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Bioefficiency of the Extracts of Azadirachta excelsa (Jack) and Xanthium italicum Moretti on the Mortality of Aphis fabae Scopoli and its Hyperparasitoids, Asaphes suspensus (Nees) and Pachyneuron aphidis Bouche (Hymenoptera: Pteromalidae)

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Abstract

Toxicity of ethanolic extracts of two plants (*Azadirachta excelsa* Jack) and *Xanthium italicum* Moretti to the bean aphid, *Aphis fabae* Scopoli and its main hyperparasitoids; Asaphes suspensus (Nees) and *Pachyneron aphidis* Bouche was investigated. The susceptibility of the hyperprasitoidos to *X. italicum* and *A. excelsa* was not varied. All extracts showed remarkable toxicities. The extract from *A. excelsa* has shown more pronounced toxic effect, having Ec_{50} 3.5 and 3.6 ppm compared to 90 and 22 ppm for *M. italiucum* against the aphid and its hyperparasitoids respectively. The percent mortality of *A. fabae* was found to be concentration and exposure-time dependent for A. excelsa extract. The extract of *X. italicum* was appeared selectivity of toxic action between aphid and its hyperparasitoids. These leaves extracts could have promising practical application in protection cultivated plants against attack by *A. fabae* and increasing efficiency of its primary parasitoids as biological control by reducing viability of main hyperparasitoids.

الفعالية الحيوية لمستخلص النيم Azadirachta excelsa والحسك في موت مَنْ الباقلاء Aphis fabae Scopoli وطفيلية التانويين Asaphes suspensus و Pachyneuron aphidis

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المستخلص

جرى البحث في سمية النباتين *Azadirachta excelsa Jack* والحسك *Xanthium italicum Moretti* على من الباقلاء *Aphis fabae Scopoti* وطفيلية الثانويين *Asaphes suspensus (Nees)* و *Pachyneuron aphidis Bouche* . اظهر مستخلصا النباتين اعلاه كفاءة في قتل من الباقلاء وطفيلية الثانويين. كما اظهر مستخلص اوراق الحسك تاثيرا انتخابيا بين المن والطفيلين الثانويين اعلاه. وقد كانت قيمة Ec_{50} لمستخلص اوراق شجرة *A. excelsa* 3.5 و 3.6 جزء في المليون (ج.ف.م) مقارنة مع 90 و 22 ج ف م لاوراق الحسك. وجد ان نسبة القتل للحشرات قيد الدراسة تعتمد على تركيز مستخلص اوراق شجرة *A. excelsa* وفترة بقاء الحشرة تحت تاثير ذلك المستخلص. ابدت المستخلصات استخداما عمليا واعدا في مكافحة من الباقلاء، وزيادة فاعلية الطفيليات الاولية من خلال تقليل نشاط الطفيليات الثانوية الرئيسة.

