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Comparative Study on Esterase Isozyme Patterns between the Larvae of *Bactrocera papayae* and *Bactrocera carambolae* (Diptera:Tephritidae)

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Abstract: Asian papaya fruit fly, *Bactrocera papayae* Drew & Hancock and carambola fruit fly, *Bactrocera carambolae* Drew & Hanckock (Diptera: Tephritidae) are the major agricultural pests, especially fruits and vegetables, in Malaysia. A vertical type polyacrylamide gel electrophoretic technique was used for the first time to compare the esterase isozyme banding patterns between the larvae of *Bactrocera papayae* and *B. carambolae*. Two esterase isozymes, EST-1, EST-2 were detected and their relative mobility values were 0.61 and 0.46, respectively. In both species, EST-1 and EST-2 bands present in larvae. The thickness and the staining degree of bands varied in both species; and EST-2 was comparatively thicker than the EST-1 band. There was no difference in the esterase isozyme patterns of larvae of these two *Bactrocera* species. So, the results prove that the larvae of the two *Bactrocera* species have similar non-specific esterase isozyme band patterns in the same polyacrylamide gel.

Key words: Polyacrylamide Gel · Asian Papaya Fruit Fly · Carambola Fruit Fly · Electrophoresis

INTRODUCTION

The family Tephritidae, or true fruit flies, under the order Diptera, which worldwide consists of around 4000 species [1]. According to Clarke *et al.* [2], the *Bactrocera dorsalis* complex (Diptera: Tephritidae) of tropical fruit flies contains 75 described species, largely endemic to Southeast Asia; and within the complex are a small number of polyphagous pests of international significance, including *B. dorsalis* sensu stricto, *Bactrocera papayae*, *Bactrocera carambolae* and *Bactrocera philippinensis*. In Malaysia, there are possibly at least a hundred *Bactrocera* species of which only approximately half of them have been recorded [3,4]. Of these, the *B. papayae* Drew & Hancock; *B. carambolae* Drew & Hancock; the melon fly, *Bactrocera cucurbitae* Coquillett; *Bactrocera umbrosa* Fabricius; *Bactrocera latifrons* Hendel; and *Bactrocera caudata* Fabricius are major agricultural pests that can cause serious losses and increase greatly the production cost [5]. Economic losses by the fruit flies in Malaysia were estimated at 12.8 million ringgit in the year of 1987 [6].

Asian papaya fruit fly, *B. papayae* Drew & Hancock and carambola fruit fly, *B. carambolae* Drew & Hackock (Diptera: Tephritidae) are most serious pests in Malaysia, as in other Asian countries. These two *Bactrocera* species are belonging to the *B. dorsalis* complex and considered as sibling species. Both species are polyphagous and infest mainly carambola, *Averrhoa carambola* L.; water apple, *Eugenia* spp.; sapodilla (chiku), *Manilkara zapota* L.; guava, *Psidium guajava* L.; mango, *Mangifera indica* L.; soursop, *Annona muricata* L. In addition, *B. papayae* also infests papaya, *Carica papaya* L.; banana, *Musa* spp.; brinjal, *Solanum melongena* L. var. *esculentum*; and chili, *Capsicum annuum* L. [5]. The Asian papaya fruit fly, *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) [7,8]. Its presence can cause severe damage of the agricultural produce as well as limitations to the import/export of these products.

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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, insecticide resistance, pheromones and juvenile hormone hydrolysis [9]. Esterase patterns are important tool for analysis of genetic differentiation and evolutionary relationship of insects [10]. They are also stage-specific and tissue-specific in insects [11] and are closely associated with morphological, physiological, or biochemical ontogenetic alterations [12]. The present study was undertaken to compare the esterase isozyme band patterns between the mature larvae (9 days old) of *B. papayae* and *B. carambolae* in the same polyacrylamide gel.

MATERIALS AND METHODS

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

Polyacrylamide Gel Electrophoresis (PAGE): Mature larvae (9 days old) of *B. papayae* and *B. carambolae* from the laboratory reared stock were used for the experiments. According to Bernardo and de Campos Bicudo [13], samples were homogenized in 200 µl of buffer solution (0.1 M Tris-HCl plus 10% glycerol at pH 8.8). Ten micro liter of bromophenol blue was added into each sample as a tracking dye. Homogenates were centrifuged at 10,000 rpm for 15 min at 5°C. Esterase isozyme patterns were analyzed in 10% polyacrylamide gels [14]. After the sample application (30 µl), the gels were subjected to electrophoresis (vertical type) for 4 h at room temperature (25°C) using a constant voltage of 200 V and 0.1 M Tris-glycine (pH 8.3) as the running buffer [13]. Esterase isozymes were identified in the gels following the technique described by Johnson *et al.* [15]; Steiner and Johnson [16], using α- and β-Naphthyl acetates as substrates. The esterase isozyme bands were numbered from the anodal end of the gel according to the Recommendations of the Standard Committee of Enzyme [17]. The relative mobility (Rm) value of esterase isozymes was calculated using the following formula [18].

