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Mortality of Adult Stages of the Biocontrol Agent, *Anthocoris nemoralis* (Hemiptera: Anthocoridae) Exposed to Insecticides

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Abstract: This study was conducted to investigate the mortality of adult stages of *Anthocoris nemoralis*, a predator of the pear leaf flea, *Cacopsylla pyri* (Hemiptera: Psyllidae) in nature, using four different insecticides namely; spinetoram, chlorpyrifos ethyl, diflubenzuron and spirotetramat at three different period such as 24, 48 and 72 hours. The mortality rate in male adult individuals of *A. nemoralis* proportionally increased with the exposure time in different insecticide treated environments. The lowest mortality rate of 11.25 % was recorded on 24 hours followed by 45 % and 86.67 % on 48 and 72 hours, respectively. Spinetoram caused the highest mortality (62.78 %), while diflubenzuron resulted in the lowest mortality (41.66 %) in males. The mortality rates in females were similar to those in the males, with the highest mortality seen on the 72 hours of insecticide treatment and the lowest mortality on 24 hours. Unlike in the males, the highest mortality was observed when diflubenzuron was used and the lowest mortality when using spinetoram. The male individuals were found to be more resistant than the females in terms of average period and average time+insecticide in mortality rates with respect to different biological stages of *A. nemoralis*, except for average means of insecticides. Overall, the average mortality rates in males and females were 47.64 % and 48.96 %, respectively. The findings suggest that the most harmful insecticide to adult stages of the insect was spirotetramat, while chlorpyrifos ethyl was only slightly harmful**.**

Keywords: Biocontrol, insecticide, mortality, natural enemy, predatory insect.

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ÇOBİLTUM
ÇANAKKALE ONSEKİZ MART ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ UYGULAMA VE ARAŞTIRMA MERKEZİ

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Introduction

Many species of insect pests affect the yield of pear (*Prunus* sp. L.). One of them such important and notorious insect pest is *Cacopsylla pyri* (Hemiptera: Psyllidae), which is commonly known as pear psylla. The biological stages, except eggs, of this insect pest feed on leaves, shoots and even on fruit of pear, causing economic damage. If the population of *C. pyri* is dense, the growth and development of the pear trees may stop and may also cause the shedding of pear leaves and fruit from the trees to the ground. Pear psylla causes direct and indirect damages to pear orchards. During direct damage, pear trees become weak and there is reduced production because of intense attack by this harmful insect pest. During indirect damage by psyllids, sooty molds develop and a large amount of honeydew is produced from different vegetative parts of pear trees (Civolani, 2012).

In Türkiye currently, *C. pyri* has developed resistance against several insecticides due to unconscious as well as the heavy application of insecticides to control this insect pest. This heavy application of insecticides has resulted in increased consumption of insecticides due to endurance. Hence, biological, cultural, biotechnological, and other environment-friendly alternative methods of controlling pear psylla need to be introduced. Of all the above-mentioned pest control programs, priority should be given to biocontrol of harmful insects. *Anthocoris nemoralis* (Hemiptera: Anthocoridae), the natural enemy of pear psylla, is one of the important beneficial predatory insects used in the biocontrol of this insect pest, as it keeps the population of psyllids below a level at which they could cause economic damage under natural field conditions. Naturally, *A. nemoralis* is a polyphagous predatory insect of majority of the egg, larval, nymphal, and even adult stages of different harmful insect pests. *A. nemoralis* is the most common species of the Anthocoridae family in Türkiye (Yıldırım et al., 2013). This predatory insect species is used extensively for biological control of harmful insect species of the Psyllidae family that cause tremendous damage, especially in pome fruit trees (Simionca et al., 2022). Many researchers have used different methods and techniques of releasing *A. nemoralis* on pear psylla and olive psyllid, *Euphyllura olivina* (Hemiptera: Psyllidae) (Gharbi, 2021) populations in pear and pistachio (Yanık and Unlu, 2015) orchards. Sigsgaard et al. (2006) reported that field releases of 10 or 30 *A. nemoralis* nymphs per pear tree in three different pear orchards in early May and two weeks later resulted in reductions of 31-40% in the *C. pyri* population. This species is not commercially produced in my country. In Türkiye, some taxonomic studies were conducted on *A. nemoralis*. Additionally, some paramount research studies were employed on different techniques and methods in terms of mass-rearing and releasing of this predatory insect, along with the prey–predator relationships, in Türkiye (Durlu and Uğur, 2014; Yazıcı, 2019) as well as in other parts of the world (Ferrero et al., 2014). But, in Türkiye, not any research work which will pave a way for *A. nemoralis* in the integrated pest management programs, and also the appraisement of the aftereffect of different insecticides are being used in fruit orchards against several harmful insect species, particularly psyllids.

