Zagazig Journal of Plant Protection Research 2519-2528

Zagazig J. Agric. Res., Vol. 43 No. (6B) 2016

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

EVALUATION OF SOME PESTICIDE ALTERNATIVES FOR CONTROLLING EGYPTIAN COTTON LEAFWORM, *Spodoptera littoralis* (BOISD.)

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ABSTRACT

The lethal and sublethal effects of five insecticides from different groups namely: chlorfluazuron, emamectin benzoate, pyrethrins and *Bacillus thuringiensis* subsp. *kurstaki* compared to one of the most known organophosphorous compound (chlorpyrifos) as a chemical insecticide, were evaluated on the 2nd instar larvae of a laboratory strain of cotton leafworm, *Spodoptera littoralis* (Boisd.). The toxicity effect indicated that emamectin benzoate proved to be the most effective compound among all tested insecticides. Some biological aspects were also investigated to show the latent effects of the tested compounds, such as; duration periods of larval and pupal stages, mortality percentage in pupal stage, pupation percentage, weight of pupae, percentage of adult's emergence, longevity of adult stage (male & female), sex ratio, female fecundity of eggs, incubation period and percentage of egg's hatching revealed that all compounds varied in their influences on biological aspects, and these biological aspects could have relation with toxicity of insecticides against *S. littoralis* larvae.

Key words: *Spodoptera littoralis*, chlorfluazuron, emamectin benzoate, pyrethrins, *Bacillus thuringiensis*, chlorpyrifos, toxic effects, biological aspects.

INTRODUCTION

Cotton leaf worm, *S. littoralis* (Boisd.) is the most important polyphagous pest, widely distributed all over the world (Azab *et al*., 2001). Larvae of this pest can feed on 90 economically important plant species belonging to 40 famililes and the rate of development has strong nutritional relations (Meisner and Nemny, 1992; Abd El-Atty, 1995; El-Maghraby *et al*., 1999; Khidr *et al*., 2003; Pineda *et al*. 2007; Abd El-Mageed and Shalaby, 2011).

Usually, this pest is controlled by using many conventional insecticides which often result many bad and undesirable side effects such as environmental pollution, resistance development in the target pests and leading to transformation of the economical rank of many secondary pests to become major ones in addition to suddenly harmful outbreak of the main pests and consequently many direct and indirect injurious effects on human, domestic animals as well as environmental balance may take place. Saleem *et al*. (2008) recorded that insect growth regulators display a delayed and latent toxicity on entomophagous insect populations. Also, Gogi *et al*. (2006) found that both two IGRs buprofezin at 370 and 555 g a.i./ha and lufenuron at 37 and 49 g a.i./ha, appeared safe to predator populations. On the other hand, Radwan *et al*. (2004) used biocides for controlling *S. littoralis*; these materials may not cause any kind of pollution to the environment. Also, they reported that the bioinsecticide (Xentari) demonstrated the least harmful effect on entomophagous insect populations which were significantly lower than those counted in control. The safety of bacterial bioinsecticides to insect predators was studied by Salama and Zaki 1984) and Kares (1991) and to insect parasitoid populations by Morallo-Rejesus *et al.* (1992) and Atwood *et al.* (1997). On the contrary, chemical

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insecticides reduced significantly the numbers of predaceous and parasitic species than all treatments. The present investigation aims to study the efficacy of chlorpyrifos, chlorfluazuron, emamectin benzoate, pyrethrins and *B. thuringiensis* on the 2nd instar larvae of the cotton leafworm, *S. littoralis* under laboratory conditions. Some biological aspects of *S. littoralis* as influenced by these insecticides were also studied.

