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Research Article/Araştırma Makalesi

The determination of antifeedant effect of Neem Azal T/S on almond leaf bee *Cimbex quadrimaculata* (Müller, 1766) (Hymenoptera: Cimbicidae) larvae

Neem Azal T/S'nin badem yaprak arısı, Cimbex quadrimaculata (Müller, 1766) (Hymenoptera: Cimbicidae) larvaları üzerinde beslenme engelleyici etkisinin belirlenmesi

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ABSTRACT

In this study, the antifeeding index (AFI) value of Neem Azal T/S plant extract against the larvae of the almond leaf bee Cimbex quadrimaculata Müller, 1766 (Hymenoptera: Cimbicidae) was determined under laboratory conditions. The leaf dipping method was employed, and the measurement concentrations were set at 0% (control), 0.8%, 1.0%, 1.2%, 2%, 4%, 6%, 8%, and 10%. The extract was applied to the 3rd and 4th larval period of the pest. The antifeeding indexes of different doses of the extract were calculated using both choice and non-choice methods, and the differences between the data were assessed using the Kruskal-Wallis test. The differences between the choice and non-choice methods were also statistically determined using the Mann-Whitney U test. As a result of the study, the highest AFI value in both larval stages was recorded for the 10% concentration in both choice and no-choice methods. The lowest AFI value was observed at the lowest concentration of 0.8% in both methods during the 4th larval stage of the pest. It was found that as the concentration of extract increased, the C. quadrimaculata larvae consumed fewer leaves. In conclusion, it was determined that the highest AFI values occurred at the of 8% and 10%, indicating that the effectiveness of Neem Azal T/S increased with increasing dosage.

Key Words: Neem Azal T/S, *Cimbex quadrimaculata*, larvae, Maximum antifeeding index (AFI), Efficacy

ÖZ
Bu çalışmada, badem yaprak arısı *Cimbex quadrimaculata* (Hymenoptera: Cimbicidae) larvalarına karşı Neem Azal T/S bitki ekstraktının laboratuvar koşullarında maksimum beslenme engelleyici indeks (AFI) değeri belirlenmiştir. Çalışmada, yaprak daldırma yöntemi uygulanmış ve ölçüm konsantrasyonları %0 (Kontrol), %0.8, %1.0, %1.2, %2, %4, %6, %8 ve %10 olarak belirlenmiştir. Ekstrakt, zararlının 3. ve 4. dönem larvalarına karşı uygulanmıştır. Ekstraktın farklı dozlarının anti beslenme indeksleri seçenekli ve seçeneksiz metoda göre hesaplanmış, veriler arasında farkın olup olmadığı Kruskal Wallis testi ile belirlenmiştir. Seçenekli ve seçeneksiz metotlar arasındaki farklar da istatistiki olarak Mann Whitney U testi ile belirlenmiştir. Çalışma sonucunda, her iki larva döneminde de seçenekli ve seçeneksiz metotlar içerisinde en yüksek AFI değeri % 10 konsantrasyon için kaydedilmiştir. En düşük AFI değeri ise iki metotta da her iki larva döneminde en düşük konsantrasyon olan %0.8'lik dozda ve ve zararlının 4. larva döneminde belirlenmiştir.

Ekstraktın konsantrasyonu arttıkça, *C. quadrimaculata* larvalarının daha az yaprak tükettiği saptanmıştır. Sonuç olarak, en yüksek AFI değerlerinin %8 ile %10'luk dozlarda gerçekleştiği, Neem Azal T/S'ın doz artışıyla birlikte etkililiğinin arttığı belirlenmiştir.

