

Efficacy of some Essential Oils on Cowpea Aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae)

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ABSTRACT

A trial study was managed to estimate the efficacy of neem oil and cinnamic oil on cowpea aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae) which infest a wide host range of plants caused serious damage. Due to the problems of chemical pesticides to organisms and environment, natural control replaced pesticides. The data obtained in this study showed that neem oil was more effective in controlling *A. craccivora* where LC_{50} was 125.26 ppm comparing with cinnamic oil which LC_{50} recorded 378.68 ppm. GC/ MS analysis of Neem oil showed the presence of saturated and unsaturated fatty acids by 99.34%, where it was found to contained eight different fatty acids. The linoleic acid represents the highly proportion with 34.69%.

Keywords: *Aphis craccivora*, cinnamic oil, neem oil, GC/ MS

INTRODUCTION

Cowpea aphid, *A. craccivora* is one of the most important sucking pest which infest many different host of plants caused critical damage whether directly, by sucking the host plant and cause many problems on leaves, stems and fruits (Sharma and Joshi, 2010) or indirectly, by its role in transmitting pathogens of plant diseases (Abbas *et al.*, 2009) where, it causes the transfer of 14 legume viruses at least (Thottappilly *et al.*, 1990). In addition, secreting of honeydew by this insect pest provides a medium for the growth of black sooty mold which cover on leaves resulting impair photosynthesis and causing more damage to the plant (Cranshaw *et al.*, 2000)

In order to avoid the many problems resulting from the use of traditional pesticides, including development of insecticide resistant population the interest in finding other products of effective natural alternatives in pest control is necessary. This is reflected in the continuing interest in studying the impact of the use of essential oils as pesticides for the last period where, it was found to possess many repellent and toxic capacities for various pests which show potential to control it including aphids (Gupta and Dikshit, 2010; Barkman, 2013 and Plata-Rueda *et al.*, 2018).

In cinnamon oil (*Cinnamomum verum*), bark oil consists of 80-90% cinnamaldehyde along with many other ingredients mentioned by Heath (1978) which known as an insecticidal compounds that have been studied against various insects (Isman and Machial, 2006 and Cheng *et al.*, 2008) including eugenol which changing the functioning of octopamine and leads to abruption of nervous system functioning in insects (Enan, 2005) beside linalool that affecting ion transport and release of acetyl choline esterase in insects through the work on its nervous system (Re *et al.*, 2000). Whereas, Azadirachtin is the main component of neem that may reduce insect infestations of various plants including cowpea and repel them (Sarode *et al.*, 1995; Selvasundaram and Muralaedarhan, 1999 and Lale and Kabeh, 2004).

In this study, we examined the effect of some different concentrations of both neem and cinnamon oils on *A. craccivora* to assess their effect on the pest.

MATERIALS AND METHODS

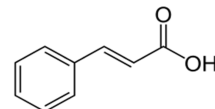
Rearing of the insect:

To obtain *A. craccivora* adults which used to start the experiments, cultures of the current insect were established by planted cowpea in pots and infested it artificially by placing leaves infested with aphids, which

were collected from unsprayed plants experimental farm of Faculty of Agriculture, Mansoura University on the planted cowpea. The colony was kept at $27\pm 2^{\circ}C$ and $65\pm 5\%$ RH and maintained for approximately two generations before the beginning of the experiments. Thereafter, the new emerged adults of *A. craccivora* were collected from the colony and used in the test.

Cinnamon oil was bought from Essential oil Extracts Center, National Research Center.

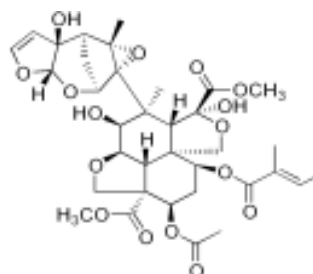
- Cinnamon oil contains cinnamic acid, $C_9H_8O_2$.



Cinnamic acid formula (Vogt, 2010)

- However, Neem oil was extracted in laboratory.

Neem contains azadirachtin $C_{35}H_{44}O_{16}$



Azadirachtin formula (Veitch, 2007)

Preparation and isolation of essential neem oil:

To extract the essential oil (volatile oil) of neem, seeds of neem plant were used. Where, it was extracted from this seeds by steam distillation apparatus found in Plant Protection Research Institute, Mansoura, Egypt. The oil has been separated dried on anhydrous sodium sulphate. Then, it was kept at $4^{\circ}C$ in refrigerator into dusky bottles until needed in the test.

Preparing stock solution of the tested plant oils:

On basis of the examined plant oil weight and the volume of the distilled water (w/v) in the presence of tween 80 (0.1%) as emulsifier, stock concentrations of each neem and cinnamic oils were prepared. It was stored under refrigeration in closed glass bottles. Stock solutions were equipped periodically. To draw the LC-P lines, five diluted concentrations were utilized for each plant oil. Four replicates were used for each treatment concentration and also for the untreated.

