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Life-stage-Dependent Secondary Responses of Some Biorational Insecticides on *Trichogramma dendrolimi* (Matsumura) (Hymenoptera: Trichogrammatidae)

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ABSTRACT

In the study, lethal and sub-lethal doses of Neem Azal (azadirachtin 10 g/l), Nimiks (azadirachtin 40 g/l), Nimbecidine (azadirachtin 0.3 g/l), Oread (spinosad 480 g/l), and Nostalgist BL (1.5% *Beauveria bassiana* strain Bb-1-) were tested on different developmental stages of the egg parasitoid, *Trichogramma dendrolimi* under laboratory conditions. The lowest melanized egg were found on the larval stage of parasitoid in the 200 ml dose of Nimiks (79.17%), on 5 ml dose of Oread (75.25 %) and on 250 ml dose of Nimbecidine (79.37 %). **An approximately 10-fold decrease in emergence rates was determined in the larval, prepupal and pupal stages of the parasitoid at doses of 5 ml and 6.25 ml of Oread.** The other doses of the same insecticides resulted in **100% of mortality.** The longest development time of *T. dendrolimi* was found on Oread with 6.25 ml (11.00 days), on Nimiks with 200 ml (11.04 days), and on Oread with 5 ml (10.90 days). No significant difference was observed in the sex ratio. **The longevity of** *T. denrolimi* was shorter than that of the control for all insecticides and doses applied to **the larval, prepupal and pupal stages of the parasitoid. Parasitism rates of F_1 and F_2 varied greatly depending on the insecticides, doses and biological stage of the parasitoid. The new crop protection strategies aim to reduce the use of chemical insecticides while supporting the combined use of biorational insecticides and natural enemies. The study offers helpful data for IPM that is focused on the ecology.**

KEYWORDS: azadirahtin, spinosad, *Beauveria bassiana*, secondary response, *Trichogramma dendrolimi*

INTRODUCTION

Egg parasitoids are known to be very effective against a number of crop pests. Egg parasitoids of the genus ***Trichogramma*** (Hymenoptera: Trichogrammatidae), are among the most important biological control agents of Lepidoptera pests worldwide (**Li, 1994; Mansour *et al.*, 2018; Guo *et al.*, 2019)** and are used annually on15 million hectares in 40 countries worldwide

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(Vinson *et al.*, 2015). *Trichogramma dendrolimi* (Matsumura) (Hymenoptera: Trichogrammatidae) has great economic importance as a biological control agent; the species has a wide host range, eg *Spodoptera litura* **(Fabricus)** (Hamada, 1992), *Mamestra brassicae* **(Linnaeus)** (Takada *et al.*, 1994), *Hyphantria cunea* **(Drury)** and also is mass-produced for biological control programs in Türkiye.

"Biorational" or "reduced risk" insecticides was initially used only for products derived from natural sources, i.e., plant extracts, insect pathogens, etc. (**Kapoor and Sharma, 2020).** Nowadays, azadirachtin is a broad-spectrum insecticide and plays an important role in agriculture worldwide **(Aribi *et al.*, 2017).** Spinosad is a fermentation product of the **Actinomycete bacterium** and is a tetracyclic macrolide component containing spinosyns A and D. Spinosad is referred to as a "bioinsecticide", "biocyclic pesticide" or "synthetic organic pesticide" because of this particular property **(Williams *et al.*, 2003). Entomopathogenic fungus *Beauveria bassiana*** (Balsamo) Vuillemin (Ascomycota: Hypocreales) can be used instead of **synthetic insecticides**. Fungal spores are able to mechanically and enzymatically penetrate the cuticle of an insect and infect insect eggs (**Al-Deghairi, 2008**).

Integrated Pest Management (IPM) programs are used worldwide to control different pests in agriculture and forestry. The combination of biorational insecticides and natural enemies is considered an important component in ecologically based IPM programs **(Volkmar *et al.*, 2008). Before using biorational insecticides in IPM, their possible negative impacts on beneficial insects must be assessed**. Detailed knowledge of the effects of different insecticides on the immature stages of natural enemies helps to determine the timing of sprays to avoid the most vulnerable stages. However, the efficacy of the parasitoid is affected by the timing of insecticide application before and after the release of the parasitoid **(Takada *et al.*, 2001). Therefore, studies on side effects are essential to improving the combined effectiveness of chemical and biological control method** (Asma *et al.*, 2018). The aim of our study is to determine the secondary responses of azadirahtin, spinosad, and *B. bassiana* on different immature stages of *T. dendrolimi* under laboratory conditions.

