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Original Research Article

Characterization of serine proteases and glycosidases using enzyme assay in the mid-gut of adult *Trachyderma philistine* (Coleoptera: Tenebrionidae)

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Abstract

The biochemical activities of some digestive enzymes produced by adult *Trachyderma philistine* beetle were studied. The mid-gut of adult stage of *Trachyderma philistine* was separated into anterior, middle, and posterior parts. The total protein of anterior mid-gut was 3.34 mg/dl, and that of middle mid-gut was 2.53 mg/dl and the total protein of posterior mid-gut was 3.21 mg/dl. The highest activity of trypsin was at pH 8 in anterior mid-gut, middle mid-gut, and posterior mid-gut. The highest activity of chymotrypsin in anterior mid-gut was at pH 6, while at pH 7 in middle and posterior mid-guts. Leucine – amino peptidase showed activity at pH 8 in 3 portions of midguts. The carboxypeptidase A showed activity at pH 8 in both the anterior mid-gut and middle mid-guts, while at pH 9 in the posterior mid-gut. The highest pH activity of carboxypeptidase B was 9 in 3 portions of midgut.The total carbohydrates of anterior mid-gut in the adult stage of *Trachyderma philistine* is 39.2 mg/ml, middle mid-gut is 34.04 mg/ml and posterior mid-gut is 34.9mg/ml .The highest activity of α-glucosidase was at pH 3.6 in anterior and posterior mid-guts while at 4.6 in middle midgut. The highest activity of β glucosidase was at pH 3.6 in anterior and posterior mid-guts while at 4.6 in middle mid-gut. The highest activity of α-galactosidase was at pH 3.6 in 3 portions of midguts. The highest activity of α – amylase is at pH 4.6 in both anterior mid-gut and middle mid-guts, while at pH 3.6 in posterior mid-gut.

Keywords: Amylase, Carbohydrases, Coleoptera, Midgut, Proteases, *Trachyder-maphilistine*

INTRODUCTION

Digestion primarily occurs in the insect mid-gut, which often has variable structural and physiological adaptations, including compartmentalization, pH differences within mid-gut regions, and variation in redox potential; all of which are adaptations to the digestive functions of the mid-gut [1,2]. Proteases are divided into exo- and endopeptidases. Exopeptidases remove amino acids from the N-terminal and C-terminal of protein molecules, known as amino- and carboxy-peptidases [3]. Endopeptidases are classified into four classes based on the nature of their catalytic site cleaving peptide bonds, namely serine, cysteine, aspartic, and metalloproteases [3]. Except for some hemipteran and Curculionid coleopterans, in most insect groups, the initial digestion of proteins relies on serine-proteases, particularly trypsin- and chymotrypsin-like proteases. These enzymes have been shown to have a high pH optimum of 8–11, which is consistent with the alkaline conditions in the midgut [4].

Glycosidases are key enzymes in central and intermediate metabolic pathways. They release terminal sugars from glycosides as oligo- or disaccharides, aryl, and alkyl glycosides. They may also have activity against

a wide array of glycoconjugates, such as polysaccharides, glycoproteins, or glycolipids [3].

Insect digestive glycosidases are essential enzymes for nutrition, regardless of taxonomic order or diet. These enzymes are essential for final digestion of sugars and have important roles in the interaction of insects with plants and pathogens [3].Glycosidase hydrolases (GH) activities have been extensively studied in different insect orders, including agricultural pests and disease vectors. They are responsible for the breaking down of molecules of plant and animal sources [3,5,6,7,8,9,10,11,12]

The aim of present study is to identify the activity profile of the digestive peptidases in midgut of adult *Trachydermaphilistine* beetle. This identification is based on hydrolysis of specific synthetic substrates and also the pH at which the maximum hydrolysis occurs. This study is also aimed at determining the biochemical characterization of carbohydrate-hydrolyzing enzymes in the digestive system of the *Trachydema philistine* to gain a better understanding of the digestive physiology of this insect.

