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## Effects of a Commercial Neem Insecticide ((Neem Azal™-T/S) on Early and Late Developmental Stages of the Beet Armyworm, *Spodoptera exiqua* (Hübner) (Lepidoptera: Noctuidae)

<sup>1</sup>Levent Efil, <sup>1</sup>Inanc Ozgen and <sup>2</sup>Erdal N. Yardim

<sup>1</sup>Plant Protection Research Institute, Diyarbakir, Turkey

<sup>2</sup>Department of Plant Protection, Faculty of Agriculture, Yuzuncu Yil University, Van, Turkey

**Abstract:** The efficacy of a neem insecticide (Neem Azal™-T/S) against early (1st instar) and late (3rd-4th instars) stage beet armyworm, *Spodoptera exiqua* (Hübner) (Lepidoptera: Noctuidae) larvae was assessed at different concentrations (0.5, 1, 1.5, 2, 2.5 ml L<sup>-1</sup>) and time of exposure in field cage and leaf dip assays using cotton plant. Overall results of both assays indicated that both level of concentration and time of exposure had significant effects on mortalities of larvae. Some treatments caused significant mortality as early as 2 days after exposure in the field cage study. However, none of the concentrations in leaf dip assay led to mortality earlier than 6 days after exposure. Neem over 1 ml L<sup>-1</sup> caused complete (100%) mortality of early stage larvae in both assays. Treatments reduced number of leaves fed by larvae significantly, thus causing weight loss in individuals. No larvae could develop to pupa in treatments that received the test material at concentration levels higher than 1 ml L<sup>-1</sup>. In general, effects occurred in a dose dependent manner. Based on our findings and evidence in the literature, the neem insecticide could be useful as a biorational product in management of *S. exiqua* in cotton agroecosystems.

**Key words:** Beet armyworm, efficacy, mortality, neem, *Spodoptera exiqua*

### INTRODUCTION

Beet armyworm, *Spodoptera exiqua* (Hübner) (Lepidoptera: Noctuidae) is a widely distributed pest of cotton in many parts of the world. Control of this pest relies extensively on the use of synthetic insecticides. The long-term use of these chemicals represents potential environmental risks because they may be indiscriminately toxic to wide range of organisms, often kill beneficial insects and destroy ecological balance between pests and their natural enemies. Additionally, insects can develop resistance to insecticides; consequently larger amounts of such toxins may be required for their control. For example, various levels of tolerance and/or resistance of beet armyworm to organophosphorus chlorpyrifos, carbamate methomyl and thiodicarb, pyrethroid cypermethrin, benzoyphenylureas diflubenzuron and spinosad have been reported<sup>[1-4]</sup>. Alternative and environmentally acceptable new insecticides are needed in case of resistance or to delay the appearance of resistance through integrating them into programs utilizing currently available insecticides.

A considerable attention has been given to neem tree, *Azadirachta indica* A. Juss (Meliaceae) because its

pesticidal properties<sup>[5-7]</sup>. A variety of preparations based on some components of neem have demonstrated satisfactory efficacy against insect species in laboratory and field studies. Results of trials using some extracts from neem seeds/neem seed kernels in Africa, Asia and Latin America have shown that diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and other important cabbage pests like *Hellula* spp. (Lepidoptera: Pyralidae) can be controlled satisfactorily in cabbage and other crucifers<sup>[8]</sup>. Seed extract of neem was effective by 93.8% against potato tuber moth, *Pthorimaea operculella* Zeller (Lepidoptera: Gelechiidae)<sup>[9]</sup>.

Leaf extract of neem (four applications of 10% (w/w)) reduced flower infestation by pod borers, *Maruca testulalis* and flower thrips *Megalurthrips sjostedti* on cowpea in Kano, Nigeria<sup>[10]</sup>. Powder of neem seeds at 1% concentration level decreased damage by *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae) in grain<sup>[11]</sup>; it also protected cowpea seeds from severe perforation by cowpea seed bruchid, *Callosobruchus maculatus* F. at 0.125-3 g /20 g seed level<sup>[12]</sup>.

