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Physiological Properties and Enzymatic Activities in Different Strains of Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann), (Diptera: Tephritidae)

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Abstract: *Background*: *Ceratitis capitata* (Wiedemann) (medfly), is considered to be one of the most destructive fruit pests because of its high capability to damage the production, its global distribution and its wide range of hosts. The aim of this study was to determine physiological properties and enzymatic activities in susceptible, tolerance, intermediate and resistant strains of Mediterranean fruit fly, *Ceratitis capitata*. *Methods*: Medflies laboratory strain (susceptible strain) was reared at laboratory conditions (26 ± 2°C, 72 ± 5% R.H.) in pests physiology laboratory, Plant Protection Institute, Agricultural Research Center, Dokki, Cairo, Egypt for many generations in (2020 & 2021). Newly emerged adults were placed into a wooden cage (9x9x10 cm^3) with four sides; two sides were covered with screen wire, front side covered with muslin to allow the females to lay eggs. Results: The present results indicated that, the total soluble protein content of resist strain of *C. capitata* adults was significantly lower than the laboratory strain of *C. capitata* adults where the average of the total protein content was 26.34 and 38.4 mg/g.b.wt, respectively. The averages of invertase activities were equal 210 and 155.33 µg glucose/min/mg proteins, respectively.

Key words: Physiological Parameters · Enzymatic Activities · *Ceratitis capitata*

INTRODUCTION

Ceratitis capitata (Wiedemann) (medfly), is considered to be one of the most destructive fruit pests because of its high capability to damage the production, its global distribution and its wide range of hosts. Several specific control methods have been developed and applied successfully in many countries against *C. capitata* [1]. Erythrosine-B showed phototoxic effects on *C. capitata* adult flies at 24 h post- exposure to white florescent neon lamp for four hours at 10 W/m fluence rate. The mortality percentage of two first generation of *C. capitata* adults was 48.47% as compared with 49.34 % in the case of control group [2]. David Nestel *et al.* [3], mentioned that the patterns of total lipid, glycogen, hemolymph-carbohydrate and protein during the larval to adult transition in the Medfly. The putative role of proteins was a source of energy during the initial stages of Medfly metamorphosis by measuring the activity of glutamate dehydrogenase (GDH) [4, 5]. The insects were not carefully synchronized Langley [6]. A constant rate of utilization of total lipids during the entire metamorphosis, [7, 8] described a different trend in total lipid content during Medfly metamorphosis. Data were not correlated with specific metamorphosis events, Municio *et al.* [7] and Pagani *et al.* [8] a small drop of total lipids during what seems to be the prepupal and pupal phases. Data also obtained that small drop during the first half of metamorphosis is followed by a more drastic utilization of lipids during the pharate-adult stage (just a few hours before adult emergence). More accurately describe the time-dependent events of metamorphosis in the Medfly as a result of a well-synchronized protocol, Municio *et al.* [7], Pagani *et al.* [8] and Rabossi *et al.* [9] confirm the

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differential lipid utilization during larva to adult transition. This accumulation of lipids at the beginning of metamorphosis coincides with the in vivo incorporation of labeled triacylglycerols reported [10] to be maximal at this stage. Municio *et al.* [7] and Pagani *et al.* [8] could not detect this lipid increase, probably because of the extraction method used or because of a less careful synchronization protocol. lipid trends may be related to the lipid-extraction method which, in this case, seems to be based on whole individuals chloroform extraction to remove "fat." This method may have created an artifact because water is also removed with this technique. Thus, the initial large lipid drop seen [11] may be a result of the observed relatively large water loss during pupariation and pupal case hardening [12].

The aim of this study was to determine physiological properties and enzymatic activities in susceptible and resistant strains of Mediterranean fruit fly, *Ceratitis capitata*.

