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Effects of thiamethoxam in sublethal concentrations, on life expectancy (e_x) and some other biological characteristics of *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae)

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ABSTRACT: *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae), is an important aphidophagous predator that is noticeable in IPM as a suitable biological control agent. In this study, influence of sublethal concentrations (LC_{10} and LC_{30}) of a neonicotinoid insecticide, thiamethoxam, was evaluated on some of life parameters of this beneficial insect. Results showed that thiamethoxam changed life expectancy (e_x), and declined age-specific fecundity (m_x). The insecticide, decreased preadult development time significantly, but it didn't have any effect on adult developmental period. Also, Eggs fecundity and fertility didn't changed in treatments. According to this study, although thiamethoxam is approximately safe for the ladybird, more care should be taken when it is used in IPM programs.

Key words: contact exposure, ladybird, life statistics, neonicotinoid insecticide, sublethal concentrations.

INTRODUCTION

Ladybirds are the most well known beneficial arthropods found in many habitats and ecosystems all around the world and in addition to the aphids they feed on various kinds of pest insects (Ali and Rizvi, 2009; Hodek and Honek, 1996). In fact, coccinellids during their adult and larval stages attack on a variety of soft bodied herbivorous pests, including aphids, whiteflies, psyllids, and scales. Moreover they ingest fungal spores, pollen and nectar as complementary or even alternative foods when the preys are rare (Ali et al., 2009).

Hippodamia (*Adonia*) *variegata* (Goeze), originally is a palaearctic coccinellid species, but now is a widespread predator by aphidophagy preference, in many agricultural ecosystems such as wheat, tobacco, cotton, vegetables and orchards in many countries including Iran (Wang et al., 1984; Pang, 1993; Yang et al., 1997; Lotfalizadeh, 2001; Franzmann, 2002; Kontodimas and Stathas, 2005; Rebolledo et al., 2009). Due to the great feeding capacity, this generalist variegated beetle, is proposed as a valuable biological control agent (Kontodimas and Stathas, 2005) in insect pest management (IPM) programs.

Integration of chemical and biological controls in IPM program is an ordinary practice and applying pesticides that are compatible with biological control agents, results in the exact concept of IPM (Elzen, 2001). In addition to the problems such as insect resistance and environmental and food contaminations, pesticides lead to reduction in population of natural enemies (Youn et al., 2003; Garrat and Kennedy, 2006). However, Physiological and behavioral characteristics of arthropods can be adversely affected by pesticides (Haynes, 1988).

In ecology, studying organism life table is one of the most important analytical tools gives us comprehensive prospect to the survivorship, development and expectation of population life history (Ali and Rizvi, 2007). Demographic analysis is an essential approach to evaluate total effects of a pesticide on organism especially natural enemies (Stark et al., 2007).

Thiamethoxam is a new neonicotinoid insecticide with stomach and contact activity has a wide spectrum of activity against aphids, whiteflie, leafhoppers and termites (Yamamoto, 1996; Senn et al., 1998; Acda, 2007). However, there are few studies about the side effects of this insecticide on predatory arthropods such as ladybirds (Youn et al., 2003; James, 2003).

Thus, this is the first report estimates effects of thiamethoxam, on the coccinellid beetle, *Hippodamia variegata* (Goeze). In the current study, some of the biological and demographic factors such as life expectancy, age-specific fecundity, development time, fecundity and fertility affected by sublethal concentrations of thiamethoxam are evaluated.

MATERIALS AND METHODS

Insect rearing

The colony of *Hippodamia variegata* obtained from laboratory of Insect Ecology from department of plant protection, university of Tehran, was reared on *Aphis fabae* and maintained in a growth chamber set at 27 ± 1° C, 70 ± 10% RH, and a photoperiod of 16:8 (L:D) for several generations before being used for life table study. Aphids were reared on broad bean plants, *Faba vulgaris*, under 22 ± 1 °C and 70 ± 10% RH, and a photoperiod of 16:8 (L:D).

Toxicity bioassays

Thiamethoxam (commercial formulation, Actara®, WG 25%, Syngenta, India) was used in this experiment. Serial dilutions (ranged from 150 to 8400 mg(ai)L^{-1}) were prepared in acetone. Acetone alone was used as control. Six concentrations of the insecticide were prepared to treat the insect. For assessing the toxicity, one microliter of each solution applied on the dorsal abdomen of the third instar larvae using micropipette. For each concentration (treatment), 79 insects were used. These larvae were obtained from six hours cohort eggs. Treated insects in groups of 4 or 5 individuals, were put in Petri dishes (60 mm in diameter × 15 mm in height) and sufficient *Aphis fabae* were put in the Petri dish in order to provide food for the beetles. Mortality was assessed 24 h after treatment.

Subletal concentrations on life history data

Three cohorts of about 100 eggs (six hours old) for each treatment were selected from the lady beetle laboratory colony (Schneider et al., 2009). Eggs were kept in a growth chamber at 27 ± 1 °C, 70 ± 10% RH, and a photoperiod of 16:8 (L:D). The eggs were checked every six hour and newly emerged larvae were transferred to new Petri dishes (60 mm diam.) into the same condition and were supplied daily by enough *A. fabae* as food sources.