Introduction

Bean aphid, *Aphis fabae* Scopoli (Homoptera: Aphididae) is one of the very important crop pest[1], causing damage by plant feeding and as vectors of plant viruses[2]. Many efforts concentrated on the control of aphids to restrict the spread of virus diseases met with little success[3]. Also, *A. fabae* have alternative hosts including weeds and cultivated plants. Black bean aphid was considered a complex of closely related host plants-associated forms[4,5].Aphidophagous predators and parasitoids play a significant role in reducing *A. fabae* populations[6-12]. *A. fabae* are parasitized as young nymphs may have no apportunities to live to reproductive age[13]. The hyperparasitoid *Asaphes suspensus* (Nees) (Hymenoptera: Pteromalidae) can be a tertiary parasitoid on its own species[14], or on other hyperparasitoids[15]. Also *A. suspensus* can also be used for destruct hosts trough host-feeding, for which the female constructs a feeding tube to feed on the host hemolymph[14]. The hyperparasitoid *A. suspensus* (Nees) attacking the host (pre) pupa after the primary parasitoid has killed the aphid and the mummy is formed[16]. *Pachyneuron aphidis* (Bouche) (Hymenoptera: Pteromalidae) has been known as cosmopolitan and polyphagous hyperparasitoid[16-18], and seemed to have a negative effect on both population and activity of primary parasitoids[19]. Jaskiewicz[20] (2004) stated that *P. aphidis* is major secondary parasitoids among hyperparasitoids of *A. fabae*.Marrago tree, Azadirachta excelsa (Jack) (Meliaceae) is one of six species in the family meliacease, which had been studied for pesticidal properties in different parts of the world[21]. Marragnin (one of the azadirachtin formulae), azadirachtin A and fatty acids are major substances purified from marrago tree[22,23]. Kanokmedhakal *et al.* (2005) isolated some Azadirachtin derivatives from seed kernels of *A. excelsa*. Margosan-O, a commercial biopesticide containing 0.1% azadirachtin affected the longevity of *A. fabae*[24]. It was found that leaves extract of *A. excelsa* was much more effective than that of *Melia azedarch* and *A. indica* on fourth instar larvae of the Mexican bean beetle, *Epidachna varivestis*[22]. Methanolic crude extract of *A. excelsa* leaves most effective mortality on diamond back moth than other tested indigenous plants. Furthermore, the surviving larvae showed unusual development of the pupal stage, darker colour, non-functional silk thread and failure of adult emergence[25].Cocklebur, *Xanthium italicum* Moretti (Asteraceae) is annual herbal weed, *xanthium* spp were found toxic for mammals, and have antibacterial, antifungal and cytotoxic properties[26-28]. *Xanthium* spp were be shown to be rich with active ingredients are peroxy compounds as terpenoid derivatives such as xanthanol, xanthatin, xanthanotides and others[26,29]. The objective of this study is to test efficacy of widely distributed weed, *Xanthium italicum* and newly implanted ornamental tree *A. excelsa*, against one of the important pests A. fabae and its main hyperparasitoids.

Materials and Methods

Insects

The colony of black bean aphids *Aphis fabae* scopoli was educated on plants of cowpea, *Vigna radiata* (Brassicaceae) which was planted in plots in spring 2006. To increase chances of parasitize aphid with

primary parasitoids and hyperparasitoids, the infested plants with stem mother aphids and their progenys, was transferred to open field for about month to obtain mummy aphids. Beside parasitoids; the mummy contains the hyperparasitoids, *Asaphes suspensus* Nees and *Pachyneuron aphidis* Bouche.

Extract Preparation

Leaves of marrago tree, *Azadirachta excelsa* (Jack) (Meliaceae) were gathered in middle June from Mosul forest. Also, leaves of the weed cocklebur, *Xanthium ilaticum* Mor. (Asteraceae) was collected in September from Tigris river banks. The leaves were washed with water and dried in shadow place and grinded, then the leaves powders were preserved in refrigerator until the beginning of experiments.The leaves powder was macerated in appropriate volume of absolute ethanolic alcohol, and preserved in refrigerator for 48 hours, then the maceration was stirred in magnetic stirrer for 24 hours. The filtrate was separated through filter paper No. 1 and washed with excess absolute ethanol, the ethanol was evaporated by leaving the filtrate in front appropriate air current. To prepare stock solution of 1000 part per million (ppm) absolute ethanol was added to the obtained extract.

Feeding experiments

Black bean aphids were seen more feeding on unripe pods of cowpea than other parts of the other vegetative parts, and the pods did not walting in contrast with leaves and twigs.For prepare the concentrations of the extracts, 50 ml of the following concentrations: 0.5, 5, 10, 15, 25, 50 and 100 ppm were done. The pods of *V. radiate* were dipped in the above concentrations as well as control for 10 seconds with moving, then left in the laboratory for drying the solvent.Statistical measurements were represented by effect of different concentrations of the extracts on aphid mortality. For each concentration of the two extracts three replications were prepared. Appropriate number of treated pods were putted in the one liter jars, then 100 insects of different nymphal stages and stem mothers were collected randomly and putted in each jar, the jars were covered with pertidishes to prevent escaping the insects and preserve the relative humidity above 50%. All the applied jars as well as control ones were examined after one day of the treatment and repeated every two days for one week.The effect of the leaves extracts on the hyperparasitoids *A. suspensus* and *P. aphidis* was estimated by spraying out door infested plants with the extracts two times for two weeks, after that, mummy aphids were collected from treated and control jars and every 25 of them were putted in each jar of the three replications. The hyperparasitoids *A. suspensus* and *P. aphidis* were counted for mortality estimation.

