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Biochemical Genetic Studies on Genotype Strains of Med-Fly *Ceratitis capitata* (Wied.)

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ABSTRACT

The biochemical studies were carried out on larvae, pupae and adult stages of four Med-fly genotypes, i.e. two laboratory strains (dark and yellow pupae), genetic sexing line (G.S.) and wild strain. Isoelectrophoresis of Alkaline phosphatase (AP), Esterase and Polyphenol oxidase enzymes were determined with further genetic variations, for their controlling of physiological reaction. Results of electrophoresis indicated that four zones of isozyming bands in Alkaline phosphatase are designated according to the migration from the origin line. The functional genes of Alkaline phosphatase isoenzymes of larvae were higher in band numbers and activities than in pupae and adult extractions in all materials. The highest activity units of bands were found in extraction of the adult male samples of (G.S.) line. No great variations were found between males and females in wild genotype and the two lab. strains. The esterase isozymes showed three banding zones designated corresponding to the migration from the origin line in all extracted materials. The genes controlling the activity of isoesterases varied from banding zone to another according to the stage of development. The first zone of larval stage, and the second zone of pupal stage were more active than the other zones, while, all the three zones were more active in adult stage. For polyphenol oxidase isozymes the two methods used of enzymatic analysis indicated that larval stage was more active than in other stages, The yellow pupae strain and (G.S.) line showed activity in polyphenol oxidase enzyme than the other genotypes.

Keywords : Med-fly, *Ceratitis capitata*, Genotype Strains, Isoenzymes.

INTRODUCTION

The Mediterranean fruit fly *Ceratitis capitata* (Wied.)**,** is a major pest in temperate and subtropical regions worldwide, attacking over 150 varieties of cultivated fruit crops Christenson and Foote,(1960). Biochemical genetic studies on the Med-fly have been undertaken with the specific purpose of providing information for direct and/ or indirect supports of the Sterile Insect Technique (SIT) and other genetic control methods Milani, *et al*. (1985). The laboratory genetic studies of the Med-fly genotypes exhibited variations in the strains as well as (G.S.) line according to the biological characters El-Minshawy, *et al*. (2000,2003).Variants of a given enzyme function are direct indicators of genetic variations, and further differences among different lines used in (SIT) are confirmed by electrophoretic mobility of these isoenzymes.

In this article we examine the biochemical genetic variations among the previous four genotypes in the different stages of Med-fly. Isozymes of Alkaline phosphatase, Polyphenol oxidase and Esterase enzymes were detected to determine the further genetic variations.

MATERIALS AND METHODS

Three different enzymes ; Alkaline phosphatase (AP), Polyphenol oxidase and Esterase isoenzymes , were assayed among larvae, pupae, and adult stages of the wild genotype and two lab. Strains (dark and yellow pupae) beside genetic sexing line (G.S.).

Electrophoretic bioassay: Polyacreylamide gel electrophoresis (PAGE) was used for analysis of the latter isoenzymes. It was carried out by utilizing vertical slab gel electrophoresis unit, SE600, from Hoffer Scientific instruments. The thickness of the slab gel was 0.75 mm. and the PAGE was 8%.

Buffer solutions used in this study after El-Minshawy, *et al*. (2003), were; (1) - 0.1M Tris HCL buffer, pH 9, (2) - 0.1 M phosphate buffer, pH 7, (3) - 0.3 M boric acidsodium hydroxide buffer, pH 8) and (4) - 0.2 M Tris-citric acid buffer, pH 8.3. Gel buffer solution was composed of 0.09 M Tris, 0.08 M Boric acid, and 0.0025 M Sodium (EDTA) pH 8.4. with extraction buffer 0.125 M Tris-boric acid pH 8.9.

Samples of all materials were homogenated in glass homogenizing apparatus containing 300 μl of 0.1 M extracting buffer adjust to pH 7.0 for 2 minutes. Homogenates were centrifuged at 14,000 rpm for 30 min. under cooling (4 °C) for polyphenol oxidase and at 10,000 rpm for 15 min. also under cooling for Alkaline phosphatase, and Esterases enzymes. The supernatant was poured through a small plug of glass wool to remove floating fat particles, and the resulting volume used as the enzyme source. The electric current was adjusted at 4 °C using 250 volt for 6 hours. Staining procedure adopted for alkaline phosphatase (AP) according to the method of Psarianos, *et al*. (1987), and for polyphenol oxidase and Esterases according to the method of Sabrah, (1980). A. L. T. C. scanner Shimadzu, Dual-wave length, Cs-910 was used at 560 nm wave lengthg for densitometric tracing of isoenzymic bands.

