

Comparison of Susceptibility of Melon Aphid populations, *Aphis gossypii* Glover (Hemiptera: Aphididae), from Seven Regions in Iran to Pirimicarb and Malathion

Mona Torkamand^{1*}, Ahmad Heidari², Hamid Ghajarieh³ and Leila Faravardeh²

1. Former Graduate student, Collage of Abouraihan, Tehran University, Iran.

2. Pesticides Research Department, Iranian Research Institute of Plant Protection, Iran.

3. Department of Plant Protection, Collage of Abouraihan, Tehran University, Iran.

Abstract: The melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is one of the most important pests of agricultural products in Iran. In this study, susceptibility of melon aphid populations collected from seven regions (Yazd, Varamin, Mohammadshahr, Esfahan, Karaj, Hashtgerd and Ramsar) 24 h after exposure to pirimicarb and malathion in 2009 was compared. Bioassay experiments on adults were carried out using leaf dipping method. Squash (*Cucurbita pepo*) leaves were dipped in different concentrations of insecticides in laboratory conditions (25 ± 0.2 °C, $60 \pm 5\%$, 16: 8 [L: D] h). Control treatment was dipped in distilled water. Each test was replicated three times. The results showed that LC_{50} values of malathion for Ramsar, Karaj, Mohammadshahr, Hashtgerd, Varamin, Esfahan, and Yazd populations were 162.99, 159.19, 117.94, 79.96, 38.81, 36.32, and 20.25 ppm, respectively. Also, LC_{50} values of pirimicarb for Yazd, Mohammadshahr, Kararj, Hashtgerd, Ramsar, Esfahan, and Varamin populations were 1414.16, 1359.34, 1057.62, 970.33, 881.87, 806.14, and 601.98 ppm, respectively. According to results, aphid populations of Varamin and Yazd had the most susceptibility to pirimicarb and malathion, respectively. Also, aphids of Yazd and Ramsar populations had the least susceptibility to pirimicarb and malathion, respectively. Regarding the fact that Yazd and Ramsar populations had the least susceptibility to pirimicarb and malathion, respectively, there is a possibility that melon aphids in these regions are susceptible to other organophosphate and carbamate pesticides with similar mode of action. Therefore, in the pest management program, careful chemical control measures for melon aphid in these regions is recommended.

Keywords: *Aphis gossypii*, pirimicarb, malathion, Susceptibility, Iran

Introduction

The Cotton aphid or Melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is one of the most important pests of cotton, cucurbits and vegetables (Blackman and Eastop, 2000). This

pest damages crops either directly by feeding which results in curling and deformation of young leaves and twigs, or indirectly by transmission of viruses and contaminating fruits with honeydew which in turn may cause the growth of black sooty mould which inhibits photosynthesis and therefore causes substantial yield loss (Jacobson and Croft, 1998; Andrews *et al.*, 2004). The melon aphid has at least 900 known host plants and transmits over 50 plant viruses (Blackman and Eastop, 2000).

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* **Corresponding author**, e-mail: mttorkamand@gmail.com

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Afshar (1939) reported this pest for the first time in Iran. *A. gossypii* is the main pest of cucumber, melon, watermelon, squash, cotton, eggplant, tobacco, tomato, and sesame, sometimes pistachio and citrus in Iran (Behdad, 2002). This pest has been spread in all regions of Iran (Farahbakhsh, 1961). Its status has changed in recent years and it has been considered as a serious pest in main cotton production areas of Iran (Darvish-Mojeni and Rezvani, 1997; Afshari *et al.*, 2006).

The effective management of melon aphid and its associated plant diseases include cultural, biological, and chemical control. Chemical treatment remains the main method of melon aphid control (Irshaid and Hassan, 2011). Many insects, and especially aphids, are known to have adapted rapidly to recent strong selective pressures such as insecticides application (Denholm *et al.*, 2002). Melon aphid has the potential to develop resistance to insecticides due to high reproductive potential (Mallet and Luttrell, 1991). Also, intensive use of insecticides often leads to resistance development by sprayed aphids, forcing farmer to increase dosage and application frequency. Misuse of chemical insecticides might be accountable for the outbreaks of the pest because extensive and intensive use of insecticides exerts heavy selection pressure on target pests and accelerates resistance development (Hirai, 1993). Resistance of *A. gossypii* to some insecticides has been reported (Andrew *et al.*, 2006). According to Insecticide Resistance Action Committee reports (2012), resistance to insecticides has been documented in many *A. gossypii* populations from China, France, Germany, Greece, Italy, Japan, Madagascar, Netherlands, Pakistan, Peru, Portugal, Spain, Zambia, the USA and UK. Also, Melon aphid has shown resistance to 41 active ingredients (Insecticide Resistance Action Committee reports, 2012) such as malathion (Shang *et al.*, 2012) and pirimicarb (Nauen and Elbert, 2003).

