Sublethal Effects of Commonly Used Insecticides in Tomato Crop on Functional Response and Biological Parameters of *Macrolophus pygmaeus* Rumber (Hemiptera: Miridae)

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ABSTRACT

The hemipteran, *Macrolophus pygmaeus* Rumber, is an effective biocontrol agent against many crop pests including tomato leaf miner, *Tuta absoluta* Meyrick. Sublethal effects of azadirachtin (Neem Azal®), indoxacarb (Avaunt®), and emamectin benzoate+lufenuron (Proclim Fit®) were studied on biological characteristics and functional response of the predatory bug in laboratory conditions at $25\pm1°C$, $6\pm5\%$ RH, and a photoperiod of 16:8 hour (L:D). For this purpose, females of the predatory bug were exposed to sublethal residues (10% of field concentration) of the insecticides. Two-sex life table and Roger's model were used to investigate effects of the insecticides on biological parameters and functional response of the predator, respectively. Results indicated that sublethal residues of indoxacarb and azadirachtin had the highest and lowest side effects on life table parameters of *M. pygmaeus*. Whereas azadirachtin has no significant effects on the bug biological parameters, total fecundity and longevity of the bug in indoxacarb treatment were significantly lower (14.6%) and higher (6.9%) than the control. The predator showed type III functional response. The type of functional response was not affected by the insecticide residues. However, the coefficient of attack rate (b) for the indoxacarb treatment (0.1521) was significantly lower than for other treatments. However, there was no significant difference between the handling Times (T_h) of the treatments. In conclusion, the experiments proved that azadirachtin is a low risk insecticide to *M. pygmaeus*, which can be integrated with biological control by the predator.

Keywords: Biological control, Chemical control, Foraging behavior, Life table, Mirid bug.

INTRODUCTION

The mired bug, *Macrolophus pygmaeus* Rumber (Heteroptera: Miridae), is an important polyphagous predator that has been reported to be effective in controlling many crop pests including whiteflies, thrips, aphids, mites and larvae of lepidopteran pests in field and greenhouse conditions (Margaritopoulos, 2003; Sylla *et al.*, 2016). This predator has been used for biological control of tomato leaf miner, *Tuta absoluta* Meyrik (Lepidoptera: Gelechiidae) (Bompard *et al.*, 2013; Zappalà *et al.*, 2013; Jaworski *et al.*, 2015; Biondi *et al.*, 2018), and actively feeds on eggs and all larval stages of *T. absoluta* (Jaworski *et al.*, 2013; Sylla *et al.*, 2016).

Insecticide application is the most commonly method used for control of *T. absoluta* (Arnó and Gabarra, 2011). Concern about adverse effect of the chemicals on non-target organisms and the environment has prompted scientists to develop Integrated Pest Management (IPM) programs (Smith, 1991). Biological control is the main preventive control strategy in an IPM program (Pedigo, 2002). Therefore, studying the impact of the insecticides, as another component of IPM, on the biocontrol agents is necessary (Wright and Verkerk, 1995; Nazarpour *et al.*, 2016). The

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sublethal effects of insecticides on physiology, biology and behavior of natural enemies must be considered for a complete analysis of their impact (Desneux *et al.*, 2007).

Indoxacarb is an oxadiazine insecticide with a great effect on lepidopteran pests (Wing *et al.*, 2000) such as *T. absoluta* (Nazarpour *et al.*, 2016). The insecticide blocks the voltage – dependent sodium canals, group 22A according to IRAC MoA classification version 9.4 (IRAC, 2020). Another effective insecticide that is applied against the pest is azadirachtin (AZ) (Nazarpour *et al.*, 2016). Products with the active ingredient AZ, a group of tetranortriterpenoids, have antifeedant, deterrent and insect growth regulatory properties. The products have been obtained from seeds and fruits of *Azadirachta indica* Juss and *Melia azaderach* L. trees, respectively (Isman, 2017; Nazarpour *et al.*, 2016). Proclaim Fit® (Syngenta Agro AG, Basel, Switzerland) is a new insecticidal product that contains 10% emamectin benzoate, a GABA and glutamate-gated chloride channel agonist (group 6 according to IRAC 2020), and 40% lufenuron, which acts by disrupting the formation of the endocuticle, group 15 according to IRAC 2020 (Copping and Menn, 2000).

Sublethal residues of insecticides can adversely affect life parameters (developmental rate, longevity, fecundity, oviposition, sex ratio), and behavior, including functional responses to prey density) of a predatory insect (Desneux *et al.*, 2007; Rahmani and Bandani, 2013). Estimation of demographic parameters through life table analysis is an essential approach to evaluate population growth and thus these studies are valuable for assessment of sublethal effects of a pesticide on both pest and natural enemies (Rahmani and Bandani, 2013). Moreover, functional and numerical responses are two main components of prey-predator interactions that can be significantly affected by insecticide residues (Martinou and Stavrinides, 2015). Effects of some insecticidal residues including AZ (Tedeschi *et al.*, 2001; Arnó and Gabarra, 2011), indoxacarb, and spinosad (Arnó and Gabarra, 2011) on biological parameters (eg.

survival rate and fecundity) as well as thiacloprid, chlorantraniliprole (Martinou *et al.*, 2014; Martinou *et al.*, 2015), abamectin, imidacloprid and chlorpyrifos (Sharifian *et al.*, 2017) on functional responses and predation rate of *M. pygmaeus* were previously studied.