MATERIALS AND METHODS

Compounds Tested

Dursban 48% EC (chlorpyrifos) an organophosphorous compound. Product (1 L/ fad.) supplied by Dow Agro Sciences. Chemical name: O, O-diethyl O-(3, 5, 6-trichloro-2-pyridinyl) phosphorothioate. Caprice 5% EC (chlorfluazuron) IGRs' compound. Product (400 cm^3/fad.) supplied by Elhelb pesticides and chemicals company. Chemical name: 1–[3, 5-dichloro – 4 – (3-chloro-5-trifluoromethyl–2–pyridyloxy) phenyl] –3– (2, 6-difluorobenzoyl) urea. Pasha 1.9% EC (emamectin benzoate) bio-insecticide. Product (250 cm^3/fad.) supplied by Elhelb pesticides and chemicals company. Chemical name: (4"R)-5-O-demethyl-4"-deoxy-4"-(methylamino) avermectin A1a + (4"R) – 5 – O-dimethyl-25- de (1-methylpropyl)-4"-deoxy-4 "-(methylamino)-25-(1-methylethyl) avermectin A1a (9:1). Pyrethrum 5% EC (pyrethrins) a plant extract. Product (440 ml /fad.) supplied by Agropharm Ltd (UK). Chemical name: [1 R-[1 α [S*(Z)], 3 β]]-2-methyl-4 –oxo-3-(2, 4-pentadienyl) cyclopenten-1-yl 2, 2-dimethyl –3– (2-methyl -1- propenyl) cyclopropanecarboxylate. Dipel DF 32.258 potency IU/mg (*Bacillus thuringiensis* subsp. *kurstaki*). Product (200 g/ fad.) supplied by Valent biosciences.

The formulated samples of the tested insecticides and the culture of *S. littoralis* were obtained from Agricultural Research Centre (ARC), Dokki, Giza. The experiments were carried out in the Laboratory of Biological Control, Plant Protection Research Institute, Sharkia Branch. The culture of *S. littoralis* was reared according to El-Defrawi *et al*. (1964).

Toxicity to the 2nd Instar Larvae of *S. littoralis*

For studying the toxicity of the insecticides; the organophosphorous compound (Dursban), IGRs' compound (Caprice) and the bio-pesticide (Pasha) against the 2nd instar larvae of *S. littoralis* laboratory strain, serial concentrations for each compound were prepared and diluted with water. The concentrations prepared were; 40, 30, 20, 15, 10 and 5 ppm for Dursban; 15, 10, 5, 1, 0.5 and 0.1 ppm for Caprice and 0.07, 0.05, 0.03, 0.01, 0.008 and 0.006 ppm, for Pasha. The selected concentrations of each compound were chosen to cause from 20-80% mortality of larvae according to preliminary tests. In this respect, the higher concentrations (recommended concentrations) of Pyrethrum and Dipel Df were 2.2 ml/l and 1 g/l; respectively, which their higher concentration applied gave mortality percentages didn't exceed about 20% after the third day of treatment.

The leaf dipping technique was used to estimate the larvicidal action of the tested insecticides against one-day-old of the 2nd instar larvae. Leaf disks (3 cm diameter) of fresh castor bean leaves punched with a cork borer, were dipped in the tested concentrations for 10 seconds. Treated disks were left to dry under normal conditions then offered to the larvae. Larvae were allowed to feed on treated disks for 48 hr., at the rate of one larva per disk in plastic cylinder tube of 3.4 cm diameter and 7.0 cm height. The tubes were covered with a perforated plastic lid to permit good ventilation. A filter paper was placed on the bottom to absorb any excess moisture. Three replicates of 10 larvae were made (30 larvae for each concentration). Control experiments involved using leaf disks dipped in water. This trial was carried out in the incubator at 26±1ºC and 65±5% RH. Larval mortality was scored: if no movement was observed, larvae were considered as dead after 24 hr. for chlorpyrifos and 72 hr., for the remaining insecticides.

Mortality counts were recorded and LC_{50} and LC_{90}, slope, toxicity index and relative potency values were calculated.

Toxicity index (T.I.) was determined by using Sun's equation (1950) as follows: Toxicity index = LC_{50} or LC_{90} of the highest efficient

compound / LC_{50} or LC_{90} of the other compound × 100.

Relative potency (R.P.) values were measured according to the method described by Zidan and Abdel-Megeed (1988) as follows:

Relative potency (fold) = LC_{50} or LC_{90} of the lowest efficient compound / LC_{50} or LC_{90} of the other compound×100.

The 1/10 LC_{50} values were also calculated for the tested insecticides to study their latent effect on some biological aspects of the survived larvae.