According to Agricultural Statistics of Iran, chemicals are applied annually on around 12 million hectares for controlling all agents that

damage plants in Iran (Anonymous, 2011). In Iran, selected insecticides are commonly used to control various aphids on different crops, not only in the fields and gardens, but also in greenhouses (Tabasian *et al.*, 2010). The current insecticides registered and recommended for chemical control of melon aphid in Iran include imidacloprid, pirimicarb, pymetrozine, thiometon, dichlorvos, malathion, and heptenophos (Meschi, 2006).

Pirimicarb and malathion are commonly used for controlling melon aphid in Iran (Meschi, 2006). Pirimicarb is a selective aphicide that acts as an acetyl cholinesterase inhibitor (Menozzi *et al.*, 2004). This aphicide was imported for the first time to Iran in 1979. Then, pirimicarb has been registered for aphids on rose, tobacco, cotton, and vegetables (Meschi, 2006). The registered formulations of pirimicarb in Iran are WP 50% and DF 50% (Meschi, 2006). Malathion is an organophosphate insecticide which binds irreversibly to acetyl cholinesterase (AChE) (Tomlin, 2009). Malathion is active in insect's body and is less poisonous to mammals. "Safe" is a term often used to describe malathion. However, recent research shows that malathion poses serious hazards (Anonymous, 2003). The registered formulations of malathion in Iran are EC 57%, EC 50%, WP 57%, D 4%, O 11.4% and TC 96% (Meschi, 2006).

Pesticide resistance management requires an investigation of pest resistance development. Aphids' high potential for development of resistance to pesticides necessitates studying their state of susceptibility to pesticides. Therefore, in this study we determined the susceptibility of melon aphid from seven regions in Iran, to conventional chemical insecticides pirimicarb and malathion, that are to be applied in those areas.

Materials and Methods

Plant rearing

Squash plants, *Cucurbita pepo* (variety Royal), were used as host for melon aphid culturing. Two to three seeds of squash were planted in each plastic cup (9 cm diameter by 12 cm depth) in sterilized soil (45% sand, 45% silt, and 10%

clay) and perlite with a 10: 1 (soil: perlite) ratio. After planting, the cups were watered and placed in a greenhouse at 25 ± 1 °C, RH: 55-60% and 16: 8 (L: D) h conditions in the Iranian Research Institute of Plant Protection. Planting date was noted on each cup. Plants were watered four times a week. When seeds germinated, N: P: K (20: 20: 20) fertilizer was used on plants once a week. Squash plants at 3 to 4 leaf stage were used as host plants for aphids.

Melon aphid rearing

Seven populations of *A. gossypii* were collected from Varamin (Tehran province), Hashtgerd (Tehran province), Karaj (Alborz province), Mohammadshahr (Alborz province), Ramsar (Gilan province), Esfahan (Esfahan province), and Yazd (Yazd province) regions in Iran in 2009. Leaves with heavy infestations of melon aphid were removed from plants in each field, placed in plastic bags, and transferred to the laboratory in the Iranian Research Institute of Plant Protection. Each population of melon aphids was kept on the squash plants in separate plastic cages (60 × 60 × 60 cm) covered with cloth screen and cages were maintained at 25 ± 1 °C, RH: 55-60% and 16: 8 (L: D) h conditions.

To get homological aphids in bioassay tests, twenty adult aphids of each population were released on squash plants in separate cages for 24 h. Then the adult aphids were removed from plants and first instar aphids were left to become adults for about one week and were used for bioassay tests.

Insecticides

The applied insecticides in this study were pirimicarb (Pirimi G[®]) (WP 50%, Giyah Company) and malathion (Malathion[®]) (EC 57%, Shimi Keshavarz Company).