MATERIALS AND METHODS

Collection of insects

*Trachyderma philistine*were collected from Cairo and Siwa in Egypt.

Dissection of Organs

Mid-gut of adult stage of *Trachyderma philistina* were dissected using fine entomological needles under a stereoscopic microscope at 4X magnification in phosphate-buffered saline [PBS; 10 mM Na2SO4, 145 mM NaCl (pH 7.2)] and transferred to a micro centrifuge tube with a small volume of PBS (1ml for 5 dissected insect). The mid-guts were divided into anterior (AM), middle (MM) and posterior (PM) portions.

Preparation of tissue homogenate

The mid-gut from adult stage of *Trachyderma philistina* was divided into anterior, middle and posterior parts and each portion (5 mid-guts in 1 ml distilled water) washomogenized, centrifuged and the supernatant was collected and stored at 4 °C for enzyme assay.

Determination of total protein

Total protein was measured for different parts of mid-gut homogenate (AM, MM and PM) by using the Folin-phenol reagent according reported method [13].

Determination of pH optimal activity of trypsin enzyme

Preparation of appropriate buffers used

0.2 M of phosphate –citrate buffer at pH (6 and 7) and 0.05 M of tris–HCL at pH (8 and 9) were prepared.

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.

Procedure

The activity of trypsin was measured according to a modified method of [14]. The reaction medium contained 0.5 ml of appropriate buffer at pH values (6, 7, 8 and 9), 30 µl of mid-gut homogenate and 30 µl of BAPNA.

The reaction medium was incubated for 10 minutes at room temperature and then reaction was stopped by adding 0.5 ml of 30 % glacial acetic acid. Control contained all components and was under same conditions but without enzyme solution. The change in activity was measured at 410 nm (using JASCO- V 630 spectrophotometer).

Determination of pH optimal activity of chymotrypsin enzyme

Preparation of appropriate buffers

0.2 M of phosphate – citrate buffer at pH (6 and 7) and 0.05 m of tris – HCL buffer at pH (8 and 9).

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.

Procedure

The activity of chymotrypsin was determined according to the modified method of [15]. The reaction medium contained 0.5 ml of buffer at pH values (6, 7, 8 and 9)

and 200 µl of mid- gut homogenate. Reaction 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 adding 0.5 ml of 30 % glacial acetic acid.

Control contained all components and was under the same conditions but without enzyme solution. The change in activity was measured at 410 nm (using JASCO-V630 spectrophotometer).

Determination of pH optimal activity of leucine–amino peptidase (lap) enzyme

Preparation of appropriate buffers used: As previously described for chymotrypsin.

Preparation of substrate used

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

Procedure

Activity of lap was determined according to reported method [16]. The reaction medium contained 50 µl of appropriate buffer at pH values (6, 7, 8 and 9), 10 µl of mid-gut homogenate and 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. The reaction was left for a while till the solution became clear.

Control contained all components and was under the same conditions but without enzyme solution. The change in activity was measured at 410 nm (using JASCO-V630 spectrophotometer).

Determination of pH optimal activity of carboxypeptidase A and B

Preparation of appropriate buffers used

As previously described for chymotrypsin.

Preparation of substrates used

Hippuryl–DL–phenyl lactic acid (HpLA) (Sigma Chemical Company) was used for the 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.

Procedure

The activity of carboxypeptidase A and B was measured according to the modified method [17, 18]

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

The reaction was activated by 0.1 ml of 1.5 mM Zn ions (as ZnSO4) and was left for 5 minutes at room temperature. Control contained all components but without enzyme solution. The change in activity was measured at 254 nm (using JASCO-V630 spectro-photometer).

Determination of Total Carbohydrate

The total carbohydrate content was determined according to the reported **method [19]**. The procedure was carried out at Mycology Center, Faculty of Science, and Al-Azhar University.

Determination of pH Optimal Activity of Glucosidases

Preparation of Mid-Gut Homogenate

The selected insects 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 was homogenized in 8 ml cold distilled water and centrifuged at 9000 g for 10 minutes at 4 °C.

