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Botanical extracts influence the protein and free amino acid concentration of fat body in the mango leaf webber *Orthaga exvinacea* Hampson (Lepidoptera: Pyralidae)

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Abstract
Orthaga exvinacea is a major defoliator of mango trees causing reduction in crop yield. Considering the importance of the pest a study was initiated to control the particular pest using botanicals. Evaluation of the potential of methanolic leaf extracts of *Hyptis suaveolens* and *Vitex negundo* was done on protein and amino acid concentration in fat body of *Orthaga exvinacea* under laboratory conditions. Botanical and methanol treated mango leaves were used for feeding experimental and control larvae respectively. Compared with Control, the mean values of the total fat body protein and amino acid were reduced in larvae treated with both plant extracts. A significant decrease was observed in fat body protein and amino acid contents of larvae treated with *Vitex negundo*, at the highest dose of 5%. Since both botanicals can effectively reduce fat body protein and amino acid content it will add a new spectrum in the control measures of *Orthaga exvinacea*.

Keywords: Botanicals, total protein, free amino acid, mango pest, fat body.

1. Introduction
Orthaga exvinacea, defoliator pest of mango trees is a lepidopteran insect responsible for low productivity. Severe infestation was caused by the caterpillars of the pest in September [1]. The caterpillar pertains in the webbed leaves of mango tree and feed on the green matter of leaves causing defoliation. Adult moth is greyish with dark patches on the wings. The eggs are laid singly or in small clusters on the silken webbing or ribs of leaves. In the initial stage of this pest a webbed cluster of leaves may harbour several larvae. Initially the larvae feed by scraping the leaf surface gregariously. The last instar larvae are found to feed individually on the whole leaf lamina leaving only the midrib. They remain feeding within the tunnels of these webs. Verghese reported that the severe infestation causes a burnt look for the trees and it results in complete failure of flowering [2]. Severe infestation by this pest affects the flower as well as the growth of new flush [3]. As a result of heavy infestation, the fully defoliated shoots will not fruit in the coming season [4].
Phytochemicals are considered as ideal alternatives to hazardous and non-biodegradable inorganic pesticides [5]. Many plants have chemical substances with tremendous insecticidal properties which provide resistance against pests and herbivores [6]. Many alkaloids and flavonoids have been reported in many plants with pesticidal properties [7]. Since they possess such phytochemicals usage of plant materials in powder or crude extract form should be accepted ecologically as biopesticides [8]. Many of the plant components are reported to act as antifeedants, depressants and growth regulators or to impair the immune system of the insects [9-12].
Plant based insecticides are reported to have the capacity to influence the proportion of various biochemical components like carbohydrates, proteins and lipids in the body of insects; thus interrupting the metabolism of the insect leading to their reduced activity and mortality [13]. Synthesis, storage and consumption of proteins and glycogen differ during different developmental stages of an insect. Insect fat body is having an essential role in energy storage and utilization. Fat body cells are responsible for the synthesis of most of the haemolymph proteins and circulating metabolites. Keeley reported that large quantity of relevant proteins, such as storage proteins used as an amino acid reservoir for morphogenesis, lipophorins involved in the lipid transport in circulation or vitellogenesis for egg maturation are

synthesized by fat body [14]. Chapman noticed that stored proteins are consumed during adverse conditions like starvation [15]. Hence in the present study an attempt was made to evaluate the changes in total protein and amino acid concentration of fat body of last instar larvae of *O. exvinacea* after treatment with leaf extracts of *H. suaveolens* and *V. negundo.*

2. Materials and methods

2.1 Culturing of *Orthaga exvinacea*

Since heavy infestation was observed during June to September the study was carried out at that time. Larvae of *O. exvinacea* were collected from their natural habitat, mango trees in Calicut district, Kerala, India and were transferred to plastic basins kept in insect rearing cages and reared by providing fresh mango leaves. The colony was maintained at 28±2 ºC and 70–80% relative humidity. Adult moths were fed with 50% honey. First instar larvae were fed with tender leaves of mango. Last instar larvae were separated from the colony and used for further experiments.

