Esterase Isozyeme as an Indicator for the Genetic Variations of *Bactrocera zonata* (Saunders)

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ABSTRACT

The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is a serious pest in the last decade attacking a wide range of fruits in Egypt. The esterase isozyme patterns during the three different stages (larvae, pupae and adults (males and females) of three wild populations (Alexandria, El-Fayoum and Siwa) and laboratory strain were observed in agar-starch-polyvinylpyrolidine (P.V.P) gel. Two esterase isozyme (Est.a1and Est.a2) in anodal and three bands in cathodal (Est.c1, Est.c2 and Est.c3) for all stages were found. The esterase activity of female was more than that of male in the three tested wild populations and laboratory strain. The population of Siwa had greater esterase activity than those of El-Fayume and Alexandria. Generally, the wild populations were found to have more esterase activity than the laboratory strain.

Key words: *Bactrocera zonata*, esterase isozyme patterns, different stages, wild populations.

INTRODUCTION

The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a serious pest of fruits in many parts of the world, which originates in South and South-East Asia. This insect is found in the tropical Asia, India, Indonesia (Sumatra, Moluccas), Loes, Sri Lanka, Vietnam, Thailand (White and Elson-Harris,1992), Burma, Nepal, Bangladesh and probably all of South-East Asia (Kapoor,1993). Invasion of new areas by *B. zonata* is most probably linked to globe warming as this insect, as other species of the same genus, is favored by a warming up of the climate (Duyck *et al.,* 2004). Four hundred species belonging to the genus *Bactrocera* are widely distributed in tropical Asia, South pacific and Australia, with very few species in Africa and Europe (Drew, 1989). *Bactrocera zonata* was recorded in Egypt in 1999, where it caused a severe damage to a wide range of fruits including guava, peach, apricot and mango (El-Minshawy *et al*., 1999).

Esterase patterns are important tool for revealing the genetic differentiation and evolutionary relationship of insects (Nascimento and De Campos Bicudo, 2002). Esterases are classified as hydrolases, a large and diverse group of enzymes that catalyze the hydrolysis of a wide range of aliphatic and aromatic esters, choline esters and organophosphorous compounds (Dauterman, 1985).

The esterases are capable for hydrolyzing ester bonds generate and produce acid and an alcohol as metabolites. A wide range of insecticides contain ester bonds and they are susceptible to hydrolysis by esterase activity that insects develop as a mechanism of defense (Sogorb and Vilanova, 2002). Most esterases belong to the carboxylesterase (Punta *et a*l., 2012).

Individual resistance insect can be detected through increased quantities of an enzyme compared to their susceptible counterparts (Brown and Brogdon, 1987; Hemingway, 1989; Hemingway *et al.,* 1995; Hemingway & Karunaratne 1998). Therefore, the esterase isozyme analysis can be used as an indicator for genetic variations between different insect populations of different areas since the resistance is a genetic phenomenon.

The present investigation is concerned with determination of genetic variation expressed by the changes in the expression of esterase patterns during the developmental stages of the peach fruit fly, *Bactrocera zonata* of three wild populations (Alexandria- El-Fayoum and Siwa) and laboratory strain. These results may be useful determination of insecticide resistance of this pest species in nature and to find the more suitable alternative insecticide that can be used within the chemical control program or another control measures.

MATERIALS AND METHODS

Sample collections

Samples of peach fruit fly *Bactrocera zonata* were collected from different locations in Egypt (Alexandria, El-Fayoum and Siwa) from guava trees and the laboratory strain reared on artificial diet was brought from the Plant Protection Research Institute, Dokki, Giza. All stages Larvae, pupae and adult (males and females) were kept in refrigerator at -20 $^{\circ}$C.

Electrophoresis and Isozyme Technique:

1- Buffer solutions

A- 0.3M boric acid –sodium hydroxide buffer of pH= 8.0 was prepared according to Shaw (1965) where18.55 g. Boric acid and 50ml. of 1 M sodium hydroxide were added to distilled water to give a final volume of one liter of this buffer.

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B- 0.1 M phosphate buffer (pH=7.0) was prepared according to El-Metainy *et al.* (1977) (54.3 ml. of $KH_2PO_4$and 108.6 ml. K_2HPO_4 were completed to one liter with distilled water).