Preparation of Appropriate Buffers Used

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

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 and 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) [20]. All substrates were from Sigma Chemical Company.

Procedure

The activities of glucosidases were determined according to reported method [21]. 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) and 50 µl of enzyme solution were incubated at 35°C for 30 minutes, then the reaction was stopped by adding 0.15 ml of 0.2 M Na2CO3. The blank contained all components and was under the same conditions but without enzyme solution. The change in activity was measured at 410 nm.

Determination of pH Optimal Activity of Amylase

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.

Preparation of Appropriate Buffers Used

As previously described for glucosidases.

Preparation of Substrate Used

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

Procedure

The activity of amylase was determined according to reported method [22]

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 the same conditions but without enzyme solution. The change in activity was measured at 410 nm.

Statistical analysis

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

RESULTS

The mid-gut of adult stage *Trachyderma philistine* was separated into anterior, middle, and posterior parts. The optimum pH of trypsin, chymotrypsin, leucine amino peptidase, carboxypeptidase A and B was studied. The total protein of anterior mid-gut was 3.34 mg/dl, and that of middle mid-gut was 2.53 mg/dl and the total protein of posterior mid-gut was 3.21 mg/dl (Table 1).

The highest activity of trypsin was at pH 8 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 6 (Table 2), while it showed the highest activity at pH 7 in middle and posterior mid-gut (Table 2).

The highest activity of leucine – amino peptidase was at pH 8 in anterior mid-gut, middle mid-gut, and posterior mid-gut (Table 2).

The highest activity of carboxypeptidase A in the anterior mid-gut was at pH 8 (Table 2), in the middle mid-gut was at pH 8 (Table 2), while in the posterior mid-gut it was at pH 9 (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 adult stage *Trachyderma philistina* is 39.2 mg/ml, middle mid-gut is 34.04 mg/ml and posterior mid-gut is 34.9mg/ml (Table 3).

The highest activity of α- glucosidase was at pH 3.6 in anterior and posterior mid-guts (Table 4) while at 4.6 in middle mid-gut. The highest activity of β glucosidase was at pH 3.6 in anterior and posterior mid-guts (Table 4) while it was at 4.6 in middle mid-gut. The highest activity of α- galactosidase was at pH 3.6 in anterior, middle, and posterior mid-gut (Table 4).

The highest activity of α – amylase was at pH 4.6 in anterior mid-gut and middle mid-gut (Table 4), while it was at pH 3.6 in posterior mid-gut.

Table 1. Total protein of mid-gut homogenates of adult stage *Trachyderma philistina* in AM :anterior mid-gut, MM: middle mid-gut and PM: posterior mid-gut.

	AM	MM	PM
Total protein (mg/dl)	3.34	2.53	3.21

Table 2. Activity of some proteases produced by adult stage of *Trachyderma philistina* at different pH values. The variances significant at P < 0.05.

ENZYME	PH	AM MEAN ± SE	MM MEAN ± SE	PM MEAN ± SE
Trypsin	6	0.2450±0.005958	0.2762±0.005435	0.2112±0.005757
	7	0.2712±0.01838	0.2526±0.005591	0.2272±0.005044
	8	0.3274±0.005192	0.3636±0.008594	0.3636±0.008594
	9	0.1874±0.03943	0.2604±0.003982	0.1608±0.03233
Chemotrypsin	6	0.09386±0.06131	0.03872±0.002067	0.03488±0.002099
	7	0.0433±0.0009127	0.04632±0.0009510	0.09828±0.06122
	8	0.02163±0.0002673	0.02434±0.0009031	0.02434±0.0002561
	9	0.01858±0.0002673	0.0243±0.0007155	0.01282±0.0001585
Leucine amino peptidase	6	0.03146±0.01026	0.02756±0.003118	0.0149±0.001335
	7	0.04618±0.003611	0.05056±0.003483	0.0288±0.001068
	8	0.07074±0.01305	0.09368±0.001033	0.06822±0.006153
	9	0.01506±0.001305	0.03671±0.001209	0.02344±0.0008903
Carboxypeptidase A	6	0.04728±0.002299	0.04796±0.003234	0.0084±0.001111
	7	0.0335±0.001642	0.05786±0.0005124	0.02404±0.0004377
	8	0.02478±0.001427	0.06686±0.001751	0.03166±0.005446
	9	0.01454±0.001862	0.06432±0.0008564	0.0411±0.005515
Carboxypeptidase B	6	0.01122±0.0001828	0.01148±0.0001463	0.01166±0.0001691
	7	0.02822±0.001143	0.02766±0.001186	0.02704±0.0009196
	8	0.04224±0.001216	0.04392±0.001514	0.03534±0.002358
	9	0.06298±0.001962	0.06412±0.0005083	0.0624±0.002621

