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# Side Effects of Some Insecticides and Acaricides on Different Beneficial Insects in Laboratory Conditions

# Bazı İnsektisit ve Akarisitlerin Laboratuvar Koşullarında Bazı Faydalı Böcekler Üzerindeki Yan Etkilerinin Araştırılması

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# SIDE EFFECTS OF SOME INSECTICIDES AND ACARICIDES ON DIFFERENT BENEFICIAL INSECTS IN LABORATORY CONDITIONS


## ABSTRACT

Side effects studies are important for the development of Integrated Pest Management (IPM) strategies, especially due to the adverse impacts of broad-spectrum pesticides on natural enemies. This study was conducted to evaluate the side effects of commonly used pesticides on *Chilocorus bipustulatus* L. (Coleoptera: Coccinellidae), *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae), and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) under laboratory conditions. The results revealed varying mortality rates among the natural enemies, with Abamectin exhibiting the highest mortality rates of 65.00%, 71.50%, and 78.75% respectively. Spirodiclofen (S1) showed the lowest mortality rate of 17.50% for *C. bipustulatus*, while Buprofezin (B) and Spirodiclofen (S2) showed rates of 18.78% and 18.93%, respectively for *A. pseudococci*. For *A. swirskii*, Spirodiclofen (S1 and S2) recorded the lowest mortality rates of 21.25% and 23.75% respectively. According to the IOBC classification, Spirodiclofen (S1 and S2) was classified as harmless (N,) against all three natural enemies. Abamectin (A) was classified as slightly harmful (M) for all three species, while Buprofezin was rated as slightly harmful (M) for *C. bipustulatus* but harmless (N,) for *A. pseudococci* and *A. swirskii*. This research highlights the differential effects of pesticides on different natural enemies and underlines the importance of considering such impacts in pest management strategies.


***Keywords:*** Side Effect, Citrus, Insecticide, Acaricide, Natural Enemy.

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# BAZI İNSEKTİSİT VE AKARİSİTLERİN LABORATUVAR KOŞULLARINDA BAZI FAYDALI BÖCEKLER ÜZERİNDEKİ YAN ETKİLERİNİN ARAŞTIRILMASI


## ÖZ

Yan Etki çalışmaları, özellikle geniş spektrumlu pestisitlerin doğal düşmanlar üzerindeki olumsuz etkileri nedeniyle Entegre Zarar Yönetimi (EZY) stratejilerinin geliştirilmesi için önemlidir. Bu çalışma, laboratuvar koşullarında yaygın olarak kullanılan pestisitlerin *Chilocorus bipustulatus* L. (Coleoptera: Coccinellidae), *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) ve *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) üzerindeki yan etkilerini değerlendirmek amacıyla yapılmıştır. Sonuçlar, doğal düşmanlar arasında değişen ölüm oranlarını ortaya koymuş olup, Abamectin'in sırasıyla %65,00, %71,50 ve %78,75'lik en yüksek ölüm oranlarını gösterdiği tespit edilmiştir. Spirodiclofen (S1), *C. bipustulatus* için %17,50'lik en düşük mortalite oranını gösterirken, Buprofezin (B) ve Spirodiclofen (S2) sırasıyla %18,78 ve %18,93 oranlarında *A. pseudococci* için belirlenmiştir. *A. swirskii* için ise Spirodiclofen (S1 ve S2) sırasıyla %21,25 ve %23,75'lik en düşük ölüm oranlarını tespit edilmiştir. IOBC sınıflandırmasına göre, Spirodiclofen (S1 ve S2), üç doğal düşmana karşı zararsız (N,) olarak sınıflandırılmıştır. Abamectin (A), üç tür için de hafif zararlı (M) olarak sınıflandırılmış, Buprofezin ise *C. bipustulatus* için hafif zararlı (M), ancak *A. pseudococci* ve *A. swirskii* için zararsız (N,) olarak derecelendirilmiştir. Bu araştırma, pestisitlerin farklı doğal düşmanlar üzerindeki farklı etkilerini vurgulamakta ve zararlı yönetimi stratejilerinde bu tür etkilerin dikkate alınmasının önemini vurgulamaktadır.

