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# Effect of Some Neem Based Insecticides on Wing Shape and Pigmentation in Lemon-Butterfly, *Papilio demoleus* L.

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**Abstract:** Neem is an important source of environment-friendly bio-pesticides. The unique feature of neem products is that they do not directly kill the pests, but alter the life-processing behavior in such a manner that the insect can no longer feed, breed or undergo metamorphosis. Nowadays, eco-friendly methods are being adopted that are rather specific for the target species without harming the other organisms. In the present study, the neem based insecticides (NBIs) (multineem and neemark) were applied topically on to *Papilio demoleus* larvae to see their bio-efficacy. Both the NBIs induced defecation and jerking of the body sporadically. The NBIs caused heavy pigmentation in V instar larvae, disappearance of red spot, a change in wing shape and specific pigmentation pattern characteristic of the butterfly. Highly significant reduction in the length of ovariole, number of oocytes per ovariole and body weight of treated larvae was also recorded.

**Key words:** Neem-based insecticides · Wing · Pigmentation · Oocytes · Ovariole

## INTRODUCTION

For insect pests' control, man has employed several means chiefly chemical insecticides which, however, created several serious problems like air and water pollution, interruption of natural balance, health hazards, killing of beneficial insects, secondary pest outbreaks, pest resistance, pest-resurgence etc. [1-3]. Neem based insecticides (NBIs) are environment-friendly bio-pesticides having unique feature to control the insect pests by altering the life-processing behavior in such a manner that the insect can no longer feed, breed or undergo metamorphosis. Nowadays, eco-friendly methods are being adopted that are rather specific for the target species without harming the other organisms. NBIs are known to disrupt several biological functions in insects (4, 5). Most of the studies on effect of NBIs on lemon butterfly *Papilio demoleus* (serious pest of lemon plant) have been limited to molting deformities and hemocyte profile without having much correlation with pigmentation, length of ovariole, number of oocytes per ovariole and wing shape [6-11]. This communication comprises the results of our investigation on wing shape, pigmentation and oocyte number in lemon-butterfly, *Papilio demoleus* after NBIs application.

## MATERIALS AND METHODS

**Insect Culture:** The early larval instars of the lemon-butterfly, *Papilio demoleus* L. (Lepidoptera: Papilionidae) were collected from lemon nurseries and lemon plants. The larvae were reared on fresh leaves of the said plant in a B.O.D. incubator at 27±1°C, 75% RH and 16L: 8D photoperiod [11, 12]. Works were carried out during the Year 2008-2009 at Department of Zoology, K.N. Govt. P.G. College, Gyanpur – 221304, S.R.N. Bhadohi, India. Late IV and about 24 hr old V instar larvae from the said lab culture were used.

**Neem Based Insecticides (NBIs) Treatment:** The NBIs used in present study were Neemark (neem oil based E.C. containing azadirachtin 0.03% EC 300 PPM minimum, West Coast Herbochem Ltd. Mumbai, India) and Multineem (seed extract containing 0.03% azadirachtin, Multiplex Fertilizers Pvt. Ltd., Bangalore, India). To examine their effect on sensitivity, wing shape, pigmentation, oocyte number and body weight, various concentrations (2.5, 5, 10 and 25%) of NBIs to be used were prepared by diluting with acetone. All the dilutions of insecticides were applied topically in different doses with the help of glass

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micropipette on the dorsal and ventral surfaces at three different body regions (cephalic, thoracic and abdominal) of V instar larvae.

**Microscopy and Staining:** The experimental and acetone treated control insects were dissected under binocular microscope in insect Ringer solution [13] and their ovaries were taken out and fixed in Bouin's fluid. A few of the ovaries were stained in borax carmine solution and permanent slides were made to measure the size/number of oocytes and ovarioles with the help of calipers and meter scale under microscope. The body weight of the larvae of both the groups was also recorded at 24 h interval till pupation to assess the toxic effect of NBIs.

**Statistical Analysis:** Thirty insects were used for each experimental and control group separately. Data was statistically analyzed using Student's test.

## RESULTS

The effect of aforesaid insecticides in different concentrations and doses on insect's sensitivity, pigmentation, wing shape, oocyte number and body weight are described here briefly.

**Effect on Sensitivity:** Topical application of NBIs on ventral surface of cephalic and thoracic regions of the larvae was more effective than that on dorsal surface of abdominal region. The treatment caused movement of the head on the wall of rearing glass vials, increased defecation and jerking of the body intermittently.

**Effect on Pigmentation:** Out of 30, only six V instar larvae showed heavy pigmentation following treatment with 50µl of 2.5% multineem (cf Figures 1, 2). In few neemark treated insects, the adult butterflies showed disappearance of red spot on the hindwing. Apical and anal margins of both the wings were occupied by a single row of enlarged (7-15 mm) white-yellowish pigment spots in experimental insects whereas in controls, the pigment spots are smaller in size (1-8 mm) arranged in many rows and in specific pattern characteristic of the butterfly.

**Effect on Wing Shape:** Apical and anal margins of the fore- and hindwings were very much torn giving a grotesque shape to the wing in neemark treated butterflies (cf Figures 3, 4) while in multineem treated insects, wings were moderate and few much crumpled. These experimental butterflies were unable to fly and copulate.

