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**RESEARCH ARTICLE**

# IMPACT OF *Syzigium lineare* WALL. (MYRTACEAE) LEAF EXTRACTS ON HAEMOLYMPH PROTEIN PROFILE OF THE ARMYWORM, *Spodoptera litura* (FAB.) (LEPIDOPTERA: NOCTUIDAE)

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**ABSTRACT**

Quantitative and qualitative changes of haemolymph protein profile of *S. litura* was studied under the stress of subfractions isolated from *S. lineare.* The study was conducted at Entomolgy Research Institute, Loyola College, Chennai, India. Standard protcol was followed to estimate qualitative and quantitatiive changes of haemolymph protein. The estimation was done after fourth instar larvae of *S. litura* fed on castor leaves treated with 25, 50, 75 and 100ppm concentration of promising sub fractions II, III, IV and VI for 24h. Protein content in haemolymph of *S. litura* was reduced significantly ($P<0.05$; by LSD) in all treatments. The reduction of protein in sub fraction II and positive control azadiracthin did not show any statistical significance ($P>0.05$; by LSD). Maximum qualitative changes were observed only on storage proteins with the molecular weight of 68KD. The concentration of storage protein was reduced maximum in sub fraction II and III at 25ppm concentration and it was increased in sub fraction III at 100ppm and sub fraction IV at 25ppm concentration respectively compared to control. The dissimilarity in qualitative and quantitative changes may be associated with differences in biological and biochemical activities in the larvae of *S. litura* to overcome the stress of *S. lineare.*



## INTRODUCTION

Proteins have always been an interesting biochemical tool because of their prominent role in development, morphogenesis and the intermediary metabolic pathways in insects (Naga Jyothi and Suneetha, 2010). In addition to structural and functional maintenance of the cells, play a vital role in reproduction, enzyme catalysis, transport materials, regulation of metabolism, movement and in body defense. According to Vijayaraghavan *et al.* (2010) plant derived insecticides have the ability to influence the proportion of various biochemical compounds such as carbohydrates, lipids, proteins etc., in the body of insects, thus disturbing the internal metabolism of insects, causing their reduced activity or mortality. The biochemical compostion of haemolymph is highly variable among the insect species at different developmetnal stages (Florkin and Jeuniaux, 1974). The 4% dosage of *Brassica nigra* and mineral oil, Kemesol 95% dissolved in petroleum ether and acetone extracts remarkably decrease total haemolymph and fat body protein of *Spodoptera littoralis* (Khatter and Abldahb, 2010). The diet of *Tribolium castaneum* incorporated with 2.4%< concentration of methanol extract of *Peganum harmala* seeds decreases protein and lipid concentration (Jbilou and Sayah, 2007). Armyworm, *Spodoptera litura* is a serious polyphagous pest causing severe damage by defoliation and the damage has been estimated to an extent of 80 to 100 per cent in tobacco nurseries and 72 to 98 per cent in soybean (Chari and Ramaprasad, 1987; Singh, 2000). Botanical preparations are used since time immemorial for insect pest control program. The degree and mode of actions are mainly based on the bioactive principles. The bioactive compound azadirachtin treated at 1ppm concentration significantly affects ten proteins in 4th instar larvae of *S. litura* (Huang *et al*., 2004). In our earlier report confirmed significant antifeedant and growth inhibitory activities of *Syzygium lineare* crude extracts and purified fractions against *S. litura* (Jeyasankar *et al.,* 2010). According to Duraipandiyan *et al.*(2008) methanol and acetone extract of *S. lineare* is having antifungal activity. In addition, ethylacetate, acetone, methanol and water extracts are also proved to have antibacterial activities. There is no other published work on impact of various subfractions isolated from the *S. lineare* against *S. litura.* Therefore, quantitative and qualitative changes of haemolymph protein of *S. litura* treated with various subfractions of *S. lineare* is a first hand information for scientific publication.

