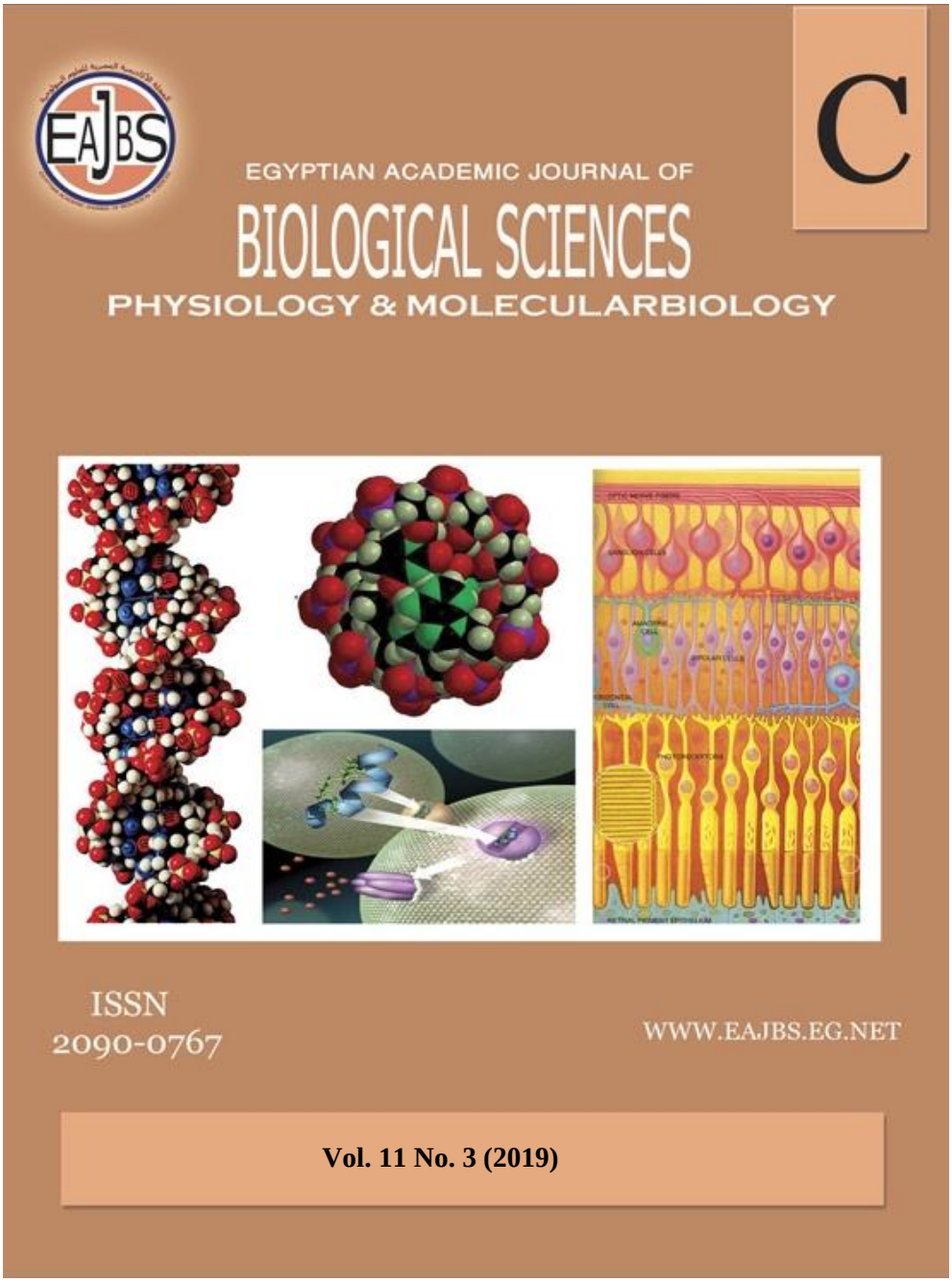

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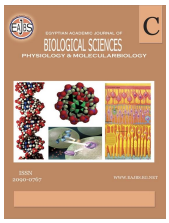

Biochemical Characterization of Some Digestive Enzymes in the Midgut of *Eristalis megacephala* (Diptera: Syrphidae)

Nancy Taha Mohamed; Mohamed Salah Mohamed; Ahmed S. El-Ebiarie and NourhanGamal

Zoology & Entomology Department, Faculty of Science, Helwan University.
11795 - Helwan, Cairo (Egypt).
E.Mail: *nancyt0000@yahoo.com*

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ABSTRACT

The enzymes of some parts have been studied and it shows that the highest pH of trypsin was at pH 6 in the anterior midgut, central midgut and posterior midgut. The highest pH of chymotrypsin in the anterior midgut was at pH 7, nearly similar in the median midgut at all pH values chosen but slightly higher at pH 7, while chymotrypsin activity in the posterior midgut of *Eristalis* larval stage was at pH 8. Considerable activity of leucine-aminopeptidase was at pH 9 in the frontal midgut, at pH 6 in the median midgut and at pH 7 in rear midgut. Intense activity of carboxypeptidase B pH was 9 in the fore, central and caudal midguts.

Some glycosidase's enzyme action was studied, and it shows that the highest pH of alpha-glucosidase in the anterior, middle, and posterior midgut was at pH 5.6. The highest pH of β glucosidase in the anterior, middle, and posterior midgut was at pH 6. The highest pH of β -galactosidase was highest in the anterior midgut at pH 6 and in the middle and posterior midgut at pH 5.6. The highest pH of alpha-galactosidase was highest in the anterior, middle, and posterior midguts at pH 6. The highest pH of alpha-amylase is pH 3.6 in the anterior midgut, pH 6 in the middle midgut and pH 5.6 in the posterior midgut.

