

EFFECT OF *VITEX NEGUNDO* ON THE RNA LEVELS IN THE FAT BODY OF *CORCYRA CEPHALONICA*

M. Madhavi^{1*} and S. Sabita Raja²

^{1,2}Department Zoology, Nizam College, Osmania University, Hyderabad-500 001, Andhra Pradesh, India

E-mail: prsmadhavi@gmail.com

ABSTRACT

Corcyra cephalonica is a menace to agricultural crop produces infesting cereals, and many other food products, hence an attempt was made to control the stored products pest by using medicinal plant extract *Vitex negundo*. The RNA levels in the fat body increased gradually in the larvae, pupae and the adults of *Corcyra cephalonica*, whereas in the *Vitex negundo* treated resultant larvae there was a prominent decrease in the protein content when compared with the controls.

Key words : *Vitex negundo* , *Corcyra cephalonica*, RNA, fat body, 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, K.J, 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). RNA 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 RNA are proportional to the increase in cell size. The studies on various tissues/organs show that there is a relationship between cellular RNA synthesis and capacity of the cells for

differentiation (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 RNA synthesis in various tissues (Anitha et al., 1999; Manjula, 2001, Anuradha et al., 2010).

Vitex negundo is a small shrub or tree belonging to the family Verbenaceae. Leaves of this plant yield an essential oil used as a tonic and vermifuge and also in smoking for relief from catarrh and headaches. They are also used as insect repellents. (Dharmasri et al., 2003; Umamaheswari et al., 2007). *Vitex negundo* induces morphological changes and biochemical changes (Ignacimuthu 1998). The RNA levels in the fat body of *Corcyra cephalonica*, were studied in the *Vitex negundo* treated instars.

MATERIAL AND METHODS

A rich standard culture of this insect was maintained in the laboratory on normal dietary

medium composed of coarsely ground jowar (*Sorghum vulgare*) inside a glass container at $26\pm 1^{\circ}\text{C}$ temperature and $65\pm 5\%$ Relative humidity.

Preparation of crude leaf extract of VN:

Fresh leaves of *Vitex negundo* were collected, shade dried for a week and pulverized. The material was cold extracted in different solvents of Petroleum ether, Methanol, diethyl ether and acetone separately at room temperature for 24hrs and the extract was evaporated to dryness under reduced pressure. The extract was weighed, re-dissolved in a known volume of acetone for making different concentrations of the extract. Preliminary studies showed that the methanol extract to be most effective among all the three solvents. Hence the follow up study were conducted using methanol extracts.

Freshly moulted IV and V instar larvae were treated on the abdominal region with $1\mu\text{g/larva}$ of VN dissolved in $2\mu\text{l}$ 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 $2\mu\text{l}$ 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.

Statistical Analysis:

The experimental data was analyzed statistically, mean and standard Deviation was calculated. The RNA levels in the fat body were estimated in the control of larval stages, pupa and Adult.

RESULTS

Fat body RNA

Larval stages:

The RNA content in the fat body of *Corcyra cephalonica* showed a gradual increment. The RNA content on the first day of the III instar larvae (9 day old) larvae to the last day of the V instar larvae (20 day old) larvae ranged between

0.292 ± 0.019 mg/gm weight of the tissue and 0.782 ± 0.052 mg/gm weight of the tissue. The 2nd day of the III instars larvae (10 day old) larvae showed 0.324 ± 0.021 mg/gm weight of the tissue. It further increased to 0.390 ± 0.026 mg/gm weight of the tissue on the 3rd day of the III instar larvae (11day old) larvae. The RNA content increased to 0.420 ± 0.028 mg/gm weight of the tissue on the 1st day of the IV instar larvae (14 day old) larvae. It steadily increased from 0.542 ± 0.036 mg/gm weight of the tissue on the 1st day to 0.611 ± 0.040 mg/gm weight of the tissue on the 2nd day of the V instar (16 day old) larvae. The 3rd day of the V instar larvae (17 day old) larvae recorded a value of 0.636 ± 0.042 mg/gm weight of the tissue (Figure-1).

Pupal stage:

There was a increase in the fat body RNA content during the pupal period. The recorded value in the freshly pupated pupa was 0.812 ± 0.054 mg/gm weight of the tissue and increased to 0.884 ± 0.058 mg/gm weight of the tissue on the 2nd day of the pupal period. The fat body RNA content drastically decreased to 0.720 ± 0.061 mg/gm weight of the tissue on the 3rd day. The 4th day recorded a value of 0.690 ± 0.066 mg/gm weight of the tissue and increased to 1.080 ± 0.072 mg/gm weight of the tissue on the 5th day (Figure-1).

Adult:

The fat body RNA content in the freshly emerged adult was 1.110 ± 0.074 mg/gm weight of the tissue and it decreased to 1.024 ± 0.074 mg/gm weight of the tissue on the 2nd day of the adult life. The third day and on the fourth day recorded a value of 1.001 ± 0.066 mg/gm weight of the tissue and 0.824 ± 0.054 mg/gm weight of the tissue respectively (Figure-1).

