Effect of Some Insecticides on the Egg Parasitoid, *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae)

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Abstract

In this study, the effects of three insecticides including: Fenvalerate, Thiacloprid and Fenoxycarb on egg parasitoid, *Trichogramma evanescens* were investigated. The toxicity of insecticide were tested using two laboratory tests: (a) direct spray on *Ephestia cautella* eggs containing the parasitoid for effects on parasitism and emergence of the adult parasitoids (b) adult parasitoid exposure to dry insecticide films residue in glass vials. All insecticides were prepared according to the manufacturer's recommendations for field application. Results showed that these insecticides varied in toxicity and have different degrees of effect regarding the emergence and parasitism of the parasitoid. Fenoxycarb was non-toxic or low toxic to *T. evanescen* and did not affect immature stages development and adult emergence. Whereas, Fenvalerate was highly noxious to the parasitoid and significantly reduced the percentage of emergence and parasitism of *T. evanescens* developing in host eggs. The effects of Thiacloprid were slightly toxic to *T. evanescens* as it fell into the class 2 when parasitoids were directly exposed to chemical residues. While, Fenvalerate classified as moderately toxic and caused higher mortality than Fenoxycarb and Thiacloprid in both glass surface residues and direct spray of host eggs. Therefore, obtained data provided useful data for implementing both chemical and biological control strategies in integrated pest management.

Keyword**:** Egg parasitoid, *Trichogramma evanescens*, Insecticides.

Introduction

Many countries in the world including Iraq turned towards integrated pest management (IPM) strategies as an effective and complement to chemical insecticide. Naturally, most biological control agents are commonly susceptible to insecticide applications and in order to consider insecticide as suitable for IPM, it must cause minimal influence on the activity of beneficial species when applied. Methods used to study the selectivity of insecticides to natural enemies have been coordinated by the International Organization for Biological and Integrated Control (IOBC) since 1974 to ensure reproducible results and obtain data on insecticide recently used.

Egg parasitoids, *T. evanescens* are common biological control agents that have been successfully used in biological control programs for a variety of lepidopteran pests attacking corn, rice, sugarcane, cotton, vegetables, sugar beets, fruit trees, pine and spruce trees [1]. Most releases are to control corn borers, sugarcane borers, and cotton bollworm [2]. Nearly 200 species of *Trichogramma* have been identified, of which 25 are used for pest management in 34 crops in 30 countries [3].These parasitoids attack the egg stage of their hosts, mainly Lepidoptera, and prevent larvae reaching the larval stage which cause crop damage [4]. *Trichogramma* used widely in IPM on many important pests crops including spiny bollworm *Earias insulana* Boisd. (Lepidoptera: Phalaenidae), a major serious pests on cotton crop which caused decrease in cotton yield in Iraq [5] and worldwide [6]. Their effectiveness can be significantly lowered by insecticide applications that interfere with parasitism and parasite population growth. Negative effects of insecticides on populations of the parasitoid *Trichogramma* have been reported [7, 8] whereas some studies show that insecticides may increase the performance of natural enemies [9]. Another studies showed that the sublethal effects of insecticides can severely reduce the performance of biological control agents [10].

Fenvalerate is the most widely used compound of the cyanophenoxy-benzyl group of the synthetic pyrethroid pesticides and is registered for use in agriculture to protect a wide variety of crops including cotton, soybeans, corn, vegetables, apples, peaches, pears and nuts from insect pests [11]. It is a mixture of four optical isomers which have different insecticidal activities. The Esfenvalerate, is the most insecticidally active isomer. Fenvalerate consists of about 23% of this isomer. It acts by contact. In previous studies [12] conducted a field studies in Egypt to determine the release of *T. evanescens* with bioinsecticide applications and the recommended chemical insecticides (Trichlorfon, Fenvalerate and Carbaryl) to control the cotton bollworms *Pectinophora gossypiella* and *E. insulana*.

