

Lethal and sublethal effects of imidacloprid and pirimicarb on the melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) under laboratory conditions

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Abstract: The toxicity of imidacloprid and pirimicarb for all stages of the melon aphid, *Aphis gossypii* Glover, were investigated under laboratory conditions (25 ± 1 °C, $65 \pm 5\%$ R.H. and a photoperiod of 16:8 (L: D) h.) using a leaf dipping method. These pesticides were very toxic for first instar nymphs of *A. gossypii* with LC_{50} values of 17 and 220.2 ppm for imidacloprid and pirimicarb, respectively. For other nymphal instars, values of 23.9 to 70.5 ppm and 308.8 to 781.7 ppm were recorded for imidacloprid and pirimicarb respectively. Their LC_{50} values for adults were 90.1 and 983.1 ppm, respectively. Toxicity decreased with increasing age. Imidacloprid was more toxic than pirimicarb for all stages of development. The effect of applying sublethal concentrations of imidacloprid and pirimicarb was evaluated, also, using demographic toxicology. Longevity and population growth parameters, including intrinsic rate of increase (r_m), net reproductive rate (R_0), generation time (T_c) and finite rate of population increase (λ), were affected negatively by both insecticides. The r_m values for control, imidacloprid and pirimicarb exposed populations were 0.438, 0.150 and 0.335 female offspring per female per day, respectively. The doubling time (DT) also, was affected by imidacloprid. Overall, these results suggest that imidacloprid and pirimicarb can be effective against *A. gossypii*.

Keywords: *Aphis gossypii*, Carbamate, Demographic toxicology, Neonicotinoid

Introduction

The melon aphid, *Aphis gossypii* Glover is an important pest of vegetables and ornamentals in fields and greenhouses (Leclant and Deguine, 1994). It is a cosmopolitan, polyphagous species widely distributed in tropical, subtropical and temperate regions (Kresting *et al.*, 1999). *A. gossypii* causes both direct and indirect

damage to plants, the former by sucking phloem sap and the latter by either secreting honeydew or transmitting viruses. This aphid species is a vector of 76 viral diseases across a very large range of plants (Chan *et al.*, 1991).

Generally, chemical control has been the major tool for the control of aphids (Parrella *et al.*, 1999). Even though resistance of *A. gossypii* to some insecticides has been documented (Delorme *et al.*, 1997; Herron *et al.*, 2001; Wang *et al.*, 2002), the use of selective insecticides is needed for successful integrated pest management (IPM) programs (Talebi Jahromi, 2007).

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Imidacloprid is one of the neonicotinoid insecticides with systemic activity that acts as an agonist of insect nicotinic acetylcholine receptors, causing a reduction in, or complete cessation of, feeding and movement. It is particularly effective against aphids, whiteflies and planthoppers (Boiteau and Osborn, 1997; Elbert *et al.*, 1998; Nauen *et al.*, 1998).

Pirimicarb is a selective carbamate insecticide that inhibits acetylcholinesterase (AChE) activity in the insect nervous system. It is used to target aphids in particular (Hassall, 1990; Talebi Jahromi, 2007).

Estimating of lethal concentration appears to be incomplete measure of the toxic effects of insecticides because of the limited number of endpoints examined (mortality) and due to the often short duration of the tests (Walthall and Stark, 1997). The use of the intrinsic rate of increase of population (r_m) for toxicological studies has been recommended as a means of obtaining a complete measure of sublethal effects (Stark and Wennergren, 1995; Banks and Stark, 1998; Stark and Banks, 2003).

Demographic toxicology is an ecotoxicological technique that incorporates life table parameters in the context of toxicology. Life table parameters of populations exposed to various concentrations of a pollutant and those of unexposed populations are compared. Since ecological and toxicological parameters are combined, the general assumption is that predictions of the effects of pollutants at the population level can be made (Stark and Wennergren, 1995).

The characterization of differential susceptibility among life stages is critical to the assessment of pesticides and other xenobiotic on sensitive species (Stark and Wennergren, 1995). Acute toxicities and sublethal effects of imidacloprid and pirimicarb have been investigated on adult stage of *A. gossypii* (Moore *et al.*, 1996; Rongai *et al.*, 1998; Nauen and Elbert, 2003; Khaloobagheri *et al.*, 2006; Gerami *et al.* 2007; Tabacian *et al.*, 2011, Gerami *et al.*, 2012; Shi *et al.*, 2012; Torkamand *et al.*, 2013). However there is no literature about the effects of these insecticides

on immature stages of *A. gossypii*. Imidacloprid and pirimicarb are recommended in Iran for controlling aphids in fields and glasshouses.

