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ORIGINAL ARTICLE

Bioactivity of azadirachtin against *Scrobipalpa ocellatella* Boyd. (Lepidoptera: Gelechidae) on sugar beet

Somaye Allahvaisi[1]*, Mahdi Hassani[2], Bahram Heidari[3]

[1] Plant Protection Research Department, Hamedan Agriculture and Natural Resources Research and Education Center, AREEO, Hamedan, Iran

[2] Sugar Beet Research Department, Hamedan Agriculture and Natural Resources Research and Education Center, AREEO, Hamedan, Iran

[3] Department of Plant Production and Genetics, School of Agriculture, ShirazUniversity, Shiraz, Iran

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*Corresponding address:
allahvaisis@yahoo.com

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Abstract

The use of environmentally friendly bio-pesticides is crucial for higher root and sugar yield in sugar beets. The economic importance of beet moth [*Scrobipalpa ocellatella* Boyd. (Lepidoptera: Gelechidae)] losses in the field and storage highlight the need for evaluation of appropriate, environmentally friendly methods for pest control. The aims of the present study were to i) assess azadirachtin (AZN) effects on the life cycle and activity of the pest, and ii) manage the beet moth on roots under laboratory conditions. For the experiments, the main concentrations were prepared on the basis of the field-recommended dose of this pesticide (1–1.5 l/1000 l water). The LC_{50} was estimated for 3rd instar larvae. Later, at sublethal concentrations, the relative time for the emergence of each developmental stage was determined. The mean female fecundity was 37% (±4) for treated tests at the lowest AZN concentration (0.5 ml · l^{-1}). Assess azadirachtin at 0.5 ml · l^{-1} concentration resulted in 62 (±4) deposited eggs per plant for the treated roots and 326 (±1) for roots in the control test. Mortality increased in response to increased AZN concentrations. The results revealed that after 72 h, the highest AZN concentration (2.5 ml · l^{-1}) caused 100% repellency and 82% (±1.38) mortality on 3rd instar larvae. According to our findings, a concentration of 2 ml · l^{-1} AZN (20 gr active ingredient per 1 hectare) after 4 days affected 1st instar larvae and the larvae with no further development had 92.2% (±1.2) mortality. In conclusion, the results revealed that AZN as a biorational pesticide can significantly minimize the losses of *S. ocellatella* on sugar beet crops.

Keywords: azadirachtin, life cycle, management, sugar beet moth

Introduction

Sugar beet (*Beta vulgaris* L.) is an economically strategic crop and with 12–21% root sugar content it is the basic source of Iran's sugar supply. In Iran during the cropping season of 2018, this crop was grown on more than 121,849 ha with a total annual production of 8.1 million tons (Anonymous 2020). By 2017, the annual production of sugar accounted for 1,141,841 tons in Iran (Anonymous 2020). Yield losses in harvest and postharvest stages are the main concerns of sugar beet farmers (Fugate and Campbell 2009). There are several pest insects which are root damaging agents during growth stages in beet crops (Bazazo and Mashaal 2014). In recent years, the sugar beet moth [*Scrobipalpa ocellatella* Boyd. (Lepidoptera: Gelechiidae)] has become a serious threat for sugar beet production (Rashidov and Khasanov 2003; Amin *et al.* 2008; Al-Keridis 2016). This specific pest is an oligophagous insect which is found in almost all sugar, fodder and wild beet growing areas in Iran where it was reported for the first time in Karaj city in 1936 (Kheiri *et al.* 1980; Kheiri 1991;

Ganji and Moharramipour 2017). The sugar beet moth has three to six generations during one vegetation season, depending on temperature variations, geographical region and sowing date (Kheiri 1991). *Scrobipalpa ocellatella* prefers warm, dry weather and initially attacks at the edges of fields (Fajt 1951). Annually, the average losses from this pest on sugar beet farms of Iran is almost greater than 10% (Anonymous 2020). Bazok (2010) reported that the economic threshold of *S. ocellatella* damage during various stages of plant phenology differs. During the phenophase of 6–8 leaves there is 0.5 caterpillar per plant, while at the beginning of the formation of root crops 0.8–1 caterpillar can be found per plant and at the beginning of the withering away of leaves there are 2 caterpillars per plant. In Iran, the pest contamination rate ranges from 20 to 25% under field conditions and can reduce root yield by 2.3 to 3.8 tons per hectare with 0.5 to 1.15% sugar loss (Razini *et al.* 2016). Then, an 1% increase in sugar content of tubers will lead to an 8% raise in the price of the sugar beet consignments in the country (Abdollahian-Noghabi *et al.* 2014).