Bioassay method

A leaf dip bioassay similar to that described by Koziol and Semtner (1984) was used to determine the susceptibility of different populations of melon aphid to pirimicarb and malathion. Leaf discs (50 mm diameter) were cut from squash leaves. Then leaf discs were dipped into pirimicarb or malathion

solutions for five seconds and allowed to dry in room condition for 30 minute. Once the leaves had dried, they were placed individually into Petri dishes (50 mm diameter) and 15 adult aphids reared as described above were released into each Petri dish. Control treatment was dipped in distilled water only. The dishes were held closed with a rubber band and were kept in growth chamber at 25 ± 0.2 °C, RH: 55-60% and 16: 8 (L: D) h condition. The range of malathion concentrations for preliminary test was 10-40 ppm for Yazd, 10-60 ppm for Esfahan, 15-60 ppm for Varamin, 20-300 ppm for Hashtgerd, 50-300 ppm for Mohammadshahr, 50-500 ppm for Ramsar, and 50-700 ppm for Karaj populations. Also, the range of pirimicarb concentrations for preliminary test was 300-1200 ppm for Varamin, 400-1700 ppm for Esfahan, 200-2000 ppm for Ramsar, 500-1600 ppm for Hashtgerd, 500-1600 ppm for Karaj, 700-2000 ppm for Mohammadshahr, and 700-2100 ppm for Yazd populations.

Each concentration was replicated three times. Mortality was assessed at 24 hours after treatment. Aphids were considered dead if they could not take a coordinated step after being gently stroked several times with a fine paint brush. The selected ranges of concentrations for pirimicarb and malathion were examined in preliminary tests in order to establish mortality ranging from 20% to 85%.

Data analysis

POLO-PC software (LeOra software, 1987) was used for determining LC₅₀ and other related parameters. The POLO-PC software was used to estimate LC₅₀. The results were considered statistically significant when the *P* value was < 0.05 (Robertson and Preisler, 1992) and there were no overlaps of confidence limits (95%) of LC₅₀ values (Adams *et al.*, 1990) for both pesticides.

Results

Susceptibility to pirimicarb

The results of the comparison of LC₅₀ values in different populations of *A. gossypii* after 24h

showed that different populations had various susceptibility to pirimicarb (Table 1). For adults of *A. gossypii* exposed to pirimicarb, LC₅₀ values ranged from 601.98 ppm to 1414.16 ppm. The aphids populations of Varamin and Yazd had the most and the least susceptibility to pirimicarb, respectively. There was significant difference in susceptibility of Varamin and Yazd populations ($\chi^2 = 7.77$; df = 12; $P < 0.05$).

Aphids collected from Karaj were moderately more tolerant to pirimicarb compared with those from Esfehan and there were no overlaps of confidence limits of LC₅₀ values. Also, comparing the responses of the seven populations revealed that Hashtgerd and Ramsar populations were moderately less susceptible to pirimicarb compared with Esfehan, but there were considerable overlaps in individual values (LC₅₀).

The susceptibility of different population form the highest to the lowest was in the following order Varamin > Esfehan > Ramsar > Hashtgerd > Karaj > Mohammadshahr > Yazd populations had the highest susceptibility to pirimicarb after 24h, respectively.

The results showed that there were no overlaps of confidence limits of LC₅₀ values in Varamin population compared to other

populations. The aphids of Varamin (the most susceptible) were 2.3-fold more susceptible than aphids of Yazd (the least susceptible) populations after 24h.

Susceptibility to malathion

For adults of *A. gossypii* exposed to malathion after 24h, LC₅₀ values ranged from 20.25 ppm to 162.99 ppm (Table 2). The results of current study revealed that aphid populations of Yazd and Ramsar had the highest and the least susceptibility to malathion after 24h, respectively. There was significant difference in susceptibility of Yazd and Ramsar populations ($\chi^2 = 8.37$; df = 9; $P < 0.05$). In addition, aphids of Hashtgerd population had moderate susceptibility to malathion. The results of the comparison of LC₅₀ values in different populations of *A. gossypii* to malathion showed that aphids of Yazd > Esfehan > Varamin > Hashtgerd > Mohammadshahr > Karaj > Ramsar populations had the highest susceptibility, respectively. In fact, susceptibility of aphid population from Yazd (the most susceptible) was 8 times greater than that of aphid population from Ramsar (the least susceptible).

Table 1 Susceptibility of seven *Aphis gossypii* populations from Iran 24 hours after exposure to pirimicarb.

