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Araştırma Makalesi

Lethal Effects of Delthamethrin on *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae) and *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae)

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Anahtar Kelimeler

Depo zararlıları,
Letal etki,
Toksisite,
İnsektisit

Öz: Çalışmada kullanılan depo zararlılarından *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae) fasulyede önemli derecede ekonomik kayba neden olmaktadır. Diğer depo zararlısı *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) depolanmış ürünlerin başlıca zararlılarındandır. Bu zararlılarla mücadelede piretriod bazlı insektisitlerin farklı formülasyonlarının etkili olduğu bilinmektedir. Ayrıca bu kimyasalların düşük dozlarının zararlılar üzerindeki etkilerinin ortaya çıkartılması oldukça önemlidir. Bu amaçla bu çalışmada da laboratuvar koşullarında uzun zaman insektisite maruz kalmayan depo zararlıları (*A. obtectus* ve *O. surinamensis*) üzerinde Deltamethrin etkili insektisitin letal etkileri ortaya çıkartılmış ve LC_{30}, LC_{40} ve LC_{50} değerleri belirlenmiştir. Çalışma genelinden elde edilen verilere göre denemede kullanılan insektisitin *A. obtectus* ve *O. surinamensis* mücadelesinde kullanılabileceği, ancak düşük dozlarının zararlılar üzerindeki etkilerinin belirlenmesinin de önemli olduğu sonucuna varılmıştır. Elde edilen verilerin uygulanan insektisitin subletal dozlarının zararlılar üzerindeki etki çalışmalarına destek sağlayacağı düşünülmektedir.

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Abstract: *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae) used in the study causes significant economic losses in beans. The other storage pest, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), is a major pest of stored products. It is known that different formulations of pyrethroid-based insecticides are effective in controlling these pests. In addition, it is very important to reveal the effects of sublethal doses of these chemicals on pests. For this purpose, in this study, lethal effects of Deltamethrin

Keywords

Storage pests,
Lethal effect,
Toxicity,
Insecticide

effective insecticide on storage pests (*A. obtectus* and *O. surinamensis*) that have not been exposed to insecticide for a long time under laboratory conditions were revealed and LC_{30}, LC_{40} and LC_{50} values were determined. According to the data obtained from the study in general, it was concluded that the insecticide used in the trial can be used in the control of *A. obtectus* and *O. surinamensis*, but that it is also important to determine the effects of low doses on pests. It is thought that the obtained data will support the studies on the effects of sublethal doses of the applied insecticide on pests.

