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ESTERASE VARIATION AND ORGANOPHOSPHEROUS RESISTANCE IN COTTON APHID, *Aphis gossypii* (GLOVER)

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ABSTRACT

Cotton aphid, *Aphis gossypii* (Glover) adults were bioassed with Malathion organophosphorus (OP) insecticide. The roles of carboxyesterase activity in resistance to Ops are discussed along 15 generations. Five substrates (α-naphthyl acetate, Indoxyl acetate, Lurate actate, Myristate acetate and Acetylthiocholine iodide) were screened. The results showed that, esterase activity, measured by using α-naphthyl acetate and Indoxyl acetate can detect the level of OP resistance and used as a marker for OP resistance in field population. Bands No. 1, 3 and 6 were classified as carboxyesterase in resistant strain. The diagnostic resistance-specific esterase bands have been used as a resistance marker to detect the population resistance in field for enhancing the control strategy.

INTRODUCTION

Esterase in general, have been noted to play a number of significant roles in resistance to insecticides, particularly organophosphates (Needamand and Sawicki1971, Hama 1976, Huges and Devonshire 1982, Oppenoorth 1982, de Malkenson *et al.* 1984). Specially, carboxylesterase production has been implicated in organophosphate resistance of the green peach aphid, *Myzus Persicae* (Sluzer). Esterase may contribute to resistance by hydrolysing the Pesticide (Anber and Oppenoorth 1989; Soderlund and Bloomquist 1990) or by temporary binding to the pesticide when the catalytic activity is relatively low (Devonshire 1989). In *Bemisia tabaci* (Genn.) organophosphate (OP) and pyrethroid resistance is correlated with high naphthyl esterase activity (Dittrich *et al.*,1990).

The present work was designed to study the relation between EST activity and resistance to Malation (an OP insecticide), and to explore the possibility of using EST activity as a marker for insecticide resistance in *A.gosspii*.

MATERAIL AND METHODS

Insect strain:

A Malation selected ressistant strain, as well as a Malthion susceptible strain, reared on cotton, (*Gossypium hirsutum* var.) were used for the study. The resistant aphids had been selected repeatedly with Malathion for 15 generations up to 11 fold resistant as compared with the susceptible insects kept without insecticides exposure. LC50 was measured by introducing samples of adults on cotton treated leaf on petri dish in four repictes (Each replicate had 100 adults). Mortality on all leaves was determined after an exposure period of 24 h.

Polyacralamid Gel Electrophoresis:

Esterase were separated by PAGE according to the methods of Davis (1964) on a 1.5 mm thick vertical slab polyacrylamide gel (Sturdier Slab Gel Electrophoresis Unit SE 400).

Buffers were as follows:

0.5 M Tris pH 6.8 (Stacking gel), 1.5 M Tris, pH 8.8 (resolving gel), Acrylamide solution (30%), 1% ammonium persulphate and TEMED. The electrode buffer was Tris-glycine (1.5M, pH 8.3). 0.5M Borate buffer, pH 4.1, Phosphate bufferA, pH 6.5, Phosphate buffer B, pH 6.5

Casting of gel:

The measurements of the glass plates of electrophoresis were 16 ×18Cm.

Preparation of gel:

The running gel concentration was 7% and was prepared by mixing 12.4 ml distilled water, 6.3 ml of Tris 1.5 M pH 8.8, 5.8 ml of Acrylamide solution (30%), 20 µl TEMED, 250 µl of % ammonium persulphate.

The stacking gel (4%) was made by 4.1 ml distilled water, 5.0 ml of Tris 0.5 M pH 6.8, 1 ml of Acrylamide solution (30%), 10 µl TEMED and 60 µl of 1% ammonium persulphate. Phosphate buffer (0.1 M, pH 6.5) was prepared by taking 46 ml of solution A added to 40 ml of solution B and the mixture was completed to one liter using distalled water.

Preparation of tissue samples:

Esterase electrophoresis analyses were taken from the whole body tissues of the insect. 0.03g. of each sample was homogenized in a cold porclain mortar containing 125 µl of distilled water. Each sample was then centrifugated and the supernatant were used.

Substrates :

α-naphthyl acetate, Indoxyl acetate, Lurate actate, Myristate acetate and Acetylthiocholine iodide .