A formulated neem extract (Margosan-O or Azatin) has performed intermediate to good level effectiveness

against variety of sap feeding insects and mites on common greenhouse crops<sup>[13]</sup>. NeemAzal-T/S® (NA, at 20 g a. I. ha<sup>-1</sup>) caused similar mortality of third instar nymphs of *Jacobiasca lybica* to that of Omethoate at 200 g a. I. ha<sup>-1</sup> in greenhouse and also exerted similar mortality to fenvalerate (20% EC at 140 g a. I. ha<sup>-1</sup>) in *J. lybica* and *Bemisia tabaci* populations in potato fields in Sudan<sup>[14]</sup>.

Azadirachtin, the major principal of neem, has been reported for its toxic, antifeedant, growth and molt disrupting effects on *Schistocerca gregaria*, *Spodoptera littoralis*, *Oncopeltus fasciatus*<sup>[15]</sup>; it reduced the population growth rate of pea aphid, *Acyrtosiphon pisum* Harris 4 day after treatment<sup>[16]</sup> and adversely affected the fecundity, fertility and adult emergence of the melonfly, *Bactrocera cucurbitae* and oriental fruitfly, *Bactrocera dorsalis*<sup>[17]</sup>. Using azadirachtin in cabbage against cabbage looper, *Trichoplusia ni*, Hübner, diamondback moth, *Plutella xylostella* L. and silverleaf whitefly, *Bemisia argentifolii* (Bellows and Perring) resulted in lower insect damage and higher marketable head weights<sup>[18]</sup>.

The primary objective of present study was to assess the efficacy of the neem insecticide (Neem Azal™-T/S) at various concentrations against beet armyworm to utilize it for control of this pest when necessary and also in resistance management strategies in an integrated pest management context because neem seems promising for this approach.

## MATERIALS AND METHODS

**Field cage study:** Egg masses of beet armyworm were collected from a cotton field that was planted on 7 May 2001 and received no pesticides and they were reared in a growth chamber kept at 25±1°C and 65% R.H under a 16L:8D photoperiod.

STV-453 variety cotton seeds were planted in the field on 7 June 2001 and plants were grown on single raised beds (20 cm high) with a 75 cm between row and 20 cm within row-spacing. The soil received N (6 kg da<sup>-1</sup>) and P<sub>2</sub>O<sub>5</sub> (6 kg da<sup>-1</sup>) at planting and NH<sub>4</sub>NO<sub>3</sub> (9 kg da<sup>-1</sup>) before the first irrigation. Irrigation was applied as required. Experiments were conducted in a Complete Randomized Block Design, including six treatments each with three replications (plants) using early stage (1-d-old first-instar) and advanced stage (7-d-old 3rd-4th instars) larvae of *S. exiqua* in separate assays. Five concentrations (0.5, 1, 1.5, 2 and 2.5 ml L<sup>-1</sup>) of water of a commercial neem seed kernel extract (Neem Azal™-T/S, Verim Ltd Co., Istanbul), which contains 1% azadirachtin were used in trials. Control plants received

only distilled water. Plants were checked for other arthropods and all the pest and natural enemies were removed from cages before placement of larvae. Five newly hatched larvae (neonates) were transferred on an individual plant via a paintbrush. Plants were sprayed with the test solutions using a hand sprayer on 11 July 2001. Each plant received the same amount of spray solution and was covered with a 45×45×85 cm cage following the applications. The number of larvae on plants was recorded 2, 5 and 7 days after exposure to assess larval mortality (%). Mortality levels in the control were corrected using Abbott's formula<sup>[19]</sup>. On the seventh day of exposure, each larva was weighed and number of leaves fed by larvae was recorded.