Population	n	Slope \pm SE	LC ₅₀ (ppm)	LC ₉₀ (ppm)	Chi-square	Df
			Confidence Limits 95%			
Hashtgerd	315	2.54 \pm 0.44	970.33 (834.68-1106.6)		3.62	5
Mohamadshahr	315	3.81 \pm 0.55	1359.34 (1238.8-1488.91)		4.62	5
Karaj	315	4.14 \pm 0.55	1057.62 (969-1150.54)		2.24	5
Varamin	315	3.92 \pm 0.4	601.98 (542.8-660.6)		1.81	5
Ramsar	360	2.2 \pm 0.23	881.87 (755.15-1026.58)		4.34	6
Esfahan	360	3.41 \pm 0.36	806.14 (728.2-887.82)		0.74	6
Yazd	360	3.16 \pm 0.43	1414.16 (1279.66-1577.79)		4.26	6

n: Number of insects per each experiment.

Table 2 Susceptibility of seven *Aphis gossypii* populations from Iran 24 hours after exposure to malathion.

Population	n	Slope \pm SE	LC ₅₀ (ppm)*	LC ₉₀ (ppm)*	Chi-square	Df
			Confidence Limits 95%			
Hashtgerd	270	2.76 \pm 0.29	79.96 (68.14-92.91)	233.09 (188.04-314.76)	3.11	4
Mohamadshahr	270	3.86 \pm 0.43	117.94 (105.61-132.19)	227.8 (197.39-277.93)	3.85	4
Karaj	270	2.37 \pm 0.25	159.19 (131.56-188.89)	554.17 (436.06-775.62)	0.78	4
Varamin	270	1.83 \pm 0.38	38.81 (31.96-50.28)	197.16 (115.22-637.43)	0.41	4
Ramsar	270	2.45 \pm 0.3	162.99 (138.33-190.94)	544.52 (418.52-813)	3.46	4
Esfehan	270	2.7 \pm 0.41	36.32 (31.27-41.76)	108.48 (83.09-172.85)	3	4
Yazd	270	3.02 \pm 0.44	20.25 (17.76-22.91)	53.74 (42.62-79.62)	3.65	4

n: Number of insects per each experiment.

Discussion

By ranking the susceptibility of *A. gossypii* collected from seven regions in Iran, it clearly showed that susceptibility to pirimicarb and malathion varied among the seven populations. The results of current study revealed that susceptibility to pirimicarb was on the whole less than that to malathion in all populations. Actually, malathion had more lethal effect on melon aphid than pirimicarb in Iran. Also, there was significant difference between confidence limits in malathion LC₅₀ values and pirimicarb LC₅₀ values in each population [Esfahan ($x^2 = 6.14$; df = 11; $P < 0.05$), Hashtgerd ($x^2 = 6.93$; df = 10; $P < 0.05$), Karaj ($x^2 = 11.04$; df = 10; $P < 0.05$), Mohammadshahr ($x^2 = 4.98$; df = 10; $P < 0.05$), Ramsar ($x^2 = 8.35$; df = 11; $P < 0.05$), Varamin ($x^2 = 6.51$; df = 10; $P < 0.05$), and Yazd ($x^2 = 7.96$; df = 11; $P < 0.05$)].

These results were similar to findings of Wang *et al.*, (2007) who found that resistance of different populations of *A. gossypii* collected from five regions in China varied greatly during the years (1985-2004), and among the five regions (Binzhou, Heze, Liaocheng, Dezhou,

and Taian) in response to six pesticides (fenvalerate, omethoate, imidacloprid, acetamiprid, carbosulfan, and endosulfan).

The results of the present experiment showed that aphids from Varamin region had highest susceptibility to pirimicarb, whereas aphids collected from Yazd were more susceptible to Malathion. Also, aphids of Yazd and Ramsar populations had the least susceptibility to pirimicarb and malathion, respectively. These results are similar to studies by Herron *et al.*, (2000) who found that melon aphids collected from New South Wales were susceptible to pirimicarb and many organophosphate pesticides. Whereas, aphids collected from north and west Australia such as Queensland were more susceptible.

The investigation of pest susceptibility in different regions and years is one of the best methods for susceptibility monitoring. Khalobagheri (2003) in Iran examined the susceptibility of *A. gossypii* collected from Mehrshahr Karaj (Alborz province), to pirimicarb. His finding revealed that LC₅₀ value of pirimicarb in Mehrshahr population was 375.73 ppm after 24h. Comparing the results of Khalobagheri's (2003), we found that the

tolerances of Mohamadshahr and Karaj populations (Alborz province) were increased in 2009. In fact, the susceptibility of the Alborz province populations of melon aphid to pirimicarb had decreased 2.8 to 3.6 folds from 2003 until 2009. Probably continuous application of pirimicarb for aphid control in Karaj region has contributed to reduced susceptibility of aphids to pirimicarb.

