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EFFECT OF NEEM OIL ON THE DNA LEVELS IN THE OVARY OF CALLOSOBRUCHUS CHINENSIS (COLEOPTERA: BRUCHIDAE)

M. Madhavi*

Assistant Professor, Department of Zoology, Nizam College, Osmania University Hyderabad-500001.

***Corresponding Author: M. Madhavi**

Assistant Professor, Department of Zoology, Nizam College, Osmania University Hyderabad-500001.

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ABSTRACT

Callosobruchus chinensis is a menace to agricultural crop produces infesting pulses, and many other food products, hence an attempt was made to control the stored products pest by using medicinal plant extract Neem oil. The DNA levels in the ovary increased gradually in the larvae, pupae and the adults of Callosobruchus chinensis, whereas in the Neem oil treated resultant larvae there was a prominent decrease in the protein content when compared with the controls. The Neem oil is safe, cheap, residue free and ecofriendly that can fit into the IPM package of stored grain pests of pulses.

KEY WORDS: Neem oil, *Callosobruchus chinensis*, DNA, ovary, larvae, pupae and adult.

INTRODUCTION

Proteins are the first biological factors making their manifestation during development. During metamorphosis of an insect, process like destruction of certain larval tissue and rejuvenation and remoulding of various tissues into adult. One is bound to take place involving synthesis and consumption of the macro molecules as well (Venugopal & Dinesh Kumar 1997). The Fat body tissue plays a key role in storage proteins. Storage proteins increased during successive stages of development (Kanost *et al*., 1990 Rajathi *et al*. 2010).

DNA synthesis is a key biosynthetic pathway which operates actively during early larval development in holometabolous insects and is thought to be an important preparatory mechanism for active metabolic functions to be carried out later by different organs during late larval development (Dean *et al*., 1985). The resultant increase in nuclear volume and DNA are proportional to the increase in cell size. The studies on various tissues / organs show that there is a relationship between cellular DNA synthesis and capacity of the cells for differentiation (Bowers and Williams 1964; Krishna kumaran et al., 1967).Mitosis is necessary for a cell to change from one developmental stage to another, presumably to a more mature stage, Coccinelled beetles, Mosquito (Dittman et al.,1989) show that during each larval moult cycle there is a definite temporal pattern of DNA synthesis in various tissues (Anitha *et al*., 1999; Manjula, 2001, Anuradha *et al.*, 2010).

Red gram is an important pulse crop in India which is the major source of dietary protein for most of the vegetarian population of India. Post harvest losses during red gram storage are severe due to pulse beetle, *Callosobruchus chinensis* (F.) (Coleoptera:Bruchidae). It causes 30- 55 % grain losses in terms of both qualitative and quantitative constituents. *C. chinensis* is a serious cosmopolitan and polyphagous pest of stored pulses such as green gram, black gram, red gram, bean, cowpea, lentil, chickpea or other legumes. In the recent past, the preservation of pulses has relied heavily upon the insecticides to control the storage pests. But the increasing problems of resistance and residues of pesticides and contamination of biosphere have led the need for safer and eco friendly biodegradable pesticides. The present trend is towards the use of alternative environmental friendly and non-toxic control methods that pose no threat to the health of operator or consumer. It is demanding to develop the alternative methods that are economically feasible and ecologically safer to control the storage grain insects. The use of botanical pesticides is considered as one of the alternative substitute to hazardous chemicals. Among the botanicals, Neem is visualized as an eco-friendly pesticide having rich source of bioactive chemicals with a greater potential for use as successful pest control agent which can affect insects in several ways: they may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phago-stimulants, antifeedants or ovipositional deterrents, also retard or accelerate development or interfere with the life cycle of the insects. Hence, the present study was conducted to evaluate the efficacy of neem oil treated instars on the DNA levels in the Fat body of *Callosobruchus chinensism.*

MATERIALS AND METHODS

A rich standard culture of this insect was maintained in the laboratory. Freshly harvested, insect free and clean red gram variety was used for experimental purpose. Neem oil was used for experimentation. The Neem oil is diluted with acetone to obtain the required doses 1, 2, 3, 4, 5% concentration for the evaluation. Freshly moulted IV and V instar larvae were treated on the abdominal region with 1µL/larva of Neem oil dissolved in 2µl of acetone with the help of Hamilton micro syringe. 50 larvae were treated each time and the experiments were replicated 5 times. Controls were treated with 2µl of acetone. After treatments a suitable time gap of 5 minutes was given and they were transferred into diet. The treated larvae were observed daily to note the changes. Fat body is dissected and rinsed free of haemolymph with Ringers solution. 10% homogenate was prepared for the estimation of proteins and the protein was estimated by the method of Lowry *et al* (1951).

RESULTS

Statistical Analysis of the Data: The experimental data was analyzed statistically, mean and standard Deviation was calculated. The DNA level in the Ovary was estimated in the control of larval stages, pupa and Adult.

Ovarian DNA

Larval stages

On the first day of the V instar larvae (18 day old) larvae the DNA content in the ovaries recorded a value of 0.052±0.003 mg/gm weight of the tissue. There was a slight increase in the DNA content on the 2nd day showing 0.086±0.005 mg/gm weight of the tissue. On the 3rd day the DNA content further increased to 0.129±0.008 mg/gm weight of the tissue (Graph 1).

