Indian Journal of Entomology 84(4): 770-773 (2022) DoI No.: 10.55446/IJE.2021.74

PROTEIN AND AMINOACID PROFILE IN DIAPAUSING LARVAE OF PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA (*SAUNDERS)

Kiranamaya Pradhan*, M Bheemanna and Saroja N Rao[1]

*Department of Agricultural Entomology, College of Agriculture;
[1]Pesticide Residue and Food Quality Analysis Laboratory,
University of Agricultural Sciences, Raichur 584104, Karnataka, India
*Email: kiranamayapradhan95@gmail.com (corresponding author)

ABSTRACT

The pink boll worm *Pectinophora gossypiella* (Saunders) is a threat to cotton cultivation. A study conducted in the NFSM laboratory of University of Agricultural Sciences, Raichur focused on the protein and amino acid profile of the diapausing larvae. Amino acid composition of diapause and non-diapause larvae revealed that out of 17 amino acids, 14 showed a significant difference. In diapausing ones total amino acid content was found to be 603.23± 5.10 µg/ g which was significantly higher compared to non-diapausing ones at 534.91± 3.19 µg/ g. Likewise, protein content was more in diapausing larva destined to become female (27.15± 2.27 mg/ 100 g) as compared to non-diapausing ones (20.29± 2.61 mg/ 100 g). Thus, when larvae undergo diapause, the protein and amino acid content in larval body increases, implying that the larvae store dietary food for its survival during resting phase.

Key words: *Pectinophora gossypiella*, larvae, haemolymph, protein, amino acids, diapause, non-diapause, female male difference, dietary food, physiology

Cotton is infested by a number of insect pests of which the pink bollworm *Pectinophora gossypiella* (Saunders) is a major pest causing severe losses, in spite of several rounds of insecticidal applications. The locule damage by this pest is to an extent of 55% with reduction in seed cotton yield to the extent of 35 to 90% (Narayanan, 2016). During the developmental it enters a state of arrested growth called diapause. Diapause always occurs during the end of crop season, when maximum number of mature bolls are present, and larvae often form their hibernacula inside seeds. Hibernacula may occupy single seeds or double seeds. Survival of the pest from one season to another is entirely through hibernating larvae in seeds, soils and plant debris. Sharma (1999) reported sticks carrying green bolls, unopened and half opened bolls carried about 82% diapause larvae, while seeds carried about 12% diapausing larvae. Before insect become inactive in diapause there is usually a buildup of reserve food substances particularly in the fat body, with a consequent reduction in the proportion of water in the body. Comparison of diapause or non-diapause show that those destined for diapause buildup bigger food reserves. In adult *Pyrrhocoris apterus* diapause associated storage proteins accumulate in the haemolymph, and in *Bombyx* eggs destined for diapause receive about 10% more lipid during vitellogenesis than non-diapause eggs (Chapman, 2013). Proteins are the key factors within the cell influencing growth and development accompanied by variation in free amino acids (Singh and Baquaya, 1971). Pink bollworm conserves sufficient quantity of energy reserves during diapause stage to be utilized during pupal and adult stage (Jeffery et al., 1974). The present study attempts determining the quantitative pattern of total protein and free amino acids in haemolymph of diapausing larva of *P. gossypiella*.

MATERIALS AND METHODS

The diapausing larvae were collected from NFSM, laboratory, UAS, Raichur. Male and female larvae were sexed by observing the presence of testis at 8th abdominal segment of male larvae that are visible outside as black dots which is absent in female larvae (Dhara Jothi et al., 2010). The haemolymph sample was collected in an Eppendorf tube containing a pinch of phenyl thiourea from diapauses larvae by puncturing the prolegs after surface sterilization. The haemolymph was then centrifuged at 10,000 rpm in a refrigerated centrifuge at 4°C for 5 min. The clear supernatant was transferred to another Eppendorf tube. Thus, purified clear plasma samples were deep frozen at -20°C until estimations were done. HPLC system of Waters Series having separation module (2695) equipped with photodiode array detector (2996) and a Phenomenex Luna dC18 250×4.6 mm column were used. The haemolymph supernatant were filtered through a polyvinyl difluoride

filter (PVDF; Millipore, Millex-GV, filter 0.22 m dia.). The samples were eluted using isocratic solvent system at a flow rate of 0.8 ml/min, run time was 50 min, and the compounds were detected at 266 nm with 2 µl injected volume of the extract. The mobile phase was 10 mM potassium dihydrogen phosphate +10 mM dipotassium hydrogen phosphate and acetonitrile: methanol (60:40).