Spectrophotometer bioassay: Samples of different genotypes of Med-fly were homogenated for 2 min. in 300 μl. of 0.1 M phosphate buffer, pH 6.5 and the source of polyphenol oxidase enzyme was prepared as mention before. An enzyme reaction tube contained 29.5 mg. methyl DOPA in 2.95 ml. of 0.1 phosphate buffer, pH 6.5 and 50 μl. of enzyme source was incubated for 5 min. at 30 °C. The intensity of the color produced was measured by light absorbance of 0.01/ min at 475 nm. in Spectronic 20 D colorimeter.

RESULTS AND DISCUSSION

Alkaline phosphatase

Fig. (1) shows the photograph and its diagram of alkaline phosphatase (AP) isozyme patterns of different stages of Med-fly genotypes. Isoziming bands containing thirteen bands at maximum distributed into four zones, l, ll, lll, and lV, according to the migration from the origin line.

The first Zone includes three bands numbers (1,2,and 3); Zone ll, includes two bands numbers (4,and 5); Zone lll, includes five bands numbers (6, 7, 8, 9, and 10); Zone lV, includes three bands numbers (11, 12, and 13). The first zone characterizing the larval stage, the third zone exhibit the pupal stage, especially the band No.10, and the fourth zone with the two larval and pupal stages. The pupal extracts of the two lab. strains were marked by the presence of the band No. 10. Moreover, the (G.S.) line had the maximum number of isozyming bands in immature and mature stages more than the other genotypes. The bands No. 2, 3, 11 and 12 were absent in either two adult sexes of the wild genotype and the two lab. strains compared with the males of (G.S.) line extracts, while band No. 13 was absent in the two adult sexes of wild genotype only.

The densitometric scan units of the (AP) isozyming bands showed in Table (1) and Fig. (2), which illustrated that the larvae of the four tested strains have higher units of density scan more than other stages. Also, data showed that bands No. 4and 5 of the second zone were the highest ones approximately in all extracted samples. The larval and pupal samples of wild genotype were less in bands activity than that of other strains. While the two adult sexes of the lab. strain (yellow pupae) were lowest in the activity units than two sexes of other strains and the highest units found in adults sample of the male (G.S.) line.

Moreover, the functional genes of (AP) extracted from larvae of all genotypes, were higher in band numbers and activities than that of pupae and adult extractions in all strains. No great variations were found between males and females in wild genotype and the two lab. strains. Bands No. 4 and 5 in zone 2 of male(G.S.) line were more active than the other bands. Therefore, the gene controlling the (AP) isozymes were descendingly in numbers from larval stage to the adult sexes, and have approximately the same activities.

In fact, these results indicated that the functional genes of Alkaline phosphatase isoenzymes of larvae were higher in band numbers and activities than in pupae and adult extracts of all materials. The results were supported by those of Gakhar and Vandana (1993) who reported that Alkaline phosphatase of *Anopheles stephensi* Liston appeared at maximum isoenzymes heterogeneity in larval stage than the other stages. While, the present results my be in agreement with those of Psarianos *et al*. (1987), in which they noticed that two peaks of Alkaline phosphatase enzyme activity were found in Med-fly; one just perior to pupation and the other during eclosion.

