J. BIOL. ENVIRON. SCI.,
2019, 13(37), 39-47

Original Research Article

Side Effects of Azadirachtin On Some Important Beneficial Insects in Laboratory*

Mehmet Sadık Cura and Nimet Sema Gençer†
Department of Plant Protection, Faculty of Agriculture, Bursa Uludag University, 16059, Bursa, TURKEY

Received: 15.04.2019; Accepted: 03.04.2019; Published Online: 25.05.2019

ABSTRACT

The toxic effect of azadirachtin (0.3 g/L) in recommended dose, its half and twice doses were tested on *Encarsia formosa*, *Aphidius colemani*, *Orius laevigatus* and *Nesidiocoris tenuis* under laboratory conditions. The mortalities of nymphs and adults were evaluated by the IOBC toxicity rating scale for pesticides. As a result of the study, the product was harmful to *O. laevigatus* and *N. tenuis* adults and *E. formosa* pupa. Slightly and moderately harmful on *N. tenuis* nymph and *A. colemani* at recommended and twice doses. In recommended dose it was harmless only on *N.tenuis* male and *E. formosa* pupa at 24 h. Twice dose of the product was very toxic to all of the beneficial insects. The adulticide effects on males and females of *O. laevigatus* and *N. tenuis* in half dose at 48 h were found to be 83.33% and 75.00% and 78.26% and 76.20%, respectively. Additionally, little toxic effect (28%) was detected for *A. colemani* at half dose after 48 h. Similarly, at this dose, the mortality rates indicated less toxic effects on *N. tenuis* nymphs. In conclusion, it was understood that the recommended and twice doses of azadirachtin had negative effects on natural enemies. It was suggested that azadirachtin should be used carefully in pest control programs.

Keywords: Azadirachtin, Beneficial insects, Insecticidal effect, Toxicity, Side effect

INTRODUCTION

Pesticides and agrochemicals became an important component of worldwide agriculture systems during the last century, allowing for a noticeable increase in crop yields and food production (Carvalho 2017, Alexandratos and Bruinsma, 2012, Ngowi et al. 2007). Pesticides not only kill the pest organisms but also affect many natural enemy populations (Cloyd 2012). However, continuous use of pesticides may result in some potential ecological problems, including resistance and secondary pest epidemics (Ruberson et al.,1998). The adverse effects of pesticides against the human, animals and the environment cause alternative methods of pest control. In this case, one of the alternative pesticides used against pests is herbal compounds. Some studies have shown that the use of plant-based insecticides can keep insect pests below economic damage levels (Tepe 2010). Natural products having insecticidal effects have been used in the struggle of pests since ancient times (Isman 1997, Ujvary 2001). Neem is one of these plant-derived insecticides. Approximately 150 compounds have been detached from different parts of the neem tree *Azadirachta indica* A. Juss (Meliaceae) (Girish and Shankara 2008, Singh et al. 2010). Azadirachtin was a native compound derived from seeds and leaves of neem tree. It was known to act as a growth regulator (Mordue and Nisbet, 2000). Also it has mortality effect on insects, as well as negative impacts on the feeding development, behaviour and fertility (Hanning et al. 2009, Isman 2006, Waghmare et al. 2007). Azadirachtin was used against many pests found in greenhouses and open-field plant growing. It was believed that the IPM programs will have a selective effect on natural enemies (Schmutterer 1995, Simmonds et al. 2002, Santolamazza-Carbone and Fernández de Ana- Magán 2004). The effect of azadirachtin has been studied with many pest species (Schmutterer 1985, Lynn et al. 2012, Pavela et al. 2013, Tomé et al. 2013) and some natural enemies (Raguraman et al. 2004, Cordeiro et al. 2010, Celestino et al. 2014) and it has been found safer than other conventional insecticides.

More than 550 harmful species can be controlled by the neem extract even if they are resistant to synthetic pesticides (Whalon et al. 2008, Ascher 1993). Among them, Western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), and aphids were key pests of many agricultural plants that cause extensive economic losses in greenhouses and open-field area (Reitz et al. 2011, Minks and Harrewıjn 1987). The generalist predator, *Orius* spp. (Hemiptera: Anthocoridae), is the efficient predators of thrips, aphids, spider mites, whiteflies and moths (Blaeser et al. 2004, Silveira et al. 2004, Islam et al. 2010). Besides, parasitoid, *Aphidius colemani*

* This article is part of the MSc thesis of the first author.
† Corresponding author: nsgencer@uludag.edu.tr

Viereck (Hymenoptera: Braconidae), is used commercially for biological control of aphids in greenhouse crops. It has a host range of over 41 aphid species (Starý 1975).

