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Research Paper

Bioefficacy of leaf extracts of *Clerodendrum infortunatum* L. and *Eupatorium odoratum* L. on both quantitative and qualitative analysis of midgut protein of sixth instar larvae of *Orthaga exvinacea* Hampson (Lepidoptera: Pyralidae)

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Abstract

The impact of methanolic leaf extract of *Clerodendrum infortunatum* and *Eupatorium odoratum* on protein concentration and protein profile in the midgut tissue of sixth instar larvae of *Orthaga exvinacea* were studied under laboratory conditions. The different concentrations (1%, 2%, 3%, 4%, and 5%) of each botanical treated mango leaves were fed to the sixth instar larvae. After 48 hours larvae were sacrificed to collect midgut tissue and analysis were done. The results of quantitative and qualitative estimation of protein content in the midgut tissue of both botanicals treated and control showed that there was some considerable decrease in the amount of protein and alterations in protein profile were observed in treated tissue compared to control. The decrease in level of protein concentration was correlated with the increase in concentration of botanicals. Formation of new protein bands and disappearance of some protein bands were noticed in protein profile of treated tissues with increase in botanical concentrations. Among the botanicals tested *E. odoratum* was more effective than *C. infortunatum* and this reveals the potency of both botanicals to be used as natural biopesticides.

Keywords: *Orthaga exvinacea*, *Clerodendrum infortunatum*, *Eupatorium odoratum*, midgut tissue, protein

Introduction

O. exvinacea, the mango leaf webber is a serious pest of mango trees as caterpillar cause defoliation and reduction in crop yield. The larvae web the leaves and terminal shoots into clusters. The larvae are initially gregarious and feed by scraping the leaf surface and later they feed whole lamina leaving only the midrib. Heavy infestation by this pest adversely affects the flowering and fruit formation.

In recent years the use of synthetic organic insecticides in crop pest control programs around the world has resulted in pest resurgence, pest resistance to insecticides, lethal effects on non-target organisms, and environmental pollution. Synthetic insecticides can leave potentially toxic residues in food products and can be deleterious to non-target organisms in the environment[1]. Consequently an intensive effort has been made to find out alternative methods of control. Botanical insecticides and microbial pesticides are highly effective, safe and ecologically acceptable. Biopesticides are considered to be safe to natural enemies and free from residue problem on the crop and in the environment[2].

Over the last 50 years, more than 200 plant species belonging to different families and genera have reported to contain toxic principles which are effective against insects[3]. Plant derived insecticides are reported to have the ability to influence the proportion of various biochemical components (carbohydrates, lipids, proteins etc.) in the body of insects, thus disturbing the internal metabolism of the insect, causing their reduced activity and mortality[4]. Many of the plants possess chemical substances which are known to act as antifeedants, depressants and growth regulators or to impair the immune functions of insects[5]. *C. infortunatum* and *E. odoratum* are locally available plants which have insecticidal property. Pesticidal effect of *C. infortunatum* on the fat body of *Oryctes rhinoceros* causes drastic changes like reduction in the lobes of fat body and their derangement together with the disintegration of cell membrane, shrunken and scattered nucleus[10]. *E. odoratum* leaves mixed with soil in sweet potato beds before planting reduces weevil infestation[11]. Methanolic extracts of *E. odoratum* leaves caused disruption of oocyte development and vitellogenesis in *Oryctes rhinoceros*[12]. The present study was undertaken to assess the impact of *C. infortunatum* and *E. odoratum* on the midgut tissue protein of sixth instar larvae of *O. exvinacea* Hampson.

Materials and Methods

Culturing of *O. Exvinacea*

The pupae and larvae of *O. exvinacea* were collected from the field, reared and maintained in laboratory conditions. The larvae were reared in plastic troughs closed with muslin clothes and kept inside rearing cages. Fresh mango leaves were given till the pupation of the larvae. Adult moths emerged were sorted out for their sexes and kept in plastic jars in the ratio of 1:1 and fed with 50% honey. When the egg hatched, young larvae were fed with fresh tender mango leaves. Laboratory reared sixth instar larvae were used for the experiment.

