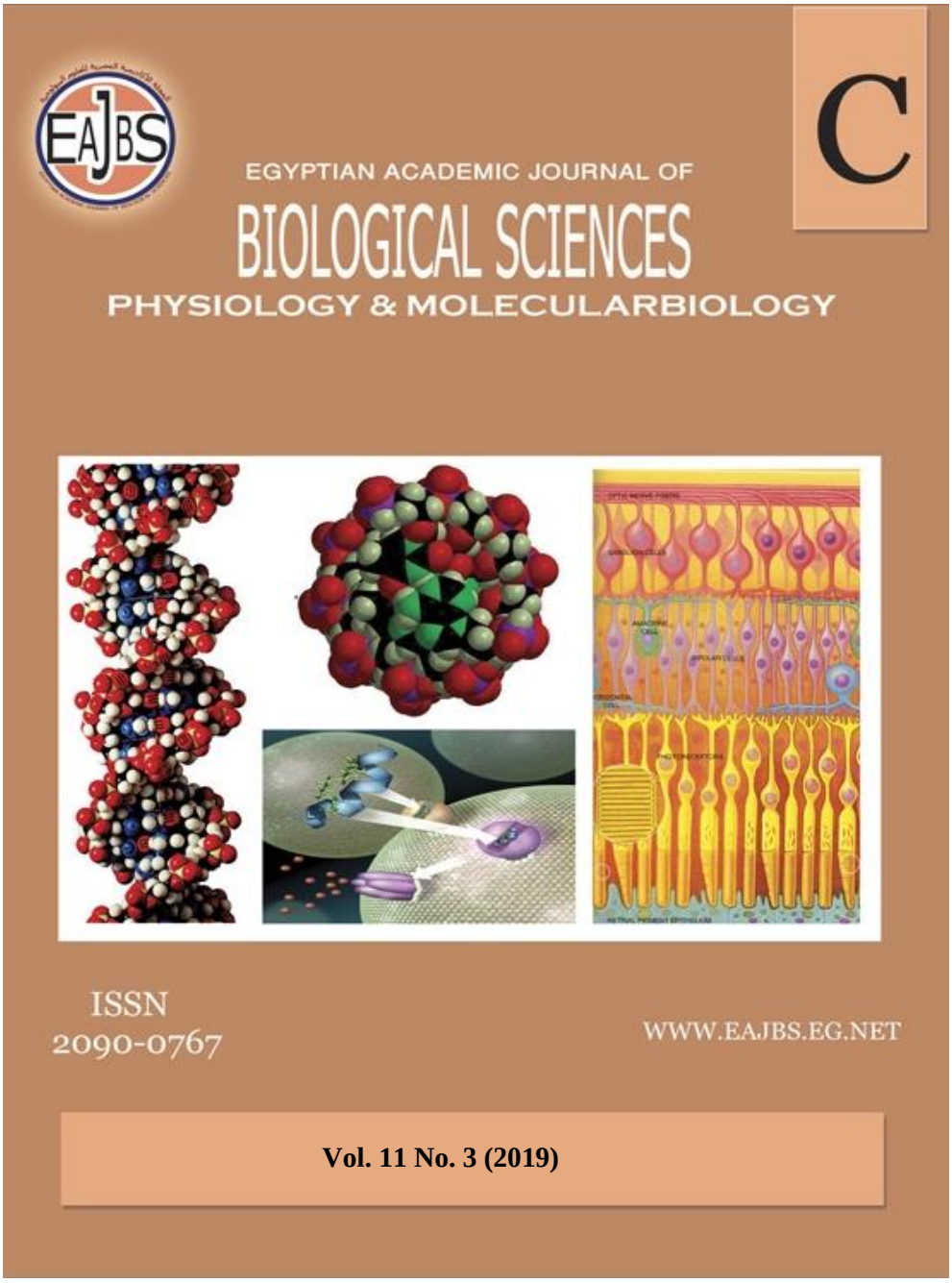

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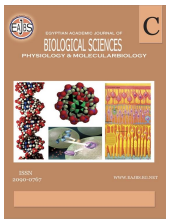

Biochemical Characterization of Some Digestive Enzymes in the Midgut of *Eristalis megacephala* (Diptera: Syrphidae)

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ABSTRACT

The results revealed the activity of some digestive enzymes in the anterior, middle, posterior portions of midgut of third instar larvae of *Eristalis megacephala* and it showed that the highest pH of trypsin was at pH 6 in anterior mid-gut, middle mid-gut, and posterior mid-gut. The highest pH of chymotrypsin in anterior mid-gut 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 mid-gut of the larval stage of *Eristalis megacephala* was highest at pH 8.The highest activity of leucine – aminopeptidase was at pH 9 in anterior mid-gut, at pH 6 in middle mid-gut and at pH 7 in posterior mid-gut. The highest 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 highest pH activity of carboxypeptidase B was 9 in anterior, middle and posterior mid-guts.