The recommended dose of pirimicarb for melon aphid control is 0.5-0.7 kg/ha in Iran (Meschi, 2006). The ratio of calculated LC_{50} value to recommended concentration of pirimicarb after 24h in different populations of melon aphid was 2, 2, 1.5, 1.4, 1.2, 1.2, and 0.8 in Yazd, Mohamadshahr, Karaj, Hashtgerd, Ramsar, Esfahan, and Varamin populations, respectively. However, this relation in the Karaj population was 0.53 in 2003 (Khalobagheri, 2003).

Also, the recommended dose of malathion for aphid control is at the concentration of 0.2% in Iran (Meschi, 2006). The relation of calculated LC_{50} values to recommended concentrations of malathion in different populations of melon aphid were 0.08 (Ramsar and Karaj), 0.06 (Mohamadshahr), 0.04 (Hashtgerd), 0.02 (Esfahan and Varamin), and 0.01 (Yazd).

The results of this study indicated that the aphids of Yazd population were less susceptible to pirimicarb than other populations. Whereas, this population (Yazd) was more susceptible to malathion. Differences between susceptibility of different populations to pirimicarb and malathion in this study are similar to results of Miller (2003) who found that differences in susceptibility to malathion, diazinon, and dimethoate in varied populations of aphids may be due to variation in dosage rate and frequency of application of pesticides by different farmers.

Devonshire (1989) suggested that *A. gossypii* had high tolerance to pirimicarb because of existence of a mutant form of acetylcholinesterase that is less sensitive to inhibition by pirimicarb. Takada and Murakami (1998) showed that high esterase activity in melon aphid plays an important role in resistance to malathion and pirimicarb.

As the applied insecticide imposes more pressure on insect population and causes more mortality, the individuals carrying the resistance gene increase, thereby the resistance trend will accelerate. Regression analysis in different populations of melon aphid showed that slope of pirimicarb is steeper than the slope of malathion in Karaj (Slope \pm SE = 4.14 ± 0.55), Varamin (Slope \pm SE = 3.92 ± 0.4), Esfahan (Slope \pm SE = 3.41 ± 0.36), and Yazd (Slope \pm SE = 3.16 ± 0.43) populations. Highest slope line indicating heterogeneity is higher. Also, highest heterogeneity in these populations (Karaj, Varamin, Esfahan, and Yazd), indicates a high potential for increasing resistance to pirimicarb. In fact, these populations need to be exploited in managerial programs for preserving susceptibility.

Hardman *et al.*, (1959) and Kuperman *et al.*, (1961) suggested that the slope of the probit regression reflects the quality of the enzyme systems that detoxify insecticides in an insect's body. Thus, parallel lines may indicate that organisms have qualitatively identical, but quantitatively different, levels of detoxification enzymes (Robertson and Preisler, 1992). The present study proved that probit regression slopes of pirimicarb and malathion were parallel in Esfahan, Ramsar, Mohammadshahr, Hashtged, and Yazd. Whereas, slopes of probit regression of pirimicarb and malathion were non-parallel in Pardis (Karaj) and Varamin populations.

Populations of melon aphid have demonstrated the ability to develop resistance to several insecticides especially for organophosphate and carbamate insecticides (Longly, 1999). According to results of current study, malathion is more effective than pirimicarb for controlling melon aphid in Iran.

Probably the greatest factor contributing to the development of insecticide resistance in pests is long term and frequent use of a single insecticide or a class of insecticides (Tang *et al.*, 1988).

Mojeni and Rezwani (2000) investigated effect of imidacloprid, pymetrozine, benfuracarb, and methamidophos on *A. gossypii* in two regions of Golestan Province in Iran.

Their results revealed that imidacloprid, pymetrozine, and benfuracarb had the highest effect on melon aphid.

Therefore, use of different insecticides that have different mode of action is recommended for management of resistance to insecticides. However, reducing the number of applications is the best resistance management strategy for preserving susceptibility. Integrated pest management (IPM) tactics such as monitoring pest levels, use of biological and cultural controls, insecticide application when aphids reach the action threshold, and using the most selective insecticides, have been suggested for melon aphid control and management of insecticide resistance of this pest (Praat *et al.*, 1996). Also, proper IPM program must be established to control this pest, depending on integration between parasitoid application and a non-persistent pesticide spraying (Irshaid and Hassan, 2011).

