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Vitex negundo induced protein changes in the haemolymph of *Tribolium confusum* (Coleoptera: Tenebrionidae)

[*] Dr. M Madhavi, Dr. V Srinivasa Rao
Department of Zoology, Nizam College, Osmania University, Hyderabad, Telangana, India

Abstract
Tribolium confusum is a Serious pest to agricultural crop produces infesting cereals, and many other food products, thus causing heavy damage to the food stuffs and useless for human consumption. 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 *Tribolium confusum*, 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, Tribolium confusum*, 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 [5, 11]. The Fat body tissue plays a key role in storage proteins. Storage proteins increased during successive stages of development [4, 9]. Proteins are synthesized in the fat body and released into the haemolymph to be incorporated later into various organ including ovaries [10].

Vitex negundo is used as a folk medicine. Medicinal properties of V.negundo have attracted the attention of plant physiologists and chemists. *Vitex negundo* is known to be insect repellent, insecticidal [7], larvicidal, mosquito repellant [9] and antifeedant anti-bacterial, anti-fungal. The fat body protein content of *T. confusum*, 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 pulses, green gram inside a glass container at 26±1^{0}C temperature and 65±5% Relative humidity.

Plant material and Extraction of *Vitex negundo* crude leaf Extract
Fresh leaves of the *Vitex negundo* (VN) were collected, shade dried for a week and pulverized. The material was cold extracted in different solvents of petroleum ether, methanol, diethylether, and acetone separately at room temperature for 48 hrs 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 studies 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 [6].

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
Estimation in control insects
Haemolymph proteins
IV instar larva
The protein content of the haemolymph of Tribolium confusum was estimated in the IV instar larva; from the 1st to the 7th day. A gradual increase in protein content was observed. On the 1st day of the IV instar 1.025±0.028 mg/ml of proteins was recorded in haemolymph. The value recorded on the 4th day was 1.250±0.031 mg/ml which further increased to 2.0620±0.035mg/ml on the 7th day of the IV instar (Figure 1).

V instar
The 1st day of the V instar showed a value of 2.075±0.0353mg of protein /ml. It increased to 2.625±0.0369mg/ml on the 6th day. It futher increased to 2.9375±0.0375mg/ml on the 9th day and is slowly declined to 2.350±0.034mg of protein /ml on the 10th day (Figure 1).

Pupa

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

Adult

The freshly emerged adult recorded a value of 0.724±0.024mg/ml of haemolymph proteins. The value decreased to 0.321±0.019mg/ml 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 (Figure 1).

Estimation of Haemolymph proteins in the larvae of *Tribolium confusum* treated with extract of *Vitex negundo*

Treated Insects

Haemolymph Proteins

IV instar larva

The effect of extract of *Vitex negundo* on Triboilum confusum larvae showed a decrease in haemolymph proteins when compared to the control.

The haemolymph proteins started increasing from the 3rd day. The recorded value was 1.03±0.0281 mg/ml. The value recorde on the 5th day was 1.058±0.0284mg/ml as compared to 1.642mg/ml in control. The protein content on the 7th day was 1.12±0.0289mg/ml (Figure 1).

V instar

The haemolymph protein content steadily increased till the 9th day of the larva. The 1st day of larva showed 1.124±0.0284mg/ml of protein content. The protein content increased to 1.324±0.02951mg/ml on the 5th day of the V instar. It reached the maximum on the 9th day,1.381±0.032mg/ml and decreased to 0.9254±0.029mg/ml on the last day of the V instar (Figure 1).

Pupa

There was a steady decrease in the protein content of the pupa. The value recorded on the 1st day was 0.921±0.0281mg/ml. It decreased to 0.201±0.0185 mg/ml on the last day of the pupa (Figure 1).

Adult

The treated resultant adults' showed a decrease in haemolymph proteins when compared to control adults. The recorded value was 0.183±0.0189mg/ml on the 1st day and 0.11±0.014mg/ml on the 2nd day and 0.095±0.099 mg/ml on last day (Figure 1).

Haemolymph Proteins

Estimation in control insects

V instar

The haemolymph proteins of the V instar of *Tribolium confusum* estimated from the 1st day of the instar to the 10th day. On the 1st day of the 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 2.9375±0.0373 mg/ml on the 9th day. There was a decrease on the 10th day and the values recorded were 2.350±0.036 mg/ml (Figure 2).

Pupa

The recorded value on the 1st day of the pupa was 1.984±0.031 mg/ml. The haemolymph protein content steadily decreased and observed value on the 7th day was 0.985±0.027 mg/ml (Figure 2).