Table 3. Total carbohydrates of mid-gut homogenates of adult stage *Trachyderma philistina* in AM :anterior mid-gut, MM: middle mid-gut, PM: posterior mid-gut.

Mid-gut	AM	MM	PM
Total carbohydrates (mg/ml)	39.2	34.04	34.9

Table 4. Activity of some carbohydrases produced by adult stage *Trachyderma philistina* at different pH values. AM is anterior mid-gut; MM is middle mid-gut and PM is posterior mid-gut. The variances significant at P < 0.05.

ENZYME	PH	AM MEAN ± SE	MM MEAN ± SE	PM MEAN ± SE
α – glucosidase	3.6	0.7386±0.01108	0.5467±0.1354	0.6284±0.005879
	4.6	0.6922±0.01840	0.5836±0.007447	0.5222±0.009303
	5.6	0.5630±0.007752	0.3480±0.01648	0.2586±0.02553
	6	0.2614±0.01724	0.2028±0.0008602	0.1640±0.009127
β – glucosidase	3.6	1.598±0.06086	1.265±0.04185	0.6284±0.005879
	4.6	1.080±0.04117	1.435±0.3921	0.5222±0.009303
	5.6	0.7462±0.01116	0.6680±0.03377	0.2586±0.02553
	6	0.4832±0.04206	0.4908±0.01615	0.1640±0.009127
α – galacosidase	3.6	0.6590±0.01601	0.6114±0.005335	0.4897±0.01167

Table 4. Continue

	4.6	0.5330±0.01263	0.4876±0.004946	0.3798±0.01312
	5.6	0.4116±0.006345	0.2808±0.005625	0.2334±0.009729
	6	0.2680±0.01188	0.2230±0.005263	0.1720±0.01638
α – amylase	3.6	1.935±0.03017	1.714±0.02904	1.534±0.01432
	4.6	2.119±0.06071	1.976±0.01252	1.046±0.006838
	5.6	1.764±0.01851	0.8104±0.004523	0.7362±0.01009
	6	1.666±0.008517	0.7078±0.02091	0.5944±0.003444