MATERIALS AND METHODS

Rearing Technique of Susceptible Strain *Ceratitis capitata*: Medflies laboratory strain (susceptible strain) was reared at laboratory conditions (26 ± 2°C, 72 ± 5 % R.H.) in pests physiology laboratory, Plant Protection Institute, Agricultural Research Center, Dokki, Cairo, Egypt for many generations. Newly emerged adults were placed into a wooden cage (9x9x10 cm^3) with four sides; two sides were covered with screen wire, front side covered with muslin to allow the females to lay eggs. The other opposite side from each cage was provided with a plug to provide the cage with food and water. Pans full of water to 1.5 cm height were surrounding the cage to receive the deposited eggs. Adult flies were provided with a food consisting of sugar and brewer's yeast (3:1) diet in Petri dish. Eggs were collected daily and scattered on the surface of rectangular plastic trays containing larval artificial medium. The larval medium used in the present study consisted of 100 g wheat bran, 25 g brewer's yeast, 30 g sucrose, 0.5 g sodium benzoate and 80 ml tap water; the larval containers were covered with white muslin cloth and tight with a rubber band to ensure maintenance suitable humidity. Infestation of the Drosophila spp. and other foreign flies controlled by distributing bottles containing baits consists of agar, yeast, corn (or wheat), molasses and water. This bait was prepared according to the method mentioned [13]. The trays were placed in a wooden cage with sand at the bottom to allow the jumping larvae to pupate [14]. Rearing technique of wild strain *Ceratitis capitata*: A wild Medfly, *C. capitata* flies, was initiated from infested all citrus varieties samples that collected from two Governorates, El-Beheyra (Noubarya zone), El-Giza (Atfih zone), during 2021 – 2022, the collected fruits were kept in plastic containers covered with a thin con layer of the fine sand and kept inside an environmental chamber at about (26 ± 2°C, 72 ± 5 % R.H), the mature larvae will be jumped out from the infested fruits to the fine sand for pupation. Pupae of the healthy larvae were collected by sieving sands and transferred to the adult screened' & cages (9X 9Xl 0 cm^3) Emerging Flies were supplied with sucrose and brewer's yeast (3:1) diet in Petri dish with adequate fresh water on sponge for drinking.

Experimental Design: Using of (Lab. strain), untreated of selective resistant strain, tolerance strain, inter mediated strain adult flies from selective governorates, frozen alive and stored in - 20°C. 25 adult flies / strain. Total soluble proteins were determined by the method of Bradford [15]. Amylase, invertase and trehalase activities were assayed by determining the free aldehdic group of glucose formed after starch, sucrose and trehalose digestion, respectively, using 3, 5-dinitrosalicylic acid reagent. Activity of Non-specific esterases was measured with the method of Van Asperen[16] using P-naphthyl acetate as a substrate. Peroxidase activity was detennined at 30°C by a direct spectrophotometric method described by Hammerschmidt and Kuć [17] evaluate the histopathological effects of xanthyne dyes on treated 3-7 days-old virgin *C. capitata* adults with selective photosensitizer at LC_{50} and susceptible strain was dissected in Ringer's saline solution to examine the effects of this concentration on the female integument.

Biochemical Experiments on *Ceratitis capitata*: Samples for biochemical experiments were collected from both control (Lab. strain), untreated of selective resistant strain, tolerance strain, inter mediated strain adult flies from selective governorates, frozen alive and stored in - 20°C for further biochemical tests. At least, three replicates for each test.

Twenty frozen adult flies for each strain were homogenized by using a chilled glass Teflon tissue grinder in 1 ml 50 mM phosphate buffer (pH 7.4) and a few crystals of phenylthiourea was added to inhibit

"'tyrosinases [18]. In sample prepared for determination of oxidizing enzymes, lmM EDTA was used as alternative of phenylthiourea to avoid the inhibition of oxidizing enzymes. All samples were centrifuged at 70 000 g for 10 min at 4°C in cooling centrifuge. The resulting supernatant was either used immediately or refrigerated at - 20 until required.

Total Soluble Proteins: Total soluble proteins were determined by the method of Bradford [19]. Sample solution (50µ1) was pipette into test tube. The volume in the test tube was adjusted to 0.1 ml with phosphate buffer (pH 6.6). Five ml of protein reagent was added to the test tube and the contents mixed by inversion or overtaxing. The absorbance at 595 nm was measured (spectrophotometerically) after 2 min. and before 1 hour against blank prepared from 0.1 ml of phosphate buffer (pH 6.6) and 5 ml of protein reagent. Protein concentration was calculated from the standard curve made using Bovine serum albumin as a standard. Protein reagent was prepared by dissolving 100 mg. Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol. To this solution 100 ml 85% (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 1 liter.

