Serangga 17(1): 57-66
ISSN 1394-5130 © 2012, Centre for Insect Systematics,
Universiti Kebangsaan Malaysia

ELECTROPHORETIC BANDING PATTERNS OF ESTERASE ISOZYMES IN THE ADULT FLY OF *BACTROCERA PAPAYAE* AND *B. CARAMBOLAE* (DIPTERA: TEPHRITIDAE) BY USING POLYACRYLAMIDE GEL

M. Hasanuzzaman[1,2] & A. B. Idris[1]
[1]School of Environmental and Natural Resource Sciences,
Faculty of Science and Technology, Universiti Kebangsaan Malaysia,
43600 Bangi, Selangor Darul Ehsan, Malaysia
[2]Institute of Food and Radiation Biology (IFRB),
Atomic Energy Research Establishment,
Bangladesh Atomic Energy Commission, Ganakbari,
Savar, Dhaka, Bangladesh
Email: mhasanuzzaman72@yahoo.com

ABSTRACT

Asian papaya fruit fly, *Bactrocera papayae* Drew & Hancock and carambola fruit fly, *B. carambolae* Drew & Hackock (Diptera: Tephritidae) are the major agricultural pests, especially fruits and vegetables in Malaysia. A polyacrylamide gel electrophoretic technique was used for the first time to study the esterase isozyme patterns in the male and female adult flies of these two *Bactrocera* species. Two esterase isozymes, EST-1, EST-2 were detected and their relative mobility values were 0.46 and 0.15, respectively. EST-$1^{0.46}$ was highest mobility and EST-$2^{0.15}$ was lowest mobility and close to the cathode. Two bands, EST-$1^{0.46}$ and EST-$2^{0.15}$ were present in *B. carambolae*, whereas only one band, EST-$2^{0.15}$ was observed in *B. papayae*.

The thickness and the staining degree of bands varied in the adult flies of these two species. EST-$2^{0.15}$ band was thick and highly stained than the band of EST-$1^{0.46}$ in *B. carambolae*. There was no significant difference in male and female adults, when comparing among the same species. The results prove that different species have different esterase bands on polyacrylamide gel. This technique can be used to distinguish the adult fly of the two sibling species as well as helpful to develop environment friendly control methods for these pests.

Key words: Polyacrylamide gel, Asian papaya fruit fly, Carambola fruit fly, Electrophoresis, Esterase isozymes

ABSTRAK

Lalat buah papaya asia, *Bactrocera papayae* Drew & Hancock dan lalat buah belimbing, *B. carambolae* Drew & Hackock (Diptera: Tephritidae) adalah serangga perosak pertanian yang penting terutamanya pada buah-buahan dan sayuran di Malaysia. Teknik gel elektroforesis *polyacrylamide* digunakan buat pertama kali untuk mengkaji corak isozim esterase dalam jantan dan betina dua spesies lalat *Bactrocera* tersebut. Dua isozim esterase EST-1 dan EST-2 telah dikenalpasti dan nilai mobiliti relatif masing-masing 0.46 dan 0.15. EST-$1^{0.46}$ ialah mobiliti tertinggi manakala EST-$2^{0.15}$ ialah mobiliti terendah dan berdekatan dengan katod. Dua jalur, EST-$1^{0.46}$ dan EST-$2^{0.15}$ hadir pada *B. carambolae*, tetapi hanya satu jalur EST-$2^{0.15}$ kelihatan pada *B.papayae.* Ketebalan dan darjah perwarnaan jalur berbeza dikalangan lalat buah dewasa kedua-dua spesies ini. Jalur EST-$2^{0.15}$ adalah tebal dan perwarnaan sangat tinggi berbanding EST-$1^{0.46}$ dalam *B. carambolae.* Tiada perbezaan secara signifikan di antara jantan dan betina dewasa di antara spesies yang berlainan. Keputusan membuktikan spesies berlainan mempunyai jalur esterase yang berbeza di atas gel *polyacrylamide*. Teknik ini boleh digunakan untuk membezakan lalat buah dewasa spesies beradik dan juga membantu dalam mengembangkan teknik kawalan serangga perosak ini sambil dapat mempastikan persekitaran yang lebih mesra alam.