Thiacloprid a neonicotinoid insecticide (belonging to the chloronicotinoid chemical class) is targeted to control sucking and biting insects in cotton, rice, vegetables, pome fruit, sugar beet, potatoes and ornamentals. Pests controlled include Lepidoptera, beetles, aphids and whitefly. It acts as acute contact and stomach poison, with systemic action. It works by disrupting the nervous system by acting as an inhibitor at nicotinic acetylcholine receptors mode of action. In a standard IOBC glass plate assay, residues of Thiacloprid (SC 480) severely affected adult stages of the cereal aphid parasitoid *Aphidius rhopalosiphi* whereas pre-imaginal stages (which develop within the host) revealed a high tolerance against spray treatment with the same insecticide [13]. Also [14] studied the selectivity of pesticides including Thiacloprid on cotton *Gossypium hirsutum* to *T. pretiosum* relared on two laboratory-reared hosts.

Fenoxycarb is a carbamate insect growth regulator. It is used to control butterflies and moths, scale insects, and sucking insects on olives, vines, cotton and fruit in addition to control these pests on stored products. Fenoxycarb mode of action is different from most of the IGR's, which are chitin synthesis inhibitors or molting accelerators. It disrupts the insect transformations and therefore has the same biological effect as natural insect growth regulators, with lepidoptera; it disrupts the transformation from egg to larva (ovicidal effect) and from larva to pupa (morphogenetic effect). Fenoxycarb acts by contact and ingestion. [15] mentioned that the insect growth regulator insecticides and the insect growth inhibitors (Fenoxycarb, Diflubenzuron and Lufenuron) were harmless to pupae, slightly harmful to eggs of the parasitoid *T. cacoeciae* Marchal, which was reflected in the highest rate of adults emerged (81.27%) compared with the control, later in another studies the insecticides were harmless to the adults of the parasitoid *Ascogaster quadridentata* Wesmael and classified as class 1 [16].

Hence, chemical control is, still, largely used and therefore it is necessary to study the use of insecticides with parasitoids and predators. A study was designed to investigate the effect of three commonly insecticides used in vegetable crops in Iraq, Fenvalerate, Thiacloprid and Fenoxycarb on egg parasitoids, *T. evanescens* developing in *E. kuehniella* eggs in the laboratory.

Materials and Methods

Insects Rearing

Both egg parasitoid *T. evanescens* and the almond moth, *Ephestia cautella* eggs were used in all experiments obtained from Integrated Pest Control Center / Directorate of Agricultural Research / Ministry of Science and Technology- Baghdad- Iraq. They were reared as describe below:

Host

Cultures of *E. cautella* were obtained from a laboratory rearing maintained on artificial diet of groats (crushed wheat) (81%) mixed with dates syrup (6 %), dry yeast (1%) and glycerin (12%) [17] at a constant temperature of 27 ± 2°C, 60 ±10% relative humidity (RH) and 14:10 (L: D). The adults were moved to new rearing media every 2 days, this proce dures allowed to obtain *E. cautella* used in tests.

Parasitoid

T. evanescens population were reared on *E. cautella* eggs glued onto blue cartoon cards (60 × 8 mm) impregnated with Arabic gum diluted in 30% of water in a laboratory condition 23 ± 2ºC, 75 ± 5% RH and 16:8

(L:D) in glass tubes (8.5 cm×2.5 cm diameter) containing honey droplets as a source of food for emerged adult [18]. The parasitoids were maintained for 4–5 generations before they were used in the experiments.

The Tested Insecticides

The study includes three insecticides commonly used in Iraq (Table (1)). All insecticides were applied at concentrations recommended for field application by manufacturers.

Table (1)
Insecticides screened for toxicity to T. evanescens.

Insecticide	*Trade name*	*Formulation*	*% of active ingredient*	*Field recommended concentrations [ppm]*	*Manufacturer*
Thiacloprid	Calypso	SC	48	600	Bayer
Fenoxycarb	Insegar	WP	250	400	Greenriver Industry Co., Ltd.
Fenvalerate	Fantom	EC	20	140	Chemet Chemicals Pvt. Limited

ppm – parts per million
SC – Suspension Concentrates
WP – Wettable powders
EC – Emulsion Concentrates

Effects on Immature Stages

Fresh *E. cautella* eggs aged less than 24 h. were glued onto cartoon cards (60×8mm) each contained nearly 200 eggs. These cards were placed in glass tubes. Newly emerged *T. evanescens* adults were transferred to the glass tubes containing *E. cautella* egg-cards for oviposition and kept for 24 h. at 25 ± 2 ºC, 70 ± 5 % RH, and a photoperiod of 16:8 (L:D) then wasps were gently brushed off with a camels-hair brush, and eggs were stored at the conditions mentioned above.