Most *S. ocellatella* eggs are laid on the central bud and the root collar. The eggs are oval in shape and pale yellow. At the pre-hatching stage the eggs are orangish (Kheiri 1991). The feeding of the 1st and 2nd larvae instars results in curly, discolored black leaves. Older larvae of later ages feed on terminal petioles and the central shoot which is the insect's main habitat. Damaged shoots help the larvae to penetrate through the roots' apexes. All these changes interfere with root morphology, sugar content and sugar extraction. Furthermore, the holes created by this insect allow for penetration of pathogenic infestations (Kheiri 1991; Al-Keridis 2016). This larvae habitat can provide appropriate conditions for storage pathogens to enter, such as *Penicillium claviforme* Bain., *Phoma betae* Fr. and *Botrytis cinerea* Pers. leading to substantial postharvest losses (Fugate and Campbell 2009). With increasing *S. ocellatella* damages in the field, the control of this pest particularly during preharvest is a major priority of growers for reducing losses. Various approaches have been used for sugar beet moth control in Iran. The main cultural methods have used resistance varieties and chemicals with organophosphates (Razini *et al.* 2017). The largest population densities of *S. ocellatella* larvae can be observed at harvest time (Ganji and Moharramipour 2017). The application of green food technology and green control technology assists reducing the adverse effects of intensive agriculture which is associated with heavy use of synthetic insecticides that threatens vital ecosystems and food safety (Mordue *et al.* 2005; Amoabeng *et al.* 2019).

In recent years, there has been an increasing use of eco-friendly natural insecticides which are of plant origin, known as botanical and biological pesticides. These pesticides are inexpensive, easily degradable and target-specific with low toxicity on vertebrata (Dreistadt 2004; Lopez *et al.* 2005; Senthil-Nathan 2013; Sharma *et al.* 2019). Azadirachtin (AZN) belonging to NeemAzaln components, disrupts insect biological fitness and lifespan (Wilps 1989; Lucantoni *et al.* 2006). It also affects juvenile hormone titers and 20-hydroxyecdysone in the treated insects (Mordue *et al.* 2005; Abedi *et al.* 2014). It has been shown that AZN has cytotoxic effects (Radhika *et al.* 2018), is an oviposition deterrent (Dhar *et al.* 1996; Liu and Liu 2006), causes antifeeding or low absorption of nutrients (Wilps 1989; Tome *et al.* 2013; Qiao *et al.* 2014; Bezzar-Bendjazia *et al.* 2017; Qin *et al.* 2019) and reproduction (Garcia *et al.* 2006; Pineda *et al.* 2009; Ismadji *et al.* 2012). Reports reveal the effects of this compound on the sexual behavior of insects in response to mating pheromones or spermatogenesis in males (Dorn *et al.* 1987; Shimizu 1988). AZN causes high mortality in insects and reduces the larval population (Dorn *et al.* 1987; Raman *et al.* 2000).

In recent years, several studies have demonstrated the suppressive effects of AZN on 250 species of insect pests, especially Lepidoptera (Martinez and Emden 2001; Liang *et al.* 2003; Seljasen and Meadow 2006; Pineda *et al.* 2009; Ünsal and Güner 2016; Boadu *et al.* 2011; Darabian and Yarahmadi 2017; Zhong *et al.* 2017; Heibatian *et al.* 2018; Qin *et al.* 2019; Betz and Andrew 2020). The objectives of the present study were to i) determine an efficient AZN concentration for pest management by dose dependent AZN toxicity and ii) assess the life cycle of *S. ocellatella* on sugar beet plants exposed to sublethal concentrations of AZN.