DISCUSSION

The digestive enzymes such as proteolytic enzymes play important roles in insect growth, development and reproduction, enzyme activation, toxin activation/ detoxification, and inflammation processes [23]. In this research we characterized the digestive proteases in the gut of *Trachyderma* beetle and the results revealed that the highest activity of trypsin was at pH 8 in anterior mid-gut, middle mid-gut and posterior mid-gut .The pH value of trypsin (pH 7.8–10) of other insects recorded by different authors *e.g. Pterostichus melanarius* [18],*Tenebrio molitor*[24], *Vespa crabo* [25], *Attagenus megatoma* [26], *Hypoderma lineatum* [27], *Bombyx mori* [28], *Aedes aegypti* [29], *Costelytra zealandica* [30],*Locusta migratoria* [31], *Musca domestica* [32],*Thrombi domestica* [33],*Choristoneura fumiferana* [34],*Nauphoeta cinerea* [35], *Mamestra configurata* [36], *Osphranteria coerulescens* [37], *Choreutisnemorana* Huber [38] and *Spodoptera littoralis* [39]. Trypsin-like enzymes (EC 3.4.21.4), along with chymotrypsins, belong to the trypsin superfamily of serine endoproteinases characterized by the H-D-S catalytic triad, i.e., three amino acids in the active site involved in catalysis namely histidine, aspartate, and serine. Whereas trypsins cleave polypeptide chains on the carboxyl side of arginine and lysine, aromatic amino acids are the preferential cleavage site for chymotrypsins. Trypsins appeared early in the evolution of organisms and their structure is highly conserved. Molecular size, catalytic mechanism, and inhibition by natural and synthetic inhibitors are very similar in most animal groups, even phylogenetically distant ones such as insects and vertebrates, although there are differences in substrate specificity, activation by calcium ions, responses to proteinaceous inhibitors, and stability in acidic pH [3,40].As the most ancestral proteinase, trypsin is wide-spread in the digestive tract of insects from various orders and with different feeding habits, ranging from strict phytophagy to strict zoophagy. Besides digestion, trypsin-like enzymes take part in a wide range of other physiological processes, such as molting [40], tissue remodeling [42] innate immunity [43] diapause [44], fertilization [45], regulation of female post mating behavior [46], and activation of enzyme precursors of trypsin, chymotrypsin [47]chitinase [48], and phenol oxidase [49] In addition, trypsin can affect the biology of pathogenic organisms and may have antiviral effects [50] or activate viral infectivity by cleavage of envelope proteins[51]. It is also involved in the activation of *Bacillus thuringiensis* Berliner (Bt) protoxin [52] and Bt toxin degradation [53]. Trypsin may also have a role in preventing infection by parasites [54,55]or assist in parasite passage through the peritrophic membrane [56]. Generally, it can be said that distribution of trypsin activity along the digestive tract is more dependent on taxon than on feeding habit of an insect species [3]. There are many examples in which phylogenetically distant species, despite having similar feeding habits, exhibit large differences in compartmentalization and midgut pH gradients [1]. The highest trypsin activity or amounts of trypsin-encoding mRNAs occur in midgut caeca of Orthoptera [57], the anterior midgut of Phthiraptera [58], Phasmida [59], Hymenoptera [60], and Lepidoptera [61,62], the anterior and/or posterior midgut of Coleoptera [63,64,65,66,67] and Diptera [68,69,70,71], and the posterior midgut of Dictyoptera [35,72]

The present study revealed that the highest activity of chymotrypsin in anterior mid-gut was at pH 6, while it showed the activity at PH 7 in middle and posterior mid-gut.

Chymotrypsin is a serine proteinase. [73] 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 like that of trypsin [74]. 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 chymotrypsin were isolated [75]. This pH value is similar to those recorded (pH 8 – 10) in other insects *e.g. Pieris brassicae* [76], *Vespa orientalis* [77], *Glossina morsitans* [18] ,*Locusta migratoria* [78], females of *Anopheles* [79], *Nauphoeta cinerea* [35],*Mamestra configurata* [36], *Choreutis nemorana* Huber [38] , *Hyphantria cunea* [80] and *Spodoptera littoralis* [39].

The highest activity of leucine – amino peptidase was at pH 8 in anterior mid-gut, middle mid-gut, and posterior mid-gut in the present study.

The pH values of leucine amino peptidase in adult *Trachyderma* lie within the range of the pH optima of the

other insects' amino peptidases activity that lie within an alkaline range 7.2–9.0 [1].

However, in *Acanthoscelides oblectus* [81] it ranges between 5.5 and 8.0.The occurrence of only endoproteinases in the insect digestive system would not complete the hydrolysis of ingested proteins to small peptides and amino acids.