Using study of life table parameters, a comprehensive description of population dynamics including the multiple sub-lethal effects of insecticides on insects can be achieved (Zhang *et al.*, 2014). Due to some limitations of traditional female age-specific life table, two-sex life table method was developed. The method can be applied to age-stage-structure two-sex populations, and is able to include variation in pre-adult developmental time, therefore, survival and fecundity curves will be more accurate (Chi and Getz, 1988). Moreover, functional response models and parameters have been studied for the predator foraging and exploring their dynamics and provide a conceptual understanding of prey-predator relationships. The response can be affected by sublethal residues of insecticides (Martinou and Stavrinides, 2015).

There has been limited information the effects of AZ, indoxacarb and emamectin benzoate+lufenuron on functional response and biological characteristics of *M. pygmaeus*. Therefore, this study aimed to evaluate effects of these three insecticides on life table parameters and functional responses of this predatory bug.

MATERIALS AND METHODS

Rearing of Macrolophus pygmaeus

The initial colony of *M. pygmaeus* was provided from Koppert Biological System Co. representative in Iran, Giah Co., Tehran. The insects were reared on flour moth eggs, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), and date palm pollen as daily diet, and bean pod as oviposition substrate at 25±1°C, 60±5% RH, and 16:8 hours (Light:Dark) in an incubator. The insects (30-70 individuals) were kept in Plexiglas

cylinders (18 cm high, 7.5 cm diameter) covered with a fine gauze, 100 meshes, lid on the top and margin for ventilation. Pieces of paper were placed in the rearing cylinder for resting the predator and reducing cannibalism.

Experimental Design

The experimental insecticides were azadirachtin A (Neem Azal® 1%EC, Trifoli Co., Germany), indoxacarb (Avaunt®15%SC, Giah Corp., Iran) and emamectin benzoate+lufenuron (Proclim Fit® 50% G, Syngenta Co., Austria). The insecticides were selected because they are currently applied to control *T. absoluta* in greenhouses of tomato (Derbalah *et al*., 2012). Standard methods to study side effects of insecticides on beneficial organism, developed by IOBC/WPRS Working Group, were used for the trials. The insects were exposed to 10% of field concentration in the experiments according to preliminary tests. The tests showed that the concentration caused about 18-20% mortality in the predator population. The concentrations were 400, 250, and 50 PPM for indoxacarb, azadirachtin and emamectin benzoate+lufenuron (Proclim Fit®), respectively. It should be noted that each treatment was separately carried out at 25±1°C, 65±5% RH, and a photoperiod of 16:8 hour (L:D) in an incubator that was well ventilated to avoid interference of different treatments.

Effect of Insecticides on Life Table Parameters

For each treatment, fifty glass Petri dishes (10 cm in diameter) were directly treated with the insecticides and allowed to dry (approximately 1-hour after application). Fifty pairs (male and female) of *M. pygmaeus*, which were obtained from first progeny (G1) of reared insects, were separately placed in each dish, which was then covered with a piece of Parafilm M® to avoid escaping of the bugs. To facilitate ventilation, a hole was made

on top of the dishes (2.5 cm diameter) and covered with fine net. After 24 hours, 20 pairs of the bug were chosen and transferred to a new rearing cylinder, with bean pods, for oviposition. After 24 hours, the bugs were removed. Females oviposit in plant tissues, leaf and stem. Due to difficult detection of the bug eggs in the plant tissues, the first twelve instar nymphs, 1 day old, were used for experiments as cohort. The life table was constructed based on 60 individuals of 1st nymphs as cohort. Each of the first instar nymphs was placed in Plexiglas Petri-dish, 9 cm in diameter, at 25±1ºC, 60±5% RH, and 16:8 hours (Light:Dark) in an incubator. The insect was daily checked and number of alive bugs and their life stages were recorded. Upon adult emergence, bean pods were removed to record the laid eggs and replaced with fresh one. The observation was continued until death of the last individual. In control, the males and females were not exposed to any insecticides. Each treatment had 12 replications.

Theory of age-stage (two sex life table), developed by Chi and Getz (1988) was used to analyze the raw life history data of *M. pygmaeus* by "TWOSEX-MS Chart for the Windows operating system" software, available at http://140.120.197.173/Ecology/prod02.htm (Chi, 2017). According to the theory, the parameters of age-specific survival rate (l_x), age-stage specific survival rate (s_{xj}; where, x= Insect age and j= Life stage), life expectancy (e_{xj}; where, x= Insect age and j= Life stag), mean generation Time (T), age-specific fecundity (m_x), age-stage specific fecundity (f_{xj}), gross reproductive rate ($GRR = \sum m_x$), net Reproductive rate (R_0), intrinsic rate of increase (r), as well as finite rate of increase ($\lambda = e^r$) were estimated. Also, the Adult Pre-Oviposition Period (APOP: The period between the emergence of an adult female and her first oviposition), Total Pre-Oviposition Period (TPOP: The time interval from birth to the beginning of oviposition) were also calculated using the experimental data. Bootstrap technique, using 1,000,000 re-sampling, was used for estimating standard

errors and variance of the life table parameters (Tibshirani and Efron, 1993). Moreover, the software was used for estimating the population consumption projected by using the life tables and predation rate of the original cohort, the cohorts constructed based on the 2.5 and 97.5% percentiles of finite rate (λ) and net Reproductive rate (R) in different experimental treatments. Sigma plot 12.5 was used to generate graphs.