Approximately 72 h after exposure to *T. evanescens*, the host eggs that their chorion changed to shiny black were counted as parasitized by the parasitoids, and the ones that did not change their color were counted as unparasitized.

Parasitized eggs were divided into groups each containing 30 eggs. Each egg group was placed in a petri dish containing single filter paper (5.5 cm diameter; Whatman No.1 filter paper), and saturated with insecticide solution at the concentrations recommended for field application by manufacturers. All spray solutions were prepared immediately before application. The exposed eggs were treated with insecticides by a 0.5 L sprayer containing 100 ml of the insecticide solutions and ensured that all eggs within dishes were sufficiently and similarly exposed to insecticides sprays, with excess solution being absorbed by the filter paper [19]. The control group was sprayed with distilled water only. Filter papers and eggs were allowed to dry for 1 h in a fume hood, and then 30 eggs from each insecticide treatment and control were transferred individually with a camel-hair brush into plastic vials containing a streak of 50% honey solution.

Vials containing eggs were then plugged with cotton and held at the same condition. The eggs were observed daily until the emergence or death of the adult parasitoids. Three parameters were then evaluated: the number of parasitised eggs, the emergence percentage and the reduction in parasitism capacity. The reduction in parasitism capacity was compared with the control treatment and was calculated using the formula:

PR= [1− (P/p) ×100], where PR is the percentage of parasitism reduction, P is the average value for each parasitism insecticide and p represents the average parasitism rate for the control treatment [20].

A final assessment of emergence was made 14 day after initial parasitism. Host eggs were inspected under a microscope. Each treatment was replicated four times.

Residual Acute Toxicity on Adults

Evaluation followed the method prescribed by IOBC/WPRS to determine the effects of the dried residues of three insecticides on *T. evanescens* over time by treated glass surfaces with insecticides [21]. Laboratory bioassays were conducted at 25 ± 2 ºC, 70 ± 5 % R.H. and a photoperiod of 16:8 (L: D) h. The *T. evanescens* used for experiment were randomly chosen as the parasites in this life stage are more susceptible to pesticide [22]. Lethal effects of contact toxicities of insecticides were evaluated by sprayed insecticides in glass petri dishes (1cm in depth and 10 cm in diameter). The lid and bottom of each glass petri dish were sprayed with 1 ml of field rates of each insecticide and water (control) with sprayer. The sprayed petri dishes were kept for 2 h. to dry, forming a dry insecticide film. Then 20–30 unsexed parasitoid adults (24–48 h. old) were introduced into each petri dish for 24 h. adults. The mortality rate was determined after 2, 4, 6 and 24 hours from treatment application. Insects that no longer strong enough to stand up and continue its activities were considered as dead. The calculation of mortality rate was corrected for control mortality according to Abbott’s correction [23].

Based on the results of this study, each insecticide was classified according to IOBC criteria [22]:

Class 1 = non-toxic (<30% reduction in parasitism capacity);
Class 2 = slightly toxic (30–79 % reduction in parasitism capacity)
Class 3 = moderately toxic (80–99% reduction in parasitism capacity)
Class 4 = toxic (>99% reduction in parasitism capacity)

Statistical Analysis

The experiment was designed as a randomized complete block with four treatments (three for insecticides and one for control), four replicates were used of each treatment in addition to control. Results were expressed as means ± Standard deviation (SD). All data were subjected to analysis of variance and means compared using SPSS software [24].

Results and Discussion

Effects on Immature Mortality

The results showed that adult emergence of *T. evanescens* were affected by the insecticides used. The percentage of emergence of adult parasitoid with Fenvalerate was lower than the percentage with Thiacloprid (Table (2)), while the adult emergence was not affected by Fenoxycarb as there were no significant differences between the adults emerged of *T. evanescens* in Fenoxycarb and water control. The percentage of reduction rate in adults emergence compared to control ranged from 88.62, 60.75 and 7.86 % respectively for Fenvalerate, Thiacloprid and Fenoxycarb (Table (2)).