Carbohydrate Hydrolyzing Enzymes Assay: Amylase, invertase and trehalase activities were assayed by determining the free aldehdic group of glucose formed after starch, sucrose and trehalose digestion, respectively, using 3, 5-dinitrosalicylic acid reagent according to the method described by Lshaaya and Swiriski [20]. Twenty microliter of sample was taken in d1y test tubes, 0.5 ml 0.2 M phosphate buffer (pH 5. 7), 0.5 ml distilled water and 0.5 ml 1 % (W/V) soluble starch for amylase, sucrose for invertase or trehalose for trehalase was carefully mixed in each tube. The reaction was initiated by incu~ation at 37°C for one hour, then te1minated by adding 1 ml alkaline dinitrosalicylic acid reagent and then placed immediately into a boiling water bath. Colour was fully developed after 5 min then tubes were removed from the bath and allowed to cool, then read spectrophotometrically at 540 nm against boiled 0.5 ml enzyme extract (which was treated similar to the sample). Activities of amylase invertase and trehalase were calculated from the standard curve made using known concentrations of glucose as a standard and expressed as specific activity (mg glucose released/hr/mg protein).

Non-Specific Esterases: Activity of Non-specific esterases was measured with the method of Van Asperen [21] using P-naphthyl acetate as a substrate. A liquate of 100µ1sample was transferred to test tube and the volume was adjusted to 1.5 ml with phosphate buffer (pH 7.4). Substrate was added (0.5 ml of 4 mM P-naphthyl acetate dissolved in 1 % acetone and buffer) to give a reaction concentration of 1 mM in 2 ml volume. After a 15 min reaction at 25°C, 0.25 ml of stop reagent (0.8% fast blue B in 3.4% SDS) was added. Color was developed for 10 min and read spectrophotometrically at 490 nm. The enzyme activity was calculated from the standard curve made using known col.}Centrations of P-naphtol as a standard and expressed as specific activity (µmole/mg protein).

Acetylcholinesterase (AChE) Assays: An estimate of AChE activity was detennined according to a method modified from Ellman *et al.* [22]. Aliquots (100 µl) of the supernatant were added to a cuvette containing 800 µl of 0.1 M phosphate buffer (pH 7-7.4 and 1 ml 2 mM 5, 5-dithio-bis- (2-nitrobenzoic acid) (DTNB). The reaction was initiated by the addition of 100 ul 20 Mm acetylthiocholine iodide (ATCI) giving final concentrations in the cuvette of 1 mM DTNB and ATCI. The mixtures were incubated at 20°C and absorbance at 412 nm was measured at 10-second intervals for 200 seconds at room temperature.

Peroxidase: Peroxidase activity was detennined at 30°C by a direct spectrophotometric method described by Hammerschmidt and Kuæ [23]. The reaction mixture consisted of 0.5 ml insect extract, 1.5 ml of 0.05 M pyrogallol and 0.5 ml of 1 % hydrogen peroxide (H_2o_2). The reaction was incubated in a water bath and absorbance at 420 nm was recorded at 39 S intervals. The enzyme activity was expressed as change in absorbance of reaction mixture (i'iA420) /min /mg protein.

Histopathological Technique: To evaluate the histopathological effects of xanthyne dyes on treated 3-7 days-old virgin *C. capitata* adults with selective photosensitizer at LC_{50} and susceptible strain was dissected in Ringer's saline solution to examine the effects of this concentration on the female integument. Integument of both control and treatment were removed and fixed in alcoholic Bouin's fluid for 24 h, washed several times in 70% ethyl alcohol to remove most of the fixative and then dehydration takes place by using

ascending series of ethyl alcohol; 70, 80, 90 and 95% followed by two changes of absolute ethyl alcohol (30 min. for each) then transferred to a mixture of absolute ethyl alcohol and xylene and then to xylene. After that, infiltration with wax takes place by transferring to xylene-paraffin mixture for 30 min. and then to three successive paraffin baths at 58°C - 60°C, then embedded in pure paraffin.