Latent Effects of Chlorpyrifos, Chlorfluazuron, Emamectin Benzoate, Pyrethrins and *B. thuringiensis* on some Biological Aspects of *S. littoralis*

Four replicates of 10 larvae each were used (40 larvae for each concentration of one-day-old of the 2nd instar larvae) fed for 24 hr., on castor bean leaves disks treated with 1/10 LC_{50} of chlorpyrifos, other batches were fed for 48 hr., on disks treated with other compounds, then transferred to untreated disks. Percentages of larvae that completed larval duration post treatment and pupated were recorded. Larval and pupal duration, mortality percentage in pupal stage, pupation percentage (based on the number of survived 6th instar larvae), weight of pupae, percentage of adults emergence (calculated as percent of adult emerged from normal pupae), longevity of adult stage (male and female), sex ratio among emerged adults (♂: ♀), fecundity, incubation period of eggs laid and percentage of egg's hatching were recorded.

Statistical Analysis

All the obtained data were statistically analyzed according to Tukey's HSD (1949). Data were subjected to statistical analyses using a software package Costat Statistical Software (2005).

RESULTS AND DISCUSSION

Toxicity of the Tested Materials

Data presented in Table 1 summarize the efficacy of different tested insecticides against the 2nd instar larvae. The second larval instar showed higher level of susceptibility towards the tested insecticides.

The results revealed that, at LC_{50} and LC_{90} emamectin benzoate was more toxic to the 2nd instar larvae than chlorfluazuron and chlorpyrifos. At LC_{50}, the toxicity of emamectin benzoate was 1411.99 times more toxic to the 2nd instar larvae than chlorpyrifos. Korrat *et al.* (2012) found that emamectin benzoate was the most effective insecticide (LC_{50}=0.017 ppm) among all tested insecticides.

Biological Effects

The aim of this experiment was to study the biological changes produced in this insect after exposure the 2nd larval instar to the tested insecticides. The insecticides used were tested at the level of 1/10 LC_{50} with the exception of Dipel DF and pyrethrins were tested at the recommended concentrations.

Data in Table 2 show that all the tested insecticides prolonged the total larval duration comparing to the control. The larval duration slightly prolonged to 10.10±0.08 days for chlorfluazuron and maximally prolonged to 10.95±0.31days for *B. thuringiensis* whereas untreated larvae recorded 9.75±0.17 days, with a significant difference between both insecticides (Table 2). The tardiness of the larval duration may be explained by Radwan *et al*. (1986) who noticed reduction in the consumption of food and considerable decrease in growth rate. Many authors mentioned the same result when tested different insecticides against *S. littoralis* larvae (Abd El-Aziz, 2000; Mohamady, 2000; Dutton *et al*., 2003). Mohamed *et al*. (2005) reported that the longest larval period 13.30±0.12 days was recorded when treated the 2nd instar larvae of *S. littoralis* with Dipel-2x (*B. thuringiensis* subsp. *kurstaki*).

All the tested compounds caused a significant elongation in the pre-pupal period as compared to control. This increase ranged between the minimum value 1.11±0.13 days for chlorfluazuron to the maximum one 1.80±0.11 days for emamectin benzoate. Pre-pupa of control recorded 1.05±0.06 days (Table 2). The increase of pupal duration may be attributed to the feeding of larvae on leaves treated with the insecticides.

Table 1. Acute toxicity of chlorpyrifos, chlorfluazuron and emamectin benzoate on the 2nd instar larvae of *S. littoralis* at 26±1ºC and 65±5% RH

Insecticide	LC_{50} ppm	LC_{90} ppm	Slope ± SE	Toxicity index at		Relative potency (fold) at	
				LC_{50}	LC_{90}	LC_{50}	LC_{90}
Chlorpyrifos	21.74	51.17	0.87±1.70	0.07	0.09	1.00	1.00
Chlorfluazuron	2.23	23.55	0.94±0.46	0.69	0.20	9.75	2.17
Emamectin benzoate	0.0154	0.047	0.99±0.0016	100.00	100.00	1411.99	1086.38

Toxicity Index and Relative Potency based on LC_{50}.