Adult

The haemolymph protein content of the adult on the 1st day was 0.724±0.0269 mg/ml. The protein values recorded showed steady decrease and it was 0.19±0.013 mg/ml on the 5th day (Figure 2).

Estimation of protein in treated resultant *Tribolium confusum* larva.

Estimation in the treated insects

V instar

V instar treated with crude bulb extract and the resultant pupa and adult showed a decrease in protein content as compared to the control. The recorded value of haemolymph protein on the 1st day was 2.075±0.034 mg/ml. The 6th day recorded a value of 2.399±0.036 mg/ml and it decreased to 2.041±0.0315 mg/ml on the last day of the V instar (Figure 2).

Pupa

There was a steady decrease in haemolymph proteins in the treated resultant pupa stage. The value recorded on the 1st day was 1.062±0.029mg/ml. it decreased to 0.912±0.020 mg/ml on the 6th day and further decreased to 0.541±0.018 mg/ml on the 7th day (Figure 2).

Adult

The recorded value of haemolymph proteins on the 1st day was 0.12±0.013 mg/ml which steadily decreased to 0.058±0.0031 mg/ml on the 4th day and to 0.04±0.0026 mg/ml on the last day (Figure 2).

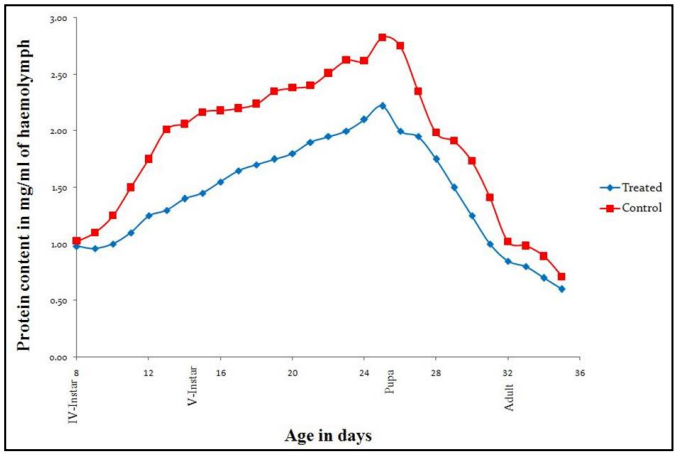

Fig 1: Quantitative changes in the protein content of the haemolymph of the IV, V instars, Pupa and Adult of the control insect and leaf extract of *Vitex negundo* treated IV instar insect during the development of *Tribolium confusum*

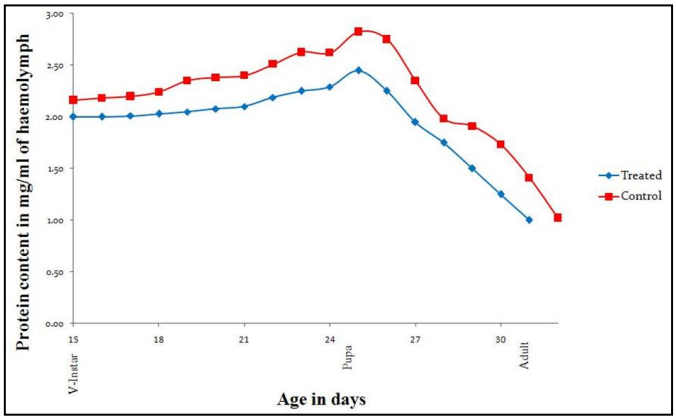

Fig 2: Quantitative changes in the protein content of the haemolymph of the V instar, pupae and Adult of the control insect and leaf extract of *Vitex negundo* treated V instar insect during the development of *Tribolium confusum*

Discussion

Tribolium confusum V instar larva were treated with *V.negundo* treated resultants showed a decline in the protein content when compared to the control larvae. This may be due to the *Vitex 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 *T. confusum* exhibited a steady increase and the increase was markedly accelerated during the pre-pupal stage of development on the contrary, the protein concentration [3] 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 [1, 2] there was a gradual decline in the protein content of the treated resultant *T. confusum* during the course of development. The disturbance in the hormonal imbalance inhibited protein synthesis in the ovary these results are in concurrence with that of the [6, 8]. Administration of *Vitex negundo* controlled the stored product pest *T. confusum* by influencing the moulting hormone. Thus, raising hope for its practical application in the stored grain pest management.

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