Kata kunci: gel *polyacrylamide,* Lalat buah papaya asia, lalat buah belimbing, elektroforesis, isozim esterase.

INTRODUCTION

Asian papaya fruit fly, *Bactrocera papayae* Drew & Hancock and carambola fruit fly, *Bactrocera carambolae* Drew & Hackock (Diptera: Tephritidae) are major agricultural pests, especially fruits and vegetables. In Malaysia, there are at least a hundred *Bactrocera* species of which only about half have been recorded (Chua et al. 2010). *B. papayae* has been recorded from 193 host plant species in 114 genera and 50 families in Asia; *B. carambolae* attacks more than 151 kinds of fruits and vegetables; and they are distributed in Southeast Asia (Malaysia, Singapore, Indonesia, Thailand, Brunei) and South-America (Suriname, Brazil, French Guiana) (Drew and Hancock 1994; Allwood et al. 1999). Its presence cause severe damage to the agricultural produce as well as halted/hindered the export/import of these products (Hasanuzzaman and Idris 2012).

Esterase comprises a multi-functional and heterogeneous group of enzymes which have as a shared characteristic participation in ester hydrolysis. In insects, they are related to several metabolic processes, such as food digestion, degradation of insecticides/insecticide resistance, pheromones and juvenile hormone hydrolysis (Campbell et al. 2003). Esterase patterns are important tool for analysis of genetic differentiation and evolutionary relationship of insects (Nascimento and de Campos Bicudo 2002). They are also stage-specific and tissue-specific in with morphological, physiological, or biochemical ontogenetic alterations (Cohen et al. 1977). A polyacrylamide gel electrophoretic technique was used to study the esterase isozyme patterns in the adult flies of these two *Bactrocera* species. The results prove that different species have different esterase bands on gel. This technique can be used to distinguish the adults of the two species as well as helpful to develop an environment friendly control method for these pests.

MATERIALS AND METHODS

Rearing of Insects

Initially the pupae of *Bactrocera papayae* and *B. carambolae* were collected from the Malaysian Agricultural Research and Development Institute (MARDI). Then the culture has been maintained generation wise in the laboratory, Universiti Kebangsaan Malaysia. Mixture of yeast and sugar (1:3) served as adult diet and water was supplied through soaked cotton. Fresh star fruits were used for egg laying as well as larval media. Rearing was maintained at 25±2ºC with 70-80% relative humidity and 14h light: 10h dark cycle.

Polyacrylamide Gel Electrophoresis (PAGE)

Male and female adult flies of *B. papayae* and *B. carambolae* from the laboratory reared stock were used for the experiments. According to Bernardo and de Campos Bicudo (2009), two individuals of each sample were homogenized for a better visualization of the bands in the gel, at 0ºC in 25 µl of buffer solution (0.1 M Tris-HCl plus 10% glycerol at pH 8.8). Ten micro liter of bromophenol blue (0.05 mg/ml) was added into each sample as a tracking dye. Homogenates were centrifuged at 10,000 rpm for 15 min at 5ºC. Esterase patterns were analyzed in polyacrylamide gels using a 10% separating gel and a 4% stacking gel (Laemmli 1970). After the sample application (10 µl), the gels were subjected to electrophoresis for 4 h at room temperature (25ºC) using a constant voltage of 200 V and 0.1 M Tris-glycine (pH 8.3) as the running buffer (Bernardo and de Campos Bicudo 2009). Esterases were identified in the gels following the technique described by Johnson et al. (1966); Steiner and Johnson (1973), using α- and β-Naphthyl acetates as substrates. The technique involved gel incubation for 45 min at room temperature (25ºC) in 50 ml of 0.1 M sodium phosphate at pH 6.2, and staining reaction in the dark for 1 h with a solution containing 20 mg of α-Naphthyl and 15 mg of β- Naphthyl acetates dissolved in 1 ml of acetone, used as substrates, 60 mg of Fast Blue RR and 5 ml of N-propanol in 50 ml of sodium phosphate buffer solution. The esterase isozyme bands were numbered from the anodal end of the gel according to the Recommendations of the Standard Committee