Side effects of azadirachtin were studied on some natural enemies, include larvae of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), eggs, nymphs and adults of *Amphiareus constrictus* (Stal) and *Blaptostethus pallescens* Poppius (Hemiptera: Anthocoridae), coccinellid predators *Coccinella undecimpunctata* L, *Adonia variegata*, egg parasitoid, *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) and aphid parasitoid, *A. colemani* (Medina et al. 2003, Gontijo et al. 2015, Swaminathan et al. 2010, Abudulai and Shepard 2003, Atalla et al. 2009, Schmutterer 1997, Stara et al. 2011). However, some toxicity effects of Neem Azal on some *Tuta absoluta* (Meyrick,1917) (Lep.: Gelechiidae) predators, *Nesidiocoris tenuis* Reuter and *Macrolophus pygmaeus* Rambur (Het.: Miridae) (Arno and Gabarra 2011). Additionally, behavioral and mortality effects of some botanical insecticides on the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hem: Aleyrodidae) and its parasitoid *Encarsia formosa* Gahan (Hym: Aphelinidae) (Simmonds et al. 2002). Particularly, in recent years, there has been an increase in the use of biological control agents in IPM against pests in the greenhouses. Therefore, in this study, it was planned to study the side effect of different doses of a commercial formulation of azadirachtin on various biological stages of some natural enemies which can be found in natural environments or used in biological control methods.

MATERIALS AND METHODS

Host plant rearing

In this study, common bean (*Phaseolus vulgaris* L.) (Fabaceae) (Magnum) were used as the test plant. To provide green leaves, bean plants were grown in plastic pots (12 x 11 cm) containing a mixture of vermiculite and soil.

Natural enemies

Commercial strains of predatory bugs, *Orius laevigatus* (Fieber) (Het.: Anthocoridae) and *N. tenuis*, Whitefly parasitoid, *E. formosa* and aphid parasitoid *A. colemani* were provided by Koppert Biological Systems (Antalya, Turkey). En-strip product has 3000 *E. formosa* pupae in one package. One-hundered ml bottle of Nesibug product had 500 adults and nymphs of *N. tenuis*. Tripor-L product was packed in bottles containing 500 *O. laevigatus* individuals dispersed in vermiculite. Aphipar product was mixed with wood-chips in one bottle containing 1,000 *A. colemani* mummies.

Pesticide doses

The commercial formulation of azadirachtin, nimbecidine (0.3 g/L azadirachtin) (Agrobest, Turkey), were tested at the recommended field dose (500 ml/100 L water) which is registrated for the western flower thrips in pepper, and two other doses (half and twice of the recomended doses) in the experiment.

Contact or Residual method

The method used in this study was adapted from the method stated by Simon (2014). In this method, formulated insecticide was diluted in a solvent (water) and the insecticide solution filled in water spray container. In the experiments, plastic Petri dishes (10 cm diameter) with 25 small air holes were used in the upper cover. Firstly, water-soaked paper towel were placed at the bottom of Petri dishes in order to prevent the bean leaves dry in a short time. Also, fully expanded leaves were selected to prevent leaf deterioration in a short time. Then the bean leaves approximately 4 cm diameter were placed on paper towel one by one.

The azadirachtin solution was then sprayed on to the bean leaf in the Petri dishes and allowed to dry in room temperature at about 30-40 minutes. Mobile insects were released on the treated surface and thus got exposed to insecticide. But immobile stages (pupa) and first pupae were placed and then insecticide sprayed.

Experimental design

We separated female, male and nymphes of predatoy bugs under stereomicroscobe. A total of two or three days old adults (10 female+10 male) and nymphes were selected and transferred using a fine paintbrush on to the threatened bean leaves.

Exposure of *E. formosa* pupae to azadirachtin by direct contact+residues led to mortality in laboratory bioassays. Twenty *E. formosa* pupae and female were used for experiments. In the *E. formosa* pupae experiment first we put pupae on bean leaves then we treated with azadiracthin. *E. formosa* pupae on cards in glass tubes put into the climatic condition room (25±5°C, 65±5% R. H. and 16 L: 8 D hours) to provide adult emergence in a short time. They were released on to the bean leaves and the upper cover was closed.

In *A.colemani* test, 10 females were taken into the glass tubes. Since parasitoids were very mobile, they were left in the refrigerator for 5 minutes in order to reduce movements. Thus, the release of parasitoids into the petri dishes was easier. After releasing 10 individual of parasitoid wasp into the dishes.

In all experiments, we surrounded the dishes by parafilm in order to avoid the insects escape. The control bean leaves were treated with distilled water. There were three replications for each doses of pesticide. The results were controlled after 24, 48, 72 hours and the number of live and alive individuals were noted. Adult individuals were examined for mobility, however deformation of the pupae was examined.

For the toxicity rating of pesticides (based in the total effect caused in the enemy), the IOBC classification for laboratory standard tests (residual exposure) (Hassan, 1994): 1 (harmless, <30%), 2 (slightly harmful, 30-79%), 3 (moderately harmful, 80-99%), 4 (harmful, >99%) has been used in the study.

Statistical Analysis

Toxic effects were analysed by two-way ANOVA. The means were compared with Tukey post-hoc test and the results were displayed in the form of letters. Variables were displayed as mean with 95% confidence interval (CI). Mortality rates were arranged according to Abbott's formula (Abbott,1925). Statistical analysis were performed using JMP program.