MATERIALS AND METHODS

Study insects

The Mediterranean flour moth, *Ephestia küehniella* (Zeller) (Lepidoptera: Pyralidae) was reared in plastic boxes **(15 x 20 x 7.5 cm)** containing corn flour: wheat bran (1:1) and maintained in climate chamber under controlled conditions (25 ± 1 °C, 60 ± 10% RH, and 16:8 L: D). A 300-gram sterilized diet mixture was weighed and added to disinfected plastic boxes

(19 × 24 × 7 cm). On average, 1500-2000 host eggs were added to the diet mixture. After the development, the adults were collected and transferred to a box. This process was repeated every day. *T. dendrolimi* was reared in a climate chamber at 27 ± 1ºC, 70 ± 5% RH, and 16:8 L: D. The eggs of ***E. kuehniella*** were used for the production of ***T. dendrolimi*****.** Host eggs were examined under a stereomicroscope and only healthy eggs were used for parasitoid production. An average of 250 healthy flour moth eggs (0-24 hours) were diluted with arabic gum (**30 ml water/1 gr arabic gum)** on paper strips (1x10 cm) and offered to mated parasitoids in tubes (3 x 10 cm). The parasitoids were removed one day after parasitism and the parasitized host eggs were allowed to develop in the climate chamber (Kandil, 2022).

Biorational İnsecticides

Neem Azal T/S, Trifolio–M GMBH, Lahnau-Germany-VitVerim Beyoğlu-İstanbul (azadirachtin 10 g/l) 200, 300, 100 and 150ml, Nimiks®4.5 Certis USA, LLC- Agrikem Ziraat İlaçları ve Endüstri Ürünleri San ve Tic AŞ, İzmir -Türkiye (azadirachtin 40 g/l) 125 , 150, 200, 62.5, 75 and 100ml, Oread® Hektaş Ticaret T.A.Ş, Gebze-Kocaeli, Türkiye (spinosad 480g/l) 10, 12.5, 25, 30, 5, 6.25 and 15ml, Nimbecidine (azadirachtin 0.3g/l) **Agrobest Grup** – İzmir, Türkiye 500 and 250ml and Nostalgist BL®, **Agrobest Grup**– İzmir, Türkiye (***B. bassiana*** strain Bb-1 %1.5, 1x108 cfu/ml min) 250 and 125ml were used in the experiments. The hosts of ***T. dendrolimi*** are lepidopteran eggs and therefore we use commercial application concentrations **(ml per 100 liters)** and half concentration of the commercial concentrations of the products against the lepidopter in the field.

Experiments

Twenty healthy host eggs (0-24 hours) were glued with arabic gum to 1x 9 cm paper strips and placed in 1.5x10 cm tubes. Two honey-fed, mated and non-parasitizing female parasitoids were placed in these tubes. After 24 hours, **the adult of *T. dendrolimi*** were removed from the tubes. Two ml of each biorational insecticides were sprayed onto the larval, prepupal, and pupal stages of *T. dendrolimi* (Lu *et al*., 2019) and left to dried for 15-20 minutes. **After parasitism, the parasitoid reaches the larval stage after 48 hours, the prepupal stage after 96 hours, and the pupal stage after 144 hours**. Pure water was used for the control application. Fifteen replicates were performed for each treatment. The aim of this study is to determine the proportion of melanized eggs, emergence rate, development time, longevity, sex ratio and also the parasitism ratios of F_1 and F_2.

Data analysis

All data were analysed with one-way analysis ANOVA followed by Tukey's multiple comparison test. All analyses were carried out considering a significance level of 5%. Statistical analyses were performed using Minitab version 17. The percentage data were normalized using an arcsine transformation (p0 = arcsine√p) (Zar, 1999).

RESULTS

The effect of biorational insecticides on the melanized eggs of *T. denrolimi* in generations F_0

In the analysis of variance, the difference between the means of the melanized eggs was found to be significant (df= 65, F= 2.94, P≤ 0.05; Table 1). After the larval stage applications, the lowest melanized eggs were found (79.17 %) with the 200-ml dose of Nimiks, (75.25 %) with the 5-ml dose of Oread, and (79.37 %) with the 250-ml dose of Nimbecidine (Table 1). After the prepupal stage applications, the lowest melanized eggs (80.56 %) were observed with a 200 ml dose of Nimiks (80.45 %) and (80.83 %) with a 500 ml dose of Nimbecidine at the pupal stage (Table 1).

