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LC$_{30}$ effects of thiamethoxam and pirimicarb, on population parameters and biological characteristics of *Macrolophus pygmaeus* (Hemiptera: Miridae)

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Abstract

Chemical pesticides have important role in integrated pest management strategies. However, they can adversely affect on natural enemies as non-target organisms, even in sublethal concentrations. In this study, sublethal effects of two insecticides, thiamethoxam and pirimicarb, were examined on demographic parameters of an important predator, *Macrolophus pygmaeus*. Bioassay results indicated that LC$_{30}$ of thiamethoxam and pirimicarb, applied on the third instar larvae, were 451.6 and 2013.4 mg (ai) L^{-1}, respectively. The two insecticides extended the pre-adult duration, significantly. Demographic parameters were analyzed by two-sex life table. The results showed that all of the main demographic traits (r, λ, R$_0$ and T) have been changed significantly and there are also some changes in other parameters such as age-specific survival rate (l_x) and life expectancy (e_x). Intrinsic rate of increase in control was 0.15 but it reduced to 0.10 and 0.99 day^{-1} in thiamethoxam and pirimicarb treatments, respectively. Also, finite rate of increase in control, thiamethoxam and pirimicarb treatments was 1.11, 1.08 and 1.03 day^{-1} respectively. Reproductive rate in control showed 36.75 offspring/individual but this statistic in thiamethoxam and pirimicarb treatments was 19.62 and 18.24, respectively. Mean generation time was 22.69 days in control but it extended in both treatments and illustrated 27.79 and 31.24 days in thiamethoxam and pirimicarb treatments, respectively. Thus, obtained results in this study showed that although pirimicarb and thiamethoxam are selective insecticides, they have potential to affect on the predator, *M. pygmaeus* severely, and need to take care in IPM programs.

Keywords selective pesticides; predator; sub-lethal concentration; life table; biological trait.

1 Introduction

Combination of selective pesticides with biological control agents (predators and parasitoids) is one of the

major purposes of IPM strategies (Elzen, 2001; Desneux et al., 2006). Therefore, measuring side effects of both of lethal and sublethal effects of chemicals on natural enemies is essential prior to IPM programs execution (Desneux et al., 2007; Stark et al., 2007). Insecticides in sublethal concentrations, can adversely affect physiological and biological parameters such as longevity, fecundity, oviposition, sex ratio, developmental rate, behavior, mobility, weight, feeding, etc. (Desneux et al., 2003, 2007; Galvan et al., 2005). Many studies in the last few years have reported both lethal and sublethal adverse effects of pesticides on non-target beneficial organisms (Schneider et al., 2004, 2008, 2009; Desneux et al., 2007; Cloyd and Bethke, 2011; Fogel et al., 2013; Rahmani and Bandani, 2013; Martinou et al., 2014; Gontijo et al., 2014; Yao et al., 2015). One of the most important statistical parameters shows overall toxicity of pesticides more precisely, is population growth rate (r_m) evaluating by demographic analysis (Kim et al., 2004; Stark et al., 2007; Schneider et al., 2009). In fact, r_m in ecotoxicology, explains complex relationships between toxic compounds and population dynamics to predict the ecotoxicological effects, especially when sublethal effects are expected (Billoir et al., 2007).

The predator, *Macrolophus pygmaeus* (Rambur) is native to the Mediterranean region. Due to its potential as predator, it has been commercially mass produced and successfully released in temperate and Mediterranean crops (Martinou and Wright, 2009). *Macrolophus pygmaeus* is a polyphagous insect and stay alive in the absence of prey (whiteflies, aphids, mites, thrips, and eggs and larvae of lepidopterous pests) by feeding on plant sap (Urbaneja et al., 2009, 2012; Perdikis et al., 2011; Martinou et al., 2014).