Sectioning takes place by using the Rotary microtome at thickness of 5 µ. Paraffin ribbons were affixed on clean glass microscopal slides and flattened by the usage of the hot plate for a period of 15 min. and then staining takes place by using double stain, i.e. haematoxylin and eosin. The staining procedure takes place as follows; dewaxation carried out by passing the slides through xylene I, xylene II and then to a mixture of xylene and absolute ethyl alcohol (5 min. for each) and then hydrate to water by running through descending series (100, 95, 90, 80, 70, 50 and 30%) at ethyl alcohol and then to distilled water (2 min. for each). Sections were put in haematoxylin for 5 min and rinsed in distilled water. Destained in 70% acid alcohol until light pink colour of specimen was appeared and then neutralized in tap water, stained with eosin (15 sec.) and washed in 2 changes of 95% ethyl alcohol then in absolute ethyl alcohol (5 min. for each). Clearing in 2 changes of xylene (5min. for each) and then mounted with DPX, covered with clean glass covers and left the slides to dry in an electric oven at 37°C. Ovaries of both species undergo the same previous technique; however, they differ in certain steps in which, after fixation and dehydration transferred from 95% ethyl alcohol to methyl benzoate for clearing until it settled in the bottles bottom, washed with benzene for 5 min., then transferred to benzene and wax mixture and then running to three bathes of paraffin wax (30 min. for each) and then complete the routine technique that previously described in testes.

Electron Microscope: This technique was carried out in electron microscope unit, Faculty of science, Ain shams university. Two samples of *C. capitata* adults 3-7 days-old resistant adult strain and susceptible strain were dissected to isolate the integument and fixed the integument in gluteraldhyde 3% over night then washed in phosphate buffer PH (7-2) for 30 min, dehydration ascending serious of ethanol 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95 % respectively and absolute each step for 15 min, Sample were impeded 1: 1 alcohol: acetone for 20 min. then impeded in absolute acetone for 20 min. sample in 1 resin: 2 acetone 1 hour then impeded the sample in 1 part resin: 1 pa1t acetone overnight, transfer the sample to 2part resin: 1 part acetone for 4 hours followed by pure resin overnight then put the sample in embedded mold and in oven at 70°C for 18 hours.

Sections of the tested samples were cut by ultra-microtome (Leica EMUC6) then stain with uranyl acetate and lead acetate. The examination takes place in Research Park, faculty of agriculture, Cairo University using Jeol TEM - 1400 electron microscope. The sections were photographically using Optoelectronic AMT CCD camera with 1632 pixel format as side mount configuration.

Statistics: All toxicity data were corrected for control mortalities according to Abbott's equation [24]. The LC_{50} and LC_{90}, the fiducial limits at P = 0.05 level and the slope of concentration/mortality regression were estimated by probit analysis [25] using a software package "LD-Pline", Copyright of Ihab. M. Bakr, Plant Protection Research Institute, Egypt.

Data of the other experiments were evaluated statistically using ANOVA and means compared using T-Test and Fl Test at P<0.05). The relationship between the mortality of *C. capitata* and both different concentrations and different successive 16 generations. Results are recorded as mean± standard deviation (SD).

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RESULTS

Physiological Properties and Enzymatic Activities in Different Strains of *C. capitata* Adults: Biochemical bioassay carried out on selected El-Fayoum (Tolerance), (El-Menyia) Intermediate, El- Beheyra (Resistant) strains of wild *C. capitata* adults without treatment of phloxine -B compared with laboratory strain (susceptible strain) under same conditions.

Table 1: Bioassay analysis of total soluble protein in different statins of *C. capitata* adults

Strains	Mean ± SD (mg/g.b.wt)	Change %
Susceptible strain	38.42 ±2.43[a]	-
Tolerance strain	35.14 ± 0.41 [a]	-8.52
Intermediate strain	56.25 ± 2.83 [b]	+46.38
Resistant strain	26.33 ± 1.51 [c]	-31.44

Table 2: The amylase activity in different strains of *C. capitata* adults

Strains	Mean ± SD (µg glucose/min/protein)	Change %
Susceptible strain	19.30±5.55[a]	-
Tolerance strain	23.63 ± 4.03 [a]	+22.40
Intermediate strain	32.67 ± 3.20 [b]	+68.95
Resistant strain	67.30 ± 5.12 [c]	+248.30

Table 3: Invertase activity in different strains of *C. capitata* adults

Strains	Mean ± SD (µg glucose/min/protein)	Activity %
Susceptible strain	155.33 ±5.51[a]	-
Tolerance strain	127.67 ± 7.77[b]	-17.81
Intermediate strain	168.67 ± 7.76 [c]	+7.70
Resistant strain	210.00 ± 21.79 [a]	+35.48

Table 4: Trehalase activity (µg glucose/min/protein) in different strains of *C. capitata* adults