Some glucosidases were studied and it showed that the highest pH of α- glucosidase was at pH 5.6 in anterior, middle and posterior mid-gut .The highest pH of β- glucosidase was at pH 6 in anterior, middle and posterior mid-gut. The highest pH of α- galactosidase was highest at pH 6 in anterior mid-gut and in the middle and posterior mid-gut at pH 5.6 .The highest pH of β- galactosidase was highest at pH 6 in anterior, middle and posterior mid-guts .The highest pH of α – amylase is at pH 3.6 in anterior mid-gut, pH 6 in middle mid-gut and pH 5.6 in posterior mid-gut. The present study also revealed that the activity of acid – phosphatase was high in anterior mid-gut that middle and posterior mid-gut while the activity of alkaline phosphatase was nearly similar in anterior, middle and posterior mid-gut but slightly higher in anterior mid-gut of larval stage of *Eristalis megacephala*.

INTRODUCTION

The Syrphidae (commonly known as hoverflies, a reflection of their mode of flight) comprise one of the largest and most sharply defined families of Diptera. The adults feed on nectar and pollen of flowers, especially of Compositae, and are good pollinators. The larvae exhibit a wide range of feeding habits: saprophagous, phytophagous and predaceous, while a few are scavengers in the nests of social insects.

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).

The digestive tract of insects can be divided to fore-, mid- and hindgut (Snodgrass, 1935). Most digestion occurs in the midgut, where a variety of enzymes is available in abundance (Hori *et al.*, 1981). In certain insects, digestion commences in the foregut by virtue of salivary gland secretions or enzyme regurgitation from the midgut. Rare instances of extra-intestinal digestion have also been reported in some insects (Chapman, 1972). Insect digestive enzymes are all hydrolases, showing general similarities to mammalian enzymes and being classified using standard nomenclature based on the reactions they catalyze. A wide range of digestive enzymes has been recorded in the alimentary canal of insects (Terra and Ferreira, 1994).

The nature of the enzymes secreted is related to the nature of the meal that an insect can assimilate (Torres and Boyd, 2009). Herbivorous insects secrete more carbohydrase (Agrawal and Bahadur, 1978), carnivorous insects secrete mainly proteases (Gooding and Rolseth, 1976). Digestive enzymes specific for zoophagous animals include proteases (*e.g.*, trypsin, chymotrypsin, cathepsin), hyaluronidases and phospholipases (Cohen, 2000). Digestive enzymes specific for phytophagous animals include amylases and pectinases (Cohen, 1996).

In this study, we investigate the digestive enzymes present in the midgut of third instar larvae *Eristalis megacephala* to clarify the mode of feeding of these larvae that live in a much-polluted area.

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 alimentary canals of third instars larvae of *Eristalis megacephala* were dissected using fine entomological needles under a stereoscopic microscope at 4X magnification in distilled water. The mid-gut was separated in distilled water.

1. Preparation of Tissue Homogenate:

The mid-guts from third instar larvae of *Eristalis megacephala* were divided into anterior, middle and posterior parts, each portion (10 mid-guts in 1 ml distilled water) were homogenized and centrifuged, the supernatant was collected and stored at 4 °C for enzyme assay.

2. Determination of Digestive Enzymes:

1. Determination of Total Protein:

Total protein was measured for different parts of mid-gut homogenate (anterior midgut, middle midgut, posterior midgut) of third instar larvae of *Eristalis megacephala* by using the BIOSCOPE diagnostic kit of total protein.

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–nitroanilide HCl (BAPNA) (Sigma Chemical Company) was used for the determination of trypsin activity.

The substrate was prepared by dissolving 5 mg (BAPNA) in 1 ml dimethyl sulphoxide (DMSO). The temperature of the stock solution was not allowed to fall below 25 °C.

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

The activity of trypsin was measured according to a modified method of Erlanger *et al*. (1961). The reaction medium contained 0.5 ml of appropriate buffer at pH values (6, 7, 8 & 9), 30 µl of mid-gut homogenate and 30 µl of BAPNA.

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

The change in activity was measured at 410 nm.

