Efficiency of Three Plant Oils on the Black Legume aphid, *Aphis craccivora* Koch (Homoptera: Aphididae) and some of its Biological Aspects

M. E. Tawfeek, Amany M.H.Abu-shall and Anar A. Bakr
Applied Entomology Dept., Fac. of Agric., Alexandria Univ., Egypt.

ABSTRACT

The toxic effect of three natural plant oil extracts (Clove bud oil, linseed oil and Fennel seed oil) and Selecron as a pesticide compound on the 3rd instar larvae of the cowpea aphid, ***Aphis craccivora*** Koch, was studied at four concentrations after 24 hrs period under laboratory conditions. Results of Probit analysis showed that the Clove oil gave the highest effect against the 3rd instar larvae followed by Linseed oil then Fennel oil.

Also, the effect of the lowest concentration of each of the tested oils on some biological aspects was studied. The treated individuals of the black legume aphid, by Fennel oil (2000 ppm) survived for only 6 - 7 days and completely died in 2nd larval instar. In case of Linseed oil (1000 ppm) the aphids survived between 7 and 10 days and died in the 3rd instar larvae, while they survived for 9 - 12 days and died in the 4th instar larvae when reared on the treated faba bean with Clove oil (100 ppm).

In view of the potentiality reported here, at least under indoor conditions, it was obvious that certain plant oils such as Fennel, Linseed and Clove oils could be used successfully to inhibit the build-up of cowpea aphid population at economically low rate of application.

Data showed that Clove oil caused the lowest mortality percentage on the adult stage of ***Coccinella undeciumpunctata*** L. after 24hours of treatment (33.3%) followed by Linseed oil (66.7%), then Fennel oil (100%). Whereas all adults of the minute wasp parasitoid, ***Diaeretiella rapae*** (M'Intosh) succeeded in emergence from the mummy aphids, which were treated with all concentrations of the tested plant oils.

Keywords: plant oils, control, biology, natural enemies, *Aphis craccivora* .

INTRODUCTION

The black legume aphid or cowpea aphid, ***Aphis craccivora*** Koch. (Homoptera: Aphididae) is considered among the economically important pests of Leguminous crop plants. The direct damage when plant shoots are heavily infested is the reduction of extension growth and the debilitating effect of sap extraction by large numbers of aphids can reduce crop size (**Habib and El-Kady, 1961; Darwish *et al.*, 1989; Darwish and El-Sheikh, 1991; El-Hawary and Abdel-Salam, 2005**). Also, this species infests some fruit trees in Egypt (**Darwish *et al.*, 1994** and **Tawfeek, 2001**).

It damages the infested plants by feeding on the sap and it also known as a vector of about 30 plant virus diseases (**Blackman and Eastop, 1984; Sanz *et al.*, 2001**). At heavy infestation, the secretion of honey dew causes growth of black sooty mould causing interference with the vital processes of plants (**Darwish, 1998** and **Summers *et al.*, 2004**).

In spite of widespread public concern for long-term health and environmental effect of synthetic pesticides, botanical insecticides have long been considered as acceptable alternatives to synthetic chemical insecticides for pest management as they have low persistence in the environment, little mammalian toxicity and resulting in good selectivity and wide public acceptance (**Isman, 2000, 2005; Sampson *et al.*, 2005** and **Digilio *et al.*, 2008**). One alternative method to control aphids is the use of essential oils and extracts. These products are described as complex mixtures of natural substances made by plants (**Moschetti, 2006**).

The present study aims to evaluate the activity and efficiency of three commercial plant oils (Fennel, Linseed and Clove oils) and comparing their effect with an conventional insecticide on the black legume aphid, ***A. craccivora***, besides on one of each of its predators and parasitoids under laboratory conditions.

MATERIALS AND METHODS

A- culture of the tested aphids:

The collected ***A. craccivora*** individuals were obtained from Abbeas Farm, Faculty of Agriculture, Alexandria Univ., Egypt. Samples of infested faba bean plants were transported from the field and used to infest clean faba bean plants, which were cultivated in pots under semi-field conditions at the Department of Applied Entomology, Faculty of Agriculture in Alexandria. The aphids were reared for several generations.