Anahtar Kelimeler: Neem Azal T/S, *Cimbex quadrimaculata*, Larva, Maksimum beslenme engelleyici indeks (AFI), Etkinlik

Introduction

Almond leaf bee *Cimbex quadrimaculata* Müller, 1766 (Hymenoptera: Cimbicidae) is a significant pest both in Türkiye and worldwide. This pest causes considerable damage in almond orchards. In a study conducted in the provinces of Diyarbakır, Elâzığ, and Mardin, the almond leaf bee *C. quadrimaculata* was identified as the dominant species at a rate of 51% (Bolu et al., 2005). This pest damages not only almonds but also cherries, apricots, peaches, and pears in the region. There is no effective application for controlling this pest. Therefore, the absence of a licensed pesticide against this pest has led to random and unconscious chemical control practices among farmers. In addition to determining the biology and population dynamics of this pest, studies have been conducted on the important factors affecting its populations, the identification of significant natural enemies, and alternative chemical control methods using some pyrolysis wood vinegar products (Özgen et al., 2021 a, b, c; Özgen et al., 2022 a, b; Koç et al., 2024).

In this study, the antifeeding effect of Neem Azal T/S, which has insecticidal, repellent, and antifeeding effects against pests, was investigated against the larvae of *C. quadrimaculata.* Neem extracts are known to have insecticidal effects on over 550 pests worldwide, and the number of pests affected is increasing day by day (Saxena and Basit, 1982; Isman, 1999; Durmuşoğlu et al., 2003; Whalon et al., 2008; Özgen and Karsavuran, 2011; Cura and Gencer, 2019).

Neem Azal T/S contains 1% active ingredient azadirachtin. Azadirachtin is a triterpenoid found in the neem tree, scientifically known as *Azadirachta indica* A. (Juss) (Meliaceae). The importance of this active ingredient is increasing due to its low toxicity to mammals and minimal environmental harm. The established efficiency of this active ingredient against various pests will contribute to organic and sustainable agricultural approaches in combating this pest, which is increasingly damaging almonds, a significant hard-shelled fruit in the Eastern and Southeastern Anatolia regions. As there is no licensed pesticide recommended in technical instructions for chemical control of this pest, the determination of the effectiveness of this plant-based product, used in organic farming, against this pest for the first time will contribute to integrated pest management of *C. quadrimaculata* in almonds.

Material and Method

The study was conducted in the laboratories of the Department of Bioengineering at Fırat University. The larvae, all from the same habitat, were collected in their first larval stage from Sütlüce village, Central district of Elazığ province. They were reared in the laboratory until they reached the third and fourth larval stages, which were the targeted biological stages for the study, and the experiments were initiated at the appropriate biological stage. While separating the third and fourth larval stages of the pest, the publication by Bolu (2016) was taken into consideration. Neem Azal T/S (1%) preparation containing active ingredient azadirachtin was used as insecticide for the pest larvae. The leaf dipping method was used in the experiments (Park et al., 2002). The measurement concentrations were set at 0% (Control), 0.8%, 1.0%, 1.2%, 2%, 4%, 6%, 8%, and 10%. "The concentrations have been mixed with pure water. Concentrations should be given as ppm or mg l^{-1}.

In the study, leaf disks with a diameter of 3 cm were immersed in extract dilutions for 30 seconds and dried in a fume hood for 1-2 hours. They were then placed on a damp filter paper inside a plastic container (7 cm in diameter and 3 cm high) to prevent the leaves from drying out. Ten disks were used in each trial, and three replicates of leaf disks were used for each treatment. For each dose application, one individual was placed in each container, and leaf consumption was recorded with 10 replications. A total of 340 larvae (chııce: 160/ non choice: 180) of the same biological stage (3rd or 4th instar larvae of the same age) were used during each trial. Experiments were conducted on a total of 340 larvae. The larvae were starved for 4 hours before being placed on the extract-treated leaf disks using both choice and no-choice options. The feeding areas of every ten larvae were summed according to the dose, and the average consumption area for each dose was calculated using the arithmetic mean.