Acid-phosphatase activity was high in the frontal midgut, than median and posterior midgut, while alkaline phosphatase activity was nearly similar in the anterior, middle, and posterior midgut, but slightly higher in the anterior midgut of the *Eristalis megacephala* larval stage

INTRODUCTION

The Syrphidae (commonly referred to as hoverflies reflecting their flight modes) make up one of the largest and most well defined Diptera families. Adults especially composites, feed on nectar and pollen from the flowers, and are good pollinators. The larvae display various feeding practices: saprophagous, phytophagous and predaceous, and a few of them are scavengers in social insects' nests.

Saprophagous larvae live in dung, wet decaying vegetation, wood, and tree sap. There are three larval instars, and the length of the life cycle varies greatly among species, taking less than two weeks in some, and possibly up to five years in others (Gilbert, 1993). The rat-tailed maggots (Syrphidae) actually prefer organically polluted sites and thrive in sewage and carrion.

Species of *Eristalis* all have filter-feeding saprophagous larvae, known as rat-tailed maggots from their extensible telescopic posterior respiratory process. This 'tail' enables the larva to descend deep into the water without losing contact with the atmosphere (Hynes, 1960).

In the fore, middle, and hind parts the digestive tract of insects can be divided (Snodgrass, 1935). The majority of digestion occurs in the midgut, which has a wide range of enzymes (Hori *et al.*, 1981). In some insects, digestion starts in the foreground due to salivary gland secretions or regeneration of the midgut. Some insects have also reported rare cases of extra-intestinal digestion (Chapman, 1972). All hydrolases in insect digestive enzymes show general resemblances to mammalian enzymes and are classed according to the reactions they catalyze using standard nomenclature. The insects' food channel has recorded a wide range of digestive enzymes. (Terra and Ferreira, 1994).

Whilst herbivores insects separate more carbohydrases (Agrawal and Bahadur, 1978), carnivores' insects mainly secrete proteases. The structure of the selected enzymes is related to the form of meal an insect may assimilate (Torres and Boyd) (Gooding and Rolseth, 1976). Protease (e.g., trypsin, chymotrypsin, cathepsin), hyaluronidases and phospholipases are digestive enzymes special to zoophage animals (Cohen, 2000). Amylases and pectinases include digestive enzymes special to phytophagous animals (Cohen, 1996).

This study investigates the digestive enzymes of *Eristalis megacephala* in the midgut of third larvae in order to clarify their mode of alimentation in a highly polluted region.

MATERIALS AND METHODS

Sampling Aquatic Larvae of *Eristalis megacephala*:

On the basis of ecological consideration, two sites (Map 1) located in Rosetta Branch, El- Rahway Village, Giza Governorate were chosen to carry out the present study. Site I (30°12'29.6"N 31°02'04.9"E, used as reference point), located 200 meters south and upstream of El-Rahway drain, before mixing of both river and the drain water in Rosetta branch. Site II (30°12'23.3"N31°01'51.9"E; study point), located 200 meters north to the mouth and downstream of El-Rahway drain (the site at which water of the river and the drain are mixed) in Rosetta Branch. The main human activity at both sites is fishing and agriculture. Aquatic larvae of *Eristalis megacephala* were collected from site (II) during May and June 2013. Sampling was performed with a square hand- net, with 30 cm^2 and a mesh size of 250 μm. The hand net stirs the substrate and vegetation strongly to a depth of several inches to dislodge any *Eristalis megacephala* larvae. The collected larvae were kept alive in plastic bottles and were identified according to Zumpt (1965).

Dissection of Organs:

The food canals of the *Eristalis megacephala* larvae were cutup under a stereoscopic Microscope with a 4x magnifying of fine entomological needles. The midgut was separated in distilled water.

1. Preparation of Tissue Homogenate:

The midguts for *Eristalis megacephala's* third instar larvae were divided into anterior, middle and posterior sections, each of these parts was homogenized and centrifuged (10 midguts in 1 ml of distilled water), supernatant was collected and stored at a temperature of 4 C for the enzyme test.

2. Determination of Digestive Enzymes:

1. Determination of Total Protein:

A total of three instar larvae of *Eristalis megacephala* by were assessed with the use of the BIOSCOPE diagnostic Kit of Total Protein for various sections of midgut homogenous (anterior midgut, central midgut and posterior midgut).

2. Determination of pH Highest Activity of Trypsin Enzyme:

2.1. ***Preparation of Appropriate Buffers Used:***

0.2 m of phosphate–citrate buffer at pH (6–7), also 0.05 M of tris-HCl at pH (8–9) was prepared.

2.2. ***Preparation of Substrate Used:***

N–benzoyl–DL–arginine–p–nitroanilideHCl (BAPNA) (Sigma Chemical Company) was used for the determination of trypsin activity.

Dissolving 5 mg (BAPNA) of the dimethyl sulfonate in 1 ml of the substrate was achieved (DMSO). The stock solution's temperature did not drop under 25 C.

2.3. ***Procedure****:*

Trypsin activity was calculated using Erlanger *et al's* updated procedure (1961). A suitable pH buffer (6, 7, 8 and 9), 30 µl midgut homogeneous, and 30 µl BAPNA was found in the reaction medium.

The reaction medium was incubated for 10 minutes at room temperature and then reaction was stopped by 0.5 ml of 30% glacial acetic acid. Control contained all components and was under same conditions but without enzyme solution.