Results and Discussion

Mortality of Aphis fabae Scop.

The percent mortality of *A. fabae* population at 1,3,5 and 7 days after treatment with *A. excelsa* leaves extract are presented in Table 1. This Table is illustrate that percent mortality was depended on extract concentration, therefore, it was found mortality beginning since first day after the treatment with 25 up to 100 ppm. While, the mortality at the concentrations 5, 10 and 15 ppm was late until the third day of the treatment. Finally, percent mortality were appeared at the fifth day of the treatment with 0.5 and 1.0 ppm. Percent mortality in relation to *A. excelsa* extract concentrations is presented in Fig. 1, whereby *A. fabae* mortality 20% at 0.5 ppm, and the

following concentrations 1.0, 5, 10 ppm were caused increasing in mortality, but high percent mortality was reported at the concentrations 15 up 100 ppm. The toxicity of *A. excelsa* leaves extract and the bioinsecticides containing azadirachtin derivatives were previously reported on diamondback moth[25], Mexican bean beetle *Epilachna varvestis*[22] and *Aphis. fabae*[24]. Treating insect food with azadirachtin derivatives will disturpt the insect feeding, growth and metamorphosis is not completed[3].The effect of *X. italicum* leaves crude extract on *A. fabae* population are given in the Table 2. When the infested pods were applied with the concentrations 25, 50 and 100 ppm, dead aphids were observed after one day of treatment. Also, percent mortality by the concentrations 15 ppm and down were increased with the propagation of examining date.Fig. 1 was revealed percent mortality within bean aphid population after 7 days of treatment, applied pods with 0.5 ppm was caused 16.9 percent mortality, but the treatment with higher concentrations were weakly effected on the aphids population, so that, percent mortality was increased 8.0 only between the treatments 25 and 100 ppm(Fig. 1). The toxic properties of cockulebur was previously reported on swine[28], cattles[27] and as antibacterial and antifungal action[26]. Although, the toxic activity of *X. italicum* and its action mode in insects is not yet investigated, *X. italicum* activity may be attributed to its high content of biologically active peroxides[26], and they behaves as insect growth regulators as Fig.1 summarized this behaviour.

Mortality of hyperparasitoids

It has been found that the spraying of infested cowpea pods with *A. excelsa* leaves extracts led to failure of emergence the hyperparasitoids *A. suspenses* and *P. aphidis*. Fig. 1 is shown that mortality of the hyperparasitoids was 13.6% in case of treatment with 0.5 ppm. The mortality was nearly folded at each of the concentrations 1.0 and 5 ppm. But the percent mortality at the concentrations 10 and 15 ppm were less proportional with proceeding concentrations. High concentrations (25, 50 and 100 ppm) were not increased mortality percentage more than 8.0% collectively.The hyperparasitoids were sensitive to 0.5 ppm of *X. italicum* leaves extract and caused mortality 32.4%. But the mortality of the hyperparasitoids were very low affected by the concentration factor more 1.0 ppm (Fig. 1).The mechanism were by the extract constituents which caused mortality of hyperparasitoids are attributed to aphid feeding on spraying cowpea pods, accumulation of active ingredients in primary parasitoids bodies or they were penetrated through mummy integument.

Effective concentration (EC50)

Ec_{50} was calculated by log concentration probit lines (Fig. 2). The Ec_{50} values of *A. excelsa* for *A. fabae* and its hyperparasitoids were 3.5 and 3.6 ppm respectively. While Ec_{50} of *X. italicum* leaves extract for previous insects were 95.0 and 22.0 ppm respectively. Fig. 2 is shown that Ec_{50} values were depended on extracts sources and targeted insects, for this mention, extract of *A. excelsa* was found highly effective than that of *X. italicum* for more than 27 and 6 folds against *A. fabae* and its hyperparsidtoids respectively. On the other hand, *A. fabae* and their hyperparasitoids had the same sensitivity for *A. excelsa*, whereas the difference in sensitivity between them for *X. italicum* more than four folds.

