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# Toxicological studies of certain insecticides on peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) in Sohag governorate, Egypt

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## Abstract

Peach fruit fly, Bactrocera zonata (Saunders) (Diptera: Tephritidae), considered a serious pest in the last decade which attacking a wide range of fruits in Egypt. Control strategies for B. zonata in Egypt are mainly based on the use of conventional chemical pesticides. The aim of this study is to evaluate the efficiency of five pesticides i.e., malathion, methomyl, lambodcyhalothrin, imidacloprid, and spinosad against the three field strains of B. zonata, in addition to susceptible strain. The results showed that, the tested insecticides against the adult stage of susceptible strain of B. zonata arranged descendingly according to LC50 values as followed: λ-cyhalothrin, imidacloprid, methomyl, malathion and spinosad and the corresponding LC50 values were 0.5, 0.5, 0.76, 1.04, and 1.09 ppm, respectively. While when evaluating the same pesticides on the three field strains from three different locations in Sohag governorate (Maragah, Tahta and Shatwra), the results showed that,in Maragah strain was more susceptible than other strains to pesticides λ-cyhalothrin, imidacloprid, and malathion with LC50 values, 15.22, 31,40, and 30,49 ppm, respectively. While Shatwra strain was the most susceptible to spinosad and Tahta strain to the methomyl pesticide. Likewise, results indicated that the order of the tested field strains of malathion, λ-cyhalothrin and imidacloprid based on the degree of resistance was in descendingly arranged as followed: Tahta, Shatwra, and Maragah, respectively, whereas the corresponding resistance degrees were as follows; (76.95, 51.62, and 29.38)., (84.53, 64.23., and 30.28)., and (241.17, 101.68, and 62.45) – fold. On the other hand, for methomyl and spinosad, the arrangement differed, as the three field strains were arranged according to their degree of resistance to methomyl as follows: in Shatwra was (462.09 fold), Maragah was (307.94 fold) and Tahta was (178.95 fold), respectively, while for spinosad it was in Tahta (27.08), Maragah (26.16) and Shatwra (19.88) - fold. The development of resistance to spinosad was observed for the peach fruit fly, thus, we recommended looking for other alternative insecticides in integrated pest management programs.

**Keywords:**
Peach fruit fly, insecticides, toxicity, resistance ratio

# INTRODUCION

The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the most destructive pests of horticultural crops. *B. zonata* is an origin polyphagous pest in South and South-East Asia. and spread to other parts of the world (Pena *et al*., 1998; Agarwal *et al*., 1999; Dhillon *et al*., 2005). It has numerous host plants (more than 50 hosts) (EPPO, 2005). *B. zonata* is considered the key-pest on many horticultural crops such as guava, mango, and peach fruits. (El-Gendy and Nassar, 2014). Essentially, in Egypt, the peach fruit fly was detected for the first time in 1924 in Port-Said (Efflatoun, 1924). Currently, it was recorded in plentiful areas in Egypt, even the dry desert areas, Oases and North Sinai, where the host plants are exsited (El-Minshawy *et al.*, 1999; Hashem *et al.*, 2001; Draz *et al.*, 2002; Mosleh *et al.*, 2011; El-Gendy and ElSaadany, 2012). *B. zonate* is one of the most economically important insect pests that cause economic losses by damaging fruit and by interfering international horticultural trade (Shehata *et al.*, 2008). Female flies lay eggs in the fruits and the larvae feed on the pulp. Subsequently, fruits became vulnerable to secondary bacterial and fungal infections and infested fruits drop down (Abdel-Galil, 2007; Amro and Abdel-Galil, 2008; Mosleh *et al*., 2011). In Pakistan, it causes losses of to 100 % in different fruits, where the damage in guava fruits reached to 25-50% (Siddiqui *et al*., 2003; Kakar *et al*., 2014). EPPO (2005) recorded the annual costs of damage in the Middle East by 320 million EUR and 190 million EUR in Egypt. In this interim, several cultural control methods (e.g., pruning, weeding, and collection of fallen fruits) are relatively considered by farmers for reducing the population on the pest (El-Heneidy, 2012). In general, farmers rely on cover sprays for the management of fruit fly with least success in its control. The unwise use of these pesticides against fruit flies is greatly affected in Egypt and this application of insecticide is increasing rapidly. In this regard, control strategies for *B. zonata* in Egypt mainly depened on the use of conventional chemical pesticides for example, organophosphates, carbamates, synthetic pyrethroids and new chemistry are being indiscriminately used by farmers as cover sprays (Stonehouse *et al.*, 1997; Alston, 2002; El-Aw *et al.*, 2008). Tree trunks are either partially sprayed or baited. Killing bags are used in semi-isolated orchards and in areas with moderate population densities (WHO, 1986). Thus, the aim of this study is to evaluate the toxicity and resistance ratio of five insecticides belonging to different groups which are differing in their mode of action against the *B. zonata*, (malathion , methomyl, λ-cyhalothrin ,imidacloprid and Spinosad)