***Anahtar Kelimeler:*** Yan Etki, Turunçgil, İnsektisit, Akarisit, Doğal Düşman.

## 1. INTRODUCTION

Citrus is one of the most important agricultural products for Türkiye both in terms of production and consumption (Anonymous, 2023). Several limiting factors, including crop protection problems, affect citrus yield and fruit quality, with 89 pests, 155 weed species, 34 diseases, and 16 nematode species identified as significant challenges (Uygun and Satar, 2007). Notable pests such as the citrus mealybug (*Planococcus citri* Risso), citrus scale insects (*Aonidiella aurantii* (Mask.) and *Aonidiella citrina* (Coq.)), Mediterranean fruit fly (*Ceratitis capitata* Wied.), citrus rust mite (*Phyllocoptruta oleivora* Ashm.) and citrus red spider mite (*Panonychus citri* McGregor) are economically significant species causing damage in Turkish citrus orchards (Anonymous, 2017).

Conventional control methods have been used against citrus fruit pests, but they often lead to negative results in terms of human health and the environment. Consequently, alternative control methods, such as the use of biological and biotechnical approaches within Integrated Pest Management (IPM), have been proposed to provide more sustainable and environmentally friendly strategies against these pests (Şimsek and Uygun, 2013).

Numerous predators and parasitoids have been identified in citrus orchards in Türkiye (Kansu and Uygun, 1980; Uygun et al., 2001). Uygun et al. (2001), reported the presence of nine predator+parasitoid species for citrus mealybugs, nine predator species and six parasitoid species for citrus scale insects, and nine predator species for citrus spider mites. These results showed that there are many natural enemies of citrus in Türkiye; therefore, chemical control should be applied carefully, and broad-spectrum pesticides should not be used in citrus orchards.

Furthermore, the side effects of insecticides on beneficial organisms should be thoroughly studied in order to develop effective IPM strategies for pest management in citrus orchards (Simsek and Uygun, 2013; Satar et al., 2012; Serdar et al., 2018). Laboratory and field studies to assess the side effects of insecticides against natural enemies have to be applied according to IOBC/WPRS standards before their registration by the Ministry in Türkiye (Anonymous, 2018).

This study aimed to detect the side effects of some acaricides-insecticides in citrus orchards, specifically focusing on Spirodichloen, Buprofezin, and Abamectin, against *Chilocorus bipustulatus* L. (Coleoptera: Coccinellidae), *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae), and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) in laboratory conditions. The results of this study are expected to contribute to the development of IPM strategies for citrus production in Türkiye.

## 2. MATERIAL AND METHODS

The laboratory trials were conducted according to Standard test methods for the side effects of pesticides on beneficial organisms by the General Directorate of Agricultural Research and Policies (TAGEM). (Anonymous, 2018). Tested insecticides and acaricides under laboratory conditions have been given in Table 1.

**Table 1.** The used insecticides and acaricides in side effect experiment

| Applications | Types of Pesticides | Active Ingredient (g/l) | Recommended Doses (100 lt water) |
|---|---|---|---|
| W (PositiveControl) | | Water | |
| A | Insecticide + Acaricide | Abamectin 18 g/l | 25 ml |
| S1 | Acaricide | Spirodiclofen 240 g/l | 20 ml |
| S2 | Acaricide | Spirodiclofen 240 g/l | 20 ml |
| B | Insecticide | Buprofezin 400 g/l | 65 ml |
| D (Negative Control) | Insecticide | Dimethoate 400 g/l | 150 ml |

### 2.1. Insect Rearing

Three natural enemies, which were used in experiments, were reared in climate rooms at the Biological Control Research Institute in Adana/Türkiye during 2017-2018. Potato tubers (*Solanum tuberosum* L.) were used for *P. citri* and *A. pseudococci* were reared on these tubers. Additionally, *Aspidiotus nerii* Bouche (Hemiptera: Diaspididae) was used for the rearing of *C. bipustulatus* along with potatoes. *Carpoglyphus lactis* L. (Acarina, Carpoglyphidae), dried apricot fruit and *Typha latifolia* L. pollen were used for the rearing of *A. swirskii* during experiments. Climate rooms conditions were maintained at 26 ± 2 °C, 65 ± 10% RH and a 16:8 h (L:D) photoperiod