Exopeptidases such as metallocarboxypeptidases are supposed to play a major role in protein digestion and affect the breakdown of endoproteolysis products [82, 83]. Carboxypeptidase A and B activity in the digestive systems of insects has been studied [84].In the present study the highest pH of carboxypeptidase A in the anterior mid-gut was at pH 8, in the middle mid-gut was at pH 8, while at pH 9 in the posterior mid-gut. The highest pH activity of carboxypeptidase B was 9 in anterior, middle, and posterior mid-guts .

Coleopteran digestive proteases have been detected and associated with certain well-known families, where the type of digestive proteases seem not to be closely related to the current type of diet but, rather, are related to the taxonomic order as a result of adaptation to the diet throughout the evolutionary history [23,85]. For Coleoptera, serine, cysteine and metalloproteases have been reported as predominating proteases types [23,85,86,87,88,89]. For this work, results showed mainly neutral and slightly alkaline proteolytic activity in *adult Trachyderma midgut*, which is typically associated with serine proteases.

Serine proteases have been reported in a wide range of coleopteran families, which shows their functional relevance and their implications as important digestion elements. Several ideas have arisen and have suggested that serine proteases are the basal digestion elements in primitive coleopterans. These enzymes could vary in concentration or catalytic types throughout the whole intestine [90].

Carbohydrates are essential for most insects to produce nutrient materials needed for growth, development and for the maintenance of adult survival and reproduction [91]. 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.

The present study also revealed that the highest activity of α- glucosidase was at pH 3.6 in anterior and posterior mid gut while it was at 4.6 in middle midgut. The highest activity of β glucosidase was at pH 3.6 in anterior and posterior mid-guts while at 4.6 in middle midgut .The highest activity of α- galactosidase was highest at pH 3.6 in anterior , middle, and posterior mid-guts. The highest activity of α – amylase is at pH 4.6 in anterior and middle mid-gut while at pH 3.6 in posterior mid-gut of the adult stage *Trachyderma philistina.*

Glycosidases are carbohydrases – enzymes that catalyse the hydrolysis of glycosidic bonds to liberate monosaccharides and oligosaccharides of lower molecular weight. These enzymes are very widely distributed in nature and found in all organisms [92].

Starch-degrading enzymes have been broadly classified into two groups – endo-acting enzymes or endohydralases and exo-acting enzymes or exohydralases [93].

Exoglycosidases. Exoglycosidases are the enzymes, which release specific monosaccharides from non-reducing termini of oligosaccharides and the sugar chains of glycoproteins and glycolipids and are relatively specific for the sugar to be released as well as for the configuration (α or β) of the bond. Examples are α, β-galactosidase or α, β -glucosidase, they are different enzymes that release (α or β) linked galactose or glucose end groups [94].

Endoglycosidases are able to hydrolyze poly or oligo -saccharides in the interior of the molecule and thus all products of the reaction are usually oligosaccharides or polysaccharides of reduced molecular weight.

Examples are chitinase, which recognizes linkages of the β- 1-4 linked polymer of N-acetyl-(d) glucosamine, and lysozyme which cleaves linkages between N-acetyl-(d) glucosamine and muramic acid in bacterial cell walls [95].

Most living organisms can exploit environmental polysaccharides. α-Glucosidase (EC 3.2.1.20) is an enzyme that catalyses the hydrolysis of 1,4-α-glucosidic linkages, releasing α-glucose. This enzyme strongly hydrolyses sucrose, maltose, maltodextrin, and PNP-α-d-glucopyranoside. It can be found in the alimentary canal, salivary secretions of insects [96] and hypopharyngeal glands of some insects, such as *Apis mellifera*[75]. So far, α-glucosidases have been isolated and characterised from many insects including Dysdercusperuvianus (Hemiptera: Pyrrhocoridase), *Pyrhocoridaezeamais* (Coleoptera: Curculionidae), *Apis mellifera* (Hymenoptera: Apidae), *Drosophila melanogaster* (Diptera: Drosophilidae), and *Glyphodespyloalis* (Lep.: Pyralidae) [10].