Table 2. Changes in some biological aspects of the insecticidal survived 2nd instar larvae of *S. littoralis* and the descended-subsequent developmental stages under laboratory conditions

Treatment	Larval duration (day)	Prepupal period (day)	Pupation (%)	Pupal Duration (day)	Pupal weight (g)	Pupal mortality (%)
Control	9.75± 0.17^{d}	1.05± 0.06^{c}	100.00^{a}	9.03± 0.10^{d}	0.3366± 0.0120^{a}	0.00^{b}
Chlorpyrifos	10.54± 0.14^{abc}	1.57± 0.17^{b}	87.50^{b}	8.41± 0.13^{e}	0.2946± 0.0050^{b}	17.15^{a}
Chlorfluazuron	10.10± 0.08^{cd}	1.11± 0.13^{c}	90.00^{ab}	10.41± 0.11^{b}	0.2631± 0.0070^{c}	19.44^{a}
Emamectin benzoate	10.46± 0.10^{bc}	1.8± 0.11^{a}	87.50^{b}	10.80± 0.10^{a}	0.2961± 0.0010^{b}	14.24^{a}
Pyrethrins	10.68± 0.29^{ab}	1.47± 0.04^{b}	95.00^{ab}	9.94± 0.17^{c}	0.2605± 0.0050^{c}	10.56^{ab}
B. thuringiensis	10.95± 0.31^{a}	1.49± 0.06^{b}	97.50^{ab}	9.74± 0.08^{c}	0.2990± 0.0040^{b}	7.50^{ab}

Data expressed as means ±standard deviation (SD).

Table 2. Continued

Treatment	Adult emergence (%)	Sex ratio		Longevity ♂	Longevity ♀			Total longevity ♀
		Females (%)	Males (%)		Pre-ovi.	Ovi.	Post-ovi.	
Control	100.00^{a}	50.00^{ab}	50.00^{ab}	10.84± 0.19^{a}	2.42± 0.17^{d}	5.17± 0.19^{a}	2.92± 0.32^{b}	10.50± 0.20^{b}
Chlorpyrifos	82.85^{b}	61.76^{ab}	38.24^{ab}	11.25± 0.32^{ab}	4.5± 0.20^{bc}	2.67± 0.27^{c}	2.17± 0.19^{c}	9.33± 0.27^{cd}
Chlorfluazuron	80.56^{b}	45.09^{b}	54.91^{a}	8.56± 0.16^{c}	4.78± 0.17^{ab}	1.11± 0.16^{e}	3.11± 0.16^{b}	9.00± 0.27^{d}
Emamectin benzoate	91.67^{ab}	71.87^{a}	28.13^{b}	11.67± 0.27^{a}	4.78± 0.17^{ab}	2.33± 0.27^{cd}	4.44± 0.16^{a}	11.56± 0.16^{a}
Pyrethrins	88.76^{ab}	50.72^{ab}	49.28^{ab}	11.78± 0.16^{a}	5.00± 0.27^{a}	2.11± 0.16^{d}	4.78± 0.16^{a}	11.89± 0.31^{a}
B. thuringiensis	92.73^{ab}	55.83^{ab}	44.17^{ab}	11.00± 0.41^{b}	4.22± 0.16^{c}	4.00± 0.20^{b}	1.50± 0.20^{d}	9.72± 0.16^{c}

Data expressed as means ± standard deviation (SD).

Table 2. Continued

Treatment	No. of eggs / female	Incubation period (day)	Hatchability (%)
Control	1747.50± 93.78^{a}	2.75± 0.29^{b}	95.65^{a}
Chlorpyrifos	925.83± 74.41^{c}	3.37± 0.25^{a}	47.72^{d}
Chlorfluazuron	567.50± 78.76^{d}	0.00± 0.00^{c}	0.00^{e}
Emamectin benzoate	795.00± 60.52^{c}	3.63± 0.25^{a}	70.71^{c}
Pyrethrins	1570.00± 73.49^{ab}	3.50± 0.41^{a}	91.43^{ab}
B. thuringiensis	1445.00± 101.49^{b}	3.75± 0.29^{a}	88.54^{b}

Data expressed as means ±standard deviation (SD).

As shown in Table 2, the pupation percentages of the insecticidal treated larvae recorded 87.50, 90.00, 87.50, 95.00 and 97.50% for chlorpyrifos, chlorfluazuron, emamectin benzoate, pyrethrins and *B. thuringiensis*, respectively, while the pupation of control recorded 100%. Gaaboub *et al*. (2012) mentioned that Protecto (*Bt*) induced a decrease in pupation percentage, being 85.90% compared to control.