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

RESULTS AND DISCUSSION

The electrophoretic banding patterns of nonspecific esterase isozymes were observed on Polyacrylamide Gel Electrophoresis (PAGE) in the mature larvae (9 days old) of *B. papayae* and *B. carambolae* (Diptera: Tephritidae). Esterase iozyme patterns were shown in Fig. 1. Two esterase isozymes, EST-1, EST-2 were detected and their relative mobility values were 0.61 and 0.46, respectively. EST-$1^{0.61}$ had highest mobility and EST-$2^{0.46}$ showed lowest mobility, close to the cathode. In both species, EST-$1^{0.61}$ and EST-$2^{0.46}$ bands present in larvae. The thickness and the staining degree of bands varied in both species and EST-$2^{0.46}$ band was comparatively thicker than the EST-$1^{0.61}$ band. There was no difference in the esterase isozyme patterns of these two *Bactrocera* larvae. Many researchers have been studied on the esterase isozymes banding patterns in insects. Borja et al. [19] found four esterase isozyme bands, controlled by two loci (EST-1, EST-2) in *Bactrocera occipitalis* and *Bactrocera philippinensis*.

Hasanuzzaman [20] 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. Hasanuzzaman and Idris [21] 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 (3 and 6 days old); 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 and Idris [22] also found in the adult flies that 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*. EST-$2^{0.15}$ band was thick and highly stained than the band of EST-$1^{0.46}$ in *B. carambolae*, but there was

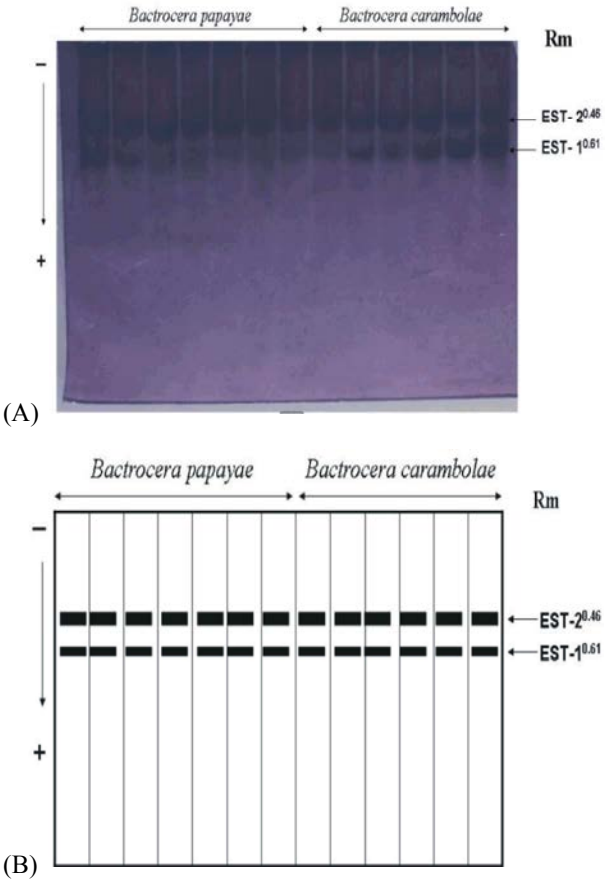

Fig. 1: Esterase isozyme patterns in the mature larvae (9 days old) of *Bactrocera papayae* and *Bactrocera carambolae* in polyacrylamide gels. (A) Photographic representation and (B) Schematic diagram.

no difference in male and female adults, when comparing among the same species. Two esterase loci (EST-1 and EST-2) were detected in the adult brown planthopper, *Nilaparvata lugens* by Bashar *et al*. [23] on PAGE gel. Cohen *et al.* [12] 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 virilis*, the maximum activity/intensity of esterase patterns was detected by Sasaki [24].

So, the vertical type polyacrylamide gel electrophoretic technique can be used to study the biochemical characterization of the esterase isozyme patterns in the larvae of the two *Bactrocera* species and also the developmental stages of the *Bactrocera* species. Furthermore, the data presented here will be helpful for future study on the phylogenetic relationships among these pest species. The information will also be helpful to develop environment friendly control methods for these pests, like sterile insect technique (SIT) which needs some basic information on the enzyme expression of the target species.

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