Strains	Mean ± SD (µg glucose/min/protein)	Activity %
Susceptible strain	98.67 ± 4.04[a]	-
Tolerance strain	85.67 ± 4.62 [b]	-13.17
Intermediate strain	75.67 ± 3.06 [c]	-23.31
Resistant strain	102.67 ± 4.73 [a]	+3.38

Table 5: β- esterases activity (µg B- naphthol/min/mg/protein) in different strains of *C. capitata* adults

Strains	Mean ± SD (µg B- naphthol/min/mg/protein)	Activity %
Susceptible strain	4.74±0.25[ab]	-
Tolerance strain	4.94 ± 0.20A[b]	+4.23
Intermediate strain	4.31± 0.24[bb]	-9.07
Resistant strain	4.77 ± 0.49 [ab]	+0.63

Total Soluble Protein: The changes in the level of total soluble proteins which considered the; main metabolites and the major biochemical components necessary for an organism to develop, grow and perform its vital activities were determined to 11 evaluate the difference between the wild strains comparable to laboratory strain (susceptible strain), (Table 1). The results indicated that, the total soluble proteins content of resist strain of *C. capitata* adults significantly low than the laboratory strain of *C. capitata* adults where the average of the total protein content was 26.33 and 38.42 mg/g.b.wt, respectively. The higher significant average of total soluble protein content was 56.25 mg/g.b.wt. in the intermediate strain. Tolerance strains of *C. capitata* adults consider the lower ones at 5 % level. The value of this biochemical parameter was 8.52% as compared to laboratory strain where the mean average of total soluble protein control was 38.42 ± 2.43, (L.S.D. = 3.8273).

Amylase: Data in Table (2) indicated that the amylase activity was significantly increased in all of the tested strains under investigation. The average of amylase activities of susceptible, tolerance, intermediate and resist strains were 19.30, 23.63, 32.67 and 67.30 µg glucose/min/mg/protein, respectively with regard to amylase activity, the increase in the activity was +22.40, + 68.95 and +248.30 %, L.S.D. = 7. 2516.

Invertase: The results in Table (3) Show that were significant difference in invertase activity of resist, tolerance and susceptible strains of *C. capitata* adults. For instance, the averages of invertase activities were equal 210, 127.67 and 155.33 µg glucose/min/mg protein, respectively. However, invertase content had no significant higher percentage content of C. *capitata* adults (168.67 ± 7.76) in intermediate strain, where the value of invertase activity was + 17.70 % compared to susceptible strain. (L.S.D. = 23.55).

Trehalase: Trehalase content of C. *capitata* in tolerance and Intermediate strains are illustrated in Table (4). The data showed that terhalase content was significantly decreased at the level• 5% (L.S. D= 7. 8389) for aforementioned strains and the percentages of inhibition were 13.17 and 23.31% than the laboratory strain groups, however, insignificant increasing of this enzyme content was observed in adults of *C. capitata* Resist strain.

Where it was 102.67 µg glucose/min/mg/protein compared with 98.67 µg glucose/min/mg/protein in laboratory strain group.

Esterases: Beta-estrseses activity was illustrated in Table (5) the data revealed that beta esterase activity through the present study revealed that there was insignificant increase in B- esterase activity in tolerance and resist strains. The percentage activity of B- esterase in tolerance and resist strains was + 4.23 and 0.63 % (L.S.D. = 0.6089), respectively, as compared to susceptible strain, However, the activity was insignificantly inhibited in the case of intennediated strains, it was 9.07% as shown in Table (5).

Acetylcholinesterase (AChE): AchE activity in different strains of *C. capitata* adults are presented in Table (6) the obtained data showed that the AchE activity of tolerance and intermediate strains of *C. capitata* adults was significantly decreased, it was - 10.7 3 and - 28.42 % (L.S.D. = 1.0843), respectively. On other hand the AchE activity was increased (+ 10.14) in C. capitata adults of resist strain.

