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Research Article

Toxicity of garlic extract on Spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae) in comparison to its two hosts

Ziba Mamduh[1*], Vahid Hosseininaveh[2], Morteza Movahedi Fazeli[3] and Khalil Talebi Jahromi[2]

1. Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.
3. Department of Plant Protection, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

Abstract: The toxicity of garlic is confirmed on some agricultural pests. The aim of this study was to comparatively evaluate the sensitivity of *Podisus maculiventris* (Say) to garlic extract and two insect pests: Mediterranean flour moth *Ephestia kuehniella* (Zeller) and Colorado potato beetle *Leptinotarsa decemlineata* (Say). LC_{50} was calculated at 8.02% for *E. kuehniella*. The results showed toxic compounds in garlic extract delayed all developmental stages and reproduction parameters of *L. decemlineata*. In contrast, there were no considerable negative effects on many of the biological parameters of the predatory insect, *P. maculiventris*. The results suggest that garlic, as a green pesticide, could be considered an environmentally suitable alternative in pest management programs.

Keywords: *Allium sativum*, Biological control agent, Botanical pesticide, *Ephestia kuehniella*, *Leptinotarsa decemlineata*

Introduction

Agricultural systems remain heavily reliant on synthetic pesticides for pest control which can cause environmental and management problems (Isman, 2006). The first step towards successful integrated pest management is to develop alternative environmentally sustainable strategies (Ekesi, 2000; Jarial, 2001; Prowse *et al*., 2006; Marie *et al*., 2009; Mobki *et al*., 2014; Anwar *et al*., 2017). Recently, botanical derivatives have been used to control many pests including insects and mites, nematodes, bacteria, and fungi due to their low toxicity on the environment and humankind (Muhsin *et al*., 2001; Bakri and Douglas, 2005; Prowse *et al*., 2006; Castillo-Sanchez *et al*., 2010; Bonsignore and Vacante, 2012; Anwar *et al*., 2017).

Garlic *Allium sativum* is considered to have insecticidal properties (Prowse *et al*., 2006; Kalu *et al*., 2010; Attia *et al*., 2012; Baidoo and Mochiah, 2016; Beltagy and Omar, 2016; Niroumand *et al*., 2016; Anwar *et al*., 2017). Toxicity of garlic was elucidatedfor many pests including Coleoptera (Lu and Liu, 2003; Beltagy and Omar, 2016); Lepidoptera (Perez-Mendoza and Aguilera, 2004; Oparaeke *et al*., 2007; Sadeghi *et al*., 2008; Baidoo and Mochiah, 2016; Rizvi *et al*., 2016); Hemiptera (Jaastad *et al*., 2009; Barati *et al*., 2013; Saeed *et al*., 2016; Mamduh *et al*., 2017); Diptera (Prowse *et al*., 2006); and also for Acari (Hincapié *et al*., 2008; Attia *et al*., 2012). Additionally, garlic and the compounds in its

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* **Corresponding author**, e-mail: z.mamdooh@gmail.com
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extract possess repellent, antifeedant, and anti-microbial properties (Rahman and Motoyama, 2000; Bakri and Douglas, 2005; Mobki *et al*., 2014; Mamduh and Movahedi Fazel, 2015; Beltagy and Omar, 2016; Niroumand *et al*., 2016; Anwar *et al*., 2017). Nowadays, the main compounds of garlic are extracted and synthesized in products with different commercial names (for example, Garlic Barrie®, Ecoguard® and Repel®); these are used as pesticides and repellents for pest control in greenhouse conditions in Iran and other countries, as cited by Moreau *et al*. (2006), Jahromi *et al*. (2011), Attia *et al*. (2012), EFSA (2012) and Kumar (2015). Also, garlic cloves has been added to pesticide registration lists in Brazil and Mexico (El-Wakeil, 2013).

Several previous studies have evaluated the toxicity of garlic on herbivorous insects; while the side effects of garlic on non-target organisms have not been completely understood. The application of garlic along with biological control agents for integrated pest management strategies may have an effect on the success of pest control. Therefore, it is important to investigate the side effects of garlic compounds on natural enemies as compared to their herbivore preys. This study aimed to investigate the side effects of Garlic extract (GE) on *Podisus maculiventris* (Say) (Hem.: Pentatomidae) in comparison with two pest species, a factitious host, *Ephestia kuehniella* (Zeller) (Lep.: Pyralidae) and insect pest, *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae) as *P. maculiventris*'s preys. The Colorado potato beetle (*L. decemlineata*) is an economically important pest in potatoes and some Solanaceaeous plants (Alyokhin *et al*., 2008). The Mediterranean flour moth (*E. kuehniella*) is one of the major pests in industrial flour mills; the larvae reduce the product quality by their presence and cause direct damage by feeding and the production of frass (Mikhaiel, 2011). *P. maculiventris* is known as an important generalist predatory bug on some pests from different orders of insects such as *L. decemlineata*, *Epilachna varivestis* Mulsant (Col.: Coccinellidae), *Pyrrhalta viburni* Paycull (Col.: Chrysomelidae), etc. (De Clercq, 2008). It was hypothesized that *Allium sativum* product is a friendly environmental pesticide as it may have low side effects on biological parameters of the key predator *P. maculiventris* as compared to herbivore insects.

