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Research Article

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Determination of sub-lethal effects of spirotetramat and chlorpyrifos on *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) by using life table

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ABSTRACT - Compatibility of pesticides and biological control agents is the main concern of Integrated Pest Management (IPM) programs. The present study focused on the side effects of spirotetramat and chlorpyrifos on the demographic parameters of *Aenasius bambawalei* Hayat (Hym: Encyrtidae). Adult parasitoids were exposed to one ppm of chlorpyrifos and 7.5 ppm of spirotetramat for 24 hours. Then, they were released on the third instar nymphs of the mealybug *Phenacoccus solenopsis* Tinsley (Hem. Pseudococcidae) and their demography was investigated. The results showed that oviposition period, fecundity, and female and male longevities adversely were affected by both insecticides. However, the adult pre-oviposition period (APOP), the total pre-oviposition period (TPOP), and the number of daily eggs of insect females were not affected significantly. The intrinsic rate of increase (*r*) was calculated to be 0.16, 0.13, and 0.12 day^{-1} for control, spirotetramat, and chlorpyrifos treatments, respectively. The results of this study revealed that chlorpyrifos at sublethal concentrations have more negative effects on *A. bamabawalei* than spirotetramat and need to be applied with caution in IPM programs.

INTRODUCTION

Biological control of the pests can be supported by using pesticides in essential situations (De Cock et al. 1996); thus, chemical insecticides play an important role in integrated pest management (IPM) in many agricultural systems (Stark et al. 2004). The compatibility of biological control agents with pesticides is a major concern in IPM programs. Insecticides may have lethal and sub-lethal effects on insects. Their lethal effects usually cause death; however, the sub-lethal effects may have different aspects (Galvan et al., 2005).

Sub-lethal effects of insecticides are considered as physiological or behavioral effects on individuals that survive after exposure. A sub-lethal concentration is described as a concentration that causes no apparent mortality in the experimental population. Estimation of sub-lethal effects of insecticides is essential for a complete analysis of their impacts (Desneux et al., 2007). Abnormality in development, shortened life span, reduced fecundity, changes in sex ratio or offspring emergence, may occur as a result of sub-lethal effects of insecticides on the physiology of parasitoids (Stark and Banks, 2003; Desneux et al., 2006). These sub-lethal effects can also be detected by calculating the life table parameters such as net reproductive rate (R_0), intrinsic rate of natural increase (r), finite rate of increase (λ), and mean generation time (T) (Desneux et al., 2007). Behavioral changes may also occur in natural enemies after chemical exposure as loss of sexual competitiveness or reduction in parasitism/predation rate (Stark et al., 2004).

Mealybugs (Hemiptera: Pseudococcidae) are among economic insect pests attacking more than 246 families of plants worldwide (Ayyamperumal et al., 2019). The species *Phenacoccus solenopsis* Tinsley is a new species of mealybugs was first reported as a pest of cotton in Texas, USA (Fuchs et al., 1991). It has also been recorded as a serious pest of cotton from Pakistan (Hodgson et al., 2008), India (Nagrare et al., 2009), and China (Wang et al., 2009). In Iran, the pest initially was seen on *Hibiscus rosa-sinensis* in Hormozgan province in January 2009 (Joodaki et al., 2018). It has been reported that the colonies of the pest seem white and cottony and usually are settled on the buds, main stems and branches of host plants (Joodaki et al., 2018). *P. solenopsis* sucks the plant sap and produces honeydew which results in the development of sooty mold that interrupts the photosynthesis process (Saeed et al., 2007). Due to the great reproductive potential of this pest in favorable climate conditions, the natural enemies are not able to keep the population of this pest below the economic injury level. Therefore, in some cases, the application of chemical insecticide is inevitable (Sahito et al., 2011). Different insecticides such as pyrethroids, organophosphates, and neonicotinoids have been used for control of this pest (Afzal et al., 2015).