Table 1. Melanized egg (%) of *Trichogramma denrolimi*, in the generations F_0 treated with insecticides during the larval, prepupal and pupal stages when developing in *Ephestia kuehniella* egg.

Insecticides and Doses	Biological stages of *Trichogramma denrolimi*		
	Larval stage	Prepupal stage	Pupal stage
Neem Azal 200 ml	85.83 **AB**	86.11 **AB**	91.11 **A**
Neem Azal 300 ml	86.94 **AB**	85.84 **AB**	84.72 **AB**
Neem Azal 100 ml	84.44 **AB**	88.89 **AB**	88.06 **AB**
Neem Azal 150 ml	87.35 **AB**	89.72 **AB**	87.78 **AB**
Nimiks 125 ml	86.11 **AB**	89.44 **AB**	91.67 **A**
Nimiks 150 ml	81.94 **AB**	90.00 **A**	92.50 **A**
Nimiks 200ml	79.17 **B**	80.56 **B**	85.83 **AB**
Nimiks 62,5 ml	95.77 **A**	94.17 **A**	93.06 **A**
Nimiks 75 ml	92.22 **A**	94.44 **A**	93.68 **A**
Nimiks 100 ml	89.17 **AB**	88.89 **AB**	93.07 **A**
Oread 10ml	88.00 **AB**	91.00 A	92.25 **A**
Oread 25ml	87.75 **AB**	83.50 **AB**	82.75 **AB**
Oread 12.5ml	90.00 **A**	86.00 **AB**	88.00 **AB**
Oread 30 ml	88.00 **AB**	89.00 **AB**	93.50 **A**
Oread 5ml	75.25 **B**	88.25 **AB**	90.00 **A**
Oread 6.25ml	84.75 **AB**	85.75 **AB**	87.25 **AB**
Oread 15ml	82.50 **AB**	87.00 **AB**	89.50 **AB**
Nostalgist 250 ml	96.12 **A**	95.56 **A**	91.94 **A**
Nostalgist 125 ml	94.72 **A**	96.11 **A**	91.39 **A**
Nimbecide 500ml	87.19 **AB**	85.00 **AB**	80.83 **B**
Nimbecidine 250 ml	79.37 **B**	90.56 **A**	85.10 **AB**
Control	91.67 **A**	93.06 **A**	87.78 **A**

Means followed by the same capital letter do not differ statistically differences, P≤ 0.05.

The effect of biorational insecticides on the emergence rate of F_0 generation parasitoids of *T. denrolimi*

The emergence rate of parasitoid was significantly reduced after insecticide treatments at the three immature developmental stages of the parasitoid (df=50, F=21.51, P≤0.05) (Table 2). that there are differences in insecticides, doses and developmental stages of the parasitoid. There was a decrease in the larval and prepupal period of the parasitoid at 200 ml dose of Neem Azal compared to the control. The emergence rate was significantly reduced after 300 ml dose of Neem Azal in the prepupal stage of the parasitoid and 150 ml dose of Nimiks in the larval stage of the parasitoid, 200 ml dose of Nimiks in the larval and prepupal stage of the parasitoid, 500ml dose of Nimbecidine in the larval and prepupal stage of the parasitoid, and in the 250 ml dose of Nimbecidine in the larval stage of the parasitoid compared to the control. After the application of Oread 5 ml and 6.25 ml doses, rate of emergence decreased by 10-fold compared to the control in all three preimajinal periods of the parasitoid. There was no emergence of the parasitoid treated with other doses of Oread.

Table 2. Emergence **rate** (%) of *Trichogramma denrolimi*, in the generations F_0 treated with insecticides during the larval, prepupal and pupal stages when developing in *Ephestia kuehniella* egg.