Because of the broad spectrum of prey, *M. pygmaeus* can be exposed to many pesticides applying on the different crops. Thiamethoxam and pirimicarb are two selective contact insecticides with systemic characteristics. The former is a neonicotinoid, acts by binding to nicotinic acetylcholine receptors and provides excellent control of a broad range of commercially important pests like aphids and whiteflies (Maienfisch et al., 2001; Acda, 2007). The late is a fast-acting carbamate aphicide operates as a cholinesterase inhibitor (Silver et al., 1995; Kwon et al., 2009).

There are several studies measuring effects of different factors on *M. pygmaeus* life table (Perdikis and Lykouressis, 2002; Perdikis and Lykouressis, 2004; Diaz et al., 2014) but focusing on effects of chemicals on this species was rare and more about lethal outcomes (Arno and Gabarra, 2011; Martinou et al., 2014). Therefore, this study was performed to recognize the effects of pirimicarb (Pirimor®) and thiamethoxam (Actara®) on some of biological and demographic parameters of *M. pygmaeus*.

2 Materials and Methods

2.1 Insect culture

Bemisia tabaci (Gennadius) as prey, was collected from the cotton fields in Golestan province, Iran, and reared in the cages (70 × 70 × 70 cm^3) at the laboratory conditions (27 ± 1 °C, 65 ± 10% RH and 16L: 8D hour photoperiod) for four generations. *Macrolophus pygmaeus* was collected from tomato fields in Hashtgerd, Alborz province, Iran, and reared on the *B. tabaci* at the above conditions, for four generations.

2.2 Chemicals and toxicity bioassays

In this experiment commercial formulation of two insecticides, thiamethoxam (Actara®, WG 25%, Syngenta, India) and pirimicarb (Pirimor®, WP 50%, Giah, Iran) were used. Toxicity of the insecticides was assessed on the third instar larvae of *M. pygmaeus*, using topical application method. Bioassay was done in five concentrations (treatments) and three replications, during several days. Serial dilutions were prepared in acetone and solvent (acetone) alone was used as control. One microliter of each solution applied on the dorsal abdomen of the larvae using micropipette. For each treatment, more than 100 insects -obtained from six hours cohort eggs- were used. The treated insects were put in Petri dishes (60 mm in diameter × 15 mm in height)

with cotton leaf infested by *B. tabaci* as food. Mortality was assessed 24 hours after treatment.

2.3 sub-lethal Effects of pirimicarb and thiamethoxam on biology and population traits

Macrolophus pygmaeus lays its eggs into the stems of cotton. A stereomicroscope was needed to detect the horn of the eggs protruding from stems. Each stem, individually, was placed into a cylinder made of transparent plastic and were checked every 24 hours until the eggs hatched (PERDIKIS and Lykouressis, 2000). Newly emerged larvae were transferred to the new Petri dishes and supplied daily by all stages of *B. tabaci* as food. Petri dishes were kept in a growth chamber at 27 ± 1 °C, 65 ± 10% RH and photoperiod of 16L: 8D hours. The experiment had three treatments including control and LC_{30} of Actara® and pirmor®. For each treatment 100 eggs were exposed and each egg was considered as one replicate (Chi and Yang, 2003; Schneider et al., 2009; Rahmani and Bandani, 2013). The experimental units were surveyed every day. When larvae reached to the third instar stage, they were topically treated with both insecticides at concentrations causing 30 percent mortality like bioassay experiment. Third instars (L_3) were chosen because of less natural mortality (Booth et al., 2007) and higher voracity (Schneider et al., 2009). Checking the larval mortality and development were continued. After the emergence of adults, males and females were paired and checked daily in order to record their survival and the numbers of laid eggs. The experiments continued until the death of all the individuals.

2.4 Data analysis

In the bioassay experiment, concentration-mortality regression for the larvae was evaluated using probit analysis (Polo-PC Probit and Logit analysis; LeOra Software, 1997) in order to determine the LCs and slopes in 95% Fiducial Limit (FL).

