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An Investigation on The Side Effects of Some Pesticides Against The Predatory Insect *Exochomus nigromaculatus* (Coleoptera: Coccinellidae) Under Laboratory Conditions

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ABSTRACT
Several harmful pest species can cause damage to apricot trees. Chemical control is the often preferred method in controlling these pests. The use of pesticides has generally resulted in pesticide resistance and elimination of natural enemies. *Exochomus nigromaculatus* is a predatory insect of globose scale and other scale insects. Inappropriate use of pesticides has been linked with adverse effects on non-target organisms (e.g., reduction of beneficial species populations and increase of pest populations). This study evaluated the side effects of five insecticides (Acetamiprid, deltamethrin, spirotetramat, sulfoxaflor, dimethoate) and a plant extract (orange oil) on immature stages of *E. nigromaculatus* using dry film method. Acetamiprid and deltamethrin caused the highest mortality rate (97.3%) besides standard toxic dimethoate. Sulfoxaflor accounted for approximately 70% mortality to *E. nigromaculatus*. In contrast, spirotetramat and orange oil caused less than 20% mortality to the predator. As a result of the dry film method applied against the pre-adult stage of *E. nigromaculatus;* dimethoate was classified as harmful (class 4), acetamiprid and deltamethrin were classified as moderately harmful (class 3), sulfoxaflor were classified as less harmful, (class 2) while spirotetramat and orange oil were classified as harmless. Thus, it was concluded that spirotetramat and orange oil did not have a negative effect on the predatory insect, *E. nigromaculatus* and could be used safely in IPM programs.

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Kayısı Bahçelerinde Kullanılan Bazı Pestisitlerin Laboratuvar Koşullarında *Exochomus nigromaculatus* (*Coleoptera*:Coccinellidae)'a Karşı Yan Etkilerinin Araştırılması

ÖZET
Kayısı ağaçlarında çeşitli böcek türleri zarara neden olmaktadır. Bu zararlılarla mücadelede kimyasal mücadele sıklıkla tercih edilen yöntemdir. Pestisitlerin uygunsuz kullanımı, hedef olmayan organizmalar üzerinde de olumsuz etkilerle sonuçlanmaktadır (örneğin, faydalı tür popülasyonlarının azalması ve haşere popülasyonlarının artması). *Exochomus nigromaculatus*, erik koşnili ve diğer koşnillerin avcı böceğidir. Bu çalışmada, beş insektisit (Acetamiprid, deltamethrin, spirotetramat, sulfoxaflor, dimethoate) ve bir bitki ekstraktının (portakal yağı) *E. nigromaculatus*'un kuru film yöntemi kullanılarak larva dönemlerine yan etkisi değerlendirilmiştir. Acetamiprid ve deltamethrin en yüksek ölüm oranına (%97.3) neden olmuştur. Sulfoxaflor, *E. nigromaculatus* için yaklaşık %70 ölüme neden olurken, spirotetramat ve portakal yağı avcılarda %20'den az ölüme neden olmuştur. *E. nigromaculatus*'un ergin öncesi evresine karşı uygulanan kuru film yöntemi sonucunda; dimethoate zararlı (sınıf 4), acetamiprid ve deltamethrin orta derecede zararlı (sınıf 3), sulfoxaflor az zararlı (sınıf 2), spirotetramat ve portakal yağı zararsız olarak sınıflandırılmıştır. Böylece, spirotetramat ve portakal yağının avcı böcek *E. nigromaculatus*'a olumsuz bir etkisinin bulunmadığı ve IPM programlarında güvenle kullanabileceği sonucuna varılmıştır.

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INTRODUCTION

Apricot is a stone fruit tree that has spread from Central Asia to a wide geography including countries neighboring the Mediterranean (Anonymous 2019). Among the world's apricot-producing countries, Turkey ranks first with a share of 23.1% (Anonymous 2020). In the year 2020, 863 thousand tons of apricot were produced in Turkey; 53% of this production was from Malatya, 12% from Mersin and 7% from Elazig (Anonymous 2021). Apricot grown in Malatya is important for both domestic consumption as well as for the country's economy as it is an important agricultural export product.