# MATERIALS AND METHODS

**Insect culture**

A susceptible reference strain was obtained from Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt in pupal stage which was reared for the last many years without insecticide exposure for comparison according to the method of (Mahmoud, 2004) and maintained at temperature of (27 ± 3ºC and 65 – 75% RH). Plastic containers were furnished with sterilized sand and the infested fruits were placed inside the containers where eggs were hatching to 1st instars, then to 2nd and 3rd instar larvae until pupation stage. Pupae were collected daily and transferred to adult rearing cages. The sides of the adult cages were coated with wire screen except one side which had a sleeve opening (for daily examination) and the cage floor was made of wooden sheet. The newly emerged flies were provided with a source of drink water and the food was sugar mixed with yeast extract (3:1), the adult cages were supplied with artificial plastic fruits that had many small pores (as an ovipositor site) except 3 cm at the bottom and one wide pore at the top which was covered with a suitable lid; these plastic fruits were filled with water to the mentioned 3 cm at the bottom to receive the eggs. The deposited eggs were collected every 24 h and placed on an artificial diet containing wheat bran (1000 g), brewer's yeast (250 g), sugar (300 g), sodium benzoate (2 g), HCl (1ml) and water (300ml)." The diet was kept in plastic containers and stored in a refrigerator" These ingredients were carefully mixed in a large plastic container. Then eggs were scattered on the surface of the diet which was placed in plastic trays of 20x10x8cm tightly covered with muslin clothes using rubber bands. After that, these trays were placed in a large plastic van with sand at the bottom to allow the jumping larvae to pupate. All pupae were separated by sieving from sand. Field populations of *B. zonata* were collected from the infested and fallen fruits of guava from Shatora, Tahta and Maragha city, Sohag governorate, Egypt, and kept under laboratory conditions for two generations to increase the number of insects and for adaptation. Rearing of field strains of *B. zonata* were carried in wooden cages on healthy guava fruits for egg laying (El- Khayat *et al.*, 2010). The infested fruits were then placed in a wooden cage having soil at the bottom for pupation. The pupae were isolated from the soil and placed in a separate cage for emergence. The newly emerged flies were provided with a source of drink water and the adults were fed on sugar mixed with yeast extract (3:1).

**Insecticides used**

The commercial formulations of insecticides belong to different groups used in bioassays were malathion (malathion 57% EC), methomyl (lannet 90% SP), λ-cyhalothrin (lambda-cyhalothrin 5% EC), imidacloprid (imi power 35% SC), and spinosad (tracer 24 % SC).These insecticides were obtained from the local market.

**Toxicological studies**

All formulation insecticides were dissolved in acetone and at least 4-7 serially diluted concentrations were prepared for each insecticide. Toxicity experiments were conducted using residual film method originally according to Plapp *et al.* (1987) with minor modifying a glass tube of 10 ml volume was washed in acetone and a 500 µl of the insecticide solution was transferred into the tube. The treated tubes were rolled on a bench-top surface until the solvent evaporated completely. Each tube received ten adult male and female flies of Peach Fruit Fly (PFF). Three replicates were used for each concentration and control tubes were treated with 500 µl acetone without insecticide. The treated and control vials were held in the same condition. Mortality was recorded after 24 hours. The average percentage of adult mortality was calculated for each concentration. The toxicity experiment of each insecticide was repeated twice and the results were corrected by Abbott's formula (Abbott 1925). The $LC_{50}$ was calculated according to Finney (1971).