The same treatments and processes were repeated for the advanced stage larvae of the same colony. Three larvae were placed on an individual plant, which then received the same neem treatments and caged. Mortality % was recorded 2 days after exposure. Number of surviving larvae was used as the effect parameter 5 days after exposure because of pupation. On the seventh day of exposure, the soil under and 15 cm around each cage was dug up and sieved for pupae; number of pupae and also leaves fed by larvae were recorded.

**Leaf-dip bioassay:** This assay was also conducted using early and advanced stage larvae of the same colony, kept at 25°C and % R.H. under a 16L:8D photoperiod in a growth chamber. Insects were assayed on leaf discs of 6 cm diameter cut from middle leaves of cotton plants. The same treatments (0.5, 1, 1.5, 2 and 2.5 ml L<sup>-1</sup> of the commercial neem seed kernel extract (Neem Azal-T/S) per liter of water and a control) with seven replicates (petri dishes) were used for both early and advanced stage larvae in separate assays. Each leaf disc was immersed in the test solution (control leaves in distilled water) for 30 sec drained and allowed to dry for 30 min on a filter paper. Leaf discs and larvae were individually introduced in Petri dishes (7 cm space diameter and 4 cm high) containing moistened filter paper. Leaf disks were replaced with ones that received the same treatments every two days for three times throughout the assay. Larval mortality (%) of early stage larvae was evaluated every two days by the eight-day after exposure. Weights (g/larvae) of advanced stage larvae was measured every 2 days until pupation. The number of pupae was recorded on the eight and tenth day of exposure till all the larvae pupated in the control.

**Statistical analyses:** Data were analyzed using repeated two-way analysis of variance (ANOVA). Group means were further separated by Duncan's Multiple Range Test at the level of p<0.05.

**RESULTS**

**Field cage study:** Both concentrations ( $F=9.537$ ;  $df=5,12$ ;  $p<0.001$ ) and time of exposure ( $F=54.462$ ;  $df=3,36$ ;  $p<0.001$ ) had significant effects on mortalities of early stage larvae (Table 1). Concentration exposure time interaction was significant ( $F=5.155$ ;  $df=15,36$ ;  $p<0.001$ ), indicating the differences between concentrations in different times of exposure and/or differences between exposure times in different concentrations. Test material at  $0.5 \text{ ml L}^{-1}$  level did not cause mortality of early stage larvae at any time throughout the assay. Treatments at 1 and  $1.5 \text{ ml L}^{-1}$  resulted in significant (66.6%) mortality of larvae 2 days after exposure. Treatments at 1.5, 2 and  $2.5 \text{ ml L}^{-1}$  concentrations caused complete (100%) mortality of larvae 5 days after exposure.

Only the highest concentration ( $2.5 \text{ ml L}^{-1}$ ) of the test material caused significant mortality 2 days after exposure (Table 1). Mortality was not recorded following this observation, the number of live larvae was evaluated instead because larvae entered in soil for pupation. Although no significant difference was observed in numbers of live larvae between treatments 5 days after exposure, numbers of pupae 7 days after exposure differed significantly ( $F=6.657$ ;  $df=5,12$ ;  $p<0.01$ ) (Table 2). Numbers of pupae in  $0.5$  and  $1 \text{ ml L}^{-1}$  treatments were significantly lower than those in the control; no larvae could develop to pupa in treatment received the test material at concentration levels higher than  $1 \text{ ml L}^{-1}$ .

Treatments had significant effects on the number of leaves fed by early stage ( $F=10.851$ ;  $df=5,12$ ;  $p<0.001$ ) and advanced stage ( $F=10.978$ ;  $5,12$ ;  $p<0.001$ ) *S. exiqua* larvae. Plants that received treatments at levels higher than  $0.5 \text{ ml L}^{-1}$  had significantly fewer leaves fed by larvae. Although  $0.5 \text{ ml L}^{-1}$  concentration did not induce mortalities in early stage beet armyworm larvae, it still caused significant weight loss in individuals ( $F=38,518$ ;  $df=5,12$ ;  $p<0.001$ ) (Table 2). Weights of larvae decreased as the concentration of treatments increased.