For the choice method of each dose application, 10 3rd or 4th stage larvae were placed on the extract-treated or untreated (control) leaf disks in a plastic container. For the non-choice method, extract-treated leaf disks and controls were placed in separate plastic containers (7 cm in diameter and 3 cm high). After 24 hours, the larvae were removed, and their feces were brushed off the leaf disks. The remaining surfaces of the leaf disks were photographed, and the areas consumed and not consumed were measured and recorded using the IMAGEJ computer program (version 1.410, available at http://rsb.info.nih.gov/ij).For the choice method, the Antifeeding Index (AFI) was calculated using the formula (AFI), (C-T)/(C+T)*%100 , while for the non-choice method, the formula (C-T)/C*%100 was used (Arivoli and Tennyson, 2013) In these formulas, C and T (cm) represent the consumed leaf area of the control and extract-treated disks, respectively. Additionally, a scale was created to number the damage rates on the leaves. The Kruskal-Wallis test was used to determine if there were differences between the data. The differences between the choice and non-choice methods were statistically analyzed using the Mann-Whitney U test (Nachar, 2008).

Figure 1. Effects of the prepared solutions and the Neem Azal T/S on the larvae in the application

Results and Discussion

The results of the antifeeding effects of Neem Azal T/S formulations in both choice and no-choice applications against *Cimbex quadrimaculata* are presented in Table 1 and Table 2.

Table 1. AFI determined after 24 hours in *Cimbex quadrimaculata* larvae using the choice method with Neem Azal T/S formulation.

Concentration	Consumed area by 3rd larval stage (T) (cm)	Consumed area (C) (cm)	AFI for 3rd instar larvae	Consumed area by 4th larval stage (T) (cm)	Consumed area (C) (cm)	AFI for 4th instar larvae
0.8%	0.22	1.49	74.26	0.29	1.59	69.14
1%	0.20	1.5	76.47	0.25	1.61	77.27
1.2%	0.16	1.46	80,24	0,24	1.6	76.7
2%	0.16	1.44	80	0.17	1.58	80.57
4%	0.16	1.48	80.48	0.16	1.55	81.28
6%	0.10	1.49	87.42	0.10	1.57	88.02
8%	0.08	1.51	89.93	0.09	1.62	89.47
10%	0.04	1.5	97.07	0.08	1.54	90.12

Table 2. AFI determined after 24 hours in *Cimbex quadrimaculata* larvae using the non-choice method with Neem Azal T/S formulation.

Concentration	Consumed area by 3rd larval stage (T) (cm)	AFI for 3rd instar larvae	Consumed area by 4th larval stage (T) (cm)	AFI for 4rd instar larvae
0.8%	0.28	83.62	0.26	85.22
1%	0.27	84.21	0.24	86.36
1.2%	0.20	88.3	0,21	88.06
2%	0.21	87.71	0.18	89.77
4%	0.19	88.88	0.17	90.34
6%	0.15	91.22	0.15	91.47
8%	0.10	94.15	0.11	93.75
10%	0.07	95.9	0.09	94.88
Control	1.71		1.76	

A normality test was conducted to determine whether there were differences between the doses in terms of larval stages within each method, as well as to identify the differences among larvae between the choice and no-choice methods. Since the obtained values did not conform to a normal distribution, non-parametric tests, specifically the Kruskal-Wallis and Mann-Whitney U tests, were performed.

Table 3. Results of the Kruskal-Wallis Test for different doses applied with choice and no-choice methods in terms of larval stages.

	Dose	**Median**	**H**	***p***	**Pairwise comparisons**
3^{rd} instar choice	0.8	73.90	71.773	<0.001	%0.8 < %6, %8, %10 %1 < %6, %8, %10 %1.2 < %10 %2 < %10 %4 < %10
	1	75.88			
	1.2	80.02			
	2	80.31			
	4	80.14			
	6	88.67			
	8	89.89			
	10	98.09			
4^{rd} instar choice	0.8	69.10	71.335	<0.001	%0.8 < %2, %4, %6, %0.8 < %8, %10, %1 < %6, %8, %10, %1.2 < %6 %8, %10
	1	77.27			
	1.2	76.20			
	2	80.57			
	4	81.31			
	6	88.02			
	8	89.60			
	10	89.85			
3^{rd} instar non-choice	0.8	83.65	63.360	<0.001	%0.8 <%6, %8, %10 %1 < %6, %8, %10 %1.2 < %10 %2 < %10 %4 < %10
	1	84.21			
	1.2	87.87			
	2	87.69			
	4	88.88			
	6	91.56			
	8	94.15			
	10	95.80			
4^{rd} instar non-choice	0.8	85.05	67.362	<0.001	%0.8 < %6, %8, %10 %1 < %6, %8, %10 %1.2 < %8, %10 %2 < %10
	1	86.36			
	1.2	88.03			
	2	89.68			
	4	90.26			
	6	91.47			
	8	93.99			
	10	94.94			

