Azadirachtin influences total head protein content of *Helicoverpa armigera* Hub. larvae

Azadirachtin, a tetranortriterpenoid from *Azadirachta indica* A. Juss (Meliaceae), is well known as an insect growth inhibitor[1]. It inhibits the feeding and growth of insects belonging to several taxa. Although its effect involves intervention in endocrinal activities[2], specific site(s) of action are unknown.

Pod borer [*Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)] is one of the most widely distributed agricultural insect pests occurring throughout the world. Being highly polyphagous, it collectively attacks a wide range of food, fibre, oil and fodder crops as well as many horticultural and ornamental crops. In fact, polyphagy, high mobility, high fecundity and facultative diapause are key physiological, behavioural and ecological characteristics which facilitate survival of *Helicoverpa* species even in unstable habitats[3]. *H. armigera* has developed resistance against most classes of synthetic insecticides, including DDT[4], endosulphan[5], pyrethroids[6] and carbamates[7]. Even transgenic crops resistant to insect pests have failed to perform as expected[8,9].

The worldwide demand for natural insecticides is increasing. Among these natural insecticides, azadirachtin has attained commercial status in some developed as well as developing countries. Despite the increasing interest in azadirachtin, we still do not fully understand manifestations of azadirachtin-feeding on insects, besides its digestion in the insect mid-gut[10]. An examination of the effect of the phytochemical on insect metamorphosis with special reference to the nervous system is important. This study aims at determining the effect of azadirachtin on head protein levels in *H. armigera* larvae.

Azadirachtin was procured from Supelco (Bullefonte, PA, USA). Bovine serum albumin (BSA), vitamins, cholesterol, streptomycin, choline choloride, sorbic acid, sucrose and yeast extract (high grade) were obtained from Hi-Media (Mumbai, India). All other chemicals and reagents were of high purity analytical grade, obtained from Cisco Research Laboratory, Mumbai or Sigma Chemicals Co. (USA). Semi-synthetic diet was used for larval rearing in the experiments, as described by Singh and Rembold[11]. Larvae of field-collected *H. armigera* Hub. were reared on semi-synthetic diet. The larvae, pupae and eggs were maintained at a photoperiod of 16 h light and 8 h dark cycle, 60–70% relative humidity and at 27 ± 1°C temperature. Adult moths for maturation, mating, and egg harvesting were kept in 10 l jars in the laboratory and maintained at 24 ± 2°C. Larvae were reared in culture tubes ($2 \times 5 \times 6$ cm) individually on a semi-synthetic diet continuously for a year.

After anesthetizing the larva with diethyl-ether, azadirachtin was applied topically (0.1 µg/g body wt) at the thoracic region of the anesthetized 7- and 20-day-old larvae on the dorsal side, using Burkard Arnold Hand micro-applicator (Rickmansworth Herts, England). Each larva was treated only once. Control larvae were applied with the corresponding carrier only. Larvae were allowed to feed on diet for 0, 24, 48 and 72 h post-treatment.

In another experiment, azadirachtin (dissolved in diluted 70% ethanol) was injected into 10-, 13- and 16-day-old larvae at the base of the second abdominal proleg using 10 µl Hamilton syringe (Hamilton Company, Reno Nevada) fitted with a 26 S pointed needle. Each larva was injected only once. The doses used were 0.05 to 0.5 µg/g body wt. The control set of larvae was injected with corresponding carrier only[12]. Larvae were fed on normal diet for 48 h post-treatment followed by head-dissection. Twenty larvae were used for each treatment, which was replicated five times.

For each treatment twenty larval heads were hand homogenized in an extraction medium consisting of Tris-HCl (pH 7.5), 1% Triton-X100 and 10% DMSO using a pestle and mortar. The homogenate was centrifuged at 15,000 *g* for 15 min at 4°C and the supernatant was precipitated with equal volume of trichloroacetic acid (12%) or cold acetone (–20°C). Precipitated protein was collected by centrifugation and dissolved in 0.1 N NaOH. Protein concentration in the solution was estimated using Lowry *et al.*s method[13] with BSA as standard.

Results show that a 0.1 µg dose of azadirachtin resulted in an optimal decline (30%) in protein level up to 72 h in 7-day-old larvae. However, the magnitude of reduction (23%) in 20-day-old larvae was found only 24 h after treatment. Twenty-day-old larvae retained the mean protein concentration (22.7 mg/g) over the 72-h period compared to the corresponding control larvae (24.11 mg/g; Table 1). Different doses of azadirachtin varying from 0.05 to 0.5 µg/g fresh larval body wt, were injected in a single administration into 16-day-old larvae. The results showed that azadirachtin decreased the total head protein in a dose-dependent manner. Injection of 0.05 µg azadirachtin/g decreased the protein level (1.65%), whereas 0.5 µg azadirachtin/g elicited maximum response (34.65%; decline) compared to control.