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 tryosine ethyl ester (BTEE) (Sigma Chemical Company) was used for the determination of chymotrypsin activity by dissolving 0.1567 gm in 50 ml of 50% methanol.

3.3. ***Procedure:***

The activity of chymotrypsin was determined according to method of Hummel (1959).

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

The reaction was stopped by 0.5 ml of 30% glacial acetic acid.

The blank contained all components and was under same conditions but without enzyme solution .the change in activity was measured at 4100 nm.

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:***

The activity of lap was determined according to modified method of houseman *et al.* (1985).

The reaction medium contained 50 µl of appropriate buffer at pH values (6, 6.6, 7, 7.6, 8, 8.6 & 9), 10 µl of enzyme solution, 5 µl of LpNA and it was shacked well till complete miscibility and was left for about 10 minutes at room temperature and the reaction was stopped by 0.3 ml of 30% glacial acetic acid and it was left for a while till solution became clear.

The blank contained all components and was under same conditions but without enzyme solution .the change in activity was measured at 410 nm.

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:***

Hippuryl–DL–phenyl lactic acid (HpLA) (Sigma Chemical Company) was used for determination of the activity of carboxypeptidase A and hippuryl–L–phenyl alanine (HA) (Sigma Chemical Company) was used for the determination of carboxypeptidase B activity, by dissolving 0.01 gm of each substrate in 20 ml of 0.15 m NaCl.

5.3*Procedure:*

The activity of carboxypeptidase A and B was measured according to method of folk *et al*. (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, 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 reaction was activated by 0.1 ml of 1.5 mMZn ions as $ZnSO_4$) and was left for 5 minutes at room temperature. The blank contained all components but without enzyme solution. The change in activity was measured at 254 nm.

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:***

The selected larvae were immobilized by placing them on ice and the alimentary canals were dissected and separated in phosphate buffer saline (PBS – 0.15 M NaCl + 0.01 M sodium phosphate, pH 7.2 and 0.24 gm of mid-gut were homogenized in 8 ml cold distilled water and centrifuged at 9000 g for 10 minutes at 4 °C.

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

0.2 M sodium acetate buffer at pH 3.6, 4.6 and 5.6 also 0.2 M phosphate–citrate buffer at pH 6 were prepared.

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).

Hydrolysis of p–nitro phenyl glycosides produces a yellow coloration in an alkaline medium. The absorbance of p–nitrophenol is pH dependent, however, as the intensity of color at a given concentration increases with increasing pH. To avoid this, p–nitrophenol solutions were diluted with 0.2 M glycine: 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. The blank contained all components and was under same conditions but without enzyme solution. The change in activity was measured at 410 nm.

6.2. Determination of pH Optimal Activity of Amylase:

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

The selected larvae were immobilized by placing on ice, the alimentary canals were dissected and separated and 0.14 gm of mid-gut were homogenized in 8 ml cold distilled water and centrifuged at 8000 g for 10 minutes at 4 °C.

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

As previously described for glucosidases.

2.3. ***Preparation of Substrate Used:***

Starch was used for detecting amylase by dissolving 50 mg in 1 ml distilled water.

2.4. ***Procedure:***

The activity of amylase was determined according to method of Snell and Snell (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 and was incubated at 35 °C for 30 minutes and then diluted with 0.1 ml of 5% potassium iodide.

The blank contained all components and was under same conditions but without enzyme solution. The change in activity was measured at 410 nm.

7. Determination of Acid and Alkaline Phosphatase:

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

The selected larvae were immobilized by placing on ice, the alimentary canals were dissected and separated in saline and 0.14 gm of mid-gut were homogenized in 8 ml cold distilled water and then centrifuged at 8000 g for 10 minutes at 4°C.

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. The change in activity was measured at 410 nm.

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).

The highest activity of α-glucosidase was at pH 5.6 in anterior, middle and posterior mid-gut (Table 4). The highest activity of β glucosidase was at pH 6 in anterior, middle and posterior mid-gut .The highest activity of α- galactosidase was at pH 6 in anterior mid-gut and in the middle and posterior mid-gut at pH 5.6 (Table 4). The highest activity of β- galactosidase was at pH 6 in anterior, middle and posterior mid-guts (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

The activity of acid – phosphatase was high in anterior mid-gut that middle and posterior mid-gut while the activity of alkaline phosphatase was nearly similar in anterior, middle and posterior mid-gut but slightly higher in anterior mid-gut of third instar larvae of *Eristalis megacephala*.(respectively; 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 major group of hydrolytic enzymes in insects that digest ingested proteins to oligo- and di-peptides (Terra and Ferreira, 2012). These enzymes are classified according to amino acid composition in their active site, pH optima, and attack positions on the protein molecule.

