

SIDE EFFECTS OF DIFFERENT DOSES OF AZADIRACHTIN ON PREDATORY MITE *METASEIULUS OCCIDENTALIS* (NESBITT) (ACARI: PHYTOSEIIDAE) UNDER LABORATORY CONDITIONS

YANAR, D.

*Department of Plant Protection, Faculty of Agriculture, Tokat Gaziosmanpasa University
60240 Tokat, Turkey*

(e-mail: durdane.yanar@gop.edu.tr, durdane.yanar@gmail.com; phone: +90-356-252-1616/2185; fax: +90-356-252-1488)

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Abstract. *Metaseiulus* (= *Galendromus* or *Typhlodromus*) *occidentalis* (Nesbitt) (Acari: Phytoseiidae) is an important and effective predator of pest mites in agricultural crops and is commercially available in the United States. Azadirachtin is a biorational pesticide, which contains azadirachtin A (10 g/l) as the basic active compound. The active compound has several properties useful for insect control (repellency, feeding and oviposition deterrence) and is considered safe for the environment. With the use of natural compound like azadirachtin A in integrated pest management programs, it is important to know whether the compounds have any side effects on predatory mite such as *M. occidentalis*. Therefore, the current study aimed to examine the toxic effect of different doses (0.1 ml (NZ-1*2), 0.05 ml (NZ-1), 0.025 ml (NZ-2), 0.01 ml (NZ-3), 0.005 ml (NZ-4) and 0.001 (NZ-5)/ 10 ml water) of NeemAzal-T/S (a.i. 10 g/l azadirachtin A) on *M. occidentalis*. The mortality rate of *M. occidentalis* was 77.5% 24 h after application of NeemAzal-T/S at 0.1 ml/10 ml water dose. The mortality caused by the recommended dose (0.05 ml/10 ml water) of NeemAzal-T/S was 62.31%, 70% and 73.07%, 24, 48 and 72 h after application, respectively. It is concluded that NeemAzal-T/S is moderately toxic against the predatory mite *M. occidentalis*. Therefore, the use of NeemAzal-T/S to control the pest mites in agricultural crops might affect the populations of predatory mites.

Keywords: *azadirachtin, spider mite, Metaseiulus occidentalis, side effect, pesticide*

Introduction

Plant protection strategies, pest management in particular, have focused on minimizing the use of pesticides in the recent years. The use of organic pesticides, i.e., derived from plants or living organisms has gained attention in this regard. However, the evaluation of the side effects of organic pesticides is required to know whether or not these pesticides are fatal to the natural enemies.

Agricultural crops are infested by several pests, some of them may be controlled by using pathogens, predators, and parasitoids in addition to pesticides. However, selective pesticides are required to achieve a satisfactory control of complexes of pests (Cloyd et al., 2006) when using living organisms for pest control. Most of the biological control agents, including predators and parasitoids can provide excellent management of many pests. There is great potential for reducing pesticide use in pest management through exploiting natural enemies of the pests (Kavousi and Talebi, 2003) as alternatives to pesticides. Alternative pest management approach includes the use of predator mites along with pesticides instead of using pesticides or biocontrol agent alone (Sato et al., 2000).

It is very important to determine the positive/negative impacts of selective insecticides/acaricides that could be used in combination with biological control agents (Martin and Woodcock, 1983) for pest management. Phytoseiidae mites are one of the most important biological control agents effective on different kinds of pests on various

greenhouse plants and orchards (Schmutterer, 1990). *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae) is one of these mites. It is a predatory mite species, specialized in the phytophagous red spider mite species (Tetranychidae) in North America (Hoy et al., 1982; Hoy, 2011). This predatory mite species has been adapted to semi-arid conditions, can produce large populations in low relative humidity environments, thus suppresses spider mites (Mc Murtry, 1982; Mc Murtry and Croft, 1997). The length of time taken from egg to adult is approximately 8.5 days. The average eggs laying capacity of adult female is 2.2 eggs/day and capable of laying 34 eggs during the entire life cycle, longevity of *M. occidentalis* females is approximately 20 days (Laing, 1969). The length of time from egg to adult varies greatly with temperature and as the temperature increases, the development time is shortened with increasing temperature. While the length of time from egg to adult is 7-10 days at 24 °C, it become 8.7, 7.4 and 5.2 days at 27 °C, 29 °C, and 32 °C respectively (Tanigoshi et al., 1975).

Phytoseiulus persimilis and *Neoseiulus californicus* are commercially available and actively used in the biological control of two-spotted spiders. *Metaseiulus occidentalis* is a predator mite belonging to the Type II group and is feed with Tetranychidae mite species and rust mites (McMurtry and Rodrigues, 1987).