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## MATERIALS AND METHODS

### Site for plant collection and identification

*Syzygium lineare* leaves were collected from Kalakad Mundanthurai Tiger Reserve Forest, Western Ghats of Tamil

Nadu, India with an latitude between 8° 25′ and 8°53′ N and longitude between 77°10′ and 77°35′ E. The identity of the plant was confirmed by Dr. D. Narasimhan, a plant taxonomist, Department of Botany, Madras Christian College, Chennai, India. The voucher specimen (MPH No. 170) was prepared and preserved at medicinal plant herbarium, Entomology Research Institute, Loyola College, Chennai, India.

**Processing of plant materials**

The plant materials were thoroughly washed with tap water and shade dried under room temperature (29.0 ± 2°C) at Entomology Research Institute, Loyola College, Chennai. The dried leaves were powdered using electric blender and sieved through kitchen strainer. Two kg of plant powder was extracted sequentially with increasing polarity of solvents (hexane, diethyl ether, dichloromethane, ethyl acetate and methanol) and filtered through Whatman No.1 filter paper. The solvents from the crude extract was evaporated by using rotary vacuum evaporator at 40°C in water bath and with 25-30 Hg pressure (Saxena and Yadav, 1983). The crude extracts were collected in clean borosil vials and subjected to isolation of fractions.

**Isolation of fractions from ethyl acetate extract of *S. lineare***

Ethyl acetate extract was proved to be effective against *S. litura* (Jeyasankar *et al.,* 2010) Therefore, Thin Layer Chromatographic (TLC) techniques (0.2mm thick pre coated TLC plates from E. Merck, Germany, 60 F254) was used to identify number of fractions from ethyl acetate crude extract. The solvent system used to run the TLC plate was 70:30, 80:20 and 90:10 ratio of hexane and ethyl acetate. After running, plate was incubated in iodine chamber and exposed to UV light. Finally, plate was treated with pancal D reagent which was prepared by the mixing 21g of Ammonium molybdate and 1g of Cerric sulphate in few ml of distilled water with the addition of 31ml of concentrated sulphuric acid ($H_2SO_4$) drop wise and making up the volume of 500ml with distilled water. Totally seven spots were identified and subjected to column chromatographic techniques for isolation.

**Isolation of fractions through Column chromatographic technique**

Glass column (size 60cm height and 3cm width) was thoroughly washed with tap water and rinsed with distilled water and hexane and fixed on the stand. The slurry of 100-200 mesh silica gel powder was prepared using hexane and carefully filled with ¾ of the column without any air bubbles and gaps. The powdered crude extract (19.5g) was thoroughly macerated with silica gel using mortar and pestle and loaded above the silica gel. The solvent system used to isolate the fractions were hexane (100%); hexane: ethyl acetate (80:20); hexane: ethyl acetate (60:40); hexane: ethyl acetate (40:60); hexane: ethyl acetate (20:80); ethyl acetate: methanol (50:50) and methanol (100%). The solvents from the fractions were evaporated using vacuum rotary evaporator and the bioactivity was tested against *S. litura* and identified fraction III and VI was promising (Jeyasankar *et al*., 2010). Further, promising fractions were subjected to isolation of sub fractions.

**Isolation of sub fractions**

The sub fractions were isolated from the promising fraction III and VI. Three sub fractions were isolated from fraction III by using Hexane: Ethyl acetate (50:50); Hexane: Ethyl acetate (30:70); and Hexane: Ethyl acetate (10:90) solvent system. In addition, four sub fractions were isolated from fraction VI by using Hexane: Ethyl acetate (30:70); Hexane: Ethyl acetate (10:90); Ethyl acetate: Methanol (40:60); Ethyl acetate: Methanol (20:80) solvent system. The vacuum rotary evaporator was used to remove the solvents from the sub fractions and tested against *S. litura.* Totally seven fractions were isolated among that II, III, IV and VI was proved to be promising bioactivities against *S. litura* (Jeyasankar *et al.,* 2010). Those promising sub fractions were used to check their impact on haemolymph protein profile of *S. litura*.