Standard samples of known amino acids (aspartic acid, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, cystine, valine, methionine, phenyalanine, leucine, isoleucine, lysine and proline) were used to spike the HPLC peaks to identify different amino acids. After identification of peaks corresponding to different amino acids, a range of concentrations for each amino acid were run through the HPLC to obtain a normal curve. The amounts of different amino acids present in the diapause and non-diapause larvae were estimated from normal curves based on peak areas. The quantitative estimation of total protein was done following the standard Bradford method using bovine serum albumin as standard and measurement of OD at 595 nm. The concentration of protein in the sample was calculated with the standard curve (10- 100 µg/ ml) with Y=0.0059X+0.0826, R^2= 0.935 and results were expressed in mg/ ml of haemolymph. Paired t-test (p=0.01) was used to determine the molar changes in amino acid composition between diapause and non-diapause larvae. Duncan's multiple range test (p=0.01) was used to determine the molar changes in protein content.

RESULTS AND DISCUSSION

The current HPLC based amino acid analysis method gave clear separation of all the 17 amino acids from the diapause and non-diapause larvae. The sequence of elution of all the amino acids in non-diapause and diapause larvae was similar to that of standards (Fig. 1, 2). Elution of some unknown amino acids was also observed within the elution time range of the test amino acids. Amino acid composition of diapause and non-diapause larvae with student t-test revealed that out of 17 amino acids analyzed 14 showed a significant difference between diapausing and non-diapausing larvae (Table 1); serine, histidine and proline did not show significant difference. Indicating that these have no role in diapause. In diapause larva tyrosine was in more quantity (70.31 µg/ g) differing significantly with that of non-diapausing larvae (56.62 µg/ g). The total amino acids concentration of diapause and non-diapausing larvae were 603.23± 5.10 and 534.91± 3.19 µg/ g, respectively, revealing that there is an increase in amino acids concentration as insect undergoes diapause. When the male and female were compared, all the amino acids show non-significant difference except for histidine; tyrosine and valine (with concentration of 39.60, 70.31, 52.46 µg/ g and 36.76, 66.65, 48.67 µg/ g in diapausing female and diapausing male, respectively) (Table 1). These results are in line with those of Jeffery et al. (1974) who reported that diapause larvae possessed more amino acids on a molar basis. Similarly, Han et al. (2008) found that the protein and amino acid contents of the hemolymph of diapausing larvae of pine caterpillar *Dendrolimus tabulaeformis* (William) were higher than that of non-diapausing larvae. The protein and amino acid content of diapausing larvae in the present study were 20.56± 1.20 mg/ ml and 4595 ± 45 µmol/ ml respectively as compared to 14.75± 0.85 mg/ ml and 3889± 13 µmol/ ml respectively in non-diapausing larvae. The amino acids have an important role in osmoregulation during diapause (Beadle and Shaw,