When larvae got to the third instar, they were treated with two sub-lethal concentrations of the insecticide (LC_{10} and LC_{30}). Mortality and development were checked every 24 hours. After the emergence of adults, males and females were paired and checked daily in order to record their survival and their laid eggs. The experiments continued until the death of all the individuals. For assessing fertility, 100 eggs separated three times during the oviposition, from each treatment and control and kept them into the above condition until hatched.

Data analyses

In the toxicity test, 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).

General linear models (PROC GLM, SAS Institute, 2003) and comparison of means using Duncan's multiple range test (DMRT) were conducted for determining the differences in life history traits among *H. variegata* exposed and unexposed to chemicals (SAS Institute Inc., 2003). The significance level was $P<0.05$.
The life expectancy (e_x) was obtained by the equivalent of: $e_x = T_x/l_x$ (Carey 1993) where l_x is individuals that survive to age x in the cohort and T_x is a total of insect-days beyond the age x.

RESULTS AND DISCUSSION

According to the results of bioassay, The concentrations caused 10 and 30 percent mortality in the third instar larvae were 48.21 (11.06-111.05) and 251.31 (108.43-425.99) mg(ai)L^{-1} respectively (Table 1).

Effect of thiamethoxam on life history showed that this insecticide increased development time of preadults in both LC_{10} and LC_{30} treatments significantly ($F = 24.48$, $df = 116$, $P<0.0001$) but it didn't have any significant effect on the development time of adult females, fecundity, and fertility (Table 2).

The age-specific fecundity (m_x) in fig. 1 shows that egg laying was started in the day 17 (in control and LC_{10}) and 18 (in LC_{30}). Also, the first obvious peak of age-specific fecundity in both control and LC_{10}, occurred on the day 19, but it happened on the day 21 in LC_{30} treatment. Fecundity peaks in control was higher than the other two treatments and LC_{30} treatment had the lowest m_x peaks. Egg laying was finished on the day 95 in

LC_{30} but in LC_{10} continues until 109's day and the egg numbers laying in this treatment (LC_{10}) were very more than control which has been shown as high length peaks after the day 90 (fig. 1).

Table 1 Toxicity of thiamethoxam on the third instar larvae of *H. variegata*

Insecticide	N[a]	LC_{10} [mg(ai)L^{-1}]	LC_{30} [mg(ai)L^{-1}]	LC_{50} [mg(ai)L^{-1}]	Slope±SE	X^2(df)
Thiamethoxam	553	48.21 (11.06-111.05)	251.31 (108.43-425.99)	788.55 (473.93-1201.66)	1.56±0.12	36.62 (22)

[a] Number of subjects

Figure 2, shows the effects of thiamethoxam in sublethal concentrations on life expectancy (e_x) of the *H. varuegata*. The life expectancy curve, in control, LC_{10} and LC_{30} is almost equal until the day 50 but after this day, this parameter in control becomes lower than the other treatments. In fact, in the day 110, life expectancy in control becomes 1, but in LC_{10} and LC_{30} treatments it is 14 and 19 days respectively. This parameter at the beginning of adulthood, in control was 60.36 whereas in LC_{10} and LC_{30} treatments decreased to 58.07 and 56.5 days respectively.

Neonicotinoid insecticides, such as imidacloprid, thiamethoxam, acetamiprid, dinotefuran and clothianidin are commonly used against a wide range of herbivorous insect pests such as aphids, mealybugs and whiteflies in greenhouses or farms. So parasitoids and predators are exposed to these chemicals (Cloyd and Bethke, 2010). In this study, sublethal effects of thiamethoxam, on the predatory variegated coccinellid beetle, *H. variegate*, has been investigated. All neonicotinoid insecticides have similar mode of action. These compounds interact with nicotinic acetylcholine receptors (nAChR) of the central and peripheral nervous systems (Yamamoto, 1996).

Table 2. Life statistics (mean±SE) of treated and untreated *H. variegata* by sublethal concentrations of thiamethoxam

statistics	preAdult (day)	Adult (day)	Fecundity (eggs/female)	Fertility (%)
Control	14.63±0.10b	60.86±3.006	709.3±59.69	86±3.78
LC_{10}	15.93±0.21a	58.15±6.32	656.5±80.38	83±3.21
LC_{30}	15.86±0.18a	56.58±7.135	524.8±93.81	89.33±1.76
P	<0.0001	0.80	0.24	0.39
F	24.48	0.22	1.44	1.06
df	116	52	52	6

Means in a row followed by different letters are significantly different (P<0.05) (Duncan).