The alpha glucosidase in the mid gut of the adult stage *Trachyderma philistina* showed high pH activity in the acidic range (pH 3.6-6)with highest activity at PH 3.6. this range is agree with those reported for Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleaptera.: Curculionide) [97] and in other insects using different substrate example: Apis mellifera [98] , α-glucosidase of *Callosobruchus maculatus* reached the highest activity at pH 5.6. , [99]. Overall, the studies on coleopteran insects indicated an optimal pH for α-glucosidase in a slightly acid region. Highest activity for sucrose hydrolyzingα-glucosidase from the mid-gut of *Dermestes maculatus* adults was reported at pH 6.3 [100] α-glucosidase in *Eurygaster integriceps puton* (Hemiptera: Scutelleridae) had acidic range of 4 to 6 [101].

β-Glucosidase hydrolyses β 1–4 linkages between two glucoses or glucose-substituted molecules (such as

cellobiose) [75]. In addition to the important digestive role of enzymes, they can also act as elicitors (triggering agents of plant defense mechanisms) in plants when they are encountered with the feeding damage of the insect pests [102]. Beta-Glucosidase (EC 3.2.1.21; beta-D-glucoside glucohydrolase) hydrolyses terminal, non-reducing 1, 4-linked-beta-glucose residues releasing beta-D-glucose from oligo or polysaccharides [103]. Other names for this enzyme are gentiobiase and cellobiase. Beta-Glucosidase has been subdivided into three classes based on substrate specificity. Class 1 includes enzymes with glycosyl beta- glycosidase and aryl beta-glycosidase activity; these enzymes can hydrolyze cellobiose, lactose, beta-p-nitrophenyl glucoside, beta-p nitrophenyl galactoside, beta-p-nitrophenyl fructoside and other similar substrates. Class 2 includes those with only glycosyl beta-glucosidase activity; therefore, they can only hydrolyze substrates such as cellobiose and lactose. Class 3 includes enzymes with only aryl (or alkyl) beta-glucosidase activity; these enzymes would have significant activity towards beta-p-nitrophenyl glucoside and similar substrate [1].

The midgut of the adult stage *Trachydema philistina* showed high activity of β-Glucosidase in the pH range (3.6-6) but with optimum pH at 3.6. ThispH value is like those pH values found in other insects like: Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleaptera.: Curculionide), maximum activity was observed at pH 5 [97].The maximum activity of β-glucosidase in the midgut of *Leptinotarsa decemlineata* (Coleoptera: *Chrysomelidae) occurred at pH 4-5.5 [104]. The maximum activity in OsphranteriacoerulescensRedt*. (COL.: Cerambycidae) digestive system was observed at pH 4 [105].

α-d-Galactosidases (EC 3.2.1.22) are exo-acting glycoside hydrolases that cleave α-linked galactose residues from carbohydrates such as melibiose, raffinose, stachyose, and gluco- or galactomannans [106].The activity of α-galactosidase in the midgut of *Trachydema philistine* showed high activity in the acidic range PH (3.6-6) with optimum PH at 3.6.The highest activity of α-galactosidase was stated at pH 5.0 in *Diabroticavirgiferavirgifera* (Coleoptera: Chrysomelidae) [107].The highest activities of α-galactosidases at pH 4 in red palm weevil [97] .Alpha -galactosidases optimum activities occurred at pH 5 in *Ectomyeloisceratoniae* [108].

In the digestive system of *Xanthogalerucaluteola* (Coleoptera: Chrysomelidae), the optimum pH for α-galactosidase activities were reported at pH 4 [109].