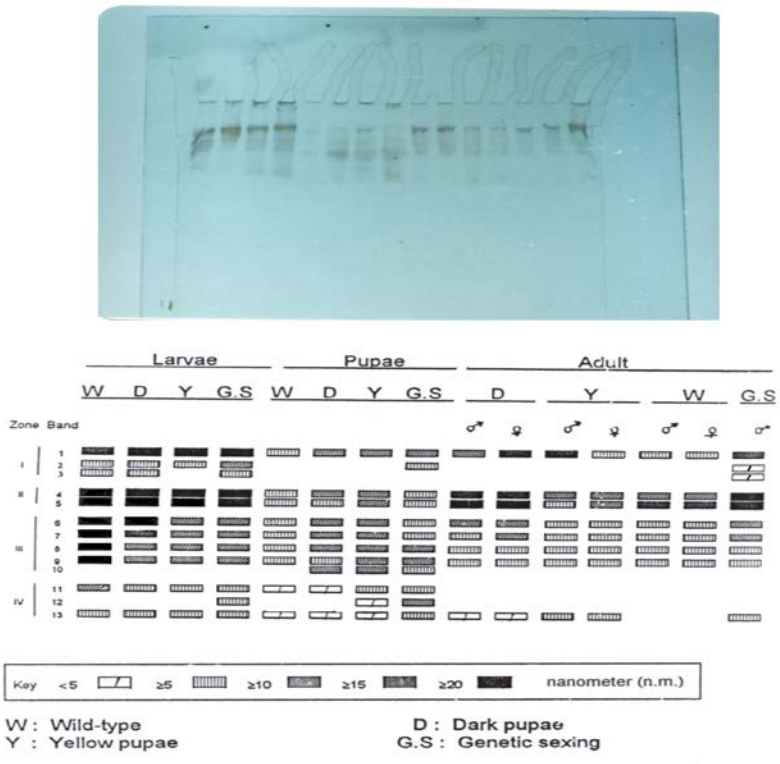

Fig. (1): Electrophoretic patterns of Alkaline phosphatase isozymes extracted from larvae, pupae, and adult individuals of Med-fly.

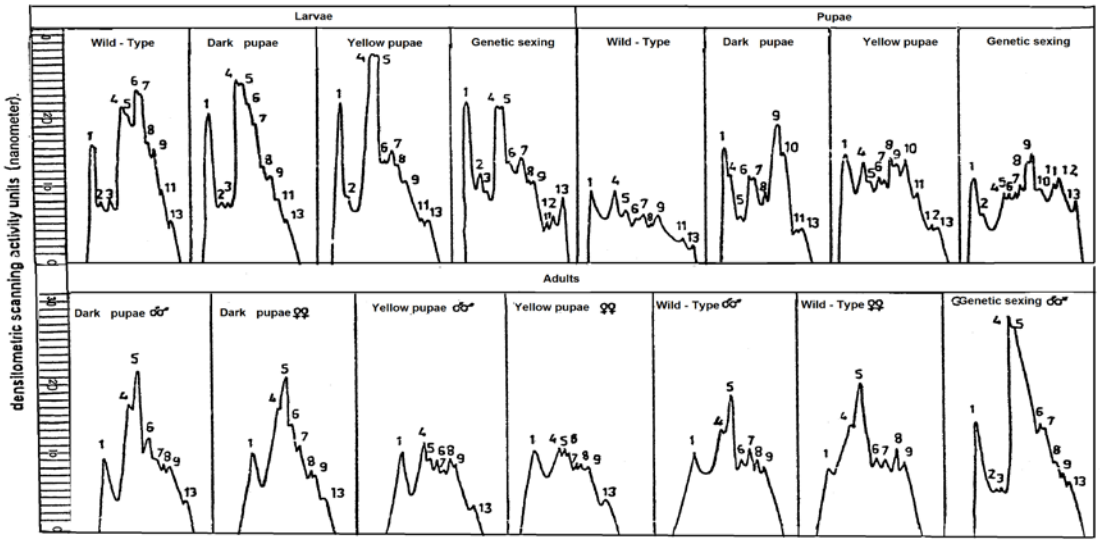

Fig. (2) : Densitometric tracing curves of alkaline phosphatase isozyme bands of Med-fly individuals extracted from immature stages (larvae and pupae) and from mature stage (adults).

Table (1): Densitometirc scan units* of alkaline phosphatase isozyme of the immature and adult stages of the four Med- fly lines.

Zone	No. Of band	Larvae				Pupae				Adult						
		W	D	Y	G.S	W	D	Y	G.S	D		Y		W		G.S
										♂	♀	♂	♀	♂	♀	♂
	1	17	21	23	22	10	15	15	11	11	11	11	10	10	8	15
I	2	9	9	9	12	--	--	--	7	--	--	--	--	--	--	--
	3	10	9	--	8	--	--	--	--	--	--	--	--	--	--	--
II	4	22	26	29	22	10	11	14	9	17	16	12	11	14	13	28
	5	21	25	29	22	8	7	11	9	21	21	10	11	18	19	26
	6	25	23	15	14	7	12	12	9	13	14	10	10	9	9	14
	7	24	20	16	15	7	12	11	10	10	12	9	9	10	9	13
III	8	17	14	14	11	6	10	14	13	9	9	10	9	9	10	10
	9	17	12	12	11	7	18	13	14	9	8	9	8	8	9	8
	10	--	--	--	--	--	15	14	9	--	--	--	--	--	--	--
	11	11	9	7	6	3	4	9	10	--	--	--	--	--	--	--
IV	12	--	--	--	7	--	--	4	11	--	--	--	--	--	--	--
	13	7	6	8	10	2	4	4	8	4	4	6	7	--	--	7

W: Wild-type **D: Dark pupae**
Y: Yellow pupae **G.S : Genetic sexing**
***nanometer (n.m.)**

