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Improvement of methanol extract of *Melia azedarach* by some additives against *Aphis fabae* Scop.

Abstract - The aphicidal effect of methanol extract of *Melia azedarach* L. against 2nd and 4th nymphal instars of *A. fabae* was evaluated. Although this extract may not be as effective as the synthetic insecticides, yet its effect can be enhanced by the use of suitable additives. The different additives used increased the toxicity of the crude extract. The most promising formulation used was methanol extract of *M. azedarach* + sesame oil. Addition of $CaCl_2$ or $MnCl_2$ to this formulation did not improve it, but, on the contrary, they had an antagonistic effect evidenced by lesser percentage of mortalities.
In choice tests, high concentration of methanol extract exhibited a high degree of deterrence, for the adults larviposited very few number of young in comparison to those landed on control plants. Addition of different additives showed various effects. Methanol extract + DMSO exhibited a stimulant effect, where more young were larviposited on treated plants in comparison to the control. On the other hand, all the other formulations tested had a deterrent effect.

Riassunto - *Miglioramento dell'efficacia insetticida con alcuni additivi di un estratto di metanolo di* Melia azedarach *su* Aphis fabae *Scop.*
È stato verificato l'effetto di un estratto metanolico di *Melia azedarach* L. nei riguardi della II e IV età di *A. fabae* Scop. Sebbene questo estratto non sia tanto efficace quanto gli insetticidi di sintesi, tuttavia il suo effetto può essere esaltato dall'impiego di adeguati sinergizzanti, che aumentano la tossicità dell'estratto grezzo. La formulazione piú promettente è stata quella a base di estratto metanolico di *M. azedarach* e olio di sesamo. Ulteriori aggiunte di $CaCl_2$ o $MnCl_2$ non aumentano l'efficacia, ma, al contrario, determinano un effetto antagonistico con minore percentuale di mortalità. Si è inoltre verificato che un'elevata concentrazione di estratto determina una forte repellenza, con scarsa deposizione di neanidi, nei confronti del testimone non trattato. Al riguardo, l'aggiunta di diversi additivi mostra effetti differenti. L'estratto metanolico con DMSO manifesta attività stimolante

con la piú elevata deposizione di neanidi sulle piante cosí trattate. Tutte le altre formulazioni saggiate hanno invece effetto deterrente.

Zusammenfassung - Die aphicide Wirkung von Methanolextract aus *Melia azedarach* L. auf das zweite und vierte Nymphenstadium von *Aphis fabae* Scop. wurde näher untersucht. Obgleich dieser Extrakt nicht so wirksam ist wie synthetische Insektizide, kann seine Wirkung doch durch Verwendung geeigneter Additive verbessert werden. Die verschiedenen verwendeten Additive erhöhten die Toxizität des Rohextraktes. Die wirksamste Formulierung war Methanolextrakt aus *M. azedarach* + Sesame oil. Zugabe von $MnCl_2$ or $CaCl_2$ zu dieser Formulierung ergab keine bessere Wirkung, sondern im Gegenteil, sie hatte einen antagonistischen Effekt, der sich in einem geringeren Prozentsatz an Mortalität zeigte. In Wahlversuchen übten hohe Konzentrationen von Methanolextrakt eine hohe Deterrenswirkung auf adulte *A. fabae* aus. Im Vergleich zu solchen, die auf Kontrollpflanzen landeten, produzierten sie nur wenig Jungtiere. Nach Zugabe von verschiedenen Additiven wurden unterschiedliche Wirkungen erzielt. Methanolextrakt + DMSO hatte einen stimulierenden Effekt, so daß auf behandelten Pflanzen mehr Jungtiere abgesetzt wurden als auf den Kontrollen. Alle anderen getesteten Formulierungen hatten eine Deterrenswirkung.

Key words: *Aphis fabae, Melia azedarach*, methanol extract, additives.

INTRODUCTION

Now, aphids are considered as key pests on various crops and vegetables in Egypt. Owing to the misusage of insecticides many aphid species are insecticide resistant; therefore, a search for new insect control substances of plant origin may be the right way to aphid control.

The plant kingdom can be a rich source of a variety of such chemicals with the potential for development as successful pest control agents. This can economically be feasible, especially if the source plant materials are available in abundance.