Matching the results found here for the effect of growth regulator Fenoxycarb with previous studies such as [15] which found the insect growth regulator insecticides and the insect growth inhibitors (Fenoxycarb, Diflubenz- uron and Lufenuron) were harmless to pupae, slightly harmful to eggs of egg parasitoid *T. cacoeciae*. Also, [25] found that the growth regulators and selective insecticides had no effect in adult emergence.

Fenvalerate decrease the parasitism and adult emergence of the parasitoid and this might have been caused by the toxicity of the insecticide and their repellent action on *T. evanescens* as reported by [26]. Since parasitoids can be olfactory orientation affected by insecticides resulting in a low capacity to found their hosts [27].

Table (2)
Parasitism and emergence percentage (mean ± standard error) of T. evanescens from E. cautella eggs treated with insecticides.

Insecticides	*Parasitism %*	*Adult emergence % ± SD*	*Reduction rate in Adults emergence % compared to control*	*Class**
Fenvalerate	10.50 ± 4.02 c	2.24 ± 0.85 c	88.62	3
Thiacloprid	36.22 ± 2.65 b	26.18 ± 10.52 b	60.75	2
Fenoxycarb	85.03 ± 2.69 a	74.05 ±12.04 a	7.86	1
Control (distilled water)	92.28 ±8.21 a	88.58 ±6.45 a	-	-

**** Evaluation categories according to IOBC classification: 1- harmless (<30%), 2- slightly harmful (30-79%), 3- moderately harmful (80- 99%), 4- harmful (>99%).- Means followed by the same letter in the column were not significantly different at P = 0.05***

Literatures also found that the effect of some insecticides on emergence of some parasitoids was related to the progression of preimaginal development at the time of exposure [28]. While, in other studies the timing of exposure to insecticides had no impact on emergence rate of *T. exiguum* [25]. [29] proposed that the developmental stage of parasitoids at the time of insecticide application appears to be important because it determines the time allowed for insecticide degradation before the emergence of parasitoids.

It has been reported that the susceptibility of *Trichogramma* to insecticides differs among *Trichogramma* species and is substantially influenced by the host egg upon which they were reared [30]. It was considered that the bioassays conducted in laboratory conditions expose the insects to the maximum possible dose and contacts they would face under field conditions and may not be realized under field conditions. However, the information from these effects of the three insecticides on laboratory study can be helpful in using insecticides but further research should focus on the impact of insecticide on field conditions.

Residue Toxicity on Parasitoid Adults

Results showed that the toxicity of glass surface residues on the adults parasitoids mortality varied significantly between the three insecticides (Fig. (1)).

The effects of Fenoxycarb on *T. evanescens* were significantly not different from that of the water control in 2, 4 and 6 h after treatment, but caused more mortality than water control after 24 h (Fig. (1)). In fact, there was no mortality in *T. evanescens* adults after 2 and 4 h. while, the effects of Thiacloprid were slightly toxic (class 2) to *T. evanescens* and Fenvalerate caused higher mortality than Fenoxycarb and Thiacloprid and classified as moderately toxic (class 3).

For the three insecticide treatments the longer period after treatment, the higher mortality caused. After 2, 4, 6 and 24 h. the residue of Fenoxycarb treatments caused 0, 0, 1, 5.5% mortality of *T. evanescens*, while Thiacloprid caused 11.75, 26.25, 41.5, 78 % and 21.5, 46.75, 61, 95 % mortality for Fenvalerate treatments.

Previous studies reveled that residues of Thiacloprid severely affected adult stages of the cereal aphid parasitoid *Aphidius rhopalosiphi* whereas pre-imaginal stages which develop within the host) remained unaffected [13]. These differences in response of *Trichogramma* to different insecticides were also reported by [31], they found that Esfenvalerate and the Carbamate methomyl were lethal to all life stages. The neonicotinoids Thiacloprid and acetamiprid demonstrated strong larvicidal and ovicidal activity but were somewhat weaker adulticides than the conventional broad-spectrum compounds while, Azinphosmethyl, phosmet, indoxacarb, Thiacloprid, and acetamiprid were all toxic to the egg parasitoid *T. minutum* Riley. Also, [14] found that Thiacloprid did not affect *T. pretiosum* emergence when eggs of *S. cerealella* enclosing pupae of the wasps were surface treated.