Esterases

Fig. (3) shows the photograph and its diagram of Esterase isozyming bands of different stages of four tested genotypes of Med-fly. Three zones, l, ll, lll, designated from the origin line of migration, the first zone contains the first five bands (No's. 1, 2, 3, 4, and 5), zone ll contain the next four bands (6, 7, 8, and 9), and zone lll contain the bands (No's. 10, 11, and 12). The lowest band numbers of isoesterases were found in larvae of wild genotype (three bands only No. 1, 7, and 12), but the highest numbers were found in adult females of yellow pupae strain (12 bands), and the adult males of (G.S.) line (11 bands). Also, larval and pupal stages of wild samples had the lowest band numbers. Again, in adult stage the lowest band numbers were found in females of dark pupae and males of wild genotype than the other material extracts. The maximum number of isoesterases was found in the adult stage especially in yellow pupae strain than the other two immature stages.

Concerning the activities of esterase isoenzymes, Table (2) and Fig. (4) illustrated densitometric scanning units, and data indicated that the higher activities were found in adult stage males and females of all materials, and some bands diffused in all the first zone which was high in activity. In the pupal stage the band No. 7 in the two lab. Strains and (G.S.) line was the highest and obvious than the other bands of other zones. Therefore, it is concluded that the genes controlling the activity of isoesterases were

more active in the first zone of larval stage. In addition, activity of genes occurred on the second zone of pupal stage and in all the three zones of adult stage, and approximately in the all strains.

Results indicated that three banding zones were found in all extracted materials and the genes controlling the activity were different from zone to another according to the stage of development. The first zone of larval stage, and the second zone of pupal stage were more active than the others, while, in adult stage the three zones were more active than the others. These results were supported by those of Cladera (1981) who found that the pupae of *C. capitata* lab. strain proved to be dimorphic structure for the first locus (Est-1), and those of Civetta *et al.* (1990) who found that wild population of Med-fly proved to be polymorphic alleles for the Est-1 locus. Also, the present results of the present work in agreement with those of Vilardi *et al.* (1991) based on the electrophoretic analysis for the adult individuals of *C. capitata* from the field, they found four loci were controlling esterase isozymes, one at least was polymorphic with two alleles, but the other three loci were expressed only in females. Results of the present work were supported too by those of Sabrah *et al.* (1995) who studied the genic polymorphism and ontogenic variation of esterase isozymes in the Med-fly *C. capitata* (Wied.) collected from different geographical zones in Egypt. When compared with dark and yellow pupae genotypes, they found that five loci are responsible for the inheritance of the esterase isozyming bands of larvae and pupae, while four loci are found in the adult males and females.