Effect of Insecticides on Functional Responses

Each treatment was separately carried out. The experimental arena consisted of a Plexiglass Petri dish (10 cm diameter×1 cm deep). For ventilation, a hole was created on top of the dishes (2 cm diameter) and covered with fine gauze. The dishes were dipped in concentrations of 400, 250 and 50 PPM of indoxacarb, AZ and emamectin benzoate+lufenuron for 30 seconds, respectively. Distillated water was used in the control. One hundred females were exposed to the insecticide residues during 24 hours and they were used for functional responses experiments.

Densities of 8, 16, 32, 64 and 128 eggs of *E. kuehniella* per Petri dish were used in the trials. A female bug (3±2 days old) was located to each Petri-dish. Before beginning the experiments, the predator was starved for 24 hours. After 24 hours of the introduction, the predators were eliminated and the numbers of preyed eggs were recorded. All experiments were done in completely randomized design with nine replications.

Logistic regression analyses were used to determine type of *M. pygmaeus* functional responses (Juliano, 2001). A polynomial function that describes the relationship between N_e and N_0 was used for fitting the data (Equation 1): All experiments were carried out at 25±1°C, 65 ± 5% RH, and a photoperiod of 16:8 hours (L:D) in an incubator.

$$\frac{N_a}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 P_0^3)}{(1 + \exp P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)} \quad (1)$$

Where, N_e is the Number of consumed eggs, N_0 is the initial Number of eggs, and P_0, P_1, P_2, and P_3 are the intercepts of the linear, quadratic, and cubic coefficients, respectively**.** These parameters were calculated using the method of maximum likelihood (PROC CATMOD, SAS Institute 2001). If $P_1 > 0$ and $P_2 < 0$, the proportion of consumed eggs is positively density-dependent, Therefore, the data describes type III of functional response, but if $P_1 < 0$ and $P_2 > 0$, the proportion of consuming eggs decreases gradually as the initial number of egg offered increases and the data indicate type II of functional response (Juliano, 2001). Because our data implicated type III functional response (see result section), in the next step, fitting Roger's type III random predator (Equation 2) was done by using nonlinear least squares regression (PROC NLIN; SAS Institute Inc. 2001) for estimating parameters of functional responses (Rogers, 1972).

$$N_a = N_0[1 - e^{(bN_0)(T_h N_e - T)}] \quad (2)$$

Where, N_a is the Number of preyed egg, N_0 is the initial Number of egg, b is coefficient for change in attack rate (a) with prey density (a= bN_0), T is the total available Time available for searching (in this experiment T= 24 hours), and T_h is the handling Time. The functional response parameters were not considered statistically different when their confidence limits (95%) overlapped (Juliano, 2001).

RESULTS

Effect of Insecticides on Life Table Parameters

Total fecundity, sex ratio, TPOP and APOP of *M. pygmaeus* in different experimental treatments are shown in Table 1. The results indicated significant differences in TPOP, naymphal periods and APOP of the bug among different treatments. TPOP in indoxacarb treatment (26.41 days) was significantly more than

Table 1. Effects of different experimental treatments on biological parameters (mean±SE) of *Macrolophus pygmeus.*[a]

Treatment	Total fecundity	Sex ratio	APOP (Days)	TPOP (Days)
Azadirachtin	54.5±5.8[a*]	4.07±0.54[b]	10.12±0.23[b]	26.02±0.19 [a]
Indoxacarb	47.2±5.5[b]	7.06±0.54[a]	10.35±0.16[a]	26.41±0.2[b]
Emamectin benzoate	55.19±4.6[a]	6.1±0.53[a]	9.52±0.3[c]	26.06±0.2[a]
Control	55.3±7.1[a]	6.7±.063[a]	10.47±0.23[a]	26.12±0.18[a]

[a] TPOP: Total PreOviposition Period, APOP: The Adult PreOviposition Period. (a-b) The same letters in each column indicate non-significant difference (P< 0.05) using paired-bootstrap method.

Table 2. Effects of sublethal dosage of azadirachtin, indoxacarb and emamectin benzoate+lufenuron on life table parameters (mean±SE) of *Macrolophus pygmaeus.*[a]

	λ	GRR	R	R_0	T
Azadirachtin	1.086±0.006[a*]	49.424±11.088[a]	0.082±0.006[a]	21.379±4.733[a]	37.18±1.014[a]
Indoxacarb	1.085±0.005[a]	55.969±7.694[a]	0.091±0.004[a]	28.539±4.819[a]	37.256±0.697[a]
Emamectin benzoate+Lufenuron	1.076±0.007[b]	43.068±8.092[a]	0.074±0.007[b]	16.049±4.215[b]	36.749±0.686[a]
Control	1.089±0.006[a]	44.043±8.573a	0.085±0.005[a]	23.522±4.777[a]	36.73±0.745[a]
P-value	0.003	0.24	0.003	0.002	0.354

[a] λ: Finite rate of increase, *GRR*: Finite Rate of increase; r: Intrinsic rate of increase; R_0: Net Reproductive rate, T: Mean generation time (Tukey–Kramer Procedure). (a-b) The same letters in each column indicate non-significant difference (P< 0.05) using paired-bootstrap.

that of the control (26.12 days). However, APOP in emamectin benzoate+lufenuron (9.52 days) was significantly less than other treatments. Sex ratio of *M. pygmaeus* was affected by azadirachtin. Moreover, indoxacarb had the least total fecundity (47.2 eggs) among all treatments. Total fecundity in azadirachtin (54.5 eggs) and emamectin benzoate+lufenuron (55.19 eggs) treatments did not differ significantly from the control (55.3 eggs). However, total fecundity in indoxacarb treatment (47.2 eggs) was significantly lower than the control.