2.2 Preparation of phytoextracts

Leaves of *H. suaveolens* (Lamiaceae) and *V. negundo* (Verbenaceae) were collected from selected areas of Calicut district and were washed with water; air dried under room temperature and pulverized into powder with an electric mixer grinder. 50 grams of leaf powder was extracted using 500 ml (100%) methanol in a Soxhlet apparatus for 72hours. The resulting extract was subjected to evaporation in an oven at 50–60 ºC. After complete evaporation 10% stock solution was prepared using methanol and different concentrations of 1%, 2%, 3%, 4% and 5% of each plant extract was prepared from the stock solution for the bioassays.

2.3 Estimation of total protein and free amino acid concentration of fat body

Five different concentrations of both extracts (1%, 2%, 3%, 4% and 5%) were used for treatment on mango leaves and were used for feeding early stages of sixth instar larvae of *O. exvinacea*. The experimental sets of larvae were pre-starved for one hour before food intake and for the control set methanol treated leaves were fed for 48hours. Five insects are formed one treatment and each experiment was replicated five times. After 48hours of feeding the larvae of each experimental set were dissected out in insect ringer separately and the fat body was collected in separate tubes. Fat body tissue was cleared off tracheae and blotted the water adhered to it. Each sample was weighed before homogenization. Protein was precipitated from homogenized samples using 80% ethanol, centrifuged and the residue was dissolved in 1N NaOH. The protein concentration in each sample was estimated spectrophotometrically using the method of Lowry *et al*. [16] The supernatant was used for estimation of free amino acids by Lee and Takahashi method using spectrophotometer [17].

2.4 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).

3. Results

The data in table 1 and table 2 shows a steady decrease in protein concentration in the fat body of the sixth instar larvae of *O. exvinacea* as the concentration of botanicals increased. The decreasing protein concentration was also expressed in graphical form (Fig. 1 and Fig. 2). Results revealed that all the treatments with biopesticides in the present study significantly reduced the fat body protein concentration of treated larvae than untreated.

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

Treatments	Protein concentration		
	mg/ml	mg/g	mg/larva
Control	2.95±0.024	22.73±3.95	0.59±0.005
1%	2.89±0.004	15.75±1.9	0.58±0.001
2%	2.6±0.07	15.72±2.53	0.52±0.014
3%	2.32±0.035	15.59±2.4	0.46±0.007
4%	2.15±0.055	13.65±2.84	0.43±0.011
5%	1.67±0.04	13.1±3.5	0.33±0.008

Values expressed as – 'Mean±S.D'. P<0.01

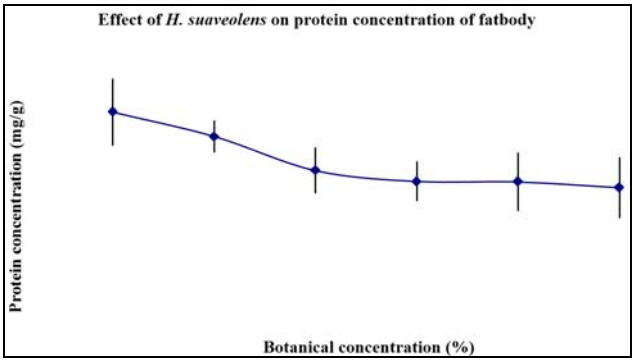

Fig 1: Showing the effect of *H. suaveolens* on protein concentration in fat body

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

Treatments	Protein concentration		
	mg/ml	mg/g	mg/larva
Control	2.95±0.024	22.73±3.95	0.59±0.005
1%	2.57±0.1	19.86±7.15	0.51±0.02
2%	2.16±0.04	15.84±2.72	0.43±0.007
3%	1.8±0.061	14.56±2.35	0.36±0.012
4%	1.64±0.06	14.5±3.43	0.33±0.012
5%	1.47±0.02	13.8±3.6	0.29±0.004