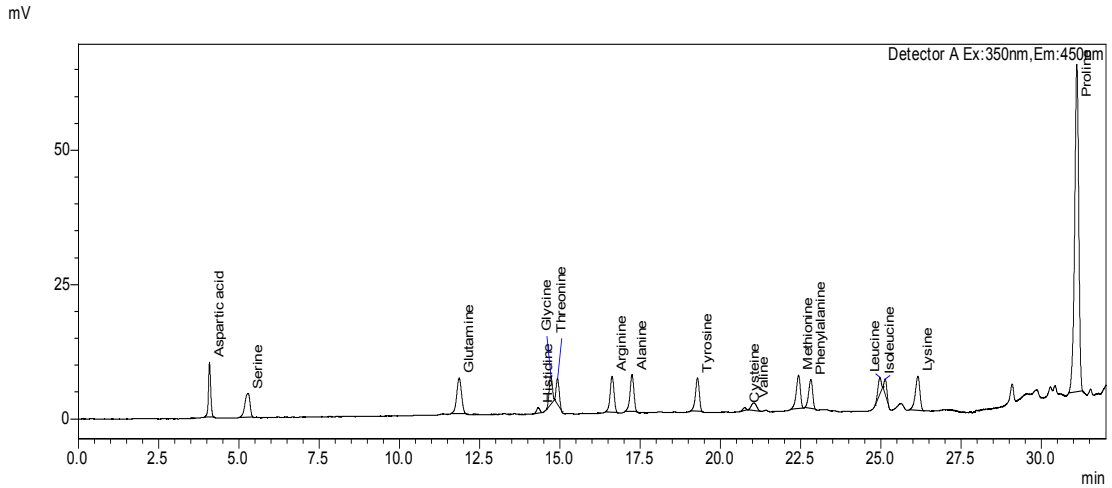

Fig. 1. Chromatogram of amino acid standards (HPLC)

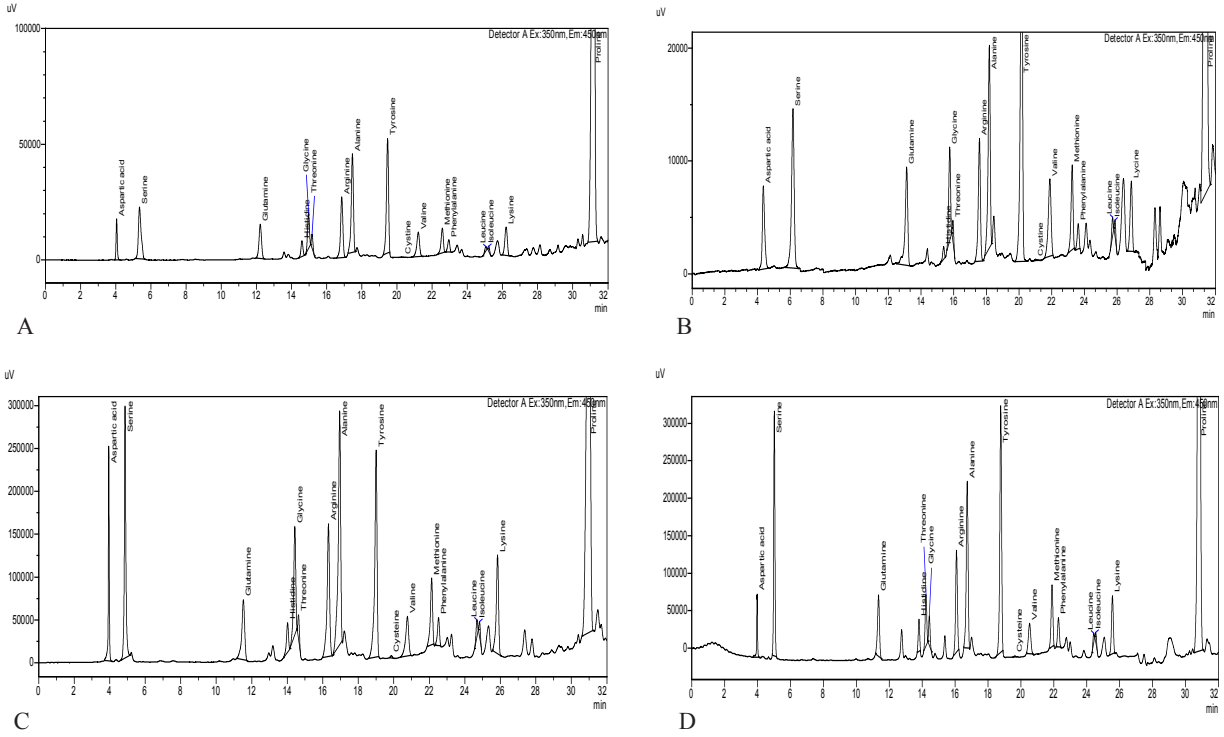

Fig. 2. Amino acid from A. diapause female B. non-diapause female C. diapause male D. non-diapause male larvae of *P. gossypiella* (HPLC)