Table 6: Acetylcholinesterase activity (µg Ach Br released /min/mg protein) in different strains of *C. capitata* adults

Strains	Mean ± SD (µg glucose/min/protein)	Activity %
Susceptible strain	8.48 ± 0.5^{ab}	-
Tolerance strain	7.57 ± 0.56^{b}	-10.73
Intermediate strain	6.07 ± 0.61^{c}	-28.42
Resistant strain	9.34 ± 0.63^{aa}	+10.14

Table 7: Peroxidase levels in different strains of *C. capitata* adults

Strains	Mean ± SD (O.D. Unit x10^3/min mg protein)	Activity %
Susceptible strain	84.33 ± 3.21^{a}	-
Tolerance strain	82.00 ± 4.35^{a}	-2.76
Intermediate strain	58.33 ± 5.68^{b}	-30.83
Resistant strain	80.33 ± 2.51^{a}	+4.74

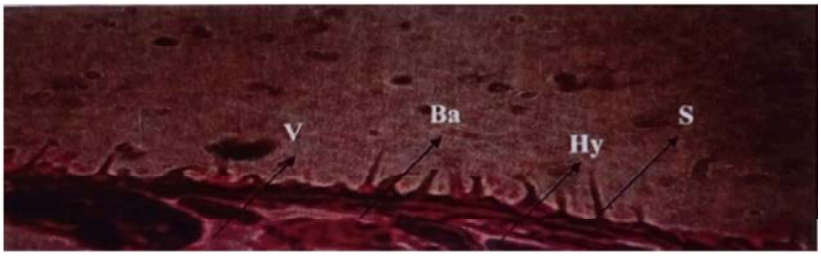

Fig. 1: Transverse (T.S.) of control integument of one week old Female *Ceratitis capitata* (X=25)

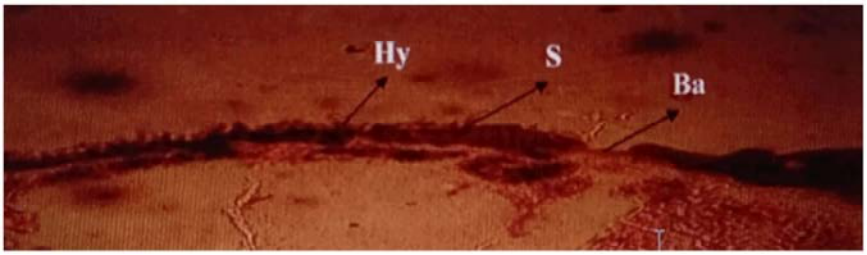

Fig. 2: Transverse section (T.S.) of treatment integument of one week old Female *Ceratitis capitata* with LC_{50} of Phloxine-B (X=50)

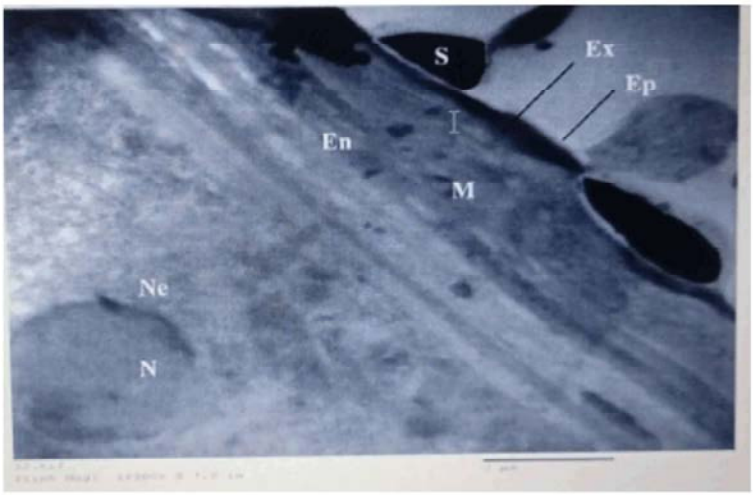

Fig. 3: Electron micrograph of the integument of *C. capitata* susceptible strain adult (16200 x)