Application method:

Adults of the cowpea aphids were used for application. Five concentrations and four replicates were

used for each treatment concentration. Twenty individuals of *A. craccivora* for each replicate were treated in plastic units (9 cm in diameter and 5 cm high) to estimate the mortality line, as well as twenty adults of the aphid were used in the untreated which was sprayed only by distilled water and tween. Different concentrations (50, 100, 200, 300 and 400 ppm) were applied on the current insect by sprayed the aphid directly. The mortality percentage was recorded after one, three, five and seven days and data were corrected relatively to control mortality (Abbott, 1925). LC₅₀ values were determined by using probit statistical analysis method of Finney (1971).

Toxicity index for LC₅₀ (Sun, 1950)

$$= \frac{\text{LC}_{50} \text{ of the most effective compound}}{\text{LC}_{50} \text{ of the other tested compound}} \times 100$$

Chemical analysis:

GC/MS analysis was conducted in Central Laboratory for Food and Feed, Agriculture Research Centre. To determine the fatty acid composition, Crude oils were analyzed as methyl ester. It was executed on Agilent Technologies 7890. There was a coupling between Gas Chromatography (GC) Systems and Mass Spectrometry (MS) detector. By using GC auto sampler, 1µl/L of fatty acid methyl ester solution was injected in the system. At the flow rate of 1 ml/min, helium was used as a carrier gas. Of 100% dimethyl polysiloxane by 30 m lengths, 0.25 mm diameter and 0.25µm thickness, the separation was procedure on non polar capillary DB-1. Initially, the GC oven temperature was set to 60°C for 3 min then; it was increased to 240°C as a maximum at the rate of 3°C/min. The temperature was maintained constant for 10 min Once 240°C were reached. The inlet temperature was set at 250°C. As a split less mode, the mass detector conditions were set, 250°C injector temperature and ion-source temperature of 230°C. To identify separate peaks, Wiley and Wiley Nist mass spectral data base was used.

Table 2. Efficiency of some plant essential oils against cowpea aphid, *A. craccivora*

Treatment	Conc. (ppm)	Corrected mortality%	LC ₅₀	LC ₉₀	Slope ± S.D.	LC ₉₀ / LC ₅₀	Toxicity index	P	R
Neem oil	50	35.1	125.26	1652.31	1.14± 0.178	13.19	100	0.274	0.956
	100	45							
	200	52.7							
	300	65.1							
	400	77.6							
Cinnamic oil	50	21.3	378.68	10338.11	0.892± 0.182	27.30	33.08	0.199	0.922
	100	33.9							
	200	36.4							
	300	40.1							
	400	57.6							

R: Regression

P: Probability

On the other side, the result showed a varied of the mortality percentages for the different concentrations. Where, although the neem oil was more effective than cinnamon oil on aphid it was noticed that the high concentrations of cinnamon oil were most effective on the target insect compared with the low concentrations. Also, all the replicates treated with both plant extracts showed an effect on the current insect through its mortality percentages compared to untreated. Johnson (2018) mentioned that all oils coating the insect, plugging their respiratory spiracles, and killing them by suffocation when used to control aphids and other pests and this indicated to the importance of the

RESULTS AND DISCUSSION

(1) Toxicity Effect:

The data in Table (1) showed the efficiency of cinnamic oil and neem oil on *A. craccivora* adults. It demonstrated that, neem oil was the most effective essential oil than cinnamic oil against the adults of the insect. The obtained results were agreed with previous researches showed that neem products have a critical role in the sucking pests control including aphids (Hussain et al., 1996; Satyanarayana et al., 2003; Roy and Gurusubramanian, 2011; Sakthivel et al., 2011 and Sreerag and Jayaprakas, 2015).

Table 1. Corrected mortality percentage of the adults of cowpea aphid, *A. craccivora* treated with neem oil and cinnamic oil under laboratory conditions (27±2°C and 65±5% RH).

Treatment	Conc. (ppm)	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Neem oil	50	7.5	11.3	10	6.3	35.1
	100	7.5	15	12.5	10	45
	200	11.3	18.8	13.8	8.8	52.7
	300	15	22.5	16.3	11.3	65.1
	400	27.5	22.5	18.8	8.8	77.6
Cinnamic oil	50	6.3	7.5	5	2.5	21.3
	100	7.5	13.8	8.8	3.8	33.9
	200	7.5	11.3	11.3	6.3	36.4
	300	8.8	15	11.3	5	40.1
	400	13.8	21.3	15	7.5	57.6

Table (2) and Fig. (1) showed that LC₅₀ was 126.26 ppm for neem oil and 378.68 ppm for cinnamic oil. Also, LC₉₀ was 1652.31 ppm and 10338.11 ppm for neem oil and cinnamic oil, respectively. These results proved the efficiency of neem oil than cinnamic oil. The probability was 0.274 for neem oil and 0.199 for cinnamic oil. Vinelina et al. (2014) proved the effectiveness of neem extract against the cotton leaf worm, *Aphis gossypii* Glover.

method of application. Barkman (2013) noted that most compounds are both repellent and toxic in high concentrations while often being toxic or repellent only in low concentrations. This is consistent with Shannag et al. (2015) who observed that application of pure neem oil and Azatrol on Larvae of *Spodoptera eridania* (Cramer) at high concentrations was more effective than lower concentrations where they noticed increasing in larval mortality percentages using high concentrations compared to low concentrations which the mortality percentages was lower. Also Masatoshi (1999a and 1999b) tested several essential oils on different species of insect including aphids

and reported the importance of exposure to high concentrations in the vapour phase to kill the insects.