In the sub-lethal concentrations experiment, fecundity, developmental time, and the life table parameters including intrinsic rate of increase (*r*), net reproductive rate (R_0), mean generation time (*T*), gross reproductive rate (*GRR*), and finite rate of increase (*λ*) were estimated. In addition to, life expectancy (e_x), age-specific survival rate (l_x), age-specific fecundity (m_x), pre-oviposition period of female adult (APOP) and total pre-oviposition period (TPOP) were calculated based on age-stage, two-sex life table (Chi, 1988) using TWOSEX-MSChart software (Chi, 2014). Calculation of the population parameters has been exposed in the following:

Net reproductive rate (R_0)

$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

Intrinsic rate of increase (*r*)

$$\sum_{x=0}^{\infty} e^{-1(x+1)} l_x m_x = 1$$

Mean generation time (*T*)

$$T = \frac{\ln(R_0)}{r}$$

Finite rate of increase (*λ*)

$$\lambda = e^r$$

General linear models (PROC GLM, SAS Institute 2003) was used for analysis of variance and the mean comparison was done by Tukey test in order to determine the significant differences in life history traits between exposed and unexposed *M. pygmaeus* individuals to insecticides. The probability level of *P*< 0.05 was used for significant difference. The means and standard errors of the life table parameters were estimated using the bootstrap techniques (Efron and Tibshirani, 1993) embedded in the TWOSEX-MSChart (Chi, 2014). Survival, fecundity and reproductive values curves were constructed using SigmaPlot 11.0.

3 Results

The bioassay results showed that LC_{50} for the third instar larva was 1041.3 (932.5-15041.2) and 3210.3 (2441.2-4113.5.5) mg (ai) / L in thiamethoxam and pirimicarb treatments, respectively (Table 1). In addition to, the concentrations and in parenthesis, the 95 percent confidence intervals, of thiamethoxam and pirimicarb, caused 30 percent mortality were 451.6 (243.2-689.2) and 2013.4 (1145.1-1318.3) mg (ai) / L, respectively (Table 1).

Table 1 Toxicity of thiamethoxam and pirimicarb on the third instar larvae of *Macrolophus pygmaeus*.

Insecticide	N^a	Concentration mg (ai) litre $^{-1}$ (95% CL)$^{-1}$		Slope ± SE	X^2 (df)
		LC_{30}	LC_{50}		
Thiamethoxam	320	451.6 (243.2-689.2)	1041.3 (932.5-15041.2)	3.01±0.13	0.51(30)
Pirimicarb	320	2013.4 (1145.1-1318.3)	3210.3 (2441.2-4113.5.5)	1.08±.05	12.09(30)

X^2 is significant (p < 0.05)
[a] Number of subjects

Long term effects of sublethal concentrations of thiamethoxam and pirimicarb (LC_{30}) changed pre-adult duration and fecundity, significantly (Table 2). In control, pre-adult period (egg to pupae) had been spent faster than thiamethoxam and pirimicarb treatments (*P*< 0.0001). Moreover, in control, females laid more eggs (about 1.5 times) than both treatments (*P*< 0.0001, *F*= 1.36, *df*= 52). However, in these three parameters, there was not any significant difference between the two experimental treatments.

Table 2 Life history statistics (Mean ± SE) of *Macrolophus pygmaeus*, after the third instar larvae, treated by LC_{30}s of thiamethoxam and pirimicarb.

Treatments	Life history parameters		
	Pre-adult duration (day)	Adult duration (day)	Fecundity (eggs/female)
Control	34.63±0.075b	32.11±2.43	198.3±19.69 a
Thiamethoxam	44.16±0.18a	29.44±1.916	111.8±13.81b
Pirimicarb	45.98±0.12a	30.12±2.14	123.5±16.08 b
P	<0.0001	0.92	<0.0001
F	29.03	0.09	1.36
df	194	122	52

Means in a column followed by different letters are significantly different (P<0.05) (Tukey test).