Preparation of leaf extracts

Fresh leaves of both plants *C. infortunatum* and *E. odoratum* were collected from the field, washed and shade dried. These dried leaves were ground into fine powder with an electric mixer grinder and sieved through a muslin cloth. This powder was used for preparing solvent extracts. 50 gm of leaf powder was extracted using 500 ml methanol in Soxhlet apparatus at 70-80ºC temperature. The extract was allowed to evaporate in a pre-weighed petridish in an oven at 50-60ºC. After complete evaporation of solvent, 10% stock solution was prepared from the weighed extract using methanol. From this stock different desirable concentrations of botanicals (1%, 2%, 3%, 4% and 5%) were prepared.

Bioassay

From the laboratory reared culture, newly moulted sixth instar larvae were used for the experiment. Fresh mango leaves were treated with different concentrations of the leaf extracts and allowed to air dry for a few minutes. This treated leaves were supplied to pre-starved experimental larvae for 48 hours. The control set was maintained with feeding larvae with methanol treated leaves. The experiments were replicated five times for each concentration and each set contained 3 larvae. After 48 hours, larvae were sacrificed to collect midgut tissue. For the sample preparation midgut tissues from 3 larvae were taken, weighed and homogenized. Protein was precipitated from the tissue homogenate by using 80% ethanol and the total protein concentration in each sample was estimated spectrophotometrically by the method of Lowry *et al.* [13]. Data were subjected to statistical analysis using student't' test and ANOVA using SPSS and expressed as Mean ± Standard Deviation. Level of significance of each experiment was analysed by Scheffe test and found to be highly significant ($p<0.01$).

Protein profile

SDS-PAGE analysis for midgut protein samples were carried out by using 4% stacking and 16% resolving gel. The resolved protein bands were visualized by Coomassie staining as per standard protocol of Schagger and Von Jagow [14].

Results and Discussion

The impact of botanicals tested on the larvae was found to be effective in reducing protein content in the midgut tissue. The total protein concentration was significantly lower in treated larvae than in the control (1.9 mg/ml). The midgut protein concentration of larvae treated with five different concentrations (1%, 2%, 3%, 4%, 5%) of *C. infortunatum* was found to be decreasing from 1.78 mg/ml to 0.53 mg/ml (Table 1) and in case of similar five different concentrations of *E. odoratum* the protein concentration decreased from 0.91 mg/ml to 0.07 mg/ml (Table 2). Among the botanicals tested the *E. odoratum* showed the maximum efficacy in reducing protein content in midgut tissue of sixth instar larvae of *O. exvinacea.* (Figure 1).

Table 1: Efficacy of leaf extracts of *C. infortunatum* on protein concentration of midgut tissue of sixth instar larvae of *O. exvinacea*.

Concentration of Botanicals (in %)	Protein Concentration		
	mg/ml	mg/gram tissue	mg/insect
Control	1.90±0.02	18.15±1.13	0.63±0.01
1	1.78±0.07	18.09±0.82	0.59±0.02
2	1.40±0.17	14.63±2.30	0.46±0.06
3	0.95±0.04	9.81±0.42	0.32±0.01
4	0.67±0.06	7.17±0.47	0.22±0.02
5	0.53±0.05	5.91±0.48	0.17±0.02
F value	236.63	111.69	245.55

Each value represent - Mean ± S.D. Significance level – P<0.01 = highly significant

Table 2: Efficacy of leaf extracts of *E. odoratum* on protein concentration of midgut tissue of sixth instar larvae of *O. exvinacea*.

Concentration of Botanicals (in %)	Protein Concentration		
	mg/ml	mg/gram tissue	mg/insect
Control	1.90±0.02	18.15±1.13	0.63±0.01
1	0.91±0.02	9.27±0.40	0.30±0.01
2	0.58±0.02	6.60±0.30	0.19±0.00
3	0.28±0.04	3.14±0.49	0.09±0.01
4	0.20±0.02	2.35±0.31	0.07±0.01
5	0.07±0.02	0.81±0.17	0.02±0.01
F value	3662.00	643.27	2690.00

Each value represent - Mean ± S.D. Significance level – P<0.01 = highly significant.

