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Research Article

VITEX NEGUNDO INDUCED PROTEIN CHANGES IN THE HAEMOLYMPH 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 protein content in the haemolymph increased gradually in the larvae, pupae and the adults of *C. cephalonica*, whereas in the *V. negundo* treated resultant larvae there was a prominent decrease in the protein content when compared with the controls.

Key words: *Vitex negundo, Corcyra cephalonica*, haemolymph, larvae, pupae.

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 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). Proteins are synthesized in the fat body and released into the haemolymph to be incorporated later into various organ including ovaries (Vallae1993).

V.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). *V. negundo* induces morphological changes and biochemical changes (Ignacimuthu, 1998). The Fat body protein content of *C. cephalonica*, were studied in the *V.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^{0}C temperature and 65±5% Relative humidity.

1. Preparation of crude leaf extract of *VN*

Fresh leaves of *V. 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.

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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.

Statistical Analysis of the Data: The experimental data was analyzed statistically, mean and standard Deviation was calculated. The haemolymph proteins was estimated in the control of IV instar larva, V instar larva, pupa and Adult.

RESULTS

1. Protein content of the haemolymph of *C. cephalonica*

IV instar larva

The protein content of the haemolymph of *C. cephalonica* was estimated in the IV instar larva; from the 1st to the 7 th day. A gradual increase in protein content was observed. On the 1st day of IV instar 1.025 ± 0.027 mg of protein / ml of haemolymph was recorded. The value recorded on the 4 th day was 1.25± 0.032 mg/ml which further increased to 2.052± 0.035 mg/ml on the 7th day if the IV instar.

V instar larva

On the 1st day of the V instar, 2.075 ± 0.0253mg of protein/ml of haemolymph were recoded. It increased to 2.711±0.0369 mg/ml on the 6th day. It further increased to 2.9375± 0.0375 mg/ml on the 9th day and is slowly declined to 2.357± 0.034mg/ml on the 10th day.

Pupa

It was observed that the protein content of haemolymph showed a steady decline. The value recorded on the 1st day was 1.984 ± 0.032 mg/ of protein /ml of haemolymph. Then it steadily decreased to 0.985±0.023mg/ml on the 7th day.

Adult

The freshly emerged adult recorded a value of 0.0724±0.024 mg of protein /ml of haemolymph proteins. The value decreased to 0.321±0.019 mg/ml of haemolymph on the 2nd day. There was a steady decrease and the last day of the adult recorded a value of 0.19±0.0154 mg/ ml of haemolymph proteins.

2. IV instar larva treated with crude leaf extract of *V.negundo*

IV instar larvae of *C. cephalonica* treated with crude leaf extract of *V. negundo* exhibited a decrease in the content of haemolymph proteins as compared to the control. The protein content in the haemolymph on the 2nd day of the treated IV instar was 1.027±0.0282mg/ml. The 5th day recorded a value of 1.056±0.02852 mg/ml of protein content. The 7th day of the IV instar showed a value of 1.102±0.0287 mg/ml of haemolymph. This value was much less when compared to the value of 2.0520±0.035 mg/ml.

V instar

There was gradual increase in protein content in haemolymph. On the 1st day, the recorded value showed 1.238±0.0289 mg/ml. It increased to 1.345±0.032mg/ml of haemolymph on the 7th day .The value was comparatively less than the control value 2.711 mg/ml of haemolymph. The 10th day of the V instar recorded a value of 1.012± 0.028 mg/ml of haemolymph indicating a decrease in haemolymph protein content.

Pupa

The resultant pupa of the treated IV instar exhibited a steady decrease in the protein content in the haemolymph. The value recorded on the 1st day being 0.987± 0.0274 mg/ml of haemolymph and 0.921±0.0235 mg/ml of haemolymph on the 4th day. It was 0.624±0.015 mg/ml of haemolymph on the 7th day of the pupa.

Adult

The haemolymph protein content in the resultant adults steadily decreased. The recorded value on the 1st day was 0.465±0.018 mg/ml of haemolymph and 0.213±0.013 mg/ml of haemolymph on the 2nd day. It was 0.092±0.095 mg/ml of haemolymph on the 5th day (Figure 1).