Proteinases (endopeptidases) are involved in primary digestion of protein and are classified based on their catalytic mechanisms as serine proteinases, cysteine proteinases, aspartic proteinases, or metalloproteinases (Bode and Huber, 1992; Terra and Ferreira, 2012). Exopeptidases are divided into two categories based on their activity on protein molecules. Amino peptidases attack protein molecules from the N-terminal end and carboxypeptidases attack from the C-terminal end (Terra and Ferreira, 2012).Insect digestive carboxypeptidases have been classified into carboxypeptidase A and B according to their hydrolytic ability in alkaline medium to HpLA or HA, respectively (Terra and Ferreira, 1994).

Serine proteinases are found in viruses, prokaryotes, and eukaryotes, which are crucial for digestion, protein activation in the melanization cascade, antibacterial activity, and insect immune responses (Gorman *et al.*, 2000a; Gorman *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 mid-gut, posterior mid-gut. The highest activity of chymotrypsin in anterior mid-gut 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 mid-gut of the larval stage of *Eristalis* sp. was optimum at pH 8.

Trypsin is serine proteinases**.** Lehninger (1970) stated that trypsin catalyzes the hydrolysis of peptide bonds in which the carbonyl function

is donated by a basic residue like lysine or arginine. Trypsin – specific substrate (N- benzoyl –DL –arginine – p – nitroanilide) has a bond that is susceptible to trypsin hydrolysis because the carbonyl function is contributed by the basic residue arginine. Trypsin-like activity has been reported in most insect species. Important exceptions are Hemiptera species and species belonging to the series Cucujiformia of Coleoptera. The optimum pH of trypsin in most insects always alkaline (mostly between 8 and 9), irrespective of the pH prevailing in mid-guts from which the trypsin were isolated. Nevertheless, trypsin isolated from Lepidoptera insects higher optimum pH corresponding to the higher pH values found in their mid-guts (Terra *et al.,* 1996).The profile of trypsin-like activity in the anterior- and posterior mid-gut of larval *Parasarcophaga hirtipes*, when measured at variable values of pH (7.5- 10.5), by use of the amidolytic substrate BApNA (according to Erlanger *et al.,* 1961), displayed maximum activity at pH 9.0 irrespective of the luminal pH of the anterior- (pH 7.5) and posterior mid-gut (pH 8) (Elmelegi *et al.,* 2006). The highest activity of trypsin in the mid-gut of the early third instars larvae of *Gasterophilus intestinalis* was at pH 8 (El-Ebiarie, 2011). Taha (2015) reported that the pH value of trypsin in alimentary canal of third instar larvae of *Eristalis megacephala* matches those values (pH 7.8–10) of other insects recorded by different authors 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).This highest pH of trypsin near the acidic range may be due to the pollution in the areas where these *Eristalis megacephala* live.

Chymotrypsin is a serine proteinase. Lehninger (1970) reported that chymotrypsin catalyzes the hydrolysis of peptide bonds in which the carbonyl function is contributed by an aromatic amino-acid residue like phenylalanine, tyrosine or tryptophan. It seems that the distribution of chymotrypsin-like enzymes among insect taxa is similar to that of trypsin (Applebaum, 1985). The optimum pH of chymotrypsin in most insects is in the range (8 – 9), irrespective of the pH prevailing in the mid-guts from which the chymotrypsins were isolated (Terra *et al.,* 1996). The pH value is similar to recorded values (pH 8 – 10) in other insects e.g. *Pieris brassicae* (Lecadet & Dedonder, 1966), *Vespa orientalis* (Jany *et al*., 1974), *Glossina morsitans* (Gooding &Rolseth, 1976), *Locusta migratoria* (Sakal *et al*., 1988), females of *Anopheles* (Hörler & Briegel, 1995), *Nauphoeta cinerea* (Elpidina *et al*., 2001), *Mamestra configurata* (Hegedus *et al*., 2003), *Choreutis nemorana Huber* (Gholamzadeh *et al*., 2013) , *Hyphantria cunea* (Aghaali *et al*., 2013) and *Spodoptera littoralis* (Abd El-latif, 2014).