The behavior shift was assessed at 410 nm (utilizing Jenway 6100 spectrophotometer).

3. Determination of pH optimal activity of chymotrypsin enzyme

3.1. ***Preparation of Appropriate Buffers:***

0.2 M of phosphate – citrate buffer at pH (6–7), also 0.05 m of tris – HCl buffer at pH (8–9).

3.2. ***Preparation of Substrate Used:***

N-benzoyl–L ester of tridosine ethyl (BTEE), Sigma Chemical Corporation, has been used in chymotryosin activity determination with the dissolution of 0.1567 gm in 50 ml by 50 percent methanol.

3.3. ***Procedure:***

Chymotrypsin activity has been assessed using a modified Hummel method (1959).

The reaction medium contained 0.5 ml of buffer at pH values (6, 7, 8 & 9) 200 µl of midgut homogenate. A 5-minute response was left to stabilize, then 200 µl BTEE was added and the reaction was incubated at room temperature for 10 minutes.

0.5 ml of 30 percent glacial acetic acid has prevented the reaction.

The control included all components and was contained under the same conditions but without an enzyme solution (utilizing Jenway 6100 spectrophotometer).

4. Determination of pH Optimal Activity of Leucine–Amino Peptidase (lap) Enzyme:

4.1. ***Preparation of Appropriate Buffers Used:***

As previously described for chymotrypsin.

4.2. ***Preparation of Substrate Used:***

Leucine–p–nitroanilide (LpNA) (Sigma Chemical Company) was used for the activity of LAP by dissolving 4 mg in 0.1 ml DMSO.

4.3. ***Procedure:***

Lap operation according to the updated houseman et al method has been calculated (1985).

The reaction medium contains 50 µl of the corresponding pH buffer (6, 6.6, 7, 7.6, 8, 8.6 & 9), 10 µl of a solution of enzymes, 5 µl of LPNA and has been closed well to full miscibility and has been left at room temperature

for around 10 minutes and a reaction of 0.3ml l of 30 % glacial acetic acid has been halted. For a while, reaction persisted until solution became obvious.

Control contained all the components and was in the same state but without an enzyme solution (utilizing Jenway 6100 spectrophotometer).

5. Determination of pH Optimal Activity of Carboxypeptidase A and B:

5.1. ***Preparation of Appropriate Buffers Used:***

As previously described for chymotrypsin.

5.2. ***Preparation of Substrates Used:***

The carboxypeptidase A and hippuryl–phenyl alanine (HA) (the Sigma Chemical Company) activity was calculated by dissolving 0,01 gm of each of the substrates in 20 mL of 0,15 m NaCl of 20 NaCl in the measurement of carboxypeptidase B activity.

5.3*Procedure:*

Carboxypeptidase A and B were calculated using adapted folk and other methods (1960) and Gooding and Rolseth (1976).

The reaction medium contained 0.3 ml of appropriate buffer at pH values (6, 7, 8, & 9), 80 µl of enzyme solution, then reaction was allowed to equilibrate for 5 minutes and then 0.3 ml of HA was added (in case of determining pH optimal activity of carboxypeptidase B) or 0.3 ml of HpLA (in case of determining pH optimal activity of carboxypeptidase A).

The 0.1 ml 1.5 mMZn ions triggered the reaction as ZnSo4) and stayed at room temperature for 5 minutes. All the components but without enzyme solution were included in the control system. Action changes were calculated at 254 nm (using Shimadzu spectrophotometer).

6. Determination of Total Carbohydrate:

The total carbohydrate content was determined according to Singh and Sinha (1977). The procedure was carried out at Mycology Center, Faculty of Science, and Al-Azhar University.

6.1. Determination of pH Optimal Activity of Glucosidases:

1.1. ***Preparation of Mid-Gut Homogenate:***

Chosen larvae were immobilized and isolated in salina (PBS - 0.15 M NaCl + 0.01M Sodium phosphate, pH 7.2 and 0.24 gm Midgut, homogenized in 8 ml cold distilled water and centrifugated at 9000 g at 4 °C for 10 minutes).

1.2. ***Preparation of Appropriate Buffers Used:***

Also 0,2 M phosphate–citrate pH 6 pH buffer was made with 0,2 M sodium acetate at pH 3,6, 4,6 and 5,6.

1.3. ***Preparation of Substrates Used:***

Substrates used were p–nitro phenyl– α- D – glucoside acting on α-glycoside linkages, p–nitro phenyl–β–D–glycoside acting on β- glucoside linkage, p–nitro phenyl – α-D galactosidase acting on α-galactosidase linkages.

All were prepared by dissolving 0.3 mg of each one in 1 ml distilled water and diluted with 2 ml of 0.2 M glycine: 2 M NaOH (pH 10.4).

P–nitrophenyle glycoside hydrolysis gives an alkaline yellow colouration. However, the p–nitrophenol absorption depends on pH, given the increase in color intensity in a given concentration of pH. P–nitrophenol solutions were diluted with 0,2 M of glycine in order to prevent this:2 M NaOH buffer (pH 10.4) (Dean, 1974).

All substrates were from Sigma Chemical Company.

1.4. *Procedure:*

The activities of glucosidases were determined according to method of Ribeiro and Pereira (1984). The reaction medium contained 50 µl of appropriate buffer at pH values (3.6, 4.6, 5.6 & 6), 50 µl of (each substrate in each time) 50 µl of enzyme solution and were incubated at 35°C for 30 minutes, then the reaction was stopped by 0.15 ml of 0.2 M Na_2CO_3. Control contained all components and was under same conditions but without enzyme solution. Action changes were calculated at 410 nm (utilizing Jenway 6100 spectrophotometer).