C- 1.0 M Tris-HCl buffer (pH= 7.0) was prepared according to El-Metainy *et al.* (1977) where a weight of 60.57 g. of Tris and 46.65 ml. of HCl were completed to one liter with distilled water.

2-Gel media

Agar-starch-polyvinylpyrolidine (P.V.P) gel was prepared according to Sabrah and El-Metainy (1985). Weight of 1.0 g. agar, 0.5 g. (P.V.P) and 0.3 g. of hydrolyzed starch were added to 100 ml. of 0.03 M boric acid –sodium hydroxide buffer (pH= 8.0) and the mixture was cooked in boiling water until the solution becomes transparent .Gel plates were prepared by pouring the solution on glass plates 20 x30 cm. to produce a smooth surface layer with thickness of 0.8-0.9 mm and they were kept at 4 °C until utilization (El-Metainy *et al*., 1977).

3- Procedures

Homogenates of collected and sampled larvae, pupae and adults (males and females) from the three different wild populations (Alexandria- El-Fayoum and Siwa) and a laboratory strain of peach fruit fly *Bactrocera zonata* were used in this study. For homogenizing, five adult individuals (either males or females) and also five larvae or pupae were randomly selected from each of the tested populations. Each sample was homogenized in cold mortar with 0.02 ml. of 0.3 M boric acid –sodium hydroxide buffer (pH 8.0) and centrifuged for 15 second. The homogenate of each sample was absorbed on strips of filter paper (2 x 8 mm), inserted on original line of gel plate and kept at 4 °C. for about 30 min and then the filter paper strips were removed.

4- Running condition

Electrophoresis was conducted in an incubator refrigerator adjusted at 4°C using a 250 volts AC electrical current, with constant voltage throughout the running period (90 minutes). The buffer (0.3M boric acid –sodium hydroxide) (pH = 8.0) was used as electrode buffer.

5-Staining solution

Ten milliliter of 0.01 M Tris –HCl buffer (pH = 7.0) and 3.0 ml. of substrate solution which contains 20 mg α-naphthyl acetate (α-NA), 20 mg β-naphthyl acetate (β-NA) dissolved in l ml acetone and completed to 5 ml by distilled water were admixed. Fifty milligrams of fast blue RR salt were dissolved in 5 ml distilled water and added after three minutes of the addition of α- and β-NA. (Shaw and Kaen, 1967). Incubation was extended for thirty minutes at room temperature and complete darkness. Plates were then distained in distilled water until a clear background of gel plate was appeared. The gel plates were replicated three times for all samples examined.

The similarity coefficients were used to construct dendogram using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) employing the SAHN (Rohlf, 2000).

RESULTS AND DISCUSSION

Esterase isozyme analysis through an electrophoresis running was done for Three different stages of peach fruit fly *Bactrocera zonata* larvae, pupae and adults (males and females)of three wild populations (Alexandria, El-Fayoum and Siwa) collected from guava trees and a laboratory strain reared on artificial diet that has been brought from Plant Protection Research Institute, Dokki, Giza. Esterase isozyme variations can be used for detecting the effect of the geographical locations and climate and thus might be of great help for developing novel control methods of this pest.

The symbols in electrophoretic analysis pattern were described in terms of Anode (a) or Cathode (c) zones according to their direction of mobility in the electrophoresis field. Each zone was assigned for a locus coding for an esterase isozyme. The locus with the least migration is designated the first; the next one is the second, and so on. Allelic variants, however, were designated according to their relative electrophoresis mobility within a locus; the allele for slow migrating band was specified by (S) and the allele for the fast one by (F). In case where no isozymes expressed at a certain locus, the symbol (N) (for null) was assumed the stained gel is showing the appearance of the detected esterase bands either toward anode or cathode.

Figure (1) illustrates the photograph of the stained gel and its diagram showing the esterase isozyme patterns of the different tested stages (larvae, pupae and adults) of the three studied wild populations (Alexandria, El-Fayoum and Siwa) of the peach fruit fly *Bactrocera zonata*. One anodal (Est. a1) band was found as common band for all the wild populations, while the results detected another anode band (Est.a2) for the all tested samples except those of Alexandria population.