Experiment

Preparation of garlic extract (GE)

GE was prepared according to Meriga *et al*. (2012) with some modifications. Briefly, 20g fresh garlic cloves (Tarom cultivar) were blended and mixed with 100 ml methanol ≥ 99.9% (Merck Schuchardt, Germany). The mixture was shaken at 70 rpm for 60 min at room temperature and then centrifuged at 4000 rpm for 30 min at 4 °C. The supernatant was removed and centrifuged twice under the same conditions. The final supernatant was subjected to rotary evaporation (Rotavapor R-114, Buchi: Swiss) in order to evaporate the solvent. The condensed matter was considered to be the stock extract (100% concentration) and was kept in a dark glass container at-4 °C. Serial dilutions of 25, 15, 10, 5, 2%, and water (as control) were prepared by using distilled water. GE was freshly prepared before each bio-assay.

Insect rearing

P. maculiventris insect predators were reared in containers (10 × 6 × 5 cm) with cotton, soaked in distilled water. They were fed on factitious host, *E. kuehniella* larvae in a growth chamber at 24 ± 1 °C, 70 ± 10% Relative Humidity, and a photoperiod of 16:8 h (Light: Dark). The number of preys (*E. kuehniella*) for each predator was increased based on the growth of *P. maculiventris* insects. During the reproduction period, the eggs were removed and placed in Petri dishes (9 cm diameter) containing a filter paper (Schleicher and Schuell, Microscience, Ref. No. 10 311 610) moistened with distilled water.

E. kuehniella were reared on artificial food (10: 2: 1 mixture of oat bran: wheat germ: yeast) in plastic containers (30 × 20 × 15 cm) at 27 ± 1 °C, 60 ± 10% RH, and 16:8 h (Light: Dark). Another host (insect pest), Colorado potato beetles were collected from the potato fields of Zanjan, Iran (37°8′N and 47°47′E), and were reared on potato leaves in greenhouse conditions at 25 ± 3 °C and 60 ± 10% RH. The 4th instar larvae in the pre-pupal stage were placed in special boxes containing sand in order to produce pupa. Fresh leaves were available to feed the larvae and adults every three days. The egg-containing leaves were removed to Petri dishes (9 cm diameter) containing a filter paper (Schleicher and Schuell, Microscience, Ref. No. 10 311 610) moistened with wet cotton soaked in distilled water.

Bioassay

Toxicity of GE on the predator and the preys

To investigate contact toxicity, the 4th instars of *E. kuehniella* (1-2 days old) were dipped for four seconds in GE dilutions of 25, 15, 10, 5, 2%, and control v/v. For each treatment, there were five replications and 15 larvae per replication. Treated insect larvae were air dried and transferred to Petri dishes containing some artificial food. Due to a majority of larval death, the mortality percentage was recorded three days after the treatment. The treated insects were kept under same rearing conditions.

Twenty-four-hour-old 4th instar nymphs of *P. maculiventris* were treated with the same method as explained above, with four replications and 15 nymphs per replication. Treated insects were air dried and transferred to plastic containers (30 × 20 × 15cm) containing filter paper (Schleicher and Schuell, Microscience) and were fed with *E. kuehniella* under the same rearing conditions. In this assay, the mortality percentage was calculated after three days.

L. decemlineata larvae and adults cause more damage by feeding. Hence, oral toxicity was tested on Colorado potato beetle larvae. Twenty-four-hour-old 4th instar larvae were placed in Petri dishes (10 cm diameter) and were fed on leaves treated with various concentrations of GE (25, 15, 10, 5, 2% v/v and water as control) in five replications with 15 larvae for each replication. The leaves were dipped in GE for five seconds and were available to the larvae after drying. The insects were kept in a growth chamber at 25 ± 1 °C, 60 ± 10% RH, and 14: 10 (Light: Dark). The mortality percentage was calculated after three days.

In toxicity experiments, insects were considered to be dead when they did not move or respond to gentle touch.

Side effects of GE on biological parameters of *P. maculiventris*

To evaluate the influence of GE on biological parameters of *P. maculiventris*, 60 4th instar nymphs (24-hour-old) were treated at different concentrations of GE for 4 seconds; they were kept in the same rearing condition until all the insects died. Data was recorded every 24 h. The total mortality percentage was calculated on the 19th day when the insects had grown into seven-day-old adults. Moreover, the developmental time, sex ratio, longevity, and reproduction parameters were measured as impact factors of fitness.

Contact toxicity of GE on eggs of *P. maculiventris* and *L. decemlineata*

Twenty egg batches were selected to evaluate the toxicity of GE on the embryonic stages of *P. maculiventris* and its prey, *L. decemlineata*. The different egg ages (one, three, and five-day-old eggs) were separated and treated in different concentrations of GE for 4 seconds as explained above, in five replications for the predator and six replications for *L. decemlineata*. After air drying, the egg batches were placed in Petri dishes containing filter paper and cotton moistened with distilled water until hatching time. The rearing condition was the same as mentioned above. The duration of the embryonic period and the hatching rate were recorded, daily.

Digestive toxicity of *L. decemlineata* larvae and adults

Different larval instars: first (one-day-old), second, third, and fourth stage larvae of *L. decemlineata* were tested separately; they were fed daily on treated leaves until they emerged as adults. Data was recorded every day and new leaves were placed for the insects. The total mortality percentage was calculated for all the immature stages in the treatment of different larval instars. After maturation, some treatments that had almost less mortality (0, 5, 15, and 25% concentrations of GE) were selected for investigating delayed effects on reproduction parameters. This experiment was done in five replications with two adults (a male and a female) per replication for four treatments. These adults were fed on non-treated leaves until the time of death. In addition to the developmental time and the daily feeding of treated larvae of *L. decemlineata*, other biological parameters such as sex ratio, longevity, pre-oviposition and oviposition period, fecundity, and fertility were measured. To investigate the toxicity of GE on adults, 24-h-old adults were separated and transferred to containers (10 × 6 × 5 cm) in five replications with six insects (three females and three males) per replicate. The insects were fed daily with the treated leaves of different concentrations of GE as explained above. The mortality percentage of the adults was calculated after seven days. Moreover, all biological parameters, measured in the experiment on larvae, were calculated. The nutritional level of daily feeding was measured by a curvimeter.