**Experimental design and haemolymph collection**

Ten fourth instar larvae of *S. litura* was allowed to feed on castor leaves treated with 25, 50, 75 and 100ppm concentration of sub fractions such as II, III, IV and VI for 24h and transferred to untreated leaves. The larvae fed with solvent and water treated leaves was used as negative control and azadiracthin (only for quantitative study) treated was used as positive control. Three days after treatment haemolymph was collected from 10 larvae for each treatment separately. The haemolymph was drawn by pricking the second proleg of the larva with sterilized needle and collected into prechilled eppendorff vials having few crystals of phenylthiocarbamide (1-phenyl-2-thiourea). Haemolymph from 10 larvae was pooled which constituted a replication. Minimum of three such replicates were maintained. The sample was centrifuged in refrigerated centrifuge at 10,000 rpm for 10 minutes at 4°C to get clear solution and stored at –20°C for quantitative and qualitative analysis.

**Estimation of haemolymph protein**

The quantitative estimation of total protein content/ml of haemolymph was estimated according to Lowry *et al*. (1951), using bovine serum albumin as the standard. The qualitative estimation of haemolymph protein profile was determined by one dimensional sodium-dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a vertical slab gel electrophoresis unit as the detail described by Laemmli (1970) with some modification.

**Preparation of SDS-PAGE**

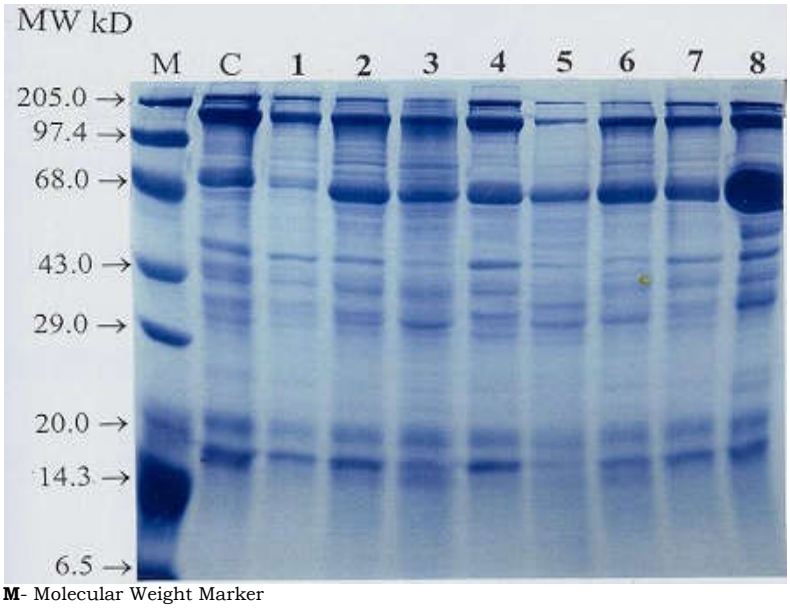
In the first phase 10 ml of 10% separating gel was prepared by adding 3.3 ml of 30% acrylamide; 2.5 ml of 1.5M Tris-HCL (pH 8.8); 0.05 ml of 10% ammonium persulphate; 0.05 ml of 10% SDS; 8 µl of tetramethyl ethylene diamine (TEMED) and 4.0 ml of distilled water. The mixture was stirred well for degassing and cast within the sandwiched glass plates leaving sufficient space for the stacking gel. The top of the separating gel was layered with distilled water to get an even surface and was kept 30 minutes for polymerization. After polymerization the surface was removed carefully and washed with distilled water before casting the stacking gel. The second phase, 4% stacking gel was prepared by the mixture of 0.67 ml of 30% acrylamide; 0.54 ml of 0.5 M Tris-HCL (pH 6.8); 0.05 ml of