All the tested compounds with the exception of chlorpyrifos (8.41±0.13 days) caused a significant elongation in the pupal stage as compared to control. The pupal duration was 9.74±0.08 days for *B. thuringiensis* and increased to the maximum period (10.80±0.10 days) for emamectin benzoate. Pupae of control recorded only 9.03±0.10 days (Table 2). The increase in the duration of pupae may be due to feeding of larvae on leaves treated with the tested insecticides causing a reduction in the food consumption and decrease in growth rate of the 2nd instar larvae of *S. littoralis*. Mohamed *et al*. (2005) reported that pupal duration of Dipel-2x (*B. thuringiensis* subsp. *kurstaki*) treated 2nd instar larvae of *S. littoralis* was elongated to 12.80 ±0.22 days.

The obtained data showed that, all the tested insecticides reduced the average weight of pupae developed from the insecticidal treated 2nd instar larvae than the control. The highest significant decrease recorded 0.2605±0.0050 g for pyrethrins pupae, whereas the lowest decrease 0.2990±0.0040 g was observed for *B. thuringiensis*, (Table 2). It is assumed that the reason for the loss of weight of pupae resulted from the insecticidal treated larvae were less able to convert ingested and digested food into body substances (Radwan *et al*., 1986; El-Ghar, 1993).

Data in Table 2 indicated that percentages of mortality of pupae descended from larvae treated with chlorpyrifos, chlorfluazuron, emamectin benzoate, pyrethrins and *B. thuringiensis* were 17.15, 19.44, 14.24, 10.56 and 7.50%, respectively. El-Aw (2003) found that Dipel 2X reduced pupa survival comparing to control.

The results indicated that there was a reduction in emergence of adults descended from the larvae treated with all used insecticides compared to control that recorded 100% (Table 2). The lowest emergence percentage was 80.56% for chlorfluazuron, while the highest emergence 92.73% was recorded for *B. thuringiensis* (Table 2). The decrease in the emergence percentages as compared to control may be due to increased percentage mortality of pupae descended from insecticidal treated larvae. The same trend was obtained after treatment of *S. littoralis* larvae with Dipel 2X (Abd El-Aziz, 2000; Mohamed *et al*., 2005) as well with chlorpyrifos (Mead, 2006).

All tested compounds affected the normal sex ratio (1:1) in comparing with control. The lowest female percentage was 45.09% for chlorfluazuron, while the highest female percentage was 71.87% for emamectin benzoate (Table 2).

The obtained results showed that the average longevity of male moths resulted from the 2nd instar larvae fed on treated castor bean leaves recorded the lowest significant value 8.56±0.16 days for chlorfluazuron, while the highest significant value was 11.78±0.16 days for pyrethrins. Control male moths lived about 10.84±0.19 days (Table 2).

Longevity of adult females included pre-oviposition, oviposition and post- oviposition periods were recorded in Table 2. Some of the tested insecticides increased longevity of females while others decreased the females longevity than control. Pyrethrins caused the longest female longevity 11.89±0.31 days. On the other hand, chlorfluazuron recorded the shortest female longevity 9.00 ± 0.27 days. Females of control lived 10.50 ± 0.20 days. The prolongation in adult longevity may be as a result of the reduction in food consumption, considerable decrease in growth rate and obvious reduction in the efficiency of converting ingested and digested food to body tissues which may lead to rejection of feeding. TianShu *et al.* (2013) mentioned that chlorfluazuron decreased oviposition period.

As shown in Table 2, the total number of eggs laid /female descended from the 2nd instar larvae fed on castor bean leaves treated with the tested insecticides was reduced in comparison with the control. Chlorfluazuron caused the highest significant reduction recording 567.50±78.76 eggs/female, while control female

laid an average of 1747.50±93.78 eggs/ female. Dipel DF caused the lowest reduction in the fecundity recording 1445.00±101.49 eggs/ female. The decrease in the fecundity may be due to the inhibition of the protein contents and its synthesis which is necessary for the nutrition of eggs. These results are in agreement with those of El-Khayat (1993) who reported that treatment of the 2nd and 4th instar larvae of *S. littoralis* with *B. thuringiensis* reduced female fecundity. Emamectin benzoate reduced fecundity of the treated 4th instar larvae of *S. littoralis* (El-Aw, 2003). Perveen *et al.* (2011) recorded that fecundity of mated adults of *S. littoralis* was reduced from 24-41% when treated by chlorfluazuron. Gaaboub *et al.* (2012) mentioned that chlorpyrifos significantly reduced the number of eggs deposited by females developed from the treated 4th larval instar of *S. littoralis*.