Glucosidases and galactosidases play an important role in insect digestion. Our results showed that these enzymes are important in the initial and final phases of food digestion of *Trachyderma philistina.*

Alpha- and β-glucosidases and α- and β-galactosidases in insects are generally the most active in neutral to slightly acid pH conditions [10,96]. Optimal pH values for glucosidases of several coleopterans are 3.0–6.5 [110]. α-glucosidase of *Callosobruchus maculatus* was the most active at pH 5.6 [99]. In other coleopterans, like in many insects, the optimal pH for α-glucosidase activity is generally in the slightly acid region. Maximal activities of sucrose hydrolysing α-glucosidase from the midgut of *Dermestes maculatus* adults occurred at pH 6.3 [111]. The pH optimum of α-galactosidase in the midgut of *Odontotermesobesus* (Isoptera: Termitidae) is 5.2. Also, in the hindgut the pH optimum of this insect was 8.4 for α-galactosidase [112].. In non-coleopteran insects, [113] it was shown that the optimal pH for the α-glucosidase from both the ventriculus and the salivary glands of *Helicoverpa zea* is 5.5(Lep.: Noctuidae). Also, it was shown that the optimal pH values for α- and β-glucosidases activity in the midgut and salivary glands of *G. pylo*alis are 7.5, 5.5, 8–9 and 8–9, respectively [10].

Alpha-amylases (EC 3.2.1.1) are glyco- syl hydrolases that break down alpha-1, 4 glycosidic bonds inside the maltopolysaccharide linear chain, mainly in starch and glycogen, resulting in maltose, maltotriose, and residual branched maltodextrins as final products. These molecules are in turn hydrolyzed into glucose by alpha-glucosidases. [114].

α-Amylase (α-1,4-glucan-4-glucanohydrolase; EC3.2. 1.1) is a hydrolytic enzyme found in microorganisms, plants, and animals. This enzyme catalyzes the endohydrolysis of long α-D-(1,4)-glucan chains, such as starch and related carbohydrates, allowing organisms to use starch as an energy source [115,116].

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 and Hemiptera . 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 haemolymph [117,118].

Endoamylases present in human saliva, pig pancreas, *Bacillus subtilis* and *Aspergillus oryzae* are known to be Ca2+-containing proteins [119]. Insect α-amylases are also Ca2+-dependent enzymes [23]. AMF-3 activity was modulated in the presence of different Ca2+ concentrations. Concentrations of Ca2+ from 0.005 to 5 mM activated *M. funereus* AMF-3. In contrast, concentrations above 10 mM were inhibitory[120].

The activity of alpha amylase in the mid-gut of *Trachyderma philistina* was high at pH 3.6.The pH value of amylase in the present study is more or less similar to those pH values (3.6-6) found in other insects example : The optimum pH for α-amylase activity was found to be within pH range of 5 in *Callosobruchus maculatus* [121].The pH optimum for α-amylase was 5-6 from *Vigna angularis*[122], and *C. chinensis*[123]. Acidic pH (4.6 to 5.2) optimum was observed for *T. castaneum* and *S. oryzae* [74,124]. Optimal amylase activity was found

between pH 4.5 and 6.5 with maximum activity at pH 5.2 in *Morimusfunereus* (Coleoptera: Cerambycidae).

Coleopteran insect -amylases are well adapted to acidic physiological environment of larval midgut, with optimum pH between 4.5 and 5.5[123]

CONCLUSION

Insects need an adequate source of protein to grow and maintain themselves. Proteins composed of amino acids and organic compounds are fundamental for growth, reproduction, and as a structural element of the cell in the insect body. Proteases are very important enzymes in the alimentary canal of insects, and they cleave the peptide bonds in the proteinous insect foods to release the amino acids that are then absorbed by epithelial cells of the insect midgut. Proteolysis plays an important role in insect physiology and food digestion, facilitated by serine, cysteine, aspartic proteinases, or endopeptidases and metalloproteinases. It is very important to study insect proteases. Future studies are recommended to study more about other different families of protease like cysteine proteases in adult stage *Trachyderma* beetles other than serine proteases. Carbohydrates are the most important source of energy essential for both optimal larval growth and for the maintenance of adult longevity in the majority of insects. The nature of the enzymes secreted is related to the nature of the meal that an insect can assimilate ,whereas herbivorous insects secrete more carbohydrases, carnivorous insects secrete mainly proteases .

Conflict of Interest

I am interested to work more in same field searching for valuable insects in the field of medicine that are not known yet.

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