6.2. Determination of pH Optimal Activity of Amylase:

2.1. *Preparation of Mid-Gut Homogenate:*

In the cold distillated water of 8 ml the selected larvae were immobilized, feeding canals dissected and segregated into saline and 0.14gm of midgut were homogenized and centrifuged in 8000 g at 4°C at a pace of 10 Minutes.

2.2. *Preparation of Appropriate Buffers Used:*

As previously described for glucosidases.

2.3. *Preparation of Substrate Used:*

Starch was used for determination of amylase activity by dissolving 50 mg in 1 ml distilled water.

2.4. *Procedure:*

Amylase behavior was determined by updated Snell and Snell methods (1953).

The reaction medium contained 100 µl of the appropriate buffer at pH values (3.6, 4.6, 5.6 & 6), 100 µl of enzyme solution and 100 µl of starch, then reaction was incubated at 35 °C for 30 minutes and then diluted with 0.1 ml of 5% potassium iodide.

Control contained all components and was under same conditions but without enzyme solution. Action changes were calculated at 410 nm (using Jenway 6100 spectrophotometer).

7. Determination of Acid and Alkaline Phosphatase:

7.1. *Preparation of Mid-Gut Homogenate:*

The selected larvae have been immobilized, food canals have been dissected and divided into saline and 0.14 g of midgut have been homogenized with 8 ml of cold, distilled water.

7.2. *Preparation of* Appropriate ***Buffers Used:***

0.2 M of phosphate–citrate buffer at pH 5 and 0.1 M tris- HCl buffer at pH 9 were prepared.

7.3. *Preparation of Substrate Used:*

P–nitrophenol was used for the determination of alkaline phosphate and acid phosphate activity by dissolving 0.69 mg of a substrate in 10 ml distilled water.

7.4. *Procedure:*

The method of John Butterworth (1971) was used to determine the activity of acid phosphate. The modified method of Bessey, Lowry, brock (1946) was used for the determination of the activity of alkaline phosphatase.

The reaction medium contained 100 µl of 0.2 M phosphate– citrate buffer at pH 5 in case of determination of acid phosphatase activity (or 100 µl of 0.1 M tris-HCL buffer at pH 9 in case of the determination of alkaline phosphatase activity), 100 µl of enzyme solution, 100 µl of p–nitrophenol solution and then reaction was incubated for 1 hour at room

temperature. The reaction was stopped by 1 ml of 0.1 N NaOH in case of acid phosphatase and by 1 ml of 0.02 N NaOH for alkaline phosphatase. The blank contained all components and was under same conditions but without enzyme solution. Action changes were calculated at 410 nm (using Jenway 6100 spectrophotometer).

8. Statistical Analysis:

Data of proteolytic activity were subjected to analysis of variance (ANOVA), and the 129 means were compared by Tukey's test. Statistical analysis was performed using the 130 software Prism. Differences among means were considered significant at $P \leq 0.05$.

RESULTS

The mid-guts of third instar larvae of *Eristalis megacephala* were separated into anterior, middle and posterior parts. The activity of trypsin, chymotrypsin, leucine amino-peptidase, carboxypeptidase A and B was studied. Also, the activity of α-glycosidase, β- glycosidase, α-galactosidase, β- galactosidase, α-amylase, alkaline and acidic phosphatase was studied.

The total protein of anterior mid-gut was 0.0236 g/dl, and that of middle mid-gut was 0.21212 g/dl and the total protein of posterior mid-gut was 0.01040 g/dl (Table 1).

The activity of trypsin was high at pH 6 in anterior mid-gut, middle mid-gut and posterior mid-gut (Table 2). The highest activity of chymotrypsin in anterior mid-gut was at pH 7 (Table 2), in the middle mid-gut it was nearly similar at all chosen pH values but slightly higher at pH 7 (Table 2), while the chymotrypsin activity in the posterior mid-gut of the larval stage of *Eristalis megacephala* was highest at pH 8 (Table 2).

The highest activity of leucine – amino peptidase was at pH 9 in anterior mid-gut, at pH 6 in middle mid-gut and at pH 7 in posterior mid-gut (Table 2).

The highest activity of carboxypeptidase A in the anterior mid-gut was at pH 6 (Table 2), in the middle mid-gut was at pH 7 (Table 2), while at pH 6 in the posterior mid-gut (Table 2). The highest activity of carboxypeptidase B was 9 in anterior, middle and posterior mid-guts (Table 2).

The total carbohydrates of anterior mid-gut in the larval stage of *Eristalis megacephala* are 11.7 µg/ml, middle mid-gut is 94.4 µg/ml and posterior mid-gut is 62.3 µg/ml (Table 3).

At pH 5.6 of the previous, middle, and rear midgut the highest activity was α-Glucosidase (Table 4). At 6 in the anterior, medium, and posterior midgut at pH the highest activity of β glucosidase was (Table 4). The highest activity of α-galactosidase was at pH 6 in anterior midgut and in the middle (Table 4) and posterior midgut at pH 5.6 (Table 4). The highest activity of β-galactosidase was at pH 6 in anterior, middle and posterior midguts (Table 4).

The highest activity of α – amylase is at pH 3.6 in anterior mid-gut (Table 4), pH 6 in middle mid-gut (Table 4) and pH 5.6 in posterior mid-gut.