Isozyme bands show dark staining in the column (4) which indicated that there is a high activity in females of the three wild populations more than that detected in larvae extractions. The number of esterase isozyme bands ranged from of 2 in Alexandria population to 5 in both the other wild populations.

For Alexandria population, only one band migrated towards the anode (Est.a1) and another band towards the cathode (Est.c3) for all stages, while there were no more bands (absent) in the other remainder locus (N) (null). Concerning Siwa population, five bands; two anodal bands (Est.a1 and Est.a2) and three cathodal bands (Est.c1, Est.c2 and Est.c3) for all stages were detected. Samples of El-Fayoum populations showed one anodal band (Est.a1) and two cathodal bands (Est.c1and Est.c2). Pupal and adult males plates revealed two anodal bands (Est.a1 and Est.a2) and three cathodal bands (Est.c1, Est.c2 and Est.c3), while in adult females samples there were two anodal bands (Est.a1and Est.a 2) and also two cathodal bands ((Est.c1and Est.c2).

The esterase isozyme analysis of the laboratory (susceptible) strain using the three stages [larvae, pupae, adults (males and females)] is shown in Fig. (2). Two anodal bands (Est.a1and Est.a2) and only one cathodal band (Est.c1) were detected for the samples of all stages.

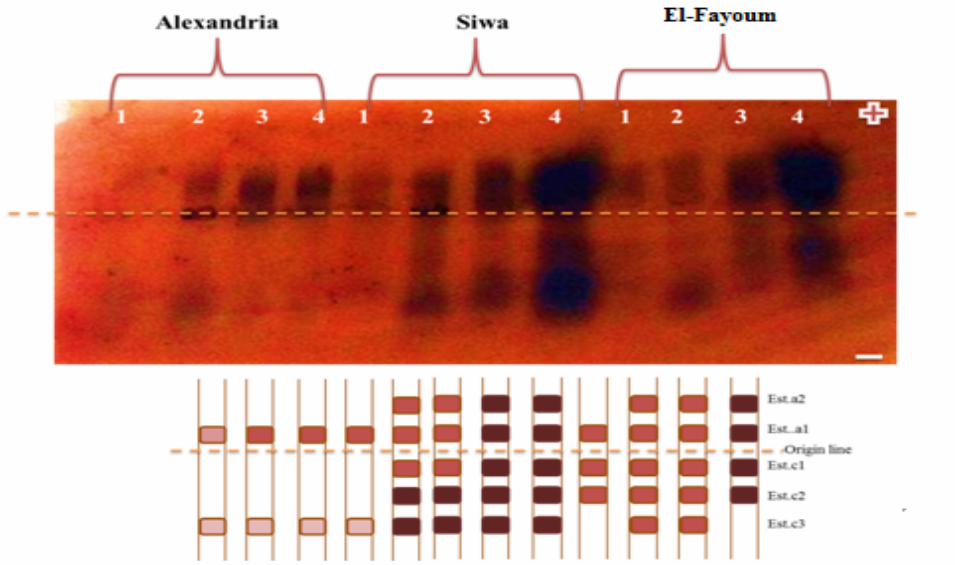

Figure 1. Zymograms (down) and photograph (up) of esterase isozymes pattern of *B. zonata* to three wild populations (Alexandria- El-Fayoum and Siwa).(1= Larvae, 2= Pupae, 3=Adult (male) and 4= Adult (female)