Insecticides and Doses	Biological stages of *Trichogramma denrolimi*		
	Larval stage	Prepupal stage	Pupal stage
Neem Azal 200 ml	74.44 **CD**	73.33 **CD**	82.22 **ABC**
Neem Azal 300 ml	77.78 **BC**	67.78**D**	76.39 **BC**
Neem Azal 100 ml	77.79 **BC**	83.61 **AB**	81.67 **ABC**
Neem Azal 150 ml	83.53 **AB**	80.00 **BC**	79.17 **BC**
Nimiks 125 ml	76.11 **BC**	79.72 **BC**	82.50 **ABC**
Nimiks 150 ml	73.61 **C**	83.60 **AB**	84.17 **AB**
Nimiks 200ml	70.28**C**	74.72 **CD**	80.28 **BC**
Nimiks 62,5 ml	88.06 **AB**	88.89 **AB**	90.00 **A**
Nimiks 75 ml	85.28 **AB**	89.44 **AB**	89.43 **AB**
Nimiks 100 ml	79.17 **BC**	81.94 **ABC**	88.33 **AB**
Oread 10ml	-	-	-
Oread 25ml	-	-	-
Oread 12.5ml	-	-	-
Oread 30 ml	-	-	-
Oread 5ml	6.2 **E**	6.1 **E**	6.6 **E**
Oread 6.25ml	7 **E**	9 **E**	10 **E**
Oread 15ml	-	-	-
Nostalgist 250 ml	88.06 **AB**	87.50 **AB**	87.60 **AB**
Nostalgist 125 ml	88.07 **AB**	91.11 **A**	87.70 **AB**
Nimbecidine 500ml	71.39 **C**	73.61 **C**	76.67 **BC**
Nimbecidine 250 ml	70.29**C**	82.50 **ABC**	78.61 **BC**
Control	88.06 **AB**	87.50 **AB**	84.44 **AB**

Means followed by the same capital letter do not differ statistically differences, $P \leq 0.05$

The effect of biorational insecticides on the development periods of F_0 generation parasitoids of *T. denrolimi*

The development times of the parasitoids were significantly affected by the treatments of insecticide (df=50, F=174.15, P≤0.05) (Table 3). The longest development time was found at 6.25ml dose (11.00 days) of Oread, 200ml dose of Nimiks (11.04 days), 5ml dose of Oread (10.90 days) and 150ml (10.53 days) dose of Nimiks. The applications of 200 ml and 300 ml doses of Neem Azal, 200 ml doses of Nimiks, and 5 ml and 6.25 ml doses of Oread to the larval stage of the parasitoid prolonged the development time of the parasitoid compared to the application to the prepupal and pupal stages. **The parasitoid *T. denrolimi*** did not show any development in the application of 10 ml, 25 ml, 12.5 ml, 30 ml and 15 ml doses of Oread to all three immature developmental stage of the parasitoid.

Table 3. Development time (± SE) of *Trichogramma denrolimi*, in the generations F_0 treated with insecticides during the larval, prepupal and pupal stages when developing in *Ephestia kuehniella* egg.

Insecticides and Doses	Biological stages of *Trichogramma denrolimi*		
	Larval stage	Prepupal stage	Pupal stage
Neem Azal 200 ml	9.71 ±0.04 **BC** n=266	9.20±0.02 **D** n=261	9.31±0.03 **C** n=292
Neem Azal 300 ml	10.21 ±0.05 **B** n=280	9.37 ±0.03 **D** n=247	9.41 ± 0.03 **D** n=274
Neem Azal 100 ml	9.10 ±0,01 **D** n=280	9.07 ±0.01 **D** n=302	9.09 ±0.01 **D** n=294
Neem Azal 150 ml	9.15 ±0.02 **D** n=283	9.11 ±0.02 **D** n=288	9.10 ±0.01 **D** n=285
Nimiks 125 ml	10.15 ±0.05 **B** n=278	9.50 ±0.03 **BC** n=288	9.22 ±0.02 **D** n=296
Nimiks 150 ml	10.53 ±0.05 **AB** n=259	9.61 ±0.03 **BC** n=301	9.17 ±0.02 **D** n=303
Nimiks 200ml	11.04 ±0.06 **A** n=253	9.76 ±0.04 **BC** n=269	9.51 ±0.03 **BC** n=289
Nimiks 62.5 ml	9.15 ±0.02 **D** n=317	9.08 ±0.01 **D** n=319	9.14 ±0.01 **D** n=325
Nimiks 75 ml	9.22 ±0.02 **D** n=305	9.13 ±0.01 **D** n=323	9.12 ±0.01 **D** n=323
Nimiks 100 ml	10.10 ±0.04 **B** n=287	9.59 ±0.03 **BC** n=293	9.30 ±0.02 **D** n=319
Oread 10ml	-	-	-
Oread 25ml	-	-	-
Oread 12.5ml	-	-	-
Oread 30 ml	-	-	-
Oread 5ml	10.90 ± 0.1 **A** n=10	10.36 ±0.24 **B** n=11	10.08 ±0.22 **B** n=12
Oread 6.25ml	11.00 ±0.00 **A** n=7	10.41 ±0.14 **B** n=12	10.40 ±0.13 **B** n=15
Oread 15ml	-	-	-
Nostalgist 250 ml	9.16 ±0.02 **D** n=320	9.15 ±0.02 **D** n=315	9.18 ±0.02 **D** n=315
Nostalgist 125 ml	9.13 ±0.02 **D** n=318	9.12 ±0.01 **D** n=324	9.10 ±0.01 **D** n=315
Nimbecidine 500ml	9.15 ±0.02 **D** n=254	9.16 ±0.02 **D** n=265	9.17 ±0.02 **D** n=272
Nimbecidine 250 ml	9.13 ±0.02 **D** n=236	9.21 ±0.02 **D** n=297	9.07 ±0.01 **D** n=284
Control	9.08 ± 0.01 **D** n=317	9.03 ± 0.03 **D** n=324	9.09 ±0.01 **D** n=314

Means followed by the same capital letter do not differ statistically differences, P ≤ 0.05.