1.Introduction

Acanthoscelides obtectus Say (Coleoptera: Chrysomelidae), an important bean pest, is a widespread species worldwide and causes economic damage to dry beans (*Phaseolus vulgaris* L.) (Alvarez et al., 2005; Soares et al., 2015; Njoroge et al., 2017). In addition, it also causes damage to beans, chickpeas, and cowpeas (Güvenç and Güngör, 1996). When looking at the global distribution of *A. obtectus*, it has been reported to be found throughout Europe, as well as in Asia, North and South America, Africa, and Australia (Atak, 1975; Borowiec, 1987). Considering the global climate changes, it is anticipated that species, including *A. obtectus*, will particularly focus on Europe and its surroundings (Adler et al., 2022). Studies have shown that losses due to *A. obtectus* in production areas range from 70-90%, with losses up to 40% being defined as low damage (Berim, 2007). Female individuals of this insect lay their eggs in the drying pods of beans, and the first-stage larvae emerging from the eggs select and settle inside the seeds. Therefore, they are transported with the harvested seeds to storage conditions, further increasing the damage (Tuda, 2007). The structure of the first-stage larvae of this pest causes losses in agricultural products both before and after harvest (Parsons and Credland, 2003). When examining the damage caused by this pest in legumes, it is known that the larvae especially damage by consuming the proteins in the seeds (Southgate, 1979). The adults of this pest have a short lifespan and do not feed. The first individuals seen in the crops are multiplying to increase the population (Baier and Webster, 1992; Tucić et al., 1996; Soares et al., 2015). It is known that this pest causes significant damage directly or indirectly in fields and storage (Schmale et al., 2002; Rees, 2004; Hagstrum and Subramanyam, 2006), and it is reported that maintaining the economic damage threshold (4% damaged beans) is very important (Baier and Webster, 1992; Hagstrum and Flinn, 2014). Studies show that in the control of *A. obtectus*, there is a trend towards chemical, biological, mechanical, and cultural methods, with successful results (Abate and Ampofo, 1996; Boyer et al., 2012; Mutungi et al., 2015; Velten et al., 2008; Yankova and Sofkova, 2013). Despite resistance developed by stored product pests (Boyer et al., 2012; Collins and Schlipalius, 2018) and the residue problem, insecticides are still commonly used (Daglish et al., 2018). Some insecticide formulations that are effective on this pest and cause residue problems reduce the impact on pests in stored products (Toews and Subramanyam, 2003; Hagstrum and Subramanyam, 2006; Athanassiou and Arthur, 2018; Daglish et al., 2018). In some studies, it has been reported that different formulations of pyrethroid-based insecticides are effective on stored product pests (Toews and Subramanyam, 2003; Hagstrum and Subramanyam, 2006; Yu, 2015; Athanassiou and Arthur, 2018; Daglish et al., 2018; Andric et al., 2019; Sağlam et al., 2022). However, studies on the effect of these insecticides on *A. obtectus*, which is harmful to beans, are limited. In one study, it was reported that synthetic pyrethroids were less effective than organophosphates against *A. obtectus* in long-term storage (24 and 36 weeks) (Daglish et al., 1993). Additionally, another study found that the deltamethrin-PBO (synergist piperonyl butoxide) mixture showed 40-87% toxicity on *A. obtectus*, and the remaining individuals did not produce offspring (Kljajić et al., 2002). Some studies have reported that *A.* obtectus, which was briefly exposed to insecticides with different active ingredients (especially biological pesticides such as spinosyns and spinetoram), was negatively affected, and the chemicals used have high potential on the pest (Sağlam et al., 2022). In a study investigating the effects of deltamethrin on *A. obtectus*, it was found that different insecticides (malathion and spinosad) caused a higher mortality rate than deltamethrin. According to the obtained data, individuals treated with deltamethrin did not produce offspring and caused no damage (Kljajić et al., 2022; 2023).

Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae) is a globally distributed pest and one of the main pests of stored products (Tazbian, 2016). Morphologically, before 1956, *Oryzaephilus mercator* (Fauvel) (Coleoptera: Silvanidae) was known as *O. surinamensis* (Howe, 1956). However, subsequent studies have shown that they can be easily distinguished from each other. *O. surinamensis* has larger eyes, a wider area immediately behind the eyes, and a more triangular head shape compared to *O. mercator* (L.) (Haines, 1991). *O. surinamensis* is a secondary pest and generally feeds on damaged grains and processed cereal products. Additionally, it causes economic damage to chickpeas, lentils, canola, rice, nuts, yeast, sugar, tobacco, dried fruits, and oilseeds (Hill, 2003; Mahroof and Hagstrum, 2012; Govindaraj et al., 2014; Nika et al., 2020; Awadalla et al., 2021; Eldeghidy et al., 2022). This species, which is distributed worldwide (Hagstrum and Subramanyam, 2009), is also known as a significant pest of dates in storage in countries such as the UAE, Tunisia, Saudi Arabia, Jordan, Egypt, Algeria, Sudan, and Pakistan (Kousar et al., 2021). The larvae of *O. surinamensis* cause pre- and post-harvest losses on agricultural products. Additionally, they reduce the germination power of seeds and decrease the nutritional value of the product (Heather and Wilson, 1983; Trematerra and Sciarretta, 2004). Female individuals lay their eggs one by one, in groups, or in rows, with a total of 300-400 eggs, laying 6-10 eggs per day (Howe, 1956; Arbogast, 1976; Beckett and Evans, 1994). Depending on environmental conditions, the egg-laying period and total egg production of the species vary (Breese, 1961; Beckett and Evans, 1994; Nika et al., 2020). The number of eggs laid by female individuals increases with higher temperature and humidity (Beckett and Evans, 1994), but it is reported that fertility decreases under low humidity and high temperatures (Curtis and Clark, 1974; Beckett and Evans, 1994). According to some studies, more than 50 insecticides have been reported to be effective on the mentioned pest (Gourgouta et al., 2023). Among these, active ingredients such as cypermethrin (Gourgouata et al., 2019; Paloukas et al., 2020), cyphenothrin (Karanika et al., 2016), and deltamethrin (Sehgal et al., 2014) have been highlighted. However, several studies have reported that *O. surinamensis* has developed resistance to different insecticides. Therefore, evaluations of alternative insecticides are being made (Barson, 1983; Wallbank and Collins, 2003; Al-Jabr, 2006; Gautam et al., 2020). General control practices have shown that pyrethroids are effective in controlling *O. surinamensis* (Collins and Wilson, 1987; Watson and Barson, 1996). In one study, it was reported that even after 4 months of cypermethrin application, 63% mortality occurred (Gourgouta et al., 2019). Additionally, the application of alpha-cypermethrin was found to be very important in the control of *O. surinamensis* (Agrafioti and Athanassiou, 2018). Some neonicotinoids, such as thiamethoxam and imidacloprid, have also been reported to show high toxicity against this species (Arthur et al., 2004; Daglish and Nayak, 2012; Athanassiou et al., 2013; Tsaganou et al., 2021a; b). Pyrethroids, including deltamethrin, and some other insecticides have been found to have no lethal effect on agricultural pest insects at low doses and cause different negative effects (Kidd et al., 1996; Desnuex et al., 2004, 2005; Quan et al., 2016; Xiao et al., 2016; Qu et al., 2020; Afza et al., 2021; Alfaro-Tapia et al., 2021; Graily-Moradi et al., 2021; Tan et al., 2021; Shi et al., 2022). Therefore, determining the sublethal doses of insecticides tested on agricultural pests is very important. In this study, the lethal effects of deltamethrin, an effective insecticide, on *A. obtectus* and *O. surinamensis* under laboratory conditions were investigated, and the LC_{30}, LC_{40}, and LC_{50} values were determined.

2. Material and Method

2.1. Main Materials

In this study, an insecticide containing the active ingredient Deltamethrin (25 g/L, Deltharin®/HEKTAŞ) was used to determine its effect on two different stored product pests, dried bean beetle *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae) and the saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae).

2.2. Insect Rearing for the Experiments

The *A. obtectus* individuals used in the experiments were obtained from a mass rearing system that had not been exposed to insecticides for two years. The individuals (male-female) taken from this mass rearing system were placed into plastic containers (10x15x10 cm) containing an excessive amount of bean seeds, sufficient for feeding, and left to lay eggs. Afterward, the individuals that emerged from the eggs were allowed to mature into adults, and only individuals of the same age were used in the experiments. The rearing of this pest was carried out in climate chambers with a temperature of 25 ± 1 °C and relative humidity of 60 ± 5%.

The other stored product pest, *O. surinamensis*, used in the experiments, was also obtained from a mass rearing system without insecticide exposure for two years. The individuals taken from this mass rearing were placed into rearing containers containing a mixture of 5:5:1 oats:flour:yeast and allowed to lay eggs. The adult individuals were then removed, and the eggs were allowed to hatch, with the emerging individuals reaching adulthood. Only adults of the same age, that emerged simultaneously, were used in the experiments. These rearing procedures were carried out in climate chambers at a temperature of 25 ± 1 °C and relative humidity of 60 ± 5% (Aulicky et al., 2017; Awadalla et al., 2021).