The intrinsic rate of increase (r), net Reproduction rate (R_0), Gross Reproductive Rate (GRR), finite rate of increase (λ), and the mean generation Time (T) were estimated using the bootstrap method and are shown in Table 2. No significant dereference was observed between the GRR and T values of *M. pygmaeus* in insecticidal treatments and control. In indoxacarb treatment, the λ, r and R_0 values were 10.09, 12.94% and 31.77% lower than those in the control, respectively.

The adult, female and male longevity as well as nymphal and adult durations are shown in Table 3. The longest and shortest adult longevities were observed in indoxacarb (46.9 days) and control (43.9 days), respectively. The insecticide treatments had no effects on male and female longevities. Similarly, nymphal and adult duration times were not affected by the insecticidal treatments. The lowest nymphal duration (25.02) was observed in emamectin benzoate+lufenuron.

The curves for the age-stage specific Survival rate (S_{xj}) indicate the probability of a newborn individual surviving to age *x* and stage j, are presented in Figure 1 for different treatments. The S_{xj} curves of the control, azadirachtin and indoxacarb are somewhat similar, but emamectin benzoate+lufenuron causes reduction in survival rate of all developmental stages. Age-specific survival rate (l_x), age-specific fecundity of total population (m_x) and age-specific net maternity ($l_x m_x$), specific survival rate for different treatments are shown in Figure 2. Curves of l_x, m_x and $l_x m_x$ in AZ treatment are similar to those in

Table 3. Developmental time (mean±SE) of different stages of *Macrolophus pygmaeus* in different treatments.[a]

Treatment	Adult longevity (Days)	Female longevity (Days)	Male longevity (Days)	Nymph	Adult
Azadirachtin	45.8±1.6[ab*]	44.89±2.36[a]	47.09±.18[a]	25.63±0.21[ab]	19.87±1.56[a]
Indoxacarb	46.9±1.6[b]	45.52±2.63[a]	44.57±2.63[a]	26.16±0.16[a]	18.75±1.51[a]
Emamectin benzoate+Lufenuron	45.1±1.54[a]	43.3±2.68[a]	45±3.01[a]	25.02±0.22[b]	17.94±1.88[a]
Control	43.9±1.98[a]	44.74±2.24a	47.04±2.23a	27.08±0.19[a]	19.68±1.51[a]
P-value	0.002	0.78	0.92	0.001	0.83

[a] (a-b) The same letters in each column indicate non-significant difference (P< 0.05) using paired-bootstrap method.

Table 4. Maximum-likelihood estimates from logistic regressions of the proportion of *E. kuehniella* eggs eaten by *M. pygmaeus* female on initial prey density.

Treatment	Type	Coefficient	Estimate	SE	χ^2	P-value
Azadirachtin	II	Constant	-0.2516	0.605	17.3	<0.0001
		Linear	0.00641	0.00209	9.44	0.0021
		Quadratic	-0.00007	0.000028	6.93	0.0085
		Cubic	2.92×10^{-7}	1.2×10^{-7}	5.63	0.0179
Indoxacarb	II	Constant	-0.2508	0.604	17.27	<0.0001
		Linear	0.00637	0.00208	9.34	0.0022
		Quadratic	-0.00006	0.000028	6.8	0.0091
		Cubic	2.87×10^{-7}	1.23×10^{-7}	5.45	0.0196
Emamectin benzoate+Lufenuron	II	Constant	-0.2501	0.0602	17.28	<0.0001
		Linear	0.000639	0.00208	9.41	0.0022
		Quadratic	-0.00007	0.000028	6.78	0.0088
		Cubic	2.9×10^{-7}	1.23×10^{-7}	5.51	0.0189
Control	II	Constant	-0.2528	0.0605	17.48	<0.0001
		Linear	0.00646	0.00209	9.6	0.0019
		Quadratic	-0.00007	0.000028	7.04	0.008
		Cubic	2.93×10^{-7}	1.23×10^{-7}	5.67	0.0173

control. The highest reduction in l_x and $l_x m_x$ values were observed in emamectin benzoate treatment. The curves of the life expectancies (e_{xj}) of *M. pygmaeus* for the control, azadirachtin, indoxacarb and emamectin benzoate+lufenuron are given in Figure 3. Values of e_{xj} in all experimental treatments decreased with time. The reduction was gradual in the control and AZ treatment. There was no difference between e_{xj} curves in AZ and control. However, the age-e_{xj} curves rapidly fall down in emamectin benzoate and indoxacarb treatments, especially during days 10-20.

There was no difference between the reproductive value (v_{xj}) curves of nymphal and female in the different experimental treatments (Figure 4). However, (v_{xj}) of nymphal stage in emamectin benzoate+lufenuron was relatively more than the other treatments.