Therefore, this study was designed to investigate the lethal effect of these two insecticides on immature and adult stages of *A. gossypii* in addition to their sublethal effect on the life table parameters of the pest. The results of this research would be important for more effective use of these insecticides in management programs of *A. gossypii* through improved understanding of their activity profiles.

Materials and Methods

Insect rearing

The colony of *A. gossypii* used in this research was established from insects originally collected from cucumber fields, in Ahvaz, Southwest Iran, in April 2011. It was reared on glasshouse cucumber plants (*Cucumis sativus* L. cultivar Negin) in a muslin-walled cage (150 × 65 × 65 cm) maintained in a growth chamber at 25 ± 1 °C, 65 ± 5% R. H. and a photoperiod of 16:8 (L: D) h.

In order to obtain synchronous cohorts of the experimental aphids, some apterous adults were placed on 5 cm diameter cucumber leaf discs. Each leaf disc was set upside down on a layer (4-5 mm) of 1.2% agar into a 9 cm diameter × 1 cm plastic Petri dish with three screened holes (1.2 cm diameter) in its lid for ventilation. After 12 h, apterous adults were removed from each Petri dish. Then the first instars were reared to the desired stage of development.

Insecticides

Commercial formulations of the two insecticides were used: imidacloprid (35SC, Gyah, Tehran, Iran) and pirimicarb (50WP, Moshkfam Fars, Iran).

Bioassay

A leaf dipping method was used in bioassay tests for each developmental stage of *A. gossypii* (Koziol and Semtner, 1984). Concentration setting experiments were done to determine the best concentrations to use for the

definitive measurements. Serial dilutions of formulated compounds were prepared in distilled water containing 500 ppm Tween 20 (as a non-ionic surfactant).

Cucumber leaves were dipped for 10 s into the insecticide solutions or into deionized water containing 500 ppm Tween 20 for controls. After air drying for 1 h, they were cut into 5 cm diameter discs and each leaf disc was placed with its adaxial side on a thin layer (2-3 mm) of 1.2% agar, into a 6 cm diameter \times 1 cm plastic Petri dish with two screened holes (1.2 cm diameter) on its lid for ventilation. Twenty aphids at the same stage of development per replicate were tested for each insecticide concentration.

Mortality was assessed 24 h after treatment. Aphids that seemed extremely lethargic or did not show any sign of movement when lightly touched with a needle were recorded as dead. Mortality data were corrected using Abbot's formula (Abbot, 1925).

Five concentrations of each insecticide plus a water control were used for each bioassay. Five Petri dishes were used per concentration (unit of replication) and each bioassay was repeated three times, *i.e.* 300 aphids per concentration. All Petri dishes were incubated at 25 ± 1 °C, $65 \pm 5\%$ R.H. and a photoperiod of 16:8 (L: D) h in laboratory conditions. LC_{50} values and their corresponding 95% fiducial limits (FL) were determined by probit analysis using the POLO-PC software (LeOra Software, 1987). LC_{50} values between developmental stages and between the insecticides on each developmental stage of *A. gossypii* were compared by relative potency test. Relative potencies were considered significant ($P < 0.05$) when their 95% confidence intervals (CI) did not include the value 1.0. (Robertson *et al.*, 2007). Furthermore, the results were considered significant when 95% confidence intervals of LC_{50} values did not overlap (Adams *et al.* 1990).