Values expressed as –'Mean±S.D'. P<0.01

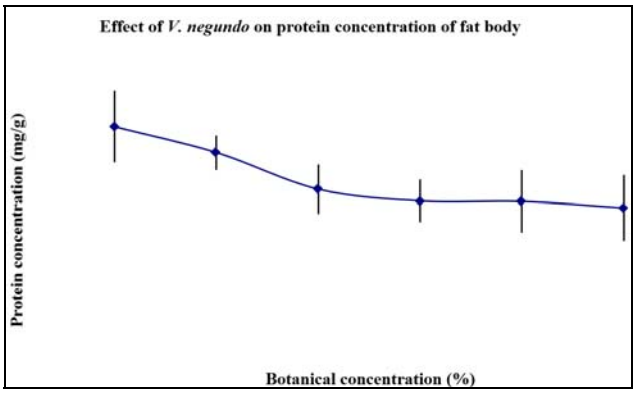

Fig 2: Showing the effect of *V. negundo* on protein concentration in fat body

The protein concentration of fat body was very high in control larvae when compared with the treated larvae. Both phytoextracts significantly reduced the total protein concentration of fat body. Among these two plants *V. negundo* was found to be more potent in reducing the fat body protein content of larvae. Results confirmed that the plant extracts of *H. suaveolens* and *V. negundo* influenced the total protein of fat body of *O. exvinacea*.

The amino acid concentration of fat body of control and experimental sixth instar larvae are given below (Table 3 & Table 4). The effect of botanicals on amino acid concentration was also expressed in graphical form (Fig. 3 & Fig. 4). The total free amino acid concentration of fat body was found to be very high in the control larvae when compared with the experimental larvae. Both botanicals were found to be highly effective in reducing the fat body amino acid content of the larvae.

Table 3: Showing the effect of leaf extract of *H. suaveolens* on amino acid concentration of fat body of sixth instar larvae of *O. exvinacea*.

Treatments	Amino acid concentration		
	mg/ml	mg/g	mg/larva
Control	15.98±0.411	74.6±1.032	3.2±0.086
1%	11.67±0.29	54.24±0.416	2.33±0.059
2%	9.92±0.2	48.33±0.853	2.002±0.066
3%	9.21±0.222	46.19±0.183	1.84±0.043
4%	6.27±0.311	35.4±0.76	1.25±0.062
5%	3.76±0.2	22.02±0.26	0.75±0.041

Values expressed as – 'Mean±S.D'. P<0.01

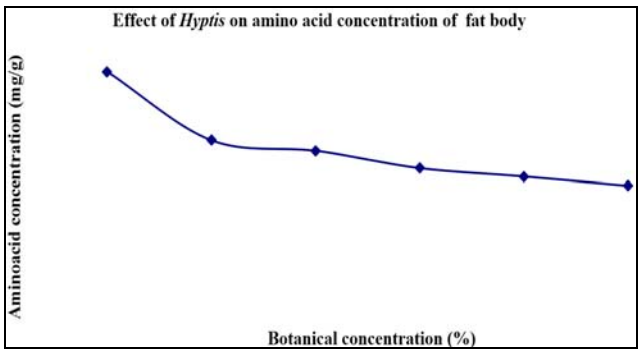

Fig 3: Showing the effect of *H. suaveolens* on amino acid concentration in fat body

Table 4: Showing the effect of leaf extract of *V. negundo* on aminoacid concentration of fat body of sixth instar larvae of *O. exvinacea*.

Treatments	Aminoacid concentration		
	mg/ml	mg/g	mg/larva
Control	15.98±.411	74.6±1.03	3.2±.086
1%	8.08±.091	51.56±0.789	1.62±.021
2%	7.59±.033	47.94±0.7	1.52±.007
3%	7.02±.083	42.14±0.416	1.40±.017
4%	6.37±.039	39.35±0.53	1.27±.005
5%	6.04±.082	36.27±0.524	1.21±.015