Thiamethoxam and pirimicarb showed several long-term effects on population parameters of *M. pygmaeus* (Table 3). In comparison with the control, the intrinsic rate of increase, finite rate of increase, net reproductive rate and mean generation time were significantly changed in treated individuals (P < 0.0001) (Table 3). The intrinsic rate of increase (*r*) in control, and LC_{30} of thiamethoxam and pirimicarb was 0.15, 0.10 and 0.99 day^{-1}, respectively. Parallel with *r*, the finite rate of increase (λ) changed significantly. It was 1.11, 1.08 and 1.03 day^{-1} in control, and thiamethoxam and pirimicarb treatments, respectively. The net reproductive rate (R_0) in control was 36.75 offspring/individual but in thiamethoxam and pirimicarb treatments it was reduced to 19.62 and 18.24, respectively. The mean generation time (T) in thiamethoxam and pirimicarb treatments increased to 27.79 and 31.24 days. However, this parameter in the control was 22.69 (Table 3).

Table 3 Life table parameters (Mean ± SE) of *Macrolophus pygmaeus* after the third instar larvae, treated by LC_{30}s of thiamethoxam and pirimicarb.

Treatments	Life table parameters			
	R_0(offspring/individual)	λ (day^{-1})	*r* (day^{-1})	*T* (days)
Control	0.15±0.00a	1.11±0.0048a	36.75±2.25a	22.69±0.28a
Thiamethoxam	0.10±0.0031b	1.08±0.0034b	19.62±1.62b	27.79±0.34b
Pirimicarb	0.99±0.02c	1.03±0.018c	18.24±0.8c	31.24±1.09c
P	<0.0001	<0.0001	<0.0001	<0.0001
F	4.42	3.5	2.5	2.5

The age-specific fecundity (m_x) of *M. pygmaeus* in treated and untreated individuals (Fig. 1) indicated that the first egg laying in control started in 14th day but in the both chemical treatments, it started four days later. Fecundity in control was more than that in the chemical treatments. In the thiamethoxam and primicarb treatments, there was not any fecundity after the day 60. However, in this day, there was a peak in egg laying in control. The reproductive value in control continues until the day 80 (Fig. 1).
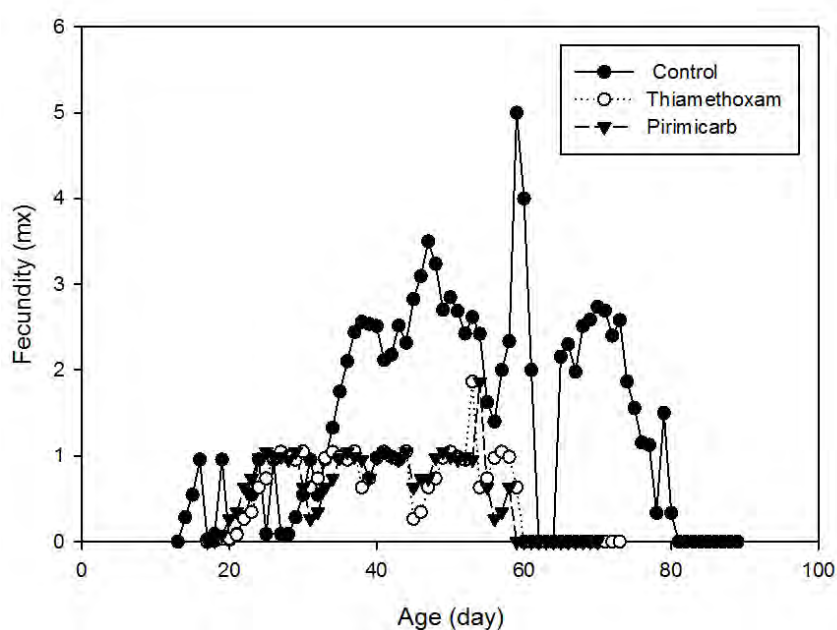