Upon examining Table 3, a statistically significant difference was found between the application dose groups for the AFI values of the 3^{rd} and 4^{th} instar larvae in both choice and non-choice methods ($p < 0.05$). The highest AFI values were observed at the 8% and 10% doses, indicating that the neem extract was quite effective. The results of the Kruskal-Wallis Test multiple comparisons for different doses applied with choice and no-choice methods in terms of larval stages are also presented in Table 3 and Figures 2-5.

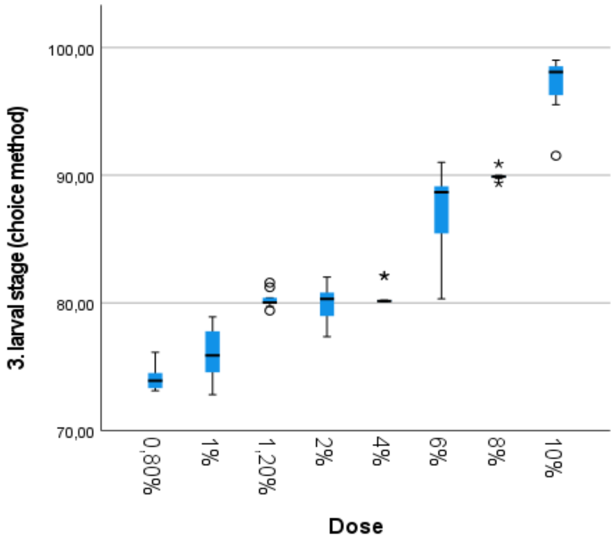

Figure 2. 3rd instar choice larvae AFI values

Upon examining Table 3 and Figure 2, it can be seen that the 6%, 8%, and 10% concentrations are significantly more effective than the 0.8% and 1% concentrations for the AFI values of the 3rd instar choice larvae ($p < 0.05$). Additionally, the 10% concentration is significantly more effective than the 1.2%, 2%, and 4% concentrations ($p < 0.05$).

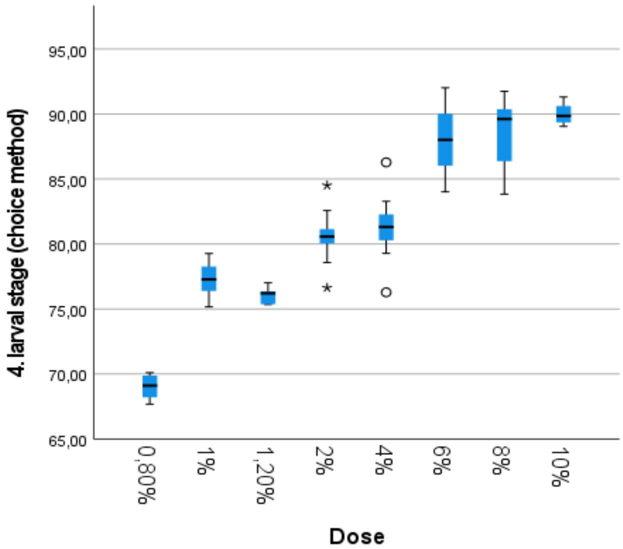

Figure 3. 4th instar choice larvae AFI values

Upon examining Table 3 and Figure 3, it can be seen that the 2%, 4%, 6%, 8%, and 10% concentrations are significantly more effective than the 0.8% concentration for the AFI values of the 4th instar choice larvae ($p < 0.05$). Furthermore, the 6%, 8%, and 10% concentrations are significantly more effective than the 1% and 1.2% concentrations ($p < 0.05$).