Materials and Methods

Moth rearing and sampling on sugar beet plants

Scrobipalpa ocellatella larvae were carefully collected from sugar beet root heads (Shokoofa cultivar) during September and October 2020 in Hamedan, Iran (52°34'N 48°32'E, 1730 m above sea level) and reared under laboratory conditions at 26°C (±2), 70% (±5) relative humidity (RH) and 16 light : 8 dark photoperiod. Before the start of the experiments, we planted sugar beet plants from the same cultivar (Shokoofa) in 85 pots covered with non-woven cotton covers in a greenhouse. Eight weeks after emergence of the first leaves, the roots with four leaves were used for the tests. Some of the emerged adult moths, eggs and larvae were selected for tests.

Preparation of AZN concentrations

No concentration of AZN is registered against sugar beet moth in Iran. According to reports of the Plant Protection Organization of the Ministry of Jihad Agriculture in Iran, this pesticide is registered for *Liriomyza sativae* Blanch., *Trialeurodes vaporariorum* Westw., some Lepidopteran larvae and many insects in the Homopteran order. Therefore, concentrations were prepared on the basis of the higher amount of the field-recommended concentration for other crop pests of this pesticide (0.5–2 l · 1 ha^{-1}). A control treatment of no AZN and concentrations of 0.2, 0.5, 1, 1.5, 2, 2.5 and 3 (ml · 1 l^{-1} water), i.e., concentrations equivalent to 2, 5, 15, 20, 25 and 30 gr active ingredient per hectare, respectively, were used, followed by Azadirachtin-A (EC 1%)-T/S (Trifolio-M GmbH/Germany). AZN was diluted with water and then 1 ml of Tween 80 (CRODA, Singapore) was added as an emulsifier.

Effect of AZN on developmental stages

The insects throughout different developmental stages used for bioassay tests were kept at 26°C (±2) and photoperiod of 16 h light : 8 h dark. A selected plant was immersed into AZN for 30 s as treatment and another plant ws dipped into deionized water as the control. Treated plants were immersed into two sublethal concentrations (0.5 and 1 ml · l^{-1}) of AZN with five replicates. After immersion, the excess moisture of the plants was removed by placing them on paper towels for 15 min. Aluminum netting supported (0.2 mm mesh) cages (77 × 70 × 68 cm) were used. Each cage was designed for one plant. After 24 h, the adult moths which emerged (3 days old) were released onto healthy sugar beet plants with two primary leaves in the cages as an oviposition site at 26°C (±2), 70% (±5) RH and 16 light : 8 dark photoperiod. To stimulate natural conditions in a field, four female moths (3 days old) laying eggs for 24 h and four males (3 days old) were carefully released into each cage (Seljasen and Meadow 2006)

After 72 h, the adult moths were removed from the cages and the number of eggs laid on the surface of leaves and root heads was counted. The data showed the fecundity percentage. Within 5 days, newly hatched neonate larvae were counted daily. This method was performed on subsequent biological stages of *S. ocellatella* on control and treated plants for determining the developmental time.

Bioassay for the repellency effect of AZN on female oviposition preference

The inhibition effect of AZN on oviposition and feeding was tested in the treated plants. One treated and one untreated plant were selected as in the method mentioned in the previous tes. In this test, two treated plants and two control plants were placed in one cage (95 × 95 × 95 cm). Then, four female moths (3 days old) that had been ovipositing for 24 h were released onto the plants in the cages designed for determining repellency of AZN at concentrations of 0.5, 1 and 2 ml · l^{-1}. To give sufficient time of for laying eggs, after 48 h, the plants were removed and the eggs which had been laid on each plant, were counted. Five replicates were used for this experiment. The percentage repellency (*PR*) values were computed according to Schreck's (1977) equation (Eq. 1).

$$PR = \left(C - \frac{T}{C}\right) \times 100, \quad (1)$$

where: *C* and *T* stand for the number of eggs in the control and treated plants, respectively.