One of the botanical pesticides is Azadirachtin (10 g/L). Azadirachtin (10 g/L) is the commercially available plant originated pesticide; It has been reported that it is effective on more than 200 plant pests, such as aphids, White flies, leafminers, Thrips, Colorado potato beetle, woolly Apple aphid, scale insects, mealy bugs, leafhopper, Noctuids, Coleopters, Yponometua species, American White fly, Pine processionary caterpillar, Oriental fruit moth, Olive fruit fly, Cherry fruit fly, leaf tortrix moths, and spider mites. It is also widely used in organic farming (Schmutterer et al., 1981; Isman, 2006). The active substance diffuses into the leaves and is partly systemically dispersed in the plant; insects take this active substance by mouth (by sucking or chewing) during feeding. Azadirachtin inhibits the feeding and molting of the exposed insects. At the same time, it decreases the fecundity of insect and may causes infertility (Isman, 2006).

This study was aimed at evaluation of different doses of Azadirachtin on *Metaseiulus occidentalis*.

Materials and methods

Tetranychus urticae

The adults, nymphs and especially eggs of *Tetranychus urticae* are needed to feed the predator mites. Therefore *T. urticae* was reared on green bean plants, *Phaseolus vulgaris*. The bean plants used in the experiment were grown at 25 ± °C and 16:8 h L:D photoperiod in the growth chamber (Fig. 1).

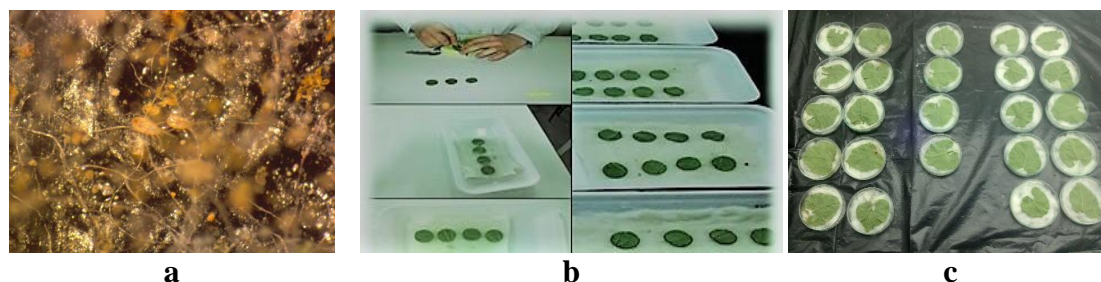


Figure 1. *a* Predatory mite *Metaseiulus occidentalis* culture. *b, c* View from experiments

Neem formulation

Neem Azal is a commercial formulation of neem seed kernel extract containing 10 g/L Azadirachtin.

Effect of azadirachtin on *Metaseiulus occidentalis*

The predatory mites *Metaseiulus occidentalis* were obtained from Emeritus Prof. Dr. Marjorie Hoy, University of Florida, USA and fed with *T. urticae*. *M. occidentalis* is nonnative to Turkey; therefore, necessary permission was obtained from Turkish Agricultural Ministry to work with this predatory mite species. The experiments were carried in Petri dishes (90 mm in diameter) by placing ten adult females of *M. occidentalis* per Petri dish. Dry film method was used to determine the toxicity of azadirachtin on *M. occidentalis* (Birah et al., 2008). Inner sides of Petri dishes were coated with 60 µl solution of six different doses (0.1, 0.05, 0.025, 0.01, 0.005, 0.001 ml/10 ml of water) of azadirachtin (10 g/l) (Table 1). The solution was spread uniformly and allowed to dry at room temperature for 30 min. Ten adult females of *M. occidentalis* were released onto the film in each Petri dish and the dishes were wrapped with parafilm to prevent evaporation. Thereafter; the predatory mite was exposed to the solution for a period of 24 h. Thereafter the adult females were transferred to bean leaf disk (2 cm in diameter) which was placed inside a petri dish on moist cotton. The experiments were carried out at 25 ± 2 °C and 16:8 h L:D photoperiod and $45 \pm 5\%$ humidity in the growth chamber. Each treatment was replicated 5 times. The experiments were repeated two times. The number of living and dead *M. occidentalis* adults was monitored 24, 48, 72, 96, 120 and 144 h after exposure to azadirachtin. The number of eggs laid by each female after 24 h period was recorded to investigate the effects of tested doses of azadirachtin on eggs laying capacity of the female.