Fig. 1

1cm

Fig. 2

1cm

Fig. 1: V instar larva showing heavy pigmentation after treatment with 50 µl dose of 2.5% multineem.

Fig. 2: Normal V instar larva

Fig. 3
1 cm

Fig. 4
1 cm

Fig. 3: Adult showing grotesque shaped wings, disappearance of red spot and enlarged white-yellowish pigment spots after treatment with 25 μl of 10% neemark.

Fig. 4: Normal adult.

Table 1: Effects of NBIs on the length of ovariole and number of oocytes per ovariole in V instar larvae of *P. demoleus* (Values are mean ± SD for 30 insects)

| Treatments | Conc.(%) | Dose (μl) | Length of ovariole (mm) | No. of oocytes per ovariole | % reduction in oocytes number |
|---|---|---|---|---|---|
| Neemark (0.03% Aza.) | 5.0 | 25 | 11.2±0.5* | 44±3* | 29 |
| | 5.0 | 50 | 10.6±0.3* | 38±2** | 38 |
| | 25.0 | 25 | 10.2±0.3* | 35±3** | 43 |
| | 25.0 | 50 | 8.6±0.2** | 28±4** | 55 |
| Multineem (0.03% Aza.) | 5.0 | 25 | 11.8±0.4* | 48±5* | 23 |
| | 5.0 | 50 | 10.7±0.5* | 39±4* | 37 |
| | 25.0 | 25 | 10.4±0.3* | 38±6** | 38 |
| | 25.0 | 50 | 8.2±0.6** | 26±3** | 58 |
| Control (Acetone treated) | | - | 13.2±0.8 | 62±6 | - |

Note: P values: *< 0.05; **< 0.01

**Effect on Oocyte Number:** Topical application of aforesaid NBIs caused significant reduction in the length of ovariole as well as number of oocytes depending upon dose and concentration (Table 1).

**Effect on Body Weight:** Late IV instar larvae (0.130g) treated with 50 μl dose of 2.5% of these NBIs moulted into V instar with a reduced body weight (0.100g). Such a V instar larva attained the maximum body weight of 0.850g and moulted into a miniature pupa with a delay of a day or two. Whereas newly emerged normal V instar larvae after treatment with above NBIs attained a maximum body weight of 1.180g in contrast to 1.530g in acetone treated controls and molted into pupae with reduced body weight.

## DISCUSSION

The neem based insecticides show varied effects on *P. demoleus* including regional variation with respect to their sensitivity on the insect body. The wing deformity observed in present study following application of NBIs is a kind of developmental abnormality and is assumed to be caused by disturbance in the endocrine functions of the treated insects [4, 9, 11, 14]. Regarding coloration, the pigment synthesis is to be influenced by juvenile hormone (JH), ecdysteriods and neurohormones as reported earlier by many researchers [15, 16]. Green body coloration is known to depend on corpus allatum (CA) and hence JH in many insects while darkening (melanization) is caused by neurosecretory cells (NSCs) of the central nervous system. It has been reported that JH deficiency inhibits melanization in *Leucania* [17] while decline of haemolymph ecdysteriod correlates with onset of melanization [18]. It is also reported that in lepidopterans, haemolymphatic ecdysone titer plays a decisive role, when it is low, pigments (melanin) are synthesized but when increased pigments lyse. Recently, Koch *et al.*, [19] reported that butterfly wing colour patterns are determined during late larval and early pupal development, a part of metamorphosis controlled by ecdysone hormone. It is reported that the synthesis and release of prothoracicotropic hormone (PTTH) by the brain was deficient in azadirachtin treated *Rhodnius prolixus* nymphs [20]. On the basis of their experiments Tiwari *et al.*, [9] found that NBIs treatment as a chemical stress affecting NSCs in the brain causing necrosis and death of hemocytes in *Dysdercus koenigii*. The heavy pigmentation (black, melanin) in NBI treated V instar larvae and a change in pigmentation pattern of wings observed in *P. demoleus* are thus suggested to be caused by the negative effects of these NBIs on NSCs of the brain and, in turn, CA and prothoracic gland activity and their hormones, JH and ecdysone respectively. Since heavy pigmentation was observed in only few fifth instar larvae, it is presumed that the time of release of respective hormone is more crucial. It might also be due to individual physiological difference amongst these larvae.

Further, our results reveal the reduction of body weight in NBIs- treated larvae. Late IV instar larvae attain a little body weight-gain in comparison to V instar larvae treated just after their emergence probably due to their exposure to these NBIs for a longer time. The reduction in body weight is also suggestive of their antifeedant property as reported earlier for azadirachtin treated cut-worm, *Peridroma saucia* [21] and NBI-treated plain tiger butterfly, *Danais chrysippus* [11]. The role of brain hormone (BH) released from insect brain and juvenile hormone (JH) from corpora allata in regulation of egg maturation by modulating vitellogenin synthesis (a female specific yolk protein from fat body which tends to be released later into haemolymph) and its incorporation in developing oocytes is well known [22-26]. Therefore, reduction in oocyte number is assumed to be caused by interference of NBIs with the above physiological processes by interrupting the release of these two hormones.

Conclusively, it is suggested that NBIs as a kind of chemical stress act negatively on insect endocrines and other vital organs. Besides, they cause abnormality in butterfly wings which are unable to copulate and hence threatening insect survival.


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