Various diseases and pests impact apricot cultivation in Malatya. The peach twig borer and flat-headed root-borer are the main pests of apricot (Anonymous 2017). Apart from these, for years, several other harmful insect species directly affect apricot production and cause significant input costs for crop production in the world and in Turkey (Viggiani 1989; Cravedi and Molinari 1995; Ulusoy et al. 2001; Öztürk et al. 2004; Anonymous 2008; Uygun et al. 2013; Öztürk and Ulusoy 2014; Anonymous 2017). Many researchers have stated that *Sphaerolecanium prunastri* (Boyer de Fonscolombe) (Hemiptera: Coccidae) is a harmful insect pest species of stone fruits in Turkey (Bodenheimer 1953; Lodos 1982; Ülgentürk et al. 2001; Özgen and Bolu 2009; Apak 2021). The pest overwinters as first and second instar larvae in apricot trees in Malatya province. This pest sucks plant sap from the trunks of trees, especially from one or two-year branches, causing intense fumagin formation in the trees; this affects the quality and quantity of fruits produced and weakens the trees. Continued introduction and feeding of species belonging to the Scolytidae family kill weakened trees (Anonymous 2008; Uygun et al. 2013). Reportedly, this pest is found on other plants belonging to the Rosaceae family in all geographical regions of Türkiye (Bodenheimer 1953; Soydanbay 1976; Öncüer 1977; Ülgentürk et al. 2001; Özgen and Bolu 2009; Yiğit and Tunaz 2015).

Several natural enemies including predators and parasitoids from different groups have been recorded against the globose scale in Turkey. Among them, *Discodes aeneus* and *Coccophaegus* sp. are important parasitoids whereas *Exochomus quadripustulatus* and *E. nigromaculatus* have been identified as predators (Özgen and Bolu 2009; Yiğit and Tunaz 2015; Keçeci et al. 2020). A recent study that detected intense infestation with the globose scale pests of apricot orchards in Kale, Kuluncak and Akçadağ districts of Malatya province, found high rates of parasitoid *D. aeneus*, and predatory insects *E. quadripustulatus* and *E. nigromaculatus* which are the natural enemy of globose scale, in an apricot orchard in Kale district in which chemical pesticides are not utilized. When chemical pesticides were unused in the orchard, the pest were suppressed in a short period of two years by these beneficial organisms (Keçeci et al. 2020). It is reported that the application of broad-spectrum synthetic insecticides leads to the resurgence of harmful insects due to their toxic effects on beneficial insects, and has negative impacts on their success (Karacaoğlu et al. 2020; Bibi et al. 2021).

Although *E. nigromaculatus* is naturally found in the orchards, pesticide use reduces its populations hence an increase in the harmful insect population. In order to carry out sustainable agriculture, the application of pesticides with less adverse effects on beneficial insects and their application at appropriate doses are important within the scope of integrated control. In literature, there have been many studies that have sought to analyze the impact of side effects of pesticides on commercially available and widely used benefits (Hassan et al. 1985; Dalcı et al. 2009; Portakaldalı and Satar 2015a, b; Kaya and Keçeci 2021). However, the most challenging situation is the lack of knowledge of predator or parasitoid which are naturally occurring and suppressing the pest population. The aim of this laboratory study was to determine the side effects of acetamiprid, deltamethrin, spirotetramat, sulfoxaflor, dimethoate and orange oil on immature stages of *E. nigromaculatus*.

METHODS

Branches infested with globose scales as well as larvae of its predator were brought to and cultured in the laboratory after surveys carried out in Malatya Apricot orchards. *Exochomus nigromaculatus* was mass-reared in plexiglass cages 30 × 30 × 50 cm (LWH) with the two sides covered by netting to allow ventilation. Citrus mealybug (*Planococcus citri*) (Hemiptera: Pseudococcidae) with potato sprouts were offered to predators as prey. The dry film method was applied against the pre-adult stages of *E. nigromaculatus*, which is one of the plant protection products that farmers mainly use in their orchards. IOBC methods were taken into account in determining the effects of these chemicals (Hassan et al. 1985; Candolfi et al. 2000). In the experiments, the pesticides were sprayed onto glass plates of 12 cm diameter with the help of a spray tower, and a thin film layer of chemical-infused liquid was formed with a thickness of 2±0.2 mg/cm2 on surface of the glass. Then, the treated surfaces of these plates were placed upside down and 2 cm high, 13 cm diameter, 5 cm diameter five-chamber cells made of fiber class were used as the test unit. Tap water was used to prepare the pesticide solutions for the experiment. For the control group only tap water was applied. The experiment was carried out in a randomized plot design with six groups (four pesticides, a standard toxic compound and water as control) with 4 replications. Ten 2nd stage larvae were used, one per cell in each replicate. These larvae were kept in these cells until they reached the adult stage (Karacaoğlu and Satar 2010; Satar et al. 2012). Food was provided to the larvae in the cells every day and the number of live-dead individuals were monitored and recorded.