Among various parasitoid species reported on *P. solenopsis*, the solitary parasitoid, *Aenasius bambawalei* Hayat (Hym.: Encyrtidae), has been found as a key natural enemy with 30-60% parasitism under field conditions (Fand and Suroshe, 2015). Moreover, higher parasitism rate, up to 95%, has been reported in southwestern Iran when insecticide application was suspended (Mossadegh et al., 2015). According to a previous study, *A. bambawalei* adults mainly prefer the third instar nymphs of *P. solenopsis* for oviposition (Fand et al., 2011).

The sub-lethal effects of insecticides can severely reduce the performance of biological control agents and have a key role in forecasting the prosperity of natural enemies in IPM programs (Desneux et al., 2007; Asadi et al., 2019). Studies on the sub-lethal effects of insecticides on the demography of various parasitoids have been reported by several researchers (see below).

It is important to estimate the compatibility of commonly used insecticides for controlling mealybugs with their parasitoids. Previous studies have investigated the demographic effects of chemical and botanical insecticides on some parasitoids including *Encarsia formosa* Gahan (Gholamzadeh et al., 2012); *Habrobracon hebetor* Say (e.g. Mahdavi et al., 2011; Rafiee-Dastjerdi et al., 2012; Faal-Mohammadali et al., 2014; Asadi et al., 2019; Rostami et al., 2018), *Trichogramma chilonis* Ishii (Wang et al., 2012), *Trisolcus grandis* Thompson (Saber et al., 2005), *Diaeretiella rapae* (Rezaei et al., 2018), *Encarsia inaron* (Walker) (Sohrabi et al., 2012), *Eretmocerus mundus* (Sohrabi et al., 2013), and *Lysiphlebus fabarum* Marshall (Mardani et al., 2016). In some other studies, the effects of botanical and conventional insecticides on the mortality of *A. bambawalei* were investigated (e.g. Nalini & Manickavasagam, 2011; Suroshe et al., 2014; Badshah et al., 2017; Ullah et al., 2017). Hadian et al. (2020) studied the effect of spirotetramat and chlorpyrifos on the functional response of *A. bambawalei.* Moreover, sub-lethal effects of some chemical insecticides including dimethoate, imiaclopride, and thiodicarb were investigated on demographic traits of *A. bambawalei* (Rafatian et al., 2021). In the present study, sub-lethal effects of spirotetramat and chlorpyrifos were assessed on pre-immaginal development and life table parameters of *A. bambawalei*, the most important parasitoid of *P. solenopsis.*

MATERIALS AND METHODS

Insects

Twigs of China rose, *Hibiscus rosa-sinensis*, infested with different life stages of *P. solenopsis* were collected from shrubs available at the campus of Agricultural Sciences and Natural Resources University of Khuzestan. The nymphs and adults of *P. solenopsis* were transferred to young sprouts (0.5-1.5 cm length) of potato, *Solanum tuberosum* L., as a laboratory host. Then, the potato sprouts were transferred to a container (24×10×16 cm) covered with fine mesh. When the new colony of mealybugs was established, they were used in the experiments.

To establish a colony of *A. bambawalei*, the parasitized nymphs of *P. solenopsis* were collected from the same *H. rosa-sinensis* twigs. The mummies were kept in a separate container until the adults' emergence. Then they were collected by an aspirator and released in containers containing 3rd instar nymphs of *P. solenopsis.* The colony of insects was kept in the incubators at 27±2° C, 65±5% R. H., and 14 L: 10 D.

Insecticides

Information about selected insecticides in the experiments is shown in Table 1. Chlorpyrifos has been widely applied to control the mealybugs in Iran. Recently, spirotetramat has been recommended for the pest control in Iran.