## 2.2. Side Effect Experiments

The experiments investigating the side effects on all three natural enemies were conducted under laboratory conditions in accordance with the Standard Test Methods for the Side Effects of Pesticides on Beneficial Organisms written by TAGEM (Anonymous, 2018). Side effect studies were carried out in climate rooms for *A. pseudococci*, the predator insect *C. bipustulatus*, and the predatory mite *A. swirskii*. The insecticides were applied in laboratory conditions at 26°C, 65% humidity, with a 16:8 h (L:D) photoperiod, using a Spray Tower (Hassan et al., 1994).

Circular test units made of plexiglass with a diameter of 13 cm (inner diameter 11.5 cm) and a height of 2 cm were utilized to determine the mortality rates of parasitoid, predator, and predatory mite adults exposed to pesticides. In the experiments, pesticides were applied onto 13 cm glass plates using a Spray Tower to achieve a thin film layer of 2±0.2 mg/cm$^2$ of the pesticide and distilled water was applied for Control. After spraying, test units were prepared for *C. bipustulatus, A. pseudococci*, and *A. swirskii*, and the pesticide-treated glass plates were placed into these units (Figure 1 and 2).

In the experiments, 3rd instar larvae of *C. bipustulatus* were used, and individuals were monitored until they reached adulthood. For *A. pseudococci*, both dead and alive individuals were counted 24 hours after exposure to the pesticide. Counts were conducted to calculate mortality rates of *A. swirskii* protonymphs 24 hours after exposure to the pesticide. The trials consisted of Control, Toxic pesticide, and 4 characters and 40 replications were used for *C. bipustulatus* and *A.pseudococi*, and 80 replicaitons were used for *A. swirskii* for each characters in the experiments. Counts of the trials were performed according to the Standard Test Methods for the Side Effects of Pesticides on Beneficial Organisms (Anonymous, 2018).

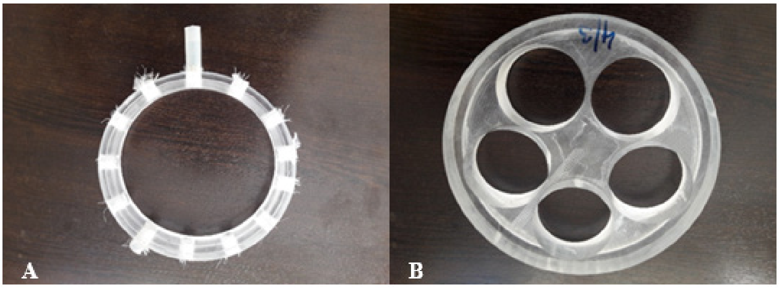


**Figure 1.** Experiment units for *Anagyrus pseudococci*(A) and *Chilocorus bipustulatus*(B)

***Şekil 1.*** *Anagyrus pseudococci*(A) ve *Chilocorus bipustulatus*(B) için kullanılan deneme üniteleri


**Figure 2.** Experimental units for *Chilocorus bipustulatus* (A), *Anagyrus pseudococci* (B), and *Amblyseius swirskii* (C)

***Şekil 2.*** *Chilocorus bipustulatus* (A) and *Anagyrus pseudococci* (B) ve *Amblyseius swirskii* (C) için kurulan denemeler

### 2.3. Statistical Analysis

The relative effect (%) was calculated using Abbott's formula (Abbott, 1925). Arcsin square root transformation was applied for the mortality rates before statistical analysis. Prior to analyses, the Shapiro-Wilk Normality Test was applied to assess whether there was a statistical difference among the mortality rates (%) between groups, a One-way ANOVA test was applied, and Duncan's multiple comparison test was conducted to determine the differences in mortality rates resulting from pesticide applications. All statistical analysis was performed using the SPSS 23 program.