of Enzyme (Webb 1964). The relative mobility (Rm) value of esterase isozymes was calculated using the following formula (Raja et al. 2009).

$$Rm = \frac{\text{Distance of isoenzyme migration (cm)}}{\text{Length of gel after staining}} \times \frac{\text{Length of gel before staining (cm)}}{\text{Distance of dye migration (cm)}}$$

RESULTS AND DISCUSSION

The electrophoretic banding patterns of nonspecific esterase isozymes were observed on Polyacrylamide Gel Electrophoresis (PAGE) in the male and female adult flies of *B. papayae* and *B. carambolae* (Diptera: Tephritidae). Esterase iozyme patterns were shown in Fig. 1.

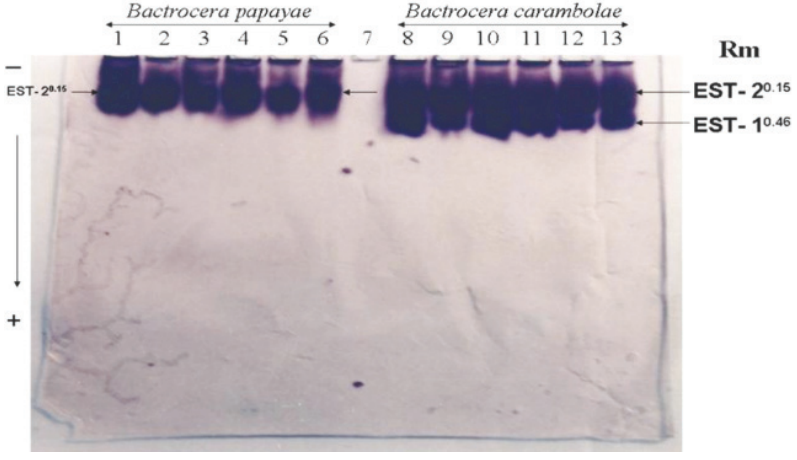

Figure 1. Result of Polyacrylamide Gel Electrophoresis showing the esterase isozyme patterns in adult flies of *B. papayae* and *B. carambolae*. Slot nos. (1,4,6) and (8,10,11) = Female; (2,3,5) and (9,12,13) = Male; 7 = Blank.

Two esterase isozymes (EST-1 and EST-2) were detected and their relative mobility values were 0.46 and 0.15, respectively. EST-$1^{0.46}$ was highest mobility and EST-$2^{0.15}$ was lowest mobility and close to the cathode. Two bands, EST-$1^{0.46}$ and EST-$2^{0.15}$

were present in *B. carambolae*, whereas only one band, EST-$2^{0.15}$ was observed in *B. papayae*. The thickness and the staining degree of bands varied in the adult flies of these two species. This observation is important because high degrees of staining and thickness are indicative of great enzymatic activity. EST-$2^{0.15}$ band was thick and highly stained than the band of EST-$1^{0.46}$ in *B. carambolae*. There was no significant difference in male and female adults, when comparing among the same species. The activation/staining patterns of esterase isozymes were shown in Table 1. For showing esterase isozyme activity, the following scale was used: - = absent, +++ = strong, +++++ = very strong. For better understanding, the schematic diagram (Fig. 2) was also provided.

Table 1 Esterase isozyme activation patterns in adult flies of *B. papayae* and *B. carambolae*.

