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Biological effects of two eco-friendly novel formulations of a pyrethroid insecticide lambda-cyhalothrin on the parasitoid wasp *Trissolcus grandis* (Thomson)

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ABSTRACT

Eurygaster integriceps Puton is an important pest of wheat and barley in Iran and the Middle East. Chemical control of the pest should be carried out by selective insecticides to conserve its natural enemies such as *Trissolcus grandis* (Thomson). In this research, the lethal effects of field recommended doses of two new formulations from lambda-cyhalothrin including Karate-zeon® (CS 10%) and Hef-lambda® (SC 5%) were studied on the mature and different immature stages of *T. grandis* under the laboratory conditions. Contact of the adult wasps with sprayed areas by both insecticides caused 100% mortality. Parasitized eggs containing first instar (at the third day of parasitism), second instar (at the fifth day of parasitism) and pupa stage (at the eighth day of parasitism) were dipped into insecticide solutions. At all immature stages and for both insecticides, the highest mean percent emergence of *T. grandis* female was observed in SC 5% treated eggs. Treatment of eggs with both insecticides at different immature stages of parasitoid did not show any negative effect on the parasitism activity. However, Hef-lambda®, was proved to be more selective insecticide, because it showed less negative effect on the number of adults that emerged after egg treatment. According to the results of present study, Hef-lambda® can be considered as a less hazardous selective and eco-friendly insecticide in the Sunn pest management.

Key words: *Eurygaster integriceps*, egg parasitoid, lambda-cyhalothrin, selective insecticide, *Trissolcus grandis.*

Introduction

Sunn pest, *Eurygaster integriceps* Puton (Hemiptera; Scutelleridae), which is the most destructive insect pest of wheat and barley in Iran and many other Middle Eastern countries, is currently controlled by synthetic chemicals (Radjabi 2000, Parker *et al.* 2011). However, repeated utilization of the chemical insecticides has created different side-effects such as environmental pollution, losses of natural enemies and Sunn pest populations' resistant (Davari and Parker 2018). Despite these negative effects, unfortunately synthetic chemicals are still used as the main agents for the Sunn pest management (Popov *et al.* 1996). Therefore, the introduction of eco-friendly agents to alternate with detrimental chemicals is necessary.

Egg parasitoid wasps are significant natural enemies of the Sunn pest and their natural parasitism sometimes reaches up to 90% (El-Bouhssini *et al.* 2004). In Iran, various species of *Trissolcus*, *Gryon*, and *Ooencyrtus* have been reported as the egg parasitoid wasps of the Sunn pest and other shield bugs. *Trissolcus grandis* (Thomson) is the most common egg parasitoid wasp of the Sunn pest in hot highland and cold lowland areas (Radjabi 2000). Widespread use of synthetic chemicals has eliminated or reduced the population of this beneficial wasp (Desneux *et al.* 2007, Biondi *et al.* 2012). Accordingly, the use of selective insecticides with lower side-effects on the natural enemies has been recommended in combination with utilization of natural enemies for integrated management of Sunn pest

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(Hassan *et al.* 1994, Critchley 1998).

Karate-zeon® is an insecticide with a contact-digestive effect and is a member of the pyrethroid group (Perrin *et al.* 1998). In Zeon technology, the active substance of lambda-cyhalothrin is surrounded by microcapsules. This insecticide rapidly penetrates in the insect integument, causes damage to its nervous system and then muscle activities are impaired and paralysis and death occurs (Perrin *et al.* 1998). Hef-lambda® is a pyrethroid insecticide with contact-digestive and non-systemic effects that paralyzes the nervous system of the insects (Anonymous 2018). These insecticides are used as selective pesticides for the Sunn pest control in Iran and in other countries (Kocak and Babaroglu 2006, Mohammadipour *et al.* 2015).

The main objective of present study was to compare the insecticidal effects of two new formulations of lambda-cyhalothrin including Karate-zeon® and Hef-lambda® on the mature and different immature stages of *T. grandis* and to introduce a less hazardous insecticide to the egg parasitoids of the Sunn pest.

MATERRIALS AND METHODS

Sunn pest eggs

Overwintered Sunn pest insects were collected from Gaskogen cultivar of wheat fields in Busajin village (37°57'48.87"N 48°14'38.44"E), Ardabil province, Iran, during May to Jun 2016 by insect nets and/or manually. Insects were reared on the wheat seeds (Gaskogen cultivar) in a growth chamber that was set at 25 ± 1 °C, 60 ± 5 % RH and 16:8 (L:D) h photoperiod in a plastic cylindrical oviposition cage (20 × 30 × 9 cm). Inside the cage, paper strips were set vertically for oviposition and every 24 hours, strips containing egg masses were collected and replaced with new strips (Sheikhi-Garjan *et al.* 2005).