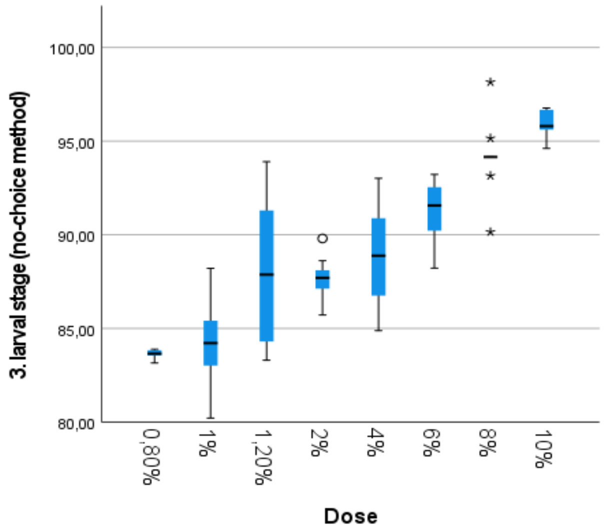

Figure 4. 3rd instar no-choice larvae AFI values

Upon examining Table 3 and Figure 4, it can be seen that the 6%, 8%, and 10% concentrations are significantly more effective than the 0.8% and 1% concentrations for the AFI values of the 3rd instar no-choice larvae ($p < 0.05$). Additionally, the 10% concentration is significantly more effective than the 1.2%, 2%, and 4% concentrations ($p < 0.05$).

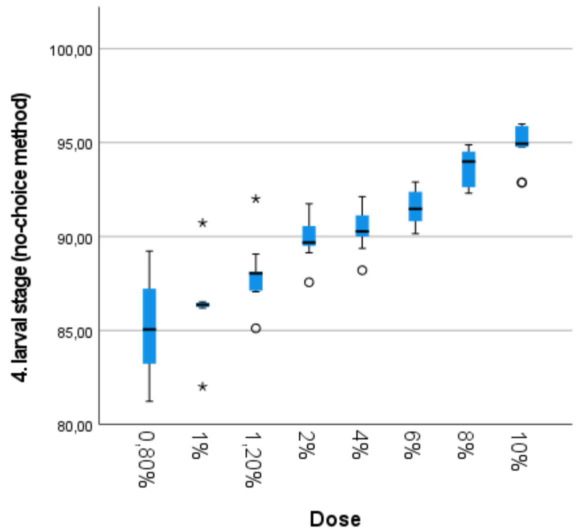

Figure 5. 4th instar no-choice larvae AFI values

Upon examining Table 3 and Figure 5, it can be seen that the 6%, 8%, and 10% concentrations are significantly more effective than the 0.8% and 1% concentrations for the AFI values of the 4th instar no-choice larvae ($p < 0.05$). The 8% and 10% concentrations are significantly more effective than the 1.2% concentration ($p < 0.05$). Additionally, the 10% concentration is significantly more effective than the 2% concentration ($p < 0.05$).

Upon examining Table 3 and Figure 5, it can be seen that the 6%, 8%, and 10% concentrations are significantly more effective than the 0.8% and 1% concentrations for the AFI values of the 4th instar no-choice larvae ($p < 0.05$). The 8% and 10% concentrations are significantly more effective than the 1.2% concentration ($p < 0.05$). Additionally, the 10% concentration is significantly more effective than the 2% concentration ($p < 0.05$).

Table 4. Results of the Mann-Whitney U Test determining the differences in larval stages between choice and no-choice methods

	Dose	Median	U	*p*
Choice	3rd term 4th term	80.27 81.11	2961.00	0.415
Non-choice	3rd term 4th term	88.74 90.15	3554.50	0.226

Upon examining Table 4, there is no significant difference between the choice 3rd instar and choice 4th instar ($p > 0.05$). Similarly, there is no significant difference between the no-choice 3rd instar and no-choice 4th instar ($p > 0.05$).

Table 5. Results of the Mann-Whitney U Test determining the differences in larval stages (3rd and 4th Instars) between choice and no-choice methods

	Dose	Median	U	*p*
3rd term	Choice Non-choice	80.27 88.74	4855.50	<0.001
3rd term	Choice Non-choice	81.11 90.15	5440.50	<0.001

Upon examining Table 5, there is a significant difference between the choice 3rd instar and no-choice 3rd instar ($p < 0.05$). It appears that the no-choice 3rd instar is better than the choice 3rd instar. Additionally, there is a significant difference between the choice 4th instar and no-choice 4th instar ($p < 0.05$), with the no-choice 4th instar being better than the choice 4th instar.