Sublethal effects

To study the sublethal effects of the insecticides on *A. gossypii*, utilizing life table parameters, adult aphids that had gone through all pre-imaginal stages on untreated leaves, were exposed

to an LC_{25} of either imidacloprid (31.1 ppm) or pirimicarb (597.8 ppm). Fifty young adult aphids (< 12 h old) were placed into individual Petri dishes (of the above mentioned dimensions) containing 5 cm diameter leaf discs treated with an LC_{25} of either insecticide including 500 ppm Tween 20. Control groups were treated with distilled water plus 500 ppm Tween 20. In order to keep the leaf discs fresh they were placed on an agar bed (4-5 mm). After 24 h, 20 live females were selected randomly and were monitored daily for mortality and reproduction. Neonate aphids were removed from each Petri dish after being counted. These data were recorded every 24 h until the females died. All Petri dishes were incubated at 25 ± 1 °C, $65 \pm 5\%$ R.H. and a photoperiod of 16:8 (L: D) h in laboratory conditions. Daily schedules of mortality and fecundity were integrated into a life table format (Carey, 1993) and were used to calculate the net reproductive rate (R_0), intrinsic rate of population increase (r_m), finite rate of population increase (λ), generation time (T_c) and doubling time (DT). The Jackknife technique was used to calculate the variance of r_m and other life table parameter estimates (Maia *et al.*, 2000). The data related to the adult longevity, fecundity and life table parameters were subjected to one-way ANOVA and means were compared by use of the least significant difference (LSD) test ($P < 0.05$) (SAS Institute, 2003). No data transformation was used because there was no evidence of non-normality of the data.

Results

Toxicity bioassay

Toxicity effects of imidacloprid and pirimicarb for all stages of the melon aphid are shown in Table 1. Toxicity of both insecticides was greater for first instars than for other stages and decreased with increasing age (Table 1). There was not significant difference in susceptibility of first and second instars of *A. gossypii* to imidacloprid ($P > 0.05$) (Table 2). Although, first instars of the melon aphid were more susceptible than second instars of *A. gossypii* to pirimicarb ($P < 0.05$). There was significant

difference in susceptibility of second and third instars ($P < 0.05$), third and fourth instars ($P < 0.05$) and fourth instars and adults of *A. gossypii* ($P < 0.05$) to imidacloprid and pirimicarb (Table 2).

The results of relative potency test showed that imidacloprid was 15.08 times more toxic than pirimicarb for first nymph instars. Both insecticides were effective against other nymphal instars of the aphid, but imidacloprid was about 14.51, 15.42 and 12.03 times more toxic than pirimicarb to the second, third and fourth nymphal instars, respectively (Table 3). Imidacloprid and pirimicarb were effective against adults with LC_{50} values of 90.1 and 983.1 ppm, respectively (Table 1). Imidacloprid was almost 11.36 times more toxic than pirimicarb on adults (Table 3).

Sublethal effects

Imidacloprid and pirimicarb significantly reduced adult longevity ($F = 43.9$; $df = 2, 57$; $P < 0.0001$) and number of offspring per female per day ($F = 106.4$; $df = 2, 57$; $P < 0.0001$) in comparison with the control (Table 4). The population parameters described by intrinsic rate of increase (r_m) ($F = 173.5$; $df = 2, 57$; $P < 0.0001$), net reproductive rate (R_0) ($F = 228.2$; $df = 2, 57$; $P < 0.0001$), generation time (T) ($F = 107$; $df = 2, 57$; $P < 0.0001$) and finite rate of population increase (λ) ($F = 194.6$; $df = 2, 57$; $P < 0.0001$) were affected by both insecticides in comparison to the control (Table 5). Doubling time (DT) also, was affected by imidacloprid ($F = 32.3$; $df = 2, 57$; $P < 0.0001$) (Table 5).

Table 1 Toxicity of imidacloprid and pirimicarb to pre-imaginal and adult stages of *A. gossypii*.

Stage	Imidacloprid				Pirimicarb			
	n	Slope (SE)	LC_{50} ppm ^a (95%FL)	χ^2 (df)	n	Slope (SE)	LC_{50} ppm (95%FL)	χ^2 (df)
1 st instar	1800	1 (0.5)	17 (11-27)	7.42 (3)	1800	2.12 (0.1)	220.2 (186.3-263.2)	5.22 (3)
2 nd instar	1800	1.06 (0.5)	23.9 (17-34.3)	5.29 (3)	1800	2.59 (0.1)	308.8 (274.1-348.9)	3.71 (3)
3 rd instar	1800	1.27 (0.6)	37.2 (26.7-53)	6.50 (3)	1800	2.78 (0.1)	527.2 (450.6-620.1)	7.51 (3)
4 th instar	1800	1.40 (0.6)	70.5 (53.1-95.9)	6.29 (3)	1800	3.22 (0.2)	781.7 (712.4-859.4)	3.58 (3)
Adult	1800	1.46 (0.7)	90.1 (80.4-101.4)	2.33 (3)	1800	3.12 (0.2)	983.1 (862.8-1122.7)	6.68 (3)

^a Lethal concentrations and 95% fiducial limits (FL) were estimated using logistic regression (LeOra Software, 1987).