Ten adult individuals that newly emerged were collected and placed into a 0.5 liter plastic rearing cage to mate. The number of eggs laid per female for 10 days after the adult individuals were placed in the cage was recorded daily to evaluate the effect on reproduction. From here, the effect on reproduction was calculated using the formula (R) =(1-(Rt/Rc))*100 (R: Reduction in fecundity, R_t: Egg number in the insecticide treatment, R_c: Egg number in the control treatment) (Anonymous 2010). Fecundity was used only as a quality criterion, while toxicity was determined only by considering mortality rates in pre-adult stages. Fecundity was not evaluated for characters with a corrected mortality rate of less than 50% compared to the control in the treated unit. The experiments were carried out in the climate rooms of the Plant Protection Department in Malatya Turgut Özal University. Conditions in the room were 25±1 °C temperature, 65±5% relative humidity and 14/10 hours of light/dark conditions. The active substance, trade names, formulation and dose values of the plant protection products included in the trial are given in Table 1.

Table 1. The active substance, trade names, formulation and dose values of the plant protection products used in the experiments against the pre-adult stages of *Exochomus nigromaculatus*

Çizelge 1. Exochomus nigromaculatus'un ergin öncesi dönemlerine karşı yapılan denemede kullanılan bitki koruma ürünlerinin etken maddesi, ticari adları, formülasyonu ve dozları

Active ingredient and formulation*	Trade name	Chemical group	Applied rate
Acetamiprid, (20 g/L, SP)	Mospilan 20	Neonicotinoids	40 gr /100 L
Deltamethrin (25 g/L, EC)	Decis EC 2.5	Pyrethroids	30 ml /100 L
Spirotetramat (100 g/L, SC)	Movento SC 100	Tetronic and Tetramic Acid Derivatives	100 g /100 L
Sulfoxaflor (240 g/L, SC)	Breaker 240	Sulfoximines	40 ml/100 L
Orange oil (60 g/L, EC)	Orange oil	Unknown	200 g /100 L
Dimethoate** (400 g/L, EC)	Poligor	Organophosphates	150 ml/100 L

* EC, emulsion concentrate; SC, suspension concentrate; SP, water soluble powder
** Dimethoate is included as a standart toxic.

Table 2. Classification of plant protection products according to their toxicity (IOBC)

Çizelge 2. Bitki koruma ürünlerinin toksisitelerine göre sınıflandırılması (IOBC)

Effect (%)	IOBC value	Class
0-30	1	Harmless
31-79	2	Slightly harmful
80-98	3	Moderately harmful
99-100	4	Harmful

Mortality data obtained from daily counting of the live and dead individuals were calculated and corrected using the Abbott formula (Abbott 1925). Arc-sin square root transformation was applied to the percent mortality values obtained and analysis of variance was applied to the transformed data (SPSS package statistical program, version 13.0) The differences between the applications were separated using Tukey Test (Efe et al. 2000). The plant protection products were classified according to the toxic categories developed by the IOBC (Table 2) (Hassan et al. 1985), taking into account the mortality rate (Abbott 1925).

RESULTS

The side effects of some plant protection products used in apricot orchards were assessed using the dry film method on the pre-adult stages of *E. nigromaculatus* and mortality data and IOBC classification are given in Table 3.

Acetamiprid and deltamethrin caused 97.3% mortality of predator larvae and were found to be as harmful as in standard toxic dimethoate. However, another active ingredient, sulfoxaflor, was produced 72.5% mortality on *E. nigromaculatus* immature stages and was classified as slightly harmful. Spirotetramat and orange oil presented 10.3% and 16.2% activity, respectively, and were found to be harmless and grouped in IOBC category 1. Moreover, the effect of these active ingredients on egg reproduction was accepted as zero, whereas spirotetramat 10.5% effective followed by orange oil with 12.2%.

Table 3. Classification of the effects of some plant protection products on the pre-adult stages of *Exochomus nigromaculatus* according to Abbott and IOBC

Çizelge 3. Exochomus nigromaculatus'un larva dönemlerine bazı bitki koruma ürünlerinin etkilerinin sınıflandırılması (Abbott ve IOBC)

Treatments	N*	Mortality rates of immature stages (%)	% Effect (Corrected using Abbott)	IOBC category	Reduction on reproduction capacity
Control	40	7.5±2.50a	-	-	-
Acetamiprid	40	97.5±2.50c	97.3	3	-**
Deltamethrin	40	97.5±2.50c	97.3	3	-
Spirotetramat	40	17.5±2.50a	10.3	1	10.5
Orange oil	40	22.5±4.78a	16.2	1	12.2
Sulfoxaflor	40	72.5±4.78b	70.3	2	-
Dimethoate	40	100.0± 0.00c	100.0	4	-

*Shows the number of replicates.