The effect of biorational insecticides on the sex ratio and longevity of F_0 generation parasitoids of *T. denrolimi*

In this study, the sex ratio (females %) was determined after each insecticide application at each immature developmental stage of the parasitoid. Difference between the means was not significant (df=44, F=0.40, P=0.999) (Table 4). The longevity of *T. denrolimi* was shorter than that of the control for all insecticides and dosages applied to all immature developmental stages of the parasitoid (df=50, F=75.67, P<0.05). **The Oread doses of 5 ml and 6.25 ml caused the fastest reduction in longevity** (Table 5).

Table 4. Sex ratio (% female) of *Trichogramma denrolimi*, in the generations F_0 treated with insecticides during the larval, prepupal and pupal stages when developing in *Ephestia kuehniella* egg.

Insecticides and Doses	Biological stages of *Trichogramma denrolimi*		
	Larval stage	Prepupal stage	Pupal stage
Neem Azal 200 ml	70.87	74.03	67.92
Neem Azal 300 ml	71.32	70.23	70.95
Neem Azal 100 ml	75.45	68.65	74.94
Neem Azal 150 ml	72.22	79.12	76.39
Nimiks 125 ml	74.75	73.57	76.49
Nimiks 150 ml	75.20	71.01	70.73
Nimiks 200ml	72.14	71.22	74.47
Nimiks 62,5 ml	74.07	71.62	73.91
Nimiks 75 ml	76.86	73.86	74.83
Nimiks 100 ml	66.83	70.51	75.56
Oread 10ml			
Oread 25ml			
Oread 12.5ml			
Oread 30 ml			
Oread 5ml			
Oread 6.25ml			
Oread 15ml			
Nostalgist 250 ml	75.54	78.07	75.05
Nostalgist 125 ml	78.19	78.65	73.58
Nimbecide 500ml	73.89	72.83	76.26
Nimbecidine 250 ml	72.22	72.92	79.79
Control	74.31	75.33	75.59

Table 1. Longevity (±SE) of *Trichogramma denrolimi*, in the generations F_0 treated with insecticides during the larval, prepupal and pupal stages when developing in *Ephestia kuehniella* egg.

Insecticides and Doses	Biological stages of *Trichogramma denrolimi*		
	Larval stage	Prepupal stage	Pupal stage
Neem Azal 200 ml	7.24 ±0.20 **F** n=266	7.75± 0.15 **E** n=261	10.31 ±0.18 **C** n=292
Neem Azal 300 ml	6.77 ±0.13 **F** n=280	7.61 ±0.12 **E** n=247	9.24±0.19 **D** n=274
Neem Azal 100 ml	9.22 ±0.15 **D** n=280	10.04 ±0.15 **C** n=302	10.10 ±0.15 **C** n=294
Neem Azal 150 ml	8.10 ±0.15 **E** n=283	8.20 ±0.14 **E** n=288	9.75±0.14 **C** n=285
Nimiks 125 ml	9.08 ±0.15 **D** n=278	9.20±016 **D** n=288	9.98 ±016 **C** n=296
Nimiks 150 ml	8.37 ±0.17 **E** n=259	9.32 ± 0.17 **D** n=301	9.90 ± 0.18 **C** n=303
Nimiks 200ml	7.68±0.16 **E** n=253	8.36 ± 0.15 **E** n=269	8.53 ± 0.13 **D** n=289
Nimiks 62.5 ml	9.11 ±0.13 **D** n=317	9.73 ±0.13 **C** n=319	10.23 ±0.14 **C** n=325
Nimiks 75 ml	9.26±0.13 **D** n=305	9.66 ± 0.15 **C** n=323	9.96±0.15 **C** n=323
Nimiks 100 ml	8.96 ±0.14 **D** n=319	9.45 ±0.15 **D** n=293	9.97 ±0.14 **C** n=319
Oread 10ml	-	-	-
Oread 25ml	-	-	-
Oread 12.5ml	-	-	-
Oread 30 ml	-	-	-
Oread 5ml	1.0 ± 0.00 **G** n=10	1.0 ± 0.00 **G** n=8	1.0 ± 0.00 **G** n=12
Oread 6.25ml	1.0 ± 0.00 **G** n=4	1.0 ± 0.00 **G** n=6	1.0 ± 0.00 **G** n=9
Oread 15ml	-	-	-
Nostalgist 250 ml	9.16 ±0.14 **D** n=320	10.37 ±0.15 **C** n=315	10.69 ±0.13 **B** n=315
Nostalgist 125 ml	10.68 ±0.13 **B** n=318	11.04 ±0.13 **B** n=324	11.64 ±0.15 **A** n=315
Nimbecide 500ml	7.44 ±0.11 **F** n=254	7.78 ±0.13 **E** n=265	9.19 ±0.13 **D** n=272
Nimbecidine 250 ml	8.03 ±0.14 **E** n=236	7.96 ±0.14 **E** n=297	9.13±0.13 **D** n=284
Control	12.06 ±0.13 **A** n=317	11.90 ±0.13 **A** n=324	12.11 ±0.15 **A** n=314