Statistical analysis

The statistical program SPSS v.22.0 (2012) was used for statistical analysis. Before ANOVA, a normality test was done by using the Kolmogorov-Smirnov test. The data were set up in randomized complete designs. Normality data related to the mortality percentage were transformed in arcsine √x and other data were transformed in Ln. One-way ANOVA was used for transformed data and multiple mean comparisons were performed using Tukey-Kramer's test (Tukey, 1953; Kramer, 1956). The data related to contact toxicity of GE on the eggs were carried out in a completely randomized two level factorial design; GE concentration (0, 2, 5, 10, 15 and 25%) and embryonic stage (1, 3, 5 day). Abbott's correction formula was used to assess natural mortality in untreated insects as control samples, and probit analysis was applied to estimate LC_{25} and LC_{50} values of *E. kuehniella* by using Polo-plus software (Robertson *et al*., 2007).

Results

Toxicity of GE on the predator and the preys

Data analysis showed a significant difference in the mortality percentage of the treated 4th instar larval of *E. kuehniella* ($F_{(5,29)} = 26.68$, $p < 0.01$). LC_{25} and LC_{50} values were 2.59% and 8.02%, respectively, in the treated larvae after 48 h (Table 1). Total mortality percentage values after three days are shown in Fig. 1a. It was observed that the mortality rate increases with increasing GE concentration (Fig. 1a). Moreover, the effect of GE on the nymphs of *P. maculiventris* and the means of mortality percentage were significantly different as compared with the control insects (Fig. 1c). The mortalities of *E. kuehniella* and *P. maculiventris* at 25% GE concentration were 76% and 27%, respectively (Fig. 1). These results confirmed that the sensitivity of *E. kuehniella*is to contact toxicity was more than the predator. Fig. 1b shows the oral toxicity to *L. decemlineata* larvae. The highest mortality (36%) was observed at concentration of 25% GE after reducing of control samples mortality and which was followed by 5%. In contrast to *E. kuehniella*, the toxicity of GE on *L. decemlineata* and *P. maculiventris* didn't show a dose-dependent relationship and higher mortalities were obtained at some lower concentrations (Fig. 1).

Table 1 Probit analysis of the efficacy of garlic extract (GE) on 4th instar larvae of *Ephestia kuehniella*.

Time (h)	n	Intercept	Slope	$\chi 2$	LC_{25} (%)	LC_{50} (%)
48	450	5.13 ± 0.05	1.37 ± 0.28	2.5	2.59	8.02

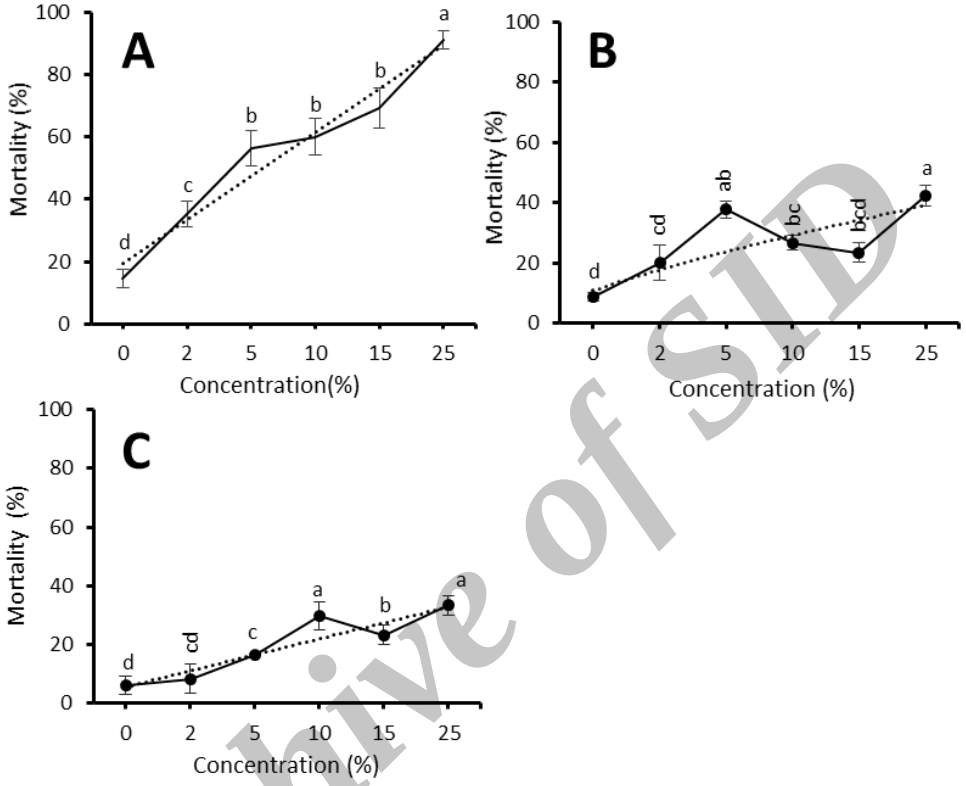

Figure 1 The efficacy of garlic extract (GE) on a predator insect, *Podisus maculicentris* in comparison to two insect pest, *Leptinotarsa deceplineata* and *Epestia kuehniella*. Mean of mortality of (A) 4th instar larvae of *E. kuehniella* and in contact toxicity (r = 0.97); (B) 4th instar larvae of *L. decemlineata* in oral toxicity (r = 0.86); (C) 4th instar nymph of *P. maculiventris* in contact toxicity treated (r = 0.95) by different concentrations of the GE after 3 days. Means followed by different letters are significantly different (Tukey-Kramer test, $P < 0.05$).

