Impact of Selected Chemical and Botanical Insecticides on *Trissolcus grandis* (Hymenoptera: Platygastridae), a Parasitoid of *Eurygaster integriceps* (Hemiptera: Scutelleridae)[1]

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Abstract The lethal effects of three synthetic (deltamethrin + thiacloprid, lambda-cyhalothrin, deltamethrin) and two botanical (Palizin®, Sirinol®) insecticides were assayed in laboratory conditions against adult and immature stages of *Trissolcus grandis* (Thomson), an egg parasitoid of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae). Contact bioassays determined that the synthetic insecticides caused 100% mortality of the adult wasps, while the two botanical insecticides had no lethal effects on the adults. The highest level of emergence of *T. grandis* females from *E. integriceps* eggs treated with the synthetic insecticides was observed after treatment with lambda-cyhalothrin, and the lowest adult emergence occurred following treatment of the eggs with deltamethrin + thiacloprid. Exposure of *E. integriceps* eggs with Sirinol impacted parasitoid emergence, but the lowest mean percentage of emergence of adults was from eggs treated when the parasitoids were second instars or pupae. Treatment of eggs with Palizin had no impact on the emergence of adults or on the activity of the parasitoid after emergence. Of these materials assayed, Palizin appears to be an excellent candidate for use in integrated pest management programs for *E. integriceps*.

Key Words egg parasitoid, *Trissolcus grandis*, synthetic insecticides, botanical insecticides

Eurygaster integriceps Puton (Hemiptera; Scutelleridae) is an important pest of early-season growth of wheat and barley in Iran and the Middle East (Radjabi 2000). The pest is controlled mainly with applications of synthetic pyrethroids, such as lambda-cyhalothrin and deltamethrin (Critchley 1998).

Egg parasitoids are significant natural enemies of *E. integriceps* (Amir Maafi et al. 2001, El-Bouhssini et al. 2004). *Trissolcus grandis* (Thomson) (Hymenoptera: Platygastridae) is reported as the most common, especially with high pest populations of *E. integriceps* in the hot highland and cold lowland areas (Radjabi 2000). Rates of parasitism have been reported at <10% in cold regions (Haghshenas 2004, Nozad Bonab and Iranipour 2009) and as high as 100% in warm regions (El-Bouhsinni et al. 2004).

Widespread use of chemical insecticides aimed at controlling *E. integriceps* has eliminated or reduced the impact of these beneficial parasitoids. Applications of

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Table 1. Synthetic and botanical insecticides included in study.

Common Name	Trade Name	Concentration*
Deltamethrin + thiacloprid	Proteus	2.5
Lambda-cyhalothrin	Karate	0.2
Deltamethrin	Decis	0.8
Botanical extract	Palizin	2.5
Botanical extract	Sirinol	2.5

* Concentration is ml/liter.

synthetic pyrethroids, such as lambda-cyhalothrin and deltamethrin, are routinely employed (Critchley 1998); yet, selective insecticides might prove to have fewer nontarget impacts on these egg parasitoids and other natural enemies (Biondi et al. 2012, Croft 1990, Desneux et al. 2007, Guedes et al. 2016, Hassan et al. 1994, Turchen et al. 2015) and might be used in combination with natural enemies in an integrated pest management (IPM) program (Critchley 1998, Theiling and Croft 1989).

Essential oils and botanical extracts, with their relatively low mammalian toxicity and higher rate of environmental degradation, could be used as alternatives for synthetic chemical insecticides (Daoubi et al. 2005, Haddi et al. 2015, Kim et al. 2005) and have been increasingly explored for use in IPM programs. The objectives of the current study were to compare the insecticidal effects of two botanical insecticides, Palizin® and Sirinol® (Kimia Sabzavar Co., Tehran, Iran), and three synthetic insecticides, deltamethrin + thiacloprid (Proteus®, Bayer Crop Science Co., Monheim am Rhein, Germany), lambda-cyhalothrin (Karate®, Syngenta Crop Protection Inc., Greensboro, NC), and deltamethrin (Decis®, Bayer Crop Science) on the egg parasitoid *T. grandis* under laboratory conditions.

Materials and Methods

Insecticides. The deltamethrin + thiacloprid (Proteus OD 11%) used in the study was an oil dispersion containing deltamethrirn (pyrethroid) at 10 g active ingredient (AI)/liter + thiacloprid (neonicotinoid) at 100 g AI/liter (Bayer Crop Science 2017). The lambda-cyhalothrin (Karate CS 10%) with Zeon technology was a capsule suspension containing lambda-cyhalothrin (pyrethroid) at 100 g AI/liter (Perrin et al. 1998). The deltamethrin (Decis EC 25%) was an emulsifiable concentrate containing 25 g AI/liter (Talebi Jahromi 2008). The botanical Palizin was a coconut soap (65%) containing mint and eucalyptus extracts with insecticidal and acaricidal contact effects (Kabiri and Amiri Besheli 2012). Sirinol, the other botanical, was a garlic extract in emulsifiable oil (EC 80%) (Danay Tous et al. 2014). Concentrations of each were recommended field rates suspended in distilled water with 0.01% Triton™X-100 T8787 (Sigma-Aldrich, Germany) as an emulsifier or wetting agent (Table 1). The control was distilled water with Triton X-100.