** Since more than 50% mortality was observed in these characters, the effect on reproduction was not evaluated. Means with the same letter in the same column were not statistically significant according to the Tukey test (P>0.05). SD= 6.21, F: 84,070 Sig: 0.000

Based on the data obtained from the side effect experiments on *E. nigromaculatus*, dimethoate, acetamiprid and deltamethrin were produced similar effect, and clustered in the same statistical group whereas sulfoxaflor was in a different group. Spirotetramat and orange oil were found to be harmless after applications. Thus, the both were statistically included in the same group as the control.

DISCUSSION

The aim of the present study was to determine the side effects of five insecticides and a plant extract to *E. nigromaculatus.* As a result of the study, acetamiprid and deltamethrin were harmful to the larva of *E. nigromaculatus*. Despite the Class II IOBC categories (slightly harmful), sulfoxaflor caused approximately 70% mortality to the predator. Spirotetramat and orange oil were harmless with 10.3 and 16.2 mortality, respectively.

Currently, no studies have determined the side effects of pesticides against *Exochomus* species. Erkılıç et al. (1994) assessed the effects of buprofezin and summer white oil against *Chilocorus bipustulatus* in their study. They stated that these active substances were harmless or less harmful to the adults of the insect. In the current study, orange oil was similarly harmless against the immature stages of *E. nigromaculatus*, which belongs to the same family as *C. bipustulatus*. Başpınar et al. (2002) tested the effects of the recommended doses of deltamethrin, azadirachtin, azadirachtin water extract, summer white oil and chlorfenapyr against adults and larvae of *Cryptolaemus montrouzieri* using spraying and dry film method. According to their results, deltamethrin was highly toxic against adults and larvae of *C.montrouzieri* with the dry film method. In another study, deltamethrin was also found highly toxic on *Discodes aeneus* Dalman (Hymenoptera: Encyrtidae), the parasitoid of globose scale found in apricot orchards (Karacaoğlu et al. 2020). We report that deltamethrin was moderately toxic to the pre-adult stages of *E. nigromaculatus*.

Brück et al. (2009) stated that spirotetramat is harmless against *Stethorus* spp, *Coccinella* spp. and *Chilocorus nigritus*. Planes et al. (2013) investigated the effects of spirotetramat, chlorpyriphos and pyriproxyfen against *Cryptolaemus montrouzieri* larvae and adults, and reported spirotetramat as harmless to *C. montrouzieri* larvae and that adults continued to reproduce after exposure. Likewise, we have determined that the active substance of spirotetramat gave similar results against the pre-adult stages of *E. nigromaculatus* belonging to the same family. Karacaoğlu et al. (2013) evaluated the effects of spirotetramat and dimethoate on the immature stages of *Chilocorus bipustulatus, Anagyrus pseudococci,* and *Amblyseius swirskii* and found that the mortality rates of *C. bipustulatus* after exposure to these chemicals were produced 11.11% and 100% mortality, respectively. Similarly, in the current study, dimethoate caused 100% mortality while spirotetramat exhibited 10.50%. Satar et al. (2018) examined the side effects of sulfoxaflor against *C. bipustulatus* (L.) (Coleoptera: Coccinellidae) under laboratory conditions and classified it according to IOBC standards. They stated that this active compound caused 71.16% mortality effect against *C. bipustulatus*. Likewise, sulfoxaflor had a similar effect on the pre-adult stages of *E. nigromaculatus* in this study. Kahraman and Öztop (2019) assessed the side effects of dimethoate, which is the active ingredient of buprofezin pesticide used in citrus orchards against *C. bipustulatus* and found that this compound was 100% effective, thus was classified as harmful according to the IOBC categories. Dimethoate was also 100% against *E. nigromaculatus* in the present study.

Bibi et al. (2021) tested the effects of orange oil against *Cryptolaemus montrouzieri* and *Chrysoperla carnea* under laboratory conditions. They reported that orange oil caused 25.9% *C. montrouzieri* larva mortality. In this study, a mortality rate of 22.5% was determined for orange oil against the second stages of *E. nigromaculatus*.

CONCLUSIONS

In conclusion, it was determined that the presence of plant protection chemical products particularly acetamiprid and deltamethrin have a negative effect on a beneficial organism *E. nigromaculatus* by causing 97.2% insect mortality. This shows that these two insecticides are not compatible for use in IPM programs. It should be emphasized that inappropriate insecticide selections easily disrupt the natural balance and can cause pest population to rise. Incorrect practices cause damage to the agricultural ecosystem as well as economic losses for the producer. Currently, spirotetramat, a temporarily recommended pesticide against the harmful mealy plum aphid (*Hyalopterus pruni*) in apricots, had low side effects against *E. nigromaculatus* and therefore it is compatible with use in IPM programs.

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Author's Contributions

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

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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