**Leaf dip assay:** Leaf dip assay on early stage *S. exiqua* larvae yielded similar results with the field cage study in that concentrations ( $F=13.01$ ;  $df=5, 36$ ;  $p<0.001$ ) and exposure time ( $F=130.79$ ;  $df=4, 144$ ;  $p<0.001$ ) had significant effects on mortality of larvae and concentration exposure time interaction was significant ( $F=8, 124$ ;  $df= 20, 144$ ;  $p<0.001$ ) (Table 3). None of the treatments in leaf dip assay led to mortality earlier than 6 days after exposure. However, some treatments caused significant mortality as early as 2 days after exposure in the field cage study. On the sixth day of exposure, only higher concentrations (2 and  $2.5 \text{ ml L}^{-1}$ ) led to significant mortality of larvae. As in the cage study, concentrations over  $1 \text{ ml L}^{-1}$  caused (100%) mortality. One different from the results obtained in the cage study was the fact that the lowest concentration ( $0.5 \text{ ml L}^{-1}$ ) exerted significant mortality of larvae 8 days after treatment.

Weights of advanced stage larvae significantly differed between treatments ( $F=23, 015$ ;  $df=5, 36$ ;  $p<0.001$ ) and between exposure times ( $F=11,99$ ;  $df=2, 72$ ;  $p<0.001$ ). Concentration time exposure interaction was also significant ( $F=5, 25$ ;  $df=10, 72$ ;  $p<0.001$ ). Treatments at 1, 1.5 and  $2.5 \text{ ml L}^{-1}$  levels caused significant weight loss in larvae in two days. All treatments suppressed larval weight gains, generally in a dose dependent manner on the fourth day of exposure and thereafter. Larvae in the control gained weight progressively overtime; however, no significant differences occurred in weights of larvae in any neem treatment between second day of exposure and thereafter.

These effects were reflected by significantly reduced numbers of pupae or complete inhibition of pupation in the treatments ( $F=26,18$ ;  $df=5, 56$ ;  $p<0.001$  for effects of treatments;  $F=9, 818$ ;  $df= 1, 36$ ;  $p<0.01$  for effects of exposure time;  $F=4.58$ ;  $df=5, 36$ ;  $p<0.01$  for concentration exposure time interaction). Nearly half of the larvae in the control pupated 8 day after treatments while no pupa was seen in any neem treatments. Hundred and twenty nine percent of larvae became pupa in the control and

Table 1: Effects of neem insecticide on percent mortality (mean±SEM) of *Spodoptera exiqua* larvae in response to different concentrations and exposure time in field cage study

Exposure time (days)	Concentrations ( $\text{ml L}^{-1}$ )					
	0 (control)	0.5	1	1.5	2	2.5
<b>Early stage larva</b>						
0	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)
2	0.00±0.00a (a)	0.00±0.00a (a)	66.67±16.67b (b)	66.67±16.67b (b)	50.00±0.00ab (b)	50.00±28.87ab (b)
5	0.00±0.00a (a)	0.00±0.00a (a)	83.33±16.67b (b)	100.00±0.00b (c)	100.00±0.00b (c)	100.00±0.00b (c)
7	0.00±0.00a (a)	0.00±0.00a (a)	83.33±16.67b (b)	100.00±0.00b (c)	100.00±0.00b (c)	100.00±0.00b (c)
<b>Advanced stage larva</b>						
0	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)
2	0.00±0.00a (a)	11.60±11.10a (a)	22.20±11.10ab (a)	22.20±11.10ab (a)	22.20±11.10ab (a)	55.50±11.10b (b)

(within a row, different letter(s) indicate significant difference in effects of concentrations at  $p<0.05$  level; within a column, different letter(s) in parenthesis indicate significant difference between exposure time at  $p<0.05$  level)

Table 2: Effects of neem insecticide on observed parameters (mean±SEM) of *Spodoptera exiqua* larvae in response to different concentrations in field cage study

