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Research article

VARIATIONS OF PROTEIN CONTENT IN DIFFERENT TISSUES OF *SPODOPTERA LITURA* FABRICIUS (LEPIDOPTERA: NOCTUIDAE) REARED ON THREE DIFFERENT HOST PLANTS

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ABSTRACT: *Spodoptera litura*, an economically important polyphagous insect, is a serious threat to a large number of cultivated crops all over the world. In the present study, the quantitative analysis of protein in haemolymph, midgut, and fat body of the fifth instar larvae of *Spodoptera litura* was carried out by giving three different host plants as food materials. A comparative study of the tissue protein with respect to the feeding of host plant leaf protein was also carried out. The data obtained were statistically analysed using one-way ANOVA ($P<0.01$).The results revealed that there was a significant variation in the protein concentration in each tissue with respect to the feeding material. The haemolymph showed a higher concentration of protein than the mid gut and fat body. Similarly, the castor fed larval tissues showed a significantly higher concentration of protein than the tissues of colocasia and papaya fed larvae. A positive correlation was found in the protein content of the host plant leaves and the insect tissues.

Key words: *Spodoptera litura*, host plants, mid gut, haemolymph, fat body, protein concentration.

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INTRODUCTION

The dependence on agriculture for the livelihood of Indian population is an important criterion for the need of pest management. For several years man facing the problem with serious insect pest attack. For the pest control, effective measures should be adopted. Nowadays farmers face serious problems with pest populations, among which majority includes lepidopteran insects which cause serious damage to the crops and reduce the crop productivity, hence the control of the pest population is essential to improve the crop yield.

Spodoptera litura is a polyphagous insect which defoliates almost 120 economically important plant species all over the world [1]. It causes an economic fall in crop yield of 25.8-100%, based on the stages of the crop and its infestation level in the field [2]. The life cycle of *Spodoptera litura* includes larval, pupal and adult stages. The eggs are laid by the female moth beneath the host plant leaves. The damage to the crop arises mostly from extensive feeding by larvae and it causes the complete stripping of the plants. Usually, these larvae are leaf eaters, but sometimes they act as a cutworm on crop seedlings. Severe infestation of the pest on a young plant leads to the stunted growth and the late development of fruits [3]. For late harvesting crops, the attack by this pest is severe and that leads to complete damage of the plant and considerable reduction in the crop yield. It becomes resistant to many insecticides such as carbamates, indicating the failure of effective control measures [4, 5, 6, 7].

The growth and development of larvae always depend on the active synthesis of protein in the tissues [8, 9]. Proteins are the structural components of the cell and they perform the duties encoded in the specific genes. So the proper understanding of changes in protein concentration of different tissues during the larval stage is inevitable. From the previous works, it was revealed that the dietary protein quality has a significant impact on insect-plant relation [10, 11]. The aim of the present work was to analyze the total protein content present in the mid gut, haemolymph and fat body of *Spodoptera litura* in order to compare the changes in the biochemical components of the insect with respect to the utilization of the different host plants as the food materials. The information from this work will be very useful in the mixed cropping system as a control measure for this economically important pest.

MATERIALS AND METHODS

INSECT CULTURE

Under laboratory conditions, a colony of *S.litura* was established from the pupae obtained from the insect rearing centre ICAR Bangalore. The pupae were kept in a wooden cage, the sides of which were covered by thin gauze. As soon as the adult emerged, they were transferred into the plastic containers covered with muslin cloth and allowed them to mate. Absorbent cotton ball soaked in 20 % honey-water was provided for the adult during the oviposition period. The eggs laid by the adult along the sides of the plastic containers were kept for incubation. Hatched larvae were transferred to the plastic trough and were fed with fresh host plant leaves. 25-30 larvae were cultured in each trough. Each day the leaves were replaced with fresh one. The utilization of the host plant leaves and the larval growth and development was observed. The fifth instar larvae from the second generation were used for the experiment.

HOST PLANTS

Three host plants used in this study were castor *(Ricinus communis)*, colocasia *(Colocasia esculenta*) and papaya (*Carica papaya*).These plants were selected because they are the primary host plants of *S. litura* and are important as economic crops. The plant leaves were collected from the nearby fields.