# RESULTS AND DISSCUTION

The toxicity of lambda-cyhalothrin, imidacloprid, methomyl, malathion and spinosad against the adult stage of susceptible strain of *B. zonata* is demonstrated in Table (1) and Fig. (1): Data clearly indicated that the order of the efficiency of the tested insecticides based on $LC_{50}$ values was descendingly arranged as followed: lambda-cyhalothrin, imidacloprid, methomyl, malathion and spinosad. The corresponding $LC_{50}$ values were 0.5, 0.5, 0.76, 1.04, and 1.09 ppm, respectively. While when evaluating the same pesticides on the three field strains from three different regions in Sohag Governorate (Maragah, Tahta and Shatwra), the results revealed that, the Maragah strains were more susceptible than other strains to pesticides lambda-cyhalothrin, imidacloprid, and malathion based on $LC_{50}$ values (15.22, 31.40, and 30.49 ppm, respectively). Whereas, Shatwra strain was the most susceptible to the pesticide spinosad and Tahta strain to the methomyl pesticide. Importantly, it was obviously that, the difference in toxicity between the tested strains is due to insect strain type, degree of resistance, type of pesticide, chemical composition, mode of action, rate of use, frequency of pesticide use, type of crop and weather conditions prevailing in the study area. The present findings are consistent with El-Aw *et al.* (2008) which they reported different toxicity rates of pesticides against PFF adults, where methomyl was the most effective insecticide, followed by thiamethoxam, spinosyn, and malathion.

Further, data in Table (1) demonstrated the response of the adults of different strains of *B. zonata* to the selected insecticides. Out of the five insecticides used, methomyl was found to be the least toxic with $LC_{50}$ values of 350.35, 233.47 and 135.68 ppm Shatwra, Maragah and Tahta, respectively compared to susceptible strain. λ-cyhalothrin showed to be the most toxic with $LC_{50}$ values of 15.22 ppm against Maragah strain. Furthermore, in comparison to susceptible strain spinosad shows high toxicity with $LC_{50}$ values of 21.67, 28.51 and 29.52 ppm to Shatwra, Maragah and Tahta, respectively. Malathion shows high toxicity to Maragah, Shatwra and Tahta with $LC_{50}$ values of 30.49, 53.57 and 79.86 ppm respectively compared to susceptible strain. Imidacloprid shows high toxicity to Maragah, Shatwra and Tahta with $LC_{50}$ values of 31.40, 51.12, and 121.25 ppm respectively compared to susceptible strain.

Furthermore, results in Table (1) and Fig. (1), indicated that, λ-cyhalothrin and imidacloprid were the steepest toxicity line and spinosad had the flattest one, methomyl and malathion lies in between, this reflects the superiority of λ-cyhalothrin and imidacloprid and inferiority of spinosad.

Moreover, the results in Table (1) showed that the level of resistance of the three field strains (Maragah, Tahta and Shatwra) of the peach fruit fly to the tested pesticides which compared to the susceptible strain was as followed adults of the resistant field strains (Shatwra, Maragah and Tahta) demonstrated the highest resistance ratio to Methomyl (462.09, 307.94 and 179.95-fold for Shatwra, Maragah and Tahta strains respectively). However, the corresponding values of $LC_{50}$ values were 350.35, 233.47 and 135.68 ppm. Likewise, adults of the resistant field strains (Shatwra and Tahta) exhibited an increase in resistance ratio to imidacloprid (101.68, and 241.16-fold, respectively) with $LC_{50}$ values of 51.12, and 121.25 ppm, respectively. In contrast, compared to the susceptible strain, adults of the resistant field strains Shatwra, Maragah and Tahta achieved the lowest resistance ratio against spinosad (19.88, 26.15 and 27.88-fold, respectively) with $LC_{50}$ values of 21.67, 29.52 and 28.51ppm, respectively. Furthermore, Maragah strain showed low resistance ratio against Malathion and λ-cyhalothrin (29.37 and 30.28-fold) with $LC_{50}$ value of 30.49 and 15.22 ppm, respectively. Furthe, adults of the resistant field strains (Shatwra and Tahta) showed moderate resistance ratio against Malathion and λ-cyhalothrin (51.62, 76.95, and 84.52,64.23-fold, respectively) with $LC_{50}$ value of 53.57,79.86 and 32.29and 42.50 ppm, respectively. Also, Maragah displayed moderate resistance ratio against Imidacloprid (62.44-fold) with $LC_{50}$ value of 31.40 ppm.

