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Bio-2 Vitality Improvement of the Mediterranean Fruit Fly, *Ceratitis capitata* Wied. II. Measured By Using ME and AT Pase Enzyme Activities and Total Protein Content.

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ABSTRACT

The present investigation aims at producing sterile adult Mediterranean fruit fly, *Ceratitis capitata* Wied. having the best possible vitality through the use of irradiation and /or a mutagenic substances to be used in a sterile insect technique program.

Several types of mutagenics that were thought to cause mutations were used as IGR's, temperature, fromaldhyde, colchicine, alcohols, several types of larval rearing media and γ- rays.

In a common pathway, malic enzyme (ME) activity, adinosine triphosphatase (ATPase) enzyme activity and the total protein contents are studied as direct parameters for measuring vitality of the insect.

It was found that there is an increment at the levels of these parameters due to the treatment of egg stage by the previously mentioned treatments specially the usage of the rice hulls as a bulking component in the larval rearing media alone or followed by irradiation of the pupal stage with 90Gy.

Key Words: Ceratitis capitata/ Sterile Insect Technique/ IGR's/ Heat/ Chemicals/ Larval Rearing Media/ Insect Vitality/ Energy Enzymes/ ME/ ATPas / Protein .

INTRODUCTION

The Mediterranean fruit fl,y *Ceratitis capitata* (Wied.) is one of the most serious pests of fruits and vegetables in tropical and subtropical areas of the world, because the fly continues to threaten important fruit production centers with subtropical or Mediterranean climates.

It was efficiently controlled from some areas by the use of integrated pest management (IPM) programs involving the sterile insect technique (SIT) (Fisher, 1985[1]; Hendrichs et al., 1983[2]). The objective of the present study was to improve the vitality of the released insects in the (SIT) program to be able to compete with the native insects. To achieve this purpose, two IGR's, some chemicals, gamma radiation, heat and larval rearing media were used as mutagenics and the activity of two energy enzymes (ME, ATPase) and protein content were measured.

MATERIAL AND METHODS

A- Rearing and Irradiation

The rearing technique described by Wakid (1975)[3] was followed. To irradiate eggs, 1cm^3 of eggs (2000 eggs) were put on a wet piece of cotton covered with a black cloth in a Petri-dish (8 cm in diameter) which was introduced into the gamma cell chamber to be irradiated. Three doses were chosen; 2.5, 5 and 10 Gray to be applied on the eggs. Irradiation of pupae was made through introducing them into a small plastic vial to be irradiated, with the sterilizing dose (90 Gy), in a CO^{60} gamma cell having a dose rate of 3.5-6.0 Gy / minute .

B- Quantitative Determination of Enzymes .

The estimations of ME were achieved according to the procedures described by Mukiama, 1980[4] using SPECOL II spectrophotometer, while the ATPase enzyme assay was performed using the method of Fisk and Sabharow (1925)[5] . For measuring the total protein content in the whole fly tissue, the Bio-Rad protein Assay Kit was used.

C- Statistical Analysis

The obtained data were manipulated statistically with the help of a microstate soft ware (specific computer's program).

Table (1): Effects of insect growth regulators (IGR) on the pupal protein and two energy enzymes (ME and ATPase) of the medfly, *Ceratitis capitata* when eggs were treated with different concentrations and pupae were irradiated with the sterilizing dose (90 Gy) before adult emergence.

Treatments	Conc in ppm	Enzyme activity (µgm/ml)					
		Non Irradiated pupae			Irradiated pupae		
		ME	ATPase	Protein	ME	ATPase	Protein
Control	0.0	27.5 ± 0.11	51.67 ± 0.8	0.048 ± 5.77	8.53** ±0.11	59.27** ± 0.63	0.044* ± 1.52
IKI	500	24.82 ± 0.07	64.30 ± 0.63	0.054 ± 5.78	17.83** ± 4.66	54.3** ± 0.55	0.0563 ± 4.16
	750	19.34 ± 0.17	32.43 ± 1.01	0.055 ± 1.3	10.01** ± 0.08	75.30* ± 1.06	0.0579* ± 7.22
	1000	16.74 ± 0.14	12.63 ± 0.84	0.056 ± 1.68	8.99** ± 7.05	90.12** ± 1.19	0.058* ± 5.33
Control	0.0	27.5 ± 0.11	41.24 ± 0.37	0.04 ± 5.5	8.53** ± 0.11	60.13** ± 1.15	0.036 NS ± 1.53
SL	500	27.33 ± 8.51	68.63 ± 0.67	0.042 ± 5.77	12.40** ± 6.92	51.97** ± 0.67	0.043 NS ± 1.25
	750	24.56 ± 0.25	22.35 ± 1.14	0.054 ± 1.2	15.67** ± 0.05	89.33* ± 1.8	0.044** ± 3.51
	1000	16.82 ± 6.92	13.92 ± 0.98	0.051 ± 2.64	17.56** ± 5.29	94.98** ± 0.85	0.04* ± 8.81