Table 2 Relative potency of imidacloprid and pirimicarb to compare the susceptibility of developmental stages of *A. gossypii* to each insecticide.

Relative potency or LC_{50} estimates ratio	Imidacloprid	Pirimicarb
LC_{50} (2 nd instar): LC_{50} (1 st instar) (95% CI) ^a	1.42 (0.97-2.09)	1.43 ^b (1.17-1.75) ^b
LC_{50} (3 rd instar): LC_{50} (2 nd instar) (95% CI)	1.61 ^b (1.1-2.39) ^b	1.71 ^b (1.49-1.98) ^b
LC_{50} (4 th instar): LC_{50} (3 rd instar) (95% CI)	1.96 ^b (1.38-2.80) ^b	1.49 ^b (1.29-1.73) ^b
LC_{50} (Adult): LC_{50} (4 th instar) (95% CI)	1.29 ^b (1.02-1.62) ^b	1.26 ^b (1.12-1.40) ^b

^a 95% Confidence interval of relative potency.

^b Relative potency considered significant when its 95% confidence interval did not comprise the value 1.0.

Table 3 Relative potency of imidacloprid and pirimicarb to compare toxicity of both insecticides on each developmental stage of *A. gossypii*.

Stage	Relative potency or LC ₅₀ (Pirimicarb): LC ₅₀ (imidacloprid)	95% Confidence interval of relative potency
1 st instar	15.08 ^a	(5.64-40.99) ^a
2 nd instar	14.51 ^a	(4.90-43.40) ^a
3 rd instar	15.42 ^a	(6.30-36.99) ^a
4 th instar	12.03 ^a	(5.50-25.74) ^a
Adult	11.36 ^a	(5.85-21.69) ^a

^a Relative potency considered significant when its 95% confidence interval did not comprise the value 1.0.

Table 4 The mean rates of longevity and fecundity (SE) of *A. gossypii* adults treated with LC₂₅ of imidacloprid and pirimicarb^a.

Treatment	Longevity (days)	Number of offspring per female/day
Control	14.95 (1.25) ^a	4.33 (0.23) ^a
Imidacloprid	3.05 (0.22) ^c	0.98 (0.07) ^c
Pirimicarb	6.55 (0.97) ^b	2.47 (0.15) ^b

^a Means in a column followed by different small letters are significantly different ($P < 0.05$, LSD, SAS Institute, 2003).

Table 5 The means rates of stable population parameters (SE) of *A. gossypii* adults exposed to LC₂₅ of imidacloprid and pirimicarb^a.

Treatment	R_0	r_m	λ	T_c	DT
Control	55.68 (3.06) ^a	0.438 (0.00) ^a	1.55 (0.01) ^a	9.16 (0.17) ^a	1.58 (0.02) ^b
Imidacloprid	2.54 (0.23) ^c	0.150 (0.01) ^c	1.16 (0.02) ^c	6.21 (0.05) ^c	4.56 (0.48) ^a
Pirimicarb	11.11 (1.13) ^b	0.335 (0.01) ^b	1.39 (0.01) ^b	7.19 (0.18) ^b	2.07 (0.07) ^b

^a Means in a column followed by different small letters are significantly different ($P < 0.05$, LSD, SAS Institute, 2003). R_0 = net reproductive rate; r_m = intrinsic rate of increase; λ = finite rate of increase; T_c = generation time; DT = doubling time.

Discussion

According to the results obtained, the level of sensitivity of *A. gossypii* to the insecticides tested depended on developmental stage with first and second nymphal instars being the most susceptible and susceptibility decreasing with increasing age. The critical time for treatment with insecticides is, therefore, during the earliest instars. Others have similarly reported the higher sensitivity of aphids (Lowery et al. 2005;

Walthall and Stark, 1997) and other sap-sucking pests (Prabhaker et al., 2006; Sohrabi et al. 2011) to insecticides in their earliest instars.