Effects of AZN on egg hatching, larva feeding and molting ability

Three plants carrying 50 eggs for each AZN concentration (0.5, 1 and 2 ml · l^{-1}) and the control were tested for the effects of AZN. The pre-eclosion eggs were 4 days old. The number of eggs hatched after 2 days, larval development and plant damage were recorded 1 week after hatching. Thus, the number of holes on roots showing moth contamination was evaluated in roots both with and without AZN. The hatching rate (%) was computed as the number of hatched eggs with respect to the total number of eggs (Godinho 2007). Subsequently, the percentage of eggs hatching-inhibition (*PIHE*) was calculated by using equation 2.

$$PIHE = \left(1 - \frac{N_t}{N_c}\right) \times 100, \quad (2)$$

where: N_t and N_c denote the number of hatched eggs on the treated and control roots, respectively.

Effect of AZN concentrations on mortality of third instar larvae

A preliminary dose setting experiment was performed to evaluate the LC_{50} of AZN toxicity and concentrations resulting in 20 to 80% mortality. No live larva were observed in 3 ml · l^{-1} AZN. Therefore, 0, 0.2, 0.5, 1.5, 2 and 2.5 ml^{-1} AZN concentrations were used for dose-response tests. For this experiment, the plants were dipped in the AZN solution for 1 min as treatment. For the control test, the plants were immersed in deionized water. After 3 h, each plant was transferred into a cage and the insects were placed on them. Five replicates were used for each treatment. A brush was used to transmit 25 larvae of 3rd instar (3 days old)

to each plant. The number of deaths was counted after 72 h. The probit model was used to estimate the LC_{50} values (Finney 1971).

Statistical analyses

Analysis of variance (ANOVA) was performed using the completely randomized design (CRD) model. The arcsine square root was used to transform the mortality data which are expressed in percentages using IBM SPSS Statistics 22.0 (SPSS, USA) (Osborne 2010). The data were analyzed by one-way analysis of variance and Tukey's post hoc tests ($p \leq 0.01$) to compare treatments. The LC_{50} and LC_{95} values were subjected to the probit model (Fong *et al.* 2016). Standard error (± standard error) of the means was computed for the traits tested.

Results

Effect of AZN on developmental stages

In the treatments with AZN, the duration of larval instars, pupa and the total developmental time varied (Fig. 1). In this experiment, AZN had no significant impact on adult longevity. The range for deposited eggs per untreated root was 48–85 (±5) and per treated root was significantly less, 31–44 (±3) and 18–29 (±3), respectively, in 0.5 and 1 ml · l^{-1}. In the control tests, mean female fecundity was 78% (±12). This value decreased to 37% (±4) in treated tests at the lowest concentration (0.5 ml · l^{-1}) and 25% (±2) at a concentration of 1 ml · l^{-1}. Here, the number of adult males was nearly twice the number of females. Twisted or defective wings were found in a few adults, especially in females.

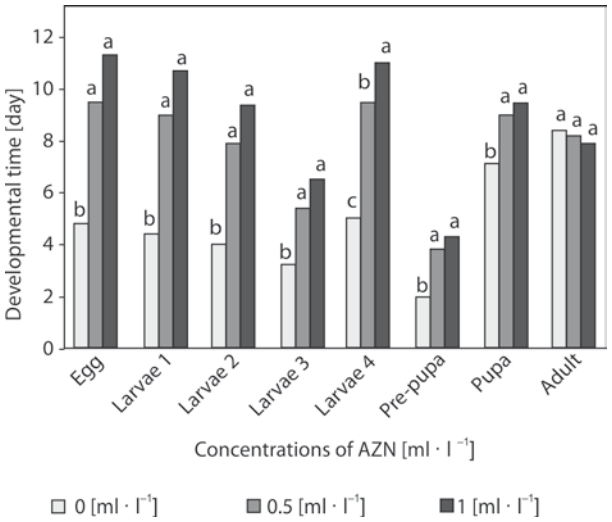

Fig. 1. Effect of azadirachtin (AZN) concentrations applied to different stages of *Scrobipalpa ocellatella* on the duration of development ($p \leq 0.01$)