In this study, within the optional method, the maximum antifeedant index (AFI) value was recorded at a concentration of 10%. Additionally, in the experiments conducted with the optional method, the antifeedant index for 3rd larvae was determined to be at its lowest at a concentration of 0.8%. In clear terms, as the concentration of Neem Azal T/S extract increased, *C. quadrimaculata* larvae consumed less leaf material. This situation was also observed in 4th larvae (Table 1). In the study, although there was an increase in the area of almond leaves consumed by 3rd and 4th larvae, this increase is believed to be directly proportional to larval size.

In the no-choice method study, the area of leaves consumed on leaves that were not treated by 3rd instar larvae was measured at concentrations of 0.8, 1, 1.2, 2, 4, 6, 8, and 10% as follows: 0.28, 0.27, 0.20, 0.21, 0.19, 0.15, 0.10, and 0.07 cm (Table 2). The highest AFI values occurred at concentrations of 6%, 8%, and 10%. The lowest AFI value was observed at concentrations of 0.8% and 1%, where the concentration of the insecticide was low.

Despite a tenfold increase in the dose of Neem Azal T/S, an effectiveness difference of 9.36% was found between the lowest dose of 0.8% and the 10% dose for the AFI value (94.88-85.22=9.36%). It was determined that the antifeedant and repellent effects against the pest were quite high at all doses. Similarly, in no-choice method studies, it was determined that the area of leaves consumed increased with the dose in the 4th larval stage (Table 2).

Overall, it was determined that the highest AFI values occurred at the highest doses of 8% and 10%, and that the effectiveness of the neem extract generally increased with the dose against the pest. In all applications, maximum leaf consumption was observed on untreated leaves. Although the amounts of leaf consumption between doses were similar, the lowest leaf consumption was observed with a dose of 10% in the optional method and again with a dose of 10% in the non-choice method during the 4th larval stage. These values were higher in both methods due to the greater control leaf consumption by the 4th instar larvae, while the control consumption in the 3rd instar larvae was somewhat lower.

The antifeedant effects of neem extracts have been tested on many pests other than the one studied here and positive results have been found. Particularly, repellent and antifeedant effects have been identified on Lepidoptera pests, certain species belonging to the Heteroptera order (*Nezara viridula*), and species from the Cicadellidae family (Isman, 1999; Saxena and Basit, 1982; Durmuşoğlu et al., 2003). The results of this study in the literature indicate that it provides antifeedant and repellent effects in parallel with increased doses applied to the plant (Sharma and Gupta, 2009). Some other studies on larvae should be given with doses. In the study conducted to determine the effectiveness of Neem Azal T/S, increases in the amount of AFI effect of the pest was observed with increasing doses.

As a result, these data indicate that the extract of *A. indica* prevents the consumption of almond leaves and has a significant antifeedant effect against *C. quadrimaculata.* This stimulatory effect is referred to in entomology as "Stimulodeterrent diversionary.", and it was determined in this study that it acts against *C. quadrimaculata* with "push-pull" activity (Charleston et al., 2005).

As suggestions;

Therefore, in orchard studies, concentrations of 6%, 8%, and 10% will be applied to trees against both 3rd and 5th instar *C. quadrimaculata* larvae to determine the antifeedant and repellent effects of neem extract against the larvae.

These results are also supported by the statistical results that will be specified below, showing differences due to the increase in doses among the applications. In these applications, the effects of neem extract on the natural enemy fauna (predators and parasitoids) will also be observed, and differences will be compared with the control plots. Additionally, individuals that transition to the pupal stage from trees treated with different doses will be collected from the field and brought to the laboratory to determine the effects of the applied doses on parasitism rates.

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Conflict of interest

Author declares that no financial or competing interest.

Author's Contributions

The authors declare that they have contributed equally to the article.

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