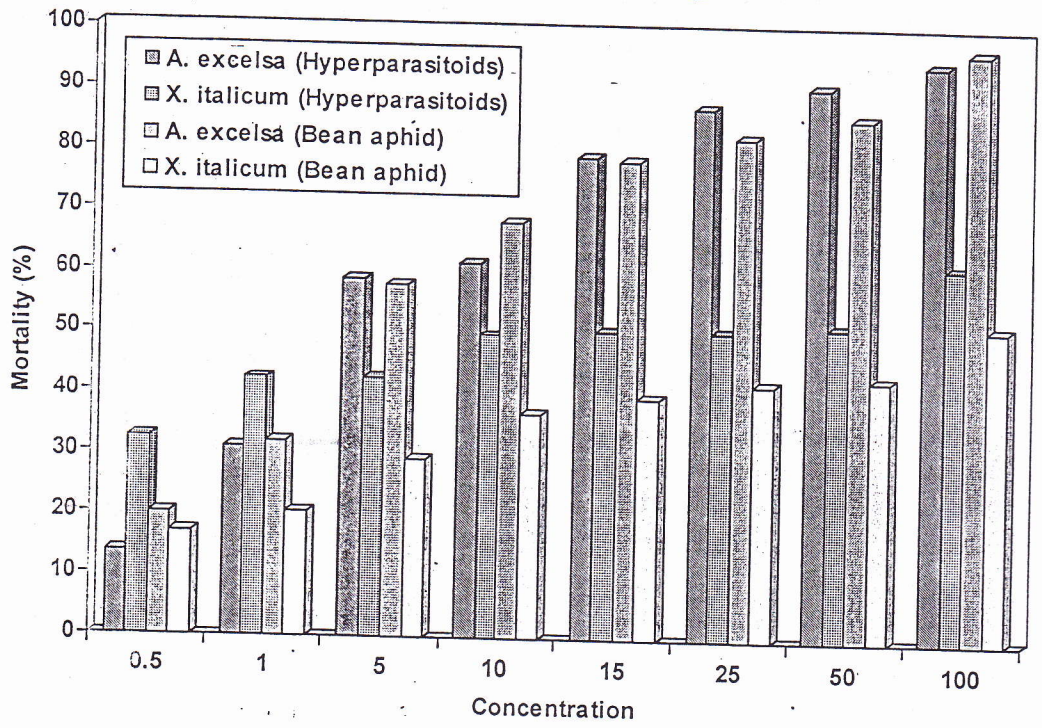

Figure (1): Mortality of *Aphis fabae* and its hyperparasitoids *Asaphes suspensus* and *Pachyneuron aphidis* which caused by leaves extracts of *Azadirachta excelsa* and *Xanthium italicum*