In contrast [32] reported that the pyrethroids tested in their study (Cypermethrin, Fenvalerate, and Deltamethrin) had relatively low residual toxicity to *T. brasiliensis* Ashmead while carbaryl (carbamate) had the highest residual activity. [33] observed that the fecundity of *T. pretiosum* was significantly reduced by Esfenvalerate in two consecutive generations, despite the fact that only the first one contacted with pyrethroid-treated host eggs.

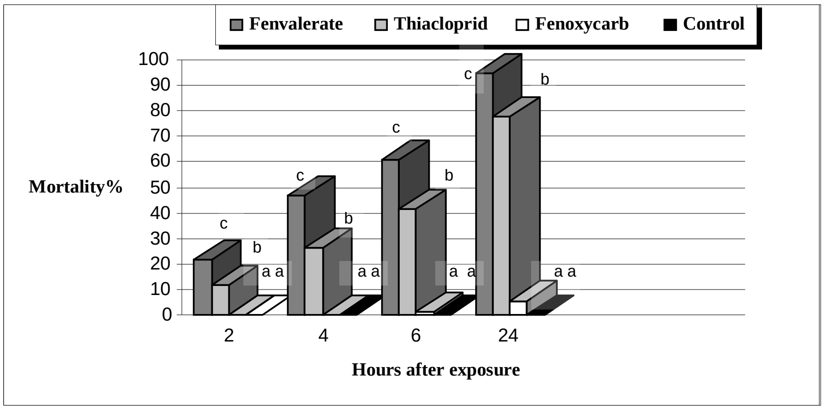

Fig. (1) Effects of glass-surface residues of Fenvalerate, Thiacloprid, Fenoxycarb and control on adults of T. evanescens. Similar letters above the bars denote that no significant differences between group means could be found at P=0.05.

As pesticides in the categories non-toxic and slightly toxic can be considered for use in integrated pest management (IPM) so that the growth regulator Fenoxycarb which have the lowest effect on *T. evanescens* and classified as non-toxic, and Thiacloprid which was slightly toxic can be used in integrated pest management programs of Lepidoptera pests for conserving *T. evanescens*. Also, according to our result Fenvalerate was moderately toxic (80–99%) to the *T. evanescen*, its application should be restricted in biocontrol field.

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الخلاصة

درس تأثير ثلاثة مبيدات حشرية هي فينفاليرات، ثايكلوبرد وفينوكسي كارب في طفيل البيض *Trichogramma evanescens* West. تم اختبار سمية المبيدات مختبريا بطريقتين: الاولى رش بيض المضيف الحاوي على الطفيل مباشرة لدراسة تاثير المبيدات في معدل التطفل وانبثاق بالغات الطفيليات (ب) تعريض بالغات الطفيل لبقايا سطح زجاجي معامل بالمبيد. حضرت المبيدات جميعها وفق توصيات الشركة المصنعة للتطبيق الحقلي. أظهرت النتائج اختلاف المبيدات الحشرية المستخدمة في سميتها وتاثيرها، اذ وجد ان الفينوكسي كارب غير سام أو منخفض السمية لطفيل البيض ولم يؤثر على نمو الاطوار غير الناضجة أو على ظهور البالغات. في حين كان مبيد الفينفاليرات ضارا للغاية في الطفيل وسبب انخفاضا معنويا في نسبة الانبثاق وتطفل *T. evanescens* على بيض المضيف. فيما كان تاثير الثايكلوبرد قليل السمية للطفيل وصنف في الدرجة الثانية عند تعريض الطفيل مباشرة إلى بقايا المبيدات. صنف الفينفاليرات بانه معتدل السمية وسبب هلاكات اعلى من مبيد الفينوكسي كارب والثايكلوبرد في كل من تعريضها الى بقايا المبيد على سطح الزجاج وطريقة الرش المباشر على بيض المضيف. قدمت الدراسة بيانات مفيدة لتنفيذ استراتيجيات كل من المكافحة الكيميائية والحياتية في الإدارة المتكاملة للآفات.