Acid – phosphatase activity was high in frontal midgut than median and rear midgut while the activity of alkaline phosphatase was nearly similar in anterior, middle, and posterior midgut but slightly higher in anterior mid gut of larval stage of *Eristalis megacephala.* (Table 4).

Table (1): Total protein of mid-gut homogenates of third instar larvae of *Eristalis megacephala* AM: anterior mid-gut, MM: middle mid-gut, PM: posterior mid-gut.

	AM	**MM**	**PM**
Total protein (g/dl)	0.0236	0.21212	0.0104

Table (2): Activity of some proteases produced by larval stage of *Eristalis megacephala* at different pH values. Significance at $P < 0.05$.

Enzyme	**pH**	**AM Mean ± SE**	**MM Mean ± SE**	**PM Mean ± SE**
Trypsin	**6**	0.05483 ± 0.002358	0.1995± 0.06655	0.04807 ±0.002788
	7	0.0254 ± 0.02021	0.07765± 0.05869	0.0085± 0.002277
	8	0.04007 ± 0.005865	0.1850± 0.1444	0.01673±0.001539
	9	0.0207 ± 0.005872	0.02632± 0.004454	0.02511±0.005295
Chymotrypsin	**6**	0.1360 ± 0.01189	0.0745 ± 0.01028	0.09517 ± 0.006675
	7	0.1502 ± 0.008284*	0.07595 ± 0.002193	0.08117 ± 0.008765
	8	0.1262 ± 0.004527	0.07367 ± 0.004566	0.1110 ± 0.003173*
	9	0.09142 ± 0.005894	0.07462 ± 0.009768	0.08733 ± 0.006820
Leucine amino peptidase	**6**	0.04402 ± 0.004743	0.03694± 0.006862*	0.02304±0.009187
	7	0.0600 ± 0.003578	0.0290± 0.005057	0.05432±0.009441
	8	0.0101 ± 0.003012	0.00878± 0.001814	0.01538±0.002136
	9	0.09406 ± 0.06196	0.0241± 0.007225	0.04244± 0.02404
Carboxypeptidase A	**6**	0.4124 ± 0.03297*	0.2148±0.03386	0.4690±0.05505*
	7	0.3642 ± 0.01446	0.3810±0.03213*	0.3918±0.05242
	8	0.1568 ± 0.04033	0.1546±0.02803	0.1112±0.02388
	9	0.0816 ± 0.01238	0.0722±0.02235	0.1484±0.01852
Carboxypeptidase B	**6**	0.5862 ± 0.05308	0.6702±0.03273	0.6860±0.03825
	7	0.3528 ± 0.01961	0.4358±0.01730	0.3844±0.02924
	8	0.2964 ± 0.01199	0.2368±0.02150	0.2988±0.01313
	9	1.300 ± 0.1508*	1.088±0.02035*	1.202±0.02990*

Table (3): Total carbohydrates of mid-gut homogenates of larval stage of *Eristalis megacephala*

Mid-gut	**AM**	**MM**	**PM**
Total carbohydrates (µg/ml)	117.5	94.4	62.3

Table 4: Activity of some carbohydrase produced by larval stage of *Eristalis megacephala* at different pH values. Significance at $P < 0.05$.

Enzyme	pH	AM Mean ± SE	MM Mean ± SE	PM Mean ± SE
Alpha glucosidase	3.6	0.1299±0.007135	0.09968±0.02351	0.1103±0.01623
	4.6	0.8260±0.05994	0.7780±0.03752	0.7008±0.05376
	5.6	1.010±0.1210*	1.194±0.09683*	0.7714±0.2213*
	6	0.3482±0.07821	0.4526±0.04404	0.4514±0.03812
Alpha beta glucosidase	3.6	0.01964±0.003545	0.0246±0.01223	0.0495±0.02120
	4.6	0.02532±0.006515	0.009056±0.001867	0.01782±0.007538
	5.6	0.08914±0.01364	0.08702±0.03220	0.09434±0.006967
	6	0.1262±0.02747*	0.1690±0.007676*	0.1698±0.01268*
Alpha galactosidase	3	0.0410±0.009386	0.04078±0.01049	0.0744±0.03098
	4	0.0508±0.01907	0.01622±0.005014	0.05898±0.01973
	5	0.02544±0.01008	0.0658±0.02139	0.1146±0.01945
	6	0.1387±0.1070	0.0251±0.009518	0.03362±0.01160
Alpha-beta galactosidase	3	0.0186±0.005872	0.09720±0.02529	0.07492±0.01256
	4	0.0396±0.01007	0.03356±0.008142	0.0496±0.01374
	5	0.1768±0.03039	0.1500±0.02274	0.1706±0.01343
	6	0.2400±0.01106*	0.2246±0.02285	0.2130±0.02019*
Alpha amylases	3	1.495±0.3167	1.282±0.3788	1.255±0.2846
	4	0.5255±0.1201	1.132±0.2850	1.218±0.3247
	5	0.7602±0.1633	1.393±0.2693	1.747±0.2905
	6	1.457±0.1420	1.505±0.05733	1.448±0.05342
Acid phosphatase	5	0.03582±0.01221	0.0236±0.006843	0.02455±0.01186
Alkaline phosphatase	9	0.06003±0.008615	0.05768±0.02062	0.0530±0.01142

DISCUSSION

Digestive proteases are the largest hydrolytic group of enzymes in insects that digest oligo- and di-peptide-ingested proteines (Terra and Ferriera, 2012). The enzymes in their active sites, their optima pH and their attacking positions on the protein molecule are categorized according to their composition.

