

## **Efficacy of *Azadirachta Indica* Leaf Extract on the Biochemical Estimation of a Lepidopteran Pest *Pericallia ricini* (Lepidoptera: Arctiidae)**

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**Abstract:** The present investigation reports on the impact of the leaf extract of *Azadirachta indica* on total protein, carbohydrate and lipid level of fifth instar larvae of *Pericallia ricini*. Fifth instar larvae of *P.ricini* was orally treated with the 200, 400, 600 and 800ppm concentration of acetone leaf extract of *A.indica*. Protein, carbohydrate and lipid were estimated by the methods as Lowry, Nelson and Bragdon described, respectively. After treatment with *A.indica* leaf extract, total protein levels were reduced to 0.22mg/g from 0.72mg/g when compared with protein content of control larvae. The carbohydrate level was decreased gradually from control (0.58mg/g) to higher concentration (0.46mg/g). Lipid content in the *P.ricini* larvae also slowly decreased from 200ppm (1.59mg/g) to 800ppm (1.08mg/g) compared with control (2.13mg/g). From this results, this extract produce significant alteration in the biochemical profile of *P.ricini* larvae. Leaf extract of *A. indica* appear to be the most efficacious in controlling the *P. ricini* especially when they are administrated along with castor leaves providing suitable alternative to synthetic pesticides.

**Key words:** *Azadirachta indica* • *Pericallia ricini* • Antifeedant

### **INTRODUCTION**

The polyphagous wooly bear moth *P.ricini* (Lepidoptera: Arctiidae) is a serious pest with a wide range of hosts in India and many parts of the world and it is a serious pest on *Ricinus communis* commonly called castor plant. Castor, *R.communis* is one of the cash cultivated in dry lands as monocrop or mixed crop with ground nut, chilly, cotton and cowpea. Castor is an important crop grown in tropical and sub-tropical regions of the world. India is the world's largest producer of castor seed and dominates the international castor oil trade [1]. Among the several factors that contribute to low productivity of castor, the insect pests constitute the major factor. After the onset of the northeast monsoon in number *P. ricini* lays eggs on the leaves of the normal host plant *R. communis*. It is consider as a major pest in south Indian by agriculture entomologist. The larvae cause heavy damage to many economical important crops in the larval duration of 20 to 30 days. It is the major pest of castor, gingelly cotton, country bean, bringal, drum stick, coccina, banana, calotropis, sunflower, oleander, tea, sweet potato, pumpkin [2] and vanilla [3].

The chemical insecticides are very effective against the target insect pest but brutally eliminate other non target arthropods in the field. Indiscriminate use of chemical pesticide results in biomagnifications through food chain subsequently causing various hazardous effects. In recent years, a new approach has been made to use plant-derived materials which are safer and effective against phytophagous insects. The increasing number of investigations on plant-insect chemical interactions in the last few decades unveiled the potential of utilizing secondary plant metabolites, or allelo chemicals, as pest control agents. One of the efforts is the development of botanical insecticides as a novel and safer alternative strategy. Identification of phytochemicals which mimic insect morphogenetic hormones or have growth regulating activity and synthesis of potent hormone agonists and antagonists in the recent past have led to their consideration as components of biorational approach to pest management.

The well known nature products, Azadirachtin from the neem tree *Azadirachta indica*, cause an anti- ecdyson effect and interfere with insect ecdysis [4]. It is a powerful insect anti-feedant and repellents [5]. They may also

disrupt growth; inhibit moulting [6] and oogenesis [7]. It could be readily biodegradable, selective, non-mutagenic, with low toxicity to mammalian, causing minimal effects on the environment [8]. Neem seed kernel extracts were found to act both as ovipositional deterrent and repellent to insects including mosquitoes [9, 10]. The neem tree *A.indica* has shown promising results for the control of insects, including key pests of agriculture are susceptible to various behavioural and physiological effects of neem [11].

In view of this investigation on the effect of plant extracts, which could be used to check insect pests at various stages of development, the present investigation was undertaken to study the effect of *A. indica* plant extracts on the biochemical estimation of polyphagous lepidopteron pest, *P.ricini*.