*Significant (P<0.05)
** Highly significant (P< 0.001)
ME= Malic enzyme
ATPase= Adinosine triphosphatase
IKI = Atabron
SL = Sumilarv

Table (2): Effect of formaldhyde and colchicine on pupal protein and two energy enzymes (ME and ATPase) of the medfly, *Ceratitrs capitata* when eggs were treated with different concentrations and pupae were irradiated with the sterilizing dose (90 Gy) before adult emergence.

Treatments	Conc in ppm	Enzyme activity (µgm/ml)					
		Non Irradiated pupae			Irradiated pupae		
		ME	ATPase	Protein	ME	ATPase	Protein
Control	0.0	18.99 ± 0.12	132.45 ± 1.09	0.04 ± 3.84	20.44** ± 6.67	75.47** ± 0.56	0.048** ± 5.77
	0.0001	17.81 ± 7.8	122.4 ± 1.36	0.036 ± 3.53	20.55** ± 6.67	85.33** ± 1.07	0.046** ± 3.25
Formaldhyde	0.005	18.99± 0.00	101.80± 1.89	0.0462 ± 6.06	20.75** ± 2.40	79.29** ± 0.41	0.051* ± 6.64
	0.01	18.86± 8.72	102.97± 0.63	0.0442± 5.51	20.87** ± 1.76	95.25* ± 2.31	0.0494* ± 8.72
Control	0.0	18.99± 0.12	132.45± 1.09	0.048± 5.78	20.49** 6.67	75.47** ± 0.56	0.043** ± 9.13
	250	20.29± 3.33	10.60± 1.06	0.0437± 8.68	20.57** ± 1.76	90.73** ± 0.84	0.0608** ± 1.30
Colchicine	500	20.29± 0.044	11.13± 1.21	0.0407± 1.20	20.57* ± 3.33	20.53** ± 0.99	0.0708** ± 8.14
	1000	20.46± 3.99	30.61± 0.89	0.0493± 8.81	2071* ± 1.33	88.11** ± 1.03	0.0563* ± 9.87

*Significant (P<0.05) ** Highly significant (P< 0.001)
ME= Malic enzyme ATPase= Adinosine triphosphatase
IKI = Atabron SL = Sumilarv

Table (3): Effects of two temperature degrees (0 and 40°C) on pupal protein and two (ME and ATPase) energy enzymes of the medfly, *Ceratitis capitata* when eggs were exposed to heat for three different time intervals then pupae were irradiated with the sterilizing dose (90Gy) before adult emergence.

Temp. degree (°C)	Time in min	Enzyme activity (µgm/ml)					
		Non Irradiated pupae			Irradiated pupae		
		ME	ATPase	Protein	ME	ATPase	Protein
25°C (control)	0.0	11.03 ± 0.14	51.98 ± 0.65	0.010± 1.20	11.81* ± 3.28	60.01** ± 0.71	0.39** ± 7.36
	30	17.01 ± 0.12	37.26 ± 0.51	0.011± 1.17	11.25** ± 0.1	32.98** ± 1.39	0.048** ± 1.73
40°C	90	13.84± 3.06	40.34± 1.30	0.012± 5.77	10.46** ± 0.07	41.95^{NS} ± 0.69	0.0607** ± 1.20
	120	11.99± 0.10	45.53± 0.60	0.012± 5.77	8.40** ± 5.29	51.65** ± 0.80	0.0593** ± 8.82
25°C (control)	0.0	11.03 ± 0.14	41.31± 0.49	0.035± 2.60	11.81* ± 3.28	58.93** ± 0.90	0.0367^{NS} ± 2.60
	30	10.41 ± 9.26	95.41 ± 2.96	0.0414± 1.17	18.29** ± 7.51	24.03** ± 0.50	0.0189** ± 1.05
0°C	90	9.97± 2.91	30.98± 3.70	0.0447± 2.96	17.00** ± 0.13	31.97^{NS} 0.89	0.043^{NS}± 2.28
	120	9.86± 3.06	41.25± 0.44	0.0517± 1.76	16.75** ± 5.70	52.27** ± 0.77	0.0507 ^{NS} ± 8.82