Table 1. Amino acid profile of diapause and non-diapausing larvae of *P. gossypiella*

Amino acids	Diapause amino acid conc. (µg/ g)	Non-diapause amino acid conc. (µg/ g)	t-test $p=_{0.01}$	Diapause female amino acid conc. (µg/ g)	Diapause male amino acid conc. (µg/ g)	t-test $p=_{0.01}$
Aspartic acid	31.54± 0.68	26.54± 0.45	4.27E-07*	31.54± 0.68	31.34± 0.02	0.261
Serine	60.08± 0.73	59.45± 0.36	0.082	60.08± 0.73	59.65± 0.04	0.134
Glutamine	20.34± 0.27	16.62± 0.24	7.29E-09*	20.34± 0.27	20.06± 0.42	0.125
Histidine	39.60± 0.49	39.15± 0.21	0.187	39.60± 0.49	36.76± 0.08	7.34E-07*
Glycine	48.03± 0.17	42.86± 0.15	1.51E-11*	48.03± 0.17	47.91± 0.04	0.124
Threonine	18.83± 0.64	16.45± 0.46	7.77E-05*	18.83± 0.64	18.36± 0.42	0.109
Arginine	45.61± 0.42	40.23± 0.18	2.5E-09*	45.61± 0.42	45.61± 0.32	0.448
Alanine	60.45± 0.29	55.67± 0.45	2.19E-08*	60.45± 0.29	60.21± 0.17	0.079
Tyrosine	70.31± 0.44	56.62± 0.16	1.85E-12*	70.31± 0.44	66.65± 0.01	4.94E-08*
Cystine	8.25± 0.09	3.99± 0.05	1.49E-13*	8.25± 0.09	8.14± 0.12	0.063
Valine	52.46± 0.31	48.1± 0.08	8.49E-10*	52.46± 0.31	48.67± 0.11	3.31E-09*
Methionine	21.46± 0.29	17.81± 0.19	6.98E-09*	21.46± 0.29	20.96± 0.52	0.054
Phenylalanine	10.23± 0.04	9.01± 0.03	3.51E-11*	10.23± 0.04	10.33± 0.25	0.196
Leucine	9.56± 0.11	4.89± 0.05	3.11E-13*	9.56± 0.11	9.46± 0.09	0.119
Isoleucine	9.78± 0.03	7.94± 0.04	1.04E-12*	9.78± 0.03	9.63± 0.18	0.060
Lysine	29.36± 0.03	22.34± 0.02	2.86E-18*	29.36± 0.03	28.97± 0.50	0.064
Proline	67.34± 0.07	67.24± 0.07	0.135	67.34± 0.07	67.32± 0.01	0.249
Total	603.23± 5.10	534.91± 3.19	2.5E-09*	603.23± 5.10	590±3.30	3.31E-09*

*Individual amino acids statistically compared with paired t-test (p=0.01) LOS

1950), and also amino acids are the building block of proteins which act as a reserve source of energy during diapause (Buck, 1953).

The protein concentration of diapausing female (27.15±2.27 mg/ ml) was significantly different from that of the non-diapausing female (20.29±2.61 mg/ ml). Similar trend was observed in diapausing and non-diapause males with proteins (26.79± 2.27 and 19.42± 2.51 mg/vml, respectively (Fig. 3). The present results are in concurrence with those of Han et al. (2008) that the hemolymph proteins of larvae in *D. tabulaeformis*