Anthocoris nemoralis is naturally found in ecosystem and has been used to control the infestation of pear psylla and other harmful insect species in orchards (UCIPM, 2023). This study was conducted aim to determine the mortality ratios of the male and female biological stages of *A. nemoralis*, a natural enemy of pear psylla, when exposed to four different commonly used insecticides in pear orchards. Hopefully, this research study will contribute to the resolution of integrated pest management (IPM) programs that could be primarily manipulated in the biocontrol of insect pests in Türkiye. Furthermore, it would help producers/farmers select insecticides to control harmful insect species.

Materials and Methods

Male and female adult stages of *Anthocoris nemoralis* (F.) (Hemiptera: Anthocoridae), and the fresh eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were used as materials during the research work in 2014-2015. The side-effects of the active ingredients of four tested insecticides, namely chlorpyrifos ethyl, spirotetramat, spinetoram and diflubenzuron, on the male and female biological stages of *A. nemoralis* were determined. The mortality rates of the insect biological stages when exposed to the insecticides for 24, 48 and 72 hours were also recorded. The adults of *A. nemoralis* were collected from the unsprayed and neglected pear trees located in the Çubuk District of Ankara, Türkiye, and then transported to the Biological Control Laboratory of the Department of Plant Protection, Faculty of Agriculture, Ankara University, Türkiye. The collected insects were reared under the following laboratory conditions:

25±1 °C temperature, 70±10% R.H., 16:8 hours (L:D) of photoperiod, and 2500-lux light intensity. The insects were fed with fresh eggs of *E. kuehniella*.

The insecticides were prepared at appropriate concentrations. A spraying tower was used adopting the spraying method of and then the insecticides were sprayed in empty glass petri dishes, at the rate of 2 ml/petri dish, before transferring the insects into the dishes (Potter, 1952). After that, the insecticide-treated petri dishes were left to dry for 30–45 minutes. Later, the newly emerged (within 0–24 hours) males and females of *A. nemoralis* were selected randomly from the stocked insect culture – 'Çubuk' – a district of Ankara in Türkiye. After that, 10 insects from each sex were shifted to each insecticide contaminated petri dish for further studies. During the entire research studies, the fresh eggs of *E. kuehniella* were adhered to black cardboard strips (1×1 cm) with the help of distilled water and kept at -4ºC for 72 hours, before presenting them to *A. nemoralis* as food daily. These prepared eggs of *E. kuehniella* were given as food to the male and female individuals of the predatory insect after every 24 hours for the entire duration of the research according to the method reported by Karakuş (2018). Experimental trials were established using 6 replications for each insecticide along with a control treatment, with 10 insect individuals (male or female) placed in each replication (insecticide-treated petri dish). Just after the experiments, all living and dead individuals were counted under a stereo microscope and the mortality rates resulted because of the application of four tested insecticides were recorded regularly after 24, 48 and 72 hrs. The experimental trials were carried out separately for the male and female individuals of *A. nemoralis*. Result evaluations were calculated over the total number of dead individuals (male and female) of the predatory insect. The side-effects of the treated insecticides were calculated by Abbott's formula [Population % = ({pre-treatment population – post-treatment population} ÷ population without insecticide application) × 100 %] (Abbott, 1925; Karman, 1971). The results were evaluated according to the standards adopted by the IOBC working group for the side-effects of insecticides on beneficial insects in laboratory and classified according to the toxicity effects of insecticides in Table 1 (Boller et al., 2006).

