

## Studies on Biochemical Changes in the Total Protein and Free Aminoacid Content of Haemolymph of Sixth Instar Larvae of *Orthaga exvinacea* Hampson Due to the Effect of *Hyptis suaveolens* and *Vitex negundo*



## Science

**KEYWORDS :** Haemolymph, *Orthaga*, botanicals.

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### ABSTRACT

*Among the pests of mango trees, the mango leaf webber, Orthaga exvinacea is a major defoliator pest. Considering the economic loss caused by the pest, due to low productivity, a study was carried out to estimate the influence of two botanicals on protein and aminoacid concentration of haemolymph. Five different concentrations of methanolic extracts of Hyptis suaveolens and Vitex negundo leaf extracts were used for treating mango leaves to feed experimental larvae. Haemolymph was collected from both control and treated larvae for estimation of protein and aminoacid content. Compared with control, leaf extracts of both plants can significantly reduce protein and aminoacid concentration of haemolymph of experimental larvae. Vitex negundo extract was more efficient in decreasing the total protein and free amino acid content of haemolymph than Hyptis suaveolens. Since these botanicals affect the biochemical profile of this pest they may be used in the management of Orthaga exvinacea.*

### INTRODUCTION

The harmful effects of chemical insecticides have facilitated the search for ecofriendly approach for pest control. Botanicals or plant derived pesticides degrade rapidly and are recommended as safer alternatives to common synthetic chemical pesticides. Hillock & Bolin reported that these botanicals either act as contact poison when sprayed on the pest or as stomach poison when applied through the feeding material of the pest. The action of botanicals is very quick by causing cessation of feeding of plant material by the insect pests, but they may not cause immediate death of the pest. Scott *et al.* (2003) reported that negative effects of chemical insecticides on non-target organisms caused a general perception that plant derived compounds are better alternatives or Generally Regarded As Safe (GRAS).

*Orthaga exvinacea*, the mango leaf webber is a serious harmful pest of mango trees. Heavy infestation by the pest results in lowest crop productivity and also complete failure of flowering (Vergheze, 1998). The infestation of *Orthaga* starts from June and continues up to December and heavy infestation is observed in September (Reddy, 2013). Heavy infestation by the pest makes trees without healthy leaves and this in turn significantly decreases the photosynthesis leading to low productivity. In order to manage the particular pest, a study was conducted to analyse the efficacy of two botanicals on the protein and amino acid content of haemolymph of *O. exvinacea*.

### MATERIALS AND METHODS

#### Rearing of *Orthaga*

The larvae and pupae of *Orthaga* were collected from the field, reared and maintained in laboratory conditions (28±2°C: Rh-70-80%) feeding with mango leaves. The larvae were reared in plastic troughs and bottles kept inside rearing cages.

#### Preparation of plant extracts

The botanicals used for the study were *Vitex negundo* and *Hyptis suaveolens*. Leaves of both plants were collected, washed with water and shade dried. These dried leaves were crushed into fine powder form in an electric mixer grinder and sieved through a muslin cloth. This powder was used for preparing solvent extracts. Methanol is used as a solvent for leaf extraction, using Soxhlet apparatus.

#### Method of extraction

About 50gms of leaf powder was used for extraction with methanol as solvent in a Soxhlet apparatus. The extract was evaporated in a glass trough and the final paste was weighed and 10% stock solution was prepared. From this stock solution different concentrations (1%, 2%, 3%, 4% & 5%) were prepared using methanol.

#### Estimation of total protein and amino acid content in haemolymph.

Early stages of sixth instar larvae were used for collecting haemolymph samples. Haemolymph samples of treated and control were collected using capillary tubes by amputating thoracic legs. Experimental larvae were fed with five different concentrations of botanicals treated mango leaves and control larvae were fed with mango leaves treated with methanol. 0.1ml of haemolymph sample was taken for analysis of total protein and total free amino acid content. Each experiment was replicated five times. The protein in the sample was precipitated and centrifuged. The residue was used for analysis of protein by Lowry's protocol (Lowry *et al.* 1951) using spectrophotometer. The supernatant was collected for estimation of free amino acids by Lee and Takahashi method (Lee and Takahashi, 1966) using spectrophotometer.