In order to obtain aphids of known age and high uniformity adequate number of apterous viviparous females were transferred from the stock colony to clean untreated faba bean leaves for 24h and then removed. The offspring were maintained under 25°C ± 2 and 75% ± 5 R.H. until reaching the 3rd instar larvae, the appropriate stage for testing.

B- The tested substances:

The commercial plant oils were a fixed oil (Linseed oil, ***Linum usitatissimum***) and two essential oils (Fennel oil, ***Foeniculum vulgare*** and Clove oil, ***Eugenia caryophyllata***). The extractions in all experiments were preformed in water by adding Tween-20D as emulsifier. For each

plant oil four concentrations were used i.e Linseed oil (1000, 3000, 6000 and 9000 ppm), Fennel oil (2000, 5000, 8000 and 10000 ppm), and Clove oil (100, 500, 900 and 2000 ppm). The comparison insecticide Selecron (72%) [common name: (Profenofos) [IUPAC name: 0-(4-bromo-2-chlorophenyl)-5-ethyl-S-propyl phosphorothioate] as used at concentration rate of 0.1, 0.5, 1, 10& 100 ppm.

The prepared aqueous solutions of the tested concentrations were sprayed on free-infested faba bean leaves to the point of runoff, using a hand pressure sprayer fitted by a low volume nozzle. Treated faba bean leaves were left for dryness before transferred to petri-dishes. For each concentration, a total number of 30 individuals of 3rd larval instar cowpea aphids were bio-assayed in three replicates each of ten individuals. While the water treated faba bean leaves were used in the control treatment. For conserving faba bean leaves fresh, each petri-dish was furnished by water moistened filter paper below the tested faba bean leaflets.

C- Effect of plant oils on some biological aspects of *A. craccivora*:

Furthermore, the biology of ***A. craccivora*** was studied at the lowest concentration of each plant oils. For each of these lowest concentrations of tested oils a total number of 30 individuals were bio-assayed in three replicates of each ten individuals of the 1st larval instar. Three petri-dishes, equipped with 30 individuals on water treated faba bean leaflets were used as control. The reproductive, pre- and post-reproductive periods, fecundity and total life span were determined according to the estimation of Darwish **(1983 a, b)** and the application by **Tawfeek (2001).**

D- Effect of plant oils on some natural enemies of aphids:

Culture of the ladybird, ***Coccinella undeciumpunctata*** L. (Coleoptera: Coccinelidae) was established and maintained in the laboratory. Field-collected larvae were reared into adults in glass petri-dishes (9.0 cm diameter) containing infested faba bean leaflets by aphids on moistened filter paper. The highest prepared concentration of each plant oil was applied upon the predator involved adults in petri dishes, in addition to untreated check. Each petri dish contained five ladybirds on a faba bean leaflet infested with first/second-instar larvae of aphids. Each treatment and the control were tricely replicated. Prior to treatment, the ladybird adults were starved for 12 hrs. To determine mortality; observations were made 24 hrs post treatment. Also, as to the abovementioned method, the parasitoid species ***Diaeretiella rapae*** (M`Intosh) (Braconidae, Hymenoptera) was exposed to the same tested plant oils as five mummies

of aphids; replicated three times. The emerged adults of the parasitoid from parasitized aphid's mummy are was investigated and counted.

RESULTS AND DISCUSSION

1. Assay:

The effect of three natural plant oil extracts (Clove bud oil, Linseed oil and Fennel seed oil) and Selecron as an organophosphorus compound on the 3rd instar larvae of the black legume aphid, ***A. craccivora***, was studied at four concentrations after 24hs of treatment under laboratory conditions.

As shown in Table 1, results of Probit analysis show that among the three plant oils used, the Clove oil gave the highest effect against the 3rd instar larvae of ***A. craccivora*** (Lc_{50} = 549.9 ppm) followed by Linseed oil (Lc_{50}= 2863.5 ppm) then Fennel oil (Lc_{50} = 4780.9 ppm).