**Table 1: Impact of different promising sub fractions on total protein concentration (mg/ml) in the haemolymph of *S. litura***

| Bioactive sub fractions | Concentrations tested | | | |
|---|---|---|---|---|
| | 25ppm | 50ppm | 75ppm | 100ppm |
| Sub fraction II | 97.36 ± 4.10$^{e}$ | 50.21 ± 2.36$^{a}$ | 48.24 ± 3.25$^{b}$ | 31.13 ± 1.22$^{a}$ |
| Sub fraction III | 90.86 ± 2.36$^{d}$ | 90.25 ± 4.77$^{d}$ | 75.46 ± 3.57$^{e}$ | 60.00 ± 2.41$^{d}$ |
| Sub fraction IV | 71.74 ± 7.80$^{b}$ | 67.08 ± 6.01$^{b}$ | 41.19 ± 4.25$^{a}$ | 36.67 ± 3.25$^{b}$ |
| Sub fraction VI | 80.64 ± 10.14$^{c}$ | 76.99 ± 6.21$^{c}$ | 62.07 ± 7.42$^{d}$ | 55.78 ± 5.44$^{c}$ |
| Azadirachtin (50ppm) | 53.85 ± 3.05$^{a}$ | 53.85 ± 7.20$^{a}$ | 53.85 ± 9.14$^{c}$ | 53.85 ± 6.38$^{c}$ |
| Solvent control | 98.04 ± 1.05$^{f}$ | 98.04 ± 5.44$^{e}$ | 98.04 ± 1.47$^{f}$ | 98.04 ± 4.00$^{f}$ |
| Control | 98.25 ± 6.38$^{f}$ | 98.25 ± 8.36$^{e}$ | 98.25 ± 2.96$^{f}$ | 98.25 ± 8.50$^{f}$ |

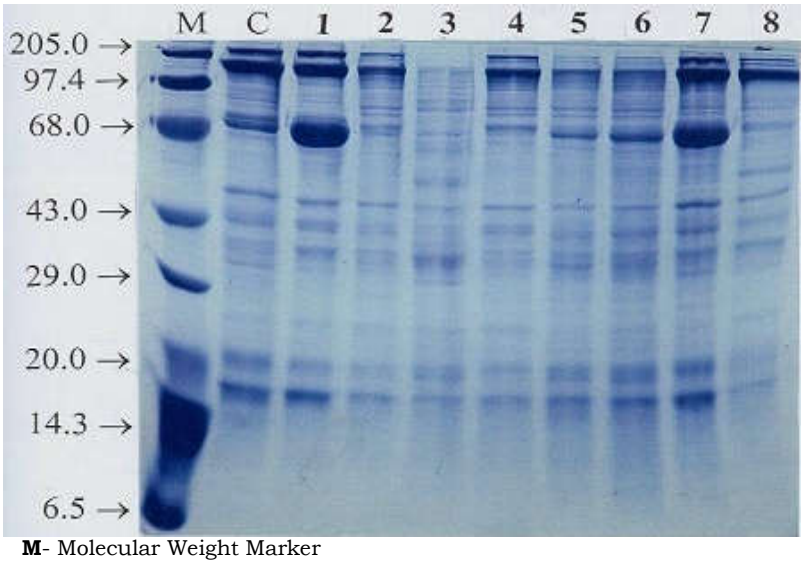
Values are mean of five replications. Within the column similar alphabets are statistically not significant (p>0.05 by LSD).

**Figure 1: Haemolymph protein pattern of IV instar larvae of *S. litura* exposed to different concentaions of subfraction II and III isolated from *S. lineare***




**M**- Molecular Weight Marker
**C-** Control
**Lane** 1-4 haemolymph collected from the larvae exposed to sub-fraction II (1. 25ppm; 2. 50ppm; 3. 75ppm and 4. 100 ppm)
**Lane** 5-8 haemolymph collected from the larvae exposed to sub fraction III (5. 25ppm; 6. 50ppm; 7. 75ppm and 8. 100ppm)