Table 1. Different doses of azadirachtin used in the study

Treatments doses (ml per 10 ml water)	
Control (C)	Distilled water
NZ - 1*2	0.1
NZ - 1	0.05
NZ - 1/2	0.025
NZ - 3	0.01
NZ - 4	0.005
NZ - 5	0.001

The results were expressed as percent mortality with correction for untreated mortality using Abbott's formula (Abbott, 1925). The data were subjected to ANOVA tests and the means were compared by Tukey test, using SPSS 17.0 software program (SPSS, 2008).

Results and discussion

One of the practical and environmentally safe biological pesticides are plant-derived products, due to their bio-degradability and target-specific nature (Birch, 2011). The

combined effect of both botanical pesticides and predators or parasitoids as biological control agents is expected to have greater effects on target insects than both approaches opted alone. The predatory mites can be combined with safe doses of botanical pesticides, which have minimum negative impacts on natural enemies and provide enough control over target pests. Therefore, the knowledge of safe dose of a botanical pesticide is required to maintain the pest population below an economic threshold level without effecting the population of predatory mite. Toxicities of different doses of azadirachtin (10 g/l) on predatory mite *M. occidentalis* were evaluated in the current study and results are summarized in *Table 2*.

Table 2. Mean mortality rates (% ± SE) of predatory mite (*Metaseiulus occidentalis*) 24-144 h after exposure to azadirachtin (10 g/l)

Doses	Mean mortality % ± SE					
	24 h	48 h	72 h	96 h	120 h	144 h
C	6 ± 2.45a*	6 ± 2.45a	10 ± 3.16a	17.78 ± 3.64a	23.33 ± 3.72a	28.89 ± 5.12a
NZ -1*2	77.5 ± 4.53e	82.50 ± 5.26e	86.25 ± 4.60e	82.5 ± 4.79d	85 ± 2.89d	92.50 ± 2.50d
NZ - 1	62.31 ± 3.23d	70 ± 2.53de	73.08 ± 3.47de	77.78 ± 6.19cd	80 ± 6.67cd	84.44 ± 6.26cd
NZ - 2	48.46 ± 2.49c	58.46 ± 3.37cd	62.30 ± 3.78cd	70 ± 5.27cd	77.78 ± 6.40cd	84.44 ± 6.48cd
NZ - 3	46.92 ± 2.37c	53.08 ± 3.08cd	61.54 ± 3.37cd	61.11 ± 4.55cd	71.11 ± 6.55cd	76.67 ± 7.26cd
NZ - 4	40.83 ± 1.48bc	42.5 ± 1.79bc	49.17 ± 3.12bc	53.75 ± 4.98bc	53.75 ± 4.98bc	61.25 ± 7.18bc
NZ - 5	32.14 ± 3.17b	35.71 ± 3.43b	36.43 ± 3.25b	35 ± 2.89ab	37.50 ± 2.50ab	40 ± 4.08ab

*Means followed by the same letter in each column are not significantly different (P < 0.05)

Females of *M. occidentalis* were more sensitive to double (0.1 ml/10 ml water) and recommended (0.05 ml/10 ml water) doses of azadirachtin than the other doses tested in the study. The female mortality was decreased with decreasing dose. At double dose, the mortality rates were 77.5, 82.5 and 86.25% after 24, 48, and 72 h of exposure, respectively. Similarly, recommended dose of azadirachtin caused a significant reduction in the viability of *M. occidentalis* 24 to 72 h after exposure, highlighting the negative effect on predatory mite. Mortality of *M. occidentalis* ranged from 62.31 to 73.8% at 0.05 ml/10 ml water concentration 24 to 72 h after exposure (*Tables 2 and 3*). These results are consistent with the findings of Mourao et al. (2004) who reported that neem cake caused 88% mortality of *Iphiseiodes zuluagai* Denmark and Muma (Acari: Phytoseiidae) adults. Several studies showed the compatibility of neem-based products with predatory mites, e.g., azadirachtin at 4.5% concentration was found compatible with *Phytoseiulus persimilis* (Bernardi et al., 2013; Duso et al., 2008; Cote et al., 2002). The effects of neem-derived compounds can vary, depending on the predatory mite species, formulation of pesticide or the plant part used.

Our study indicates that mean longevity of alive adult females of *M. occidentalis* was also decreased with increasing dose of azadirachtin. While the longevity of female adults was 7.55 days in control treatment and with the same category at 0.01, 0.005 and 0.001 ml/10 ml water concentrations statistically, at 0.01, 0.05 and 0.025 ml/10 ml concentrations it was 3.42, 3.62, 5.62 days respectively (F = 114.49; df = 6; P < 0.001) (*Table 4*).