Generally, data clearly indicated that the order of the tested field strains of malathion, λ-cyhalothrin and imidacloprid according to the degree of resistance was in descendingly arranged as follows: Tahta, Shatwra, and Maragah, respectively, and the corresponding resistance degrees were as followed; (76.95, 51.62, and 29.38)., (84.53, 64.23., and 30.28)., and (241.17, 101.68, and 62.45) – fold. On the other side, for methomyl and spinosad, the order of insecticides was dissimilar , as the three field strains were coordinated based on their degree of resistance to methomyl as followed: Shatwra (462.09 fold), Maragah (307.94 fold) and Tahta (178.95 fold), whereas, for spinosad they were Tahta (27.08 fold), Maragah (26.16 fold) and Shatwra (19.88 fold) . These results are in harmony with Radwan (2012) who found that, a field population of *B. zonata* was highly resistant to malathion. Whereas, in Pakistan, Nadeem *et al*. (2014) found moderate level of malathion resistance in some field population of *B. zonata*. Nasouri (2017) evaluated the capacity of three resistant strains of peach fruit fly, *B. zonata*, to develop resistance to malathion, lambda-cyhalothrin, and Spinosad, as well as the mechanisms of resistance. The result desgnated that, malathion-resistant (M-R) (RR: 52-fold after eight generations of selection), ʎ- cyhalothrin-resistant (L-R) (RR: 12-fold after six generations of selection), and spinosad-resistant (S-R) (RR: <3-fold after six generations of selection).

Furthermore, results evidently intimated that, Tahta strain was the most resistant to all tested insecticides except for methomyl, while Shatwra strain was the most resistant. The reason behind that owing to Tahta strain is considered (multiple resistance), i.e., resistance to several different insecticides, due to the presence of more than one mechanical resistance. These results are agreement with Nadeem *et al.* (2014) who evaluated seven insecticides, including trichlorfon, malathion bifenthrin, lambda cyhalothrin, spinosad, cypermethrin and chlorpyrifos against three field strains, M1, M2 and SWL which acquired from twenty-nine *B. zonata* field populations to determine the level of resistance. The results indicated that in three selected strains, high resistance ratios to trichlorfon (80.81-, 35.91-, and 69.92-fold) were developed, whereas, moderate resistance ratios to malathion and chlorpyrifos were found (28.15, 20.96, 27.22 and 25.52, 17.79, 24.81-fold). In the selected strains (M1, M2, and SWL), resistance ratios to pyrethroids (bifenthrin, lambda-cyhalothrin, cypermethrin) and microbial insecticides (spinosad) was low to moderate. In comparison to pyrethroid insecticides (bifenthrin, lambda-cyhalothrin, and cypermethrin) and microbial insecticides (Spinosad), the three field selected strains developed higher resistant level to organophosphates (trichlorfon, malathion, and chlorpyrifos). Shehab (2011) reported that, malathion (up to 78-fold) and methomyl populations were the highest level of resistance, whereas pyrethroid fenvalerate populations was the least. However, resistance to fenitrothion classified in between. Behira strain was the highest level of resistance, followed by Gharbia, while Giza strain was the last one.

**Table 1. Toxicity and resistance ratio of certain insecticides against different strains of *B. zonata* collected from the infested and fallen fruits of guava from Elmaragah and Tahta cities and Shatwra village in Sohag governorate during 2019-2021Season.**