Proteinases (endopeptidases) are involved in primary protein digestion and categorized as serine proteinases, cysteine proteinases, aspartic proteinases or metal proteinases according to their catalytical mechanisms (Bode and Huber, 1992; Terra and Ferriera, 2012). Exopeptidases are classified into two groups based on protein molecules activity. Amino peptidases target N-terminal protein molecules and C-final carboxypeptidoses (terras and Ferriera, 2012) attack. Digestive carboxypeptidases in the insect were categorized into carboxypeptidases A and B in alkaline medium to HpLA and HA respectively. carboxypeptidases (Terra and Ferreira, 1994).

Serine proteinases are present in viruses, prokaryotes and eucaryotes essential for digestion, protein activation in the cascade of melanisation, antibacterial activity and the response of insect immunes. (Gorman *et al.,* 2000a; Gormanm *et al.,* 2000b; Ma and Kanost, 2000).

The present study revealed that the highest activity of trypsin at pH 6 in anterior mid-gut, middle midgut, and posterior mid-gut. The highest activity of chymotrypsin in anterior midgut was at pH 7, in the middle mid- gut it was nearly similar at all chosen pH values but slightly higher at pH 7, while the chymotrypsin activity in the posterior midgut of the larval stage of *Eristalis* sp. was optimum at pH 8.

Serine proteinases are trypsin. Lehninger (1970) suggested that trypsin catalyzes the peptide bond hydrolysis in which a fundamental amino acid residue such as lysine, or arginine gives the carbonyl role. Trypsin, a particular

substrate (N-benzoyl-DL –arginine – p – nitroanlide) has a bond that is susceptible to hydrolysis of trypsin because arginine is an important residue contributing to carbonyl function. Most insect species have recorded digestive trypsin-like behavior. Species and species from Coleoptera series Cucujiformia are important exceptions. In most insects, the ideal pH of trypsin is often alkaline (mostly 8 to 9), regardless of the pH in midguts from which trypsins are isolated. The trypsin-like activity profile in anterior and posterior larval *Parasarcopaga hirtipes* when calculated by varying values of pH (7.5-10.5) by the use of the amidolytic substrate BApNA (according to Erlanger et al., 1961), is more optimum for the lepidoptera insect, and therefore corresponds to the highest pH values found in their midguts (Terra *et al.,* 1996) (Elmelegi *et al.,* 2006). The larvae from *Gasterophilus intestinalis* were the highest active in the early third instar midgut at pH 8 (El-Ebiarie, 2011). Taha (2015). Trypsin's pH value in third-instar *Sarcophagi aegyptiaca* dietary channel larvae corresponds to those values in other insects (pH 7.8–10), reported with different authors, for example. e.g. *Pterostichus melanarius*(Gooding and Rolseth, 1976),*Tenebrio molitor* (Levinsky *et al.,* 1977), Vespa crabo (Jany et al., 1978), *Attagenus megatoma* (Baker, 1981), *Bombyx mori* (Sasaki and Suzuki, 1982), *Aedes aegypti* (Graf and Briegel, 1985), *Costelytra zealandica* (Christeller *et al.,* 1989*), Locusta migratoria* (Sakal *et al.,* 1989), *Musca domestica* (Lemos and Terra , 1992), *Thrombi domestica* (Zinkler and Polzer, 1992), *Choristoneura fumiferana* (Milne and Kaplan, 1993), *Nauphoeta cinerea* (Elpidina *et al.,* 2001), *Mamestra configurata* (Hegedus *et al.,* 2003), *Osphranteria coerulescens* (Sharifi *et al.,* 2012), *Choreutis nemorana Huber* (Gholamzadeh Chitgar *et al.,* 2013) and *Spodoptera littoralis* (Abd El latif, 2014).

Chymotrypsin-style proteinase sequences are identical to vertebrate chymotrypsin, which were determined from *Vespa orientalis* and *Lucilia cuprina* (Jany *et al.,* 1983; Casu *et al.,* 1994). Also, insect chymotrypsin is identical to vertebrate chymotrypsin on glucagon and B-chain of oxidized insulin. Some properties, however, of chymotrypsin insects are opposed to those of chymotrypsin vertebrate, for example their pH-acid volatility and their strong soybean trypsin inhibitor. For trypsin-like chymotrypsin-like proteases pH 9–10 have been shown by Sorkhabi-Abdolmalekiet al. (2013). A pH of 11 and 8 was observed in Sharma *et al.* (1994) in *Tipula* abdominalis larvae for optimum activity values of trypsin and chymotrypsin. In the *Mamestra configurata* Walker (Lepidoptera: Noctuidae) midgut, the optimum pH of trypsin and chymotrypsin-like proteases were also found to be between 7.5–11.5. for both enzymes (Hegedus et al., 2003). There have been no unique activities with housefly for trypsin and chymotrypsin-like enzymes (Blahovec *et al.,* 2006). In *Chilo suppressalis* Walker Midgut (Lepidoptera: Crambidae) Zibaee (2012) reported optimum pH of general proteolytic activity at 9–10, both as azocasein as substrates and hemoglobin in the medium. For trypsin and chymotrypsin-like proteases, the results were identical. In the anterior, central and posterior midgut of the *S. aegyptiaca* 3rd-instar larvae, Taha (2014) recorded higher pH of trypsin at pH 7, and at pH 6 for chymotrypsin. In the present study the highest activity of trypsin in the acidic range and the highest activity of chymotrypsin is neutral and nearly alkaline range may be due to the pollution in the areas where these *Eristalis megacephala* live.