Esterase Isozymes (with Rm)	*Bactrocera papayae*		*Bactrocera carambolae*	
	Male	Female	Male	Female
EST-$1^{0.46}$	-	-	+++	+++
EST-$2^{0.15}$	+++++	+++++	+++++	+++++

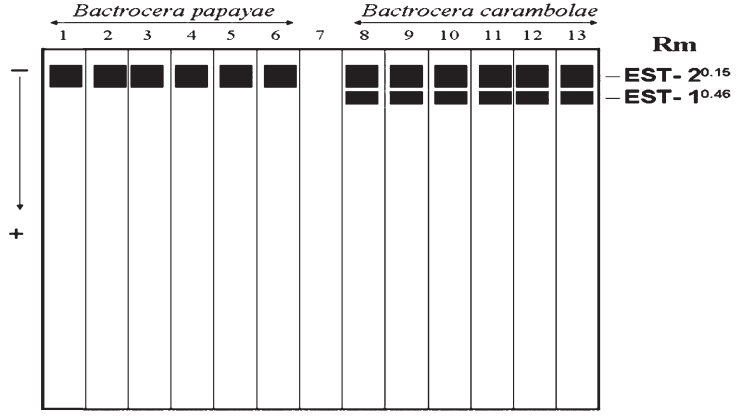

Figure 2. Esterase isozyme patterns in adult flies of *B. papayae* and *B. carambolae*. Slot nos. (1,4,6) and (8,10,11) = Female; (2,3,5) and (9,12,13) = Male; 7 = Blank.

Many researchers have been studied on the esterase isozymes banding patterns in insects. Borja et al. (2010) found four esterase isozyme bands, controlled by two loci (EST-1, EST-2) in *Bactrocera occipitalis* and *B. philippinensis*. Hasanuzzaman and Idris (2012) observed three esterase isozymes (EST-$1^{0.61}$, EST-$2^{0.46}$ and EST-$3^{0.15}$) on polyacrylamide gel electrophoresis (PAGE) during the different life stages of *Bactrocera carambolae*. EST-$1^{0.61}$ and EST-$2^{0.46}$ were present in larvae; EST-$2^{0.46}$ and EST-$3^{0.15}$ were observed in adults; whereas EST-$3^{0.15}$ was found in pupae; and esterase activity was not detected in eggs. Hasanuzzaman (2003) reported seven esterase bands, controlled by two esterase loci (EST-1 and EST-2) during the different life stages of *Bactrocera cucurbitae* on PAGE gel and their relative mobility values were 0.17, 0.27, 0.37, 0.46, 0.58, 0.87, 1.00. Two esterase loci (EST-1 and EST-2) were detected in the adult brown planthopper, *Nilaparvata lugens* by Bashar et al. (2002) on PAGE gel. Cohen et al. (1977) studied the expression of esterases during ontogenegis of the flour beetle *Tribolium castaneum* in PAGE gel. Two nonspecific esterases were detected and designated F (fast) and S (slow) according to their relative migration distances. In the adults of *Drosophila dirilis*, the maximum activity/intensity of esterase patterns was detected by Sasaki (1974).

The findings of the present research can be used for identifying these pests at the adult stages and also strengthen the necessity for a comparative study between other *Bactrocera* species in Malaysia and may impose a significant impact on the tropical environment. Furthermore, this study will be helpful for future study on esterase isozyme polymorphism in natural population; the phylogenetic relationships of the pest; and also in determination of insecticide resistant and non-resistant pest species in the population.

ACKNOWLEDGEMENTS

Authors would like to give sincere thanks to Prof. Dato' Dr. Mahani Mansor Clyde for providing the laboratory facilities to carry out this research program.

REFERENCES

Allwood, A.J., Chinajariyawong, A., Drew, R.A.I., Hamacek, E.L., Hancock, D.L., Hengsawad, C., Jinapin, J.C., Jirasurat, M., Krong, C., Kritsaneepaiboon, S., Leong, C.T.S. and Vijaysegaran, S. 1999. Host plant records for fruit flies (Diptera: Tephritidae) in South-East Asia. *The Raffles Bulletin of Zoology, Supplement No.*, 7: 1-92.