Bioassay for repellency effect of AZN on female oviposition preference

The mean percentage of eggs laid by each female in the AZN-treated plants at the lowest concentration (0.5 ml · l^{-1}) was 64.6% (±2.7) which was significantly lower than 97.2% (±1.8) as seen in the control. The mean number of deposited eggs per plant was 62 (±4) for the treated roots at the 0.5 ml · l^{-1} concentration and 326 (±1) on control plants. There was no significant difference between the mean RP (%) at 1 and 2 ml · l^{-1} concentrations of AZN. Oviposition preference of *S. ocellatella* females to plants treated with lower concentrations of AZN was significantly more. There was 80.2, 95 and 100%, respectively, repellency caused by three concentrations, 0.5, 1 and 2 ml · l^{-1} of AZN.

Effects of AZN on egg hatching and larva feeding and molting ability

The egg hatching rates were significantly different between the AZN-treated and control roots by Tukey's post hoc tests ($p \leq 0.01$) (Table 1). The percentage of hatching eggs exposed to AZN was significantly lower than the control. The means for inhibition hatching eggs (IHE) (%) were significantly different for the various concentrations (Fig. 2). Egg hatching percentage in control samples was 96.5% (±1.0). After 4 days, 2 ml · l^{-1} AZN concentration affected 1st instar larvae and the larvae without any development had 92.2% (±1.2) mortality. Although the 2nd instar larvae survived in the low AZN concentration, they showed delayed molting and development. Consequently, after 6 days all the survived larvae were not able to molt into 2nd instar. The numbers of holes created by 1st larvae on treated roots were significantly less than on the control roots (Fig. 2).

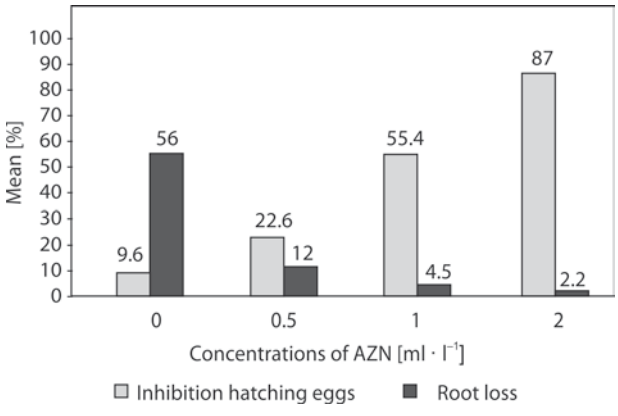

Fig. 2. Effect of azadirachtin (AZN) concentrations on hatching rate and root damage created by 1st instar larvae of *Scrobipalpa ocellatella*

Table 1. Analysis of variance (ANOVA) and mean comparison for Inhibition Hatching Eggs (%) of *Scrobipalpa ocellellata* in response to AZN treatment

Source	*df*	Mean square	*F*
AZN concentration	2	5249.1	315.78*
Error	12	16.6	
Tukey tests of the number of hatched eggs at various concentrations of AZN			
Concentration [ml · l^{-1}]	*N*	(Mean ± SE)	
		treated plant	non-treated
0.5	50	35.2 ± 2.00 a	45 ± 0.52 a
1	50	20.4 ± 1.35 b	46.8 ± 0.32 a
2	50	5.6 ± 0.71 c	43.8 ± 0.32 a

*significant ($p \leq 0.01$), N – number of total eggs at various concentrations
Lowercase letters indicate the significant differences between concentrations ($p \leq 0.01$) (Tukey test)

Effects of AZN concentrations on mortality of third instar larvae

Mortality increased in response to increases in AZN concentration (Fig. 3). The average mortality percentage at the highest concentration (2.5 ml · l^{-1}) was 82% (±1.38) which significantly differed from those in other concentrations. The results of probit analysis showed 1.2 (0.85–1.45) ml · l^{-1} and 5.74 (3.7–12.6) ml · l^{-1} fiducially for LC_{50} and LC_{95}, respectively (Fig. 3). A color change (as dark spots) was also observed on the cuticle of the survived treated larvae. A totally blacked cuticle of larvae was observed at higher AZN concentrations.