RESULTS AND DISCUSSION

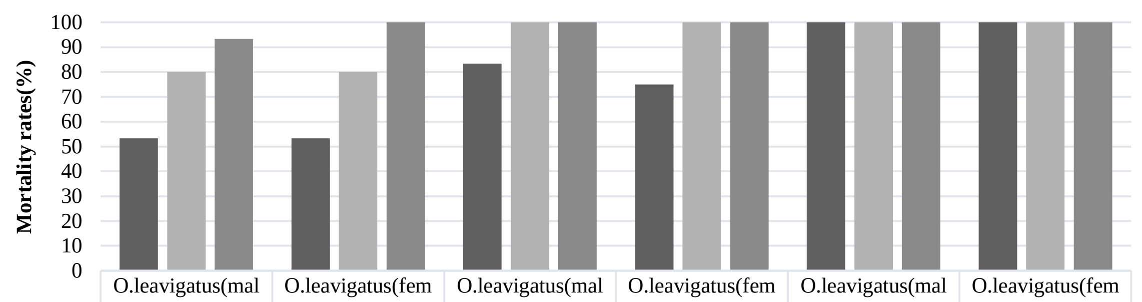
The toxicity of azadirachtin on *Orius laevigatus*

The adulticide effects of half, recommended and twice doses of azadirachtin on adult females and males of *O. laevigatus* were shown in Fig.1. At the the half dose, mortality rate was 53.33% in female and male of *O. laevigatus* within 24 hour period (Fig.1). But mortality rate increased within 48 and 72 hours. Similarly, Bonsignore and Vacante (2012) found that the botanical insecticides rotenone and neem decreased number of adult *O. laevigatus*. In contrast to our results, Angeli et al. (2005) stated that azadirachtin had no significant effect on the predator *O. laevigatus*. However, other studies with botanical insecticide-acaricide (based on plant extracts of castor bean, chicalote and berberis) was evaluated on two pollinators-bees and bumblebees-and on the predators *Chrysoperla carnea* and *Orius insidiosus* was slightly toxic (>%25) (Luna-Cruz et al. 2018).

The toxicity of azadirachtin on *Nesidiocoris tenuis*

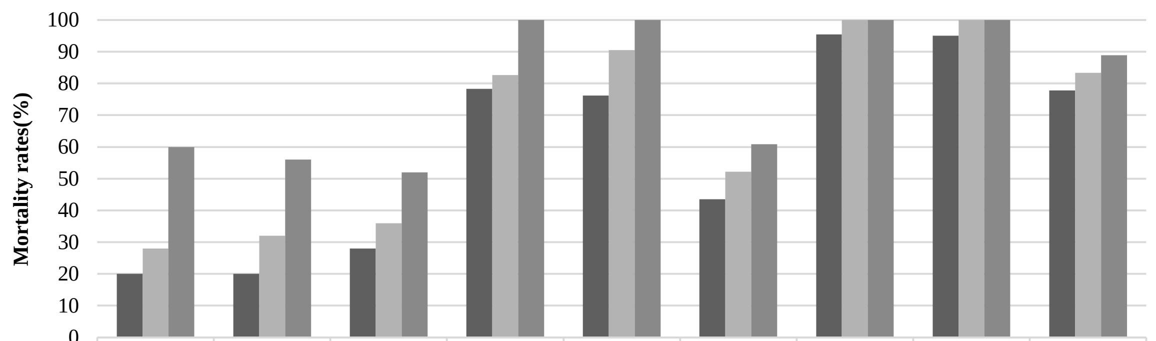
The effect of these different three doses of azadirachtin on adult females, males and last instars nymphes of *N. tenuis* were given in Fig. 2. Half dose of azadirachtin was only slightly toxic (20 %) on predatory bug *N. tenuis* male and female one day after treatment. At the recommended dose, mortality rate was 90.48% in female and 82.6% in male of *N. tenuis* within 48 hours. The significantly highest mortality rate (100 %) occured at the recommended and twice doses of the azadirachtin after 72 hours on predatory adult bugs. Also *N. tenuis* nymphal mortality was high in twice dose of azadirachtin within 72 hours. Also, the increase in doses causes an increase in death of natural enemies, as was observed by Momen et al. (1997). In agreement with Zanuncio et al. (2016) it was suggested that the mortality rates of different *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) nymph stages increased with increasing neem oil concentrations.

Similarly, Arno and Gabarra (2011) found negative effect of azadirachtin on the *Macrolophus pygmaeus* Rambur (Hem.: Miridae) and *N. tenuis*. Also, Gontijo et al. (2015) found that azadirachtin affected the mortality of adult, *A. constrictus* and *B. pallescens* important anthocorid predators of the tomato pinworm *T. absoluta*. Additionally, the same investigators reported that azadirachtin prevented the capacity of predator nymphs to reach the adult stage.