Insect colonies. Overwintered *E. integriceps* were collected from wheat (*Triticum aestivum* L. var. 'Gaskogen') fields near Busajin village in the southern Ardabil province of Iran (N 37°57′48.87″; E 48°14′38.44″) between 7 May and 10 June 2016 using sweep nets and manual collection. Insects were transported to the laboratory and were reared on wheat seedlings in cylindrical oviposition cages (30 × 20 × 9 cm) in an environmental chamber maintained at 25 ± 1°C and 60 ± 5% relative humidity (RH) on a 16:8 light:dark (L:D)–h photoperiod regime. Paper strips were vertically suspended in each cage for oviposition, and strips with egg masses were collected and replaced with new ones every 24 h as per Sheikhi Garjan et al. (2005). Egg masses were placed in 9-cm-diameter petri dishes and placed in a refrigerator at 5 ± 1°C until used for rearing of the egg parasitoid *T. grandis* (Safavi 1973).

Trissolcus grandis specimens used in this study were from a colony originally established by placement of *E. integriceps* egg masses on wheat plants grown near Busjain village on 10 May 2016. One week later, egg masses were collected and transported to the laboratory where the masses were placed individually in tubes (10 × 1.5 cm) to allow for emergence of egg parasitoids. Wasps identified as *T. grandis*, according to the key of Kozlov and Kononova (1983), were collected and reared on *E. integriceps* egg masses, which had been stored in the refrigerator. During the first 7 d, each wasp was provided with two egg masses (28 eggs) per day along with honey as a supplemental nutrition source. After that, one egg mass (14 eggs) was offered to each wasp each day until the wasp expired. Wasps from the first generation of rearing were used in the bioassay of contact toxicity of insecticides on the adult wasps. Second-generation wasps were used in bioassays of the insecticides applied to *E. integriceps* eggs containing different stages of development of immature *T. grandis* as described by Sheikhi Garjan et al. (2005).

Adult *T. grandis* contact toxicity. Bioassay enclosures were constructed of two glass plates (10 × 10 cm each) using a frame measuring 10 × 10 × 1 cm onto which the glass plates were secured by glue. Three openings on each frame were covered with 60-mesh organza screen to allow air circulation. A honey strip was placed inside each cage for parasitoid feeding. Recommended field concentrations of the insecticides (Table 1) were sprayed on the glass surfaces using a hand-operated dispenser. Controls were sprayed with distilled water and Triton X-100 only. All plates were allowed to air-dry for 3 h before releasing 60 1-day-old adult female wasps into each enclosure as described by Saber et al. (2005). Wasp mortality was recorded after 24 h. These bioassays were conducted in a completely random design with four replications for each treatment.

Toxicity of insecticides on immature *T. grandis* developing within host eggs. *Eurygaster integriceps* eggs parasitized 3 d earlier (containing first-instar *T. grandis*), 5 d earlier (containing second-instar *T. grandis*), and 8 d earlier (containing *T. grandis* pupae) were immersed for 5 s in the insecticide solutions as described by Sheikhi Garjan et al. (2005). Controls were immersed in distilled water. Immersed eggs were then air-dried for 3 h, placed on filter paper in petri dishes, and held in an environmental chamber at 25 ± 1°C, 60 ± 5% RH, and 16:8 (L:D) h. After 15 d, the number of female and male wasps that had completely emerged were counted and recorded. This bioassay also was conducted with a completely random design with 10 replications for each treatment.

The ability of adult *T. grandis* emerging from these treated eggs to parasitize *E. integriceps* eggs was also assessed by placing 10 newly mated females individually into petri dishes containing two egg masses (28 eggs). After 24 h, the egg masses were collected and placed in test tubes which were placed in the environmental chamber. Two new egg masses were placed in the petri dishes. This process was continued for a total of 5 d. After 15 d, the numbers of parasitized eggs were counted and recorded as described by Sheikhi Garjan et al. (2005).

Statistical analysis. Percentage data were arcsine transformed followed by analysis of variance using the SAS version 9.1 software (SAS Institute 2003). Treatment mean comparisons were made with the Student-Newman-Keuls (SNK) test and, in some cases, by orthogonal comparisons ($P \leq 0.05$).