It is suggested that even where *A. gossypii* is susceptible to pirimicarb (such as Varamin population) or to malathion (such as Yazd population); growers should adopt pest management strategies such as application of different insecticides (with different mode of action) alternatively. The conserving insecticide susceptibility takes the development of knowledge and research on insecticide. In addition, susceptibility of *A. gossypii* to pirimicarb and malathion in Iran must be considered as an important factor in management planning for future.

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مقایسه حساسیت هفت جمعیت مختلف شته جالیز (*Aphis gossypii* Glover (Hemiptera: Aphididae) نسبت به سموم پیریمیکارب و مالاتیون در هفت منطقه ایران

مونا ترکمند*، احمد حیدری، حمید قاجاریه و لیلا فرآورده

- ۱- فارغ التحصیل مقطع کارشناسی ارشد حشره‌شناسی کشاورزی، دانشکده گیاه‌پزشکی، دانشگاه تهران، پردیس ابوریحان
 - ۲- دانشیار بخش تحقیقات آفت‌کش‌ها، مؤسسه تحقیقات گیاه‌پزشکی کشور، تهران
 - ۳- دانشیار گروه گیاه‌پزشکی، دانشکده گیاه‌پزشکی، دانشگاه تهران، پردیس ابوریحان
 - ۴- دانشیار بخش تحقیقات آفت‌کش‌ها، مؤسسه تحقیقات گیاه‌پزشکی کشور، تهران
- * پست الکترونیکی نویسنده مسئول مکاتبه: mttorkamand@gmail.com
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چکیده: شته جالیز، *Aphis gossypii* Glover (Hemiptera: Aphididae)، یکی از آفات مهم محصولات کشاورزی در ایران می‌باشد. در این تحقیق حساسیت جمعیت‌های مختلف شته جالیز که از هفت منطقه (یزد، ورامین، محمدشهر، اصفهان، کرج، هشتگرد و رامسر) در سال ۱۳۸۷ جمع‌آوری گردید، نسبت به سموم پیریمیکارب و مالاتیون بعد از گذشت ۲۴ ساعت مقایسه شد. آزمایشات زیست‌سنجی روی شته‌های بالغ با استفاده از روش غوطه‌ور نمودن برگ کدو (*Cucurbita pepo*) در غلظت‌های مختلف حشره‌کش‌ها و در شرایط آزمایشگاهی (دما 25 ± 0.2 °C، رطوبت نسبی $50 \pm 5\%$ و دوره نوری ۱۶:۸ [تاریکی:روشنایی]) انجام شد. نمونه‌های شاهد نیز در آب مقطر غوطه‌ور گردیدند. هر آزمایش سه مرتبه تکرار شد. نتایج حاصله نشان داد که مقادیر LC_{50} مالاتیون برای جمعیت‌های رامسر، کرج، محمدشهر، هشتگرد، ورامین، اصفهان و یزد به ترتیب ۱۶۲/۹۹، ۱۵۹/۱۹، ۱۱۷/۹۴، ۷۹/۹۶، ۳۸/۸۱، ۳۶/۳۲ و ۲۰/۲۵ پی‌پی‌ام بود. همچنین مقادیر LC_{50} پیریمیکارب برای جمعیت‌های یزد، محمدشهر، کرج، هشتگرد، رامسر، اصفهان و ورامین به ترتیب ۱۴۱۴/۱۶، ۱۳۵۹/۳۴، ۱۰۵۷/۶۲، ۹۷۰/۳۳، ۸۸۱/۸۷ و ۸۰۶/۱۴ پی‌پی‌ام بود. براساس نتایج حاصله، شته‌های جمعیت ورامین و یزد به ترتیب بیشترین حساسیت را نسبت به پیریمیکارب و مالاتیون نشان دادند. همچنین در جمعیت‌های یزد و رامسر به ترتیب کمترین حساسیت نسبت به پیریمیکارب و مالاتیون مشاهده شد. با توجه به اینکه جمعیت‌های یزد و رامسر به ترتیب حساسیت کمتری به پیریمیکارب و مالاتیون نشان دادند، احتمال اینکه شته‌های جالیز در این مناطق نسبت به سایر سموم ارگانوفسفره و کاربامات (نحوه عمل مشابه) حساس باشند، وجود دارد. بنابراین پیشنهاد می‌شود در این مناطق برنامه مدیریت آفات برای کنترل شیمیایی شته جالیز با دقت بیشتری صورت گیرد.

واژگان کلیدی: شته جالیز، پیریمیکارب، مالاتیون، حساسیت، ایران