**Figure 2: Haemolymph protein pattern of IV instar larvae of *S. litura* exposed to different concentaions of subfraction IV and VI isolated from *S. lineare***




**M**- Molecular Weight Marker
**C-** Control
**Lane** 1-4 haemolymph collected from the larvae exposed to sub-fraction IV (1. 25ppm; 2. 50ppm; 3. 75ppm and 4. 100 ppm)
**Lane** 5-8 haemolymph collected from the larvae exposed to sub fraction VI (5. 25ppm; 6. 50ppm; 7. 75ppm and 8. 100ppm)

10% ammonium persulphate; 0.05 ml of 10% SDS; 6 µl of TEMED and 2.7 ml of distilled water. The mixture was thoroughly mixed and poured over the separating gel; a comb made of Teflon was inserted to form wells in to which samples were loaded later. After polymerization comb was removed and wells cleaned with electrode buffer using a syringe. After cleaning, plates were fixed in to the electrophoretic tanks.

**Preparation of haemolymph samples**

After the quantification, 100µg of haemolymph protein was mixed with 30µl of sample buffer (1 ml of glycerol; 0.5 ml of mercaptoethanol; 150 mg of SDS; 1.25 ml of 0.5 M Tris-HCL, pH 6.8, a pinch of bromophenol blue and distilled water made up to 10 ml). The sample was digested in a boiling water bath for 2-3 minutes, cooled and loaded 20µl to the wells. The electric power supply was adjusted to 60 volts when the marker dye entered into the separating gel after that the voltage was increased up to 120 volts. The electrophoretic run was stopped when the dye front reached the anodic end of the gel. The known molecular weight marker protein was also loaded along with sample proteins separately for comparison.

**Staining procedure**

The gel was stained with coomassie brilliant blue R-250 with the following composition, 200mg of coomassie brilliant blue powder, 7 ml of acetic acid, 50 ml of methanol, and 43 ml of distilled water. The gel was kept in the stain overnight and destained with a solution of 7 ml acetic acid and 30ml methanol and 63 ml distilled water until the background was cleared. The destained gels were scanned using laser densitometer scanner and stored at 7% acetic acid.

## RESULTS

**Impact of sub fraction on haemolymph protein**

Protein quantification data on *S. litura* haemolymph was quantified after exposed to promising sub fractions are presented in Table 1. Generally, protein content in haemolymph of *S. litura* was reduced significantly ($P<0.05$; by LSD) in all treatments. The reduction of protein in sub fraction II and positive control azadiracthin did not show any statistical significance ($P>0.05$; by LSD). However, reduction from sub fraction III, IV and VI are statistically significant compared to control at 50ppm concentration. The reduction of protein was based on the concentration of the sub fraction. Irrespective of concentration the reduction of protein was recorded at the maximum of 31.3mg/ml at 100ppm when compared to control (98.25mg/ml). Generally, protein concentration was decreased when the concentration of treatment increased. The ANOVA test followed by post hoc test LSD showed statistically significant ($P<0.05$) difference in fluctuation of protein concentration within the treatment.

**Electrophoretic analysis of haemolymph protein**

Qualitative changes of haemolymph protein profile in the larvae of *S. litura* on control and treatment of sub fractions were verified by using laser densitometer after the completion SDS-PAGE. Totally 12 clear fraction was detected in control. However, the larvae exposed to bioactive fraction II at 25ppm number of fraction was 9; 50ppm 13; 75ppm 12 and 100ppm 13. When the larvae exposed to sub fraction III number of fractions was 11which is almost constant in all the tested concentrations (Figure 1). The number of fractions detected from treatment of sub fraction IV was 11 at 25ppm and 100ppm concentration. However, the number of detection in 50ppm and 75ppm treatment was 12. The larvae exposed to sub fraction VI, number of protein bands detected at 25ppm and 75ppm concentration was 11 and at 50ppm 12 bands were clearly detected. However, minimum number of 10 bands was detected only in 100ppm treatment (Figure 2). In general, a slight variation was observed in the quality of haemolymph protein of larvae treated with different concentrations of sub fractions. Maximum qualitative changes were observed only on storage proteins with the molecular weight of 68KD. The concentration of storage protein was reduced maximum in sub fraction II and III at 25ppm (Figure 1 lane no. 1 and 5) and it was increased in sub fraction III at 100ppm (Figure 1 lane no. 8) and IV at 25ppm (Figure 2 lane no.1) compared to control. In sub fraction VI treated larvae; storage protein concentration was reduced at 25, 50 and 100ppm concentration and increased in 75ppm (Figure 2 lane no. 7) concentration. Generally high molecular weight polypeptide concentration was gradually reduced in all the sub fractions treatment compared to control.