Side effects of GE on biological parameters

The efficacy of GE was evaluated on nymphs of *P. maculiventris* and larvae of *L. decemlineata* during the period of their development. Analyzed data showed increasing toxicity during that time (Fig. 2). However, the increasing slope of toxicity of GE on *L. decemlineata* was more than that of *P. maculiventris* (Fig. 2). The mortality percentage was recorded from the time of treatment in 24-hour-old fourth instar nymphs of the predator and 24-hour-old first stage larvae of *L. decemlineata* until the emergence of seven-day-old adults. According to Fig. (2), the highest mortality was recorded in 10% concentrations of both insects. Fig. (3) shows the mean of total mortality of the immature stages of *L. decemlineata* (24-hour-old first larvae to emergence of adults) treated in different larval instars (1th-4th instar). The results in Fig. (3) showed a significant difference between GE concentrations in the treatment of 1st larvae ($F_{(5,29)} = 7.80$, $p < 0.001$), 2nd larvae ($F_{(5,29)} = 10.56$, $p < 0.001$), 3rd larvae ($F_{(5,29)} = 7.95$, $p <$

0.001), and 4th stage larvae ($F_{(5,29)}$ = 3.08, p = 0.028) of *L. decemlineata*. Analyzed data in Table 2 determined that GE affects the developmental time of the Colorado potato beetle and increases this parameter ($F_{(5,71)}$ = 5.09, p = 0.001).

Results showed no significant difference in the number of daily eggs, and in the total number of eggs per female of *P. maculiventris* during the oviposition period and their embryonic duration. However, there was a significant reduction in the hatching percentage ($F_{(5,232)}$ = 10.14, p < 0.01) and the longevity of female adults ($F_{(5,35)}$ = 2.91, p < 0.05), as shown in Table 3. ANOVA analysis showed no significant difference in developmental time, percentage of adult emergence, sex ratio, pre-oviposition period, oviposition period, and longevity of male adults of the predator insect. After maturation, abnormality was observed in the tarsus area of one or two legs of some *P. maculiventris* adults. Although, there were no significant differences in the percentage of abnormality between treated and control insects. Additionally, the pre-oviposition period, oviposition period, fertility, fecundity, and longevity of the abnormal adults were not significantly different as compared to the normal insects.

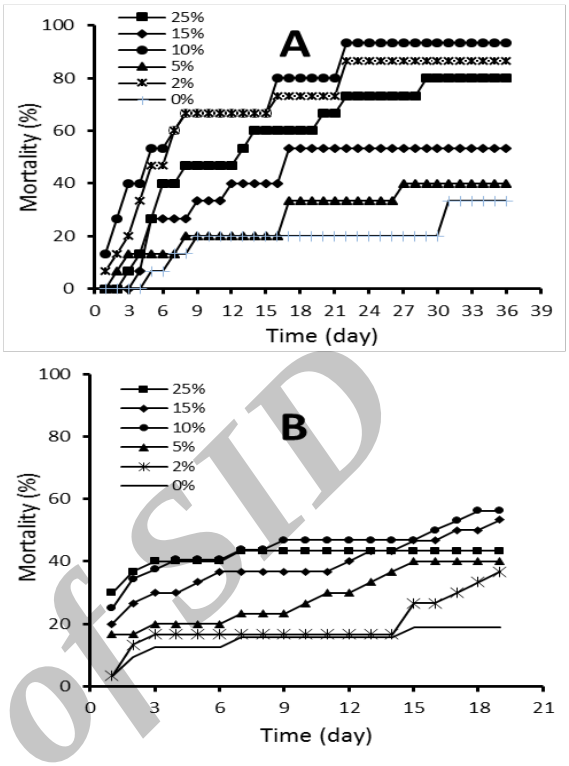

Figure 2 Cumulative mortality of (A): host, *Leptinotarsa decemlineata* [1st instar (1-d old) to adult (7-d old)] and (B): predator insect, *Podisus maculiventris* [4th instar (1-d old) to adult (7-d old)] treated by garlic extract (GE).

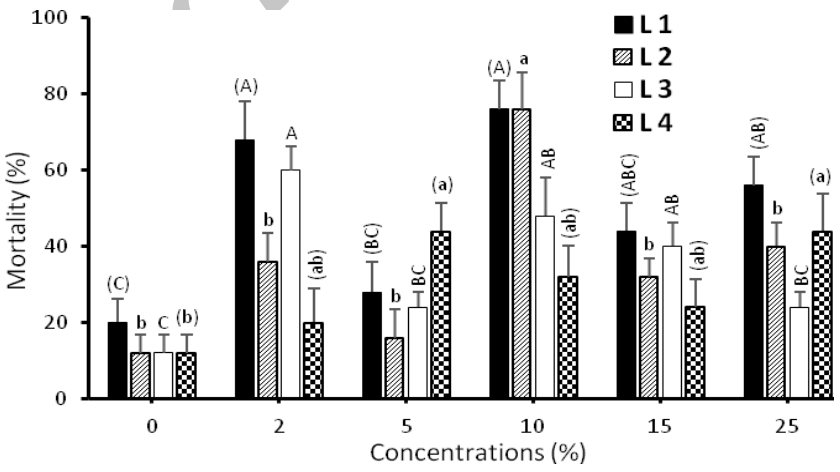

Figure 3 Mean mortality of total immature stages of *Leptinotarsa decemlineata* (1 day old first larvae to emergence of adult) treated in different larval instars (L1: first, L2: second, L3: third and L4: fourth instar larvae) by garlic extract (GE) concentrations. Means in each instar followed by different letters are significantly different by Tukey-Kramer test ($P < 0.05$).