Collection and preparation of tissue sample

Protein estimation was carried out in the haemolymph, mid gut and fat body of fifth instar larvae of *S.litura* with five replications for each sample type.

Haemolymph

The haemolymph was collected from fifth instar larvae of *S.litura* by amputing the thoracic legs and it was collected into a clean pre-cooled 1.5 ml plastic vials containing 0.5 ml of 10% sodium tungstate to prevent oxidation. After that, 1ml sodium tungstate and 1 ml 2/3N sulphuric acid were added, and then the samples were centrifuged at 2000 rpm for 5-10 minutes.The residue was used for protein estimation.

Midgut Tissue

Mid gut tissue was collected from fifth instar larvae of *S.litura* by dissecting out the alimentary canal. Mid gut tissue was isolated by removing the fat body and trachea, washed and homogenized in insect ringer using a glass homogenizer. To the tissue homogenate, 1 ml 80% ethanol was added and kept for 5 minutes for precipitation and centrifuged at 10000 rpm for 10 minutes. The residue was collected and used as the sample for protein estimation.

Fat Body

The fat body was collected from fifth instar larvae of *S.litura* by dissecting out and it was collected in pre - weighed plastic vials containing insect ringer. It was homogenized in insect ringer using a glass homogenizer. The homogenate was centrifuged at 10000 rpm for 10 minutes. The residue was used for the estimation of protein.

Estimation of Total Protein

Quantitative estimation of protein was done spectrophotometrically in different tissue samples by the method of Lowry *et al*. [12] using bovine serum albumin (BSA) as standard.

Statistical Analysis

One -way analysis of variance (ANOVA*)* was used to test the significance of differences between mean values of protein content in the mid gut, haemolymph and fat body of fifth instar larvae of *S.litura*. Comparisons were performed to find out the significant difference in the protein concentration of different tissues depending on the feeding rate of different host plants. The difference was significant at $p < 0.01$.

RESULTS

Rearing of *S.litura* with three different host plants showed, fast and maximum growth by the larvae fed with castor leaves than those fed with papaya and colocasia.

Changes of protein concentration in host plant leaves.

The protein content in different host plant leaves is expressed in Table 1. The castor leaves showed high protein content (1.32±0.19) than the other two host plants, papaya and colocasia(0.59±0.04mg/ml&0.28±0.05mg/ml).

Table 1. Changes in the protein concentration of three different host plants

Host Plant Samples	Protein concentration					
	Castor		Colocasia		Papaya	
	mg/ml	mg/gm	mg/ml	mg/gm	mg/ml	mg/gm
Sample1	1.3959	2.792	0.6394	1.2788	0.2403	0.4806
Sample2	1.5675	3.135	0.6137	1.2274	0.2789	0.5578
Sample3	1.0740	2.148	0.6223	1.2446	0.2918	0.5836
Sample4	1.2060	2.412	0.5343	1.0686	0.3626	0.7252
Sample5	1.3841	2.768	0.5536	1.1072	0.2618	0.5236
*Mean±SD	1.32± .19	2.65 ± .38	0.59±.04	1.18±.09	0.28±.05	0.57± .09