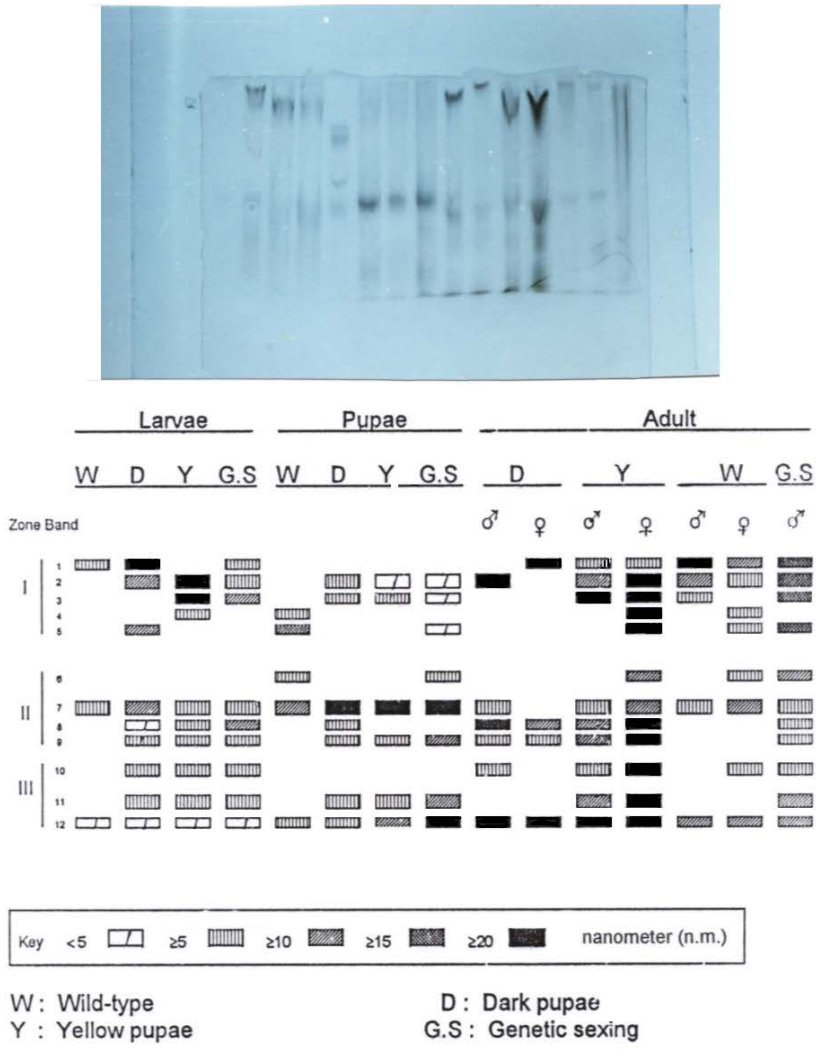

Fig. (3): Electrophoretic patterns of Esterases isozymes extracted from larvae, pupae, and adult individuals of Med-fly.

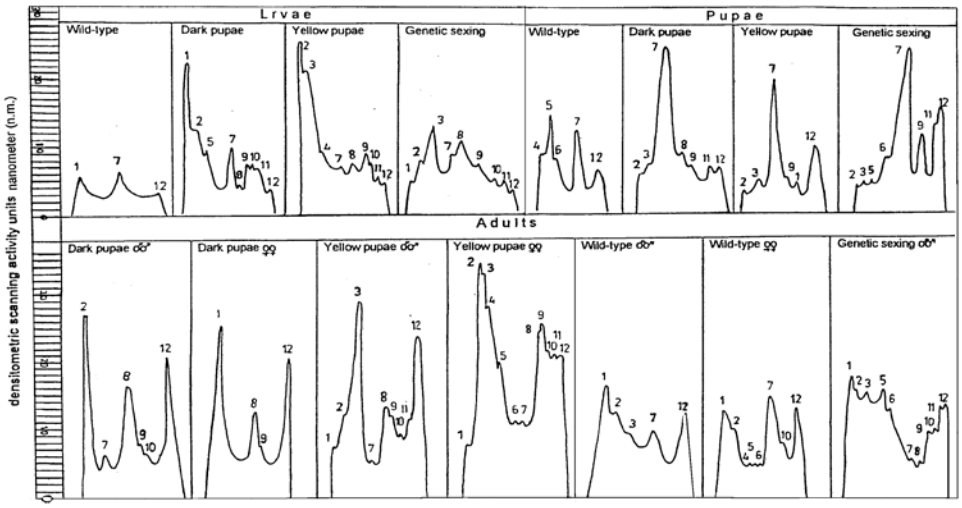

Fig. (4) Densitometric tracing curves of esterases isozyme bands of Med-fly individuals extracted from immature stages (larvae and pupae) and from mature stage (adults)

Table (2): Densitometirc scan units* of Esterases isozymes of the immature and adult stages of the four Med- fly lines.

Zone	No. Of band	Larvae				Pupae				Adult						
		W	D	Y	G.S	W	D	Y	G.S	D		Y		W		G.S
										♂	♀	♂	♀	♂	♀	♂
	1	6	23	--	5	--	--	--	--	--	25	7	7	16	12	17
	2	--	13	26	8	--	6	4	4	26	--	11	34	12	9	15
I	3	--	--	21	14	--	7	5	4	--	--	28	32	9	--	15
	4	--	--	9	--	9	--	--	--	--	--	--	27	--	5	--
	5	--	10	--	--	15	--	--	4	--	--	--	19	--	5	15
	6	--	--	--	--	8	--	--	8	--	--	--	11	--	5	12
II	7	6	10	7	9	13	25	20	25	6		5	11	9	15	5
	8	--	4	8	11	--	9	--	--	16	12	13	23	--	--	5
	9	--	7	9	7	--	7	5	12	7	7	11	25	--	--	9
	10	--	7	7	5	--	--	--	--	6	--	8	20	--	7	9
III	11	--	6	5	5	--	7	5	14	--	--	11	20	--	--	12
	12	3	3	4	3	7	7	10	16	20	20	23	20	12	13	12

W: Wild-type **D: Dark pupae**
Y: Yellow pupae **G.S : Genetic sexing**
***nanometer (n.m.)**

polyphenol oxidase

Fig. (5) shows the photograph and its diagram of polyphenol oxidase isozyming bands of different stages of four Med-fly genotypes. The patterns were distributed into two zones, l, ll, designated from the origin line. The first zone contains the first band only and the second zone contains two bands (No's. 2 and 3). These bands were found in immature stages only; the first band was found in larvae and pupae, while the bands of the second zone appeared in larvae of the two lab. strain and (G.S.) line only. On the other hand, the bands of the second zone were found in yellow pupae strain and (GS) line but, in dark pupae strain there was only one band (No. 2).