## DISCUSSION

Proteins constitute basic entities in the living being and undergo both quantitative and qualitative changes during development (Engelmann, 1979) and also act as essential component of structural material and are in a state of continuous flux with regard to their synthesis and degradation. In the present study the concentration of protein was decreased in the larvae of *S. litura* under the stress of *S. lineare* sub fractions. The quantitative changes in the haemolymph protein of treated larvae might be due to the interference of plant compounds and the reduction of protein might be due to toxic stress of sub fractions related to reduction of protein synthesis by deranging the protein machinery or it might be due to faster rate of proteolysis. Similar observations were reported by Bhagwan *et al.* (1992), Reddy *et al.* (1993), Krishnayya and Rao (1995), Chockalingam *et al.* (1987), Chitra and Rao, (1996) and Anitha *et al.* (1999). Decreased protein content due to botanical treatment was reported by many workers (De Man *et al.,* 1981; Sandhya Jadhav and Ghule, 2003; Rabinder Kaur and Rup, 2003). Ayyangar and Rao (1990) working with azadirachtin on *S. litura* also reported decrease in the haemolymph protein of the treated insects. Vijayaraghavan *et al* (2010) suggested that reduction of protein under the influence of plant extracts was attributed with factors like reduction in synthesis of proteins or increased protein breakdown to detoxify the active principles present in the plant extracts. In addition, they have further suggested that decreased protein content may be due to the mechanism of lipoprotein formation which is used to repair damaged cells and tissue organelles. Obviously it is true insects under the stress condition energy demand is higher which may lead to the protein catabolism. Present study clearly demonstrates that larvae under the stress of bioactive sub fraction of *S. lineare* protein depletion were higher compared to control. In the present study qualitative changes in haemolymph protein was observed in larvae due to the effect of sub fractions. The

protein concentration of an insect is dependent on its synthesis, breakdown and water movement between tissues and haemolymph. Haemolymph volume changes under the stress resulting in fluctuations in the protein pattern. The appearance or late appearance of protein bands in the haemolymph in the treatment indicated the developmental changes due to toxic stress of sub fractions. The variation in protein bands occurred depending upon the compound and the concentration tested. Among the four sub fractions tested maximum variation was recorded on storage protein at 68kD at higher concentration (100ppm). The variation and fluctuation of protein band might be due to the susceptibility of the larvae to sub fractions. This is in agreement with the reports of Rajasekhara Rao and Devaprasad (1999), Boreddy *et al.* (2000), Padmaja and Rao (2000), Joseph Rajkumar and Subrahmanyam, (2000).

The intensity of storage protein at 68kD may not be uniform in all the treatment. The increased intensity of the protein band associated with increased protein content. The protein increment was attributed with increased activity of protein biosynthesis by its tool (aminoacids) or increased synthesis of new proteins by the fat body, haemolymph and other tissues (Shoukry *et al*. 2003). Our finding is also agreed with their suggestion. In addition, increased amount of storage protein in the haemolymph may also be a kind of detoxification mechanism under the stress condition. In conclusion, intensity of protein bands showed some variation in control and treated larvae it may be associate with differences in biological and biochemical activities under the stress condition. The disturbance of biochemical activities associated with either antifeedant activities (Kalavathi *et al.,* 1991) or enzyme activities (Chun *et al*., 1994) or malate dehydrogenase and malic enzyme activities (Mostafa, *et al.*, 1995) under stress condition. Our finding is also agreed with their possible suggestion and we also confirmed decreased antifeeding activity of *S. litura* under the stress of *S. lineare* subfractions (Jeyasankar *et al*., 2010).