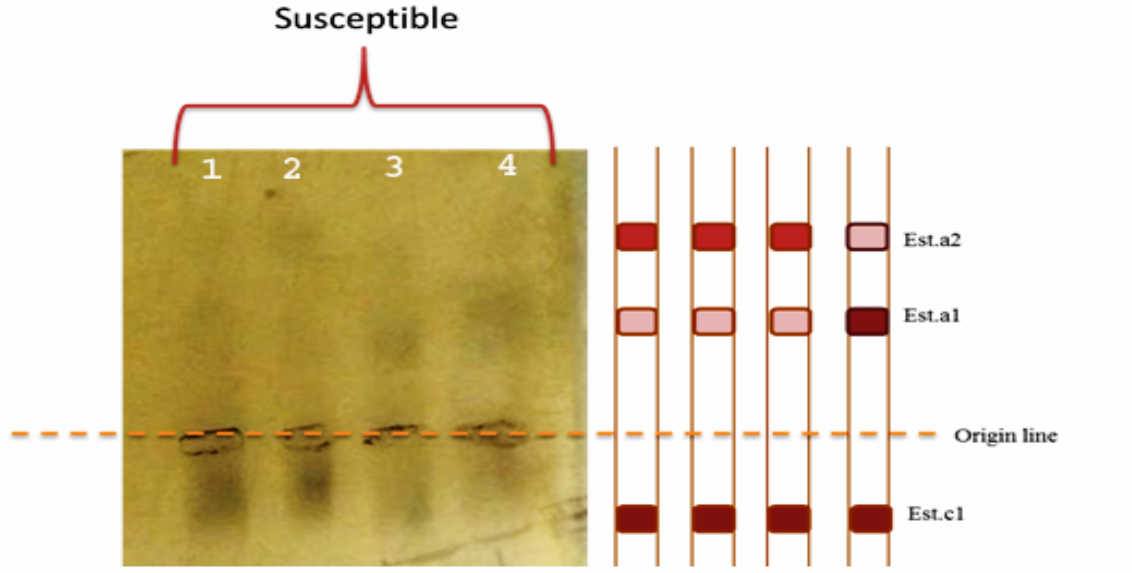

Figure 2. Zymograms (right) and photograph (left) of esterase isozymes pattern of *B. zonata* laboratory (susceptible) strain (1= Larvae, 2= Pupae, 3=Adult (male) and 4= Adult (female)

Generally, the alleles of cathodal isozyme banding were found to be more active than the alleles of anodal isozyme banding.

Figure (3) illustrates a dendogram with two major groups .the first group represents the laboratory strain (susceptible strain); the second group represents Siwa population that divided into two clusters (El-Fayoum and Alexandria) with a similarity percentage of 66%. The presented dendogram shows also similarities between the field populations and laboratory strain by 50 %.

Electrophoretic variations are generally suitable genetic markers in the study of genes linkage of different populations. The exhibited variation of the esterase isozymes which control the isozyme number and the isozyme activities can be useful for distinguishing individuals of different populations.

Patterns of polymorphism were observed between the three developmental stages [larvae, pupae and adult (male, female)] of the three wild populations (Alexandria, El-Fayoum and Siwa) and a laboratory strain. These patterns were differed in isoesterase numbers and density. These differences were found between the wild populations and laboratory strain.

The presented results are in the same trend with those of Hasanuzzaman and Idris (2012a) who observed that there were 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*. Esterase activity was not detected in eggs, increases in the larva, declines dramatically in the pupa and rises sharply in the adult stage. Hasanuzzaman and Idris (2012b) compared the electrophoretic banding patterns of esterase isozyme between the pupae of *Bactrocera papayae* and *Bactrocera carambolae* by using polyacrylamide gel. One esterase band, EST-1 was detected and the relative mobility value was 0.15 in cathode of both *Bactrocera* species. Hasanuzzaman (2003) also 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. Borja *et al*. (2010) found four esterase isozyme bands, controlled by two loci (EST-1 and EST-2) in *Bactrocera occipitalis* and *Bactrocera philippinensis*.