In the laboratory experiments, pesticides were categorized into four groups based on relative effect (%), which was calculated by the Abbot formula, according to IOBC: N: harmless (< 30%); M: slightly harmful (30-79%); T: moderately harmful (80-99%); (Hassan et al., 1994; Candolfi et al., 2000; Boller et al., 2006).

## 3. RESULTS AND DISCUSSION

The insecticides/acaricides tested in the experiments exhibited varying side effects on *C. bipustulatus, A. pseudococci* and *A. swirskii*. The highest mortality rates among the three natural enemies were recorded as 65.00%, 71.50%, and 78.75%, respectively, for Abamectin (A). Conversely, the lowest mortality rate of 17.50% was observed for Spirodiclofen (S1). For *A. pseudococci,* the lowest mortality rates were 18.78% and 18.93% for Buprofezin (B) and Spirodiclofen (S2), respectively. Furthermore, the lowest mortality rates for *A. swirskii* were 21.25% and 23.75% for Spirodiclofen (S1 and S2) respectively. Dimethoate (D) served as a negative control for toxic insecticide exposure for *C. bipustulatus, A. pseudococc*i and *A. swirskii* during the experiments, resulting in 100% mortality rates (Table 2).

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**Table 2.** The mortality rates (%) of *Chilocorus bipustulatus*, *Anagyrus pseudococci*, and *Amblyseius swirskii* for different insecticides and acaricides

***Tablo 2.*** Farklı insektisit ve akarisitlerin *Chilocorus bipustulatus*, *Anagyrus pseudococci*, ve *Amblyseius swirskii* için ölüm oranları (%)

| | Mortality rate(%) | | |
|---|---|---|---|
| **Applications** | ***Chilocorus bipustulatus*** | ***Anagyrus pseudococci*** | ***Amblyseius swirskii*** |
| W | 10.00±6.70* | 0.00±7.42a | 7.5±1.44a |
| A | 65.00±2.88c | 71.50±2.80c | 78.75±4.26c |
| S1 | 17.50±4.78ab | 22.17±4.82b | 23.75±6.57b |
| S2 | 25.00±2.88b | 18.93.6.14b | 21.25±3.75b |
| B | 52.50±8.53c | 18.78±1.73b | 30.00±4.08b |
| D | 100.00±6.70d | 100.00±7.42d | 100.00±7.16d |

*Means in columns followed by the same letter are not significantly different (p<0.05)

**Table 3.** IOBC Classification and relative effect (%) of tested insecticides-acaricides for three natural enemies

***Tablo 3.*** *Denemelerde kullanılan insektisit ve akarisitlerin Yüzde etkileri (%) ve IOBC'ye göre sınıfları*

| IOBC Classification RP(%) | | | | | | |
|---|---|---|---|---|---|---|
| **Applications** | ***Chilocorus bipustulatus*** | ***Relative effect(%)*** | ***Anagyrus pseudococci*** | ***Relative effect(%)*** | ***Amblyseius swirskii*** | ***Relative effect(%)*** |
| W | - | - | - | - | - | - |
| A | M | 61.11 | M | 72.82 | M | 77.05 |
| S1 | N | 8.33 | N | 25.24 | N | 17.39 |
| S2 | N | 16.67 | N | 20.89 | N | 14.84 |
| B | M | 47.22 | N | 18.78 | N | 24.27 |
| D | T | 100 | T | 100 | T | 100 |

The IOBC classification of each insecticide/acaricide used in the experiments was determined in this study. The relative effect (%) compared to the control was calculated using the Abbott formula. Spirodiclofen (S1 and S2) was classified as harmless (N) for *C. bipustulatus*, *A. pseudococci*, and *A. swirskii*. Abamectin (A) was classified as slightly harmful (M) for *C. bipustulatus, A. pseudococci* and *A. swirskii*. Buprofezin was classified as slightly harmful (M) for *C. bipustulatus* but harmless (N) for *A. pseudococci* and *A. swirskii*. In addition, dimethoate was classified as harmful (T,) for all three natural enemies in this study (Table 3).