Melia azedarach L., a native tree of Egypt and variously called Persian lilac or Chinaberry or Pride of India is not subjected to insect attack and is widely distributed all over the country. The insect antifeedant properties of the Indian neem tree and Persian lilac are well known. Extracts from various plant parts, particularly the seed, have been tested and found to deter feeding by a large number of cutting and chewing type insects such as locusts, armyworms, processionary caterpillars and storage pests (Ascher et al. 1984; Singh, 1984; Tanzubil, 1987; Breuer & Devkota, 1990; Breuer & Schmidt, 1990). However, limited studies of the biological activity of neem or Persian lilac have been

made on sucking insects, eg. the water lily aphid, *Rhopalosiphum nymphaeae* L. (Goyal et al., 1971), *Acyrthosiphon pisum* (Harris) and *Aphis fabae* Scop. (Schauer, 1984). Scop. Siddig (1981) reported that aqueous leaf and seed extracts of neem reduced feeding by *A. gossypii* Glover in Sudan.

In view of the above perspectives, various concentrations of methanol extract of *Melia azedarach* and its formulations with different additives were taken to evaluate their pesticidal, antifeedant and anti-larviposition properties for *Aphis fabae*.

MATERIALS AND METHODS

Stock culture of *Aphis fabae* were reared on *Vicia faba* seedlings at 23 $\pm 2°C$ and 50 $\pm 10\%$ relative humidity with a 16 hours photoperiod.

A cold 50% *Melia azedarach* extract in 80% methanol, previously prepared by Breuer & Schmidt (1990) from fruits collected on 31.III.1988 near Patras/ Greece, was used in the present study. Different concentrations were prepared and samples of known concentrations were bioassayed for toxicity as well as for orientation tests. Young *Vicia faba* plants, 7 days old, were sprayed with 0.5 ml of different concentrations and offered to either 2nd or 4th nymphal instars. Five nymphs were placed on treated bean plant which served as one replicate and maintained under a glass cylinder to avoid escape of aphids.

The different formulations were prepared as follows: a 50% methanol extract was dissolved in methanol and diluted further with distilled water to obtain the required concentration (1%). Aqueous solutions of the additives $CaCl_2$ and $MnCl_2$, each 0.5% and a 1% dimethyl sulphoxide (DMSO) solution were prepared. Sesame oil had to be emulsified with 10% Triton x-100; its concentration in the mixture was 2.5% in the ratio of 4 parts to 1 part of methanol extract. Formulation with $MnCl_2$ or $CaCl_2$ were prepared in the ratio of 1 part extract to 4 parts sesame oil to 10 parts $MnCl_2$ or $CaCl_2$. In case of DMSO, the ratio was 1 part extract to 10 parts DMSO. Twenty replicates were conducted for each toxicity test.

In choice experiments, orientation of *A. fabae* adults towards bean plants treated with different concentrations or formulations of methanol extract of *M. azedarach* in alternation with the control plants was observed in an arena described by Dimetry & Schmidt (1992). For this purpose, 30 virginoparous females were transferred in the middle of the Petri-dish to see the preferential selection of either the control plants or treated ones. The number of adults present on each plant was counted after 1/2, 1, 2 and 24 hours. Five replicates were conducted for each treatment. After 24 hours, the percentage of young born on each plant was calculated as $100\ e.E^{-1}$, e being the number of young

deposited on one plant and E the total number laid on both plants. Any difference in the percentage of young deposited on the two plants was considered to be due to inhibition of larviposition by the extract. All the mortalities were corrected using Abbott's formula (1925).

RESULTS

1. *Toxicity test*

a) Using different concentrations

The results obtained in Table 1 indicate that very high concentrations i.e. 25 and 12.5% methanol extract of *M. azedarach* caused 100% mortality of the 4th nymphal instar after 96 hours of treatment. As the concentration of the crude extract decreased, the mortality also decreased. The mortality reached the lowest value (37%) when using the extract at 1.25%. The major mortalities happened after the first 24 hours, especially at high concentrations.

b) Using different formulations

The efficacy of 1% extract of *M. azedarach* was compared with 1% of the same extract plus different additives against the 2nd and 4th nymphal instar of *A. fabae*. The results obtained in Table 2 show that the efficacy of methanol extract was increased significantly by the different additives added. Sesame oil proved to be the most promising additive, as it greatly increased the mortality of *A. fabae* in comparision to either *M. azedarach* extract alone or extract plus DMSO. The efficiency of methanol extract plus sesame oil decreased when either $CaCl_2$ or $MnCl_2$ was added. All multiple combinations resulted in lower mortalities than those achieved when adding only sesame oil to the methanol extract. This may be due to the antagonistic interaction between the additives.