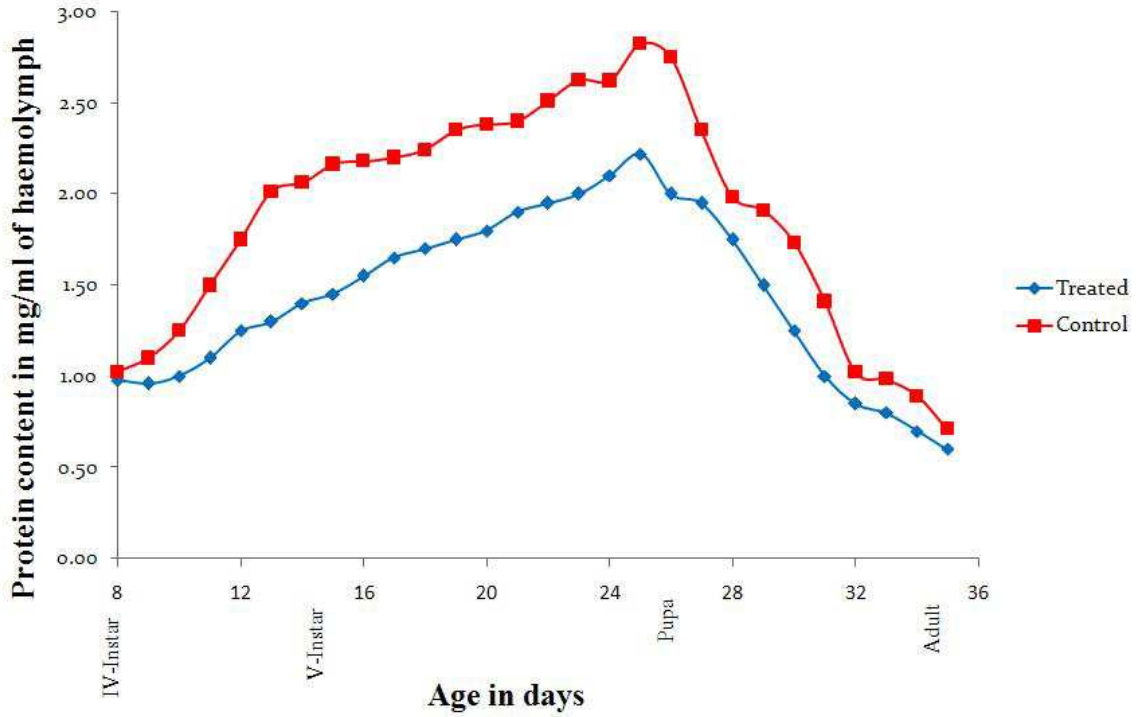

Figure 1. Quantitative changes in the protein content of the Haemolymph of the IV, V instars, pupa and adult of the control insect and crude leaf extract of *V.negundo* treated IV instar insect during the development of *C. cephalonica.*

3. Haemolymph proteins in control insects

V instar

The haemolymph proteins of the V instar of *C. cephalonica* were estimated from 1st day of the instar to the 10th day. On the 1st day of the V instar larva, the protein content recorded was 2.075 ±0.034 mg/ml.

There was a slow increase in the haemolymph content, the values being 2.565± 0.037 mg/ml on the 5th day and 2.935± 0.0373 mg/ml of haemolymph on the 9th day. There was a decrease on the 10th day and the value recorded was 2.350± 0.036 mg/ml of haemolymph.

Pupa

The recorded value on the 1st day of the pupa was 1.984± 0.031 mg/ml of haemolymph. The haemolymph protein content steadily decreased and the observed value on the 7th day was 0.985±0.027 mg/ml of haemolymph.

Adult

The haemolymph protein content of the adult on the 1st day was 0.724±0.0269 mg/ml. The values recorded showed steady decrease and it was 0.19± 0.013mg/ml of haemolymph on the 5th day.

4. Haemolymph proteins treated V instar larva with crude leaf extract of *V.negundo*

The V instar of *C. cephalonica* treated with crude leaf extract of *V.negundo* showed a decrease in haemolymph protein content as compared to the control. The value recorded on the 1st day was 2.075±0.034mg/ml of haemolymph. There was significant difference from 6th day onwards, the

value recorded was 2.195± 0.035 mg/ml of haemolymph in the treated as against 2.625 mg/ml of haemolymph in the control. The haemolymph proteins decreased on the 10th day and it was 2.030±0.032 mg/ml of haemolymph.

Pupa

The values recorded showed a steady decrease in haemolymph proteins from the 1st day of the pupal stage. The 1st day recorded 0.983±0.027 mg/ml of haemolymph and the 7th day 0.15± 0.009 mg/ml of haemolymph in control pupa.

Adult

The resultant emerged adult showed 0.09±0.009 mg/ml of haemolymph protein. It decreased further and the estimated value on the 5th day was 0.020± 0.0025mg/ml of haemolymph (Figure 2).

