BIOMONITORING OF THE GENOTOXIC AND OXIDATIVE THE EFFECT OF NEEMON MORTALITY AND PHYSIOLOGICAL INDICES OF *HYPHANTRIA CUNEA* DRURY (LEPIDOPTERA)

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ABSTRACT: The fall web worm, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) native insect to North America has recently been introduced into Iran resulting in severe damage to trees and agricultural production. An experiment was conducted to examine insecticidal effect of a commercial product of neem extract, Achook® (Godrej india) containing 0.03% of effective material,Azadirachtin, against third instar larva of this pest. The first instar larvae were collected from *Platanus orientalis* hosts and reared on fresh mulberry leaves in growth chambers (28± 2 °C, RH 75± 5 and 14:10 LD). Third instar larvae of next generation were used in the present study. The dipping method was used where mulberry leaves of similar sizes were dipped in the solution of neem and provided to the larvae. About 70 percent mortality was observed in treated insects after 48 h. The controls fed 51.93 more than treated larvae. The measurement of physiological factors (cholestrol, uric acid, urea urea, protein, glucose) showed reduction in treated larvae in comparison to control insects. The possible significance of these changes is discussed.

KEY WORDS: Neem, Azadirachta indica, fall web worm, *Hyphantria cunea*, physiology.

The fall web worm, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) is an insect native to North America that is presently distributed in many areas in the northern hemisphere (Warren & Tadic, 1970) and New Zealand (Kean & Kumarasinghe, 2007). It has been introduced to different areas of Europe and Asia (Li et al., 2001). Since, 2002, *H. cunea* established itself in northern areas of Iran, causing severe damage to trees. It is a multivoltine pest feeding on leaves of trees and hibernates as pupa in soils around the damaged forest and shade trees. Research has been conducted to look for natural plant protection compounds such as botanical insecticides, antifeedants and microorganisms (Zibaee et al., 2010).

The compounds from the neem tree have a number of properties useful for insect pest management. These include toxicity, repellence, feeding and oviposition deterrence, insect growth regulatory activity, etc. (Ascher & Meisner, 1989; Schmutterer, 1990; Mordue & Blackwell, 1993; Mordue, 2004) and more than 140 active compounds have been identified in the neem tree (Koul, 2004). The key insecticidal ingredient is azadirachtin, a steroid-like tetranortriterpenoid, responsible for both antifeedant and toxic effects in insects (Govindachari et al., 2000; Koul, 2004). Furthermore, azadirachtin blocks the synthesis and release of moulting hormones from the prothoracic glands, leading to incomplete ecdysis in immature insects (Isman, 2006). Different insect species show varying degrees of sensitivity to various extracts and components. Naumann & Isman (1995) used three concentrations of an oil-free neem seed extract to deter oviposition in noctuid moths and found that these neem-based products were not effective. According to Turcani (2001), the neem seed kernel suspension reduced the damage by *Pieris brassicae* L. larvae but failed to give any significant protection to castor, *Ricinus communis* L. leaves against the red hairy caterpillars, *Amsacta albistriga* Walker. Neem preparation showed a positive effect in controlling the turnip root fly, *Delia floralis* (Fall.) and the cabbage moth, *Mamestra. brassicae* (Karelina et al., 1992; Meadow & Seljasen, 2000; Seljasen & Meadow, 2006), However Pats & Isman (1998) found no effect on hatching or larval development for the cabbage root fly, *D. radicum* (L.) and Neemix, which kills and repels a variety of insects, had no effect on populations of the beet armyworm, *Spodoptera exigua* (Hübner) larvae. These controversial results suggest that the efficacy of any neem-based insecticides on pests should be investigated before widespread use in the field.

This research addresses the need for finding effective options for managing the insect pest *Hyphantria cunea* in Guilan province in the face of the declining popularity of conventional chemical insecticides. In this study, we have been interested in the effects of Neem EC, on deterrence and some physiological aspects of this lepidopteran pest.

MATERIAL AND METHODS

First instar larvae of *Hyphantria cunea* were collected from *Platanus orientalis* host and reared separately on mulberry leaves to reach 3th instar larvae in the laboratory at 28 ± 2 °C under a 14 h light:10 h dark photoperiod and relative humidity of 65 ± 5 percent. The third instar larvae of next generation were used to initiate the experiments.