## REFERENCES


Anitha, B., Arivalagan, M., Nalini Sundari, M.S. and G. Durairaj, 1999. Effect of alkaloid abrine, isolated from *Abrus precatorius* (Linn.) seeds on mealy bug *Maconellicoccus hirsutus*. *Ind. J. Exp. Biol.*, 37:415-417.

Ayyangar, G.S.G. and P.J. Rao, 1990. Changes in haemolymph constituents of *Spodoptera litura* (Fabr.) under the influence of azadirachtin. *Ind. J. Ent*., 52:69-83.

Bhagawan, C.N., Damodar Reddy, K. and Kumuda Sukumar, 1992. *Annona anamolies* and protein depletion in red cooten bug *Dysdercus koenigii*. *Ind. J. Exp. Biol*., 30: 908-912.

Boreddy, Y., K.C. Chitra, and Eswar Reddy, 2000. Studies on sublethal concentration of *Annona* seed extract on total proteins of *Spodoptera litura* (Fab.). *Entomon*, 25: 351-355.

Chari, M.S. and G. Ramaprasad, 1987. Integrated control of pests on tobacco. In :*Integrated pest control- Progress and Perspectives*. *Proc. National Symp, Association for Advancement of Entomology*, Trivandrum, India. pp.42-52.

Chitra, K.C. and S. R., Rao, 1996. Effect of certain plant extracts on the consumption and utilisation of food by *Prodenia litura* F. *J. Insect Sci.,* 9: 55-58.

Chockalingam, S., Nalini Sundari, M.S. and S. Thenmozhi, 1987. Alternatives to synthetic insecticides. *Proc. Symp.,* CRME. Madurai.

Chun, J., C.L. Goodman, W.C. Rice, A.H. Mcintosh, G.M. Chippendale and K.R. Schubert, 1994. *Pentaclethra macroloba* seed effect on larval growth, cell viability and midgut enzyme activity of *Helicoverpa zea* (Lepidoptera: Noctuidae). *J. Econ. Entomol.,* 87(5): 1754-1760.

De Man, W., A., De Loof, T. Briers, and R. Huybrechts, 1981. Effect of ascorbic acid on vitellogenesis in *Sarcophaga bullata*. *Entomol. Exp. et Appl*., 29:259-267.

Duraipandiyan, V., S. Ignacimuthu and M. Valanarasu, 2008. Antibacterial and antifungal activities of *Syzygium lineare* Wall. *Int. J. Integr. Biol*., 3(3):159-162.

Engelmann, F. 1979. Insect vitellogenin: Identification, biosynthesis and role of vitellogenesis. *Adv. Insect Physiol*., 14:49-108.

Florkin, M. and C. Jeuniaux, 1974. Haemolymph composition. In: The physiology of insects Rockstein M. (Ed.) Academic press, New York p255-307.

Huang, Z., P. Shi, J. Dai and J. Du, 2004. Protein metabolism in *Spodoptera litura* (F.) is influenced by the botanical insecticide azadirachtin. *Pesti. Biochem. Physiol.*, 80(2): 85-93.

Jbilou, R. and F. Sayah, 2007. Effects of *Peganum harmala* (Zygophyllaceae) seed extracts on the development of *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Int. J. Trop. Insect Sci*., 27: 199-209.

Jeyasankar, A., N. Raja and S. Ignacimuthu, 2010. Antifeedant and growth inhibitory activities of *Syzygium lineare* Wall (Myrtaceae) against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). *Curr. Res. J. Biol. Sci*., 2(3): 173-177.