NS = Non significant

*Significant (P<0.05) ** Highly significant (P< 0.001)
ME= Malic enzyme ATPase= Adinosine triphosphatase
IKI = Atabron SL = Sumilarv

Table (4): Effect of two alcohols on pupal protein and two energy enzymes (ME and ATPase) of the medfly, *Ceratitis capitata* when eggs were exposed to different concentrations and pupae were irradiated with the sterilizing dose (90Gy) before adult emergence.

Type of Treatment	Percent concent-ration	Enzyme activity (µgm/ml)					
		Non Irradiated pupae			Irradiated pupae		
		ME	ATPase	Protein	ME	ATPase	Protein
Control	0.0	18.31 ± 0.09	21.84 ± 0.53	0.0365± 4.26	17.31^{**}± 0.41	23.31^{NS}± 0.56	0.0377^{NS}± ± 2.96
Ethyl alcohol	1	18.71 ± 0.14	51.33 ± 0.53	0.0366± 3.21	18.11^{*}± 0.15	31.60^{**}± 0.55	0.0610^{NS} ± 1.61
	2	17.23 ± 0.16	62.17 ± 0.63	0.0364± ± 3.38	18.11^{*}± 0.14	42.27^{**}± 0.94	0.0817^{**} ± 1.76
	3	18.49 ± 3.71	26.72 ± 0.43	0.0358± 2.40	17.87^{**}± 6.90	17.24^{**}± 0.64	0.0920^{**} ± 1.73
Control	0.0	18.31± 0.09	42.14± 0.53	0.0365± 4.26	17.29^{**}± 0.12	56.57^{**}± 1.23	0.0387^{NS} ± 1.53
Pentyl alcohol	1	15.64± 0.19	80.52± 0.99	0.0376± 8.29	18.21^{**}± 4.81	18.48^{**}± 4.54	0.0320^{*} ± 1.73
	2	13.13 ± 0.09	44.89 ± 0.84	0.0610± 1.15	17.44^{**}± 0.11	151.74^{**} ±1.62	0.0297^{**} ± 1.76
	3	11.37 ± 8.51	29.91 ± 1.43	0.0460± 2.65	16.70^{**}± 3.46	114.14^{**} 2.33	0.0280^{**} ± ± 1.15

NS = Non significant

*Significant (P<0.05)
** Highly significant (P< 0.001)
ME= Malic enzyme
ATPase= Adinosine triphosphatase
IKI = Atabron
SL = Sumilarv

Table (5): Effect of two different larval rearing media on pupal protein and 2 energy enzymes of the medfly, *Ceratitis capitata* when eggs were cultivated in them and pupae were irradiated with the sterilizing dose (90 Gy) before adult emergence.

Type of Treatment	Enzyme activity (µgm/ml)					
	Non Irradiated pupae			Irradiated pupae		
	ME	ATPase	Protein	ME	ATPase	Protein
Control (Bran)	17.79 ± 1.10	41.58± 0.52	0.0412± 3.64	16.30^{NS}± 0.34	57.35^{**}± 0.24	0.0433^{NS}± 2.43
Hulls	17.10 ± 8.82	42.14 ± 0.31	0.0498 ± 6.37	16.79^{*} ± 3.36	107.10^{**} ± 2.87	0.0610^{*} ± 3.05
Bran : Hulls 1 : 1	16.48 ± 0.11	61.56 ± 0.65	0.0428 ± 9.91	15.40* ± 0.18	77.78^{**}± 0.95	0.0495^{**}± 7.42

NS = Non significant

*Significant (P<0.05)
** Highly significant (P< 0.001)
ME= Malic enzyme
ATPase= Adinosine triphosphatase
IKI = Atabron
SL = Sumilarv

Table (6): Effect of irradiation with δ- rays on pupal protein and 2 energy enzymes of the medfly, *Ceratitis capitata* when eggs were irradiated with (2.5, 5, 10 Gy) then pupae were irradiated with the sterilizing dose (90Gy) before adult emergence.