2.3. Setting up the Experiments

In determining the dose of the insecticide used in the experiments, the lethal concentration (LC_{90}) that caused 90% or more mortality in the populations of *A. obtectus* and *O. surinamensis* was established. After this, the LC_{90} dose was diluted to half, and six doses (excluding the control) were prepared and applied to the specified stored product pests. The lethal effects of the Deltamethrin-based insecticide on the pests in the experiment were then assessed.

For the experiments, 9 cm diameter plastic Petri dishes containing food (bean seeds for *A. obtectus*, and a mixture of oats:flour:yeast in a 5:5:1 ratio for *O. surinamensis*) were used to maintain the survival of the stored product pests. The prepared doses were sprayed onto the individuals using a hand sprayer, ensuring that all individuals came into contact with the applied dose. Afterward, the individuals were transferred to Petri dishes containing food, and the edges of the dishes were sealed with parafilm to prevent escape.

Seventy-two hours after setting up the experiments, live and dead individuals were recorded, and the effects of the insecticide on *A. obtectus* and *O. surinamensis* were assessed. In this phase of the experiments, 10 Petri dishes were used for each dose, and 10 pests were placed in each Petri dish. Distilled water was used as the control. The experiments were carried out in rooms with the same conditions as the climate chambers where the insects were reared. This phase was repeated separately for each insect species.

2.4. Statistical Analyses

To determine the mortality rates based on the live and dead individuals, the Abbott formula was used, and the percentage of mortality was calculated (Abbott, 1925). The obtained results were subjected to variance analysis (ANOVA), and if the difference between the means was statistically significant, the level of significance was determined using the TUKEY multiple comparison test. The lethal concentrations (LC_{30}, LC_{40}, LC_{50}) of the insecticide on *A. obtectus* and *O. surinamensis* were determined using the obtained mortality rates. PROBIT analysis was employed to determine these concentrations.

$$\text{Percentage effect} = \left(\frac{\text{Number of live individuals in control} - \text{Number of live individuals in application}}{\text{Number of live individuals in control}}\right) \text{X } 100$$

3. Results and Discussion

According to the data obtained from the study, it was observed that when Deltamethrin, an effective insecticide, was applied at high doses, it exhibited high toxicity on the stored product pests in the experiment. For both pests, it was determined that the two highest doses applied (75 and 50 µl L^{-1}) caused high mortality, and the mortality rate from the 75 µl L^{-1} dose was found to be statistically different from the others ($P<0.05$). When the data were examined, the lowest mortality rate caused by the insecticide on *A. obtectus* and *O. surinamensis* was found to be 44% and 36%, respectively, at the 3.125 µl L^{-1} dose (Figure 1).