Inhibitors:

Eserine, DDVP, Azodrin and Paraoxon.

Procedure:

A volume of 45 µl supernatant of each sample was applied in 10 µl of 15% sucrose solution and a drop of 0.1% bromophenal blue as a tracking dye. Power supply was adjusted to 10 mA for 10 minutes, and then the current was raised up to 30 mA until the tracking dye migrated to the end of the gel. After electrophoresis, the gel was soaked in borate buffer for 90 minutes at 4 °C (Sims, 1965). The gel was then rinsed towice rapidly in distilled water. The gel was stained for esterolytic activity by incubation at 25 °C in a freshly prepared soluation consisting of 50 mg of substrate and 50 mg fast blue RR in 100 ml of Phosphate buffer (0.1M, pH 6.5). The effects of the above mentioned inhibitors on cotton aphid tissue esterases were examined before being incubated with the substrate and coupler. The gels were placed in phosphate buffer pH 6.5 containing inhibitor for 30 minutes at 25 °C.

RESULTS AND DISSUCATION

I-Esterase patterns of the whole body tissue of cotton aphid

Table (1) represents the result of esterase bands produced by five substrates.

1- Lurate and Myristate acetate coud not able to hydrolyzed any esterase band.
2- Acetylthiocholine iodide showed single bands in both susceptible and resistant strain (band No.7).
3- α-naphthyl acetate and Indoxyl acetate produced 3 bands (No.2, 8 and 9) in susceptible strain and 8 bands (No. 1,2,3,4,5,6,8 and9) in resistant strain. Bands No.2, 8 and 9 were commmon to both susceptible and resistant strains. The α-naphthol and Indoxyl which were released on hydrolysis of the substrae, couped with the dye salt to produce an insoluble pigment at the site of enzyme activity.

Table (1): Staining behaviour of esterase bands

Band No.	Rm.	Substrate used in both susceptible (S) and resistant (R) strains									
		α-naphthyl acetate		Indoxyl acetate		Lurate acetate		Myristate acetate		Acetylthiocholine iodide	
		S	R	S	R	S	R	S	R	S	R
1	0.05	-	+	-	+	-	-	-	-	-	-
2	0.17	+	+	+	+	-	-	-	-	-	-
3	0.45	-	+	-	+	-	-	-	-	-	-
4	0.58	-	+	-	+	-	-	-	-	-	-
5	0.69	-	+	-	+	-	-	-	-	-	-
6	0.77	-	+	-	+	-	-	-	-	-	-
7	0.82	-	-	-	-	-	-	-	-	+	+
8	0.86	+	+	+	+	-	-	-	-	-	-
9	0.91	+	+	+	+	-	-	-	-	-	-

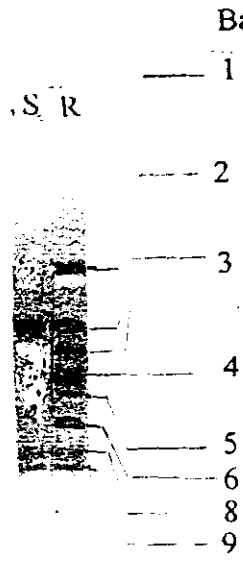

Fig.(1). Polyacrylamide gel zymogram detected by α-naphthyl acetate of esterase pattern of *A. gossypii* susceptible and Malathion selected resistant strain.

2- Characterization of esterase

Estrases have been classifed according to their actions with various specific enzyme inhibitiors to three classes (cholinesterase, carboxyesterase and arylesterase) (Bush *et al.*,1970) -i.e., cholinesterases inhibited by carbamates and organophosphates, carboxyesterases (aliesterases) inhibited by organophosphates only, and aromatic esterases (arylesterases) not inhibited by carbamates or organophosphates. The characteristics of esterases resulted from inhibitor specificity analysis are presented in Table(2).