Effect of Insecticides on Functional Responses

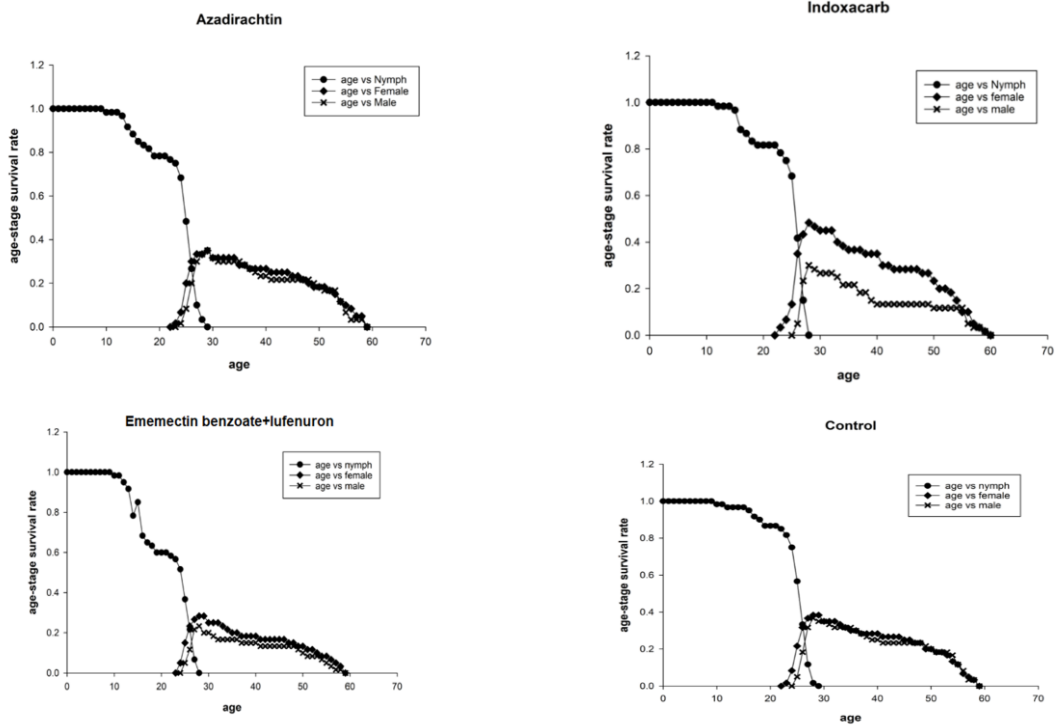Functional response curves of the predatory bug to the various densities of *E.*

Figure 1. Age-stage survival rate (s_{xj}) of *Macrolophus pygmaeus* in different experimental treatments.
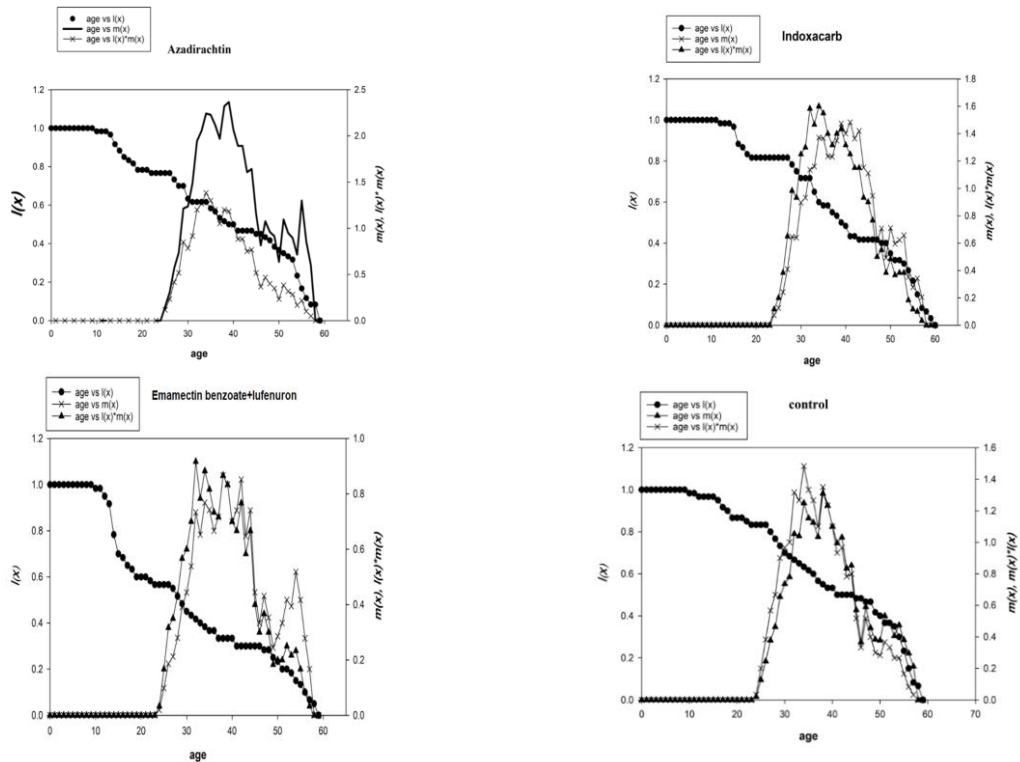