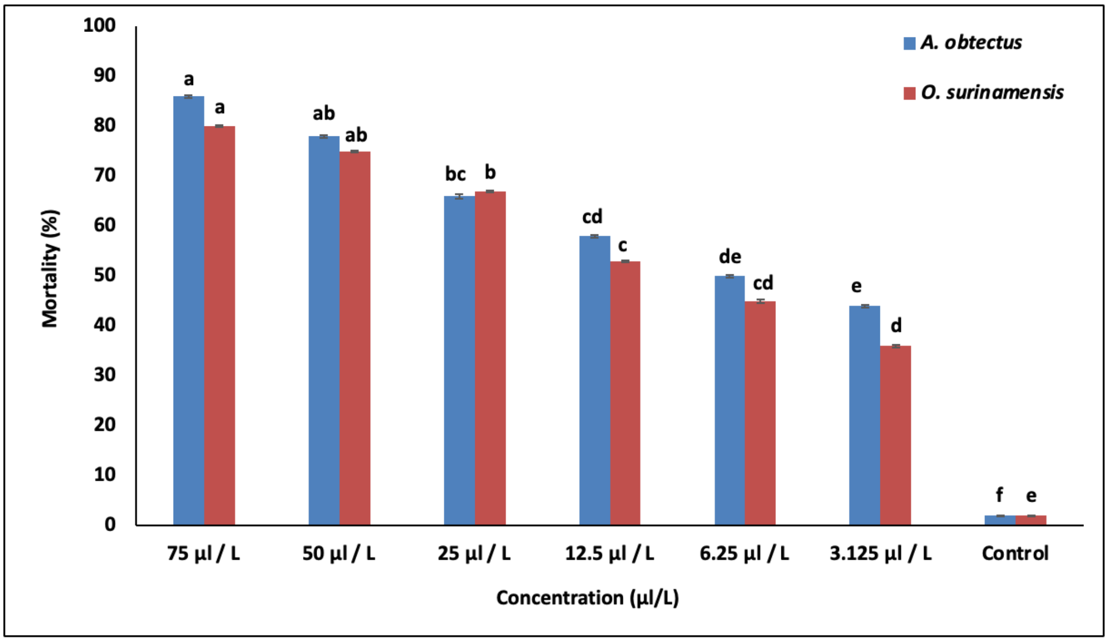

Figure 1. Mortality percentage of *Acanthoscelides obtectus* and *Oryzaephilus surinamensis* exposed to different concentrations of delthamethin for 72 h. Means above columns followed by different letters were significantly different according to Tukey ($F_{A.obtectus}$:92.38; $df_{A.obtectus}$:6; $P_{A.obtectus}$:0.001 / $F_{O.surinamensis}$:116.22; $df_{O.surinamensis}$:6; $P_{O.surinamensis}$:0.001).

Based on the mortality rates obtained from the doses applied in the study, different lethal doses (LC_{30}, LC_{40}, and LC_{50}) were determined. Probit analysis was performed, and the calculated concentrations for *A. obtectus* were 1.640, 3.449, and 6.911 µl L^{-1}, respectively, while for *O. surinamensis*, the corresponding values were 2.483, 5.078, and 9.991 µl L^{-1}. Additionally, after 72 hours, all the data related to the toxicity of deltamethrin on these two stored product pests are presented in Table 1.

Table 1. Toxicity of Deltamethrin on *Acanthoscelides obtectus* and *Oryzaephilus surinamensis* after 72 h

	Slope±SE[a]	**LC_{30} µl L^{-1} (95% CI)**[b]	**LC_{40} µl L^{-1} (95% CI)**[b]	**LC_{50} µl L^{-1} (95% CI)**[b]	**$x^2(df)$**[c]	***P* value**
Acanthoscelides obtectus	0.843±0.201	1.640 (0.661-4.068)	3.449 (1.391-8.555)	6.911 (2.786-17.142)	0.987 (5)	0.001
Oryzaephilus surinamensis	0.872±0.193	2.483 (1.040-5.928)	5.078 (2.127-12.123)	9.911 (4.151-23.662)	0.999 (5)	0.001

[a] Standart error; [b] 95% confidence intervals; [c] Chi-square value (x^2) and degrees of freedom (*df*)

Pyrethroids, which play a significant role in the control of agricultural pests, act as neurotoxic insecticides. These chemicals are known as synthetic analogs of pyrethrins (Pyrethrins I) and pyrethric acid (Pyrethrins II) esters, which are found in the flowers of *Chrysanthemum cinerafolis* (Asterales: Asteraceae) (Casida and Quistad, 1995). Large-scale production of pyrethrins from these flowers began in the mid-19th century. However, the first products were limited in agricultural use due to their low stability in air and light and high production costs. Therefore, various formulations were developed in subsequent years (Elliot, 1980). In later years, the first synthetic pyrethroids, such as permethrin, followed by cypermethrin and deltamethrin, were introduced (Ohno et al., 1976; Nakayama et al., 1979). A study conducted on these chemicals reported that synthetic pyrethroids act by slowing down the function of insect nerve cells through sodium ion channels (Davies et al., 2007). In recent years, it has been reported that applying low concentrations of pyrethroids and some insecticides on certain agricultural pests does not kill them but causes other adverse effects (Kidd et al., 1996; Desnuex et al., 2004, 2005; Quan et al., 2016; Xiao et al., 2016; Qu et al., 2020; Afza et al., 2021; Alfaro-Tapia et al., 2021; Graily-Moradi et al., 2021; Tan et al., 2021; Shi et al., 2022).