Table 2 The efficacy of garlic extract (GE) on the developmental time of *Leptinotarsa decemlineata*.

Concentrations (%)	Developmental time (day)
0	26.8 ± 0.3 b
2	28.0 ± 0.7 ab
5	29.8 ± 0.5 a
10	26.7 ± 0.9 b
15	30.7 ± 0.9 a
25	28.9 ± 0.9 ab

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

Table 3 The efficacy of garlic extract (GE) on the hatching percentage (mean ± SE) of laid eggs and longevity of females of *Podisus maculiventris* treated in nymphal instar by different concentrations.

Concentrations (%)	Egg hatching (%)	Longevity (day)
0	74.57 ± 1.22 a	52.11 ± 5.25 abc
2	60.17 ± 1.08 b	28.57 ± 3.40 c
5	35.86 ± 1.34 c	61.00 ± 14.53 ab
10	46.73 ± 2.80 bc	39.00 ± 8.85 bc
15	35.25 ± 1.62 c	75.67 ± 14.04 a
25	44.11 ± 3.84 bc	30.83 ± 3.77 bc

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

Conversely, garlic compounds had negative effects even post-maturation on *L. decemlineata* (Table 4) with immature stages treatment by GE. GE significantly decreased pre-oviposition ($F_{(3,19)} = 6.92$, $p = 0.002$) and oviposition ($F_{(3,19)} = 79.62$, $p < 0.001$) periods; fecundity ($F_{(3,19)} = 3.96$, $p = 0.036$) and fertility ($F_{(3,19)} = 56.71$, $p < 0.001$) parameters; and also, the longevity of female adults ($F_{(3,19)} = 15.02$, $p = 0.002$) (Table 4). The sex ratio showed no significant difference on this pest insect. The treatment of adults of *L. decemlineata* by GE decreased the survival rate ($F_{(5,29)} = 4.76$, $p = 0.004$), oviposition period ($F_{(5,29)} = 11.78$, $p < 0.001$), and fecundity parameters ($F_{(5,29)} = 4.93$, $p = 0.003$) (Table 5). Other biological parameters (pre-oviposition period, fertility, and longevity), however, showed no significant difference with control adults in treatment on adults. GE led to a decrease in daily feeding in treated larvae ($F_{(5,119)} = 46.32$, $p < 0.001$) and in treated adults of *L. decemlineata* ($F_{(5,179)} = 10.32$, $p < 0.001$) as shown in Fig. 4).

Table 4 The efficacy of garlic extract (GE) on reproduction parameters and longevity of females of *Leptinotarsa decemlineata* treated in larval instar by different concentrations.

Concentration (%)	Pre-oviposition period (day)	Oviposition period (day)	Fecundity (egg/female)	Fertility (%)
0	10.3 ± 0.9 a	28.8 ± 2.0 a	264.5 ± 15.7 a	95.1 ± 1.3 a
5	5.8 ± 0.5 b	7.5 ± 0.3 b	116.0 ± 26.3 ab	82.5 ± 3.3 ab
15	3.3 ± 0.3 b	7.5 ± 0.5 b	68.7 ± 10.7 ab	76.6 ± 4.1 b
25	7.3 ± 1.5 ab	6.3 ± 0.8 b	57.2 ± 12.1 b	15.7 ± 5.0 b

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

Table 5 The efficacy of garlic extract (GE) on reproduction parameters and longevity of treated adults of *Leptinotarsa decemlineata*.

Concentration (%)	Mortality (%)	Oviposition period (day)	Fecundity (eggs/female)
0	0.0 ± 0.0 b	27.4 ± 2.2 a	206.4 ± 27.3 a
2	13.3 ± 6.2 ab	15.0 ± 1.8 bc	142.2 ± 50.3 ab
5	6.7 ± 4.1 b	21.0 ± 2.4 ab	54.4 ± 5.6 b
10	20.0 ± 6.2 ab	18.8 ± 1.2 b	154.4 ± 49.8 ab
15	10.0 ± 4.1 b	10.4 ± 0.2 c	65.2 ± 11.0 b
25	36.7 ± 9.7 a	17.2 ± 1.3 bc	92.6 ± 6.8 ab