Data analyses

Data obtained from this study were subjected to variance analysis using the Minitab 15 package program. The differences between the side-effects of different insecticides on male and female biological stages of the predatory insect were evaluated within the standard error of $P \leq 0.05$, using the "Tukey Test" included in the "MSTAT" package program.

Table 1. Classification of the side-effect of insecticides established by IOBC criteria for laboratory tests.

Class value	Toxic effect (%)	Toxicity level
N	< 30	Harmless or slightly harmful
M	30–79	Moderately harmful
T	> 80	Harmful

Source: Boller et al., 2006

Results

Male Mortality Rates of *Anthocoris nemoralis* (F.)

According to the overall results of the effects of treated insecticides on male stages of the insect, it was observed that the side-effects of different insecticides on male stages of *A. nemoralis* increased in terms of the time periods (df=2, FCh=7.43, P= 0.024; df=2, FSpiro=9.38, P=0.014; df=2, FDi=6.98, P=0.027; df=2, FSpine=9.47, P=0.014). Differences between the side-effects of insecticides after the time periods of 24, 48 and 72 hours were significantly importance (df=2, F24=1.64, P=0.257; df=2, F48=5.94, P=0.020; df=2, F72=8.98, P=0.006). For the International Organization for Biological and Integrated Control (IOBC) classification (Table 2), all four insecticides fell under the moderately harmful class (M=30–79), according to the values obtained by Abbott's formula (%).

Table 2. Mortality in males of *Anthocoris nemoralis* exposed to different insecticides for the time periods of 24, 48 and 72 hours*

Insecticides	N	Mean±SE			Abbott (%)	IOBC Class
		24 hours	48 hours	72 hours		
Chlorpyrifos ethyl	6	8.33±2.77 B-a	35.00±4.38 AB-a	83.33±9.28 A-b	42.22±5.48	M
Spirotetramat	6	5.00±1.28 B-a	38.33±3.81 AB-ab	88.33±7.68 A-b	43.89±4.26	M
Diflubenzuron	6	6.66±1.92 B-a	33.33±3.03 AB-b	85.00±8.79 A-b	41.66±4.58	M
Spinetoram	6	25.00±2.98 B-a	73.33±6.55 AB-ab	90.00±6.91 A-a	62.78±5.48	M

*Difference between different uppercase letters in the same row is significantly important (Tukey, $P \leq 0.05$), Difference between different lowercase letters in the same column is significantly important (Tukey, $P \leq 0.05$).

Female Mortality Rates of *Anthocoris nemoralis* (F.)

It was found that the aftereffects of tested insecticides on the survival of female biological stages of *A. nemoralis* increased with time (df=2, FCh=14.98, P=0.005; df=2, FSpiro=17.02, P=0.003; df=2, FDi=25.41, P=0.001; df=2, FSpine=10.75, P=0.010). Differences between the side-effects of treated insecticides was recorded to be significantly important after 24, 48 and 72 hours (df=2, F24=8.88, P=0.006; df=2, F48=29.90, P=0.000; df=2, F72=11.10, P=0.003). For the IOBC classification, the overall results (Table 3) showed that all four insecticides fell under the moderately harmful class (M=30–79), according to the values obtained using Abbott's formula (%).