The experimental study of polyphenol oxidase was repeated using spectrophotometer method to determined the enzyme activity according to the reaction with substrate as colorimeter. Data in Table (3) clarified that enzyme was active in immature stages more than adult stage in all tested samples. Also, the high activity occurred in larval stage of yellow pupae strain and (G.S.) line, and in pupal stage of dark pupae strain and (G.S.) line were higher than the larval and pupal stages of wild genotype. Fig. (6) illustrate the enzyme activity as optical density (O. D) effectiveness according to stages of genotypes. It was obvious that the larval stage of all genotypes were high in enzyme activity, than pupal stage and was very low in the adult stage. Moreover, the enzyme activity of larval stages of the two lab. strains and (GS) line were higher than the wild genotype. So, the results obtained by spectrophotometer method were in accordance with those of electrophoresis method. In the present work, results of the two methods of enzymatic analysis of the Med-fly indicated that larval stage has polyphenol oxidase enzyme more active than the other stages.

These results my be well supported in comparison with results of Nation *et al.* (1995) who found that preliminary analysis of polyphenol oxidase activity was very low and difficult to detect in the first

two instars, but it increased in the third instar of larval stag of Caribbean fruit fly *Anastrepha suspense* (Loew). Also, results of the present work were supported with those of Wappner *et al*. (1995) who demonstrated that no differences or little of polyphenol oxidase activities existed in both the wild-type and white pupae of Med-fly at the beginning of pupation.

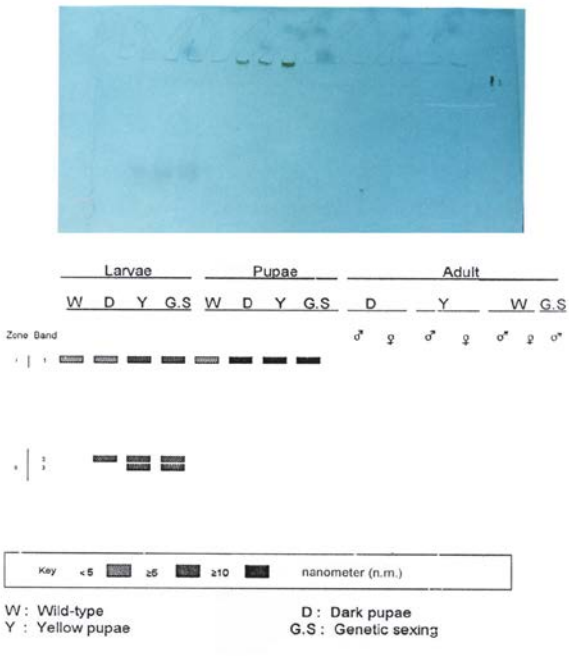

Fig. (5): Electrophoretic patterns of Polyphenol oxidase isozymes extracted from larvae, pupae, and adult individuals of Med-fly.