Figure 2. Curves of age-specific survival rate (l_x), fecundity (m_x) and maternity ($l_x m_x$) of *Macrolophus pygmaeus* in different treatments.
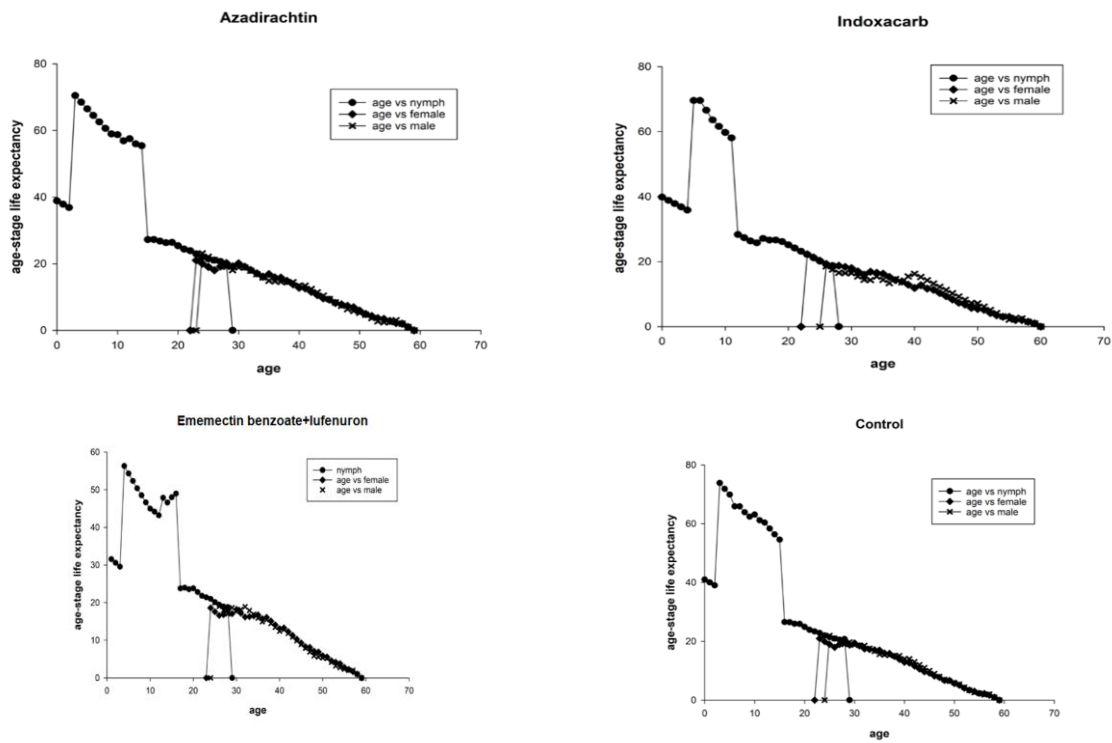
Figure 3. (e_{xj}) of *Macrolophus pygmaeus* in different experimental treatments.
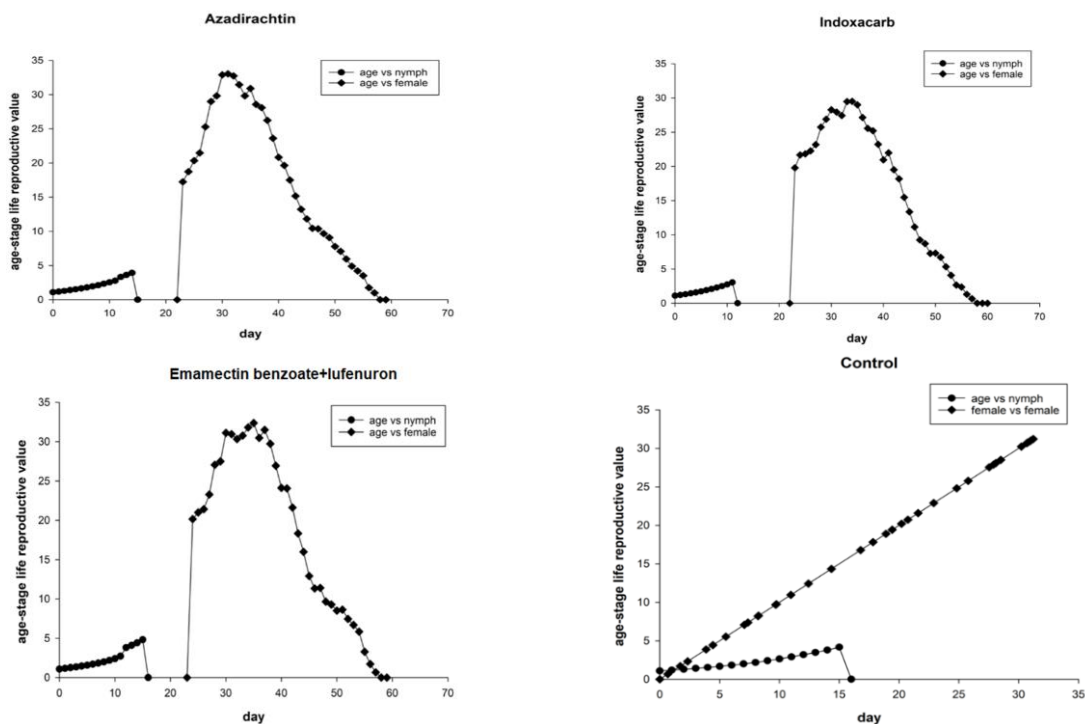

Figure 4. The age-stage life reproductive value (v_{xj}) of *Macrolophus pygmaeus* in different treatments.

kuehniella eggs are presented in Figure 5. The consumed eggs were increased by increasing the initial prey density.

The results of logistic regressions revealed a type III functional response of *M. pygmaeus* to eggs of *E. kuehniella* in the control and all insecticide treatments (Table 4). The total number of prey eggs consumed by *M. pygmaeus* increased linearly with increase in prey density. The coefficients of attack rate (b) and handling Time (T_h) for different treatments are shown in Table 5. The lowest value of the coefficient of attack rate (b) of *M. pygmaeus* females were observed in indoxacarb (0.1521). The handling Times (T_h) of the bug were 2.6471, 2.6845, 3.2226, and 2.6345 hours in AZ, indoxacarb, emamectin benzoate+lufenuron and control, respectively. There was no significant difference between the handling time values of various treatments.