Parameters	Concentrations (ml L <sup>-1</sup> )					
	0 (control)	0.5	1	1.5	2	2.5
Early stage larva						
Number of fed leaves/plant <sup>a</sup>	8.00±1.52a	7.33±1.76a	2.33±0.67b	1.33±0.33b	1.00±0.00b	0.66±0.33b
Weight (g/larva) <sup>a</sup>	4.90±0.59a	3.27±0.50b	0.33±0.33c	0.00±0.00c	0.00±0.00c	0.00±0.00c
Advanced stage larvae						
Number of live larvae/plant <sup>b</sup>	0.33±0.33a	0.67±0.33a	0.67±0.33a	0.67±0.33a	0.33±0.33a	0.33±0.33a
Number of pupae/plant <sup>a</sup>	2.33±0.33a	1.00±0.58b	1.00±0.58b	0.00±0.00b	0.00±0.00b	0.00±0.00b
Number of fed leaves/plant <sup>a</sup>	11.33±1.20a	11.00±0.57a	8.00±1.16b	6.00±0.58b	5.67±0.33b	5.33±0.67b

<sup>a</sup>Data were taken seven days after the treatments, <sup>b</sup>Data were taken five days after the treatments (within a row, different letter indicate significant at p<0.05 level)

Table 3: Effects of neem insecticide on observed parameters (mean±SEM) of *Spodoptera exiqua* larvae in response to different concentrations and exposure time in leaf dip assay

Exposure time (days)	Concentrations (ml L <sup>-1</sup> )					
	(Control)	0.5	1	1.5	2	2.5
Early stage larva						
% mortality						
0	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)
2	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)
4	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)	0.00±0.00a (a)
6	0.00±0.00a (a)	0.00±0.00a (a)	14.29±14.29ab (ac)	0.00±0.00a (a)	71.43±18.44b (c)	42.86±20.20bc (c)
8	0.00±0.00a (a)	85.71±14.28b (b)	85.71±14.28b (b)	100.00±0.00b (b)	100.00±0.00b (b)	100.00±0.00b (b)
Early stage larva						
Weight (g/larva)						
2	3.86±0.40a (a)	3.57±0.57ab (a)	2.00±0.44c (a)	2.29±0.42bc (a)	3.00±0.72a-c (a)	1.71±0.184c (a)
4	6.71±0.00a (b)	4.29±0.64b (a)	2.71±0.33bc (a)	3.00±0.44bc (a)	2.43±0.53c (a)	1.71±0.29c (a)
6	10.00±1.13a (c)	5.43±0.90b (a)	2.86±0.51c (a)	2.86±0.46c (a)	2.57±0.61c (a)	1.71±0.18c (a)
Number of pupa/petri dish						
8	0.43±0.20a (a)	0.00±0.00b (a)	0.00±0.00b (a)	0.00±0.00b (a)	0.00±0.00b (a)	0.00±0.00b (a)
10	1.00±0.00a (b)	0.29±0.18b (b)	0.00±0.00c (a)	0.00±0.00c (a)	0.00±0.00c (a)	0.00±0.00c (a)

(within a row, different letter(s) indicate significant difference in effects of concentrations at p<0.05 level; within a column, different letter(s) in parenthesis indicate significant difference between exposure time at p<0.05 level)

0.5 ml L<sup>-1</sup> neem treatments on the tenth day, respectively. None of the larvae that received higher level concentrations could develop to pupa.

## DISCUSSION

The results of both field cage and leaf dip assays clearly indicated that neem used in this study can affect *S. exiqua* larvae at a concentration level as low as 0.5 ml L<sup>-1</sup> of water and as early as two days after exposure. Effects of neem fractions or azadirachtin (AZA), the major principle of neem, on insects include direct toxicity, repellency, antifeedant, insect growth and metamorphosis (ecdysis) inhibition, malformations, reproduction (fecundity and viability) and death<sup>[20-24]</sup>.