	O.leavigatus(male)(24 hours)	O.leavigatus(female)(24 hours)	O.leavigatus(male)(48 hours)	O.leavigatus(female)(48 hours)	O.leavigatus(male)(72 hours)	O.leavigatus(female)(72 hours)
0,125	53,33	53,33	83,33	75	100	100
0,25	80	80	100	100	100	100
0,5	93,33	100	100	100	100	100

Figure 1. The mortality rates (%) (Mean ±SE) of half dose (250ml/l), recommended (500ml/l) and twice (1000ml/l) doses of *azadirachtin* on *Orius laevigatus* females, and males at different counting times (hour).



	N.tenuis(male)(24 hours)	N.tenuis(female)(24 hours)	N.tenuis(nymphe)(24 hours)	N.tenuis(male)(48 hours)	N.tenuis(female)(48 hours	N.tenuis(nymphe)(48 hours)	N.tenuis(male)(72 hours)	N.tenuis(female)(72 hours)	N.tenuis(nymphe)(72 hours)
0,125	20	20	28	78,26	76,2	43,48	95,45	95	77,78
0,25	28	32	36	82,6	90,48	52,17	100	100	83,33
0,5	60	56	52	100	100	60,87	100	100	88,89

Figure 2. The mortality rates (%) (Mean ±SE) of half dose (250ml/l), recommended (500ml/l) and twice (1000ml/l) doses of azadirachtin on Nesidiocoris tenuis females, males and nymphes at different counting times (hour).

The toxicity of azadirachtin on *Encarsia formosa*

The side effect of azadirachtin on the pupae and adults of Whitefly parasitoid *E. formosa* were shown in Fig.3. The high and significant mortality of *E. formosa* females were observed in Petri dishes treated by azadirachtin at twice doses within 48 h (97.92%) and 72 h (100%). Mortality was low (20.34%) at half doses in 24 h, but high (92.59%) in 72 h. According to Drobnjaković et al. (2018) the longevity of *E.formosa* adults exposed azadirachtin for 48 hour was shorter than that of control wasps. In contrast to us, Yankova et al. (2011) showed that azadirachtin was non-toxic to adult of the parasite *E. formosa*. Felthege and Schmutterer (1993) stated that the most suitable among the products combined *E. formosa* include azadirachtin. Mortality rates of *E.formosa* pupae were 80.64 % at half dose within 72 h. Güncan et al. (2005) stated that, neem was found to be more effective on 18 day *E.formosa* pupae (%53). Some other researchers have reported that products obtained from *A. indica* have a potential for use in IPM programs against white flies which are biologically challenged with *E.formosa* (Simmonds et al. 2002). It is known that botanicals have different mechanisms of action. Different doses of azadirachtin cause mortality, inhibits adult emergence from the pupae and survival and results in anomalies on pupae. Like to see our work, Hossain et al. (2013) determined that some of them were biologically active chemical compounds.

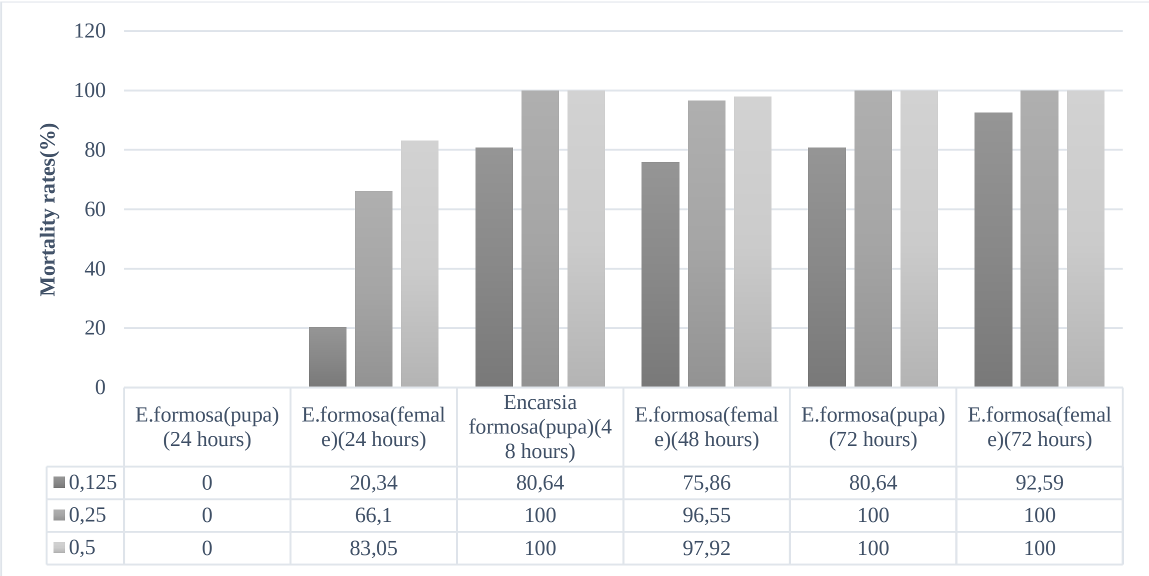


Figure 3. The mortality effects (Mean ±SE) of half dose (250ml/l), recommended (500ml/l) and twice (1000ml/l) doses of *azadirachtin* on *Encarsia formosa* pupae and females at different counting times (hour).