The optimum activity of leucine – amino peptidase was at pH 9 in anterior mid-gut, at pH 6 in middle midgut and at pH 7 in posterior midgut of larval stage of *Eristalis megacephala.*

The synthetic LpNA substrate was studied and emphasized in the leucine–amino-peptidase-like behavior in the *Parasarcophaga hirtipes* midgut (as recommended by Wachsmuth *et al.,* 1966). The maximum activity of taha (2015), respectively in anterior- and posterior-mid-gut at pH 9.0 and pH 8.5 was observed. The pH values of leucine amino peptidase from anterior and posterior midguts in third instar larvae of *Eristalis megacephala* lie within the range of pH optima of the other insects' amino peptidases activity that lie within an alkaline range 7.2–9.0(Terra and Ferreira, 1994). However, middle midgut of *Eristalis* sp. lie within the range of pH optima in *Acanthoscelides oblectus* (Osuala *et al*., 1994) it ranges between 5.5 and 8.0.

The optimum pH of carboxypeptidase A in the anterior mid-gut was at pH 6, in the middle mid-gut was at PH 7, while at pH 6 in the posterior mid-gut. The optimum pH activity of carboxypeptidase B was 9 in anterior, middle and posterior mid-guts of the third instar larvae of *Eristalis megacephala*. When carboxypeptidase A-like activity in the mid-gut of larval *Parasarcophaga hertipes* was assayed by use of HpLA, two optimal pH values (7 and 9) were observed for that of the anterior mid-gut; and an optimal pH (8) for that of the posterior-mid-gut (Elmelegi *et al*., 2006**).**Taha (2015) reported that the optimal pH of the larval *S. aegyptiaca* carboxy peptidase A was at pH 6 and carboxypeptidase B at pH 9.Also carboxypeptidase B-like activity in the mid-gut of larval *Parasarcophaga hertipes* was assayed in both the anterior- and the posterior mid-gut by use of the synthetic substrate HA, the activity rate was high at pH 7 **(**Elmelegi *et al*., 2006**).** Amino- and carboxypeptidase had a broader highest pH of 8–12.5 **(**Zibaee, 2012).

In the present study the concentration of Carboxypepidase B is higher followed by Carboxypeptidase A and then chymotrypsin. This indicates that exopeptidases are more dominant in the midgut of *Eristalis megacephala.* Only endoproteinases will not complete the hydrolyze of ingested proteins into small peptides and amino acids in the insect digestive system. Exopeptidases, such as metal carboxypeptidases, should play a major role in the digestion of proteins and influence the breakdown of endoproteolytic products (Houseman and Downe, 1981; Bayés *et al.*, 2003).

The study also showed the α-maximum glucosidase's pH at pH 5.6 in frontal, middle and back of the intestines. In anterior, median, and posterior mid-gut, the highest pH of α-β glucosidase was pH 6. In anterior medium and central and posterior midgut at pH 5, the highest pH of α-galactosidase was pH 6. The highest pH of β- galactosidase was highest at pH 6 in anterior, middle, and posterior midguts of larval stage of *Eristalis megacephala*. The highest pH of α – amylase is at pH 3.6 in anterior midgut, pH 6 in middle midgut and pH 5.6 in posterior midgut of larval stage of *Eristalis megacephala*. Most insect glycosidases particularly have optimum acidic pH operations (Gontijo *et al.*, 1998;; Jacobson *et al.,* 2007; Cançado et al., 2008Terra and Ferreira, 2005;; Tamaki et al., 2014; Moreira *et al.*, 2015,Moraes *et al.,* 2012).

Carbohydrates are important to the processing, growth, development and preservation of adult survival and reproduction of nutrients in most insects (Dadd, R.H 1985). The nutritional value of carbohydrates depends on the availability of digestive enzymes, which are then processed by epithelial cells, to hydrolyze the complex Carbs. Many carbohydrases of salivary glands and insect midguts were stated to have only alpha-amylase in long alpha-1, 4-glucan chains. (Terra, Ferreira 1996).

This enzyme mediates hydrolysis of starch and glycogen and makes it into maltose, maltotriosis and maltodextrins (Henrissat, *et al.,* 2002). Human, microorganism, bacterial, fungal and plant alpha amylase are present (Octávio *et al.,* 2000). In insect

growth and development these enzymes play an important part. A number of authors have characterized α-amylase, including Coleoptera, Lepidoptera, Hemiptera and many other orders of insects (Asadi et al., 2010, Saberi Riseh *et al.,* 2012). A-amylases are synthesized and secreted by midgut epithelial cells and salivary glands in insects but also insect hemolymph enzymes are identified (Asadi *et al.,*2010, Asadi. *et al.,* 2012). The optimum amylases pH usually corresponds to pH values in midgut lumen72. Depending on the species, the optimum amylases pH differs widely. The optimal pH of certain insect species recorded in Table 3 is shown. Coleopterans mostly exhibit optimal pH acidity for amylatic activity whereas amylases of lepidopteran are normally alkaline. Amylases of dipteran have a neutral preference (Jean-Luc Da Lage,2018).

