Research Article

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

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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 DNA 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.

Keywords: *Vitex negundo, Corcyra cephalonica*, DNA, Fat Body, Larvae, Pupae & 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 remolding of various tissues into adult. One is bound to take place involving synthesis and consumption of the macro molecules as well (Venugopal and 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).

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 DNA levels in the Fat body of Corcyra cephalonica, were studied in the *Vitex negundo* treated instars.

MATERIALS AND METHODS

A rich standard culture of this insect was maintained in the laboratory on normal dietary medium composed of coarsely ground jowar (*Sorghum vulgar*) inside a glass container at 26±1°C temperature and 65±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 1µg/larva of VN 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.

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RESULTS

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

Fat Body DNA:

Larval Stages: A gradual rise has been observed in the fat body DNA level. On the first day of the III instar larvae (9days old) larvae from 0.263±0.022mg/gm weight of the tissue on the 3rd day of the V instar larvae. On the 2nd day of the III instar larvae of the DNA content was 0.273±0.018 mg/gm weight of the tissue. It increased further to 0.278±0.012 mg/gm weight of the tissue on the final day of the III instar larvae (11 day old) larvae. The 1st day of the IV instar larvae (12 day old) larvae showed 0.292±0.013mg/gm weight of the tissue. It increased further to 0.301±0.020 mg/gm weight of the tissue on the 2nd day of the IV instar larvae (13 day old) larvae. The DNA content further increased from 0.306±0.020 mg/gm weight of the tissue on the 3rd day of the fourth instar larvae (14 day old) larvae to 0.326±0.020mg/gm weight of the tissue on the 1st day of the V instar larvae (15 day old) larvae. There was prominent increase on the second day of the V instar larva (16 day old) larvae which recorded a value of 0.329±0.021mg/gm weight of the tissue. It further increased to 0.341±0.0222mg/gm weight of the tissue on the 3rd day of the V instar larvae (17 day old) larvae. There was a slight increase in the DNA content of the V instar larvae (18 day old) larvae.

0.440±0.029mg/gm weight of the tissue was recorded on the 1st day 0.462±0.032mg/gm weight of the tissue recorded on the 2nd day and 0.493±0.032mg/gm weight of the tissue on the 3rd day of the V instar larvae (20 day old) larvae (Graph 1).

Pupal stage: The DNA level in the fat body increased to 0.505± 0.033 mg/gm weight of the tissue on the day of pupation. It steadily decreased from the 2nd day of the pupal period. On the 2nd day the recorded value was 0.502± 0.034 mg/ gm weight of the tissue. The 3rd, 4th, 5th days recorded a value of 0.494±0.032 mg/gm weight of the tissue, 0.389±0.025 mg/gm weight of the tissue 0.401± 0.026 mg/ gm weight of the tissue respectively (Graph 1).

Adult stage: The first day of the adult period recorded a DNA content of 0.380±0.0118 mg/gm weight of the tissue. The second and third days recorded values of 0.371±0.029 mg/gm weight of the tissue and 0.368±0.029 mg/gm weight of the tissue respectively (Graph 1).

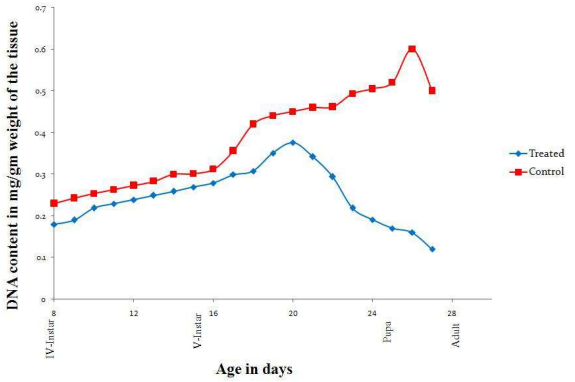
Statistical Analysis of the Data: The experimental data was analyzed statistically, mean and standard Deviation was calculated. The DNA levels in the Fat body was estimated in the treated of larval, pupa and Adult.

Larval stages: The DNA content in the fat body of the treated resultant larvae was low when compared with the controls. The DNA content in the fat body on the 1st day of the III instar larvae (9 days old) larvae recorded a value of 0.190±0.013mg/gm weight of the tissue. It slightly increased to 0.239±0.015 mg/gm weight of the tissue on the 2nd day of the III instar larvae (10 days old) larvae. The DNA content on the 3rd day of the III instar larvae was 0.249±0.0166 mg/gm weight of the tissue; it further increased from 0.258±0.0172 mg/gm weight of the tissue on the 2nd day of the IV instar larvae (13 days old) larvae. The 3rd day of the IV instar larvae (14 days old) larvae recorded a value of 0,294±0.0196 mg/gm weight of the tissue.

The DNA content further increased to 0.308±0.020 mg/gm weight of the tissue on the 1st day of the V instar larvae. The DNA content increased in the fat body from 0.312±0.0208 mg/gm weight of the tissue on the 2nd day of the V instar larvae to 0.321±0.0214 mg/gm weight of the tissue on the 3rd day of the V instar larvae (17 days old) larvae (Graph 1).

Pupal stage: The DNA level in the fat body of the treated resultant pupae recorded a value of 0.435±0.029 mg/gm weight of the tissue. It decreased to 0.342±0.0228 mg/gm weight of the tissue and on the second day DNA content further decreased from 0.294±0.0196 mg/gm weight of the tissue on the 3rd day to 0.219±0.0146mg/gm weight of the tissue on the 4th day of the pupal period. The DNA content on the last day of pupal period recorded a value of 0.191±0.0127 mg/gm weight of the tissue (Graph 1).

Adult: The DNA content in the fat body of the resultant adult showed a marked decrease of 0.093±0.0062mg/gm weight of the tissue on the first day and it further decreased to 0.082±0.0054 mg/gm weight of the tissue on the 2nd of the adult period. The third and fourth days recorded a value of 0.068±0.0034 mg/gm weight of the tissue and 0.040±0.0026 mg/gm weight of the tissue respectively (Graph 1).



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

DISCUSSION AND CONCLUSION

Corcyra cephalonica were treated with crude leaf extract of *Vitex negundo* treated resultants showed a decline in the DNA content of the fat body compared to the control larvae. According to Enesco and Leblond (1962) any increase in DNA content would reflect the growth. The fat body, changes in the amount of DNA is correlated with increase in protein content. The increased amount of DNA 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 DNA also increases. Lobbecke (1969) showed that DNA 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 DNA 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 DNA tissues of the treated resultant *Corcyra cephlonica*. The above observations clearly indicate that the fat body is major target organs for *Vitex negundo*. Similar results were observed in *Epilachna varivestis* (Schluter, 1987). The biochemical analysis of DNA, confirm the fact that *Vitex negundo* deranges the development of Corcyra *cephalonica* by interfering with the hormonal milieu.

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