Preparation and Proliferation of *T. grandis*

Sunn pest egg masses attached to the wheat plants as egg traps in a field during the spring of 2016. One week later the egg traps were collected and transferred to the laboratory to obtain *T. grandis* adults. Each egg mass was kept in a separate test tube (1.5 × 10 cm) and the emerged adults were used to identify the wasp species. After identification of the *Trissolcus grandis* species, the parasitoid wasp was reared on Sunn pest egg masses that had been stored in a refrigerator. In the first seven days each parasitoids wasp was provided with two fresh egg masses (28 eggs) per day with a strip of honey into a 6cm plastic Petri dish and after the day seven, only one egg mass (14 eggs) per day was offered to each wasp. The first in-vitro generation of *T. grandis* was used in the bioassay and the eggs parasitized by the second generation of parasitoid were used to study the effect of insecticides on immature stages of the wasp inside the Sunn pest egg (Sheikhi-Garjan *et al.* 2005).

Insecticides

Karate-zeon® (CS 10%, Syngenta Crop Protection Inc., Greensboro, NC, USA) and Hef-lambda® (SC 5%, Hef Chemical Co., Tehran, Iran) were obtained from a local distributor in Ardabil province and were used on the different immature stages and adults of *T. grandis*. The recommended field concentrations of Karate-zeon® and Hef-lambda® (75 ml/ha and 150 ml/ha correspond to 0.2 ml/lit and 0.4 ml/lit, respectively) were prepared using distilled water. A wetting drop of Triton™X-100 (T8787, SIGMA-Germany) (555ppm) was added to each emulsion and control treatment and thoroughly mixed.

Direct contact effect of insecticides on adult females

An exposure cage was used to evaluate the susceptibility of *T. grandis* adult females to the studied insecticides. The cages were made of two glass plates with 10 × 10 cm dimensions and a glass frame. The glass plates were secured by glue after being placed on the frame. Some holes were made on the frame and covered with Organza screen to allow air circulation. A honey strip was placed inside the cage for adult parasitoids feeding. Insecticides were sprayed by manual sprayer (Nangfo 2059-1L) on the glass surfaces and plates were left in the fresh air for three hours to let the surface drying (Saber *et al.* 2005). Males and females are diagnosed based on their antennae. In female antennae 11 segmented. Scapus approximately 6 times longer than its greatest width and radicle. First flagellar segment 2-2.5 times longer than its width and equal to pedicel. Males have 12-segmented antennae. First flagellar segment 1.5 times longer than its width, slightly longer than pedicel and approximately equal to following segment (Kocak and Kilincer 2003). Then, 60 one-day-old female wasps were released to each container and the mortality was recorded after 24 hours. Glass plates in some exposure cages were sprayed with water as the control. This bioassay was conducted in a completely random experiment with four replications (Sheikhi-Garjan *et al.* 2005).

Insecticidal effect on the immature stages

Parasitized Sunn pest eggs containing three different immature stages of parasitoid wasp including first instar (at the third day of parasitism), second instar (at the fifth day of parasitism) and pupa stage (at the eighth day of parasitism) were immersed in the insecticide solutions. In addition, some of the parasitized eggs were immersed in distilled water at the above stages as the controls. One egg masses of Sunn pest (14 eggs) that had been parasitized by the first generation of *T. grandis* wasps were treated with each insecticide after three days of parasitism (Sheikhi-Garjan *et al.* 2005). The parasitized eggs were dipped in insecticide for five seconds and then kept under the above stated laboratory conditions for three hours until fully dried (Saber *et al.* 2001). The treated eggs were attached with glue to the paper strip and put in Petri dishes and transferred to the growth chamber set at the above-stated conditions. After 15 days, the number of complete and incomplete emerged female and male adult wasps was recorded. The same procedure was used on 5-day and 8-day old parasitized eggs. This bioassay was also conducted in a completely random experiment with ten replications for each treatment (Sheikhi-Garjan *et al.* 2005).