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

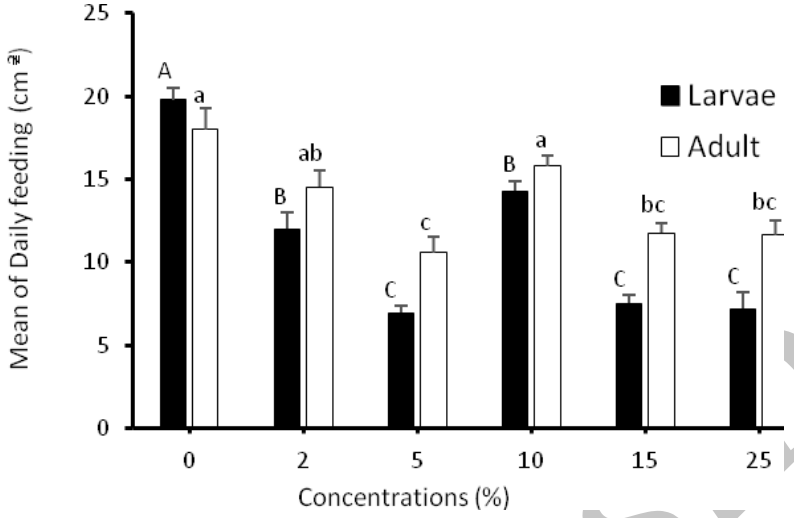

Figure 4 Mean of daily feeding (cm^2) of larvae and adult of *Leptinotarsa decemlineata* treated by different garlic extract (GE) concentrations. Means followed by different letters are significantly different by Tukey-Kramer test ($P < 0.05$).

Contact toxicity of GE on eggs of *P. maculiventris* and *L. decemlineata*

According to ANOVA results, there were no significant differences in GE concentration on embryonic stage, and interaction effects of these factors on the hatching rate of *P. maculiventris* eggs. While, GE decreased the hatching rate of *L. decemlineata* ($F_{(5,107)}$ = 6.92, p < 0.001). Additionally, results in Table 6 show that the toxicity of garlic compounds on the egg hatching percentage of the Colorado potato beetle is affected by embryonic stage ($F_{(5,107)}$ = 8.10, p = 0.001). However, the interaction of concentration × embryonic age showed no significant effects on this insect. The highest hatching percentage was in the control samples of *L. decemlineata* treated in the one-day-old egg (91.00 ± 9.61%) and the lowest percentage was observed in the three-days-old eggs treated by 10% concentration (16.33 ± 9.61%).

The embryonic period of *P. maculiventris* was not affected by GE concentrations and selection of egg ages. On the contrary, GE concentrations increased the embryonic period of *L. decemlineata*, significantly ($F_{(5,107)}$ = 4.76, p = 0.001) as shown in Table 6.

Table 6 The efficacy of garlic extract (GE) on hatching percentage and the embryonic period in treated eggs of *Leptinotarsa decemlineata*.

Entries	Egg hatching (%)	Embryonic period (day)
Concentration (%)		
0	78.61 ± 2.96 A	6.4 ± 0.19 B
2	64.50 ± 5.55 ABC	6.6 ± 0.29 AB
5	69.11 ± 5.66 AB	6.3 ± 0.32 B
10	39.22 ± 6.98 C	7.3 ± 0.30 AB
15	53.89 ± 6.81 BC	6.4 ± 0.26 B
25	47.56 ± 7.40 BC	7.6 ± 0.32 A
Age (day)		
1	70.42 ± 3.97 a	7.0 ± 0.16 ns
3	55.75 ± 5.12 b	6.7 ± 0.21 ns
5	50.28 ± 4.55 b	6.6 ± 0.25 ns

Means in a column followed by different letters are significantly different by Tukey-Kramer test ($P < 0.05$).
ns: non significant

Discussion

This study showed the toxicity of GE on a predator insect (*P. maculiventris*) and its two preys, *E. kuehniella* larvae as a model insect and *L. decemlineata* (Fig. 1). Our results were in accordance with previous studies in which garlic compounds were toxic on some insects (Singh and Singh, 1996; Prowse *et al*., 2006; Kalu *et al*.,

2010; Mikhaiel, 2011; Vassiliou, 2011; Meriga *et al*., 2012; Mobki *et al*., 2014; Mamduh *et al*. 2017). Larvicidal effects of garlic were identified on *Culex quinquefasciatus* Say (Dip.: Culicidae), *Delia radicum* Linnaeus (Dip.: Anthomyiidae), *Musca domestica* Linnaeus (Dip.: Muscidae), *Tribolium castaneum* Herbst (Col.: Tenebrionidae) and *Spodoptera litura* Fabricius (Lep.: Noctuidae) (Prowse *et al*., 2006; Kalu *et al*., 2010; Mikhaiel, 2011; Meriga *et al*., 2012; Mobki *et al*., 2014). Mobki *et al*. (2014) reported contact and fumigant toxicity of GE on larvae and adults of *T. castaneum.* The studies showed that the leaves sprayed with 7.49 mg/l concentration of GE reduced half the density of *Tetranychus urticae* Koch (Acari: Tetranychidae) (Hincapié *et al*., 2008; Attia *et al*., 2012). Field studies on cabbage looper, *Trichoplusia binotalis* Hiibner (Lep.: Noctuidae) reported minimum infestation in the plots that were treated with 3% garlic extract as compared to ginger and tobacco extracts (Rizvi *et al*., 2016). Cultivation of garlic and some other plants, intercropped between wheat strips, led to reduction in the population dynamic of *Sitobean avenae* Fabricius (Hem.: Aphididae) (Saeed *et al*., 2016). According to Table 6, GE decreased the survival rate of *L. decemlineata* eggs. These results are confirmed by other researcher that showed toxicity effects of garlic on the eggs of some species such as *S. litura* and *Helicoverpa armigera* Hübner (Lep.: Noctuidae), *Earias vitella* Sherborn (Lep.: Noctuidae), *Maruca vitrata* Fabricius (Lep.: Cerambidae), *Dysdercus koenigii* Fabricius (Hem.: Pyrrhocoridae), *Clavigralla tomentosicollis* Stål (Hem.: Coreidae), *L. decemlineata* and *D. radicum* (Gurusubramanian and Krishna, 1996; Ekesi, 2000; Prowse *et al*., 2006).