DISCUSSION

Our data indicated that sublethal concentrations of indoxacarb and AZ had the highest and lowest negative effects on life table parameters of *M. pygmaeus*, respectively. The finding is in agreement with Arno and Gabarra (2011) who showed that sublethal residue of indoxacarb causes significant mortality to *M. pygmaeus* in comparison with AZ under field condition at seven days after treatment. However, indoxacarb residue did not adversely affect the bug fecundity. The conflict results may be due to different experimental conditions and the insecticidal concentration. For instance, temperature can significantly influence insect susceptibility to sublethal residues due to physiological effects of temperature on the insect hormone systems (Glunt *et al.,* 2014; Michalko and Košulič,
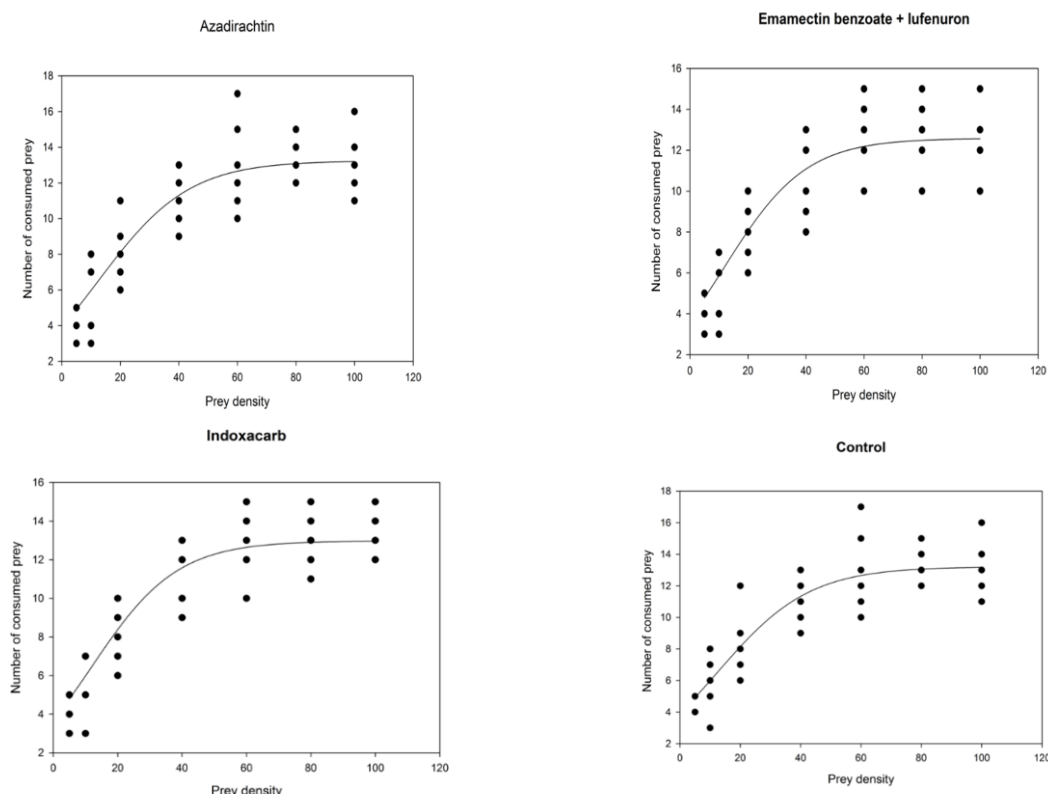

Figure 5. Number of consumed eggs of *Ephestia keuhniella* at different prey densities in various experimental treatments.

Table 5. Estimated (±SE) attack rate (*a*) and handling Time (T_h) of *Macrolophus pygmaeus* female on eggs of *Ephestia kuehniella* in different experimental treatments.

Treatment	Parameters	Estimate	Asymptotic 95% CI	
			Lower	Upper
Azadirachtin	b	0.2854	0.2753	0.2955
	T_h	2.6471	2.6470	2.6472
Indoxacarb	b	0.1521	0.1016	0.2025
	T_h	2.6845	2.5703	2.7987
Emamectin benzoate+Lufenuron	b	0.2121	0.1120	0.3121
	T_h	3.2226	0.1372	3.4988
Control	b	0.2911	0.2910	0.2912
	T_h	2.6349	2.6348	2.6349

2016). Effect of temperature on susceptibility of natural enemies to insecticide residues is well known (Desneux *et al.*, 2007). Also, using various types of substrate (petri dish or plant) may influence insecticide residue effects. Petri dish is inert substance and plant has waxy surface, trichomes, etc. The differences can significantly affect the residue effects (Yarahmadi *et al.*, 2009). Also, the results are in line with Nazarpour *et al.* (2016) who showed that indoxacarb had the highest adverse effects on population of coexisting generalist predators of *T. absoluta*, *Coccinella septempunctata* L. and *Chrysopa carnea* Stephens, in comparison with AZ and *Bacillus thuringiensis* Berlinier. Similarly, residues of neem products had fewer side effects on fecundity and survivals of *Macrolophus caliginosus* Wagner at five days after treatment (Tedeschi *et al.*, 2001). Also, it is demonstrated that fecundity and survival rate of *Orius laevigatus* Fiber (Het., Anthocoridae) were not significantly affected by AZ and indoxacarb residues (Angeli *et al.*, 2005). Moreover, indoxacarb did not show significant toxicity on *Orius insidiosus* Say (Het., Anthocoridae).