#### Data analysis

Data from different biochemical assays were statistically analyzed by ANOVA using SPSS 16 package. All experimental data were subjected to one way ANOVA to determine significant differences between samples using post hoc test (Scheffe test).

### RESULTS AND DISCUSSIONS

#### Effect on total protein concentration in haemolymph

The effect of botanicals on the protein concentration in control and each experimental sample is given below (Table 1 & Table 2). Decrease in protein concentration due to the effect of botanicals was also expressed in graphical form (Fig 1 & Fig 2).

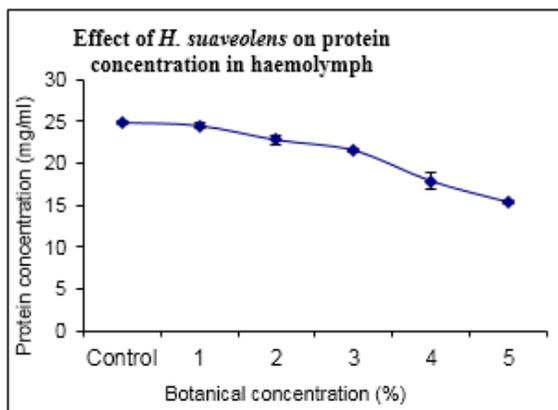


Fig.1- Effect of *H. suaveolens* on protein concentration in haemolymph

Table 1. Showing the effect of leaf extract of *H. suaveolens* on protein concentration in the haemolymph of sixth instar larvae of *O. exvinacea*.

Concentration of botanicals	Protein concentration	
	mg/ml	mg/larva
Control	24.9±0.21	3.56±0.03
1%	24.52±0.38	3.51±0.05
2%	22.81±0.58	3.26±0.08
3%	21.56±0.31	3.08±0.04
4%	17.88±0.97	2.55±0.14
5%	15.37±0.24	2.2±0.03

Values expressed as - Mean±S.D. Significance level – P<0.01

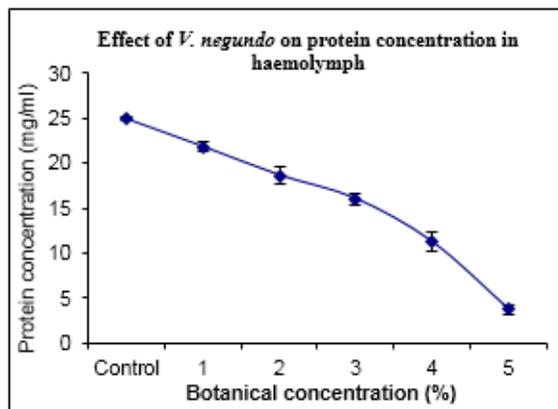


Fig.2- Effect of *V. negundo* on protein concentration in haemolymph

Table 2. Showing the effect of leaf extract of *V. negundo* on protein concentration in the haemolymph of sixth instar larvae of *O. exvinacea*.

Concentration of botanicals	Protein concentration	
	mg/ml	mg/larva
Control	24.9±0.21	3.56±0.03
1%	21.82±0.58	3.12±0.08
2%	18.65±0.96	2.66±0.14
3%	16.03±0.62	2.3±0.09
4%	11.22±1.11	1.61±0.16
5%	3.7±0.54	0.53±0.07

Values expressed as - Mean±S.D. Significance level – P<0.01

Results shows that the leaf extracts of *H. suaveolens* and *V. negundo* influence the total protein content of haemolymph of sixth instar larvae of *O. exvinacea*. Both phytoextracts significantly reduced the total protein concentration of haemolymph. Among these two plants *Vitex* was found to be more potent in reducing the haemolymph protein content of larvae.

Effect on total free amino acids in haemolymph.

The effect of botanicals on the amino acid concentration of haemolymph of control and experimental sixth instar larvae are given below (Table 3 & Table 4). Decrease in amino acid concentration due to the effect of botanicals was also expressed in graphical form (Fig 3 & Fig 4).