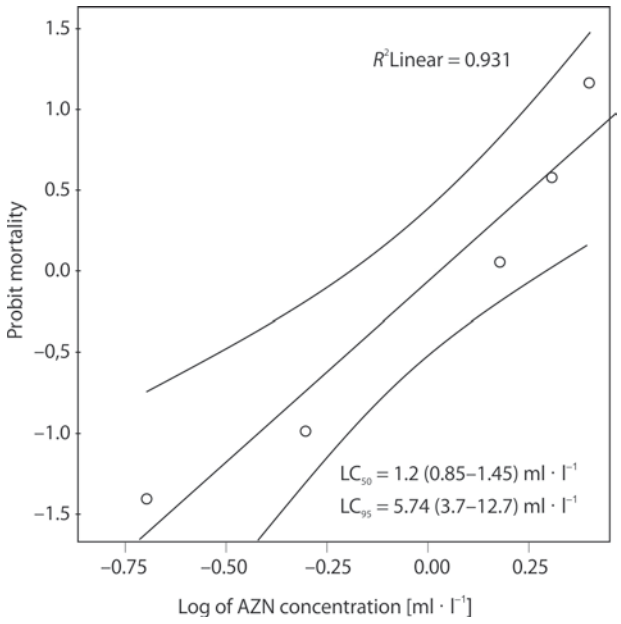

Fig. 3. Probit analysis of mortality on 3rd instar larvae of *Scrobipalpa ocellatella* for different concentrations of azadirachtin (AZN) ($\chi^2 = 3.46$; $p \leq 0.01$)

Discussion

Despite the control methods for lowering the population density of *S. ocellatella*, storage contamination continues to cause economic losses (Razini *et al*. 2017; Bazok *et al*. 2018). Obviously, sugar beet roots are targets for egg laying by *S. ocellatella* adult females. Thus, there is a need to develop an efficient method for *S. ocellatella* control to help reduce the adverse effects, including yield losses caused by this pest in sugar beet.

The use of plant-based insecticides including AZN has received a great deal of attention due to their bioefficacy and biodegradability in the environment (Butterworth and Morgan 1968; Zhong *et al*. 2017). Several other AZN effects include reduced feeding, delays in the development of larvae and nymphs, permanent larvae, incomplete ecdysis, malformed pupae and adults, sterile eggs and reduced fecundity (Morgan 2009). The effects of AZN on sugar beet moth have not been reported and the results of this current study provide additional insight into the action of this pesticide for controlling *S. ocellatella* in IPM programs. By determining the efficacy of this pesticide on sugar beet moth, we have presented the first report for control of this pest by a biopesticide in Iran.

The first instar larvae stage is a critical time for pest control because it penetrates into roots. The larvae at this stage bore tunnels into mid-ribs and enter the roots. In this study, the female adults exposed to AZN with short adult longevity had less fecundity. The results of this study suggested that AZN at sublethal concentrations significantly prolongs the developmental time of *S. ocellatella*. However, there was no significant difference in adult longevity in the moths exposed to AZN-treated roots compared to the control. The concentration of 0.5 ml · l^{-1} was not significantly different