## MATERIALS AND METHODS

**Culture of the Insects in the Laboratory:** The freshly emerged first instar larvae of *P. ricini* were collected from the castor plants cultivated in the vicinity of Madurai and kept in the laboratory at room temperature of 29 +1°C and 65%-75% R.H throughout the period of study. *A. indica* leaves (25g) were taken in soxhlet for 8h at 55°C with acetone and were evaporated to dryness. 200, 400, 600 and 800ppm concentration of the plant extract was prepared by dissolving 20, 40, 60 and 80mg of the concentrated extract in 100ml of acetone for oral application.

Leaf discs (4cm dia) of *R. communis* were used for bioassay tests. After washing it with tap water, the leaf discs were sprayed with 200, 400, 600 and 800ppm concentration of the plant extracts for twenty seconds, air dried at room temperature and kept in petri plates. The pre starved (24h) larvae were allowed to feed on the treated leaf discs. This treatment was given for three days. For each treatment, three replicates with one control were maintained. A minimum of six larvae per concentration were used for the experiments. The efficacy of the plant extract on this pest was assessed based on biochemical parameters.

**Biochemical Assay:** The treated and control larvae of fifth instar were sacrificed, oven dried and powdered. Powdered larval tissues were taken separately for analysis of protein [12], carbohydrate [13], Lipid [14]. The biochemical parameters are valuable and standard index in assessing and predicting the toxic effect on the

insects [15]. Hence, these basic biochemical parameters were selected for this present study.

**Estimation of Total Proteins:** Proteins were estimated by the method of Lowry *et al.* [12]. Proteins reacting with Folin- Ciocalteu reagent become purple blue proportional to the amount of proteins which can be read at 620nm. Total protein was calculated as:

Protein concentration

$$\text{in larvae (mg/g)} = \frac{2\pi r \text{ Concentration of test solution}}{\text{Volume of test solution taken}} \times 10$$

**Estimation of Total Carbohydrates:** Carbohydrates are estimated by the method of Nelson [13]. Proteins were removed from the tissue homogenate and the filtrate containing glucose only as reducing substrate was heated with alkaline copper reagent and subsequently treated with Arsenomolybdate reagent. The blue colour thus developed was read at 540nm. carbohydrate was calculated as:

Carbohydrate concentration

$$\text{in larvae (mg/g)} = \frac{\text{Concentration of test solution}}{\text{Volume of test solution taken}} \times 10$$

**Estimation of Total Lipids:** Total lipids were estimated by Bragdon's method [14]. Lipids separated from non-lipid components by chloroform methanol solution was estimated in the aqueous phase by the reducing action of fatty acids on a sulphuric acid dichromate mixture and the resulting green colour was then read at 600nm. Total lipid was calculated as:

Lipid concentration

$$\text{in larvae (mg/g)} = \frac{\text{Concentration of test solution}}{\text{Volume of test solution taken}} \times 10$$

## RESULTS AND DISCUSSION

Plants are a rich source of organic chemicals on earth. Already 10,000 secondary metabolites have been chemically identified. Secondary organic compounds synthesized by plants have an important role in protecting plants against insect herbivory by way of delay in larval growth and metamorphosis or antifeedants [16]. The neem in the present study clearly showed insecticidal and antifeedant effects on *P.ricini*. In this present

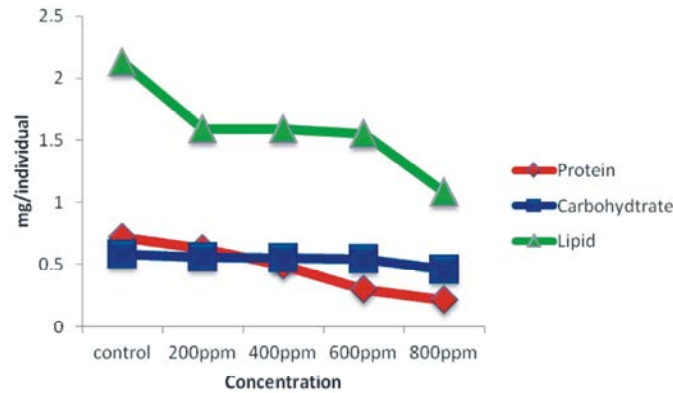


Fig. 1: Comparison of protein, carbohydrate and lipid of *P. ricini* with different concentrations of the leaf extract of *A. indica*.