Experimental Design

Fifty pairs of newly emerged wasps (less than one day old) were exposed to one ppm of chlorpyrifos and 7.5 ppm of spirotetramat for 24 hours. For this purpose, the cylindrical plastic containers (15 cm high and 7 cm diameter) were impregnated to the insecticidal solution for 30 seconds and allowed to dry for 24 h. Then the adults were exposed to insecticides in containers, having fine mesh for ventilation, for 24 h. Distilled water was used in control treatment. After 24 hours, two to three pairs of surviving wasps were randomly selected and transferred to containers containing at least 50 third instar nymphs of *P. solenopsis*. Ten replications were considered for this experiment. After 24 hours, adult wasps were removed and the parasitized 3rd nymphs of mealybug were used for experiments as a cohort. The development of parasitoid from egg to adult was monitored using a binocular dissecting microscope until adult emergence. The duration of immature stages and the number of survival parasitoids were recorded on a daily basis. After adult emergence, each pair of newly emerged female and male wasps (obtained from developmental tests) was placed in a separate container containing 30 third instar nymphs of *P. solenpsis*. Every 24 h, the parasitoids were transferred via a mini aspirator to a new container containing the 3rd instar nymphs until the parasitoid female died. The daily and the total number of parasitized *P. solenopsis* and longevity of *A. bambawalei* adults were recorded. All the aformentioned experiments were conducted in incubators at 27±2° C, 65±5% R. H., and 14L: 10D.

The data for developmental time including the data of survival rate, the longevity of male and female, as well as female fecundity of *A. bambawalei* under different insecticidal treatments, were analyzed according to the theory of age-stage, two-sex life table (Chi and Liu, 1985; Chi, 1988).

The adult pre-oviposition period (APOP: the period between the emergence of an adult female and the first oviposition) and the total pre-oviposition period (TPOP: the time interval from birth to the first oviposition) were calculated using the experimental data. The age-stage specific survival rate (s_{xj}; where x = age and j = stage), the age-stage specific fecundity (f_{xj}), the age-specific survival rate (l_x), the age-specific fecundity (m_x), and the population parameters including r, the intrinsic rate of increase; λ, the finite rate of increase ($\lambda = e^r$); R_0, the net reproductive rate ($R_0 = \sum_{x=0}^{\infty} l_x\, m_x$) ;and T, the mean generation time ($T = \frac{lnR_0}{r}$) were also calculated. The intrinsic rate of increase was estimated using iterative bisection method and Euler-Lotka equation ($\sum_{x=0}^{\infty} e^{-r(x+1)}\, l_x m_x$ =1) with age indexed from 0 (Goodman, 1982).

Bootstrap techniques (Efron and Tibshirani, 1994) were utilized to estimate the means, variances, and standard errors of the population parameters. To obtain less variable and more precise results, 10000 bootstrap iterations were performed. A paired bootstrap test was used to compare the differences among treatments using TWOSEX-MS Chart.

RESULTS

Development and Survivorship

The pre-adult developmental period, longevity and female fecundity of *A. bamabawalei* exposed to the sub-lethal concentrations of insecticides are shown in Table 2. Adult pre-oviposition periods (APOP) in spirotetramat, chlorpyrifos and control treatments were calculated to be 0.06, 0.09, and 0.05 day, and total pre-oviposition periods (TPOP) were 14.3, 13.4 and 14.2 days, respectively. There were no significant differences between treated and control wasps in APOP and TPOP (P>0.05). The oviposition period of wasps in spirotetramat, chlorpyrifos and control treatments were recorded as 14.8, 14.7 and 18.0 days, respectively which was significantly affected by insecticides (P<0.05). The highest fecundity (69.84 eggs/female) was observed in the control treatment whereas in the spirotetramat and chlorpyrifos treatments the fecundity rates were 45.05 and 44.36 eggs/female, which showed significant differences with the control treatment (P<0.05). Spirotetramat and chlorpyrifos had no significant effect (P>0.05) on the maximum daily eggs of *A. bamabawalei* females. The longevity of female parasitoids was significantly reduced in insecticide treatments (P<0.05). The maximum longevity of females was 34.4 days in control whereas, in spirotetramat and chlorpyrifos treatments, the longevity of females was reduced to 30.09 and 29.8 days, respectively. The males' longevity was also affected by insecticide treatments (P<0.05) so that in spirotetramat (29.5 days) and chlorpyrifos (28.0 days) treatments it was shorter than that in control (31.4 days) treatment (Table 2).