The toxicity of azadirachtin on *Aphidius colemani*

Neem extract at half doses was not high toxic effect at 24 and 48 hour (20.69% and 28%, respectively) in *A.colemani* (Fig. 4). In this study, *A.colemani* was relatively less susceptible compare to other species. However it was lethal to adult of *A.colemani* 80% and %100 at twice doses at 48 and 72 hours, respectively. Likewise, as also demonstrated by Stara et al. (2011) mortality rate was 100% after 48 h in adults of *A. colemani* exposed to azadirachtin. Some studies show that, after field treatment with insecticide, potential of recolonization by the parasitoid *Aphidius ervi* Haliday (Hym.: Braconidae) were reduced (Desneux et al. 2006 ab, Desneux et al. 2007, Joseph et al. 2011).

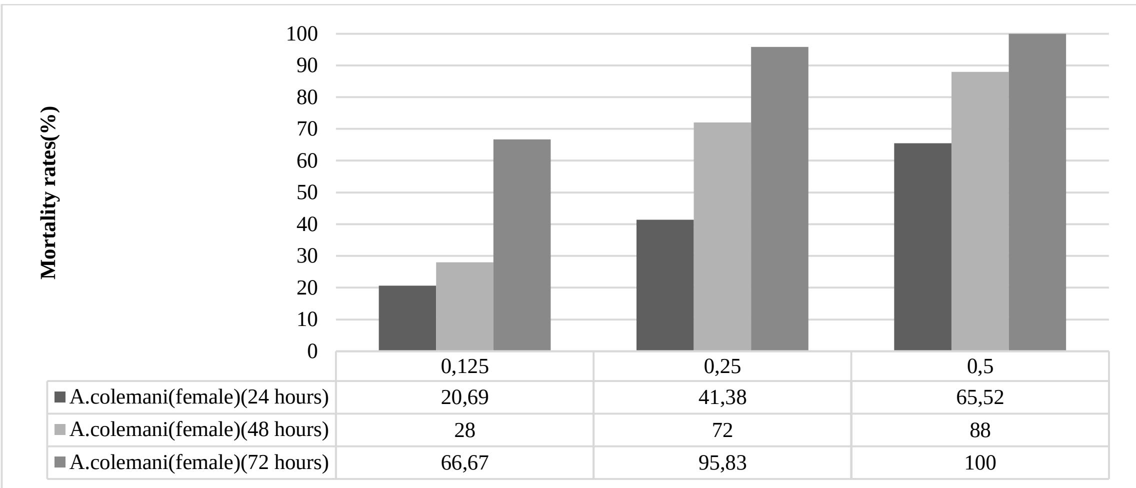


Figure 4. The mortality effects (Mean ±SE) of half dose(250ml/l), recommended (500ml/l) and twice (1000ml/l) doses of *azadirachtin* on *Aphidius colemani* females at different counting times (hour).

The toxicity of azadirachtin depending on species, dose and exposure time

The results of variance analysis in terms of species, dose and exposure time, and interactions between them were given in table 1. Main effect of azadirachtin were significant (P< 0.0001), while interaction between dose and exposure time was significant (F=13,88; df=4,4; P<0,0001). All treatments were statistically different (F=22,56; df=71,144; P<0,0001).

The effect of species, doses and time was found to be most significant in pupa of *E. formosa* and *O.laevigatus* adults (Table 1). One hundered percent mortality was observed at the 48 and 72 h at recommended and twice doses. But, *E. formosa* female was not significantly affected at half dose after 24 hour. In *O. laevigatus* females and males, 100% death was seen in 72 h. When we evaluated the effect of species and time, it was understood that *N.tenuis* male, female and nymphs was least affected by the 24th hour. When we evaluate the dose and species interaction, the females of *A. colemani* and *N. tenuis* nymphs were the least affected by the half dose of azadirachtin. Besides, we can say that *N. tenuis* nymphs are less affected by all doses. As the doses increased and time progressed, it was seen that the mortality rates increase in all species.

According to the classification of toxicity of insecticides on natural enemies for laboratory tests, according to the IOBC/WPRS (Hassan 1994), most of the results of the present work are included within the second, third and fourth category of toxicity [2 (slightly harmful, 30-79%), 3 (moderately harmful, 80-99%), 4 (harmful, >99%)]. On the other hand, the product was highly harmful to the male and female of *O. laevigatus* and *N.tenuis* and *E.formosa* pupa. But, slightly and moderately harmful on *N. tenuis* (nymph) and *A.colemani* at half, recommended and twice doses, except twice dose at 72 h in *A.colemani* . Azadirachtin was harmless on *N.tenuis* male, female and nymph and *E.formosa* pupa at 24 h. However, in recommended doses it was harmless only on *N.tenuis* male and *E. formosa* pupa at 24 h.

Table 1. Mortality effects of different azadirachtin doses on different stages of natural enemies in different exposure time.