Table 1: Effect of *A. indica* leaf extract on the total protein content of fifth instar larvae of *P. ricini* (Mean±SD).

Concentration (ppm)	Protein (mg/g)	Percentage of protein content
Control	0.72±0.015	100.00
200	0.63±0.015	87.50
400	0.49±0.020	68.05
600	0.30±0.01	41.66
800	0.22±0.011	30.50

investigation, fifth instar larva receiving different doses of *A. indica* leaf extract caused a greater depletion in protein, carbohydrate and lipid content (Fig 1). It showed that the increasing concentration of *A. indica* leaf extract seemed to diminish the protein content. The protein content was reduced from 0.72mg/g in control to 0.22mg/g in 800ppm. The reduction in protein level was comparatively higher in 800ppm. Neem extract was found to be effective in reducing the protein content of larvae and per cent decrease of protein in this treatment was 30.5, 41.66, 68.05 and 87.5 with 800, 600, 400 and 200ppm, respectively (Table 1). Protein synthesis is necessary for the maintenance of body growth and reproduction. Many factors had been implicated in the control of protein synthesis [17]. Reduction of protein content in *P. ricini* might be due to insecticidal interference of the extract with the hormones regulating protein synthesis. This view was supported by Ramakoteswara *et al.* [18] in *Spodoptera litura*. The protein content in an insect is dependent upon its synthesis, breakdown, water movement between tissues and haemolymph.

Neem extract contains Azadirachtin that has been known to affect protein amount and expression. Azadirachtin have been known to interfere with protein synthesis in *Schistocerca gregaria* [19] and *S. litura* [20]. Further, Huang *et al.* [21] reported that protein expression in *S. litura* was significantly lowered under Azadirachtin

treatment. The reduction of protein profile is probably due to structural deformities produced in larvae when they are exposed to neem extract. Brisca Renuga and Sahayaraj [22] also reported that the total head protein of *S. litura* was reduced due to the application of *Ageratum conyzoides* and *Ageratum vulgare* extracts. Animals require high energy under stress conditions and the energy demand may have led to the protein catabolism. Lack of protein caused retardation of many physiological processes in insects [23]. Similar trend was observed with chemical insecticides in the experiments conducted by Bashyia and Hazarika [24] in *Di cladispa armigera*, treated with methoprene and diflubenzuran, by Verma and Nath [25] in *S. litura* treated with carbamates and by Sak *et al.* [26] in *Pimpla turionellae* (L.) treated with cypermethrin.

Fifth instar larva treated with different doses of *A. indica* leaf extract caused great reduction in carbohydrate content (Table. 2). The carbohydrate content decreased from 0.56, 0.55, 0.54 and 0.46mg/g when treated with 200, 400, 600 and 800ppm of leaf extract of *A. indica*. The percentage of carbohydrate content was highly decreased in 800ppm treatment compared with other treatments. The plant extracts tested in the present investigation had considerably reduced the carbohydrate content of the *P. ricini* larvae. The carbohydrate level was drastically reduced in 800, 600, 400 and 200ppm of neem extracts treated larvae. This might be due to more sugars are metabolized to meet out the energy expenses during stress conditions. This could be the reason for the carbohydrate level depletion in the treated insects. Similar results were obtained by Seyoum *et al.* [27] in desert locust and by Abdul Razak and Sivasubramanian [28] in *Chelomenus sexmaculata* Fabricius and *Chrysoperla carnea* Stephens.

Table 2: Effect of *A. indica* leaf extract on the total carbohydrate content of fifth instar larvae of *P. ricini* (Mean±SD).

Concentration (ppm)	Carbohydrate (mg/g)	Percentage of carbohydrate content
Control	0.58±0.01	100.00
200	0.56±0.022	96.55
400	0.55±0.029	94.82
600	0.54±0.032	93.10
800	0.46±0.05	79.31

Table 3: Effect of *A. indica* leaf extract on the total lipid content of fifth instar larvae of *P. ricini* (Mean±SD).