Mortality of insects in response to neem treatments can result from toxic effects of the material used<sup>[25-27]</sup>. Azatin, a neem seed extract containing 3% azadirachtin has been reported to cause quick direct toxicity against mahogany shootborer, *Hypsipyla grandella* larvae at relatively high concentrations (1.0, 3.20 and 10%)<sup>[28]</sup>. Therefore, mortalities of *S. exiqua* larvae in our assays could have resulted from direct toxicity of the test material. The effect was much apparent with 100%

mortality of early stage larvae treated with concentrations over 1 ml L<sup>-1</sup> in both cage and leaf dip assays.

It has been shown that topical applications of sublethal doses (1-8 ppm) of azadirachtin does not alter acetylcholinesterase (AChE) activity in cockroaches, but rather exerts excitatory action on the electrical activity in the nervous system, possibly complementing antifeedant and growth-regulatory actions of this compound<sup>[29]</sup>. It is evidently clear from the literature that neem fractions can affect feeding activity of insects adversely. For instance, azadirachtin has been reported to deter *S. greagaria* from feeding completely at a behavioral level in simplified assays<sup>[30]</sup>. AZT, a neem extract performed antifeedant (weight loss) and repellent actions on *Plutella xylostella* L. in a choice chamber<sup>[26]</sup>. Lesser grain borer, *Rhizoperta dominica* (F.) was strongly repelled and adults made significantly fewer punctures when confined to filter paper treated with neem oil or neem-based insecticide (Margosan-O)<sup>[31]</sup>. Azadirachtin (98% AZA) and three neem extracts (48, 23 and 7% AZA) have strongly repelled stored-product insects, the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), the rice beetle, *Sitophilus oryzae* (L.) and the red flour beetle, *Tribolium castaneum* (Herbst) and reduced their numbers on

wheat<sup>[27]</sup>. This reduction was attributed to chemosensory (olfactory or gustatory) effect of neem.

Sublethal concentrations of azadirachtin can reduce the amount of food ingested by insects, decrease their ability to convert ingested food into biomass (growth), impede weight gain and prolonge the duration of immature stages<sup>[32]</sup>, which in turn affect emergence, longevity, fecundity and fertility of insects. Three commercial neem-based insecticides, Agroneem, Ecozin and Neemix, through their antifeedant action on *Plutella xylostella*, led larvae to quickly stop feeding, resulting in formation of smaller (in weight, length and diameter) larvae<sup>[10]</sup>. Extracts from neem seed, kernels and leaves increased larval and pupal duration, larval and prepupal mortality of *H. armigera* but reduced its larval weight, adult emergence, adult longevity and fecundity and egg fertility<sup>[33]</sup>. Five percent aqueous extract of neem seed powder or 5% emulsified neem oil reduced settling of banana root borer, *Cosmopolites sordidus* (Germar) adults under the corms of banana, delayed finding of feeding sites, initiating feeding and boring into pseudostem discs and caused reduction in weights of individuals up to four times and in egg hatching<sup>[34]</sup>. Two neem extracts (AZT and NEEM-AZAL) and synthetic azadirachtin (AZ) delayed the development of second instar larvae of *Plutella xylostella* L. and caused end-point mortalities between 50-90%<sup>[26]</sup>. Neem fractions delayed pupation and emergence period of *Musca domestica* L. larvae, reduced pupal weight by 70% and resulted in abnormal pupa formation<sup>[25]</sup>. A neem based insecticide slightly deterred the western cherry fruit fly *Rhagoletis indifferens* Curran from oviposition in a cherry orchard, suppressed the development of eggs and decreased survival<sup>[35]</sup>. Application of extracts of neem seed kernels with additives on *Vicia faba* at a concentration level as low as 0.004% disrupted molting of *Acyrtosiphon pisum* presumably by interfering with the molt-regulating endocrine system and led to a mortality rate of 92%<sup>[36]</sup>.