than 1 ml · l^{-1} on the developmental time of *S. ocellatella*. Alouani *et al*. (2009) revealed that *Culex pipiens* s.s. mosquito exposed to azadirachtin extracts have different developmental times than the control. AZN impairs larval development in Lepidoptera and reduces pupation, resulting in delays in molting to the next instar larvae or their mortality (Sieber and Rembold 1983; Schluter *et al*. 1985; Smith and Mitchell 1988; Jagannadh and Nair 1992; Liang *et al*. 2003; Bruce *et al*. 2004; Seljasen and Meadow 2006; Pineda *et al*. 2009; Brunherotto *et al*. 2010; Tome *et al*. 2013; Zhong *et al*. 2017). In a study on the effect of neem on fruit fly (*Bactrocera tryoni* Frog.) a decreased insect population was found that could have been due to non-lethal carryover effects in the offspring of treated adults (Wallace 2017). In another study, AZN delayed development of *Diadegma sp.* as a natural enemy of *Plutella xylostella* Lin. was demonstrated (Zada *et al.* 2018). In addition, in Vilca Malqui *et al*. (2014) study, sublethal concentrations of AZN had hormetic effects on the esterases, juvenile hormone, fecundity and other parameters of Zabrotes subfasciatus. Some botanical compounds such as AZN have the ability to affect the biological parameters and cellular immunity of insects including *Galleria mellonella* L. (Er *et al*. 2017). They are structurally similar to the insect ecdysone hormones that control the process of metamorphosis as the insects pass from larva to pupa and to adult. AZN treatment leads to morphological variations which are similar to the effects of ecdysteroid titers (Jagannadh and Nair 1992; Garcia *et al*. 2006; Gnanamani and Dhanasekaran 2013; Sami *et al*. 2016; Er *et al*. 2017; Zhong *et al*. 2017; Al-Rahimy *et al*. 2019).

The fecundity and fertility in adults exposed to treated sugar beets significantly decreased. The mean percentage of eggs per female on the AZN-treated plants at 0.5 ml · l^{-1} concentration was ≈50% less than the control which demonstrated the repellency effects of this biopesticide on beet moths. One reason for the significant reduction in eggs in AZN-treated roots even at lower concentrations could be the choice of host-plant. Results of the Ikeura *et al*. (2013) study showed the repellency effect of AZN on *Mamestra brassicae* L. In another study, the repellency and feeding deterrent effects of AZN were observed in *Agrotis segetum* Schiff. (Mochiah *et al*. 2011). Zada *et al*. (2018) found that insecticidal and repellent activities of plant parts against *P. xylostella* increased in response to increased doses and exposure times of insecticides. AZN affects tomato secondary metabolites which are attractive for leafminers (Hasan and Ansari 2011; Tome *et al.* 2013). Evaluation of the egg-laying and feeding avoidance on AZN-treated plants has been suggested for consideration for downstream experiments on the larvae. *Drosophila melanogaster* Meig. larvae exposed to AZN have shown changes in adult oviposition preference (Bezzar-Bendjazia *et al*. 2016). In several studies, the behavior of insects with respect to egg laying on plants under two outdoor and laboratory conditions differed and more eggs were observed under outdoor than caged conditions for AZN treated plants (Seljasen and Meadow 2006; Pineda *et al*. 2009; Hasan and Ansari 2011). It has been shown that AZN affects egg-laying in the tomato leafminer (Brunherotto *et al*. 2010; Tome *et al*. 2013), *M. brassicae* (Seljasen and Meadow 2006) and oviposition by the noctuid moth *Spodoptera litura* Fab. (Naumann and Isman 1995). These results raise the possibility that females avoid egg laying on host plants that have been sprayed with insecticides including which helps to identify a secure site for the next offspring. Results of several studies have shown that contact or stomach toxicity of AZN in Lepidopteran adults reduce fecundity and fertility parameters (Schmutterer 1990; Ascher 1993; Mordue and Blackwell 1993; Mordue 2004; Seljasen and Meadow 2006; Irigaray *et al*. 2010). The declining fecundity and ovipositing in *S. ocellatella* exposed to AZN may depend on the impairment of enzyme activities which Manna *et al*. (2020) demonstrated in sex and age of Acrididae grasshoppers exposed to AZN. In other research, AZN interfered with insect reproduction by altering vitellogenin synthesis and the production of healthy and defective eggs leading to failed oocyte growth and maturation (Feder *et al*. 1988; Tanzubil and McCaffery 1990; Er *et al*. 2017). Shu *et al*. (2018) demonstrated that AZN affected the digestion and absorption of nutrients which can lead to growth inhibition in *S. litura* larvae.