Life Table Parameters

The sub-lethal effects of spirotetramat and chlorpyrifos on the life table parameters of the *A. bambawalei* are shown in Table 3. The intrinsic rate of population increase (r), finite population growth rate (λ), Gross reproductive rate (GRR), and duration of one generation (T) were affected significantly in chlorpyrifos treatment but there were no significant differences between control and spirotetramat treatments in these parameters. However, the net reproductive rate (R_0) was 16.9, 12.2 and 34.9 offspring/individual in spirotetramat, chlorpyrifos and control treatments, respectively, which showed significant differences between control and treated insects. The intrinsic rate of increase (r) in control, spirotetramat and chlorpyrifos treatments was 0.16, 0.13 and 0.12 day^{-1}, respectively. The finite population growth rate (λ) also decreased in chlorpyrifos (1.13 day^{-1}) treatment compared to the control (1.17 day^{-1}) treatment but its decrease in spirotetramat treatment (1.14 day^{-1}) was not significant. Gross reproductive rate (GRR) was 34.32, 18.99 and 48.73 offspring/individual in spirotetramat, chlorpyrifos and control treatments, respectively, indicating significant decrease in chlorpyrifos treatment compared to control treatment (Table 3). The mean duration of one generation (T) in control, spirotetramate and chlorpyrifos treatments were 22.05, 21.13 and 20.40 days, respectively, with a significant decrease in chlorpyrifos.

Values for age-specific survival rate of the cohort (l_x), age-specific fecundity of total population (m_x), and age-specific net maternity (l_xm_x) are presented in Fig. 1. According to the curves, the survival rate, l_x, m_x, l_xm_x decreased by tested insecticides compared to the control treatment and the severe decrease trend was seen under chlorpyrifos treatment.

In the age-stage, two-sex life table procedure, life expectancy (e_{xj}) defines as the time an individual of age x and stage j is expected to live. Curves of age-stage life expectancy (e_{xj}) for eggs, females and males of *A. bambawalei* are presented in Fig. 2. The respective descending order of life expectancy (e_{xj}) was detected in spirotetramat, and chlorpyrifos treatments in comparison with control treatment.

Table 1. Information about experimental insecticides

Insecticide	Active ingredient	Company	Formulation	Dosage
Movento®	Spirotetramat	Bayer co., Germany	10% SC	7.5 ppm
Dursban®	Chlorpyrifos	Exir Agricultural co., Iran	40.8% SC	1 ppm

SC: Suspension Concentrate

Table 2. Sub-lethal effects of spirotetramat and chlorpyrifos on biological parameters (Mean ± SE) of *Aenasius bambawalei* reared on *Phenacoccus solenopsis* nymphs compared to the control treatment

Treatment	APOP (day)	TPOP (day)	Oviposition period (day)	fecundity	Eggs (day^{-1})	Female longevity	Male longevity	n
control	0.05±0.04^{a*}	14.2±0.43^{a}	18.0±0.96^{a}	69.8±4.81^{a}	14.1±0.43^{a}	34.4±1.01^{a}	31.4±0.64^{a}	40
spirotetramat	0.06±0.06 ^{a}	14.3±0.44^{a}	14.8±0.99^{b}	45.0±3.26^{b}	14.26±0.44^{a}	30.9±1.11^{b}	29.5±0.58^{b}	40
chlorpyrifos	0.09±0.09 ^{a}	13.4±0.33^{a}	14.7±0.93^{b}	44.4±2.82^{b}	13.36±.035^{a}	29.8±1.04^{b}	28.0±0.66^{b}	40

* Values in each column followed by the same small letters are not significantly different at 5% significant level using the paired bootstrap test.