Enzymes that extract monosaccharides from di-oligo or / or polysaccharides found in the diets of a large number of insects are digestive glycosidases or carbohydrases. These enzymes are found in several orders of insect guts and are possibly expressed according to the type of diet used by the insect. A-amylases (Ishimoto and Kitomura,1989; Lemos *et al.,* 1990; Grossi de Sa' and Chrispeels, 1997; Silva *et al.,* 1999; Titarenko and Chrispeels, 2000; Cristofoletti *et al.,* 2001) are primarily in coleopters, which use starch diet. Exoglycosidase(s), similar to b-glycosidases (Marana *et al.,*2000) and Hemipterans, both feed on the plant, primarily use b-galactosidases to digest the carbohydrates present in their diet. Lepdopterans, which feed on the leaves (Ferreira *et al.,* 1998). In the present research, amylase followed by alphabet-glucosidase was shown to be the most active in the dipteran, the main use of which is blood-feeding, the alkaline a-glucosidases during meal digestion (Dillon and Kordy, 1997) and those that feed on plants. α-glucosidine prolixus has a significant role to play in heme detoxification after a blood meal. The growth in α-glucosidase activity and the release of hema to midgut has been demonstrated by Mury *et al.* (2009). Hemin-enriched diet fed prolixus. Alpha glucosidose has a major role to play in detoxifying heme following blood meal (Mury *et al.,* 2009) and in the degradation of bacterial and fungal cell walls by the digestive glycosidase.

The present study showed that the activity of acid – phosphatase was high in anterior midgut than middle and posterior midgut while the activity of alkaline phosphatase was nearly similar in anterior, middle, and posterior midgut but slightly higher in anterior mid gut of larval stage of *Eristalis megacephala.*

Cephalopina titillator's larval centerpiece has alkaline phosphatase at pH 9 and acid phosphatase at pH 5 (Fig. 4). Alkaline phosphatase is a dipteran and lepidopteran membrane marker of the mid-basolateral membranes while the enzyme acid phosphatase is normally soluble in the cytosol in the mid-basolateral membranes. (Terra and Ferreira, 1994).

Sridhara and Bhat (1963) record steadily increasing enzyme activity at all stages of the life cycle in their studies of the variation of alkaline phosphatase activities for the silkworm *Bombyx mori*. In the gut, salivary glands and Malpigian tubules of both nymph and adults of *Periplaneta americana*, Srivastava and Saxena (1967) found alkaline phosphatase to be normal. They report that there is a role for active transport in the presence of alkaline phosphatase at such locations. In addition, Beadle (1971) found alkaline phosphatase to be associated in *Carausius morasus* with cells containing lipid droplets and reported that lipid phosphatase alkaline probably plays a role in insect lipid absorption**.** The ultrastructure results support this idea as numerous lipid spheres were found in both anterior and middle region of third instar larvae of *Eristalis*

megacephala, also these two regions possessed alkaline phosphatase activity. (Nourhan Gamal El-Din Ehsan)

Similarly, the alkaline phosphatase of two insect-species, the black carpet beetle and housefly, was compared by Nath and Butler (1973), and Barker and Alexander (1958). It takes 9 months for the long lifespan of black tapestry while house flying takes six days. The highest activity of alkaline phosphatases at the end of the larval phases.

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ARABIC SUMMERY

التوصيف البيوكيميائى لبعض الانزيمات الهاضمه فى المعى الاوسط لحشرة الاريستالس ميجاسيفالا(:ثنائية الاجنحه:سيرفيدى)

نانسى طه محمد،محمد صلاح محمد،احمد سالم الابيارى،نورهان جمال الدين
قسم علم الحيوان والحشرات- كلية العلوم- جامعة حلوان

لقد تم دراسة الانزيمات في بعض الاجزاء واظهرت ان اعلى اس هيدروجينى لانزيم التريبسين كان عند 6 فى الامعاء الوسطى الاماميه والمتوسطه والخلفيه. اعلى اس هيدروجينى للكيموتربسين فى المعى الامامى الوسطى عند 7 وكان غالبا متشابها فى الامعاء الوسطى المتوسطه عند كل الاسس الهيدروجينه المختاره ولكن اعلى قليلا عند اس هيدروجينى 7،بينما نشاط الكيموتربسين فى الامعاء المتوسطه الخلفيه ليرقة الاريستالس كان عند اس هيدروجينى 8.نشاط كبير للامينوبيبتيديز عند اس هيدروجينى 9 فى المعى الوسطى الامامى،عند اس هيدروجينى 6 فى الامعاء المتوسطه و عند اس هيدروجينى 7 فى الامعاء تم دراسة بعض الجلوكوسيديز وكان اعلى اس هيدروجينى للالفا جلوكوسيدز فى الامعاء الاماميه والمتوسطه والخلفيه للمعى المتوسط 5.6 .وكان اعلى اس هيدروجينى للبيتا جلوكوسيدز فى الامعاء الاماميه والمتوسطه والخلفيه للمعى المتوسط عند 6.اعلى اس هيدروجينى للالفا جلاكتوسيديز فى المعى الامامى المتوسط عند 6 وفى الامعاء الوسطى المتوسط والامعاء الخلفيهالوسطى عند اس هيدروجينى 5.6.اعلى اس هيدروجينى للبيتا جلاكتوسيديز فى الامعاء الاماميه والمتوسطه والخلفيه للمعى المتوسط عند 6.اعلى اس هيدروجينى للالفا اميليز عند 3.6 فى الامعاء الاماميه الوسطى،وعند اس هيدروجينى 6 فى الامعاء المتوسطه الوسطه والامعاء الخلفيه الوسطى.وكان نشاط الاسد فوسفيتيز مرتفع فى الامعاء الاماميه عن الوسطى والخلفيه للمعى المتوسط بينما الالكلين فوسفيتيز كان متشابه فى الامعاء الاماميه والمتوسطه والخلفيه للمعى المتوسط ولكن اعلى قليلا فى الامعاء الاماميه للمعى المتوسط ليرقة الاريستالس ميجا سيفالا.