Table (2): Classification of esterases bands in cotton aphid, *A.gossypii*

Inhibitor	Band No.								
	1	2	3	4	5	6	7	8	9
Paraoxon	+	-	+	-	-	+	-	+	+
Ezerine	-	-	-	-	-	-	-	+	+
Azodrine	+	-	+	-	-	+	-	+	+
DDVP	+	-	+	-	-	+	-	+	+
Esterase band type	CA	A	CA	A	A	CA	A	C	C

CA =carboxyesterase A = arylesterase C = cholinesterase

A. Resistant strain

Inhibitor specificty analysis revealed that bands No,1,3 and 6 were classified as carboxyesterase. Bands No. 8 and 9 were considered as cholinesterase. On the other hand, bands No.2,4,5 and 7 classified as arylesterase.

B- Susceptible strain

Bands No.2 was classified as arylesterase, while bands No.8and 9 were classified as cholinesterase.

Various chemicals representing insecticides (Wilkinson and Brattsten 1972, Nakatsugawa and Morelli (1976), insect hormones (Feyereisen and Durst 1987 & Eisner and Meinwald1987), and allelochemicals present in host plants (Brattsten *et al.*, 1977 and Wink 1987) induce detoxification enzyme system in insects. Numerous insect species respond to inducers by producing high levels of these enzymes (Brattsten 1988). Insects, species or even biotypes in which the polysubstrate monooxygenease (mixed funcation oxidase) system has been induced are better protected against putative toxic substances than those in which this system is not induced.

Esterases (EST) are known to be involved in the detoxification of a number of insecticides of different chemical groups currently in widespresd use: oraganophosphate, carbamates and pyrethoids (Devonshire and Moores1982; Blackman *et al.*, 1996 & Shigehara and Takada 2004). Field methods for monitoring resistance, based on EST activity, and were developed in aphids (Devonshire and Moores, 1982; Marullo *et al,* 1988). Ditrich *et al.*, (1985) found evidence for elevating EST levels (in particular α-naphthyl butyrate as the substrate and insensitivity of the enzymes to inhibition by OP insecticides in a resistant strain of *B.tabaci* from Sudan. Supporting evidence for the role of EST in resistance of *B.tabaci* to pyrethoids was found in Israel (Ishaaya *et al.*, 1987). Comared with the direct exposure methods, the enzymatic approach requires small samples (Brown and Brogdon, 1987). Dead individuals will give no or distorted pattern which mask the true variabilty.

This work suggested that the high activity can serve as a marker for the presence of resistant individuals in the field population.The association of high esterase activity concides with what is konow in peach aphid (Devonshire and Moores,1982) and in Sudances *B.tabaci* (Dittrich *et al.*, 1985) were elevated levels of EST activity is associated with pesticide resistance in mites and other organisms (Anber and Oppenoorth, 1939), and may indicated that the enzyme is high sensitive to inhibition by the pesticide.It is expected that different insect species or strains will have different resistant mechanisms. The discovery of genetic differencs in population of different insects is important in the future efforts devated in part to discover the resistant mechanisms. Among the screened esterases, we used naphthyl esterases which characterized with highly polymorphic group of enzymes widely used in studies of population genetics (Loxdale and den Hollander, 1989)

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التغيرات الإنزيمية والمقاومة للمركبات الفوسفورية العضوية في من القطن

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تم تقيم من القطن بواسطة مبيد الملاثيون (المجموعة الفوسفورية العضوية) حيث تم دراسة دور نشاط الكربوكسيل إستراز في ظاهرة المقاومة للمجموعة الفوسفورية العضوية بعد ١٥ جيلا لحشرة من القطن ٠ غربلت خمسة مواد قاعدية (سبستريت) [ألفا نافثيل اسيتيت، اند وكسيل استيت ، ليوران استيت ، مرستيت استيت واسيتيل ثيوكلين ايوديد] لاستخدام أنسبهم في قياس ظاهرة المقاومة٠ أوضحت النتائج أن النشاط الاستريزي بواسطة ألفا نافثيل اسيتيت، اند وكسيل استيت يمكن أن يستخدموا في تحديد مستوى المقاومة ويمكن أن يستخدموا مار كر لمقاومة المبيدات الفوسفورية العضوية في عشائر المن في الحقل ٠ الروابط ٦،٣،١صنفت على إنها كربو كسيل إستراز في السلالة المقاومة ٠ تشخيص الروابط الخاصة بالمقاومة يمكن استخدامهم علي انهم مار كر لتحديد العشائر المقاومة في الحقل لتحسين استراتيجية المكافحة للمركبات العضوية الفوسفورية.