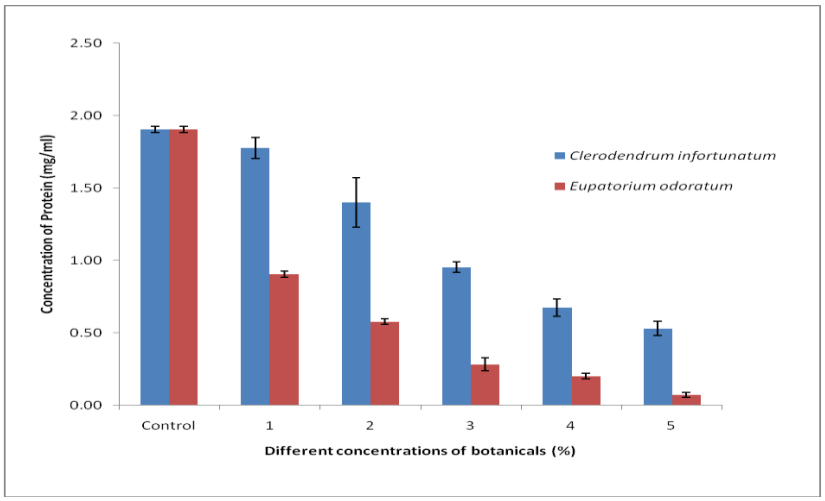

Figure 1: Graph showing the decrease in protein concentration in the midgut tissue with increase in concentrations of botanicals

The protein profiles of the midgut tissue of both untreated and treated larvae show some considerable qualitative changes. 14 protein bands (band A to N) were noticed in the protein profile of the midgut tissue of untreated (control) larvae with molecular weights in between the range of 116 kDa to 14.4 kDa (Figure 2). In *C. infortunatum* treated larvae at 1% concentration, protein profile showed appearance of two new bands (a and b) with molecular weights of 30.19 kDa and 24.54 kDa, but these were disappeared in 2%, 3% and 4% concentrations and reappeared in 5% concentration (d and e) (Figure 2). A few number of protein bands were disappeared in both 3% and 4% treated larvae but only one new band (c) having molecular weight of 33.11 kDa was formed in 4% concentration.

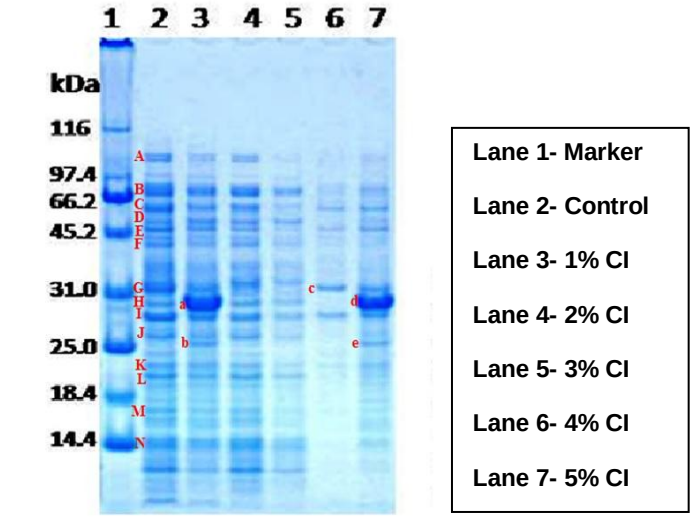

Figure 2: SDS-PAGE analysis showing protein profile of midgut tissue of sixth instar larvae treated with *C. Infortunatum*

In *E. odoratum* treated larvae at 1% concentration the protein profile of midgut tissue showed 3 new bands (a, b and c) with molecular weights of 79.43 kDa, 22.90 kDa and 19.05 kDa (Figure 3). Similarly 3 new bands (d, e and f) were also noticed in 2% concentration, having molecular weights of 89.12 kDa, 23.44 kDa and 19.49 kDa. Some bands were disappeared in 3% concentration which was already noticed in control and only one new protein band (g) was appeared with molecular weight of 19.49 kDa. Disappearance of bands as well as appearance of 4 new bands (h, i, j and k) were found in protein profile of midgut tissue of 4% concentration treated larvae, having molecular weights of 95.49 kDa, 36.30 kDa, 34.67 kDa and 19.05 kDa. At 5% concentration, a few number of protein bands were disappeared and only 3 new bands (l, m and n) were formed with molecular weights of 33.88 kDa, 28.84 kDa and 19.49 kDa.