Values expressed as – 'Mean±S.D'. P<0.01

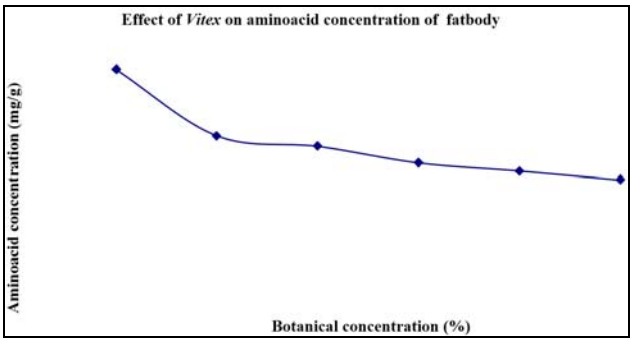

Fig 4: Showing the effect of *V. negundo* on amino acid concentration in fat body

4. Discussion

The decrease of larval protein and amino acid content might be due to the reduced synthesis of proteins or increase of breakdown of proteins to detoxify the active ingredients present in the plant extracts [13]. Studies by Renuga and Sahayaraj revealed that the total head protein of *Spodoptera litura was* reduced due to the application of *Ageratum conyzoides* and *A. vulgaris* extracts [18]. The influence of Azadirachtin on head protein of *Helicoverpa armigera* have been studied through haemolymph injection of azadirachtin into the larvae of *H. armigera* reduced the total head protein level below that of control larvae and altered the polypeptide structure [19-20]. The efficacy of leaf and flower extracts of *Calendula officinalis* on total protein and total carbohydrate contents of the hemolymph, midgut and midgut content of *S. litura* larvae were studied and suggested that *Calendula* extracts showed significant impact on the nutritional status of *S. litura* larvae [21]. Total protein level of *Anopheles* and *Culex* larvae were decreased significantly upon treatment with phytoextracts of *Artemisia annua* and *Azadirachta indica* [5]. Azadirachtin, a neem product is a well-known traditional biopesticide. Annadurai and Rembold (1993) reported that azadirachtin interfere with protein synthesis in *Schistocerca gregaria* [22]. Furthermore Azadirachtin influences the protein synthesis of *S. litura* also [23]. Again protein expression in *S. litura* was significantly lowered under azadirachtin treatment by interfering protein metabolism [24]. Likewise Qadri and Ahmed reported that the protein content of the pesticide treated *Periplaneta americana* was found to be decreased [25]. As a result of treatment with azadirachtin extract on *Culex* larvae, it damaged the body wall made of chitin protein resulting in overall decrease in protein levels of the larvae [26]. It was found that the total protein and free amino acid concentration in the fat body of sixth instar larvae was

decreased due to the effect of botanicals. The decrease in protein and amino acid content might 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 [27]. In addition to this, the antifeedant properties of botanicals also influence the reduction of nutrients in the insects. Every kind of insecticide is having an unfavourable impact on the growth and development of the insect by influencing metabolic and biochemical processes of that insect.

The accumulated nutrient reserves in the fat body regulate the rate of insect growth, timing of metamorphosis, and also egg development in insects. Since fat body is involved in multiple metabolic functions it plays important roles in the life of insects. Major function is to store and release energy in response to the energy needs of the insect. Generally high energy demands are always under adverse conditions and the energy requirement may have led to the protein catabolism. As a result of the insecticidal properties the reduced protein concentration might also be due to the consumption of stored protein for repair of damaged cells and tissue organelles [13]. Mirth and Riddiford revealed that the rate of insect growth, the timing of metamorphosis and egg development of insects life is greatly influenced by the amount of nutrient reserves accumulated in the fat body [28]. Besides all these works it was already reported that the methanolic leaf extract of *H. suaveolens* and *V. negundo* significantly lowered the midgut protein concentration of last insar larvae of *O. exvinacea* [29]. The lowering of protein content shows that these extracts can interrupt with protein synthesis hormones resulting in its decline. Rao and Subrahmanyam found out that azadirachtin induced disturbance occurs in the hormone that regulate the protein synthesis in *S. gregaria* [30].

5. Conclusions

The present study showed that both botanicals can reduce the amount of protein and aminoacid concentration of fat body tissue and thereby they may affect insect growth, metamorphosis and also egg development. So it is suggested that these botanicals can be used for the effective management of *O. exvinacea.* 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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