| Insecticides | $LC_{50}$ (95% FL) SS | Field strain of *B. zonata* collected from different Location in Sohag gouvernorat | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Elmaragah | | | | Tahta | | | | Shatwra | | | |
| | | $LC_{50}$ (95% FL) FS | RR | d.f | $\chi 2$ | $LC_{50}$ (95% FL)FS | RR | d.f | $\chi 2$ | $LC_{50}$ (95% FL) FS | RR | d.f | $\chi 2$ |
| **Malathion** | 1.04 (0.63-1.71) | 30.49 (16.83-55.23) | 29.31 | 4 | 0.11 | 79.86 (36.41-175.15) | 76.95 | 4 | 2.71 | 53.57 (24.26-118.28) | 51.62 | 3 | 0.28 |
| **Methomyl** | 0.76 (0.43-1.33) | 233.47 (160.14-340.38) | 307.94 | 5 | 0.91 | 135.68 (72.13-255.21) | 179.95 | 5 | 1.25 | 350.35 (233.88-523.60) | 462.09 | 4 | 4.74 |
| **λ-cyhalothrin** | 0.50 (0.17-1.52) | 15.22 (8.44-16.15) | 30.28 | 4 | 0.01 | 42.50 (25.40-71.09) | 84.52 | 2 | 0.10 | 32.29 (19.24-54.33) | 64.23 | 4 | 0.05 |
| **Imidacloprid** | 0.50 (0.17-1.52) | 31.40 (17.06-57.66) | 62.45 | 4 | 0.29 | 121.25 (73.30-200.82) | 241.17 | 4 | 0.53 | 51.12 (32.67-79.77) | 101.68 | 3 | 0.08 |
| **Spinosad** | 1.09 (0.69-1.73) | 28.51 (16.28-49.95) | 26.16 | 3 | 0.05 | 29.52 (17.77-49.05) | 27.08 | 3 | 0.19 | 21.67 (10.46-44.89) | 19.88 | 3 | 0.01 |

(FL): Fiducial limits; SS: Susceptible Strain; FS : Field Strain; RR: Resistance Ratio = $LC_{50}$ (FS) / $LC_{50}$ (SS); $LC_{50}$ values in each column are significantly different if their 95% FL did not overlapping.

