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Activity of midgut amylase in *Aeolesthes holosericea* Fabricius (Coleoptera: Cerambycidae)

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Abstract
Partial characterization of amylase from the larval and adult midgut of *Aeolesthes holosericea* was studied. High enzymatic activity was found at pH 5.2 and 35 °C in larva where as in adult it was at pH 5.4 and 40 °C. The linear period for enzymatic activity was 15 min in both larva and adult. The 50% inhibition at 55 °C was 1.5min and 4 min in larva and adult respectively. The Km values for larva and adult were 0.777% and 0.115% respectively. Findings of the present study would be utilized in to understand digestion process in insects and to formulate control strategies against wood boring insects.

Keywords: Midgut amylase, *Aeolesthes holosericea*

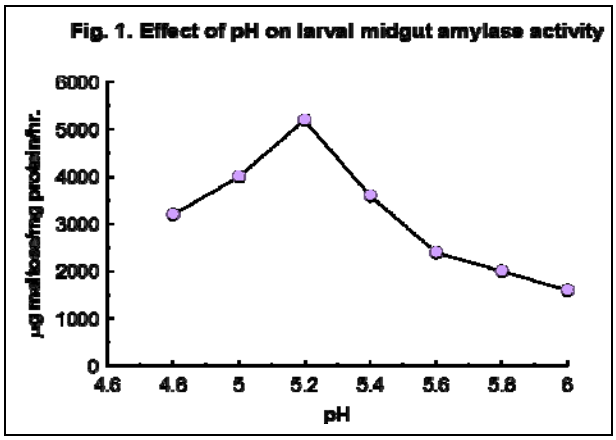
1. Introduction
Carbohydrates are the most important source of energy essential for both optimal larval growth and for the maintenance of adult longevity in majority of insects [1]. The major enzymes detected in digestive tract of insects are amylase, invertase, trehalase, protease and lipase [2, 3]. According to Terra *et al.* [4] only α-amylase has been found in insects to act preferentially on long α- 1, 4- glucan chains. The presence of amylase has been demonstrated in many insects including members of Orthoptera, Hymenoptera, Diptera, Lepidoptera and Coleoptera [5]. Applebaum *et al.*[6] and Lemos *et al.*[7] have worked on enzymological characteristics and molecular properties of α- amylase in Coleoptera. However, relatively little information [8-10] is available concerning the presence and properties of digestive enzymes in cerambycid beetles. The information available indicates no studies have been made to characterize amylase activity in the midgut of the *Aeolesthes holosericea* which is serious pest of timber. The aim of present study was to characterize amylase activity in *A. holosericea* midgut in order to gain a better understanding of the digestive physiology of this insect.

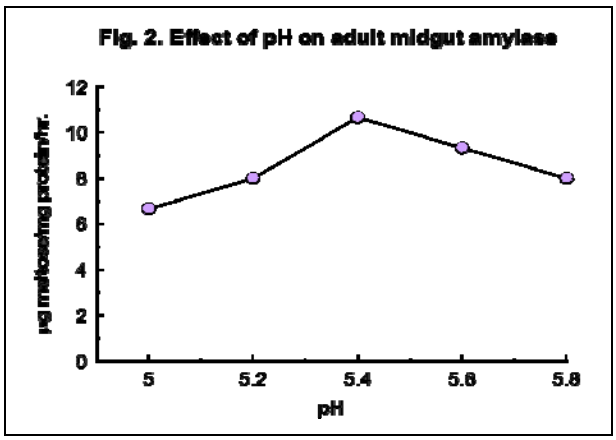
2. Material and Methods
The experiments were carried out in the laboratory from June 2016 to December 2015. The larvae and adults of *A. holosericea* were dissected in chilled insect Ringer solution [11]. Homogenate of the midgut tissue was prepared in chilled 0.9% NaCl, unless otherwise indicated, which were cold centrifuged at 3000 rpm for 20 min [12]. Aliquots of supernatant were used as enzyme source for the partial characterization of amylase. The enzyme activity was determined by using 3-5 DNSA (Dinitrosalicyclic acid) reagent [13] and measured at 540 nm spectrophotometrically [14]. The assay sample include 1 ml substrate (1% starch), 1ml 0.2M buffer with appropriate pH, 0.5 ml supernatant, 2.5 ml DNSA reagent and 2.5 ml distilled water. The standard curve was obtained by direct reaction with glucose using DNSA reagent under same assay condition. To study thermolability, supernatant was subjected to 60^0 C and the activity of residual enzyme was determined by Bernfeld [13] method. The soluble protein content of the enzyme extract was determined by Lowry *et al.* [15] using Bovine serum albumin as standard.