Joseph Rajkumar, A. and B. Subrahmanyan, 2000. Effect of Plumbagin and Azadirachtin on cuticular proteins of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Entomon*. 25:185-192.

Kalavathi, P., B.V. David and C. Peter, 1991. Evaluation of *Vitex negundo* (Verbanaceae) for the control of certain insect pests of field crop. *Pesti. Res. J.,* 3(1): 79-85.

Khatter, N.A. and F.F. Abldahb, 2010. Effect of *Ricinus communis*, *Brassica nigra* and mineral oil kemesol on some biochemical aspects of larvae stage of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *J. Egypt. Soc. Parasitol.,* 40(1): 151-158.

Krishnayya, P.V. and P.J. Rao, 1995. Plumbagin effects on *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) IV. Final instar haemolymph trehalose, cations nucleic acids. *Entomon,* 20:197–202.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.

Lowry, O.H., Rosenbrough, N.G., Farr, A.L. and R.G. Ranall, 1951. Protein measurements with folin phenol reagent. *J. Biol. Chem*., 193: 265-275.

Mostafa, Z.K., L.S. El-Sherif and M.A.A. Hewady, 1995. Effect of certain volatile plant oils on the activity of malate dehydrogenase and malic enzyme in *Pectinophora gossypiella* (Saun.) and *Earias insulana* (Boisd) larvae (Lepidoptera: Noctuidae). *J. Egypt. Ger. Soc. Zool*., 24: 155-170.

Naga Jyothi, P. and Y. Suneetha, 2010. Ultrasound induced enhancement of protein metabolism and enzyme activities

in the silk gland of fifth instar silkworms, *Bombyx mori*, L. *Global J. Biotechnol. Biochem.,* 5(1): 50-54.

Padmaja, P.G. and P.J. Rao, 2000. Effect of plant oils on the haemolymph proteins of final instar larvae of *Helicoverpa armigera* Hubner. *Entomon,* 25: 107-115.

Rabinder Kaur and P.J. Rup, 2003. Influence of some plant growth regulators (PGR) on biochemical profile in the larvae of melon fruit fly *Bactrcera cucurbitae* (Coquillett) (Diptera: Trypetidae). *Entomon,* 28: 89-95.

Rajasekhara Rao, K. and V. Deva Prasad, 1999. Host-pant induced response to insecticides and haemolymph esterase pattern in American bollworm (*Helicoverpa armigera* Hub.). *Entomon*, 24:9-13.

Reddy, K.D., Bhagwan, L.N. and K. Sukumar, 1993. Depletion of amino acids and proteins in nymphal haemolymph of the red cotton bug (*Dysdercus koenigii*) treated with *Annona squamosa* extract. *Phytophaga*, 5: 45-50.

Sandhya Jadhav and R.N. Ghule, 2003. Quantitative changes in total body proteins and haemolymph proteins due to azadirachtin in the larvae of *Corcyra cephalonica* (ST.). *Entomon*, 28:157-159.

Saxena, S.C. and R.S. Yadav, 1983. A new plant extract to suppress the population of yellow fever and dengue vector *Aedes aegyptii* L. (Diptera: Culicidae). *Curr. Sci.,* 52: 713-715.

Shoukry, I.F., Khalaf, A.F.A., Hussein, K.T. and S.K. Khater, 2003. Toxicological evaluation of some botanical oils on biochemical aspects in the Indian meal moth *Plodia interpunctella* HB. (Lepidoptera: Pyralidae). *Egypt. J. Biol.,* 5: 155-163.

Singh, U.G. 2000. Upsurge of *Spodoptera litura* Fab. on soybean in northern Madhya Pradesh. *Pest. Res. J.,* 8: 205-206.

Vijayaraghavan, C., C. Sivakumar, Zadda Kavitha and P. Sivasubramaniam, 2010. Effect of plant extracts on biochemical components of cabbage leaf webber, *Crocidolomia binotalis* Zeller. *J.Biopest*., 3(1special issue) 275-277.

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