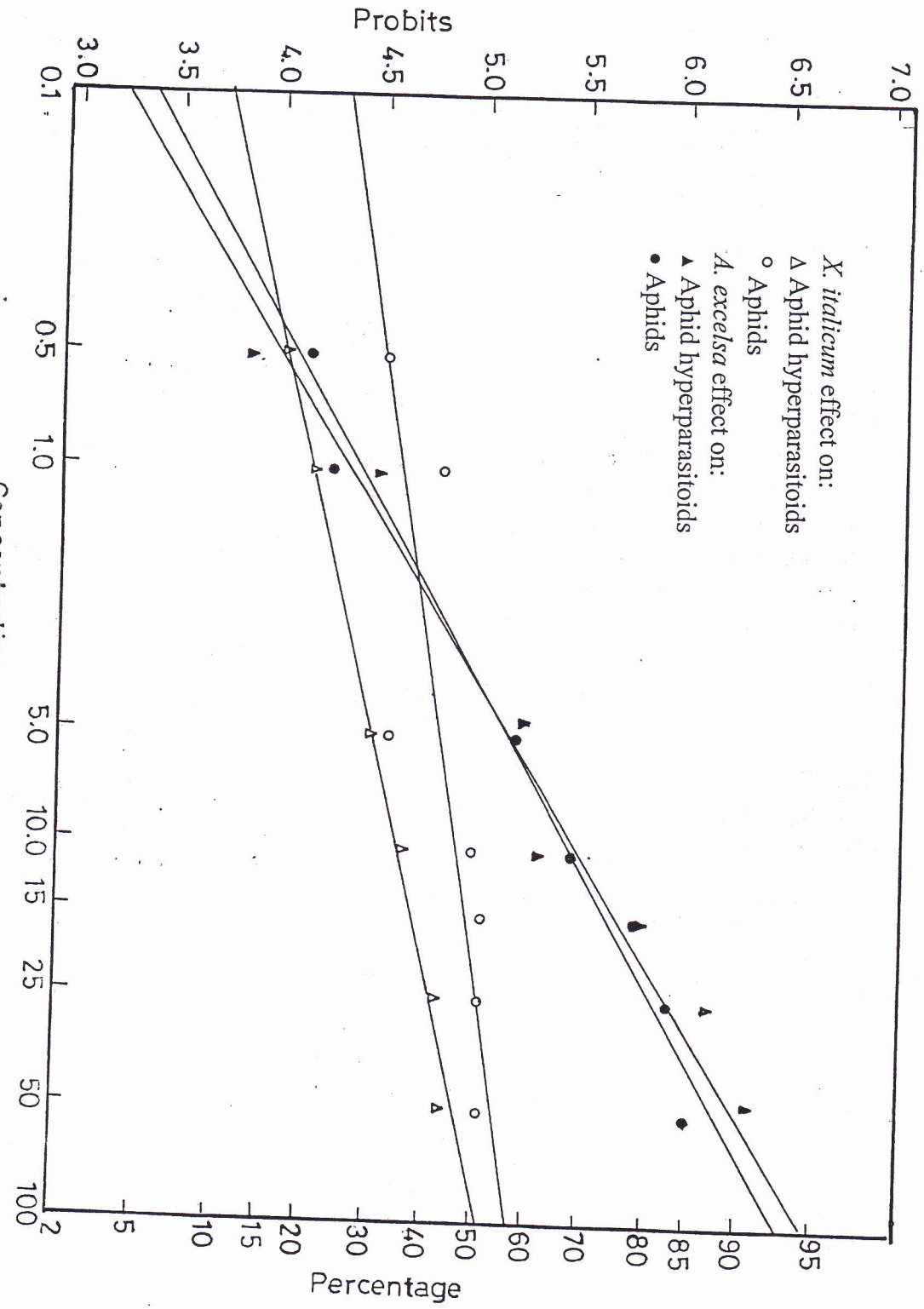

Figure (2): Log concentration probit lines for *Azadirachta excelsa* and *Xanthium italicum* against *Aphis fabae* and its hyperparasitoids *Asaphes suspensus* and *Pachyneuron aphidis*

Table (1): Percent mortality of *Aphis fabae* treated with *Azedarachita excelsa* leaves extract in related with exposure time

Concentration (ppm)	Time (days)			
	1	3	5	7
100	35.9	26.4	16.4	18.2
50	7.8	51	14.4	12.5
25	8.8	33.3	11.4	28.6
15	0	32.3	26.0	17.8
10	0	20	13.5	34.7
5	0	17.3	19.0	21.4
1.0	0	0	8.0	24.0
0.5	0	0	4.0	6.0

Table (2): Percent mortality of *Aphis fabae* treated with *Xanthium italicum* leaves extract in related with exposure time

Concentration (ppm)	Time (days)			
	1	3	5	7
100	10.0	14.1	8.6	18.7
50	8.6	13.0	7.1	14.3
25	4.0	12.2	10.6	15.2
15	1.1	10.2	11.9	16.4
10	0	9.9	7.8	19.1
5	0	7.7	11.5	13.0
1.0	0	1.1	6.4	12.8
0.5	0	1.2	6.5	9.2

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