Species	Hours	Doses		
		0.125	0.25	0.5
Encarsia formosa (pupa)	24	0.00 l	0.00 l	33.33 ı-l
	48	80.64 a-f	100.00 a	100.00 a
	72	80.76 a-f	100.00 a	100.00 a
Encarsia formosa (female)	24	20.33 kl	66.10 a-ı	83.05 a-f
	48	75.86 a-g	96.55 a-c	98.27 ab
	72	92.59 a-d	100.00 a	100.00 a
Orius laevigatus (female)	24	53.33 e-k	80.00 a-g	100.00 a
	48	75.00 a-g	100.00 a	100.00 a
	72	100.00 a	100.00 a	100.00 a
Orius laevigatus (male)	24	53.33 e-k	80.00 a-g	93.33 a-c
	48	83.33 a-f	100.00 a	100.00 a
	72	100.00 a	100.00 a	100.00 a
Nesidiocoris tenuis (female)	24	19.99 kl	31.99 ı-l	55.99 d-k
	48	76.19 a-g	90.47 a-d	100.00 a
	72	95.00 a-c	100.00 a	100.00 a
Nesidiocoris tenuis (male)	24	19.99 kl	27.99 j-l	59.99 c-j
	48	78.25 a-g	82.60 a-f	100.00 a
	72	95.45 a-c	100.00 a	100.00 a
Nesidiocoris tenuis (nymph)	24	27.99 j-l	35.99 h-l	51.99 f-k
	48	43.47 g-k	52.16 f-k	60.86 c-j
	72	77.77 a-g	83.33 a-f	88.88a-e
Aphidius colemani (female)	24	20.68 kl	38.04 h-k	65.51 a-ı
	48	27.99 j-l	71.99 a-h	87.99 a-f
	72	62.50 b-j	95.83 a-c	100.00 a

CONCLUSIONS

In conclusion, mortality rates of adult stage of *O. laevigatus*, *N. tenius*, *E. formosa* and *A. colemani* were high in twice doses compared with half and recommended doses. Additionally, females of the *O. laevigatus* and *E. formosa* were not able to survive in all doses. When all the results were evaluated, it was found that twice doses had negative effects on predators and parasitoids. In this study, in half and recommended doses, the mortality rate was low after 24 h in *N. tenuis* and aphid parasitoid *A. colemani* adults. Thus, azadirachtin should not be applied when natural enemies are released in greenhouses. Further studies are needed to determine the doses that had not any toxic effect on natural enemies in IPM programmes.

ACKNOWLEDGEMENTS

We would like to thank the Editor-in-Chief and the anonymous reviewers for the constructive comments which helped improve this manuscript. We thank Assoc. Prof. Dr. N.Alper KUMRAL (Uludag Univ. Agricultural Fac. Plant Protection Department) for their support in statistical evaluation.

REFERENCES

Abbott WS (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18:265-267.

Abudulai M, and Shepard BM (2003). Effects of neem (*Azadirachta indica* A. Juss) on *Trissolcus basalis* (Wollaston) (Hym.: Scelionidae), a parasitoid of *Nezara viridula* (L.) (Hem.: Pentatomidae). Journal of Entomological Science, 38: 386–397.

Alexandratos N, and Bruinsma J (2012). World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12-03. FAO, Rome.

Angeli G, Baldessari M, Maines R, and Duso, C (2005). Side-effects of pesticides on the predatory bug *Orius laevigatus* (Heteroptera: Anthocoridae) in the laboratory. Biocontrol Science and Technology, 15(7): 745-754.

Arno J, and Gabarra R (2011). Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). Journal of Pest Science, 84: 513-520.

Ascher K R S (1993). Non-conventional insecticidal effects of pesticides available from the neem tree, *Azadirachta indica*. Archives of Insect Biochemistry and Physiology, 22: 433-449.

Atalla FA, Shoeb MA, and Kelany IM (2009). Effect of Neem Azal T/S on some biological aspects of *Chrysoperla carnea* Steph. and *Coccinella undecimpunctata* L. and their protein contents. Egyptian Journal of Biological Pest Control, 19 (1): 17-23.

Blaeser P, Sengonca C, and Zegula T (2004). The potential use of different predatory bug species in the biological control of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). Journal of Pest Science, 77: 211–219.

Bonsignore CP, and Vacante V (2012). Influences of botanical pesticides and biological agents on *Orius laevigatus - Frankliniella occidentalis* dynamics under greenhouse conditions. Journal of Plant Protection Research, 52 (1): 15-23.

Carvalho FP (2017). Pesticide, environment and food safety. Food and Energy Security, 6(2): 48–6.

Celestino D, Braoios Gl, Ramos RS, Gontijo LM, and Guedes RNC (2014). Azadirachtin mediated reproductive response of the predatory pirate bug *Blaptostethus pallescens*. Biocontrol, DOI 10.1007/sl0526-014-9601-z.

Cloyd RA (2012). Indirect effects of pesticides on natural enemies**.** 6: 127-150.

Cordeiro EMG, Corrêa AS, Venzon M, and Guedes RNC (2010). Insecticide survival and behavioral avoidance in the laccewings *Chrysoperla externa* and *Ceraeochrysa cubana*. Chemosphere, 81:1352-1357.