n: Number of replications; APOP: adult pre-oviposition period, TPOP: total pre-oviposition period

Table 3. Sublethal effects of spirotetramat and chlorpyrifos on life table parameters (mean ± SE) of *Aenasius bambawalei* reared on *Phenacoccus solenopsis* nymphs compared to the control treatment

Treatment	λ	GRR	r	R_0	T
control	1.17±0.01^{a*}	48.73±8.68^{a}	0.16±0.09^{a}	34.92±5.97^{a}	22.05±0.56^{a}
spirotetramat	1.14±0.01^{ab}	34.32±7.32^{ab}	0.13±0.01^{ab}	16.9±3.71^{b}	21.13±0.59^{ab}
chlorpyrifos	1.13±0.01^{b}	18.99±4.62^{b}	0.12±0.01^{b}	12.2±3.20^{b}	20.40±0.52^{b}

* Values in each column followed by the same small letters are not significantly different at 5% significant level using the paired bootstrap test.

λ: finite population growth rate; GRR: Gross reproductive rate, r; intrinsic rate of population increase; R_0: net reproductive rate; T: duration of one generation

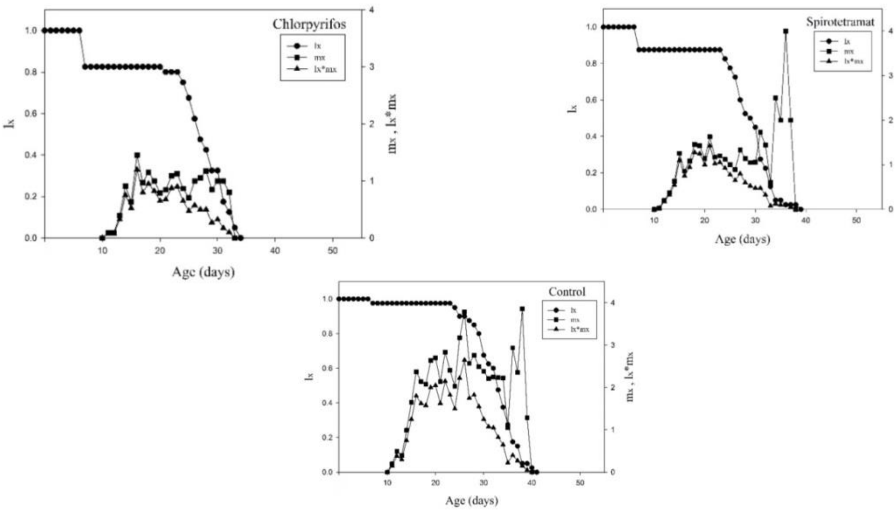

Fig. 1. Age-specific survival rate (l_x), age-specific fecundity (m_x), and age-specific maternity (l_xm_x) of *Aenasius bambawalei* in control, spirotetramat, and chlorpyrifos treatments