Results and Discussion

All adult females were dead within 24 h of exposure to three synthetic insecticides. Wasp mortality following exposure to the botanicals did not exceed 1.3% and was not significantly different from that observed with the control treatment ($F = 1.26$; df = 2, 9; $P = 0.33$). We further found that *T. grandis* wasps that emerged from the botanical treatments successfully parasitized *E. integriceps* eggs (61.66 ± 7.15% for Palizin, 62.56 ± 4.28% for Sirinol, 70.52 ± 6.2% for controls). Our results corroborate those reported by Kivan (1996), Saber et al. (2005), and Sheikhi Garjan et al. (2005). Our findings also help explain the results of Popov et al. (1980), who found that applications of synthetic insecticides to control overwintering *E. integriceps* also reduced egg parasitism from 86.9% in unsprayed areas to 8.3% in sprayed areas.

Significant differences were observed among the mean percentage of emergences of *T. grandis* wasps from eggs containing first instars ($F = 58.66$; df = 5, 76; $P < 0.0001$), second instars ($F = 170.01$; df = 5, 59; $P < 0.0001$), or pupae ($F = 67.4$; df = 5, 58; $P < 0.0001$) immersed in the insecticides (Table 2). The highest percentage of emergence of wasps occurred following immersion of the eggs in Palizin and in the distilled water control treatments. The lowest emergence was observed when eggs containing first instars were immersed in either deltamethrin + thiacloprid or deltamethrin ($F = 58.66$; df = 5, 76; $P < 0.0001$) and when eggs containing either second instars ($F = 170.01$; df = 5, 59; $P < 0.0001$) or pupae ($F = 67.4$; df = 5, 58; $P < 0.0001$) were immersed in either Sirinol or deltamethrin + thiacloprid.

Mean percentage of emergence of *T. grandis* females from eggs treated with distilled water (controls) was 65.03% for first instar exposure, 66.1% for second instar exposure, and 62.55% for pupal exposure (Table 3). The female:male ratio in each of these treatments was 2.95, 4.0, and 3.13, respectively. No males emerged from eggs treated with deltamethrin + thiacloprid while in the first and second instars and from eggs treated with Sirinol while in the second instar. Based upon these emergence data, it appears that deltamethrin + thiacloprid, deltamethrin, and Sirinol are the most deleterious of these materials tested for survival and successful emergence of *T. grandis* when *E. integriceps* eggs containing the immature parasitoids are exposed to these insecticides. Exposure of the eggs containing

Table 2. Mean ± SE percentage of emergence of *Trissolcus grandis* wasps from parasitized eggs of *Eurygaster integriceps* dipped in insecticide solutions and containing various immature stages of the egg parasitoid.*

Treatments	First Instars Exposed	Second Instars Exposed	Pupae Exposed
Deltamethrin + thiacloprid	7.83 ± 4.14cA	6.88 ± 2.82dA	7.54 ± 4.20dA
Lambda-cyhalothrin	48.66 ± 4.02bB	70.93 ± 2.70bA	44.97 ± 4.13bB
Deltamethrin	14.94 ± 4.61cA	23.61 ± 3.10cA	24.08 ± 4.00cA
Palizin	82.53 ± 4.10aA	86.52 ± 2.53aA	71.54 ± 3.91aB
Sirinol	52.33 ± 5.00bA	3.10 ± 2.94dB	5.54 ± 3.83dB
Control	87.10 ± 4.83aA	82.60 ± 3.26aA	82.55 ± 3.79aA

* Means within a column followed by different lowercase letters are significantly different, and means within a row followed by different uppercase letters are significantly different (SNK; $P \leq 0.05$).

immature parasitoids to Palizin did not significantly impact parasitoid emergence as compared to the controls (Table 3).

Based upon our contact toxicity bioassays, the three synthetic chemical insecticides evaluated in this study should not be used when *T. grandis* adult wasps are present and active in agricultural production fields. The two botanical products, however, had little toxic effect on adult wasps and might be considered for pest control even when *T. grandis* adults are present.

Overall, successful emergence of wasps was lower when eggs containing *T. grandis* pupae were immersed in the insecticides. Sheikhi Garjan et al. (2005) also found that exposure of parasitized *E. integriceps* eggs to insecticides in earlier developmental stages of the parasitoid is less harmful than exposure in later developmental stages. We might postulate that these observations could be attributed to decreased levels of insecticide residue on the host egg capsule over time. Thus, wasps emerging from their pupal stages and chewing out of host eggs are exposed to less insecticide residue when the host eggs containing first instars, compared eggs containing pupae, are treated. The host egg capsule also might lose some its integrity over time.

Wasps that emerged from *E. integriceps* eggs treated with insecticides during the immature development of the parasitoid showed varying levels of successful parasitism of *E. integriceps* eggs (Table 4). Those wasps that had emerged from the eggs treated with deltamethrin + thiacloprid at various immature developmental stages of the parasitoid died immediately after emergence and evaluation of parasitism was not possible. In addition, sufficient numbers of female wasps were lacking for evaluation of parasitism in the treatments of deltamethrin of eggs containing first instars and Sirinol eggs containing second instars.