Bean seeds are stored in different ways depending on their intended use. For sowing purposes, they are treated with various chemicals, while those intended for food consumption require protection through non-pesticide methods. Pyrethroid and organophosphate insecticides are widely preferred in the preservation of these products because they are fast, effective, and inexpensive. However, these chemicals also pose harmful effects on the product and the environment, as well as risks of human and animal poisoning, residue issues, and pest resistance problems (Rombke and Moltmann, 2000). Therefore, it is crucial to use lower doses of pyrethroid insecticides, not the recommended doses, to disrupt the biology of pests and keep the pest population below the economic injury threshold, rather than aiming to completely eliminate the pest population.

Some studies indicate that organophosphates and synthetic pyrethroids are effective against pests like *A. obtectus* that cause problems in bean production (Daglish et al., 1993; Kljajic et al., 2002; 2022). Additionally, some analyses show that products like malathion and deltamethrin reduce the damage caused by *A. obtectus* to low levels (Arthur, 1996; Hagstrum and Subramanyam, 2006; Daglish et al., 2018; Athanassiou and Arthur, 2018). Our study also shows similar results, suggesting that deltamethrin has an effective potential against this pest. Moreover, different formulations of deltamethrin (SC and EC) have been reported to exhibit a stronger effect on *A. obtectus* in later stages (e.g., egg production), rather than having a lethal effect in earlier stages. This phenomenon is thought to be due to pesticide-induced stress and a behavior of avoiding or minimizing contact (Kljajic et al., 2022). A similar phenomenon has been observed in *Sitophilus zeamais* (Motsch) (Coleoptera: Dryophthoridae) and other arthropods (Guedes et al., 2009; 2016, 2017, 2018; Velez et al., 2017). The data obtained in our study will contribute to further research on the life stages of this pest. Specifically, the application of low doses of insecticides to determine their effects on various physiological stages, rather than relying solely on direct lethal effects, is important to maintain the pest population below the economic injury threshold.

Regarding the stored product pest *O. surinamensis*, many studies have investigated the effectiveness of a large number of insecticides (54 in total) (Gourgouta et al., 2023). This species is known to have developed resistance to pesticides, and as a result, recent research focuses on the potential of alternative insecticides (Wallbank and Collins, 2003; Al-Jabr, 2006; Gautam et al., 2020). Generally, pyrethroids have been reported to be effective against this pest (Collins and Wilson, 1987; Watson and Barson, 1996). A study found that cypermethrin applied to wheat showed a lethal effect of 63% on *O. surinamensis* even after a long period (4 months) (Gourgouta et al., 2019). Additionally, although alpha-cypermethrin was found to be highly effective in controlling *O. surinamensis*, its efficacy decreased after 21 days (Agrafioti and Athanassiou, 2018). Some studies suggest that using insect growth regulators in combination with spinosad can help control O*. surinamensis* populations (Fang et al., 2002; Athanassiou et al., 2010; Hertlein et al., 2011; Sadeghi et al., 2011; Wijayaratne et al., 2018). In addition, alternative methods, especially those based on population growth, such as warehouse ventilation, fumigation, residue testing, and storage duration, have been developed for the control of this pest (Hagstrum and Flinn, 1990; Driscoll et al., 2000; Kuzmanov and Dimitrov, 2009; Agrafioti et al., 2020a; b; De Faria et al., 2022; Latifian and Rad, 2022). Literature data show that synthetic pyrethroids are effective against *O. surinamensis* and help control its population. The key point is to use insecticides at lower doses to assess their impact on different physiological events and apply them effectively, rather than focusing solely on direct lethal effects, in order to maintain pest populations below the economic injury threshold. The findings from our study will support future research on the life stages of these two pests.

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