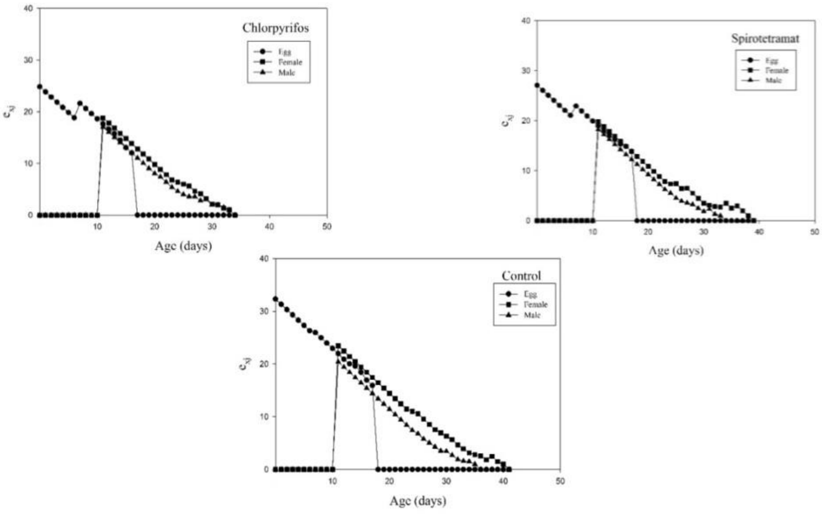

Fig. 2. The life expectancy (ex) of eggs, females and males of *Aenasius bambawalei* alive in each age-stage group (S_{xj}) in control, spirotetramat, and chlorpyrifos treatments

DISCUSSIONS

As pesticides and biological control agents are two significant elements of integrated pest management (Talebi et al., 2008), their compatibility of them for concurrent use is very important. Natural enemies may be exposed to insecticides directly through contact with toxicants, or indirectly by contact with pesticide residues or feeding on contaminated prey/host (Jepson, 1989). Therefore, demographic studies of parasitoids that are exposed to sub-lethal doses of the insecticides can provide valuable knowledge about the effects of the pesticides on the probable reduction of abilities in natural enemies (Stark et al., 2004).

In this study, sub-lethal effects of spirotetramat and chlorpyrifos on the biology of the parasitoid wasp, *A. bamabawalei*, were investigated. Both insecticides negatively affected the fecundity and longevity of the wasp. In another study (Nalini & Manickavasagam, 2011), different insecticides including chlorpyriphos were evaluated for their safety against *A. bamabawalei.* According to their results, chlorpyriphos caused 100% and 87% mortality in males and females after 1h, respectively. The mortality reached 100% in females with increasing exposure time (Nalini & Manickavasagam, 2011). In another research, studying the sublethal effects of dimethate, imidaclopride, and thiodicarb on the biological parameters of *A. bambawalei*, it was shown that all insecticides decreased the fertility of the parasitoid. In dimethoate and thiodicarb treatments, the intrinsic rate of increase (r) was significantly lower than that in the control treatment, but in imidalcloprid treatment the difference was not significant (Rafatian et al., 2021).

The results of similar previous studies on the other parasitoids and different insecticides were in agreement with the findings of the current study. For example, the biological parameters of *Habrobracon hebetor* Say including adult longevity, daily fecundity and daily fertility were affected negatively by fenvalerate, propargite, buprofezin, dayabon and palizin (Asadi et al., 2019), deltamethrin (Rafiee-Dastgerdi, 2012), and chlorpyrifos and fenpropathrin (Faal-mohammadali et al., 2014). Moreover, After treatment of *Trichogramma chilonis* Ishii (Hym.: Trichogrammatidae) with sub-lethal concentrations of fipronil and avermectins, the longevity and fecundity of treated females significantly decreased (Wang et al., 2012). According to Wang et al. (2012), the observed reduction in fecundity of treated parasitoids might be the result of their longevity reduction. On the other hand, in a study with an aphid predator, *Hippodamia variegata* (Goeze) adverse results emerged and sub-lethal concentration of thiamethoxam did not significantly affect the post-emergence adult parameters such as female and male longevity and fecundity (Rahmani and Bandani, 2013). Differences in experimental conditions, type and structure of selected pesticides, and sensitivity level of the species of the natural enemy may be the reason for different results in various studies (Asadi et al., 2019).

In the current study it was shown that the duration of pre-oviposition period described as APOP and TPOP was not significantly affected by sub-lethal concentrations of insecticides. Similar results were observed when sub-lethal concentrations of thiamethoxam and primicarb were applied on *Diaretiella rapae* (MacIntosh) (Hym: Braconidae), a parasitoid of *Lipaphis erysimi* (Kaltenbach) (Hem: Aphididae) (Rezaei et al., 2018). However, in another study conducted by Rahmani and Bandani (2013), the duration of pre-ovipostion period described as APOP and TPOP of *Hippodamia variegata* (Goeze) showed significant changes under sub-lethal concentrations of thiamethoxam. Furthermore, it has been shown that the soil application of imidaclopride increased pre-oviposition period of *Hippodamia undecimnotata* (Papachristos and Milonas, 2008).