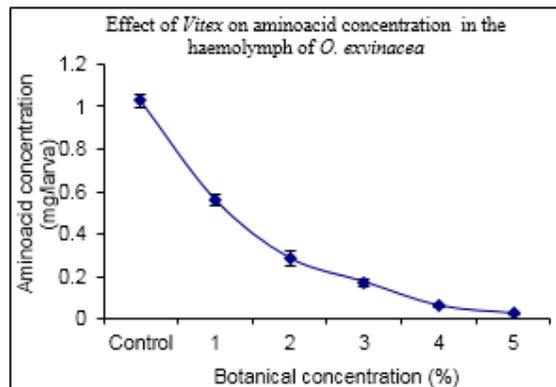


Table 3. Showing the effect of leaf extract of *V. negundo* on total free amino acid concentration in the haemolymph of sixth instar larvae of *O. exvinacea*.

Concentration of botanicals	Amino acid concentration	
	mg/ml	mg/larva
Control	5.13±0.15	1.03±0.03
1%	2.8±0.14	0.56±0.027
2%	1.41±0.19	0.28±0.04
3%	0.87±0.09	0.17±0.02
4%	0.3±0.03	0.06±0.007
5%	0.11±0.03	0.024±0.005

Values expressed as - Mean±S.D. Significance level – P<0.01

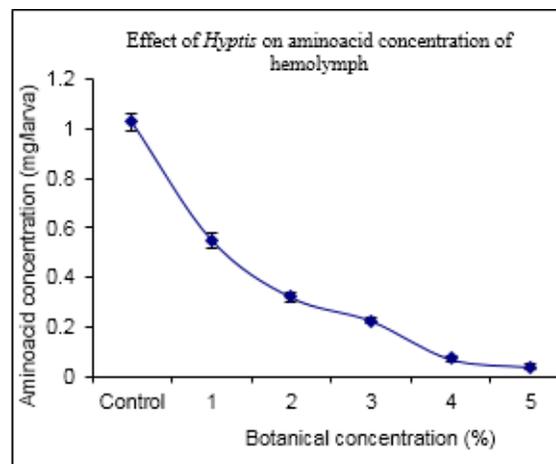


Fig. 4 –Effect of *Hyptis* on total free amino acid concentration of haemolymph

**Table 4. Showing the effect of leaf extract of *H. suaveolens* on total free amino acid concentration in the haemolymph of sixth instar larvae of *O. exvinacea*.**

Concentration of botanicals	Amino acid concentration	
	mg/ml	mg/larva
Control	5.13±0.15	1.03±0.03
1%	2.76±0.15	0.55±0.03
2%	1.61±0.09	0.32±0.02
3%	1.13±0.05	0.23±0.01
4%	0.36±0.06	0.07±0.01
5%	0.2±0.076	0.04±0.014

Values expressed as - Mean±S.D. Significance level – P<0.01

The total free amino acid concentration of haemolymph was very high in the control larvae when compared with the experimental larvae. Both plants were found to be highly efficient in reducing the haemolymph amino acid content of the larvae.

It was found that the total protein and free amino acid concentration in the haemolymph of sixth instar larvae was decreased due to the effect of botanicals. Etebari *et al.* (2005) reported that many insecticides decreased feeding potential and protein amount of an insect's body. Schmidt *et al.* (1998) revealed that treatment of *Spodoptera littoralis* and *Agrotis ipsilon* with azadirachtin decreased protein content of haemolymph. This could be due to the dissociation of protein into amino acids, these in turn enter in to TCA cycle as a keto acid, which provides energy for the insect (Nath *et al.* 1997). So protein reduction in tissues may constitute a physiological mechanism that could play a role in compensatory mechanisms for insecticidal stress to provide intermediates to the Krebs cycle by retaining free amino acids in haemolymph (Etebari *et al.* 2005). Besides this, the antifeedant properties of botanicals also influence the reduction of nutrients in the insects. Medhini *et al.* (2009) reported that petroleum ether and chloroform extracts of leaves and flowers of *Calendula officinalis* showed significant impact on the nutrient components of *Spodoptera litura*.

## Conclusions

The synthesis, storage and utilization of proteins in insects alter during different developmental stages and also during varied physiological conditions under the influence of different hormones. Morya *et al.* (2010) reported that under adverse conditions like starvation, stored proteins are utilized by the insects. Khosravi *et al.* (2011) observed a marked decline in the amount of total protein in larvae of *Glyphodes pyloalis* treated with *Artemisia annua* methanolic extract. All these indicate that plant derived insecticides have the ability to influence the proportion of various chemical components in the body of insects, thus altering the internal metabolism of the insect causing their reduced activity or mortality.

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