Effect of insecticides on parasitism

To evaluate the effect of insecticides on parasitism, the wasps that had emerged from the parasitized eggs dipped in the insecticide solution during their immature stages were assessed for 5 days regarding for their parasitic activity. To conduct evaluation, firstly, 10-one-old day mated female wasps were transferred individually into a 6 cm Petri dish containing two Sunn pest egg masses (28 eggs). After 24 hours, the egg masses were collected from the Petri dishes and transferred into test tubes and two new egg masses were replaced in Petri dishes. This process was continued for five days. The test tubes containing parasitized eggs of each day were placed in the growth chamber under the laboratory conditions. After 15 days from the first day of parasitism the number of parasitized eggs and emerged adult female and male wasps were counted and recorded (Sheikhi-Garjan *et al.* 2005).

Statistical analysis

Data were transformed to $\arcsin\sqrt{y/100}$ prior to analysis. The variance of collected data was analyzed using SAS-version 9.1 software. The mean comparison was determined with the SNK test and in some cases by orthogonal at probability level ($P \leq 0.05$).

RESULTS AND DISCUSSION

The bioassay results showed that the field recommended doses of both studied insecticides cause 100% mortality of adult wasps after 24 hours through contact effect. The mean percent complete emergence of *T. grandis* wasps from the parasitized eggs dipped in two insecticide solutions at various immature stages are given in Table 1. There were significant differences between the mean percent complete emergence of *T. grandis* wasps in both insecticides and the control for the first instar ($F = 20.48$; $df = 2, 31$; $P < 0.0001$) and pupa stage ($F = 21.30$; $df = 2, 31$; $P < 0.0001$). The highest and the lowest mean percent complete emergence from treated eggs were seen on the control and Karate-zeon® treatments, respectively (Table 1).

In both insecticides, significant differences were found among different immature stages of immersing parasitized eggs in the insecticide solutions with respect to the mean percent complete emergence of wasps ($P \leq 0.05$). Thereby, the highest mean percent complete emerged wasps was observed in Karate-zeon® ($F = 8.14$; $df = 2, 31$; $P = 0.0013$) and Hef-lambda® ($F = 2.89$; $df = 2, 31$; $P = 0.0072$) for the second instar (Table 1).

A comparison of the mean percent complete emergence of *T. grandis* adults (female and male) at all three immature stages of parasitoid showed a significant difference between insecticides treatments and the control in the mean percent of gynogenesis ($P \leq 0.05$). The highest mean percent of gynogenesis were observed in control and Hef-lambda® at the first instar ($F = 16.20$; $df = 2, 31$; $P < 0.0001$), second instar ($F = 5.16$; $df = 2, 31$; $P = 0.0137$) and pupa stage ($F = 26.37$; $df = 2, 31$; $P < 0.0001$) (Table 1).

Table 1- Mean (±SE) percent complete emergence of *Trissolcus grandis* wasps from the parasitized eggs dipped in insecticide solutions at various immature stages (first and second instars and pupa)

Treatments	First instar		Second instar		Pupa	
Karate-zeon®	48.66 ± 3.93[c] B		70.93 ± 2.84[a] A		44.97 ± 4.33[c] B	
Hef-lambda®	68.91 ± 3.81[b] AB		80.82 ± 4.02[a] A		65.48 ± 4.70[b] B	
Control	87.10 ± 4.60[a] A		82.60 ± 3.28[a] A		82.55 ± 3.96[a] A	
Treatments	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
Karate-zeon®	39.90 ± 3.04[b]	8.76 ± 2.21[b]	54.80 ± 2.66[b]	16.13 ± 2.72[a]	25.55 ± 3.88[b]	19.42 ± 4.71[a]
Hef-lambda®	57.45 ± 2.94[a]	11.46 ± 2.14[b]	66.52 ± 3.76[a]	14.30 ± 3.84[a]	54.32 ± 3.95[a]	11.16 ± 4.62[a]
Control	65.03 ± 3.55[a]	22.07 ± 2.58[a]	66.10 ± 3.07[a]	16.50 ± 3.14[a]	62.55 ± 3.55[a]	20.00 ± 4.29[a]

Means in each column followed by different small letters, or in each row by different capital letters are significantly different at 5% level (SNK test)

Number of parasitoids eggs in each instar and pupa stage: 420

Results of mean percent incomplete emergence of *T.grandis* (wasps that died while chewing the shell of an egg) from eggs that were dipped in an insecticide solution in various immature stages are given in Table 2. There were significant differences between mean percent incomplete emergence from eggs treated with the two insecticides and the control at all three immature stages ($P \leq 0.05$). The highest mean percent incomplete emergence of wasps at the first instar ($F = 18.53$; df = 2, 31; $P < 0.0001$) and pupa stage ($F = 25.70$; df = 2, 31; $P < 0.0001$) were seen in the Karate-zeon® treated eggs. At the second instar ($F = 8.76$; df = 2, 31; $P = 0.002$) the highest mean percent incomplete emergence of wasps were seen in the Karate-zeon® and Hef-lambda® treatments. In control and Hef-lambda® treatments, no significant differences were found among the parasitized eggs treated at three different immature stages of the wasp. In the Karate-zeon® treated eggs ($F = 5.56$; df = 2, 31; $P = 0.0088$) the highest mean percent incomplete emergence of wasp was observed on the eggs that were treated at the first instar and pupa stage of the parasitoid (Table 2).