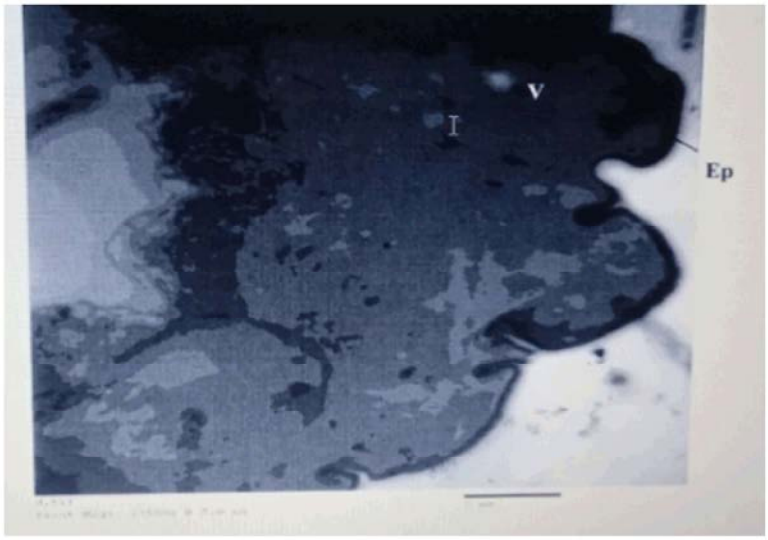

Fig. 4: Electron micrograph of the integument of *C. capitata* resistant strain adult (x13500)

Peroxidase: Peroxidase activity was indicated by optical density are presented in Table (7) the data showed that the peroxidases optical density was significant decreased (L.S.D. =7.7632) for intermediate strains by 30.83%, with respect insignificant decrease m susceptible, tolerance and resist strains, the calculating decreasing for these strains were 84.33, 82.00 and 80.33 O.D. Unitx 103/min/mg protein, respectively.

Histological Studies: The histological structure of the integument of one week *C. capitata* adult are shown in Figure (1). It is composed of single layer of hypo dermal (epidermal) cells, consists of two primary layers: the endcuticle which makes up the greater part and a thin pigmented epicuticle on the surface. In the harder region, the outer part of the endocuticle is converted into very thick layer (the exocuticle), that usually appear homogenous and structure less with the epicuticle, there are many of setae appears on epicuticle. When adults were treated with LC_{50} ofPhloxine-B, the layers of integument degeneration and the setae not obvious, cuticular melaniztion become darker in treatment adults (Figure 2).

Ultrastructure Studies: Figure 3 & 4 showed that the cuticle of *C. capitata* susceptible and resist strains characterized by presence setae appeared on the epicuticle, differences of transparency in layers of integument appears condensed pigments and the setae in resist strains rather than susceptible one (Figure 3).

DISCUSSION

To study the development of resistance and susceptibly of *C. capitata,* the enzyme systems were investigated. The total protein content is the net result of protein biosynthesis the obtained result of total protein showed significant difference in untreated four strains. These results may be attempt by the adults to variations of phototoxicity to the phloxine-B. [27] Stated that proteins help to synthesis microsomal detoxifying enzymes. Carbohydrates are very efficiently Utilized by insects and most specific derive the main part of their nourishment from there nutrients. The present biochemical parameter concerned with studies on amylase, invertase and trehalase activities in laboratory (susceptible), tolerance, intermediate and resistant strain.

This accumulation of lipids at the beginning of metamorphosis coincides with the in vivo incorporation of labeled triacylglycerols reported [10] to be maximal at this stage. Municio *et al.* [7] and Pagani *et al.* [8] could not detect this lipid increase, probably because of the extraction method used or because of a less careful synchronization protocol. Lipid trends may be related to the lipid-extraction method which, in this case, seems to be based on whole individuals chloroform extraction to remove "fat." This method may have created an artifact because water is also removed with this technique. Thus, the initial large lipid drop seen [11] may be a result of the observed relatively large water loss during pupariation and pupal case hardening [12]. Amylases hydrolyzed starch into the monosaccharide, glucose and fructose and secreted by fly salivary glands. It is also found in the crop and midgut of flies, but in smaller amounts [28]. Invertases are hydrolyzed that cleave sucrose into the monosaccharide, glucose and fructose and are secreted and worked in the gut of flies [29]. Trehalase is an important enzyme by which insect degrades trehalose to glucose for internal energy supply. Thus, the activity of trehalase might serve as an indicator of energy reserves. Trehalase present in muscles, body wall, malpighian tubules, fat body, midgut and haemolymph of insects [30]. Non-specific esterases are enzymes that catalyze the hydrolysis of esters and are found in both soluble and membrane-bound forms [31]. In insects it present mainly in hemolymph where they are involved in important physiological processes including catabolism of juvenile hormone, detoxification of insecticides and reproduction [32]. So the activity of this enzyme non significant increase for El-Fayoum governorate tolerance strains and El-Beheyra governorate resistant strains of wild *C. capitata* and the opposite trend were observed with intermediated wild strains from El- Giza governorate.