*Mean±Standard Deviation significant at $p < 0.01$

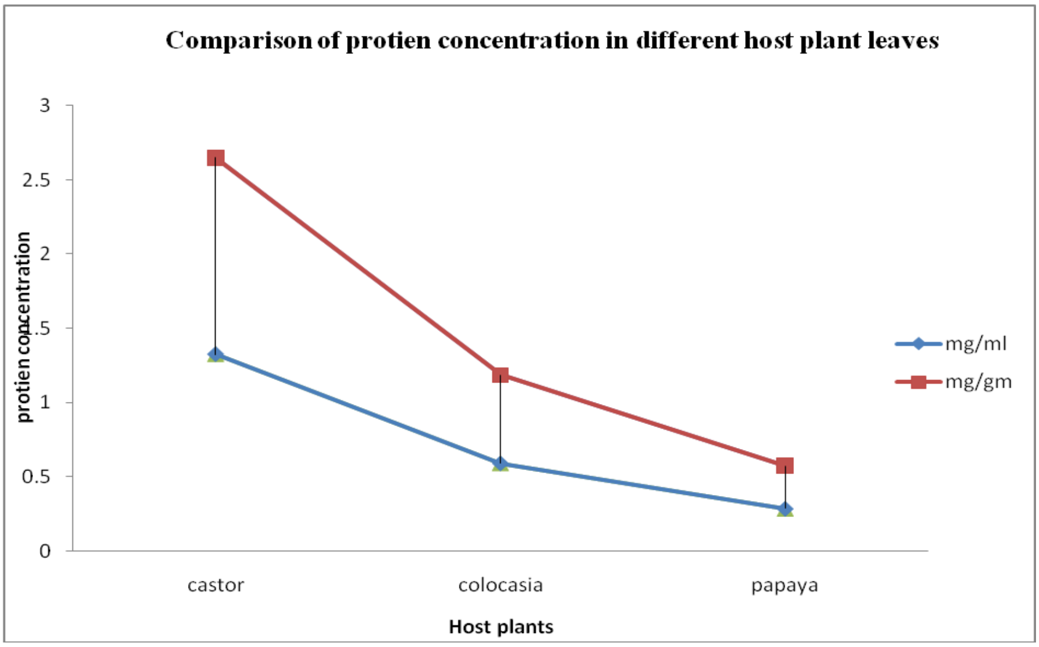

Fig.1 Comparison of protien concentration in different host plant leaves

Changes in total protein content of haemolymph, mid gut and fat body

The result of the total protein content in the haemolymph of fifth instar larvae of *S.litura* is presented in Table 2. The concentration of protein was higher in the castor fed larvae of *S.litura* (53.09±16.89 mg /ml) than in colocasia and papaya fed fifth instar larvae (28.64±11.76 and 23.24±5.30 respectively).

The changes in the protein concentration of the mid gut tissue of the *S.litura* fed with different host plants are presented in Table 2.The protein concentration in the midgut tissues of *S.litura* varies with the difference in the metabolic rate and feeding of host plants. The insect fed with castor showed significantly higher values (26.22±2.04mg/gm) of protein in the midgut than the other two host plants, papaya and colocasia (21.39±.71&12.79±2.09mg/gm)respectively.

The change in protein concentration in the fat body of *S.litura* larvae according to different host plants is presented in Table-2. The protein concentration of fat body is greater in castor fed *S. litura* larvae (5.65±.86mg/gm) compared to those fed with other two plants, colocasia and papaya (4.60±.94&3.06±.39mg/gm).

Table 2. The protein concentration in haemolymph, mid gut and fat body of fifth instar larvae of *S.litura*.

Hostplants	Protein concentration in different tissues of *S.litura*				
	Haemolymph	Mid gut		Fat body	
	mg/ml	mg/gm	mg/gm	mg/ml	mg/gm
Castor	53.09±16.89	3.46±.12	26.22±2.04	1.43±.11	5.65±.86
Colocasia	28.64±11.76	3.70±.33	12.79±2.09	0.91±.16	4.60±.94
Papaya	23.24±5.30	4.12±.39	21.39±.71	0.57±.38	3.06±.39