According to previous studies, the toxicity effects of garlic were attributed to some sulfuric compounds including thiosulfinates and disulfinates, and a main compound called allicin (Rahman and Motoyama, 2000; Prowse *et al*., 2006; Ratti *et al*., 2007; Attia *et al*., 2012; Yousaf *et al*., 2012; Anwar *et al*., 2017). Allicin is a disulfide compound induced from alliin, which can inhibit the activity of acetylcholinesterase, lactate dehydrogenase, and alkaline phosphatase (Singh and Singh, 1996; Anwar *et al*., 2017). Moreover, some proteins of garlic (as leaf and bulb lectin) disrupt certain physiological processes, especially digestion and absorption, that play an important role in garlic toxicity as mentioned by (Upadhyay and Singh, 2012; Macedo *et al*., 2015; Niroumand *et al*., 2016). It appears that some compounds of the sulfide group can penetrate into eggs and bind to sulfidryl-containing proteins, impairing vital physiological and biochemical processes in embryonic development as reported by Ekesi, (2000) and Prowse *et al*. (2006). Additionally, the prevention of egg hatching may be due to the intensification of the hardening process of egg shells by garlic compounds as confirmed by Prowse *et al*. (2006). The results of this study indicated that egg age affects the mortality *L. decemlineata* eggs; there can be two possible reasons for this-the change in thickness of egg shells of different ages, and the compounds present in the shells and inside the eggs as stated by Fogleman, (2000), Bakri and Douglas, (2005) and Meriga *et al*.(2012).

In the present study, the sensitivity range was calculated based on the maximum mortality percentage of *E. kuehniella* that showed more sensitivity than *P. maculiventris* in contact toxicity. The mean of mortality percentage of *E. kuehniella* was calculated at 76% after three days, and for *P. maculiventris* at 24% after 19 days, with deduction of the control effect (Figs. 1 and 2). Hence GE is more toxicto *E. kuehniella* than to *P. maculiventris.* Moreover, the toxicity of GE to *L. decemlineata* caused considerably increased mortality rate as compared to the predator insect during the same period of time (Fig. 2). According to the results, the efficacy of garlic compounds did not show a linear relationship among the various concentrations (Fig. 1). Hence, maximum mortality occurred in lower concentrations-10% followed by 2% (Figs. 1, 2, and 3). This contradiction was observed in previous studies (Ekesi, 2000; Prowse *et al*., 2006). Prowse *et al.* (2006) reported that one reason for this phenomenon could be the presence

of various compounds in garlic and their interaction effects in certain concentrations. According to Fig. 3, GE toxicity on the larvae of the Colorado potato beetle was affected by treated larval instar, which could be due to the different sensitivity of larval instars and their different physiological agents; these results are similar with those obtained by (Ho *et al*., 1996; Prowse *et al*., 2006). Additionally, more feeding from treated leaves causes an aggregation of toxins in the insect body. As Fig. (4) shows, the maximum daily feeding of *L. decemlineata* larvae and adults relates to 10% concentration. There are other possible reasons for lower mortality in higher concentrations, which reduce the daily feeding due to the repellent effect of garlic (Rahman and Motoyama, 2000; Hincapié *et al*., 2008; Mobki *et al*., 2014) and also, because of the connection of garlic lectins with sensory receptors of mouth parts (Fig. 4) and the amount of toxic compounds in the body (Olmstead and Shelton, 2012; Upadhyay and Singh, 2012).

The efficacy of GE on biological parameters confirms delayed effects of garlic compounds, which require enough time for the aggregation of toxic compounds and the increase in toxicity as found by other researchers like Qi *et al*. (2001); Prowse *et al*. (2006) and Mamduh *et al*.(2017). Garlic compounds increase the developmental time of the Colorado potato beetle (Table 2), which could be due to the delay in the synthesis of chitin by lectin proteins (Cutler *et al*., 2006). According to a previous study, garlic oil may show anti-ecdysone activity and disrupt the development of insects (Beltagy and Omar, 2016). Moreover, the reaction of lectins with alkaline phosphatase enzymes may have bad impact on: growth process, metamorphosis, reproduction, neural connections, hormone synthesis, elemental metabolism, diapause, and cysticosis (Jurat-Fuentes *et al*., 2011). GE has an effect on reproduction parameters and fitness factors in treated larvae and adult of *L. decemlineata* (Tables 4 and 5). In critical conditions such as contact with pesticides, insects usually oviposit sooner than under normal conditions and reduce their reproduction rate-. So the pre-oviposition and oviposition periods, and the total number of eggs per female decrease (Bandani, 2010); this was also observed in the present study. Moreover, the presence of nutritional inhibitors such as lectins in GE causes a slowdown in digestion and absorption of food, which could reduce the storage of energy sources, especially proteins, and prevent the growth of follicles (Beltagy and Omar, 2016).

Additionally, garlic lectin can disrupt metamorphosis and reproduction of insects by bonding with P450 cytochrome, halloween proteins, and alkaline phosphatase in the hemolymph, ovaries, and molting hormones, and cause embryo death, reduction of fertility, and efficacy for new cuticle synthesis (Namiki *et al*., 2005; Upadhyay *et al*., 2012; Upadhyay and Singh, 2012; Macedo *et al*., 2015).