Table 1 - Toxicity of methanol extract of Melia azedarach *against 4th nymphal instar of* A. fabae *(20 replicates).*

Conc. %	Percentage mortalities after 24	48	72	96 hrs	Corrected total mortalities after 96 hrs
25	85	15	0	0	100
12.5	80	20	0	0	100
5	28	21	25	15	89
2.5	15	20	10	5	50
1.25	10	20	4	3	37

Table 2 - The role of different additives on the toxicity of methanol extract of Melia azedarach *against 2nd and 4th nymphal instars of* A. fabae *(20 replicates each).*

Treatment	Percentage mortalities after								Corrected total mortalities after 96 hrs	
	24		48		72		96 hrs			
	2nd	4th	2 nd	4th	2nd	4th	2nd	4th	2nd	4th
Extract 1%	0	0	13	13	0	1	10	4	18.1	18
Extract 1% +0.1% DMSO	6	6	10	10	10	10	33	25	56.4	51
Extract 1% +2.5% sesame oil +0.5 $MnCl_2$	27	18	6	12	7	10	20	16	57.4	56
Extract 1% +2.5% sesame oil +2.5 $CaCl_2$	20	20	20	10	10	13	20	25	68.1	68
Extract 1% +2.5% sesame oil	10	35	7	10	7	10	50	16	72.3	71
Control	0	0	6	0	0	0	0	0	6.0	0

2. *Orientation and oviposition responses*

a) Using different concentrations

When virginoparous females of *A. fabae* were released in the central sectors of the chamber, they at once started walking and searching for the appropriate place for feeding and larviposition. In the first few hours few number of females landed on the plants treated with a very high concentration (25%) of the extract. After 24 hours, the numbers of adults present on treated plants, however, were much more than those on the control ones but all of them were dead. Inspite of the fact that high concentrations of methanol extract of *M. azedarach* exhibited an aphicidal action, yet, it had a stimulus effect as more adults were present on treated plants than on the control ones. At decreased concentration (12.5%), the reverse happened where the number of adults landed on the control plants was three times more than those on treated ones. Again, 12.5% extract also exhibited a toxic effect to the adults where all of them died. At the lowest concentration tested (1.5%) none of the adults landed on the treated plants but they preferred to settle and larviposit on the control plants. Considered the number of young deposited on either the treated or control plants, as presented in Table 3, high (25% and 12.5%) and low (1.25%) concentrations of methanol extract where found to exhibit a deterrence for larviposition. At the medium concentration tested (6.25%), it was clear that

Table 3 - Relative percentage orientation and larviposition by Aphis fabae *adults on methanol extract of* Melia azedarach *treated plants and control ones (release of 30 individuals).*

Conc. %	Orientation after 1/2		1		2		24 hrs		Aver. total No of young per 24 hrs		Aver No of young / ♀ /hrs		% young born on treated plants
	T	C	T	C	T	C	T	C	T	C	T	C	
25	2	4	3	5	1	4	9D	7	6	30	0.7	4.3	13.5
12.5	1	10	3	12	5	11	5D	15	0	70	0.0	4.7	0.0
6.25	3	13	4	16	3	15	12	13	46	48	3.8	3.7	50.7
3.12	5	10	4	10	5	9	11	17	16	32	1.5	1.9	43.5
1.5	0	7	0	7	0	8	0	11	0	11	0.0	1.0	0.0

T: treated, C: control, D: dead.

larviposition was slightly higher at the stimulus bearing plants than at the control. Thus, contact of the insects with methanol extract at a medium concentration did not reduce their larviposition as compared with that in the control.