In many cases, deleterious effects of AZ on beneficial arthropods, e.g. Natural enemies, were reported. Therefore, its safety has been a matter of debate (Arno *et al.*, 2009; Gontijo *et al.*, 2015). Moreover, the bio-pesticide exhibits some side effects including sterilant activity, in addition to anti-feedant and growth regulator activity on natural enemies and pollinators. However, different side effects on non-target arthropods can be observed based on concentration of AZ residues (Lima *et al.*, 2015). Side effect of AZ on immature stages of predatory arthropods may occur due to growth regulatory properties of biopesticide and it can also affect hormone physiology of the organisms (Pilar Marco *et al.*, 1990; Gontijo *et al.*, 2015).

Our data implicated the type III functional response of *M. pygmaeus*. Therefore, the type of functional response type was not affected by exposure to sublethal concentration of the insecticides. However, the coefficient of attack rate (b) of the predator was affected by exposure to indoxacarb residue, and no significant differences were observed among the estimated handling Times (T_h) of the treatments.

The total time of functional response includes attack rate+handling time. Therefore, increase in handling time causes decrease in available time for prey searching. It was previously reported that abiotic factors, such as pesticides, can enhance the handling time due to their behavioral effects (Li *et al.*, 2006; He *et al.*, 2012; Malaquias *et al.*, 2014; Martinou *et al.*, 2015).

Similarly, Sharifian *et al.* (2017) showed that sublethal concentration of abamectin, imidacloprid and chlorpyrifos did not significantly affect functional response type of *M. pygmaeus*. Their study revealed that

abamectin has no effect on handling time and attack rate of the predatory bug. However, these parameters were significantly influenced by imidaclopride and chlorpyrifos residues. In addition, Martinou *et al.* (2014) demonstrated that thiacloprid residue causes significant reduction in the predation rate of *M. pygmaeus*. However, no significant effect was observed for chlorantraniliprole residue on the insect predation rate. Also, it is reported that functional response parameters of *Acanthaspis pedestris* Stål (Het., Reduviidae) are negatively affected by cypermetrin (Claver *et al.*, 2003).

One of the best methods to control *T. absoluta* in the greenhouses is integrating usage of predators and selective insecticides (Arno *et al.*, 2009). Therefore, we recommend application of AZ in integration with *M. pygmaeus* release to control *T. absoluta* in greenhouse tomatoes.

CONCLUSIONS

Our laboratory study showed that biological parameters (fecundity, survival and longevities) of *M. pygmaeu* were not significantly affected by AZ residues. Moreover, functional response type and parameters were not significantly affected in AZ treatment. Therefore, the study suggests that integration of *M. pygmaeus* with AZ is a compatible and effective strategy for management of greenhouse pests. However, further studies on the compatibility and effectiveness of AZ and the predator are required under greenhouse and field conditions.

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اثرات زیرکشنده سموم رایج مورد استفاده در گوجهفرنگی روی واکنش تابعی و پارامترهای زیستی سن Rumber *Macrolophus pygmaeus* (Hemiptera: Miridae)

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چکیده

سن Rumber *Macrolophus pygmaeus* عامل موثر کنترل بیولوژیک علیه آفات بسیاری از محصولات از جمله مینوز برگ گوجهفرنگی Meyrick *Tuta absoluta* میباشد. اثرات زیرکشنده حشرهکشهای آزادیراختین (نیمآزال®)، ایندوکساکارب (آوانت®) و امامکتین بنزوآت+لوفنورون (پروکلیم فیت®) روی خصوصیات زیستی و واکنش تابعی این سن شکارگر در شرایط آزمایشگاهی در دمای ۲۵±۱، رطوبت نسبی ۶۵±۱٪ و دوره روشنایی تاریکی ۱۶: ۸ ساعت، مورد بررسی قرار گرفت. برای این منظور، مادههای این سن شکارگر در معرض بقایای زیرکشنده (۱۰٪ غلظت مزرعهای) این حشرهکشها قرار گرفتند. روش جدول زندگی دوجنسی و مدل راجرز به ترتیب برای بررسی تاثیرات این حشرهکشها روی پارامترهای زیستی و واکنش تابعی شکارگر مذبور مورد استفاده قرار گرفت. نتایج نشان داد که بقایای زیرکشنده ایندوکساکارب و آزادیراختین به ترتیب بیشترین و کمترین اثرات جانبی را روی پارامترهای جدول زندگی *M. pygmaeus* داشتند. در حالیکه که آزادیراختین تاثیر معنیداری روی پارامترهای جدول زندگی این سن نداشت، زادآوری کل و طول عمر این شکارگر در تیمار ایندوکساکارب به ترتیب به صورت معنیداری کمتر (۱۴/۶٪) و بیشتر (۶/۹٪) از تیمار شاهد بود. این شکارگر واکنش تابعی نوع دوم را نشان داد. نوع واکنش تحت تاثیر بقایای حشرهکشها قرار نگرفت. با این حال، زمان دسترسی به طعمه در تیمار امامکتین بنزوات+لوفنورون (۱/۷۴ ساعت) به صورت معنیداری طولانیتر از سایر تیمارها بود؛ ولی اختلاف معنیداری بین نرخ حمله (*a*) در تیمارهای مختلف موجود نداشت. در کل، این آزمایشات ثابت نمود که آزادیراختین یک حشرهکش کم خطر برای *M. pygmaeus* است که میتوان با کنترل بیولوژیک توسط این سن تلفیق گردد.