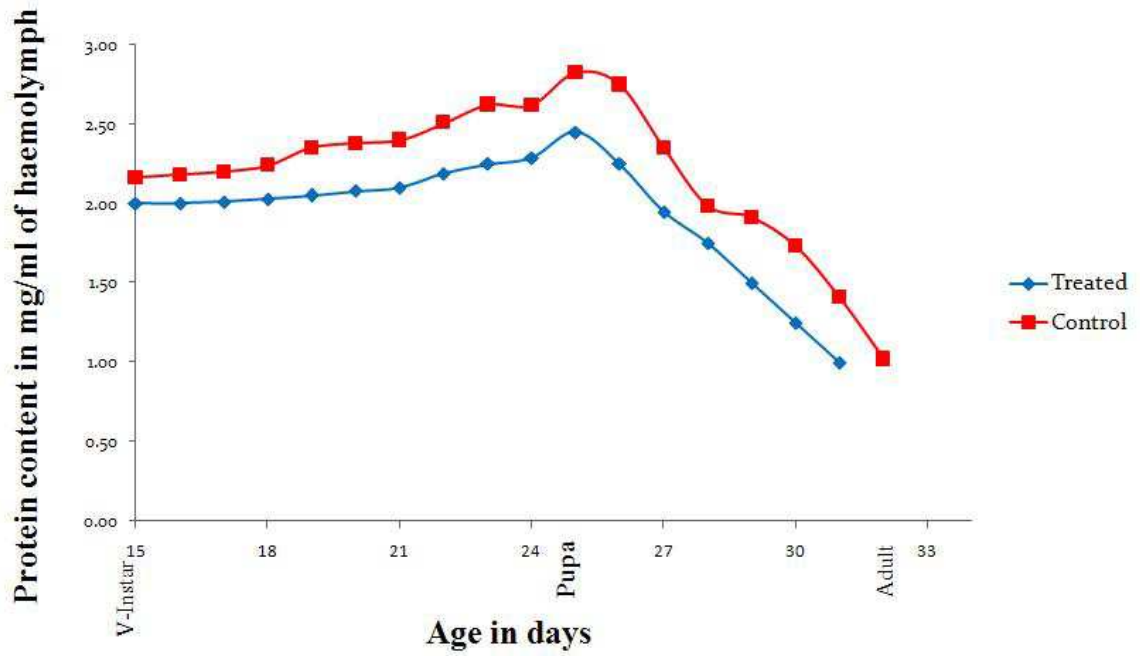

Figure 2. Quantitative changes in the protein content of the Haemolymph of the V instar, pupa and adult of the control insect and crude leaf extract of *V.negundo* treated V instar insect during the development of *C. Cephalonica.*

DISCUSSION

C. cephalonica IV instar larva were treated with crude leaf extract of *V. negundo* treated resultants showed a decline in the protein content when compared to the control larvae. This may be due to the *V.negundo* functioning as a molting hormone analogue. As such it may interfere with neuroendocrine control of molting hormone synthesis. The protein content in the haemolymph of *C. cephalonica* exhibited a steady increase and the increase was markedly accelerated during the pre-pupal stage of development on the contrary, the protein concentration of the haemolymph increased gradually during larval development and reaches its highest value in the last instar larvae but decline during the pre-pupal and early pupal stages of development. Our results are in correlation with those of (Anitha *et al.*, 2000; Banks and Malacoln, 1994) there was a gradual decline in the protein content of the treated resultant *C. cephalonica* during the course of development. The disturbance in the hormonal imbalance inhibited protein synthesis in the fat body these results are in concurrence with that of the Raja *et al.* (1986).

CONCLUSION

Administration of *V. negundo* controlled the stored product pest *C. cephalonica* by influencing the moulting hormone. Thus, raising hope for its practical application in the stored grain pest management.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

REFERENCES

Anitha, H.R., Raja, S.S., Renuka, S. and Manjula, C., 2000. Effect of precocene-II on the protein changes in the haemolymph, fat body and Ovaries of *Chilo partellus*, during ontogenesis. *Convergence*, 2(1): 18-23.

Banks, G.A., Malacoln, G.A., 1994. Temporal pattern of RNA and Protein synthesis in the ovary of *Aedes aegypti*. *J. Insects Physiol.,* 22: 299-397.

Dharmasri, M. G., Jayakody, J.R.A.C. and Galhena, G., 2003. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. *J. Ethnopharmacol.,* 87: 199-202.

Kanost, M.R., Dawooga, J.K., Ryan, R.O, Husden M.D., Zeilger, R., 1990. Insect haemolymph proteins. *Insect Physiol*., 22: 299-397.

Ignacimuthu, S., 1998. Nature's ecofriendly arsenal of pesticides. *Curr. Sci.,* 74: 1037.

Lowry, O.H., Rosebrough,, J..J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.,* 193: 263-275.

Umamaheshwari, M., Ashok Kumar, K and Somasundaram, A., 2007. Xanthine oxidase inhibitory activity of some Indian medical plants. J. *Ethnopharmacol.,* 109(3): 547-551.

Raja, S.S., Thakur, B., Kishen, R., and Kaur, A. 1986. Selective accumulation of haemolymph proteins by fat body during larval pupal transformation of *Chilo partellus*. *Entomol. Bohemoslov.,* 154:205-208.

Rajathi. A., Pandiaajan J., Krishnan,M., 2010. Effect of RH-2485 on the development, metamorphosis and synthesis of major proteins in female silkworm *Bombyx mori*. *Biologia*, 65(5): 903-913.

Vallae, D., 1993. Vitellogenesis in insects and other groups A review, *Membr Inst. Oswald, Cruz,* 88: 1-26.

Venugopal, K.J., Dinesh Kumar, 1997. Electrophoretic studies on the development profiles of protein in haemolymph, fat body and ovary of red cotton bug, *Dysdercus Koenigii*. *Entomon,* 22: 185-191.