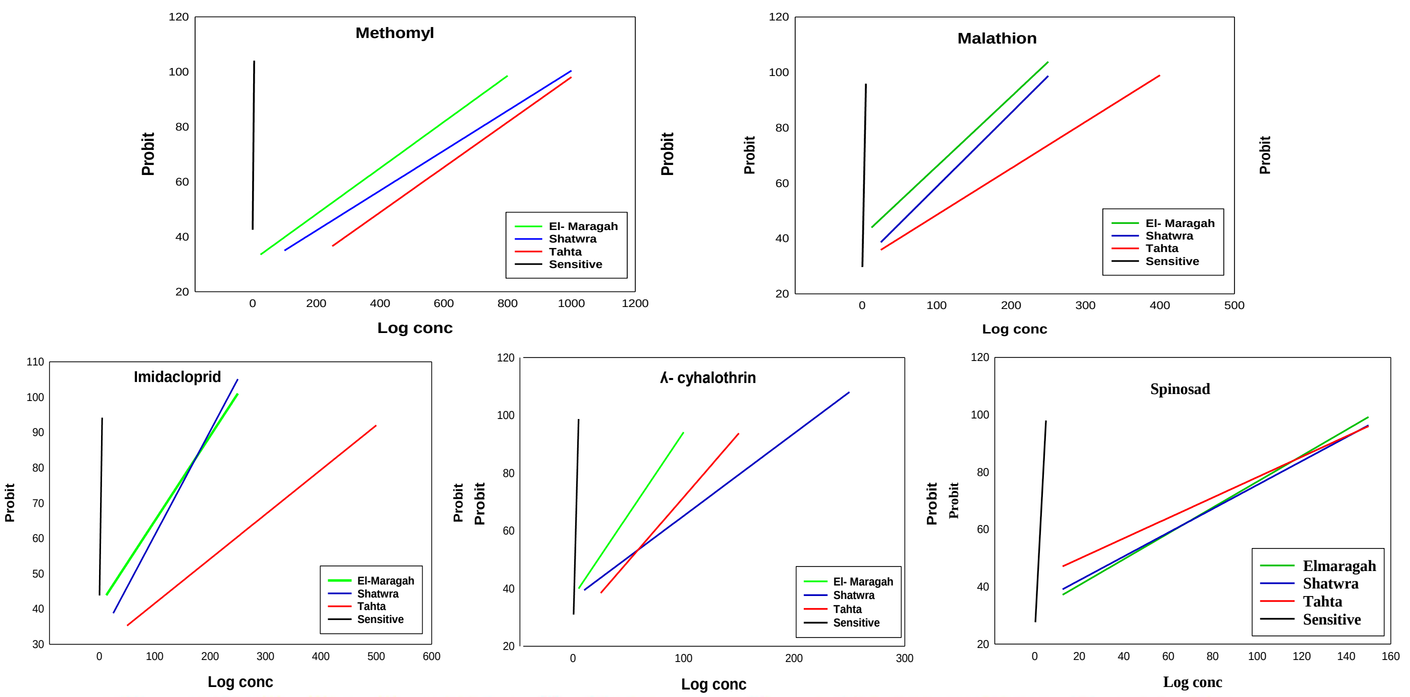


**Fig.1.** Log concentration vs probit mortality regression lines of the adults of different strains of *B. zonata* treated with different insecticides: malathion , methomyl , λ-cyhalothrin , imidacloprid, , and spinosad.

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## الملخص العربي

كانت تعتبر ذبابة الخوخ، *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) ، آفة خطيرة في العقد الماضي تهاجم مجموعة واسعة من الفاكهة في مصر. تعتمد استراتيجيات المكافحة لحشرة *B. zonata* في مصر بشكل أساسي على استخدام مبيدات الآفات الكيميائية التقليدية. الهدف من هذه الدراسة هو تقييم كفاءة خمسة مبيدات هي: الملاثيون ، الميثوميل ، اللامباداسيهالوثرين ، اميداكلوبريد ، والسبينوساد ضد السلالات الحقلية الثلاثة من *B. zonata* ، بالإضافة إلى السلالة الحساسة. أظهرت النتائج أن المبيدات المختبرة ضد المرحلة البالغة من السلالة الحساسة من حشرة *B. zonata* مرتبة تنازلياً حسب قيم التركيز النصفي المميت على النحو التالي: اللامباداسيهالوثرين ، اميداكلوبريد ، الميثوميل ، الملاثيون ، السبينوساد. كانت قيم التركيز النصفي المميت المقابلة 0.5 و 0.5و 0.76 و 1.04 و 1.09 جزء في المليون على التوالي. أثناء تقييم نفس المبيدات على السلالات الحقلية الثلاث من ثلاث مناطق مختلفة بمحافظة سوهاج (المراغة ، طهطا ، شطورة) ، أظهرت النتائج أن سلالات المراغة كانت أكثر عرضة من السلالات الأخرى لمبيدات الآفات λ-سيهالوثرين ، إيميداكلوبريد ، ملاثيون حسب قيم التركيز النصفي المميت 15.22 : و 31 و 40 و 30.49 جزء في المليون على التوالي. بينما كانت سلالة شطورة هي الأكثر تأثراً بمبيد سبينوساد وسلالة طهطا لمبيد الميثوميل. كما أشارت النتائج إلى أن ترتيب السلالات الحقلية المختبرة للملاثيون اللامباداسيهالوثرين وإميداكلوبريد حسب درجة المقاومة مرتبة تنازليًا على النحو التالي: طهطا ، وشطورة ، والمراغة ، على التوالي ، ودرجات المقاومة المقابلة كانت على النحو التالي. ؛ (76.95 و 51.62 و 29.38). و (84.53 و 64.23 و 30.28). و (241.17 و 101.68 و 62.45) - أضعاف. من ناحية أخرى ، يختلف الترتيب بالنسبة للميثوميل والسبينوساد ، حيث تم ترتيب سلالات الحقل الثلاثة وفقًا لدرجة مقاومتها للميثوميل على النحو التالي: شطورة (462.09) والمراغة (307.94) وطهطا (178.95) على التوالي ، بينما بالنسبة للسبينوساد كانت طهطا (27.08) ، مراغة (26.16) وشطورة (19.88) - أضعاف. أخيرًا ، تمت ملاحظة تطورالمقاومة لمبيد السبينوساد ضد ذبابة الخوخ ، لذلك أوصينا بالبحث عن بدائل لمبيدات حشرية أخرى في المكافحة .