3. Results
The characteristics of midgut amylase were studied using various parameters like pH, temperature, time, thermolability and substrate concentration in both larva and adult of *A. holosericea*.

3.1 Effect of pH

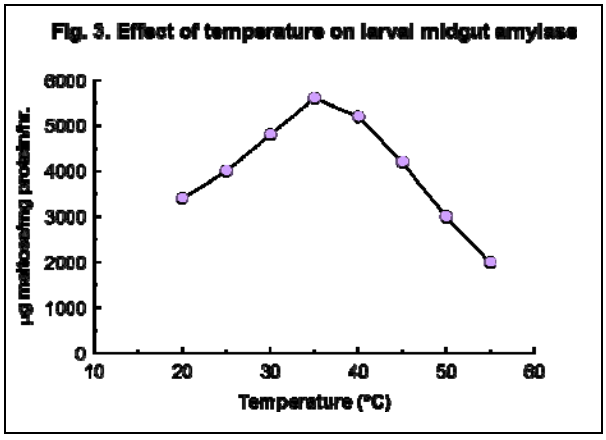
Measurement of the amylase activity in the different pH range showed the maximum activity at pH 5.2 (Fig 1) and pH 5.4 (Fig 2) in the midgut of larva and adult respectively.

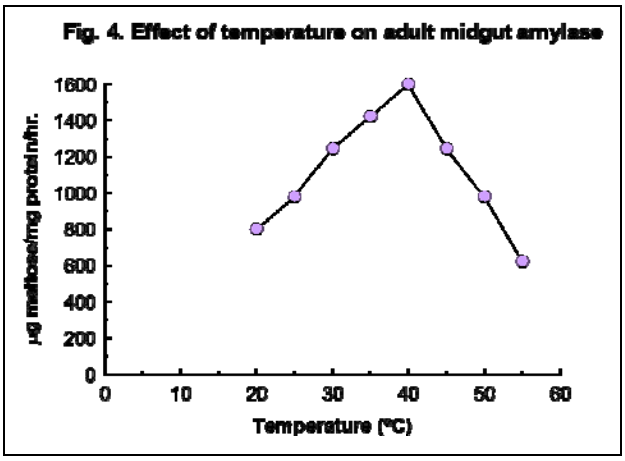




3.2 Effect of temperature

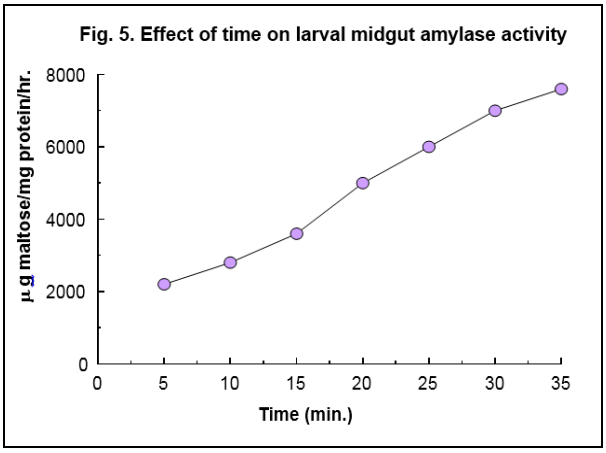
The amylase showed a steady increase in its activity by elevating of the incubation temperature from 20 °C – 35 °C in larva and from 20 °C – 40 °C in adult and then decreased till 55 °C. This observation showed that temperature optima for amylase activity were 35 °C (Fig 3) and 40 °C (Fig 4) in larval and adult midgut respectively.

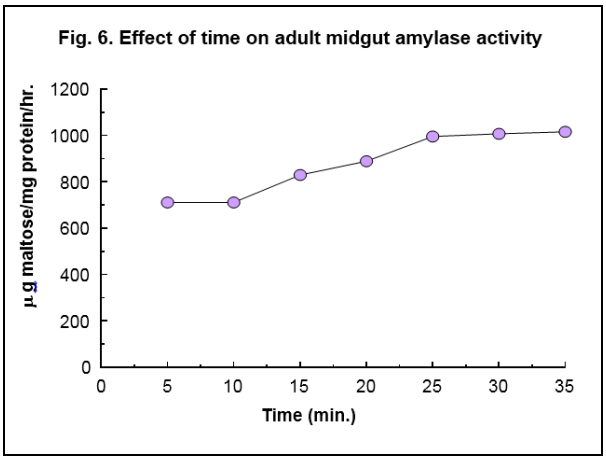




3.3 Effect of Time

The linear digestion period for the larval as well as adult midgut amylase was similar. It requires 15 min ((Fig 5 & 6)) for maximum enzyme activity.