Comparing GE toxicity on *P. maculiventris*, *E. kuehnialla*, and *L. decemlineata* showed the predator's relative resistance to the toxic compounds of garlic on mortality rate, developmental time, longevity, and reproduction factors. In this study, garlic compounds were relatively safe on the predator. Saeed *et al*. (2016) elicited similar results in which the cultivation of garlic between wheat strips reduced the population of *S. avenae*, while the number of some predator insects increased. Fand *et al*. (2012) showed that the aqueous extract of garlic caused more than 60% mortality and inhibited the reproduction process of *Phenacoccus solenopsis* Tinsley (Hem.: Pseudococcidae); while it was almost safe on two Coccinellidae predator species-*Hyperaspis maindroni* Sicard and *Cryptolaemus montrouzieri* Mulsant. Some studies showed that using some plant extracts, including garlic and hot pepper, decreased the population of some major pests of cabbage, *Brassica oleracea* L. (Brassicaceae) in compared with control samples (Baidoo and Mochiah, 2016). While, the natural enemies sampled in the botanical extract-sprayed plots were more than the insecticide-sprayed plots (Baidoo and Mochiah, 2016). Prowse *et al*. (2006) proposed that due to variations of some physiological factors of the species concerning ability of permeation of toxic compounds,

isolation and disintegration, their sensitivity to toxic compounds will be different. On the other hand, the amount of toxic agents may have been less than the threshold of toxicity (Fogleman, 2000; Jarial, 2001; Bakri and Douglas, 2005; Meriga *et al*., 2012). Moreover, the resistance of *P. maculiventris* to some insecticides was reported (Tillman and Mullinix, 2004; Cutler *et al*., 2006); this could propose that the quantity and quality of monooxygenase enzymes in this insect may cause resistance to other pesticides (Tillman and Mullinix, 2004; Cutler *et al*., 2006). Our results were contradictory to previous studies (Namiki *et al*., 2005; Cutler *et al*., 2006; Cao *et al*., 2012; Olmstead and Shelton, 2012). Hence, our results support the minimum negative effects of GE on *P. maculiventris*.

Conclusion

Garlic extract with contact and digestive toxicity can affect a range of biological function of insects. Its anti-nutritional compounds can reduce the damage of *L. decemlneata*. Although the acute toxicity effects of GE is not satisfactory on Colorado beetle, it can lengthen the embryonic and growth period, as well as reduce survival rate, weight, fecundity, fertility, oviposition period, and longevity. The GE showed high mortality on larvae of *E. kuehniella*. Conversely, this study showed lower sensitivity of *P. maculiventris*, as a generalist predator, to garlic compounds. The observations on the toxicity of different concentrations could be considered for economical use of lower yet effective concentrations. In this study, 10% and 15% concentrations may be proposed instead of higher concentrations. On the other hand, garlic extract is considered to be a safe botanical pesticide for application on the natural enemy, *P. maculiventris* in integrated pest management strategies. These results provide evidence that GE (as a botanical insecticide) has considerable promise for effective use as an environmentally friendly alternative to synthetic, chemical insecticides against various destructive pests such as the Colorado potato beetle, while still being reasonably safe to its predatory insects. Nevertheless, more research is needed to elucidate any side effects of GE on other predators and natural enemies and also to get information on physiological aspects of mortality and morbidity effects of GE on the two pest hosts and their predator.

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سمّیت ترکیبات سیر روی سن شکارگر *Podisus maculiventris* (Hemiptera: Pentatomidae) در مقایسه با دو میزبان آن

زیبا ممدوح[1*]، وحید حسینی‌نوه[2]، مرتضی موحدی‌فاضل[3] و خلیل طالبی‌جهرمی[2]

۱- گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه شهید چمران اهواز، اهواز، ایران.
۲- گروه گیاه‌پزشکی، دانشکده کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران.
۳- گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه زنجان، زنجان، ایران.
* پست الکترونیکی نویسنده مسئول مکاتبه: z.mamdooh@gmail.com
دریافت: ۱ آبان ۱۳۹۶؛ پذیرش: ۱۶ بهمن ۱۳۹۶

چکیده: سمّیت گیاه سیر بر روی گروهی از آفات کشاورزی تأیید شده است. هدف از این مطالعه، بررسی حساسیت سن شکارگر *Podisus maculiventris* (Say) به عصاره گیاه سیر در مقایسه با دو آفت کرم آرد، *Ephestia kuehniella* (Zeller) و سوسک کلرادو سیب‌زمینی، *Leptinotarsa decemlineata* (Say) بوده است. LC_{50} محاسبه شده برای کرم آرد در حدود ۸/۰۲ درصد برآورد شده است. نتایج حاصله گویای اثرات تأخیری ترکیبات سمّی بر تمامی مراحل رشد و نموی و پارامترهای تولیدمثلی سوسک کلرادو بوده است. در نقطه مقابل، بسیاری از پارامترهای بیولوژیکی سن *P. maculiventris*، تحت تأثیر اثرات منفی عصاره سیر قرار نگرفت. نتایج حاصله، عصاره گیاه سیر را به‌عنوان یک آفت‌کش سبز پیشنهاد می‌کند که می‌تواند به‌عنوان یک جایگزین مناسب در برنامه‌های مدیریتی آفات مدنظر قرار گیرد.

واژگان کلیدی: *Allium sativum*، عوامل کنترل بیولوژیک، آفتکش‌های گیاهی، *Ephestia kuehniella*، *Leptinotarsa decemlineata*