The numbers of hatched larvae on plants treated with AZN and control varied. The results of the present study indicated that the percentage of eggs of beet moth which developed to new offspring in AZN-treated plants was significantly less than the control. Therefore, AZN could cause lower survival of neonate larvae hatched from eggs. Ma *et al*. (2000) reported that neem compounds reduced egg hatching and survival off larvae of *Helicoverpa armigera* Hüb. Damage on the AZN-treated roots at 0.5 ml · l^{-1} concentration was significantly lower [$n \leq 12\%$ (±5)] than those of the control [$n \geq 56\%$ (±12)]. The number of holes and size were reduced in the AZN- treated roots. The number of holes created by *S. ocellatella* larvae on roots showed less feeding in the AZN-treated plants than those in the control suggesting an antifeeding property of AZN as a biopesticide. This was in line with the results of the Zhong *et al*. (2017) who studied the effects of AZN on *Tirathaba rufivena* Walk. which is an important pest in areca palm. To our knowledge, it appears that although the *S. ocellatella* larvae ingest AZN, it stops the moth feeding. Antifeedant properties

resulted in delayed molting. AZN indirectly reduced the efficacy of conversion of ingested and digested food (ECI and ECD) needed for growth and development of an insect (Tanzubil 1995; Shannag *et al.* 2015; Adel *et al.* 2019). In the present study, a reduction in ECI was possibly associated with the post-ingestive toxicity of AZN. It has been shown that the amount of larval food intake was significantly decreased in response to AZN treatment in *Drosophila melanogaster* (Bezzar-Bendjazia *et al*. 2017).

The bioassay test for 3rd instar larvae of *S. ocellatella* showed that higher AZN concentrations caused higher mortality. The results of tested larvae showed that AZN at sublethal concentrations had repellency properties similar to those observed at higher concentrations. The highest AZN concentration (2.5 ml · l^{-1}) caused 100% repellency while the mean for deposited eggs per plant was 62 in roots treated with the sublethal concentration (0.5 ml · l^{-1}) that was around one fifth of the number of eggs in roots without AZN treatment. The concentrations of 2 and 2.5 ml · l^{-1} are advisable for sugar beet moth mortality and management of the pest. This biopesticide at sublethal concentrations (lower concentrations, i.e., LC_{25}, LC_{10}) affects *S. ocellatella* bioactivity such as development. Consequently, the repellency action of AZN at the concentration of 500 ml · l^{-1} as the lowest concentration caused adult and egg-laying deterrence and antifeedant activity on the larvae. These results are in line with the results of Zhong *et al*. (2017) and Tome *et al*. (2013) for the toxicity effects of AZN on lepidopteran pest insects. It has been shown that other pesticides, like microbial insecticides, show synergistic effects with AZN. Spraying AZN along with Bt for *Spodoptera exigua* control on sugar beet farms gave significantly higher yields than the control plants (Darabian and Yarahmadi 2017; Orak *et al*. 2019).

Conclusions

The results of studies on the developmental time revealed that the best time for spraying AZN was 5–6 days after the pest laid eggs. During this time, newly hatched larvae fed on leaves and roots of sugar beet. AZN at 2 and 2.5 ml · l^{-1} concentrations affected significantly the mortality of *S. ocellatella*. Likewise, AZN at concentrations higher than LC_{50} (1.14 ml · l^{-1}), the properties of prolonging developmental time in larval stages, repellency, antifeeding and mortality in the tested larvae was observable, demonstrating that AZN is a suitable biopesticide that can effectively minimize postharvest losses in sugar beet.

Consequently, AZN at 2.5 ml · l^{-1} concentration can be introduced as a biopesticide for controlling *S. ocellatella*. Due to severe infection of sugar beet to this pest insect at the preharvest and postharvest stages, it can be recommended as a pesticide with defined concentrations against sugar beet moth at the end of a growth season and significantly decrease the losses. However, further studies, e.g., on the mode of action of AZN on this pest under field conditions are needed.

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