The neem (Achook®, Godrej company, India) was procured. Bioassays were performed with 3^{th} instar larvae of *Hyphantria cunea* (<24 h) using the suggested concentration of the company, 0.03 % of effective material (Azadirachtin). Leaf discs of 6cm diameter were dipped for 10 seconds and air dried leaves were provided to the larvae. Control leaves were treated with distilled water and air-dried in the same way. Three replicates of 10 larvae each were used for the bioassays with one control of 10 insects. Larvae were starved 4-5h prior to each bioassay. After 48h post-treatment the number of dead larvae was counted and the area of eaten leaves was measured by leaf area meter. For physiological indices the live larvae were randomly selected and were placed in 2 ml of distilled water or buffer related to each test and then samples were homogenized.

Protein was measured based on the Biuret's method as was described by Reinhold (1953) using a protein assay kit (Pars Azmon Co, Iran) and measuring the absorbance at 540 nm. Glucose was analyzed as described by Siegert (1987) and total cholesterol as was described by Richmonds (1973) hydrolyzing cholesterol esters with cholesterol oxidase, cholesterol esterase and peroxidase. Uric acid contents in the medium were determined using uricase as described by Valovage and Brooks (1979) at 500 nm. Urea was measured with urease-GDH kit (Biochem. Co, Iran) at 340 nm following the manufacturer's protocol.

All data were analyzed using SAS software and Tukey's studentized range (HSD) test in a complete randomized design (SAS, 1997).

RESULTS

Results showed that the mortality rate of treated larvae were 70% while no mortality was recorded for controls. The area of leaf consumed in treated leaves was 51.93 times lesser compared with the controls (Table 1).

The level of cholesterol, protein, glucose, uric acid and urea in treated larvae were reduced in the order of 0.68, 0.53, 0.92, 0.64 and 0.70 times than controls (Fig. 1).

DISCUSSION

Secondary organic compounds synthesized by plants have an important role in protecting plants against insect herbivory by way of delay in larval growth and metamorphosis or antifeedants (Isman et al., 2006). The neem in the present study clearly showed insecticidal and antifeedant effects on *Hyphantria cunea*.

Larval mortality, impaired antifeedant effect and reduced plant damage were all substantial enough to suggest that neem could be considered in the management of this insect. Yoshida & Toscano (1994) found that the relative consumption rate of *Heliothis viridescens* (Fabricius) larvae treated with neem extract was 25% that of the control. Greenberg et al. (2006) found that neem-based insecticides deterred feeding by beet armyworm (*S. exigua*). The neem tree contains different substances in its tissues, many of which are biologically active against insects as antifeedants (Jacobson, 1987; Mordue, 2004). The primary active ingredient of neem-based pesticides is azadirachtin. Inhibition of feeding behaviour by azadirachtin results from blockage of input receptors for phagostimulants or by the stimulation of deterrent receptor cells or both (Mordue & Blackwell, 1993). The Neem EC did not produce a rapid mortality. No larvae perished within the first days of the experiment; mortality began on second day, when moulting to the 4th growth stage began. Coudriet et al. (1985) detected that neem seed extract prolonged larval development, and induced larval mortality of *Bemisia tabaci* (Gennadius) on cotton foliage. In our experiments the treated larvae perished directly before moulting or in the process of moulting as they were unable to shed the old integument. The mortality observed during the molting process; support the proposition that the action of neem ingredients is connected with endocrine events in the insects. To be successful, metamorphosis requires a careful synchrony of many hormones and other physiological changes. Metamorphosis occurs when the corpora allata stops secreting juvenile hormone (Gilbert et al., 1980). It can occur only when the insect has reached a certain minimum mass characteristic of the growth stage. Neem EC caused lethal failure of larval-larval and larval-pupal ecdysis, which is typical for insecticides possessing morphogenetic activity, commonly referred to as IGR-activity. It is possible that azadirachtin, because of its similarity to ecdysone, which controls the process of metamorphosis as the insects pass from larva to pupa, will block off these vital hormones and interrupt their life cycle (Sridharan, 2007). The defects occurred in moulting into the 4th instar. Therefore it may be concluded that a higher than normal juvenile hormone level persisted in the organism, allowing growth but inhibiting metamorphosis. It is known that neem may act as an exogenous juvenile hormone or its analogue (Schmutterer, 1987). However, it is also possible that, as a result of intoxication, histopathological changes may occur in the corpora cardiaca of the insect, causing disorders in the secretion of hormones (Mordue, 2004).

Different factors may affect enzymatic and non-enzymatic processes in insects such as: climate, diet, chemicals, etc. Our results show that Achook® significantly affected biochemical process in the fall web worm larvae.