Type of Treatment	Enzyme activity (µgm/ml)					
	Non Irradiated pupae			Irradiated pupae		
	ME	ATPase	Protein	ME	ATPase	Protein
Control	18.31 ± 0.087	41.24 ± 0.38	0.0413± 3.64	17.31** ± 0.41	58.63** ± 0.81	0.040 NS ± 4.62
2.5 Gy	16.51 ± 0.17	84.40 ± 0.40	0.0246± 1.85	18.31** ± 0.09	98.57** ± 0.82	0.0123* ± 3.38
5 Gy	17.49± 7.68	41.21 ± 0.90	0.0418± 9.77	19.38** ± 3.84	58.27** ± 0.66	0.0098** ± 2.92
10 Gy	18.33± 3.33	76.46± 0.57	0.0352± 1.20	19.93** ± 2.65	87.50** ± 1.18	0.0117* ± 2.19

NS = Non significant

*Significant (P<0.05) ** Highly significant (P< 0.001)

ME= Malic enzyme ATPase= Adinosine triphosphatase

IKI = Atabron SL = Sumilarv

Gy= Gray = 100 rad

RESULTS

Malic Enzyme (ME)

The results in Table (1) revealed a highly significant decreases of ME activity due to the egg treatment with IKI with all concentrations. Irradiated pupae recorded a highly significant increase of ME activity. The sumilarv caused highly significant decreases at high concentrations while the irradiation of pupae caused highly significant increases of ME activities.

The obtained results in Table (2) showed a significant decrease due to egg treatment with formaldehyde turned to a significant increase of enzyme activity and the irradiation of pupae increased this activity.

Table (3) showed the effect of temperature on the enzyme activity. Incubation of eggs at 40°C for different time intervals caused a significant increase to the enzyme activity followed by a significant decrease of ME activity after irradiation of pupae. The significant decrease of ME activity was obtained due to the incubation of eggs at 0°C , while the irradiation of pupae caused a highly significant increase of the enzyme activity.

Table (4) revealed the decrease in enzyme activity due treatment of eggs by ethanol and pentanol while the irradiation of pupae caused a highly significant increase in the enzyme activity.

The results shown in table (5) indicated an insignificant decrease of ME activity due to the usage of hulls in the larval rearing medium , while the irradiation of pupae caused a significant increase of ME activity.

Moreover the irradiation of eggs followed by irradiation of pupae induced a highly significant increase of ME activity as recorded in Table(6).

ATPase Enzyme

Table (1) showed a highly significant increase of ATPase activity after egg treatment with IKI at the concentration of 500 ppm followed by a significant decrease at the concentrations of 750 and 1000 ppm. The irradiation of pupae led to inhibition in the enzyme activities at 500 ppm followed by significant increase of the enzyme activities at 750 and 1000 ppm. The treatment with samilarv caused a significant increase in the enzyme activities at 500 ppm, followed by a highly significant decrease at 750 and 1000 ppm. The irradiation of pupae doesn't affect the enzyme activity.

Treatment of eggs with formaldehyde and colchicine reacorded a highly significant decrease of the enzyme activity. The irradiation of pupae led to the increase of enzyme activity as recorded in Table (2)

The incubation of eggs at 40°C led to a highly significant decrease in the enzyme activity for the irradiated and non Irradiated pupae as shown in Table (3). Also the incubation of eggs at 0°C for different time intervals led to a highly significant increase of the enzyme activity at 30 minutes followed by a highly significant depression at the other time intervals. The irradiation of pupae caused depression in the enzyme activity (Table 3).

Moreover Table (4) showed the ATPase activity recorded a significant increase at all concentrations of ethyl alcohol. The irradiation of pupae increased the enzyme activity at concentrations 1 and 2% while decreased it at the concentration 3%. Also Table (4) recorded that pentyl alcohol concentrations of 1 and 2% led to a highly significant increase of the enzyme activity while the concentration 3% led to a significant decrease in the enzyme activity. The irradiation caused a highly significant increase of the enzyme activity.

The pupae produced from rice hulls media recorded an insignificant increase in the enzyme activity converted to a highly significant increase in the enzyme activity after irradiation of pupae. While the rice hulls: wheat bran media led to a highly significant increase in the enzyme activity to the irradiated and non Irradiated pupae as shown in Table (5).