Table 3. Mortality in females of *Anthocoris nemoralis* exposed to different insecticides for the time periods of 24, 48 and 72 hours*

Insecticides	N	Mean±St. Error			Abbott (%)	IOBC Class
		24 hours	48 hours	72 hours		
Chlorpyrifos ethyl	6	6.66±1.93 B-a	48.33±5.59 Ab-a	86.66±8.07 A-b	47.22±5.20	M
Spirotetramat	6	10.00±2.99 B-b	46.66±6.63 B-b	88.33±5.51 A-a	48.33±5.04	M
Diflubenzuron	6	11.66±2.77 B-b	50.00±3.88 B-b	86.66±9.63 A-b	49.44±5.43	M
Spinetoram	6	3.33±1.21 B-b	25.00±2.54 AB-b	76.66±4.61 A-b	34.99±2.79	M

*Difference between different uppercase letters in the same row is significantly important (Tukey, P≤0.05), Difference between different lowercase letters in the same column is significantly important (Tukey, P≤0.05).

Discussion

Highest mortality rate (90.00%±6.91) was recorded after 72 hours in male biological stages of the predatory insect exposed to the spinetoram active ingredient. On the other hand, the highest mortality rate in female individuals (88.33%±5.51) was noted when they were treated with the spirotetramat insecticide in this study. This indicates that the male and female biological stages of the minute pirate insect have different levels of sensitivity to spinetoram and spirotetramat active ingredients. In a study conducted by Lefebvre et al. (2012) with these two active ingredients, it was observed that spinetoram (100 %) and spirotetramat (40.20 %) caused mortality in the adult stages of *Neoseiulus fallacis* (Acari: Phytoseiidae). The mortality rates due to the application of spinetoram in this study was very close to the mortality rate due to the same insecticide in the aforementioned study, but the mortality due to spirotetramat treatments in above study was inversely correlated to the results obtained in this study.

In another study, it was observed that the usage of high dose of Spirotetramat 150 OD resulted 28.88 % mortality of predatory insect, *Chrysoperla zastrowisillemi* (Esberson Peterson) (Neuroptera: Chrysopidae). It is also stated that spirotetramat could be used within the framework of integrated pest management programs (Amala et al., 2015). In this study, spirotetramat was categorized under a slightly harmful (N) class according to the IOBC classification, whereas it was observed to be moderately harmful (M) in this research work. Al-Deeb et al. (2014) reported that chlorpyrifos ethyl caused a high mortality rate in adult biological stages of the predatory insect *Orius insidiosus* (Hemiptera: Anthocoridae). But, in case of in this study, chlorpyrifos ethyl was found to be moderately harmful (M) to both male and female adult stages of *A. nemoralis*. Rodriguez-Saona et al. (2016) found that the mortality of adult biological stages of *O. insidiosus* due to chlorpyrifos was highest, while spinetoram and six other active ingredients showed intermediate toxicities to this predatory insect.

Conclusion

This research was undertaken to determine the mortality rates in male and female biological stages of *Anthocoris nemoralis* (F.) (Hemiptera: Anthocoridae) an active predator of *Cacopsylla pyri* (Hemiptera: Psyllidae) under natural conditions when exposed to the tested insecticides for three different periods such as 24, 48 and 72 hrs. The overall lowest mortality rate of the male and female individuals of the minute pirate insect (11.25%) was recorded on the first day of application of the insecticides, followed by mortality rates of 45.00% and 86.67% on the 48 and 72 hours, respectively. The spinetoram insecticide showed the highest mortality rate in male adult individuals of *A. nemoralis* (62.78%), while diflubenzuron showed the lowest mortality rate (41.66%). The mortality rates of female biological stages of the predatory insects were similar to those of the male adult individuals, with the highest mortality rates recorded on the third day of insecticide treatments and the lowest mortality rates observed on the 24 hours of insecticide treatments. The overall average mortality rate in male individuals was 47.64 %, while this ratio was determined as 48.96 % in female individuals. Among the tested insecticides, the most harmful (T) insecticide to both the male and female biological stages of the predatory insect was spirotetramat, while the slightly harmful (N) active ingredient was chlorpyrifos ethyl.

Additional Information and Declarations

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