Selecron was the most toxic compound in comparison to the tested oils against the 3rd instar larvae of cowpea aphid as shown in Table 1, it was used as a standard in calculating the toxicity index at Lc_{50} level. No mortality was seen in the untreated controls. However, the data obtained demonstrate that the three compounds possess aphidicial activity at doses higher than that used with the chemical synthetic insecticide (Selecron). There were differences in the bioinsecticidal effects of the three plant oil extracts especially Clove oil (Table 1). The strong activity of this oil may be attributed to eugenol and methyl eugenol as major components which accounted for 60 – 80% of Clove oil (**Kim *et al.*, 2003**). Eugenol has been shown to be effective against various species of Coleoptera (**Hunag *et al.*, 2002**), Hymenoptera (**Enan, 2002**) and Isoptera (**Park and Shin, 2005**).

Many plant extracts have been reported bioactive against ***A. craccivora*** and other related species (**Ofuya and Okuku, 1994; Abdallah *et al.*, 2004** and **Tewary *et al.*, 2005**).

The second compound in toxicity for ***A. craccivora*** was Linseed oil (Table 1), our finding is in agreement with the results obtained by **El-Hadek (2007)** who found that Linseed oil was the most effective compound among seven vegetable oils tested on adults of ***A. craccivora.***

On the other hand, application of fennel (***Foeniculum vulgare***) seed oil on cabbage aphid, ***Brevicoryne brassicae*** (L.) adversely affected the daily fecundity and caused a higher offspring mortality rate (**Işik and Görür, 2009**). These findings were parallel with our results in this study which showed that, the fennel seed oil was the third active compound on ***A. craccivora*** (Table 1).

It can be concluded from the findings that the three plant oil extracts gave better results in controlling the aphid ***A. craccivora*** at higher concentrations than synthetic pesticide which was used as comparative treatment. However, the synthetic pesticide has been found significantly more effective than the natural oils not withstanding of course the dangers they possess to the environment and life. In general, the natural oils as an pesticides are safer for the environment and human health because of their low toxicity and shorter degradation time.

2. Biological study:

The delayed effects of the three tested plant oils on the developmental stages of cowpea aphids were investigated in comparison with the check of untreated aphids. The biology of different treated stage of ***A. craccivora*** was carried out under the laboratory conditions at 25°C ± 2 and 75% ± 5 R.H. by using the lowest concentrations of 100, 1000 and 2000 ppm for Clove oil, Linseed oil and Fennel oil, respectively.

Data in Table 2 show that the normal insects of untreted were survived and completed their life cycle to reach adult stage. The pre-reproductive period ranged between 14 and 17 days with an average of 15.3 days. The reproductive period ranged from 8 - 12 days and averaged 9.7 days. The post reproductive period lasted between 2 and 5 days with an average of 3.7 days. The total life span ranged between 24 - 31 days with an average of 28.7 days. Fecundity on numbers of offspring ranged between 9 and 11 with an average of 9.3 settled larvae (progenies) per one apterous adult female aphid.

Data in Table 2, clearly indicated that the used plant oils varied in their ability to disrupt the normal pattern of development to adult status in cowpea aphids initially exposed as 1st instar larvae and continuously maintained on treated faba bean leaves. Whereas, these treated aphid individuals did not reach to adult stage and died before the last moult as immature stages. Herein, the treated individuals of the black legume aphid, by Fennel oil (2000 ppm) survived for only 6 - 7 days and died in the 2nd larval instar. In case of Linseed oil (1000 ppm) the aphids survived between 7 and 10 days and died in the 3rd instar larvae, while they survived for 9 - 12 days and died in the 4th instar larvae in case of treatment with the lowest concentrating Clove oil (100 ppm).

The obtained result elucidate that each of the tested plant oils at its lowest concentration prevented the aphid individuals to reach the adult stage by inhibiting the completion of their life cycle. This phenomenon may be attributed to either the resulted unpalatability or tolerance of host plant

to the treated aphids; or may be caused by the delayed toxic efficiency of the applied concentration of each oil. In view of the potentiality reported here, at least under indoor conditions, it is obvious that certain plant oils such as Fennel, Linseed and Clove oils could be used successfully to inhibit the build-up of cowpea aphid's population at economically low rate of application.