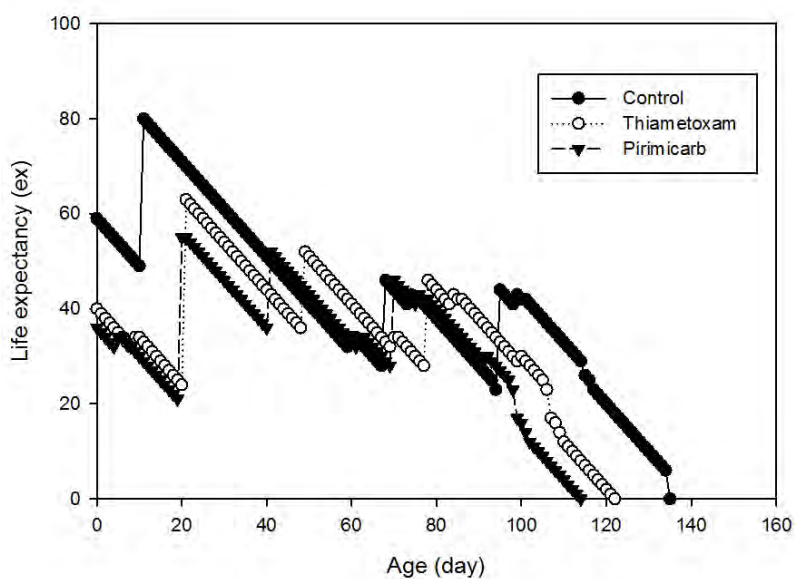**Fig. 1** The age-specific fecundity (m_x) of *Macrolophus pygmaeus* affected by LC_{30} of pirimicarb and thiamethoxam.

Fig. 2 The life expectancy (e_x) of *Macrolophus pygmaeus* affected by LC_{30} of pirimicarb and thiamethoxam.
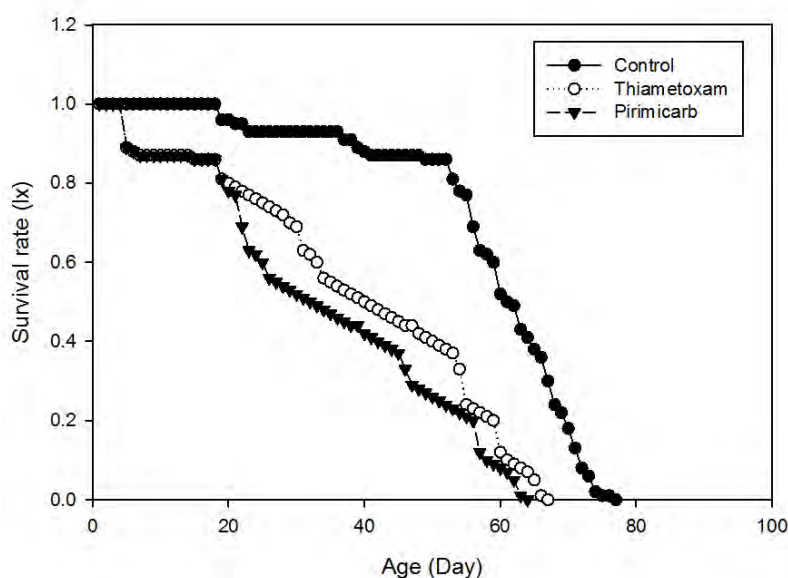

Fig. 3 Changes of survival rate (l_x) after treatment of *Macrolophus pygmaeus* by LC_{30} of pirimicarb and thiamethoxam.

The life expectancy (e_x) shows the total time that an individual of age x and stage j is expected to live (Chi and Su, 2006). Due to the high mortality during the pre-adult development, the life expectancy in both chemical treatments was lower than that of control (Fig. 2).

l_x is the probability of surviving from birth to age x. As shown in the Fig. 3, 90 percent of population in control stayed alive until the day 50. But a sharp decrease was shown in survivorship, after the day 55. Nevertheless, in thiamethoxam and pirimicarb treatments, decline in population started since the day 20. There was not any live individual after the day 70 (Fig. 3).