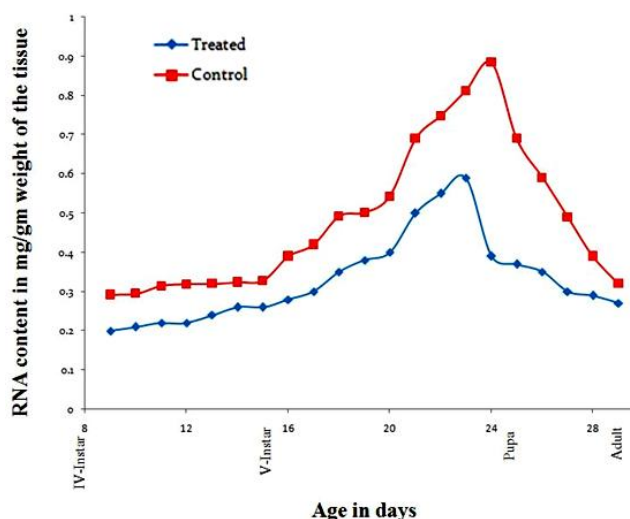
The experimental data was analyzed statistically, mean and standard Deviation was calculated. The RNA levels in the fat body was estimated in the treated of larval, pupa and Adult.

Larval stages:

The RNA content in the fat body of the treated resultant insects decreased drastically when compared to the controls. The RNA present in

the fat body of the treated insects on the first day of the III instar larvae recorded a value of 0.162 ± 0.010 mg/gm weight of the tissue. The RNA content increased from 0.184 ± 0.012 mg/gm weight of the tissue on the 2nd day of the III instar larvae to 0.202 ± 0.013 mg/gm weight of the tissue on the 3rd day of the III instar larvae. The RNA content increased further to 0.236 ± 0.015 mg/gm weight of the tissue on the 1st day of the IV instar larvae. It further increased from 0.260 ± 0.017 mg/gm weight of the tissue on the 2nd day of the IV instar larvae (13 days old) larvae to 0.272 ± 0.018 mg/gm weight of the tissue on the third day of the IV instar larvae (14 day old) larvae. On the 1st day of V instar larvae (15 days old) larvae the RNA increased from 0.293 ± 0.0195 mg/gm weight of the tissue on the 2nd day of the V instar larvae (16 days old) larvae. The RNA content further increased to 0.320 ± 0.0217 mg/gm weight of the tissue on the 3rd day of the V instar larvae (17 day old) larvae (Figure-1).

Figure-1. Quantitative changes in the RNA content of the fat body of the IV, V instar larva, pupa and Adult of the control and crude leaf extract of *Vitex negundo* treated IV instar resultant insects during the development of *Corcyra cephalonica*



Pupal period:

The RNA content on the first day of the pupal period recorded a value of 0.412 ± 0.02 mg/gm weight of the tissue. It decreased to 0.391 ± 0.018

mg/gm weight of the tissue on the second day. The third, fourth and fifth days recorded values of 0.388 ± 0.012 mg/gm weight of the tissue, 0.381 ± 0.011 mg/gm weight of the tissue and 0.294 ± 0.018 mg/gm weight of the tissue respectively (Figure-1).

Adult:

The RNA content on the 1st day of the treated adult recorded a value of 0.210 ± 0.010 mg/gm weight of the tissue. The second and third days recorded a value of 0.188 ± 0.008 mg/gm weight of the tissue and 0.102 ± 0.002 mg/gm weight of the tissue respectively (Figure-1).

DISCUSSION

Corcyra cephalonica were treated with crude leaf extract of *Vitex negundo* treated resultants showed a decline in the RNA content of the fat body compared to the control larvae. According to Enesco and Leblond (1962) any increase in RNA content would reflect the growth. The fat body, changes in the amount of RNA is correlated with increase in protein content. The increased amount of RNA in Aphid growth, suggests active mitosis. In holometabolous insect during moulting from one larval instar to next larval instar larvae involves the shedding of the cuticle, known as ecdysis. The form of the cuticle depends on the underlying epidermis, a major target organ of 20-hydroxyl ecdysone, a moulting hormone. The growth of the epidermis may occur through an increase in cell number or increase in cell size. Cell number increases just before moulting. An increase in size during larval life results, entirely from an increase in size of epidermal cells hence RNA also increases. Lobbecke (1969) showed that RNA synthesis is correlated with increased ecdysteroid titres. Lafont *et al.*, (1977), Dean *et al.*, (1985), confirmed this result.

The present study shows that the fat body RNA content increase during pupal development *Vitex negundo* acts antagonistic to that of 20-hydroxyecdysone at the target site epidermis, inhibiting ecdysis. This may be due to the fact that *Vitex negundo* inhibits mitosis thus inducing degeneration of cells, preventing

growth, resulting in reduced levels of RNA tissues of the treated resultant *Corcyra cephalonica*. The above observations clearly indicate that the fat body is major target organs for *Vitex negundo*. Similar results were observed in *Epilachna varivestis* (Achaiah, 2013); Schluter, 1987; Jayadev. D. J and Viveka Vardhani. V, 2013). The biochemical analysis of RNA, confirm the fact that *Vitex negundo* deranges the development of *Corcyra cephalonica* by interfering with the hormonal milieu.

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