All the tested compounds significantly prolonged the incubation period compared to control (Table 2). The longest incubation period was 3.75±0.29 days for *B. thuringiensis* treated larvae.

The eggs laid by females descended from chlorfluazuron didn't hatch. Data presented in Table 2 indicate that there was clear reduction in hatchability percentages for all tested insecticides compared to control. The highest percentage of hatchability was recorded for the control (95.65%); the lowest hatchability percentage (0.00%) was recorded for chlorfluazuron treatment. Hossain *et al.* (1996) mentioned that eggs hatching was reduced to almost zero at concentrations >200 ppm of chlorfluazuron and other BPUs on treated plant (*Nerium oleander*) surfaces to treat adults of *S. littoralis*. Also, El-Aw (2003) reported that egg hatchability of *S. littoralis* was significantly reduced when treated its 4th instar larvae by emamectin benzoate and *B. thuringiensis*. Shahout *et al.* (2011) elucidated that hatching rate was significantly reduced when LC_{10} of chlorfluazuron was used against the 2nd instar larvae of *S. litura*. Gaaboub *et al.* (2012) reported that chlorpyrifos caused a lower egg hatchability percentage of *S. littoralis*.

In conclusion, our studies showed that at LC_{50} and LC_{90} chlorfluazuron was more toxic to the 2nd instar larvae than chlorpyrifos and low toxic than emamectin benzoate. At 1/10 LC_{50}, chlorfluazuron caused higher pupal mortality (19.44%), more reduction in adult emergence percentage (80.56%), lowest female percentage (45.09%) as sex ratio, lowest longevity of both male and female moth (8.56±0.16 days and 9.00±0.27 days, respectively), higher reduction in the total number of eggs laid/female (567.50±78.76 eggs/female), no incubation period and no hatchability percentage for eggs laid by females descended from the treated 2nd instar larvae of *S. littoralis*. IGRs have been developed due to their high activity and selectivity against insects with inherently low toxicity to non-target wildlife. As a result of their mode of action, a subtle effect of these compounds is likely to pose a greater effect to immature stages than to adults of a number of insect species (Darvas and Polgar, 1998; Dhadialla *et al*., 1998; Smagghe *et al*., 1999; Schneider *et al*., 2003). Most compounds that belong to the IGR class are not stomach or neurotoxic poisons, but have a unique mode of action that disrupts the molting process or cuticle formation in insects (Smagghe and Degheele, 1994) or interferes with the hormonal balance of insects. They are characteristically slow acting against a narrow range of sensitive stages of the insects' life cycle with harmful effect against target pests.

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تقييم بعض بدائل المبيدات في مكافحة دودة ورق القطن

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أُجريت هذه الدراسة لتقييم التأثيرات المميتة وتحت المميتة لأربع مبيدات من مجاميع مختلفة وهي :كلورفلوزارون، إيمامكتين بنزوات، بايرثرنز وباسيلس ثورينجينسس مقارنة بواحد من أكثر مركبات الفوسفور العضوية معرفة وهو الكلوربيريفوس كمبيد حشري كيميائي على يرقات العمر لثاني لدودة ورق القطن، أثبتت دراسات التأثير السام أن الإيمامكتين بنزوات هو أكثر المبيدات المختبرة تأثيرًا على العمر اليرقي الثاني لسلالة دودة ورق القطن المرباة في المعمل، أوضحت الدراسة أن التأثيرات المتأخرة للمبيدات المختبرة على بعض المظاهر البيولوجية مثل فترة طوري اليرقة والعذراء، نسبة الموت في طور العذراء، النسبة المئوية للتعذر، وزن العذارى، نسب خروج الفراشات، فترة عمر الفراشات (ذكور وإناث)، النسبة الجنسية، إنتاجية الأنثى من البيض، فترة الحضانة ونسبة فقس البيض قد اختلفت في تأثيرها على النواحي البيولوجية وأن هذه الاختلافات ارتبطت بسمية المركبات تجاه يرقات دودة ورق القطن.

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