following recommendations are of importance; firstly is to evaluate doses higher than tested one might give higher mortality percentage; secondly more studies are highly encouraged for confirmation.

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