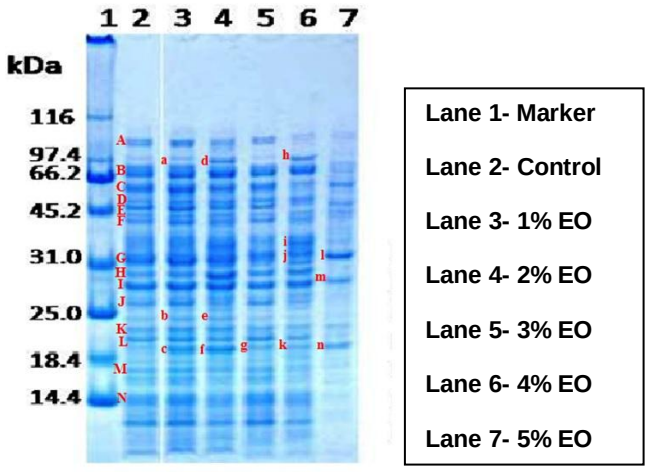

Figure 3: SDS-PAGE analysis showing protein profile of midgut tissue of sixth instar larvae treated with *E. odoratum*

Proteins are the major biological factor that plays an important role in insect growth, development and various other physiological processes[15]. The protein content in an insect is dependent upon its synthesis, breakdown, water movement between tissues and haemolymph. The reduction in protein content in larvae might be due to the reduction in synthesis of protein or increasing breakdown to detoxify the active principles present in the plant extracts**[4]**. Under stress condition an insect requires higher metabolic energy and this energy demand may have led to the protein catabolism to detoxify the toxic principles present in the *C. infortunatum* and *E. odoratum*, but the lesser consumption of food for synthesis of protein might be also the reason for the reduction of protein. Reduction of protein in *Crocidolomia binotalis* might be caused by the toxic principles present in the plant extracts[4]. Similarly the leaf extracts of *Lantana camara* reduced the protein content of *Corcyra cephalonica*[16]. Ranjini *et al.*[17] reported that the effect of methanolic leaf extract of *Hyptis suaveolens* and *Vitex negundo* reduced the amount of midgut protein of *O. exvinacea* larvae and thereby affect the growth, development and other various physiological processes. The decrease in protein content might also be due to the mechanism of lipoprotein formation which will be used to repair damaged cells and tissue organelles. Reduction in total protein content due to the botanicals might also be due to their insecticidal properties[4]. Under toxic condition the increased rate of protein catabolism has been reported in *Spodoptera litura*, because insect degrades protein to resultant amino acids in order to let them enter into the TCA cycle as keto acid for compensation for the lower energy caused by stress and also to detoxify the active principles present in the plant extract[18]. The toxic components of *Clerodendrum inerme* disrupt the process of digestion and absorption in *Aedes aegypti* larvae**[19]**.

The active principles present in both botanicals might have induced to appear new polypeptides or to disappear the existing ones, altering the protein profile. The impact of neem gold on *Cosmopolites sordidus* altered the normal physiological activity and induced the appearance of new polypeptides[15]. Any foreign particle interacting with the cellular metabolism creates stress in the cell. It may either up-regulate or down-regulate gene expression. Changes that showed in the protein profile indicated that the treatment with botanicals might have made changes at genetic level. Many researchers reported the alterations occurred in body proteins of insects after treatment with plant extracts[20-24]. The disappearance of protein bands in higher concentration might be due to the phytochemiclas present in the botanicals which down- regulates some gene expression. The protein patterns of the midgut tissue of the treated larvae showed appearance of many new small proteins which are smaller in sizes. The small sized protein might be new peptides formed or it might be the peptides which were formed by the breakdown due to the action of protease. Appearance of new set of proteins with high molecular weights and increasing or subdividing of polypeptides, deletion or denature of proteins were reported in *Oryzaephilus surinamensis* treated with some dried plants [25].

Conclusion

The present study, suggested that the active components present in both botanicals might be the cause for the reduction of protein content as well as for the alterations in the protein profile of the midgut tissue of the treated larvae. Hence higher concentration of methanolic leaf extracts of these two plants can be used as insecticides for the management of *O. exvinacea.*

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