Means followed by the same capital letter do not differ statistically differences, $P \leq 0.05$

The effect of biorational insecticides on the parasitism rates of F1 generation parasitoids of *T. denrolimi*

In the analysis of variance for the parasitism rates of the F1 generations, the difference between the means was found to be significant (df=44, F=3.48, $P \leq 0.05$). The applications of 200 ml, 300 ml, 100 ml, 150 ml doses of Neem Azal and 125 ml, 150 ml, 200 ml, 100 ml doses of Nimiks in the larval stage of the parasitoid significantly decreased the F_1 parasitism rates. In addition, the application of 200 ml, 300 ml doses of Neem Azal and 200 ml, 100 ml doses of Nimiks during the prepupal period of the parasitoid also reduced the parasitism rates of the F_1 (Table 6).

The effect of biorational insecticides on the parasitism rates of F_2 generation parasitoids of *T. denrolimi*

A similar study was performed for the F_2 generations. Application of 125, 150, 200, and 100 ml doses of Nimiks at the larval stage of the parasitoid resulted in decrease in parasitism rates of F_2. The application of 200 ml, 300 ml doses of Neem Azal, and 125 ml, 100 ml doses of Nimiks during the prepupal period of the parasitoid also reduced the parasitism rates of the F_2. In addition, the application of 75 ml dose of Nimiks to the pupal stage decreased the parasitism rate of the F_2 (Table 7) (df=44, F=1.93, $P \leq 0.05$).

Table 6. Parasitism rate (%) of *Trichogramma denrolimi*, in the generations F_1 treated with insecticides during the larval, prepupal and pupal stages when developing in *Ephestia kuehniella* egg.

Insecticides and Doses	Biological stages of *Trichogramma denrolimi*		
	Larval stage	Prepupal stage	Pupal stage
Neem Azal 200 ml	77.33 **CD**	77.67 **CD**	88.00 **AB**
Neem Azal 300 ml	74.67 **CD**	82.33 **BC**	84.00 **AB**
Neem Azal 100 ml	83.67 **BC**	89.00 **AB**	85.33 **AB**
Neem Azal 150 ml	79.33 **C**	84.33 **AB**	87.67 **AB**
Nimiks 125 ml	77.33 **CD**	81.33 **BC**	83.33 **BC**
Nimiks 150 ml	73.33**CD**	80.33 **BC**	84.67 **AB**
Nimiks 200ml	69.33 **D**	79.00 **C**	83.00 **BC**
Nimiks 62.5 ml	87.33 **AB**	89.33 **AB**	88.00 **AB**
Nimiks 75 ml	85.00 **AB**	86.67 **AB**	88.33 **AB**
Nimiks 100 ml	82.67 **BC**	81.67 **BC**	86.33 **AB**
Oread 10ml			
Oread 25ml			
Oread 12.5ml			
Oread 30 ml			
Oread 5ml			
Oread 6.25ml			
Oread 15ml			
Nostalgist 250 ml	89.00 **AB**	88.33 **AB**	87.00 **AB**
Nostalgist 125 ml	93.67 **A**	88.00 **AB**	91.67 **A**
Nimbecide 500ml	80.33 **AB**	84.33 **AB**	84.67 **AB**
Nimbecidine 250 ml	89.00 **AB**	85.67 **AB**	88.33 **AB**
Control	92.00 **A**	90.33 **A**	92.33 **A**

Means followed by the same capital letter do not differ statistically differences, $P \leq 0.05$.