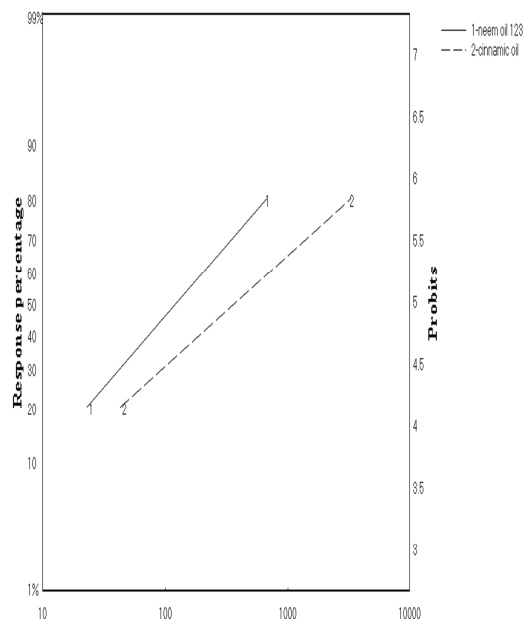


Fig. 1. LC-P line for neem oil and cinnamic oil of cowpea aphid, *A. craccivora*

(2) Chemical analysis:

GC/MS analysis of neem oil was detected and listed in Table (3) and Fig. (2) according to their percentage composition. Where, Table (3) Showed the presence of saturated and unsaturated fatty acids within eight different fatty acids found through the analysis of crude *Aindica* seed fixed oil as methyl ester. The total percentage of fatty acids which represented the relative percentage area from the sum of all specified peaks was almost 99.34%. In the present study, the presence of linoleic acid (C18: 2) was found to be 34.69% and it is the main structure of fatty acids in *aindica* seed fixed oil. Other fatty acids were present also where, the oleic - (C18:1) was found by 20.46%, stearic - (C18:0) by 20.42%, palmitic - (C16:0) by 18.66% and arachidic - (C20:0) by 3.59% beside behenic - (C22:0), lignoceric- (C24:0), and palmiticoleic acid (C16:1) which were found at the percentage of 0.80%, 0.55%, and 0.17%, respectively. Similar results were obtained by Akpuaka *et al.* (2012) and Atabani *et al.* (2013) who proved that neem oil has different fatty acids with different proportions and linoleic acid. Also, Sandanasamy *et al.* (2013) demonstrated that linoleic acid is the main component in neem oil.

Finally, it is clear that the effect on the target insect pests using several essential oils may vary according to varieties and concentrations of oil used in the control. Therefore the use of these extracts in insect pests control still needs more testing and further investigation.

Table 3. Main components of neem oil identified by GC/ MS

Fatty Acid	Systematic Name	Formula	Structure	Area (%)
Linoleic acid	9,12-octadecadienoic acid	C18H32O2	C18:2	34.69
Oleic acid	9-octadecenoic acid	C18H34O2	C18:1	20.46
Stearic acid	octadecanoic acid	C18H36O2	C18:0	20.42
Palmitic acid	hexadecanoic acid	C16H32O2	C16:0	18.66
Arachidic acid	Eicosanoic acid	C20H40O2	C20:0	3.59
Behenic acid	docosanoic acid	C22H44O2	C22:0	0.80
Lignoceric acid	tetracosanoic acid	C22H48O2	C24:0	0.55
Palmiticoleic acid	9- hexadecenoic acid	C16H30O2	C16:1	0.17

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كفاءة بعض الزيوت الطبيعية على من اللوبيا

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تهدف الدراسة الى تقدير كفاءة كلاً من زيت النيم وزيت القرفة على حشرة من البقوليات التي تصيب العديد من النباتات مسببة لها أضراراً خطيرة. تحل مكافحة الطبيعية محل المبيدات الكيماوية نظراً لما ينتج عنها من مشاكل للكانات الحية والبيئة وقد أوضحت الدراسة أن زيت النيم كان أكثر تأثيراً في مكافحة أفراد المن حيث كان التركيز النصف مميت ١٢٥,٢٦ جزء في المليون مقارنة بزيت القرفة الذي سجل التركيز النصف مميت له ٣٧٨,٦٨ جزء في المليون. أظهر التحليل الطبقي الكروماتوجرافى الذى تم عمله لزيت النيم وجود الأحماض الدهنية المشبعة والغير مشبعة بنسبة ٩٩,٣٤٪، حيث وجد أنه يحتوي على ثمانية أحماض دهنية مختلفة و يحتل حمض اللينوليك حوالى ٣٤,٦٩٪.