4 Discussion

In the integrated pest management programs, we should try to recognize selective pesticides with low adverse effects on natural enemies and natural recourses and use them beside the other control methods. In this study for the first time, sublethal effect of thiamethoxam (neonicotinoid) and pirimicarb (carbamate) insecticides was studied on the predator, *M. pygmaeus*.

LC$_{50}$ or LD$_{50}$ shows median concentration or dose, respectively, causing 50 percent mortality. In fact, this median rate is used in measuring lethal dose or concentration during short time (Desneux et al., 2007). Because in bioassay analysis, logarithm of a dose is considered in evaluation of dose-response, the rate of 50 percent mortality is very important. Actually, in this way, a minor change in dose makes a great change in response. So this rate (LC$_{50}$ or LD$_{50}$) is a critical rate.

There are many studies measure different sublethal concentrations of pesticides on target or non-target arthropods biological parameters (especially on demography) in concentrations less than LC$_{50}$. Hamedi et al. (2010) evaluated LC$_5$, LC$_{10}$, LC$_{20}$ and LC$_{30}$ of fenpyroximate on demographic parameters of predatory mite, *Phytoseius plumifer*. Alinejad et al. (2014) showed sublethal concentrations of fenazaquin caused 10, 20 and 30 percent mortality on life table statistics of *Amblyseius swirskii*. Rahmani and Bandani (2013) found thiamethoxam at concentrations of LC$_{10}$ and LC$_{30}$ have potential to affect aphid predator, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) adversely. Also, Zhang et al. (2014) studied demographic changes affected by sublethal concentrations (LC$_5$ and LC$_{20}$) of thiamethoxam on *Bradysia odoriphaga*, a major insect pest in Northern China.

In this study, we used LC$_{30}$ because the measured lethal concentrations (LC$_{50}$) of both insecticides were higher than the field rates. So, outside of the laboratory (at the field) it will be more probable that the organism will experience the concentrations very lower than its LC$_{50}$. Schneider et al. (2009) didn't show any short term effect of glyphosate on survivorship of a predator insect, *Chrysoperla externa*, while this herbicide affected the predator life table parameters during the life time.

Pirimicarb, as a dimethylcarbamate insecticide, with systemic, contact, stomach and respiratory action, and thiamethoxam, beside the other insecticides of neonicotinoid group, such as imidacloprid, acetamiprid, dinotefuran and clothianidin, are prevalent chemicals applying in greenhouse plants and the other crops in order to control a wide range of insect pests, such as aphids and whiteflies (Moura et al., 2006; Cloyd and Bethke, 2010).

However, in these agricultural areas, nearby the pests, natural enemies, i.e. parasitoids and predators, are active. Unfortunately, these beneficial arthropods are exposed to pesticides through many ways such as ingestion of pesticide-contaminated prey or hosts, and direct and/or indirect contact with pesticide residues on plant surfaces (Jepson, 1989).

According to the obtained LC$_{50}$ results of the current study, which both are higher than field recommended rate, thiamethoxam and pirimicarb are safe for *M. pygmaeus*. Pirimicarb, has been reported as highly selective and safe to coccinellid predators (James, 2003; Cabral et al., 2008; Rahmani and Bandani, 2013). This pesticide also had no significant effect on the survival and fecundity of *Orius laevigatus* (Fieber) when predators were exposed to pesticide residues by contact or by ingestion (Angeli et al., 2005). On the other hand, study about toxicity of neonicotinoids on natural enemies has been noticeable in recent times. The acute toxicity results of neonicotinoids were vary from high to harmless (Rahmani and Bandani, 2013; Gontijo et al., 2014; Tabozada et al., 2015; Yao et al., 2015). Tabozada et al. (2015) showed that thiamethoxam was safer than thiacloprid on the larval parasitoid, *Bracon brevicornis*.