Desneux N, Decourtye A, and Delpuech JM (2007). The sublethal effects of pesticides on beneficial arthropods. Annual Review of Entomology, 52: 81-106.

Desneux N, Denoyelle R, and Kaiser L (2006 a). A multi-step bioassay to assess the effect of the deltamethrin on the parasitic wasp *Aphidius ervi*. Chemosphere, 65:1697–1706.

Desneux N, Ramirez-Romero R, and Kaiser L (2006 b). Multi step bioassay to predict recolonization potential of emerging parasitoids after a pesticide treatment. Environmental Toxicology and Chemistry, 25: 2675 - 2682.

Drobnjaković T, Marčić D, Prijović M, Perić P, Milenković S, and Bošković J (2018). Sublethal effects of NeemAzal- T/S botanical insecticide on Dutch and Serbian populations of *Encarsia formosa* (Hymenoptera: Aphelinidae). Biocontrol Science and Technology, 28 (1): 1–19.

Feldhege M, and Schmutterer H (1993). Investigations on side effects of Margosan-O on *Encarsia formosa* Gah. (Hym.: Aphelinidae) parasitoid of the greenhouse white fly *Trialeurodes vaporariorum* (Westw.) (Homoptera: Aleyrodidae). Journal of Applied Entomolology, 115: 37–42.

Girish K, and Shankara BS (2008). Neem – A Green Treasure. Electronic Journal of Biology, 4 (3): 102-111.

Gontijo LM, Celestino D, Queiroz, OS, Guedes RNC, and Picanço MC (2015). Impacts of azadirachtin and chlorantraniliprole on the developmental stages of pirate bug predators (Hemiptera: Anthocoridae) of the tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae). Florida Entomologist, 98(1):59-64.

Güncan A, Durmuşoğlu E, and Yoldaş Z (2005). Bazı doğal organik insektisitlerin *Encarsia formosa* (Gahan) (Hymenoptera:Aphelinidae) pupalarına etkileri üzerinde araştırmalar. Ege Üniversitesi Ziraat Fakültesi Dergisi, 42 (2): 57-64.

Hanning GT, Ziegler M, and Marçon PG (2009). Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. Pest Management Science, 65: 969-974.

Hassan S A (1994). Activities of the IOBC/WPRS working group pesticides and beneficial organisms. IOBC/ WPRS Bulletin 17(10):1-5.

Hossain M A, Al-Toubi WAS, Weli, AM, Al-Riyami QA, and Al-Sabahi JN (2013). Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. Journal of Taibah University for Science, 7 (4): 181-88.

Joseph JR, Ameline A, and Couty A (2011). Effects on the aphid parasitoid *Aphidius ervi* of an insecticide (Plenum ®, pymetrozine) specific to plant-sucking insects. Phytoparasitica, 39(1):35-41.

Islam MT, Castle SJ, and Ren S (2010). Compatibility of the insect pathogenic fungus *Beauveria bassiana* with neem against sweetpotato whitefly, *Bemisia tabaci*, on eggplant. Entomologia Experimentalis et Applicata, 134:28–34.

Isman MB (1997). Neem and other botanical insecticides banlers to commercialization. Phytoparasitica, 25: 339–344.

Isman M (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology, 51: 45-66.

Lynn OM, Kim JE, and Lee KY (2012). Effects of azadirachtin on the development and gene expression of fifth instar larvae of Indianmeal moth, *Plodia interpunctella*. Journal of Asia-Pacific Entomology, 15:101-105.

Luna-Cruz A, Lomeli-Flores JR, Rodríguez-Leyva E, Tovar-Hernández H, Vanegas-Rico JM, and Murillo-Hernández JE (2018). Toxicity of a botanical insecticide on *Bombus impatiens*, *Apis mellifera*, *Chrysoperla carnea* and *Orius insidiosus*. Revista Mexicana de Ciencias Agrícolas, 9: 7, 1423-1433.

Medina P, Smagghe G, Budia F, Tirry L, and Vinuela E (2003). Toxicity and absorption of azadirachtin, diflubenzuron, pyriproxyfen, and tebufenozide after topical application in predatory larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environmental Entomology, 32(1): 196-203.

Minks AK, and Harrewijn P (1987). World Crop Pests, Elsevier, Amsterdam

Momen F M, Reda AS, and Amer SAA (1997). Effect of Neem Azal-F on *Tetranychus urticae* and three predacious mites of the family Phytoseiidae. Acta Phytopathologica et Entomolologica Hungarica, 32: 355-362.

Mordue AJ, and Nisbet AJ (2000). Azadirachtin from the neem tree *Azadirachta indica*: Its action against insects. An Soc Entomol Bras, 29:615-632.

Ngowi AVF, Mbise TJ, Ijani ASM, London L, and Ajayi OC (2007). Smallholder vegetable farmers in northern Tanzania: Pesticides use practices, perceptions, cost and health effects. Crop Protection, 26, 1617-1624.