The LC₅₀ value of imidacloprid for first instar nymphs was 17 ppm or 5.9 mg a.i. l⁻¹ in the study reported here. The high activity of this insecticide is also reported in pre-imaginal stages of other aphid species. Lowery and Smirle (2003) determined an LC₅₀ value of 0.064 mg a.i. l⁻¹ for imidacloprid when first instars of *Aphis pomi* (De Geer) were exposed

for three days to insecticide treated apple leaf discs. Sadeghi *et al.* (2009) reported that the same insecticide killed 16% first instar *Acyrtosiphon pisum* (Harris) treated at day 1 with 100 mg l⁻¹ in artificial diet. In other studies, the LC₅₀ value of 0.05-0.5 ppm (a.i.) for third instar *A. pomi* (Lowery *et al.*, 2005) and of 0.19-0.26 mg a.i. l⁻¹ for neonates of *A. pisum* (Walthall and Stark, 1997) are reported. The LC₅₀ value for adults of *A. gossypii* treated with imidacloprid (90.1 ppm) was in the range of that found by Tabacian *et al.* (2011) for *A. gossypii* (43-231 ppm) in Northeast Iran. In contrast, it was higher than those of *A. gossypii* populations from different locations of Europe (1-1.5 mg l⁻¹) (Nauen and Elbert, 2003). Differences between the LC₅₀ values reported here and those of other studies may be due to various factors such as pest species, host species, geographical variation in aphid populations, previous exposure and duration of exposure to insecticide, and as well as type (technical material or formulation) of insecticide used.

All LC₅₀ values reported for imidacloprid in the present study (17-90.1 ppm) were significantly lower than the rate of application of 500 ppm currently used in the field. These results demonstrate the marked effects of imidacloprid against the different stages of *A. gossypii*. As compared to pirimicarb these were greater for all stages of the aphid.

In the present study, pirimicarb affected first, second and third instars of *A. gossypii* at lower concentrations than the recommended rate of application of 700 ppm currently used in the field (Table 1). For fourth instars and adults, however, LC₅₀ values were greater than the concentration recommended for field application. The LC₅₀ value of 527.2 ppm was observed for third instars treated with pirimicarb. In another leaf-dipping bioassay, the LC₅₀ values ranged between 0.57-1.65 ppm (a.i.) for pirimicarb against Washington populations of *A. pomi* (Lowery *et al.*, 2006).

The LC₅₀ value of 983.1 ppm pirimicarb, based on formulated material or 491.5 mg a.i.l⁻¹, that we obtained for adults of *A. gossypii* was in

accordance with that (380-1038 ppm) for populations from Northeast Iran (Tabacian *et al.*, 2011) and with (755-1026 ppm) for populations from North Iran (Torkamand *et al.*, 2013) but was greater than those from England (45-62 mg a.i. l⁻¹) (Moores *et al.*, 1996) and Europe (3.8-14 mg l⁻¹) (Nauen and Elbert, 2003). According to Rongai *et al.* (1998), an Italian population of *A. gossypii* was insensitive to pirimicarb, even at the maximum dose tested (2400 µg a.i. l⁻¹). Differences between the results reported here and those of other studies may be due to various factors mentioned above.

A marked sublethal effect on the stable population parameters of *A. gossypii* was observed when an LC₂₅ of either imidacloprid or pirimicarb was administered to the adult stage. The intrinsic rate of increase in population (r_m) for the untreated control was 0.438 female offspring/female/day and is in agreement with figures of 0.42 (Zamani, 2006) and 0.47 (Shirvani and Hosseinaveh, 2004) for *A. gossypii* on cucumber cultivars Negin and Superdominus, respectively. Satar *et al.* (2005) and Van Steenis & El-khawass (1995), on the other hand, reported r_m values of 0.52 and 0.55, respectively, which are higher than ours. The r_m values obtained for treatment with imidacloprid and pirimicarb were 0.150 and 0.335, respectively; significantly lower than that of the control ($P < 0.0001$). These findings demonstrate the effects of the insecticide treatments on population growth. In contrast, Kerns and Stewart (2000) showed that the intrinsic rate of increase of population of the cotton aphid was unaffected by exposure to bifenthrin, acephate or carbofuran. Walthall and Stark (1997) noted that *A. pisum* populations exposed to sublethal concentrations of imidacloprid were able to sustain a rate of increase similar to that of the controls. Studies on the effect of imidacloprid on imidacloprid-resistant *A. gossypii* populations have shown either an increase in longevity and r_m as compared to the control (Gerami *et al.*, 2012) or no significant difference (Shi *et al.*, 2012). Our findings are not in accordance with these studies since populations of *A. gossypii* resistant to insecticide were put under additional insecticide