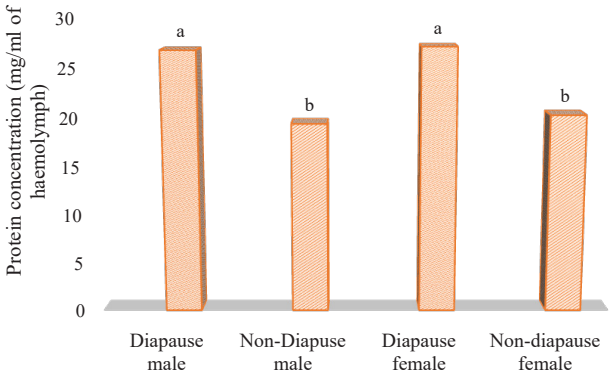

Fig. 3. Proteins- diapause and non-diapausing larvae of *P. gossypiella*

increased during diapause. Similar results was found by Rahile et al. (2015) that the protein concentration of larva of tasar silk worm *Antherarea mylitta* (Drury) increased during diapause stage. Mishra et al. (2009) observed increase in protein and amino acids during diapause in *A. mylitta*. Similar observations were also made by Agrell (1964) in *Calliphora*. Turunen and Chippendale (1979) reported that during diapause period juvenile hormone has been shown to control the synthesis and storage of diapause associated protein in the larval fat body of south western corn borer *Diatraea grandiosella* (Dyer). The increase in protein concentration in diapause stage might be due to the influence of juvenile hormone which stimulate the synthesis of diapause associated protein and also protein may act as a nutritive source of amino acids or peptides, a reservoir of proenzyme during diapause.

ACKNOWLEDGEMENTS

The first author thanks the Indian Council of Agricultural Research (ICAR), India, for providing financial assistance in the form of research fellowship during M Sc programme.

REFERENCES

Agrell I. 1964. The physiology of insect, Vol. 1, M Rockstein (ed.), Academic Press, New York. p. 91.

Beadle L C, Shaw J. 1950. The retention of salt and regulation of the non-protein fraction in the blood of aquatic larva *Sialis lutaria*. Journal of Experimental Biology 27: 96-100.

Buck T B. 1953. Physiological properties and chemical composition if insect blood. Insect physiology. K D Roeder (ed.). John Wiley. New York. pp. 147-190.

Chapman R F. 2013. The insect's structure and function. Cambridge University Press. pp. 448-450.

Dhara Jothi B, Valarmathi R, Nagarajan T, Sonai R T. 2010. Larval sex determination of pink bollworm- an easy tool for pairing of adults in mass rearing programmes, CICR Newsletter, Nagpur 26(3): 1-8.

Han R D, Gan Y L, Kong S H, Ge F. 2008. Physiological and endocrine differences between diapausing and non-diapausing larvae of the pine caterpillar *Dendrolimus tabulaeformis* (Lepidoptera: Lasiocampidae). Zoological Studies 47(1): 96-102.

Jeffery P L, Crowder L A, Watson T F. 1974. Amino acids and total nitrogen of diapause and non-diapause larvae of the pink bollworm, *Pectinophora gossypiella* (Saunders). Annals of Entomological Society of America 67(3): 473-474.

Mishra P K, Dinesh K, Jaiswal L, Kumar A B M K, Singh S K, Sharan J P P, Prasad B C. 2009. Role of haemolymph biochemical constituents and brain proteins in maintenance of pupal diapause in Daba bivoltine ecorace of tropical tasar silkworm *Antheraea mylitta* Drury, Journal of Ecophysiology 9(3): 243-252.

Narayanan E S. 2016. Biology and method of control of some important insect pests of cotton in India. Indian central committee publication. Bombay. 44 pp.

Rahile B S, Bangadkar M K, Zade S B, Saha R S. 2015. Studies on biochemical aspects of diapause preparation in the tasar silkworm, *Antheraea mylitta* D. under tropical climate of Vidarbha region of Maharashtra, India. International Journal of Current Microbiological and Applied Science 4(5): 318-326.

Sharma P D 1999. Status of pink bollworm on cotton in Haryana. In: Proceedings of one-day brain storming session on the current status of pink bollworm, CICR Regional Station, Sirsa, India, 24 September 1999. 2: 14-20.

Singh A, Baquaya M V. 1971. Amino acid in insects, changes during ontogeny and various physiological and pathological conditions. Journal of Science and Technology 9(2): 158-182.

Turunen S, Chippendale G M. 1979. Possible function of juvenile hormone dependent protein in larval insect diapause Nature 280: 836-838.

(Manuscript Received: February, 2021; Revised: August, 2021;
Accepted: August, 2021; Online Published: October, 2021)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e21053