Pupal stages

The DNA content in the ovaries of the freshly pupated pupa was 0.142±0.009 mg/gm weight of the tissue. On the 2nd day the recorded value was 0.186±0.012 mg/gm weight of the tissue of DNA was recorded. It further increased to 0.201±0.013 mg/ gm weight of the tissue on the 3rd day. The DNA content further increased from 0.262±0.017 mg/gm weight of the tissue on the 4th day to 0.280±0.018 mg/gm weight of the tissue on the 5th day (Graph 1).

Adult stage

The first day of the adult stage recorded a value of 0.310±0.020 mg/gm weight of the tissue. The second day recorded a value of 0.342±0.022 mg/gm weight of the tissue. The third day recorded a value of 0.294 ±0.019 mg/gm weight of the tissue and 0.242±0.016 mg/gm weight of the tissue on the fourth day (Graph 1).

Statistical Analysis of the Data: The experimental data was analyzed statistically, mean and standard Deviation was calculated. The DNA levels in the Ovary was estimated in the treated of larval, pupa and Adult.

Ovarian DNA

Larval stage: The DNA content in the ovaries of the treated V instar resultant larval stages showed a marked decrease than in the controls. On the first day of the V instar larvae, the ovaries contained 0.022±0.002mg of DNA/gm weight of the tissue. It changed further to 0.029±0.001mg/gm weight of the tissue on the 2nd day. The DNA content further changed from 0.032±0.0021 mg/gm weight of the tissue on the last day of the V instar larvae. (Graph 1)

Pupal stage: On the first day of the pupal period the recorded value was 0.041±0.0027 mg/gm weight of the tissue. The 2nd day of the pupal period showed a marked change of 0.048±0.0032 mg/gm weight of the tissue. The third day noted a value of 0.053±0.0035 mg/gm weight of the tissue. It further changed from 0.062±0.0041mg/gm weight of the tissue on the fourth day to 0.061±0.0040 mg/gm weight of the tissue on the last day of the pupal period. (Graph 1)

Adult: The ovaries of the 5h instar larvae treated adult showed a significant decrease in the DNA content. The first day exhibited a value of 0.058±0.003 mg/gm and 0.045±0.003 mg/gm weight of the tissue on the 2nd day of the adult life. (Graph 1)

DISCUSSION

Callosobruchus chinensis with the Neem oil treated resultants showed a decline in the DNA content of the Ovary compared to the control larvae.

The increase in the DNA content of the ovaries coincided with increase in protein content during larval, pupal and adult development in the control insects. The increased amounts of DNA in the ovaries of the controls are probably associated with mitosis of ovarian tissues during maturation of ovaries (Vanderberg, 1963 and Telfer, 1965). Lobbecke (1969) showed that DNA synthesis is correlated with increased ecdysteroid titres. Lafont *et al*., (1977), confirmed this result.

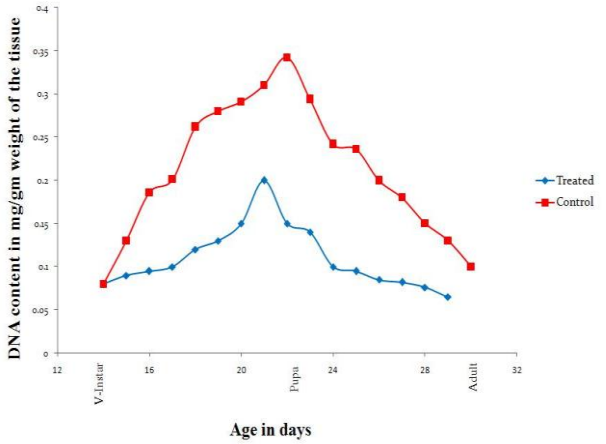
Neem oil acts antagonistic to that of 20-hydroxyecdysone at the target site epidermis, inhibiting ecdysis. This may be due to the fact that Neem oil inhibits mitosis thus inducing degeneration of cells, preventing growth, resulting in reduced levels of DNA in the tissues of the treated resultant *Callosobruchus chinensis*.

Depletion in the content of DNA in the ovaries of the resultant larvae, abnormal adults was noticed. This coincided with the reduced protein content seen in the same treated insects. The above observations clearly indicate that the ovaries are major target organs for Neem oil. Similar results were observed in *Epilachna varivestis* (Schluter, 1987).

In the Neem oil, treated insects, the cells of the ovary were smaller than the control larvae. The remnants of the

ovary cells dispersed through the haemolymph. Hence building material for further tissue synthesis was unavailable and the ovary which depends on the fat body proteins for growth, degenerated, showing abnormally reduced amounts of DNA.

The morphological observation and biochemical analysis of DNA, confirm the fact that Neem oil deranges the development of *Callosobruchus chinensis* by interfering with the hormonal milieu.



Graph.1: Quantitative changes in the DNA content of the ovaries of the control and Neem oil treated V instar resultant insects during the development of *Callosobruchus chinensis.*

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