Differences in the quantitative esterase properties of these strains were proposed as explanation of the differences in phloxine-B sensitivity and resistance agree with findings by Sukonthabhirom *et al.* [33] carried on isozymes from five wild- caught populations of *Aedes aegypti* were collected from five different locations in Bangkok, Thailand.Comparison of obtained results with the previous studies on *Culex pipiens* populations in the United State indicates that the genetic characteristics of the Florida populations of *C. pipiens* are very similar to populations from areas where ecological conditions are very different. Activities of the detoxification enzymes esterase resistance is caused by over- production of nonspecific esterases.

Acetylcholinesterase (AchE) hydrolyses acetylecholin that, when released from synaptic vesicles, briefly depolarizes the postsynaptic cell membrane. Acetylcholine is then denatured by AChE to choline and acetate. Thus, AChE regulates nerve impulse transmission across cholinergic synaptises [34]. It obvious from results For the tolerance and intermediated strain have 7.757 and 6.07 !-LAchBr released /min/mg protein, respectively, comparable to 8.48 !-LAchBr released /min/mg protein but the value for resistant strain significant ctivated that result insure the strain resist to phloxine -B, this observation agree with Magana *et al.* [35] showed the AchE specific activity of single individuals from the susceptible strain ranged between 100 and 340 nmol/min/mg protein, however, the AchE specific activity of individuals from the resistant strain presented a broader distribution ranging from 40 to 340 nmol/min/ mg protein sensitivity of AchE.Pyroxidases together with catalase appears to be the primary enzymes m insects that dediCated to removal of damaging reactive oxygen species (ROS) [36].

Pyroxidases Catalyze the Reaction:

$$SH_2 + H_2O_2 \rightarrow S + 2H_2O$$

In which SH2 is substrate that becomes oxidized and H20 2 acts as a co-substrate. General PODs can utilize many substrates [37]. The presence of such substrates, if limited, can permit enough pyroxidase activity, even over a short period of time [38]. The *C. capitata* adults bears both brown and yellow colors on its cuticle. The brown component is contributed by the pigment melanin, which

is dispersed in the brown cuticle while the yellow-colored part contains within pockets in the cuticle granules possessing a yellow pigment.

In histological studies, the layers of integument degenerated and setae not obvious by treatment *C. capitata* Sucesceptible adult strain by LC_{50} of phloxine -B. As long as the result of this histological View is expected to be that, the presence of destruction in the different sites of cell membrane and parallel sites of the nuclear membrane. El-Tayeb [35] reported that the hematoporphyrin (HP) did not accumulate inside the cell *Culex pipiens* larvae and it started its action from outside during light exposure. So HP affected on the cell membrane by singlet oxygen production. Cell membrane lost its functions and allow to the other (HP) molecules to go inside the cell. These (HP) molecules affect on the nuclear membrane and cell components. Also the effect of highly oxidative stress of singlet oxygen molecules makes holes in the cell membrane. These holes allow to HP to go inside the cell of *Culex pipiens* larvae and allow to the cell contents to go outside the cell. The effects of cell membrane holes or cell membrane destruction cause cell death. It's easy to expect that there is no chance for cell mutation formation because the cell will be dead before singlet oxygen can react with nucleus content.Now, this conclude that the lack of appearance of resistance in the rectpe successive generations. Ultrastructure on the integument of *C. capitata* susceptible and resistant strain adults, characterized by the presence of projections or papillae and setae bounded by the epicuticle. An amorphous (nonlamellate) exocuticle is found below the epicuticle. The enocuticle forms the bulk of the cuticle, with a group of procuticle lamellae. As in other insects, the multilayered cuticle overlays a single layer of epidermal cells with relatively large nuclei and mitochondria that are scattered through the cytoplasm. The same finding present in the cuticle of 4th stage larvae of *Culex pipiens* [36]. The difference is clear from the scanning electron microscope in the transparency of the integument layers characterized by condensed pigments in strains that are resistant and this explains the resistance of the insect to do the pholoxine-B (resistant strain less photosensitive than susceptible strain to the action of phloxine-B), this observation could reflect the lower rate of photosensitization process. Similarly, different concentrations of hematoporphyrin photosensitizer were tested against *B. oleae* and *C. capitata*, the results showed indicated that the adults phototoxicity of B. oleae less than C. capitata this lead to the darker pigmentation *B. oleae* body [37, 38].

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