Table 7. Parasitism **rate** (%) of *Trichogramma denrolimi*, in the generations F_2 treated with insecticides during the larval, prepupal and pupal stages when developing in *Ephestia kuehniella* egg.

Insecticides and Doses	Biological stages of *Trichogramma denrolimi*		
	Larval stage	Prepupal stage	Pupal stage
Neem Azal 200 ml	87.33 **AB**	84.67 **BC**	91.00 **A**
Neem Azal 300 ml	85.67 **AB**	84.00 **BC**	88.67 **AB**
Neem Azal 100 ml	86.00 **AB**	89.33 **AB**	87.67 **AB**
Neem Azal 150 ml	88.33 **AB**	86.67 **AB**	90.67 **A**
Nimiks 125 ml	82.33 **BC**	83.33 **BC**	84.67 **BC**
Nimiks 150 ml	82.00 **BC**	86.00 **AB**	87.00 **AB**
Nimiks 200ml	78.33**C**	88.67 **AB**	88.00 **AB**
Nimiks 62.5 ml	85.67 **AB**	92.00 **A**	90.67 **A**
Nimiks 75 ml	86.67 **AB**	88.67 **AB**	84.33 **BC**
Nimiks 100 ml	83.67 **B**	80.67 **BC**	88.33 **AB**
Oread 10ml			
Oread 25ml			
Oread 12.5ml			
Oread 30 ml			
Oread 5ml			
Oread 6.25ml			
Oread 15ml			
Nostalgist 250 ml	91.67 **A**	91.33 **A**	93.33 **A**
Nostalgist 125 ml	88.33 **AB**	90.67 **A**	89.67 **AB**
Nimbecide 500ml	85.00 **AB**	90.00 **A**	87.33 **AB**
Nimbecidine 250 ml	87.33 **AB**	89.00 **AB**	91.00 **A**
Control	92.33 **A**	89.33 **AB**	94.00 **A**

Means followed by the same capital letter do not differ statistically differences, $P \leq 0.05$.

DISCUSSION

It is important to know the different in sensitivities of the various developmental stages of parasitoids to insecticides in order to determine the proper timing for parasitoid release and insecticide treatment (Takada *et al.*, 2001). The larval stage of *T. denrolimi* was found to be more affected by egg melanization than the prepupal and pupal stages. It was found that the active substance ratio and dose are important, especially for azadirachtin. Lyons *et al.* (2003) reported that at 500 g azadirachtin/ha, the number of eggs parasitized by *T. minitum* was greatly reduced by Azatin EC (3.0% azadirachtin) and slightly reduced by Neem EC (4.6% azadirachtin) but was not reduced by an azadirachtin standard.

Researchers reported that the pre-adult stages of egg parasitoids can be protected from the negative effects of many insecticides because they are preserved in the host egg (**Orr *et al.*, 1989; Consoli *et al.*, 1998**). However, the emergence rates of the parasitoid *T. denrolimi*, especially in the larval and prepupal periods, were significantly reduced compared to the control at the Neem Azal (200 ml and 300 ml), Nimiks (125 ml, 150 ml and 200 ml) and Nimbedicine (500 ml and 250 ml) compared to the control. This result shows that the larval and prepupal stages of the parasitoid are more sensitive than the pupal stage. Saber *et al.* (2004) applied an

LC_{50} dose of Neem Azal (1330 ppm) to the larval -prepupal and pupal stages of ***T. cacoeciae*** (Hymenoptera: Trichogrammatidae) on two hosts *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) and *Cydia pomonella* L. (Lepidoptera: Tortricidae) **and the emergence rates decreased in both hosts compared to the control. Lyons *et al.* (2003) found that 500 g azadirachtin/ha reduced emergence rates of the parasitoid *Trichogramma minutum*** Riley, while 50 g azadirachtin/ha had no negative **effect. Silva and** Bueno, (2015) applied Neem oil (9.6 ppm) to the pupal stage of *T. pretiosum* and reported that there was no significant difference in the emergence rate (80.4%) compared to the control (89.8%). The parasitoid *T. denrolimi* did not show any development at 10ml, 25ml, 12.5ml, 30ml doses of spinosad (480 g/L). In 5ml and 6.25ml doses, parasitoid emergence rates ranged **from 6.2 to 10%. Shoeb (2010) reported** that there was no emergence in *Trichogramma evanescens* after application of spinosad (24%). Application of *B. bassiana* (Nostalgist 250 ml and 125 ml) to the larval, prepupa and pupal stages of *T. denrolimi* had no negative effects on emergence rates. **However, Potrich et al. (2009) reported that *M. anisopliae* reduced the emergence of *T. pretiosum*. Araujo et al., 2020 shows that the negative impacts of *B. bassiana* on *T. pretiosum* and *T. atopovirilia* biological parameters were negligible.**