Table (6) recorded a highly significant increase in the enzyme activity obtained after irradiation of the eggs with 2.5 and 10 Gy while the dose 5 Gy caused an insignificant decrease to the enzyme activity. The irradiation of pupae doesn't affect the enzyme activity.

Protein :

Table (1) revealed that the use of IKI and sumilarv induced a significant increase in the protein content. The irradiation of pupae increased the protein content too.

The lower concentrations of formaldehyde (0.0001 ug/L) caused an inhibition in the protein synthesis while the higher concentration induced a highly significant increase in the protein content. For the Irradiated pupae the protein increased at the concentrations 0.0001 and 0.01 ug/L and decreased at the concentration 0.005 ug/L Table (2). Also Table (2) recorded a highly significant decrease in the protein content due to the treatment of eggs with colchicine while the irradiation of pupae caused a significant increase in the protein content.

The incubation of eggs at 40°C for different time intervals led to an insignificant increase in the protein content changed to a significant increase after irradiation of pupae. Also the incubation of eggs at 0°C led to a significant increase in the protein content while the irradiation of pupae caused a highly significant decrease in the protein content at 30 and 60 min, followed by an insignificant increase in the protein content at 90 and 120 min as recorded in table (3).

Table (4) indicated that treatment of eggs with ethyl alochol led to a significant decrease in the protein content changed to a significant increase after irradiation of pupae. Also the treatment of eggs with pentyl alcohol led to an in significant increase except at the 1% concentration of pentye alcohol that showed significant increase in the protein content. The irradiation of pupae caused a depression in the protein content.

A highly significant increase in the protein content was obtained in the irradiated and non Irradiated pupae produced from rice hulls and rice hulls: wheat bran media as shown in Table (5)

Table (6) indicated a highly significant decrease in the protein content due to irradiation of eggs with 2.5 and 10 Gy while an insignificant increase in the protein content was obtained due to irradiation of eggs with 5 Gy. The irradiation of pupae led to a significant decreases in the protein content, at all gamma doses under study.

DISCUSSION

A group of enzymes referred to as energy enzymes (ME, α-GPDH, LDH, MDH and (ATPase) have been studied in a wide variety of insects (Yong *et al.*, 1985[6] and Paul and Mayrard, 1965)[7]. These enzymes are particularly well studied from the biochemical and genetic aspects because of they are very important tools for the investigation of insect metabolic activities during the course of development. Malic enzyme (NADP-ME), is one of the oxidative NADP enzymes that provide NADH for lipogenesis

(Wise and Ball, 1964)[8]. The results obtained recorded ME and ATPase enzyme activities converted to stimulatory effect of enzyme with the irradiation of pupae. The combined effect of irradiation and colchicine increased the enzyme activity of ME and ATPase as obtained due the treatment of for moldehyde which has mutagenic effect as 5 times less than irradiation with δ- rays (Szabad *et al*, 1983)[9].

Temperature sensitive lethal (TSL) mutations are very common in insect (Suzuki, 1967[10] and Smith, 1967)[11] as the consequence of a single amino acid substitution in apoly peptide chain (Wittmann, 1965)[12] which alter the biological activity of proteins at different temperature. (Jockusch, 1966)[13]. The treatment with heat (0°C) caused an inhibitory effect to ME and ATPase enzyme activities as recorded by Katsoris and Marmaras (1992)[14]. This effect was dimensioned due irradiation of pupae.

Alcohols have both stimulatory and inhibitory effects depending on the concentrations used. Irradiation of alcoholic treated insects had a stimulatory action on enzyme activity.

Malicmas was affected by hulls media to record inhibitory activity reversed to stimulatory effect due irradiation of pupae. This enzyme activity was decreased due δ-rays treatment as recorded by (Rechsteiner 1970)[15].

The application of formaldhyde, IKI and Sumilarv on the eggs revealed stimulatory effect on protein synthesis as reffered by Ismail (1980)[16]; Scheller and Bodenstein (1981)[17] and on the contrary of Bakr, (1986)[18] and Rutz *et al*, (1974)[19].

The colchicine cause, an inhibition for protein synthesis anatgonized by the irradiation of pupae.

The changing of the component of larval rearing media led to protein synthesis for both irradiated and non inadiated pupae, while the irradiation of eggs led to inhibition of protein synthesis as concluded by Amin *et al.*, (1996)[20].

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