The sequences of the chymotrypsin-like proteinases were determined from *Vespa orientalis* and *Lucilia cuprina* and are similar to vertebrate chymotrypsins (Jany *et al.,* 1983; Casu *et al.,* 1994). Also, insect chymotrypsins act on glucagon and B-chain of oxidized insulin in a manner similar to vertebrate chymotrypsins. However, some properties of insect

chymotrypsins contrast to those of vertebrate chymotrypsins, such as their instability at acid pH and their strong inhibition by soya bean trypsin inhibitor. Sorkhabi-Abdolmaleki *et al*. (2013) demonstrated pH 9 and pH 9–10 for trypsin-like, chymotrypsin-like proteases of *Andrallus spinidens*, respectively. Sharma *et al.* (1994) found a pH of 11 and 8 as the optimal values for activities of trypsin and chymotrypsin respectively in *Tipula abdominalis* larvae. Also, the optimal pH's of trypsin and chymotrypsin-like proteases were found to be 7.5–11.5 for both enzymes in the mid-gut of *Mamestra configurata* Walker (*Lepidoptera: Noctuidae*) (Hegedus *et al*., 2003). Low specific activities for trypsin-like and chymotrypsin-like enzymes were observed in housefly (Blahovec *et al*., 2006). Zibaee (2012) reported optimal pH of general proteolytic activity in the mid-gut of *Chilo suppressalis* Walker (*Lepidoptera: Crambidae*) to be 9–10, using both azocasein and hemoglobin as substrates. The finding was similar for trypsin and chymotrypsin-like proteases. Taha (2015) reported that the higher pH of trypsin in the anterior, middle and posterior mid-gut of third instars larvae of *S.aegyptiaca* is at pH 7, at pH 6 for chymotrypsin. In the present study, the highest activity of trypsin in the acidic range and the highest activity of chymotrypsin in 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 the middle mid-gut and at pH 7 in posterior mid-gut of larval stage of *Eristalis megacephala.*

Leucine–amino peptidase-like activity in the mid-gut of *Parasarcophaga hirtipes* was emphasized and investigated by using the synthetic substrate LpNA (as recommended by Wachsmuth *et al.*, 1966). Taha (2015) reported that the maximal activity was observed at pH 9.0 and pH 8.5 in the anterior- and posterior-mid-gut, respectively. 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).

The concentration of enzyme Carboxypeptidase B is higher followed by Carboxypeptidase A and then chymotrypsin. This indicates that exopeptidases are more dominant in the midgut of third instar larvae of *Eristalis megacephala.* The occurrence of only end- proteinases in

the insect digestive system would not complete the hydrolysis of ingested proteins to small peptides and amino acids. Exopeptidases such as carboxypeptidases are supposed to play a major role in protein digestion and affect the breakdown of end-proteolysis products (Houseman and Downe, 1981; Bayés *et al.,* 2003).

In general endopeptidases have higher proteolytic activity (trypsin &chymotrypsin) at basic pH values (>8) while exopeptidases have higher proteolytic activity at acid pH values (Fazito do Vale *et al.,* 2007). The lower activity of the endo- proteases may be due to that the larvae has extra-oral digestion during which solid materials can be broken down and nutrients can be liquefied and ingested similar to in larvae of Cyclorrhapha.Additionally,live bacteria may predigest food, change nutritional content,and/or improve digestion inside the gut(Lemaitre and Miguel-Aliaga 2013).

The present study also the highest pH of α- glucosidase was at pH 5.6 in anterior, middle and posterior mid-gut. The highest pH of α- β glucosidase was at pH 6 in anterior, middle and posterior mid-gut. The highest pH of α- galactosidase was highest at pH 6 in anterior mid-gut and in the middle and posterior mid-gut at pH 5. The highest pH of β-galactosidase was highest at pH 6 in anterior, middle and posterior mid-guts of larval stage of *Eristalis megacephala*. The highest pH of α – amylase is at pH 3.6 in anterior mid-gut , pH 6 in middle mid-gut and pH 5.6 in posterior mid-gut of larval stage of *Eristalis megacephala*. Particularly, most insect glucosidases have optimal activities in acidic pHs (Gontijo et al., 1998; Terra and Ferreira, 2005; Jacobson *et al.,* 2007; Cançado et al., 2008; Moraes *et al.,* 2012; Tamaki *et al.,* 2014; Moreira *et al.,* 2015).

Carbohydrates are essential for the majority of insects to produce nutrient materials needed for growth, development and for the maintenance of adult survival and reproduction **(**Dadd, R.H 1985). The nutritive value of carbohydrates depends on the availability of digestive enzymes to hydrolyze complex carbohydrates to their constituent monomers which are then absorbed by mid-gut epithelial cells. Many carbohydrase have been reported from salivary glands and mid-gut of insects that among them only α-amylase act preferentially on long α-1, 4-glucan chains (Terra, Ferreira 1996).