The effect of selective pesticides on predators and parasitoids, depends on natural enemy species (Roubos et al., 2014), developmental stages (Tabozada et al., 2015) and application methods (Yao et al., 2015) is

different. Yao et al. (2015) measured toxicity of thiamethoxam (Actara®) on *Serangium japonicum* Chapin, biological control agent of *B. tabaci* in China, through three exposure routes of residual contact, egg-dipping, and systemic treatment and showed that the late method had the least LC_{50} value (2.43 mg (ai) L^{-1}). In this study, contact method (topical application) was used on the third instar larvae of *M. pygmaeus*.

Demographic parameters are important in ecotoxicology due to evaluation of the total effects (lethal and sublethal) of a toxicant on organism population (Stark and Banks, 2003). In life table study, survivorship and fecundity are two important factors (Carey, 1993) and any changes in one or both of them, leads to change in demographic parameters. In this study, thiamethoxam and pirimicarb, by declining in the survival rate (l_x), and the number of female offspring (m_x), reduced the intrinsic rate of increase (*r*), the most important parameter in population statistic. Similarly, many researchers showed the same results (reduction in *r*) due to the effects of chemicals on natural enemies' life table (Stark and Banks 2003; Schneider et al., 2009; De Castro et al., 2015; Mahdavi et al., 2015). Moreover, the two other demographic parameters, finite rate of increase and net reproductive rate, decreased, significantly by the LC_{30} effects of the pesticides although the mean generation time increased, meaningfully. Reduction in R_0 illustrated that survivorship in preadult duration has been affected strongly by the pesticides. This finding, illustrated that sublethal concentrations of both chemicals, which might not be seen in the short term, have potential to produce harmful effects on the physiology of the insect during long period of time (Papachristos and Milonas, 2008).

In this study, both insecticides extended the pre-adult developmental time. There are different results about the effects of varied pesticides on arthropod developments. Developmental time of predaceous stinkbug, *Podisus nigrispinus* (Dallas), was extended by feeding on prey and plants treated with the systemic insecticide, thiamethoxam (Torres et al., 2003). In addition to, imidacloprid extended the growth time of pre-adult stages of *Hippodamia undecimnotata* (Schneider) (Papachristos and Milonas, 2008). Also, indoxicarb, pirimicarb and thiamethoxam increased pre-adult duration of the pests and their natural enemies such as *Hippodamia variegate* (Goeze) (Mahmoudvand et al., 2011; Rahmani et al., 2013). Gholamzadeh-Chitgar et al. (2015) illustrated that sublethal concentration of diazinon, fenitrothion and chlorpyrifos increased (about 1.5 times) pre-oviposition period of predatory bug, *Andrallus spinidens*. However, Schneider et al. (2009) showed that a systemic herbicide, glyphosate, decreased pre-adult stages duration of *Chrysoperla externain* (Hagen). In addition to the growth rate, in the current experiment, fecundity of the chemical treatments decreased, significantly (more than 1.5 times in comparison with the control).

Reduction in fecundity has been shown in many studies (Rahmani and Bandani, 2014; de Castro et al., 2014; Gholamzadeh-Chitgar et al., 2015; Lopez et al., 2015; Mahdavi et al., 2015). Such phenomenon shows that insecticides can affect the male and female reproductive system. Thus, the central nervous system, including the neuroendocrine system may damage leading to the hormonal regulation disruption (Moline et al., 2000).

A deformation of ovaries is another consequence of insecticidal exposure (Medina et al., 2004; Schneider et al., 2004; Gholamzadeh-Chitgar et al., 2015).

Study about the effects of pesticides on beneficial organisms, specially predators and parasitoids, gives us better picture about destiny of chemicals in environment and also applying them in IPM programs. In this project, thiamethoxam and pirimicarb, as selective insecticides, did not have risky effect on the predator bug, *M. pygmaeu* in short time. In fact, evaluated LC_{50} of both chemicals was higher than the field recommended rate. However, most of the stable population statistics and some of the biological features of this omnivorous bug that has a close relationship with plants due to feeding plant sap, in addition to the prey, were significantly affected by the two insecticides in sublethal concentration (LC_{30}) during long period of time, which must be considered in integrated pest management.

Acknowledgements

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