In the current research, significant differences were observed on life table parameters of *A. bambawalei*, among chlorpyrifos, spirotetramamt and control treatments. Chlorpyrifos had negative effects on the parameters r, λ, GRR, R_0 and T which indicated that sub-lethal concentrations of this insecticide could influence physiology of the insect, although these deleterious effects are not distinct in the short time (Papachristos and Milonas, 2008). The recommended demographic parameter for studying the sub-lethal effects of insecticides is the intrinsic rate of increase or r (Asadi et al., 2019). The higher r-value in the control and spirotetramat treatments, that was observed in the current study, compared to that of chlorpyrifos revealed the negative effect of chlorpyrifos on the biology of *A. bambawalei*. Similar results were observed with sub-lethal concentrations of chlorpyrifos treatment when applied on *H. hebetor* compared with other insecticides (Faal-Mohammadali et al., 2014). In another study, Mahdavi et al. (2015) reported that chlorpyrifos was less compatible with *H. hebetor* compared with spinosad when their lethal and sub-lethal effects of both insecticides were studied.

CONCLUSIONS

The results achieved in this study indicated that spirotetramat is more compatible with *A. bambawalei*. However, sub-lethal concentrations of chlorpyrifos may negatively influence this parasitoid wasp. Therefore, chlorpyrifos is not introduced as an appropriate choice for IPM programs that are applied on the mealybug *Phenacoccus solenopsis* and more caution should be taken in its use. Chlorpyrifos application should be restricted to the duration of inactivity of the aforementioned parasitoid in the environment.

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مقاله علمی- پژوهشی

تعیین اثرات زیرکشندگی اسپیروتترامات و کلرپایریفوس روی*Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) با استفاده از جدول زندگی

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چکیده - در برنامه های مدیریت تلفیقی آفات، سازگاری بین آفت‌کشهای مورد استفاده و عوامل کنترل بیولوژیک بسیار مهم است. در پژوهش حاضر اثرات زیرکشندگی اسپیروتترامات و کلرپیروفوس روی پارامترهای رشد جمعیت *Aenasius bambawalei* Hayat (Hym: Encyrtidae) مورد بررسی قرار گرفت. حشرات کامل پارازیتوئید به مدت ۲۴ ساعت در معرض دوز یک قسمت در میلیون (ppm) کلرپایریفوس و ۷/۵ ppm اسپیروتترامات قرار گرفتند. پس از این مدت، حشرات روی پوره های سن سوم شپشک *Phenacoccus solenopsis* Tinsley (Hem. Pseudococcidae) رهاسازی شدند و خصوصیات رشد جمعیت آنها مورد بررسی قرار گرفت. نتایج نشان داد که طول دوره تخم گذاری، باروری و طول عمر حشرات نر و ماده توسط هر دو حشره کش تحت تاثیر قرار گرفت. هرچند، طول دوره پیش از تخمگذاری بالغین (APOP) و کل دوره پیش از تخمگذاری (TPOP) و تعداد تخم روزانه ماده‌ها تحت تاثیر حشره‌کش‌ها تغییر معنی داری نشان نداد. نرخ ذاتی رشد جمعیت (r) ، در تیمارهای شاهد، اسپیروتترامات و کلرپایریفوس به ترتیب ۰/۱۶، ۰/۱۳و ۰/۱۲ در روز محاسبه گردید. نتایج این بررسی نشان داد که کلرپایریفوس در غلظت‌های زیرکشنده، در مقایسه با اسپیروتترامات اثرات منفی بیشتری روی *A. bamabawalei* داشت و لازم است در برنامه های مدیریت تلفیقی آفات با احتیاط بیشتری مورد استفاده قرار گیرد.

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اثرات زیرکشندگی
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