Concentration (ppm)	Lipid (mg/g)	Percentage of lipid content
Control	2.13±0.04	100.00
200	1.59±0.29	74.64
400	1.59±0.27	74.64
600	1.55±0.25	72.76
800	1.08±0.34	50.70

A decrease in lipid content from 2.13mg/g of control larval tissue was observed in *P. ricini* larvae when treated with *A. Indica* and it was declined to 1.08mg/g in 800ppm treated larvae (Table. 3). The lipid content of the larval tissue treated with *A.indica* was found to be dosage depended. The lipid content was reduced when increased the concentration of leaf extract of *A.indica*. The percentage of lipid content was high in untreated larvae when compared with the larvae treated with plant extracts. This reduction in lipid profile indicates a negative effect of the extract on lipid metabolism and peroxidation. The decline in lipid quantity may be due to shift in energy metabolism towards lipid catabolism as the result of insecticidal stress induced by the extract. This observation is identical to the findings of Sak *et al.* [26] who found that *Pimpala turionella* suffered lipid depletion in haemolymph, fat bodies and oocytes exposed to cypermethrin. Lohar *et al.* [29] reported the decline in lipid content due to shift in energy metabolism to lipid catabolism due to insecticidal stress induced by *Tenebrio molitor*.

It is concluded that the impact factors of extracts on carbohydrate, lipid and protein contents in treated larvae are species and specific extraction. The lowering of these biochemical components indicates that these extracts can lowered the feeding and proper digestion of food. They further interrupt with protein synthesis hormones resulting in its decline. In contrast, the increase in certain profiles demonstrates the physiological stress induced by the extract and disturbed metabolic activity of the larvae. Many insecticides that were successful at controlling pest populations in the past have negative, unintentional

consequences on the environment. These range from toxicity to non-target animals, persistence in the environment and even the possibility of breaking down into more toxic chemicals. Plant botanicals and IGRs are two classes of insecticides that have been developed as safer alternatives to organic synthetic insecticides. They address ecological concerns by being specific to insects, having low retention times in the environment and breaking down into relatively harmless compounds.

## REFERENCES

1. Sahadevan, K.G., 2002. Sagging agricultural commodity exchanges: growth constraints and revival policy options. Economic and Political Weekly., 9: 3153- 3160.
2. David, B.V. and T.N. Ananthkrishnan, 2004. General and Applied Entomology. Tata McGraw-Hill Publishing Company Limited, New Delhi.
3. Vanitha, V., K. Thiyagesan and N. Baskaran, 2011. Prevalence of intestinal parasites among captive Asian Elephants *Elephas maximus*: effect of season, host demography and management systems in Tamil Nadu, India. Journal of Threatened Taxa., 3(2): 1527-1534.
4. Sieber, K.P. and H. Rembold, 1983. The effects of azadirachtin on the endocrine control of molting in *Locustus Migratoria*. J.Insect. Physiol., 29: 523-528.
5. Butterworth, J.H. and E.D. Morgan, 1971. Investigation of the locust feeding inhibition of the seeds of the neem tree *Azadirachta indica*. J.insect Physiology., 17: 969-977.
6. Koul, O., K. Amanai and T. Ohtaki, 1987. Effect of Azadirachtin on the endocrine events of *Bombyx mori*. J. Insect physiol., 33: 103-108.
7. Naumann, K. and M.B. Isman, 1995. Evaluation of neem *Azadirachta indica* seed extracts and oils as oviposition deterrents of noctuid moths. Entomol. Ex. Appl., 76: 115-120.
8. Mordue (Luntz), A.J. and M. Nisbet, 2000. An Azadirachtin from the neem tree *Azadirachta indica* : its action against Insects. An. Soc. Entomol. Brasil., 29(4): 615- 632.
9. Dimetry, N.Z., A.A. Barakat, E.F. Abdalla, H.E. Elmetwaly and A.M.E. Abd El-Salam, 1995. Evaluation of two neem seed kernel extracts against *Liriomyza trifoli* Burg. (Dip: Agromyzidae). Anz.Schalingskde Pflanzenschutz Umweltschut., 68: 39-41.