Bashar, K., Shahjahan, R.M., Baqui, M.A. and Hasanuzzaman, M. 2002. Electrophoretic banding pattern of esterase isozymes in adult brown planthopper, *Nilaparvata lugens* (Stal) (Hom.: Delphacidae). *University Journal of Zoology, Rajshahi University*, 21: 27-29.

Bernardo, A.A. and de Campos Bicudo, H.E.M. 2009. Variability of esterase patterns in adult flies of the *saltans* species group of *Drosophila* (subgenus *Sophophora*). *Genetica*, 137: 111-124.

Borja, F.N.N., Mendioro, M.S., Medina, C. dR. and Velasco, L.R.I. 2010. Isozyme Polymorphism in the Oriental Fruit Fly [*Bactrocera occipitalis* (Bezzi) and *B. philippinensis* Drew and Hancock] from Puerto Princesa, Palawan, Philippines. *Philippine Agricultural Scientist*, 93: 232-237.

Campbell, P.M., Robi, G.C., Court, L.N., Dorrian, S.J., Russell, R.J. and Oakeshott, J.G. 2003. Developmental expression and gene/enzyme identifications in the alpha esterase gene cluster of *Drosophila melanogaster*. *Insect Molecular Biology*, 12: 459-471.

Chua, T.H., Chong, Y.V. and Lim, S.H. 2010. Species determination of Malaysian *Bactrocera* pests using PCR-RFLP analyses (Diptera: Tephritidae). *Pest Management Science*, 66: 379-384. doi:10.1002/ps.1886.

Cohen, E., Sverdlov, E. and Wool, D. 1977. Expression of esterases during ontogenesis of the flour beetle *Tribolium castaneum* (Tenebrionidae; Coleoptera). *Biochemical Genetics*, 15: 253-264.

Drew, R.A.I. and Hancock, D.L. 1994. The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. *Bulletin of Entomological Research, Supplement*, 2: 1-68.

Hasanuzzaman, M. 2003. Esterase isozyme variation and polytene chromosome analysis in the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae). *M. Phil. Thesis. Department of Zoology, University of Dhaka, Bangladesh*, p. 1-154.

Hasanuzzaman, M. and Idris, A.B. 2012. Stage-specific patterns of esterase isozymes in *Bactrocera carambolae* (Diptera: Tephritidae). *GSTF Proc. Annu. Int. Conf. Adv. Biotech. (BioTech 2012), Bangkok, Thailand, 12-13 March, 2012,* pp. 51-54.

Johnson, F.M., Kanapi, C.G., Richardson, R.H., Wheeler, M.R. and Stone, W.S. 1966. An operational classification of *Drosophila* esterases for species comparison. *The University of Texas Publication*, 6615: 517-532.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680–685. doi:10.1038/227680a0

Nascimento, A.P. and de Campos Bicudo, H.E.M. 2002. Esterase patterns and phylogenetic relationships of *Drosophila* species in the *saltans* subgroup (*saltans* group). *Genetica*, 114: 41-51.

Raja, M., William, S.J. and Hussain, K.J. 2009. Genetic diversity of *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae) populations. *International Journal of Integrative Biology*, 8: 15-18.

Sasaki, F. 1974. Esterase isozymes of *Drosophila virilis*: Developmental aspects of the α- and β-esterases and their distribution in their body parts. *The Japanese Journal of Genetics*, 49: 223-232.

Steiner, W.W.M. and Johnson, W.E. 1973. Techniques for electrophoresis of Hawaiian *Drosophila* US-IBP. *Island Ecosystems Technical Report*, 30: 1-21.

Webb, E.C. 1964. Nomenclature of multiple enzyme forms. *Nature*, 203: 821.