Many insecticides have an antifeedant effect and decrease insect feeding efficiency which affects protein biosynthesis and accumulation (Etebari et al., 2007). Present result shows that the amount of total protein decreased in larvae treated with Achook® after 48 treatments. This phenomenon is probably due to breakdown of proteins into free amino acids which are shunted into the TCA cycle as keto acids (Bizhannia et al., 2005).

Mukherjee et al. showed that higher concentrations of azadirachtin increased the amount of protein in the hemolymph of *Tribolium castaneum* and this is probably due to the increase in the activity of detoxifying enzymes. Schmidt et al. showed that treatment of *Spodoptera littoralis* and *Agrotis ipsilon* Rottenburg (Lepidoptera: Noctuidae) with azadirachtin decreased protein in the hemolymph. Zibaee et al. reported that the amount of protein decreased in mid-gut of rice stripped stem borer due to diazinon treatment.

Urea and uric acid are excreted end product of insects and their amount is correlated with the amount of protein in the insect's body. Dungern & Briegel (2001) reported that the activity level of xanthin dehydrogenase increased due to Presence of higher protein in the hemolymph. After 48 h, the amount of uric acid decreased in the hemolymph of treated larvae. This result indicates that the decrease in uric acid in the hemolymph is probably due to altered metabolic pathway after treatment that prevented the natural excretion of uric acid from the insect body (Etebari & Matindoost, 2004a).

Etebari & Matindoost (2004b) reported that if the feeding activity is normal, glucose and cholesterol amounts in the hemolymph of silkworms increases. When the feeding of larvae, however, is interrupted the amount of these metabolites are severely decreased (Etebari & Matindoost, 2004c). Several activities of insects depend on carbohydrates metabolism. The amount of glucose demonstrates the availability of this sugar for carbohydrates metabolism in insect cells (Satake et al., 2000). Our results show that the amount of glucose and cholesterol decreased in larvae that were treated with neem after 48 h. Radhakrishna and Devi showed that treatment of silkworm larvae with organophosphorus compounds decreased the amount of glucose. Nath (2003) reported that lethal and sublethal doses of ethion and fenitrothion increased the amount of glucose and trehalose in silkworm. This could be due to imbalance in the homeostasis of the silkworm. Our results similarly point to this.

In conclusion, our results demonstrates that Achook® has toxic, as well as growth regulatory, antifeedant and biochemical effects that caused 70% of mortality in treated larvae and results are promising in developing effective approaches to control insect pests, but more studies are needed to evaluate the field efficacy of Neem EC for this particular pest of economic importance.

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Table 1. Mean (± standard error) measurement of leaf area size (cm^2).

Treatments	***0.03%***	***Control***
	250±40.32a*	***4.14±0.04b***
	131.81±40.32a	***4±0.04b***
	255.39±40.32a	***4.13±0.04b***

*Data with similar letters in each column are not significantly different (α≥5%).

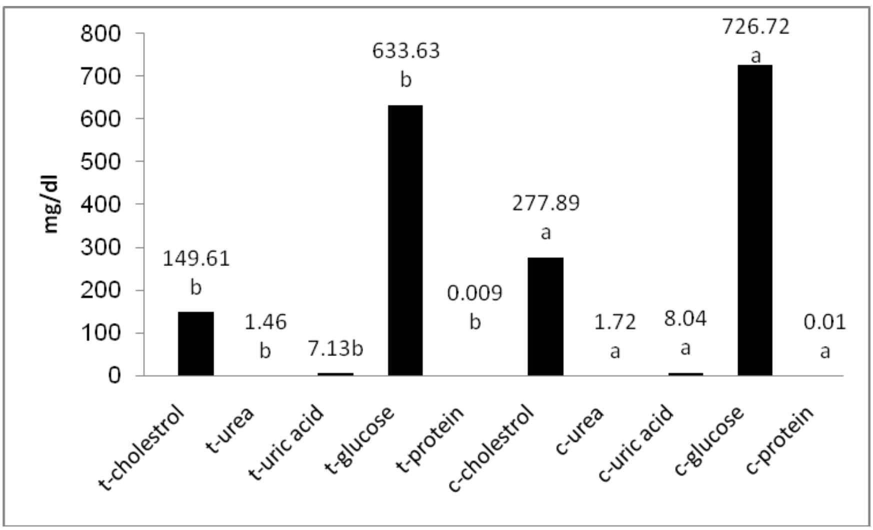

Figure 1. Comparison of mean number of physiological parameters between treatment and control.

Data with similar letters in each column are not significantly different (α≥5%) (t= Treated , c= Control).