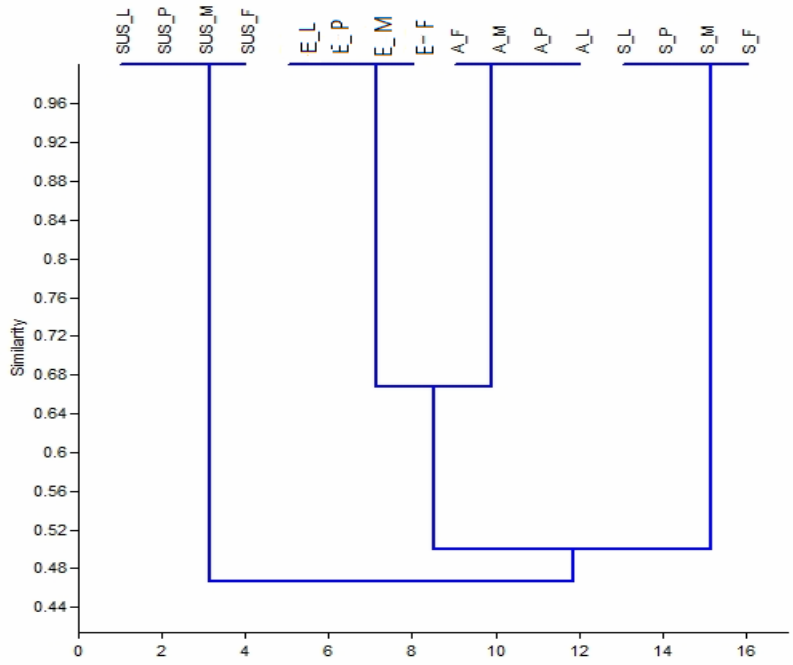

Figure 3. Similarity matrix phylogeny of esterase isozymes pattern of *B. zonata* belonging to three different wild populations (Alexandria- El-Fayoum and **Siwa) and laboratory strain (L= Larvae, P= Pupae, M=Adult (male) and F= Adult (female); (SUS = Susceptible strain, E=El-Fayoum, A=Alexandria and S= Siwa populations).**

Loukas *et al.* (1985) and Economopoulos & Loukas (1986) determined that reported the allelic frequencies of enzymes extracted from laboratory olive fruit fly (*Bactrocera olea*) strain reared on artificial substances and the wild ones were genetically differed .

The previous results indicated that the esterase enzyme bands of larvae in the three populations under study were less active in Siwa, El-Fayoum and Alexandria populations, respectively. Pupal stage esterases were the least active in Alexandria and highest in Siwa population. In adult stage, the females were found to have highest active esterase than males in the three wild tested populations and laboratory susceptible strain.

Moreover, the results of the present investigation confirmed those of Rashid *et al.* (2012) who found that the expression of these esterase isozymes increased with age in general, i.e., 2nd, 3rd-instar larvae, pupae to adult of *Bactrocera dorsalis* and *B. tau*. Pruett *et al*. (2000 and 2001) presented that the general esterase activity of the horn flies increases with aging meaning that newly emerged flies have a much lower level of general esterase activity than do more mature flies. Also Awad (1993) found that the activity of esterase isozymes in adult stage of Medfly *Ceratitis capitata* have band staining more than the other immature stages. El-Fandary (1987) obtained the patterns of esterase isozymes of Med-fly *Ceratitis capitata* and found that females have two activity bands more than males and the differences in mobility were observed between bands of females and males.

The laboratory strain of the peach fruit fly *B. zonata* used in the present investigation showed lower isozymes avtivity in the three development stages and it was relatively near to those of Alexandria population.

Montella *et al.* (2012) reported that a greater capacity to detoxify insecticide is due to an increase in the expression or activity of three major enzyme families; the most important is the esterase family that hydrolyse ester bonds, which are present in a wide range of insecticides and therefore, these enzymes may be involved in resistance to the main chemicals employed in control programs.

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الملخص العربي

استخدام مشابهات انزيم الاستريز كمؤشر للاختلافات الوراثيه لذبابه ثمار الخوخ

حسن على مصباح، نجده أحمد السيد، أحمد اسماعيل عوض، بسنت صبرى محمد البنا

اصبحت ذبابه ثمار الخوخ افه خطيره فى العقد الاخير تهاجم مدي واسع من الثمار فى مصر لذلك تم قياس نشاط انزيم الاستريز فى ثلاث مراحل تطوريه مختلفه للذبابه وهى طور اليرقه وطور العذراء وطور الحشره الكامله (الذكور- الاناث) لثلاث عشائر حقليه تم جمعها من الاسكندريه- الفيوم– سيوة بالمقارنه مع سلالة معملية باستخدام طريقة الهجرة الكهربية على جيل من نوع اجار- نشا- بولى فينيل بيروليدين اوضحت النتائج ظهور نشاط انزيمى لموقعين جينيين عند الأنود وثلاثة مواقع جينية عند الكاثود لجميع الأعمار ولوحظ أن النشاط الأنزيمى للأناث أعلى من الذكور فى الثلاث عشائر الحقلية المختبره والسلاله المعمليه وكانت عشيرة سيوة أعلى نشاط انزيمى من الفيوم والأسكندرية وعموما العشائر الحقلية كانت أعلى نشاطا مقارنة بالسلالة المعملية.