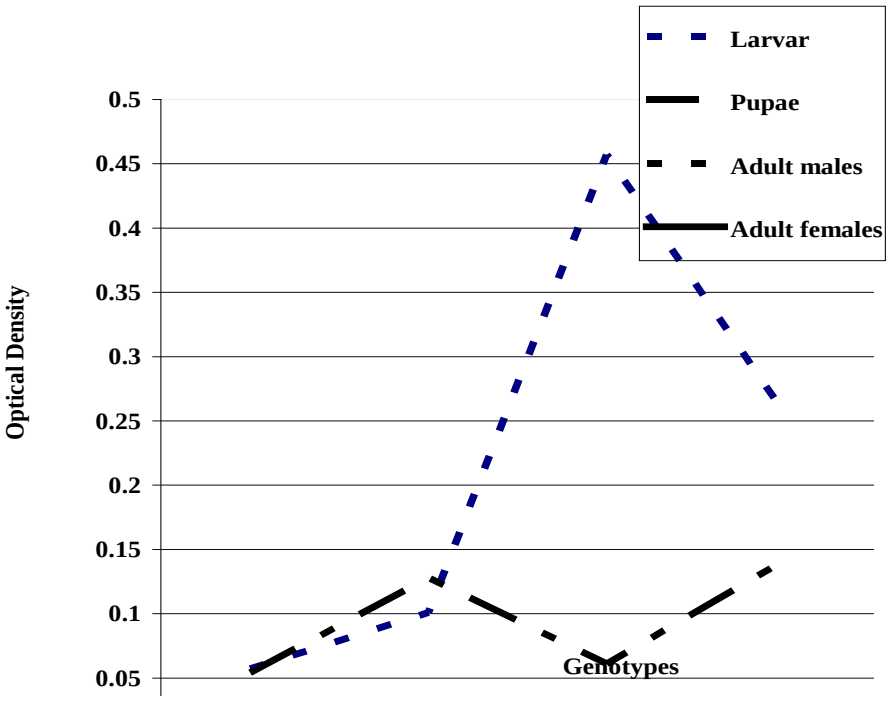

Fig. (6): Polyphenol oxidase activities as optical density units of larvae, pupae, and adults of the four Med-fly genotypes.

Table (3) : The average obtical density units of polyphenol oxidase isozymes activity in the stages of the four Med-fly genotypes.

Genotypes	O. D at 475 n.m for immature and adult stages			
	Larvae	Pupae	Adult males	Adult females
Wild- type	0.057	0.054	0.000	0.000
Dark pupae	0.101	0.128	0.018	0.000
Yellow pupae	0.457	0.061	0.014	0.024
Genetic sexing	0.255	0.142	0.000	0.000

REFERENCES

Christenson, L. D. and R. H. Foote. (1960). Biology of Fruit flies. Ann. Rev. Entomol. 5: 171-192.

Civetta, A. ; J. C. Vilardi ; B. O. Saidman ; C. A. Leanza and J. L. Cladera. (1990). Estimation of the number of ovipositing females per fruit in the Mediterranean fruit fly, *Ceratitis capitata* (Wied.). Heredity 65 : 59-66.

Cladera, J. L. (1981). Genetica de alazimas en *Ceratitis capitata* l. Dos alelos de la esterse pupal. Mendeliana. 5 : 33-38.

El-Minshawy, A. M.; N. S. Sabrah ; A. I. Awad and M. B. Hafez (2000). Biological Genetic Studies on the Mediterranean fruit fly, *Ceratitis capitata* (Wied.).Minufiya, Journal of Agricultural Research. 25 (2): 483-498.

El-Minshawy, A. M.; N. S. Sabrah and A. I. Awad (2003). Some Biological aspects on genetic sexing line of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.)treated by Gamma irradiation. The first Int. Egyptian-Romanian Conf., Zagazig, Egypt Dec., 6-8, 2003: 97-111.

Gakhar S. and K. Vandana. (1993). Development expression of alkaline phosphatases in *Anopheles stephensi* Liston (Diptera: Culicidae). Proceedings of the Indian National Science Academy Part-B, Biological Sciences, 59 6, 531-536.

Milani, R. ; G. Gasperi and A. R. Malacrida. (1985). Recent progress and perspectives in the genetical studies on *Ceratitis capitata*. In : Report on Research Coordintion Meeting on Genetic Sexing. Joint FAO / IAEA Division, Vienna. (mimeographed), pp. 1-15.

Nation, J. L. ; B. J. Smittle and K. Milne. (1995). Radiation-induced changes in melanization and phenoloxidase in Caribbean fruit fly larvae (Diptera: Tephritidae) as the basis for a simple test of irradiation. Ann. Entomol. Soc. Am. 88 (2):201-205

Psarianos, C. G. ; M. Lanpropoulou ; V. J. Marmaras. (1987). Alkaline phosphatase in the integument of *Ceratitis capitata* Developmental profile and functional properties. Insect Biochemistry 17 (4) : 619-624.

Sabrah, N. S. (1980). Genetical and Cytological studies on Maize. Ph. D. Thesis, Fac. Agric. Alex. Univ. Egypt.

Sabrah N. S.; A. M.El-Minshawy; M. B. Hafez and A. I. Awad (1995). Genic polymorphism and ontogenic variation in the Med-fly *Ceratitis capitata* (Wied.), collected from different geographical zones in Egypt based on esterase isozymes. Egypt, J. Appl. Sci. 10(4): 571-587.

Vilardi, J. C. ; A. Civetta ; B. O. Saidman and J. L. Cladera. (1991). Characterization of three isoenzymatic systems in adults of a population of *Ceratitis capitata* (Wied.) (Diptera: Tephritidae). Review of Agricultural Entomology 79 (6) abst. 5669.

Wappner, P. ; K. J. Kramer ; T. L. Hopkins ; M. Merritt ; J. Schaefer and L. A. Quesada-Allue. (1995). White pupae : a *Ceratitis capitata* mutant lacking catecholamines for tanning the puparium. Insect Biochem. Mol. Biol. 25 :365-373.