A comparison of the mean percent complete emergence of *T. grandis* adults (female and male) at the first instar ($F = 16.13$; df = 2, 31; $P < 0.0001$) and pupa stage of wasp ($F = 24.78$; df = 2, 31; $P < 0.0001$) showed a significant difference between two insecticides treatments and the control in the mean percent of gynogenesis ($P \leq 0.05$). The highest mean percent of gynogenesis was observed in the Karate-zeon® treatment (Table 2).

Table 2- Mean (±SE) percent incomplete emergence of *Trissolcus grandis* wasps from the parasitized eggs dipped in insecticide solutions at various immature stages (first and second instars and pupa)

Treatments	**First instar**		**Second instar**		**Pupa**	
Karate-zeon®	34.85 ± 3.80^{a} A		16.93 ± 2.60^{a} B		43.10 ± 4.30^{a} A	
Hef-lambda®	18.38 ± 3.56^{b} A		9.12 ± 3.54^{a} A		22.60 ± 4.52^{b} A	
Control	0.00 ± 0.00^{c} A		0.00 ± 0.00^{b} A		0.00 ± 0.00^{c} A	
Treatments	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
Karate-zeon®	29.23 ± 364^{a}	5.62 ± 2.09^{ab}	8.65 ± 2.79^{a}	8.28 ± 2.35^{a}	41.03 ± 4.16^{a}	2. 07 ± 0.96^{a}
Hef-lambda®	7.74 ± 3.41^{b}	10.64 ± 1.95^{a}	5.27 ± 3.60^{a}	3.85 ± 3.03^{a}	20.41 ± 4.55^{b}	2.19 ± 0.10^{a}
Control	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{a}

Means in each column followed by different small letters, or in each row by different capital letters are significantly different at 5% level (SNK test). Number of parasitoids eggs in each instar and pupa stage: 420

The mean percent parasitism of *T. grandis* wasps, based on 10 wasps at the start of the parasitism, emerged from eggs dipped in insecticide solutions at various immature stages are given at Table 3. There were significant differences between two insecticides and the control in the percent parasitism of wasps emerged from treated eggs at the first instar of parasitoid ($F = 3.44$; df = 2, 44; $P = 0.0373$) (Table 3).

Table 3- Mean (±SE) percent parasitism of *Trissolcus grandis* wasps emerged from eggs dipped in insecticide solutions at various immature stages (first and second instars and pupa)

Treatments	**First instar**	**Second instar**	**Pupa**
Karate-zeon®	67.31 ± 2.67^{b} A	60.66 ± 4.15^{a} A	70.11 ± 3.27^{a} A
Hef-lambda®	72.49 ± 2.74^{ab}AB	70.03 ± 4.79^{a} B	79.35 ± 3.70^{a} A
Control	79.59 ± 2.52^{a} A	72.82 ± 4.67^{a} A	75.92 ± 3.33^{a} A

Means in each column followed by different small letters, or in each row by different capital letters are significantly different at 5% level (SNK test)

The mean percent emergence of the third generation of *T. grandis* adults (female and male) from the parasitized eggs dipped in insecticide solutions at various immature stages of wasp are given in Table 4. There were significant differences between two insecticides treatments and the control in the mean percent emergence of adult wasps from the eggs that had been treated at the first instar of parasitoid wasp ($P \leq 0.05$). The highest mean percent of adult female emergence from the eggs that had been treated at the first instar ($F=4.99$; $df= 2, 44$; $P=0.0092$) were observed in control and Hef-lambda® treatments. In all treatments, the mean percent emergence of females was higher than that of the males at all immature stages (Table 4).