3. Effect of the three tested plant oils on aphid's predators and parasitoids:

Results illustrated in Table 3 show the revealed effects of evaluated plant oils, 24 hrs after treatment against the inspected cowpea aphids' predator and parasitoid. From the table, it could be seen that Clove oil gave the lowest effect on mortality of the adult stage of ***C. undecimpunctata*** (33.3%) followed by the more toxic efficient Linseed oil (66.7%), then Fennel oil (100%). Vice versa, all the adults of the minute wasp parasitoid, ***Diaeretiella rapae*** emerged from treated mummies aphids with all lowest concentrations of the tested plant oils.

As is the case with many conventional insecticides, toxicity of biologically active plant extracts on beneficial organisms can be also considered an undesirable side effect in their use for pest control. It is desirable for a candidate chemical to be selective for the pest and less toxic to predators and parasitoids. In this concern, **Ahmad *et al.* (2003)** found that first instar larvae of ***C. septempunctata*** showed a very high mortality when fed on aphids sprayed with different neem preparations. **Ofuya and Okuku (1994)** reported that acetone extracts of ***Zingiber officinale***, ***Aframomum melegueta***, ***Momordica charantia*** and ***Cymbopogon citrates*** exhibited high aphicidial activity against ***A. craccivora***. Also, **Ofuya (1997)** confirmed that these extracts have the same effects on the coccinellids, *Cheilomenes lunata* and *Cheilomenes vicina* which are predaceous on different aphids in different agroecosystems. These include toxicity to eggs, reduction in efficiency of predation in larvae and poor rate of pupation.

Adverse effects of some biologically active plant extracts on beneficial organisms have been reported by **Abramson *et al.* (2006)**, who stated that Alfazema oil has the further advantage that it is attractive to ladybugs ***Cyclonedo sanguinea*** L. (Coleoptera: Coccinelidae) a natural predator of aphids. Also, coccinellids and many other beneficial agents similarly remain unaffected by neem oil and extracts (**Schmutterer, 1990**). Presumably, at lower concentration, the extracts used in study may not have significant effects on the predaceous coccinellids tested (**Ofuya, 1997**). But of course, results gained in the laboratory and in the greenhouse are mostly different

from results under field conditions (**Herrmann *et al.*, 1997; Vogt *et al.*, 1997, 1998**). **Ahmad *et al.* (2003)** observed that foliar sprays of NKWE (neem kernel water extract) on aphids had less severe effects upon the parasitoid ***D. rapae***.

Table 1: Toxic efficiency of the three evaluated plant oil extracts and the standard insecticide Selecron against 3rd instar larvae of *A. craccivora*, after 24hrs exposure period.

Material	Slope (±SE)	Lc_{50} ppm	95% Confidence Limits	RT[a]
Clove oil	1.3 ± 0.007	549.9	356.3 – 848.2	0.003
Linseed oil	1.2 ± 0.1	2863.5	1773.01 – 4618.2	0.0005
Fennel oil	1.8 ± 0.2	4780.9	3515.6 – 6499.6	0.00003
Selecron	0.8 ± 0.001	1.6	0.84 – 3.1	1.0

[a] Relative toxicity= Lc_{50} value of insecticide / Lc_{50} value of tested material..

Nevertheless, biologically active plant extracts can be used judiciously in integrated pest management programs in several other ways as with insecticides (**Pfadt, 1985**). In a view of selectivity reported here, our results could support the use of plant oils in an integrated control program due to its safety to parasitoids and predators of aphids.

In order to develop high yielding varieties possessing resistance to insect pests, comprehensive knowledge of plant-insect-biocontrol agent and their interrelationship is necessary. Biological control of aphids has received somewhat more attention in recent years perhaps, because of the need for reducing the use of insecticides and because aphids are more commonly and easily to study and rear under laboratory conditions than other species of field economic pests. Much more research effort must go into understanding, the role which of plant resistance and natural enemies play in suppressing aphid populations (**Darwish, 1998**).

Table 2: Efficacy the tested lowest concentrations of the three evaluated plant oils on the survival potentiality and Fecundity of the cowpea aphid, *A. craccivora* under laboratory conditions.