10. Chen, C.C., Y.J. Dong, L.L. Cheng and R.F. Hou, 1996. Deterrent effect of neem seed kernel extract on oviposition of the oriental fruit fly (Diptera: Tephritidae) in guava. *J. Econ. Entomol.*, 89: 462-466.
11. Schmutterer, H., 1990. Properties and potential of natural pesticides from the neem tree *Azadirachta indica*. *Annu. Rev. Entomol.*, 35: 271-297.
12. Lowry, O.H., A.L. Farr, R.J. Randall and N.J. Rosebrough, 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
13. Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biological. Chem.*, 153: 375-380.
14. Bragdon, J.H., 1951. Colorimetric determination of fatty acids after oxidation with sulfuric acid dichromate mixture. *J. Biol. Chem.*, 190: 513.
15. Preeti, S., M. Lalit, D. Kamal Kumar and S. Chand Narayan, 2011. Status of carbohydrate, protein and lipid profile in the mosquito larvae treated with certain phyto extracts. *Asian Pacific Journal of Tropical Medicine.*, pp: 301-304.
16. Isman, M.B., 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology.*, 51: 45-66.
17. Ghoneim, K.S., K.H. Hamadah and M.A. Tanani, 2012. Protein Disturbance in the Haemolymph and Fat Body of the Desert Locust *Schistocerca Gregaria* as a Response to Certain Insect Growth Regulators. *Bull. Environ. Pharmacol. Life Sci.*, 1(7): 73-83.
18. Ramakoteswara, S., K.C. Chiia and P. Kameshwara, 1991. Studies on the effect of certain plant extracts on the protein metabolism of *Spodoptera litura* (F.) *Indian J. ent.*, 57(4): 406-408.
19. Bakr, R.F.A., K.Z. Mostafa, S.N. Badawy and F.Z. El-Sokkary, 2004. The effect of different insect growth regulators on the main metabolites in the haemolymph of the desert locust, *Schistocerca gregaria* (Forsk.). *J. Egypt. Acad. Soc. Environ. Develop.*, 5(1): 51-68.
20. Fouda, M.A. and M.S. Amer, 1990. Carbohydrate, protein and lipid in the last larval instar and pupal stage of *Spodoptera littoralis* Bois. (Lepidoptera: Noctuidae) as affected by some juvenile hormone analogues. *Egypt. J. Appl. Sci.*, 5(2): 86-91.
21. Huang, Z., Ping Shi, Jianqing Dai and Jiawei Du, 2004. Protein metabolism in *Spodoptera litura* (F.) is influenced by the botanical insecticide azadirachtin. *Pesticide Biochemistry and Physiology.*, 80(2): 85-93.
22. Brisca Renuga, F. and K. Sahayaraj, 2009. Influence of botanicals in total head protein of *Spodoptera litura* (Fab.). *Journal of Biopesticides.*, 2(1): 52-55.
23. Rajendran, B., 1977. Studies on Juvenomimetic activity of some plant extracts on the red cotton bug, *Dysdercus cingulatus* Fabricius. (Heteroptera: Pyrrhocoridae). M.Sc., dissertation, Tamil Nadu Agricultural University, Madurai.
24. Bashiya, R.L. and L.K. Hazarika, 1996. Effect of methoprene and diflubenzuron on water, lipid, protein and chitin content of *Dicladispa armigera*. *Entomon.*, 21: 7-11.
25. Verma, A. and G. Nath, 1995. Toxicity of carbaryl on haemolymph protein of late larvae and pharate pupae of *Spodoptera litura* Fab. *Indian Journal of Entomology.*, 57: 83-88.
26. Sak, O., Fevizi Uckan and Ekrem Ergin, 2006. Effects of Cypermethrin on total body weight, glycogen, protein and lipid contents of *Pimpla turionellae* (L.) (Hymenoptera:Ichneumonidae). *Belgian Journal of Zoology.*, 136(1): 53-58.
27. Seyoum, E., R.P. Bateman and A.K. Charnley, 2002. The effect of *Metarhizium anisopliae var acridum* on haemolymph energy reserves and flight capability in the desert locust, *Schistocerca gregaria*. *Journal Applied Entomology.*, 126: 119-124.
28. Abdul Razak, T. and P. Sivasubramanian, 2007. Effects of three botanical oils on carbohydrate content in *Cheilomenes sexmaculata* Fabricius and *Chrysoperla carnea* Stephens. *Asian journal of Biochemistry.*, 2(2): 124-129.
29. Lohar, M.K. and D.J. Wright, 1993. Changes in the lipid content in haemolymph, fat body and oocytes of malathion treated *Tenebrio molitor* L. adult females. *Pakistan. J. Zool.*, 25: 57-60.