*Mean±Standard Deviation significant at $p < 0.01$

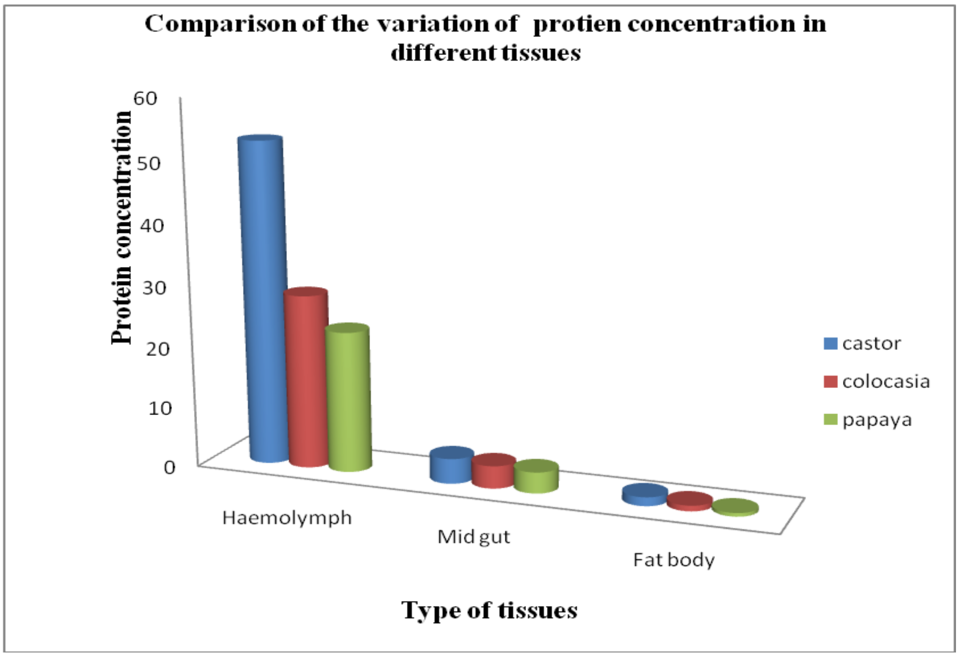

Fig .2 Comparison of the variation in protein concentration in different tissues

DISCUSSION

Nutrition is the substantial link between an animal and its surroundings; it provides all the necessary elements and energy for their survival. Depending upon the nutrient uptake the concentration of the biochemical metabolites varies in different tissues [13, 14]. The availability of different host plants plays an important role in the resurgence of polyphagous pest population [15], and the analysis of nutritional indices helps us to understand the behavioural and physiological stages of an insect in response to host plants [16].The difference in the feeding preference influences the protein content in different tissue samples. In the present study the higher feeding rate and maximum growth were observed in the case of castor than the other two host plants, papaya and colocasia. The analysis of protein content in the host plant leaves showed higher protein concentration in castor leaves than colocasia and papaya leaves. The comparison between the protein content in the plant leaf material and the insect tissues showed a positive correlation. The protein content is higher in the tissues of the castor fed larvae than the larvae fed with colocasia and papaya. The high protein content showed by the castor fed larvae may be due to the higher protein content in the castor leaves. Similar results were reported in which the growth of herbivorous insects might be limited by protein availability and quality or the availability of other primary plant metabolites [17, 18]. Some phytochemical components such as phenolic resins and tannins showed impaired herbivore performance by interfering with the digestibility of dietary proteins [19, 20].

The amount of protein content in different tissues of insects mainly depends on the metabolic activities. By comparing the protein concentration in different tissues the higher value of protein content was observed in the haemolymph than the mid gut and fat body of the insect. This indicates that the metabolic activities is higher in the haemolymph than in the mid gut tissue and fat body and may be due to the role of haemolymph as a storage reservoir of the biochemical components [21]. Protein profile changes in different tissues of insects were observed by several researchers [22, 23]. It is reported that in *Antheraea mylitta* concentration of fat body and haemolymph protein concentration showed an increase in total protein content from early instars to shortly before pupation [24].

In the present study higher concentration of protein was found in all the three tissues of the fifth instar larvae of *S.litura*. Protein concentration increases along with the development of the larvae and reaches a maximum limit at the last instar stage [25]. Earlier findings suggest that some lepidopterans show lower fitness on some host plants that may be due to the presence of some secondary metabolites in these host plants or the absence of primary nutrients required for the growth and development of insects [26].The growth of the *S.litura* larvae also showed the difference in growth rate depends on the host plants. The castor fed larvae showed maximum growth in a short period. The colocasia fed larvae showed fast growth rate than the papaya fed ones. So it is suggested that there exists a positive correlation between the metabolites in the tissues of the insect with their host preference.

CONCLUSION

Rearing of *S.litura* on different host plants showed varied host preference by the larvae. The larvae show ultimate preference upon castor plants than the other two host plants. The host preference of the larvae might be based on the nutritional components of the plant materials, they possess. As this larva is a polyphagous pest it is having negative impacts on economically important crops. Considering these aspects it is suggested that castor plant will be applicable in the agricultural fields to control the pest attack caused by the particular larvae as a biofencing tool to the economically important crops.

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