Similar findings with most of the reports discussed above were obtained in this study. Significant reductions in number of leaves fed by early (1-d-old) and advanced (7-d-old) stage *S. exigua* larvae in the field cage study could have indicated that the test material used in this study caused significant reduction in feeding activity of *S. exigua* larvae by a repellent action that led to decrease in amount of food ingested by larvae at at least 1 ml L<sup>-1</sup> concentration level. Significant reduction in weight gain in response to test material at as low as 0.5 ml L<sup>-1</sup> concentration level and as early as in two days after exposure could indicate that the test material had strong antifeedant effect on larvae. Despite the mortalities in response to test material, lack of significant difference in

numbers of advanced stage larvae between treatments five days after exposure due to pupation of some individuals in the control and also reduced pupa numbers in the cage study as well as delayed pupation and reduced pupa numbers in 0.5 ml L<sup>-1</sup> treatment in the leaf dip assay could indicate that lower concentrations extended the larval development time, thus prolonging larval duration and slowing the growth down. At higher concentrations, growth could have been completely inhibited because no larva could develop to pupa and 100% end-point mortality occurred.

Early stages of *H. armigera* larvae have been reported to be more sensitive to the exposure of neem extracts than advanced stage larvae<sup>[33]</sup>. Third instars of *A. pisum* were less susceptible to the insecticidal properties of neem than earlier instars<sup>[36]</sup>. Compared to the effects on early stage larvae, the only significant mortality of advanced stage larvae in the highest concentration (2.5 ml L<sup>-1</sup>) received treatment 2 days after exposure and lack of difference between surviving neem treated larvae numbers thereafter could indicate that advanced stage larvae were less sensitive to neem and required higher concentrations.

Generally, the effects of the test material on beet armyworm appeared to be in a dose dependent manner, increasing the dose resulted in increased mortality, reduced feeding activity, larval weight and pupa numbers. These findings are in accordance with the results of some reports discussed above indicating that effects of neem applications on insects occur in a dose dependent manner. The greater the dose was the greater the effect occurred<sup>[11,31-34]</sup>.

Time of exposure is another factor that determines the effect of neem on insects, that is, effect may appear and/or increase with increasing time<sup>[26]</sup>. In general, increased mortality of *S. exigua* larvae with time and especially significant mortality of larvae 8-d after exposure to relatively lower concentrations (0.5 and 1 ml L<sup>-1</sup>) could clearly indicate that the effects on *S. exigua* occur not only in a dose-dependent but also in a time-dependent manner.

In leaf dip assay, no mortality appeared up to the sixth day after the applications, while great mortality occurred as early as in the second day after the applications in cage feeding study. This delayed effect in leaf dip assay could be attributed to the lower temperature in the growth chamber, which is considered typical for neem products affected by environmental conditions<sup>[8,27,37]</sup>.

In addition to their diverse behavioral and physiological effects exerted on insect pests, the selectivity and safety that neem insecticides offer to

insect predators and parasitoids<sup>[5,14,38]</sup>, may facilitate their use in cotton agroecosystems as alternative insecticides. Nevertheless, neem insecticides have been found safer to the natural enemies of *Bemisia tabaci* Gennadius as compared to triazophos, monocrotophos, methamidophos, endosulfan and phosalone in cotton agroecosystems<sup>[39]</sup>. It has also been reported that bollworm complex (*Earias* spp., *Pectinophora gossypiella* and *Heliothis armigera*) can be controlled by combinations of neem, Bt and 84% reduced rate of synthetic pyrethroids in cotton. This combination provides a reduction by 75% of the load of synthetic insecticides and does not induce resurgence of whitefly populations<sup>[40]</sup>, which may occur with synthetic insecticides otherwise. Additionally, findings of Abdullah *et al.*<sup>[41]</sup> demonstrating that beet armyworm did not develop resistance sharply against a commercial formulation of neem extract (1% azadirachtin) by selection pressure for 12 generations could indicate that neem insecticide may particularly be promising in regard of resistance. Therefore, based on our findings and evidence in literature, the neem insecticide could be useful as a biorational product in management of *S. exiqua* in cotton agroecosystems.

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