Table 3. Anova tables of mean mortality rates (%±SE) of predatory mite (*Metaseiulus occidentalis*) 24-144 h after exposure to azadirachtin (10 g/l)

Between groups					
	Sum of squares	df	Mean square	F	Sig.
24 h mortality	22459.029	6	3743.172	38.661	.000
48 h mortality	27587.784	6	4597.964	38.479	.000
72 h mortality	28517.353	6	4752.892	32.208	.000
96 h mortality	24014.423	6	4002.404	20.096	.000
120 h mortality	24398.825	6	4066.471	15.499	.000
144 h mortality	25171.474	6	4195.246	12.846	.000

Table 4. Effects of azadirachtin on mean longevity of alive adult females of *Metaseiulus occidentalis*

Doses	Mean adult female longevity (day) mean ± SE
C	7.55 ± 0.10c*
NZ - 1*2	3.42 ± 0.19a
NZ - 1	3.62 ± 0.16a
NZ - 2	5.22 ± 0.13b
NZ - 3	7.46 ± 0.11c
NZ - 4	7.50 ± 0.22c
NZ - 5	7.31 ± 0.24c

*Means followed by the same letter in each column are not significantly different (P < 0.05)

Fecundity of female adults of the mite was significantly reduced as the total number of eggs laid per female were decreased at 0.1 and 0.05 ml/10 ml water concentrations, which were significantly lower than the control treatment (Table 5) (F = 45.25; df = 6; P < 0.001). These results are consistent with the findings of Irigaray and Zalom (2006), who reported significant influence of acequinocyl on fecundity of *M. occidentalis* females. Additionally, there was significant difference in the mean number of eggs deposited per mite/per day. The female exposed to double (0.1 ml/10 ml water) and recommended (0.05 ml/10 ml water) doses of azadirachtin deposited significantly lower number of eggs than the females in control treatments (p ≤ 0.05) (0.29, 0.61 and 1.30/per day/per female, respectively) (F = 26.24; df = 6; P < 0.001) (Table 6).

Table 5. The effects of Azadirachtin on fecundity (total number of eggs laid/per female) of *Metaseiulus occidentalis*

Doses	Mean egg number/adult female mean ± SE
C	10.15 ± 0.48d*
NZ - 1*2	1.08 ± 0.83a
NZ - 1	2.28 ± 0.21ab
NZ - 2	4.00 ± 0.28b
NZ - 3	8.46 ± 0.51cd
NZ - 4	6.92 ± 0.55c
NZ - 5	7.26 ± 0.63c

*Means followed by the same letter in each column are not significantly different (P < 0.05)

Table 6. Effects of Azadirachtin on fecundity (average number of eggs laid/per day/per female) of *Metaseiulus occidentalis*

Doses	Mean daily egg number/adult female mean \pm SE
C	1.30 \pm 0.05e*
NZ - 1*2	0.29 \pm 0.03a
NZ - 1	0.61 \pm 0.05b
NZ - 2	0.77 \pm 0.05bc
NZ - 3	1.08 \pm 0.06de
NZ - 4	0.88 \pm 0.06cd
NZ - 5	0.94 \pm 0.07cd

*Means followed by the same letter in each column are not significantly different ($P < 0.05$)

Hatched larvae to reaching the adulthood stage of predatory mite ranged between 66.67 and 100%, but at 0.001 ml/10 ml concentration, there was no larval hatching (Table 7). Yanar and Hoy (2017), found that surviving females held for 2 and 4 weeks cold storage laid eggs at the same rate as control females of *M. occidentalis*. Nicoli and Galazzi (1998) found that survival of predatory mites during storage decreased but the surviving females show greater longevity, fertility and fecundity.

Table 7. Effects of azadirachtin on larval hatching and reaching adulthood from larva of *Metaseiulus occidentalis*

Doses	Emerging larvae %	Larvae which can reach adulthood %
C	38.89	95.24
NZ - 1	40.00	87.50
NZ - 2	40.91	66.67
NZ - 3	50.00	100.00
NZ - 4	42.86	83.33

Conclusion

The data obtained from this study will be useful for commercial producers and pest management specialists wishing to use these predatory mites in integrated pest management programs. Considering the results of present study, it can be concluded that recommended and half doses of azadirachtin (10 g/L) should not be used in IPM programs where *M. occidentalis* is used to control pest mites. Lower concentrations (0.01 and 0.005 ml/10 ml water) can be used in combination with *M. occidentalis*, reducing the selection pressure and development of resistance (Marcic, 2007). Herron et al. (1993) and Cheon et al. (2007) suggested that acaricides at reduced rates might be used to adjust the predator/prey ratio.

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