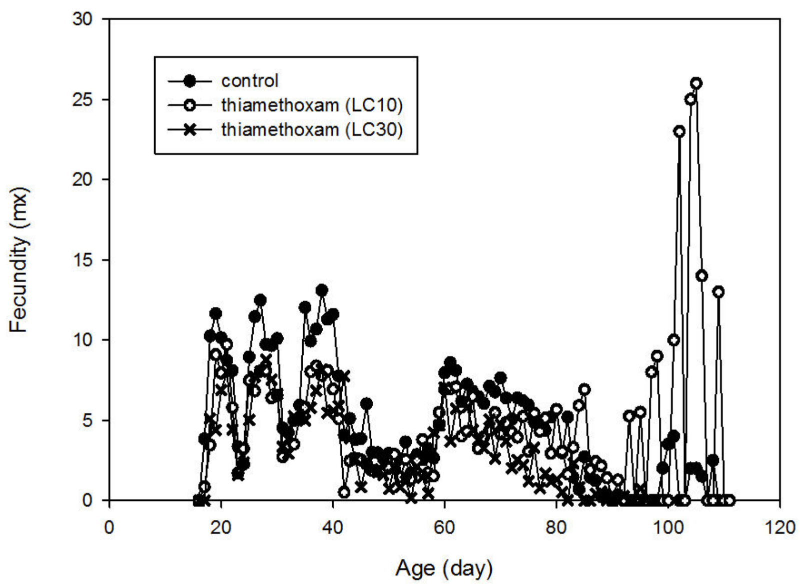

Figure 1. Effect of thiamethoxam on the age-specific fecundity (m_x) of *H. variegata*

Bioassay experiments in short time (24 hours) showed that *H. variegate* is not very susceptible to thiamethoxam. There are similar results about the coccinellid larvae, *Harmonia axyridis*, which was not susceptible to this insecticide at the field recommended rates (Youn et al., 2003; James, 2003). Our data supports by the above authors who came to the conclusion that coccinellid larvae, *Harmonia axyridis*, is not as much susceptible as its pest. Because in both studies, LC_{50} of the insecticide on the beetle was more than recommended concentration. However, thiamethoxam was toxic to the other lady beetle, *Stethorus punctum picipes* (Casey) and it produced 100% mortality at the field recommended dose (James, 2003). Nevertheless thiamethoxam on the parasitoid, *Diaeretiella Rapae* (Mcintosh), had harmful effect (Tawfiq et al., 2010). Different organisms show diverse susceptibility to the insecticides based on differences in target-site sensitivity and/or changes in enzyme activity (Cho et al., 2002).

Sublethal concentrations of thiamethoxam in this study, showed an increase in developmental period of preadult stages significantly. There are different reports about the effects of different pesticides on arthropod developments. Similar data was taken from Mahmudvand et al. (2011) when treated *Plutella xylostella* with sublethal doses of indoxcarb. Also Papachristos and Milonas (2008) found that imidacloprid extended development time of preadult *Hippodamia undecimnotata*. However, Schneider et al. (2009) showed that the herbicide, glyphosate, declined development time of *Chrysoperla externain* in the preadult stages. Thiamethoxam decreased fecundity in LC_{10} followed by LC_{30} treatments but this trend was not significant. In addition to, fertility after treatment with this chemical didn't change. Also imidacloprid didn't have any significant effect on *H. undecimnotata* (Papachristos and Milonas 2008).

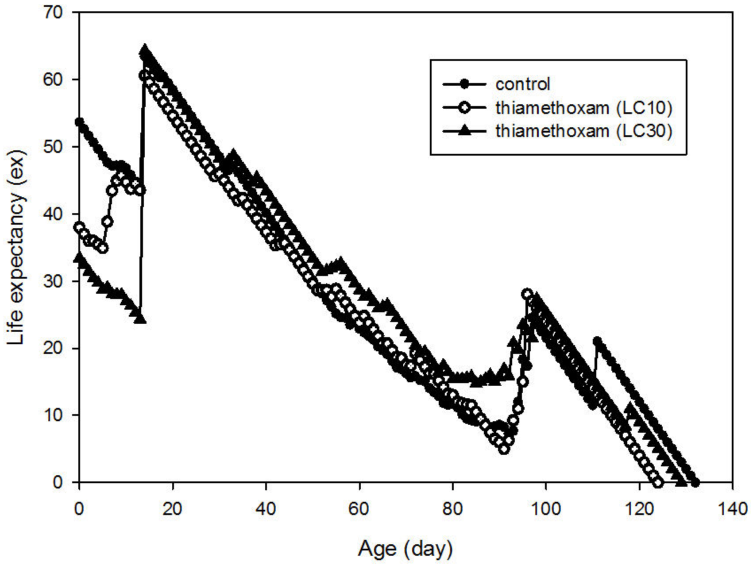

Figure 2. Effect of thiamethoxam on the life expectancy (e_x) of *H. variegata*

Fecundity and fertility are two important parameters for predatory insects in IPM programs. In this experiment, because thiamethoxam didn't show any major effect on these parameters and specially, thiamethoxam even increased life expectancy of females during mid of their ages, it seems possible we ignore the harm of this chemical during pest management. However, thiamethoxam increased significantly preadult development time and because the beetle has highest voracity during the adult stage (Farhadi et al., 2011) this subject is not proper for a biological control agent. Furthermore, it is necessary to evaluate effects of thiamethoxam on the other parameters that are important in demographic ecotoxicology.

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