دراسات وراثية بيوكيميائية على سلالات أنماط جينية لذبابة فاكهة حوض البحر الأبيض المتوسط *Ceratits capitata*

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تم إجراء التفريد الكهربى للمشابهات الإنزيمية لكل من إنزيم الألكلين فوسفاتيز والإستريزات وإنزيم البولى فينول اكسيداز بجانب إجراء التقدير اللونى لإنزيم البولى فينول اكسيداز . وقد أجريت هذه التحليلات على أطوار النمو الثلاثة لذبابة الفاكهة فى السلالتين المعمليتين (العذارى الداكنة والعذارى الصفراء) والعشيرة الحقلية كذلك السلالة المحددة للجنس وراثيا وقد تبين الآتى:١- بالنسبة لإنزيم الألكلين فوسفاتيز، فقد إختلف عدد المشابهات الأنزيمية لإنزيم الألكلين فوسفاتيز فى الأطوار الثلاثة فى جميع السلالات المختبرة ، كذلك بلغ العدد الأقصى لمشابهات الإنزيم ١٣ مشابه موزعة فى أربعة مجاميع طبقا لهجرتها من خط البداية. وكانت المشابهات الإنزيمية لجميع السلالات المختبرة مرتفعة فى العدد والنشاط فى الطور اليرقى ، ثم قلت بعد ذلك فى طور العذارى وطور الحشرات الكاملة.أما فى طور الحشرات الكاملة لم يظهر أى اختلاف فى عدد ونشاط المشابهات الإنزيمية بين الذكور والإناث لجميع العينات تحت الدراسة.٢- بالنسبة للإستريزات فقد إختلف عدد المشابهات الأنزيمية للإستريزات فى الأطوار الثلاثة لجميع السلالات المختبرة. وقد بلغ العدد الأقصى للمشابهات الأنزيمية للإستريزات ١٢ مشابه موزعة فى ثلاثة مجاميع طبقا لهجرتها من خط البداية، كذلك كان عدد المشابهات الأنزيمية للإستريزات فى يرقات العشيرة الحقلية قليلا (ثلاثة حزم فقط) بينما كان العدد مرتفعا فى ذكور السلالة المحددة للجنس وراثيا (١١ حزمة) وأيضا فى إناث السلالة المعملية العذارى الصفراء (١٢ حزمة) . أما فى طور الحشرات الكاملة أظهرت كل من إناث السلالة المعملية (العذارى الداكنة) وذكور العشيرة الحقلية أقل عدد فى المشابهات الأنزيمية للإستريزات ، بينما بلغ العدد اقصاه فى إناث السلالة المعملية (العذارى الصفراء) و ذكور السلالة المحددة للجنس وراثيا. أما المشابهات الأنزيمية للإستريزات فقد كانت كانت أكثر نشاطا فى المجموعة الأولى للطور اليرقى وفى المجموعة الثانية لطور العذارى، بينما فى طور الحشرات الكاملة زاد النشاط تقريبا فى المجاميع الثلاثة لكل العينات تحت الدراسة.٣- وبالنسبة لإنزيم البولى فينول أوكسيديز فقد بينت النتائج أنه يوجد ثلاث مشابهات لإنزيم البولى فينول أوكسيديز متوزعة فى مجموعتين طبقا لهجرتها من خط البداية. كذلك تواجدت مشابهات إنزيم البولى فينول أوكسيديز فى الأطوار غير الكاملة للحشرة فقط لكل العينات تحت الدراسة. كذلك تبين أن الحزمة الموجودة فى المجموعة الأولى وجدت فى الأطوار غير الكاملة لجميع العينات المختبرة ، بينما ظهرت حزمتى المجموعة الثانية فى يرقات السلالة المعملية (العذارى الصفراء) والسلالة المحددة للجنس وراثيا . وظهرت حزمة واحدة فقط من المجموعة الثالثة فى يرقات السلالة المعملية (العذارى الداكنة) . وقد بينت نتائج التقدير اللونى لنشاط إنزيم البولى فينول أوكسيديز أن النشاط كان عاليا فى الأطوار غير الكاملة (اليرقات والعذارى) عما عنه فى طور الحشرة الكاملة. كذلك كان نشاط إنزيم البولى فينول أوكسيديز عاليا فى السلالة المعملية (العذارى الصفراء) فى طور اليرقات ، كذلك فى السلالة المحددة للجنس وراثيا فى طور العذارى بالمقارنة مع باقى العينات طبقا للكثافة الضوئية.