pressure whereas in the study reported here, the *A. gossypii* population was maintained under insecticide-free conditions, without the opportunity to develop resistance. In fact, the use of insecticides may lead to the selection of genetically more resistant strains which reproduce more rapidly (Eggers-Schumacher, 1983), or to toxicant-resistant individuals that, when exposed to insecticides, are capable of sustaining rates of increase similar to control populations (Walshall and Stark, 1997).

Values of the other population parameters of *A. gossypii* reported here, namely generation time (T), net reproductive rate (R_0) and finite rate of population increase (λ) decreased with insecticide treatment while doubling time (DT) increased with imidacloprid treatment. These findings are similar to those reported in other studies (Gerami et al., 2007; Khaloobagheri et al., 2006; Lashkari et al., 2007).

In conclusion, our results suggest that imidacloprid and pirimicarb can make a valuable contribution to integrated pest management and will be most efficacious when directed against the first two instars of *A. gossypii*, the stages of development with greatest sensitivity to the insecticides. The possibility that these insecticides may exhibit lethal or sublethal effects on the natural enemies of *A. gossypii* cannot be overlooked and requires investigation.

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اثرات کشندگی و زیرکشندگی ایمیداکلوپرید و پیریمیکارب روی شته جالیز، *Aphis gossypii* Glover (Hemiptera: Aphididae) در شرایط آزمایشگاهی

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چکیده: اثرات سمیت ایمیداکلوپرید و پیریمیکارب برای همه مراحل شته جالیز *Aphis gossypii* Glover با استفاده از روش غوطه‌وری برگ تحت شرایط آزمایشگاهی (دمای 25 ± 1 درجه سلسیوس، رطوبت نسبی 5 ± 65 درصد و دوره نوری $16:8$ ساعت (تاریکی: روشنایی)) مورد ارزیابی قرار گرفت. پوره‌های سن اول آفت نسبت به هر دو حشره‌کش بسیار حساس بودند، به طوری که مقدار LC_{50} برای ایمیداکلوپرید و پیریمیکارب به ترتیب ۱۷ و $220/2$ پی‌پی‌ام بود. برای سایر سنین پورگی، مقادیر LC_{50} برای ایمیداکلوپرید و پیریمیکارب به ترتیب $23/9$ تا $70/5$ پی‌پی‌ام و $308/8$ تا $781/7$ پی‌پی‌ام برآورد شد. مقادیر LC_{50} آفت‌کش‌ها برای بالغین به ترتیب $90/1$ و $983/1$ پی‌پی‌ام بود. سمیت با افزایش سن کاهش یافت. ایمیداکلوپرید برای همه مراحل رشدی سمی‌تر از پیریمیکارب بود. اثر غلظت‌های زیرکشنده این حشره‌کش‌ها با استفاده از سم‌شناسی دموگرافیک ارزیابی شد. بقا و پارامترهای رشد جمعیت، شامل نرخ ذاتی رشد جمعیت (r_m)، نرخ خالص تولید مثل (R_0)، طول مدت یک نسل (T_c) و نرخ متناهی افزایش جمعیت (λ) به طور منفی توسط هر دو حشره‌کش تحت تأثیر قرار گرفت. در تیمارهای شاهد، ایمیداکلوپرید و پیریمیکارب مقادیر r_m به ترتیب $0/438$ ، $0/150$ و $0/335$ نتاج ماده به‌ازای هر فرد ماده در روز بود. مدت زمان دو برابر شدن جمعیت (DT) نیز به وسیله ایمیداکلوپرید تحت تأثیر قرار گرفت. نتایج نشان داد که حشره‌کش‌های مذکور علیه شته *A. gossypii* می‌توانند، مؤثر باشند.

واژگان کلیدی: *Aphis gossypii*، کاربامات، سم‌شناسی دموگرافیک، نئونیکوتینوئید.