The laboratory experiments revealed different impacts of insecticides and acaricides on various natural enemies. Moreover, these substances affected different stages of beneficials in different ways. For instance, Buprofezin, known as an Insect Growth Regulator (IGR), reduced the survival of adult stages and restricted the development of insects from larval to adult stages (Şimsek and Uygun, 2013; Cabral et al., 2008). Several studies have investigated the effects of Buprofezin on coccinellid larvae (Mendel et al., 1994; Olszak et al., 1994; James, 2004; Liu and Stansly, 2004). Aghajanzadeh et al. (2020), investigated the lethal and sublethal effects of mineral oils and insecticides and classified Buprofezin as "Moderately harmful" according to IOBC standards, especially due to its reduction in larval emergence and pupal development. This finding is consistent with our study, in which Buprofezin was observed to affect larval-to-adult development and was classified as "M" according to IOBC standards. Nadimi et al. (2008), investigated the side effects of some acaricides on *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), and found abamectin to be toxic. Similarly, Kolcu and Kumral (2023), investigated the toxic effects of acaricides on *A. swirskii* and found abamectin to be slightly toxic. These results are consistent with our study, where abamectin was classified as "slightly harmful" to *A. swirskii* and other natural enemies. Alimohamadi et al. (2004), studied the effects of hexaflumuron and spirodiclofen on the developmental stages of *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), and found no significant effects of spirodiclofen on the developmental stages of predator. Various studies have explored the toxicity of certain insecticides on the parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) and *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), with abamectin and spirodiclofen being classified as "slightly harmful" (Vanaclocha et al., 2013; Hoseininaveh et al., 2012). Additionally, Karmakar and Shera (2020), studied the lethal and sublethal effects of insecticides on *Aenasius arizonensis* (Girault) (Hymenoptera: Encyrtidae), classifying buprofezin as "slightly harmful" according to IOBC standards. Although different parasitoid species were used in our study, the methodology was similar and the mortality rates were comparable to previous studies. Gonzalez-Zamora et al. (2013), observed the side effects of twelve different pesticides widely used in citrus orchards in Spain on *A. melinus*, resulting in changes from harmless to slightly harmful classifications for certain pesticides. Furthermore, the sublethal effects of spirodiclofen on *A. swirskii* have been investigated, indicating that sublethal concentrations of this active ingredient may slightly affect the population parameters of *A. swirskii* (Alinejad et al., 2016).

These studies collectively demonstrate that insecticides and acaricides affect different natural enemies. In particular, Insect Growth Regulators (IGRs) are shown to affect the developmental stages of natural enemies. Despite being classified as an acaricide, abamectin can impact all predators and parasitoids, with spirodiclofen identified as the least harmful, and spirodiclofen may be classified as more environmental-friendly than abamectin in terms of natural enemies, especially predatory mites, in above studies.

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## 4. CONCLUSION

The side effects of insecticides and acaricides on natural enemies is one of the most important issues in crop protection, and research on this topic has increased significantly in recent years. This issue is of particular importance for Türkiye, given the country's dependence on citrus as a major trade and import commodity. The presence of pesticide residues poses a significant threat to trade, making it imperative to investigate the side effects of commonly used insecticides and acaricides in Turkish citrus regions. The aim of this study was to investigate the impacts of select insecticides and acaricides, widely used in Turkish citrus areas, on *C. bipustulatus*, *A. pseudococci*, and *A. swirskii* under laboratory conditions. Additionally, IOBC Classification of these pesticides was carried out as part of this study. The results of this research are fundamental, and further investigations conducted under semi-field and field conditions should help to contribute to Integrated Pest Management (IPM) strategies in Turkish citrus regions.

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### Conflict of Interest

The authors declare that there is no confict of interests

### Ethics

This study does not require ethics committee approval.

### Author Contribution Rates

Design of Study: DK (%25), ŞTK (%25), AD (%25), SEG (%25)

Data Acquisition: DK (%25), ŞTK (%25), AD (%25), SEG (%25)

Data Analysis: DK(%70), ŞTK (%20), AD(%5), SEG(%5)

Writing up: DK(%50), ŞTK (%30), AD (%10), SEG (%10)

Submission and Revision: DK(%60), ŞTK (%30), AD (%5), SEG (%5)

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