This hydrolysis of starch and glycogen converts them to maltose, malt- triose, maltodextrins (Henrissat, *et al.,* 2002). α -amylase is found in animals, microorganisms, bacteria, fungi, plants (Octávio e*t al.* 2000). These enzymes play important roles in insect growth and development. Many authors have characterized α-amylases from many different orders of insects including *Coleoptera, Lepidoptera* ,Hemiptera (Asadi *et al.,* 2010, Saberi Riseh *et al.,* 2012). In insects, α-amylases are synthesized and secreted by mid-gut epithelial cells and salivary glands, but these enzymes have been reported also from insect hemolymph (Asadi *et al* .,2010,Asadi. *et al*., 2012).Optimum pH of insect amylases varies greatly depending on the species. Coleoptera showed mostly acidic optimum pH for amylase activity, whereas Lepidopteran amylases generally have alkaline preferences. Dipteran amylases have more neutral preference (Jean-Luc Da Lage, 2018).The optimum pH of amylases generally corresponds to the pH values in the midgut lumen.

Digestive glucosidases or carbohydrase remove monosaccharides from di- oligo- and/ or polysaccharides present in the diets of many insects. These enzymes are present in the insect guts of several orders and exo-glucosidases and endo-glucosidases are probably expressed according to the kind of diet used by the insect. In Coleopterans, which feed

on a diet rich in starch, the predominant endoglycosidases are the a-amylases (Ishimoto and Kitamura, 1989; Lemos *et al.*, 1990; Grossi de Sa´ and Chrispeels, 1997; Silva *et al.*, 1999; Titarenko and Chrispeels, 2000; Cristofoletti *et al.*, 2001). In Lepidopterans insects, which feed on leaves, exoglycosidase(s)-like b-glucosidases are predominantly present (Marana *et al.*, 2000), and Hemipterans, which are plant feeders, use b-galactosidase to digest the carbohydrates present in their diet (Ferreira *et al.*, 1998). Dipterans, which feed on blood, use alkaline a-glucosidases during meal digestion (Dillon and Kordy, 1997) and those that feed on plants, use mainly b-galactosidases (Ferreira *et al.*, 1998).In the present study, the amylase followed by alpha-glucosidase showed the highest activity in the midgut of third instar larvae of *Eristalis megacephala*. This may be due to the polluted area where it lives as it was reported that *Rodinus prolixus* α-glucosidases have an important role in the detoxification of heme after a blood meal. Mury *et al.* (2009) have verified an increase in α-glucosidase activity and release of heme in the midgut of *R. prolixus* fed with hemin-enriched diet. Alpha glucosidases have an important role in the detoxification of heme after a blood meal (Mury *et al.*,2009), digestive glucosidases involved in degradation of bacterial and fungal cell walls.

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

Alkaline phosphatase is a mid-gut microvillus membrane marker in Dipteran and Lepidopteran species, although it may also occur in mid-gut basolateral membranes and even as a secretory enzyme acid phosphatase is usually soluble in the cytosol of mid-gut cells (Terra and Ferreira, 1994).
Sridhara and Bhat (1963) in their studies of the variation in the alkaline phosphatase activities of the *Bombyx mori* in all stages of life cycle reported a steady increase in the enzyme activity. Also, Srivastava and Saxena (1967) found alkaline phosphatase to be widely distributed in the gut, salivary glands and Malpighian tubules of both nymph and adults of *Periplaneta Americana*. They reported that the presence alkaline phosphatase activity at those sites indicates a role in active transportation. Moreover, Beadle (1971) found that alkaline phosphatase was associated with those mid-gut cells containing lipid droplets in *Carausius morasus* and he reported that alkaline phosphatase probably plays a role in lipid absorption in insects. The ultrastructure results supports this idea as numerous lipid spheres were found in both anterior and middle region of the instar larvae of *Eristalis megacephala*, also these two regions possessed alkaline phosphatase activity. (Unpublished Data)

Likewise**,** Nath and Butler (1973) and Barker and Alexander (1958) compared the alkaline phosphatase in the larvae stage of two insect species, the black carpet beetle and house fly. The larval longevity of black carpet beetle requires 9 months while that of house fly requires 6 days. The maximum alkaline phosphatase activity possessed towards the end of the larval stages.

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