Table 4- Mean (±SE) percent emergence of the third generation adult wasps from the parasitized eggs of the wasp that were dipped in insecticide solutions in various immature stages (first and second instars and pupa)

Treatments	First instar		Second instar		Pupa	
Karate-zeon®	57.33 ± 3.41[b] A		63.78 ± 4.26[a] A		53.71 ± 4.00[a] A	
Hef-lambda®	63.80 ± 3.94[ab] A		62.78 ± 4.16[a] A		61.17 ± 4.25[a] A	
Control	69.66 ± 3.23[a] AB		73.04 ± 4.77[a] A		61.44 ± 4.07[a] B	
Treatments	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
Karate-zeon®	41.40 ± 3.47[b]	15.93 ± 3.05[a]	54.49 ± 3.37[a]	9.29 ± 2.05[a]	48.70 ± 3.08[a]	5.01 ± 1.61[b]
Hef-lambda®	53.56 ± 3.42[a]	10.24 ± 3.31[a]	54.68 ± 3.42[a]	8.10 ± 2.60[a]	52.40 ± 3.20[a]	8.77 ± 1.94[ab]
control	55.53 ± 3.28[a]	14.13 ± 2.88[a]	60.32 ± 3.75[a]	12.72 ± 2.00[a]	50.65 ± 3.84[a]	10.79 ± 1.16[a]

Means in each column followed by different small letters, or in each row by different capital letters are significantly different at 5% level (SNK test)

In the Sunn pest contaminated areas, chemical control is considered to be the most important and effective way of controlling the outbreak population (Popov *et al.* 1996, Davari and Parker 2018). At the present time, pyrethroid insecticides due to its repellency feature on the Suun pest egg parasitoid wasps, use of low-dose, economics reason compared to organphosphorous insecticides, lower environmental pollution, rapid excretion by the kidneys and less persistent in soil have been more used in control of Sunn pest (Hill and Inaba 1991, Haghshenas 1993). Understanding the effects of insecticides on various stages of parasitoid wasp is important in the adjustment of insecticide application time for reducing their harmful effects and avoiding spraying at the most sensitive stage of the parasitoid life cycle. Our findings are in compliance with the result of Popov *et al.* (1980) who found that chemical control of overwintered adult Sunn pest reduced egg parasitism of the pest from 86.9% in un-sprayed areas to 8.3% in sprayed areas.

Lambda cyhalothrin is one of the registered pyrethroid insecticides against Sunn Pest in Iran (Mohammadipour *et al.* 2015). Mohammadipour *et al.* (2015 & 2019) and Honarmand *et al.* (2016) indicated that two new formulations of lambda-cyhalothrin including, Karate-zeon®(CS 10%) and Hef-lambda®(SC 5%) for 20 days after spraying inflicted more than 70% mortality on the overwintered adults Sunn pest.

There were some studies about the selectivity of Lambda-cyhalothrin against parasitoid wasps (Rodrigues *et al.* 2013, Aydogdu *et al.* 2017). Souza *et al.* (2014) indicated that Lambda cyhalothrin, in addition to its toxic effects, reduced the emergence success of *Trichogramma pretiosum* Riley (F1) originated from treated eggs of *Anagasta kuehniella* Zeller.

We found that the pupa stage was the most sensitive immature stage of *T. grandis* rather than all the insecticides (orthogonal comparisons) with only 64.33 percent of adult emergence. While percent emergence from the parasitized eggs that were treated in the first and second instar stages was 68.22% and 78.12%, respectively. The reason for this difference can be attributed to the longer period of time takes for first instar to reach the adult stage compared to pupa and that over time the insecticide residue on the surface of egg shell is reduced. Sheikhi-Garjan *et al.* (2005) also indicated that exposure of parasitized Sunn pest eggs to the insecticide in the earlier immature stage of parasitoid wasp was less harmful than exposure in the later stages.

Both studied insecticides were highly toxic on contact bioassay. This result is aligned with studies of Kivan (1996), Saber *et al.* (2001) and Sheikhi-Garjan *et al.* (2005). They found that contact of adult wasps by the synthetic insecticides at the recommended field doses caused 100% mortality.

Natural enemies are the key component of IPM, and they are often recommended as the first line of defense in an IPM strategy. It has been stated that the only selected pesticides that should be used are those that are the most selective and which have the lowest adverse effects on beneficial organisms (Beloti *et al.* 2005, Nasreen *et al.* 2007). The time of chemical control of overwintered adult Suun pest in the field commonly coincides with the gradual appearance of the adult *T. grandis*, therefore, the type of insecticide used will have a significant role in maintaining and conserving populations of this parasitoid wasp.

Based on the present and previous results, treatment of eggs with both insecticides at different immature stages of parasitoid has not any negative effect on the parasitism activity of this wasp. However, Hef-lambda® is a more selective insecticide to Sunn pest than Karate-zeon®, because in addition to being effective on overwintered adults Sunn pest it causes less negative effect on the emergence of parasitoid adult wasps after egg treatment.

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