In this study, the application of azadirachtin to different stages of the parasitoid affected the development time according to the **active ingredient rate** and doses. Development time was affected at high doses of Neem Azal and Neemix with high **active ingredient. Moreover**, both doses (500 and 250 mL) **of Neemix** had no negative effect on the development time of the **parasitoid *T. denrolimi*. The development** time of the parasitoid prolonged at low doses of spinosad. On the other hand, **the application** of *B. bassiana* had no negative effect on the development time. LC_{25} and LC_{50} doses of neem extract increased the development time of the larval parasitoid *Hyposoter ebeninus* G. (Hymenoptera: Ichneumonidae) (Matter *et al.*, 2002). Rahman Saljoqi *et al*. (2012) reported that 0.2%, 0.15% and 0.10% concentrations of spinosad were applied to the pupal stage of the parasitoid ***T. chilonis*****,** resulting in an increase of development time. The development time of *T. atopovirilia* was not affected by *B. bassiana* application (Araujo *et al.*, 2020).

The longevity of *T. denrolimi* was negatively affected by all insecticides and doses. Michel *et al.* (2004) reported that the longevity of males and females of the egg parasitoid *Gryon fulviventre* Crawford (Hymenoptera: Scelionidae) was not affected by the application of 5% neem solution to the larval and prepupal period of parasitized eggs. **The dose of 50 g azadirachtin/ha had no effect on the lifetime of *T. minitum* females, whereas the application of 500 g azadirachtin/ha had a negative effect (Lyons *et al.*, 2003). In our study,**

adults who completed development following the application of spinosad at doses of 5 ml and 6.25 ml lived for one day. Similar results were obtained by Shoeb (2010) for *T. evanescens*. The longevity of *Trichogramma chilonis* **decreased significantly after** the application of spinosad doses of 0.2, 0.15, 0.1 0.05 and 0.01% in the egg, larval and pupal periods of the parasitoid, respectively (Rahman Saljoqi *et al.*, 2012). **The longevity of the parasitoid** *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) was not adversely affected by the application of azadirahtin (0.03 g/l-Azamax) and spinosad (0.07 g/l- 0.07) to during pupal period of the parasitoid (Beloti *et al.*, 2015). **Martins *et al.* (2014) noted that the longevity of females of the parasitoid *Diaeretiella rapae*** McIntoch (Hymenoptera: Braconidae) decreased with the ***B. bassiana* application** in the preadult stage.

Sex ratio is an important parameter for host-parasitoid relationships and the side effect studies. In this study, the sex ratio was not adversely affected by insecticide treatments, with the exception of spinosad. **The application** of azadirachtin and spinosad to the pupal stage of the parasitoid *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) showed no difference in sex ratio (Beloti *et al.*, 2015). Similarly, it was reported that the application of 1 × 10^8 conidial mL−1 supplementation of *B. bassiana* in the pre-adult period of *T. radiata* (Waterston) (Hymenoptera: Eulophidae) caused **no significant difference in sex ratio** (Ramos Aguila *et al.*, 2021). Different applications of insecticides and doses at different stages of the F_0 generation might have a negative effect on the parasitism rate of the F_1 and F_2 generations. In this study, the application of high doses of azadirachtin in the larval and prepupal period resulted in a decrease in the parasitism rates of the F_1 and F_2 generations. Beloti *et al.* (2015) reported that 0.03 g/L azadirachtin caused a 23% reduction in the parasitization rate of F_1 generation of the parasitoid *T. radiata*. Similarly, Lyons *et al.* (2003) 500 g azadirachtin/ha application caused a decrease in parasitization rates of F_1 generation of *T. minitum* compared **to 50 g azadirachtin/ha application.**

Consequently, it was found that the effect of the biorational preparations used in this study may vary depending on the doses and the biological periods of the parasitoid. Especially, low doses of azadiractin, applied at the pupal stage of the parasitoid are the safest applications for the biological properties of the parasitoid *T. denrolimi*. *B. bassiana* can also be considered safe. All doses of spinosad had a strong negative effect on the development of the parasitoid. We believe that the results of this study can be evaluated in integrated pest management programs based on biological control.

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