Some biological aspects		Cont.	Plant oil and its concentration		
			Clove oil	Linseed oil	Fennel oil
		Water	100 ppm	1000 ppm	2000 ppm
1st instar larva	Period in days	2.3	3.0	2.3	3.0
	No. survival ind.	28	14	16	6
	% Mortality	6.7%	53.3%	46.7%	80.0%
2nd instar larva	Period in days	3.3	3.7	3	3.3
	No. survival ind.	27	7	3	0
	% Mortality	3.6%	50.0%	81.3%	100%
3rd instar larva	Period in days	3.7	3.0	3.0	-
	No. survival ind.	27	2.0	0	-
	% Mortality	0.0	71.4%	100%	-
4th instar larva	Period in days	4.0	1.0	-	-
	No. survival ind.	27	-	-	-
	% Mortality	0.0	100%	-	-
Adult stage	Period in days	15.3	-	-	-
	No. survival ind.	27	-	-	-
	% Mortality	0.0	-	-	-
Mean of pre-reproductive period (days)		15.3	-	-	-
Mean of reproductive period (days)		9.7	-	-	-
Mean of post-reproductive period (days)		3.7	-	-	-
Total life span (days)		28.7	-	-	-
Accumulated mortality	No. of dead ind.	3	30	30	30
	% Total mortality	10%	100%	100%	100%
Fecundity = No. of settled larvae	No. per 6 females	56	-	-	-
	Mean / one female	9.3	-	-	-

Abbreviation: ind. = individuals and - means that no survival individuals were found.

Table 3: Effects of the three plant oils on *C. undecimpunctata* a predator and *D. rapae* a parasitoid of cowpea aphids, *A. craccivora*

Treatment and concentration	Parasitoid after 6 days			Predatory insect after 24 hrs		
	No. of aphid mummies	% Mortality	% adult Emergence	No. of predators	% Mortality	% Survivors
Clove oil (2000 ppm)	15	0.0	100%	15	33.3	67.7
Linseed oil (9000 ppm)	15	0.0	100%	15	67.7	33.3
Fennel oil (10000 ppm)	15	0.0	100%	15	100%	0.0
Control (water)	15	0.0	100%	15	0.0	100%

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الملخص العربى

فاعلية ثلاثه من الزيوت النباتية على من البقوليات الأسود *Aphis craccivora* (متشابهة الأجنحة: عائلة المن) وبعض السمات البيولوجية

محمد السيد توفيق ، أماتى مصطفى حسن أبو شال ، أنار عبد الله بكر

قسم الحشرات التطبيقى – كلية الزراعة – جامعة الأسكندرية.

تم دراسة التأثير المميت لثلاثة من المستخلصات النباتية هى زيت القرنفل وزيت الكتان و زيت الشمر مقارنة بأحد مبيدات الآفات (السيليكرون) على العمر الثالث اليرقى لحشرة من البقوليات الأسود بإستخدام أربعة تركيزات من كل زيت لمدة 24 ساعة تحت الظروف المعملية.

أظهرت النتائج أن زيت القرنفل أعطى أعلى تأثير ضد العمر الثالث تبعه فى ذلك زيت الكتان ثم زيت الشمر. كذلك تم دراسة تأثير استخدام أقل التركيزات من تلك الزيوت الثلاثة المختبرة على بعض السمات البيولوجية لمن البقوليات حيث أظهرت النتائج ان استعمال تركيزات 100 و 1000 و 2000 جزء فى المليون لكل من زيوت القرنفل والكتان والشمر على التوالى ادى الى فقد الأفراد المعاملة القدرة على أن تكمل دورة الحياة أو حتى الوصول الى طور الأنثى البالغة.

كما تم دراسة تأثيرالتركيزات المنخفضة لهذه الزيوت الثلاثة على كل من الحشرات الكاملة للمفترس أبو العيد ذو 11 نقطة ***Coccinella undecimpunctata*** والطفيل الداخلى ***Diaeretiella rapae*** . و أوضحت النتائج ان تلك التركيزات المختبرة لم يكن لها تأثير على الحشرات الكاملة للطفيل داخل مومياء المن ، اما بالنسبة للمفترس فقد اختلفت نسبة الموت للحشرات الكاملة المعاملة حسب الزيت المختبر حيث كانت نسبة الموت 33.3 و 66.7 و 100 % لكل من القرنفل والكتان والشمر على التوالى.