In the present study, it was found that medium to low doses of azadirachtin significantly ($P < 0.01$) influenced the total head protein profile in larvae of *H. armigera*. Azadirachtin was found effective in reducing the protein concentration at 0.1 µg by topical application in the 2nd stadium. However, treated larvae did not show the same protein concentration up to 72 h compared to control. Nevertheless, azadirachtin treatment in the 6th day stadium restored protein concentration up to 72 h as compared to control (Table 1). Similarly, decrease in protein concentration has been reported in haemolymph system of *Spodoptera litura* after azadirachtin treatment[14]. On the contrary, cyclic changes in concentration have been observed in *Schistocerca gregaria*[15] and *Manduca sexta*[16].

Table 1. Head protein level of 2nd and 6th stadia of *Helicoverpa armigera* influenced by topical application of azadirachtin (0.1 µg)

	Mean* head protein level in larva (mg/g tissue) after treatment for			
	24 h	48 h	72 h	Mean
Treated 2nd stadium	4.19	4.26	4.70	4.38
Control	5.96	6.28	6.66	6.30
Treated 6th stadium	17.83	17.65	22.70	20.06
Control	23.06	23.98	24.11	23.71

*Twenty larvae/treatment and replicated five times.

Table 2. Head protein content (mg/g tissue) of 5th stadium of *H. armigera* influenced by injected dose of azadirachtin after 48 h

Dose (µg)	Protein mg/g tissue
Control	21.020
0.05	20.672
0.1	16.958
0.2	15.360
0.3	14.942
0.5	13.736

In addition, azadirachtin did not maintain reduced level of protein for 72 h, in agreement with observations by Rembold[17]. This may imply that azadirachtin may not exist in the neuroendrocrine system up to 72 h when administered topically. Similarly, ingested azadirachtin has been found accumulated in the head and other tissues of *Peridroma sausia*[18]. Azadirachtin imposed longer effects (72 h) in reducing protein in 2nd stadium. Head proteins of *Helicoverpa* are derived from storage proteins in the insect body or are synthesized by neurosecretory cells. Observed changes in head protein may arise through several possible physiological perturbations, including modulation of protein synthesis or turnover rate.

Injection of azadirachtin in the 5th stadium in increasing doses, showed dose-dependent trend in the level of protein. It also resulted in maximum decrease in protein level at 0.5 µg (Table 2). This may be due to altered feeding behaviour at higher doses[19]. It is assumed that higher doses of azadirachtin may alter the feeding in larvae towards food-avoidance and maintain body metabolism at the expense of the storage or cellular proteins. There may be more than one site of action of azadirachtin and their manifestations may depend on the mode of application of the phytochemical.

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Biodiversity of agarics from Nilgiri Biosphere Reserve, Western Ghats, India

To conserve biological diversity, several biosphere reserves have been established in India. The Nilgiri Biosphere Reserve (NBR) is one of the oldest among them. The total area of the NBR extends over 5520 km^2, comprising the whole of Nilgiri district, parts of Coimbatore plains, Mysore plateau consisting of Bandipur National Park, Wyanaad Wildlife Sanctuary, Silent Valley National Park and Nilambur plains[1]. The floristic composition of this region has been studied earlier by several workers[1–4]. It has been emphasized that understanding of the phytogeographical affinities would help policy makers in deciding the right choice of species. Similar work with reference to other groups of organisms is an urgent need.

Fungi which form an important component of the forest ecosystem have been largely neglected in any of the biodiversity studies of a given area. The present correspondence is an attempt to give a broad picture of the biodiversity of a particular group of fungi, viz. members of order Agaricales, class Basidiomycetes in the NBR.

During the last two decades, considerable work has been done on the floristic components of these fungi in the NBR[5–9]. In these studies several areas in the NBR of the Western Ghats have been covered, for the collection of different species of fungi belonging to order Agaricales. A consolidated result of several studies is presented here.

Table 1. Total number of agarics from three states forming part of the NBR

State	Area (km^2)	No. of agarics
Tamil Nadu	2537.6	195
Kerala	1455.4	28
Karnataka	1527.4	–