Pavela R, Zabka M, Kalinkin V, Kotenev E, Gerus A, Shchenikova A, and Chermenskaya T (2013). Systemic applications of azadirachtin in the control of *Corythucha ciliata* (Say, 1832) (Hemiptera, Tingidae), a pest of *Platanus* sp. Plant Protection Science 49: 27-33.

Raguraman S, Ganapathy N, and Venkatesan T (2004). Neem versus entomopathogens and natural enemies of crop pests: The potential impact and strategies, pp. 125-182 In Koul O, Wahab S [eds.], Neem: Today and in the New Millennium. Kluwer Academic Publishers, Netherlands.

Reitz SR, Gao YL, and Lei ZR (2011). Thrips: Pests of concern to China and the United States. Agricultural Sciences in China, 10:867–892.

Ruberson JR, Nemoto H, and Hirose Y (1998). Pesticides and conservation of natural enemies in Pest Management. In: Barbosa P. (ed.) Conservation Biological Control. Academic Press, San Diego, CA, p.207-220.

Santolamazza-Carbone S, and de Ana-Magan FJF (2004).Testing of selected insecticides to assess the viability of the integrated pest management of the Eucalyptus snout-beetle *Gonipterus scutellatus* in north-west Spain. Journal of Applied Entomology, 128: 620-627.

Schmutterer H (1985)**.** Which insect pests can be controlled by application of neem seed kernel extract under field conditions. Z. Angew. Entomol. 100: 468-475.

Schmutterer H (1995). The tree and its characteristics (Editör: H. Schmutterer In: The Neem Tree *Azadirachta indica* A. Juss. and other Meliaceous Plants: Sources of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes). VCH Weinheim Germany. 1-34.

Schmutterer H (1997). Side-effects of neem (*Azadirachta indica*) products on insect pathogens and natural enemies of spider mites and insects. Journal of Applied Entomology, 121: 121-128.

Silveira LCP, Bueno VHP, and Van Lenteren JC (2004). *Orius insidiosus* as biological control agent of thrips in greenhouse chrysanthemums in the tropics. Bulletin of Insectoogy,l 57:103–109.

Simon JY (2014). The Toxicology and Biochemistry of Insecticides. CRC press, 1-380.

Simmonds MSJ, Manlove JD, Blaney WM, and Khambay BPS (2002). Effects of selected botanical insecticides on the behaviour and mortality of the glasshouse whitefly *Trialeurodes vaporariorum* and the parasitoid *Encarsia formosa*. Entomologia Experimentalis et Applicata, 102: 39–47.

Singh B, Sharma D, Kumar R, and Gupta A (2010). Development of a new controlled pesticide delivery system based on neem leaf powder. Journal of Hazardous Materials, 177 (1-3): 290–299.

Stara J, Ourednickova J, and Kocourek F (2011). Laboratory evaluation of the side effects of insecticides on *Aphidius colemani* (Hymenoptera: Aphidiidae), *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), and *Neoseiulus cucumeris* (Acari: Phytoseidae). Journal of Pesticide Science, 84: 25-31.

Starý P (1975). *Aphidius colemani* Viereck: its taxonomy, distribution and host range (Hymenoptera, Aphidiidae). Acta Entomologica Bohemoslovoca, 72: 156–163.

Swaminathan R, Jat H, and Hussain T (2010). Side effects of a few botanicals on the aphidophagous Coccinellids. Journal of Biopesticides, 3(1 Special Issue) 081 - 084 81-84**.**

Tepe S (2010). Bitki korumada doğal pestisitlerin kullanımı. Derim Dergisi, 18 (3): 113-121.

Tomé HVV, Martins JC, Correa AS, Galdino TVS, Picanço MC, and Guedes RNC (2013). Azadirachtin avoidance by larvae and adult females of the tomato leafminer *Tuta absoluta*. Crop Protection, 46.

Ujvary I (2001). Pest control agents from natural products. In handbook of pesticide toxicology, 23rd (Editör: R. I. Krieger) Academic Press. San Diego, 109-179.

Waghmare JT, Ware AM, and Momin SA (2007). Neem oil as pesticide. Journal of Dispersion Science and Technology, 28: 323-328

Whalon ME, Mota-Sanchez D, and Hollingworth RM (2008). Analysis of Global Pesticide Resistance in Arthropods (Eds: M.E. Whalon, D. Mota-Sanchez & R.M. Hollingworth, Global pesticide resistance in arthropods). MRM Graphics Ltd, Winslow, UK. 5-32.

Yankova V, Masheva S, Boev B, and Toskov K (2011). Toxicity of plant protection products towards the imago of *Encarsia formosa* Gah. Agricultural Science and Technology, 3(4): 374 – 377.

Zanuncio JC, Mourão, SA, Martínez, LC, Wilcken CF, Ramalho FS, Plata-Rueda, A, Soares MA, and Serrão JE (2016). Toxic effects of the neem oil (*Azadirachta indica*) formulation on the stink bug predator, *Podisus nigrispinus* (Heteroptera: Pentatomidae). Scientific reports.