Table 4 - Relative percentage orientation and larviposition by Aphis fabae *adults towards different formulations of methanol extract of* Melia azedarach *and control ones (release of 30 individuals).*

Treatment	Orientation after 1/2		1		2		24 hrs		Aver. total No of young per 24 hrs		Aver No of young / ♀ /hrs		% young born on treated plants
	T	C	T	C	T	C	T	C	T	C	T	C	
1% MeOH-extract + DMSO	3	4	2	6	1	5	5 (4 D)	8	6	4	1.2	0.5	70.6
1% MeOH-extract + sesame oil	3	4	5	6	2	5	8 (8 D)	9	3	23	0.4	2.6	12.6
1% MeOH-extract + sesame oil + $CaCl_2$	6	8	1	9	2	6	4 (3 D)	7	2	45	0.5	6.4	7.2
1% MeOH-extract + sesame oil + $MnCl_2$	5	5	6	8	9	12	6 (2 D)	15	0	41	0.0	3.2	0.0

T: treated, C: control, D: dead.

b) Using different formulations

The results obtained in Table 4 show that the adult females of *A. fabae* preferred to settle on control plants than on those treated with extracts plus formulations where more adults were recorded on the control plants even from the beginning of the experiment.

The data show a variety of effects of different formulations upon larviposition by the adults. Methanol extract and DMSO exhibited a stimulating effect where the percentage of larvae born on treated plants reached 70.6%. In the other treatments, however, the reverse happened, where all the formulations reduced larviposition but with a varied degree. Methanol extract and sesame oil and $MnCl_2$ deterred the adults completely and no young was deposited.

DISCUSSION

The present investigation involved an attempt to determine the potential role of extract of *M. azedarach* and its formulation by adding some additives to the control of *Aphis fabae.* Recent advances in naturally occuring pest control agents have been well documented and emphasized the fact that they are not likely to cause any ecological imbalance (Hellpap, 1985). They influence the chemosensory behaviour of insects or act on the physiological processes as growth regulators. Laboratory and field experiments on Chinaberry have led to an understanding of its diverse behavioural and physiological effects on many insects (Jacobson et al., 1978; Mariappan & Saxena, 1983; Chiu, 1984; Islam, 1984; Schmutterer, 1984).

In the light of the results obtained from the present work, extract of *M. azedarach* is proved to have an aphicidal property against *Aphis fabae* and 100% mortality in the 4th nymphal instar occured after 96 hours. Decreasing the concentration, the percentage mortalities decreased i.e. mortality is concentration dependant. Addition of different additives to the crude extract resulted in increased mortalities of both 4th and 2nd nymphal instars. The most promising formulation was methanol extract plus sesame oil, which gave the highest mortality. Addition of $CaCl_2$ or $MnCl_2$ to the previous formulation antagonized the effect of sesame oil. These results are in agreement with those of Schauer (1984) who found that addition of sesame oil to the tertiary methyl butyl ether extract of neem resulted in higher mortality of *Acyrthosiphon pisum* and *A. fabae* than did the same extract without sesame oil. He also added that addition of $MnCl_2$ or $CaCl_2$ to the neem extract and sesame oil resulted in lower mortality than the extract and sesame oil alone.

Also, Lange & Schmutterer (1982) investigated the effect of added synergists

and reported that a mixture of 1:10 piperonyl butoxide with MTB/H_2O-VR increased the mortality in the first instar of *Leptinotarsa decemlineata* Say. and the activity of the extract was slightly more rapid.

Orientation and larvipositional responses of *A. fabae* adults to different concentrations of methanol extract of *M. azedarach* alone or 1% methanol extract + different additives may differ independently of each other. At 25% extract, more adults settled on treated plants. This observation suggests that the extract is not a repellent but serves as a contact larvipositional deterrent for *A. fabae*, as very low percentage of nymphs deposited on treated plants in comparision to the control. These data agreed with those obtained by Saxena & Rembold (1984) who found that neem seed oil presented at a distance did not repel *Heliothis armigera* Hbn. moths but contact with the oil inhibited oviposition.

Methanol extract of *M. azedarach* can be enhanced by the use of suitable additives. Spray formulation of methanol extract reduced larviposition in *A. fabae* adults when sesame oil and $MnCl_2$ or $CaCl_2$ were added to the crude extract. An approximately 93-100% reduction in larviposition was obtained when methanol extract was formulated with sesame oil + $MnCl_2$ or $CaCl_2$. On the other hand, when DMSO was added to the extract, an increased percentage of larvae was deposited on treated plants. This means that addition of DMSO did not improve the deterrent effect of the extract.

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