In conclusion, deltamethrin + thiacloprid, Sirinol, deltamethrin, lambda-cyhalothrin, and Palizin were categorized in groups 3, 2, 2, 2, and 1 based on the

Table 3. Mean ± SE percentage of emergence of female versus male *Trissolcus grandis* wasps from parasitized eggs of *Eurygaster integriceps* dipped in insecticide solutions and containing various immature stages of the egg parasitoid.*

	First Instars Exposed		Second Instars Exposed		Pupae Exposed	
Treatments	**% Female Emergence**	**% Male Emergence**	**% Female Emergence**	**% Male Emergence**	**% Female Emergence**	**% Male Emergence**
Deltamethrin + thiacloprid	7.83 ± 3.74c	0.00 ± 0.00d	6.88 ± 2.75d	0.00 ± 0.00c	5.45 ± 3.76c	2.09 ± 3.80d
Lambda-cyhalothrin	39.90 ± 3.51b	8.76 ± 2.34c	54.80 ± 2.56b	16.13 ± 2.12b	25.55 ± 3.52b	19.42 ± 3.71a
Deltamethrin	12.38 ± 3.32c	2.56 ± 2.40d	21.96 ± 3.00c	1.65 ± 2.32c	18.64 ± 3.60b	5.44 ± 3.60c
Palizin	68.64 ± 2.91a	13.89 ± 1.91b	63.21 ± 2.78a	23.31 ± 2.22a	57.13 ± 3.32a	14.41 ± 3.23b
Sirinol	42.10 ± 4.36b	10.23 ± 2.73c	3.10 ± 2.87d	0.00 ± 0.00c	4.64 ± 3.53c	0.90 ± 3.44d
Control	65.03 ± 4.40a	22.07 ± 2.51a	66.10 ± 3.20a	16.5 ± 2.45b	62.55 ± 3.45a	20.00 ± 3.46a

* Means within a column followed by different lowercase letters are significantly different (SNK, $P < 0.05$).

Table 4. Mean ± SE percentage of parasitism of *Eurygaster integriceps* eggs by *Trissolcus grandis* wasps that emerged from *E. integriceps* eggs immersed in insecticide solutions at various stages of immature parasitoid development.*

Treatments	First Instars Exposed	Second Instars Exposed	Pupae Exposed
Lambda-cyhalothrin	67.31 ± 2.98bA	60.66 ± 3.98aA	70.11 ± 4.40aA
Deltamethrin	—**	67.49 ± 3.51aA	65.55 ± 3.88aA
Palizin	73.53 ± 2.56abA	71.90 ± 3.44aA	69.41 ± 4.00aA
Sirinol	69.16 ± 2.33abA	—**	47.46 ± 3.90bB
Control	79.59 ± 2.81aA	72.82 ± 4.21aA	75.92 ± 4.22aA

* Means within a column followed by different lowercase letters are significantly different, means within a row followed by different uppercase letters are significantly different (SNK; $P < 0.05$).
** Denotes lack of adequate numbers of emerged wasps to conduct parasitism assay.

International Organization of Biological Control (Boller et al. 2005). The botanical insecticide Palizin appears to be a good candidate for further investigation and development as a selective control agent for *E. integriceps*. It is harmless to all growth stages of parasitoid *T. grandis* and has no effect on parasitic activity of females that have been previously exposed to the agent. Kabiri and Amiri Besheli (2012) reported similar results when pupae and adults of *Psyllaephagus pistaciae* Ferriere, a parasitoid of *Agonoscena pistaciae* Burckharat and Lauterer, was exposed to three concentration of Palizin. They also compared its toxicity with two synthetic insecticides and found concluded that Palizin is a low-risk pesticide for IPM programs with parasitoid wasps as natural enemies.

The timing of applications of insecticides to control overwintered *E. integriceps* in wheat commonly coincides with the appearance of the adult *T. grandis*. Thus, the type of insecticide used will have a significant role in maintaining and conserving populations of the parasitoids. In spite of the widespread use of deltamethrin and deltamethrin + thiacloprid for control of *E. integriceps,* we found that these insecticides are highly toxic to *T. grandis*, and their use should be avoided to control this pest. Lambda-cyhalothrin is also efficacious against *E. integriceps* (Honarmand et al. 2016, Kocak and Babaroglu 2006, Mohammadipour et al. 2015), but it also is toxic to *T. grandis* and should be avoided when the parasitoid is foraging or when parasitized egg masses are in the field. Although Palizin is less effective in controlling *E. integriceps* (Honarmand et al. 2016), it could be used as a component in an IPM program for management of *E. integriceps* in wheat in Iran.

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