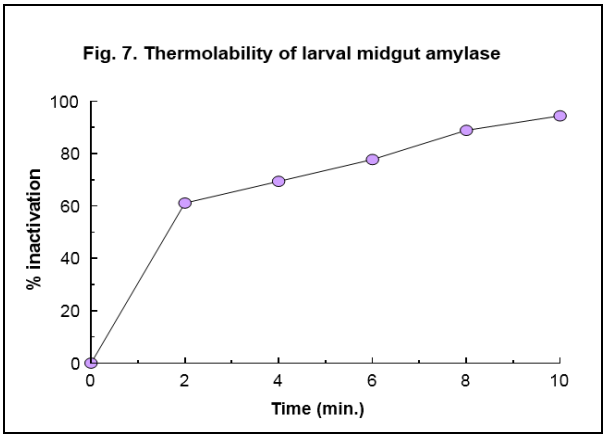


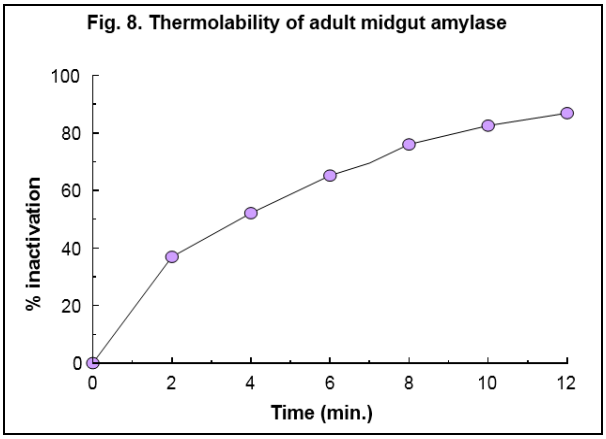


3.4 Thermolability

The theoretical duration of high temperature at 55 °C for 50% loss of activity was found to be 1.5 min (Fig 7) in larva and 4 min (Fig 8) in adult.

3.5 Effect of substrate concentration

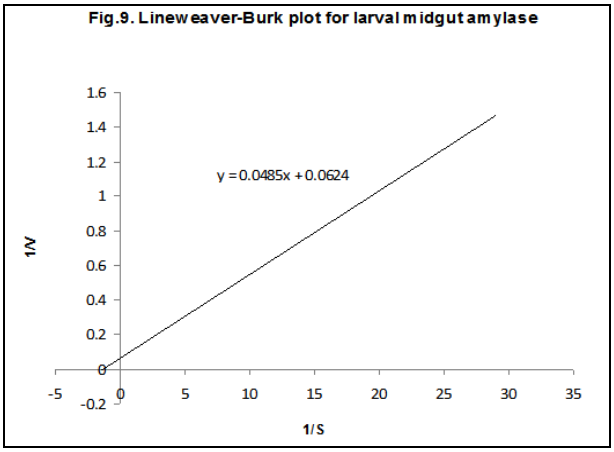


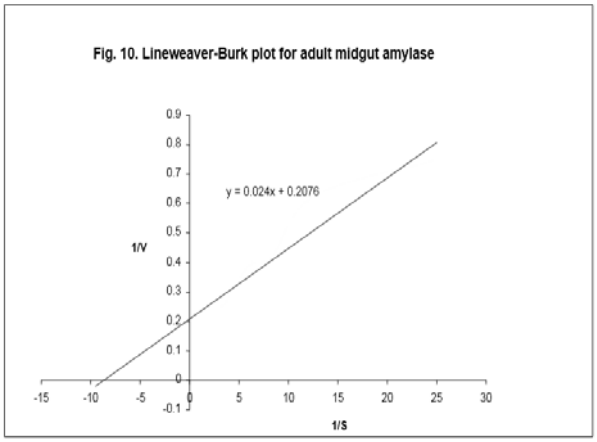


4. Discussion

The relationship between the substrate concentration (1/s) and rate of hydrolysis (1/v) was studied. The substrate concentration and rate of hydrolysis is shown in Fig 9 and Fig 10. Lineweaver- Burk plot were employed to calculate Km values by using regression equation $Y = ax + b$. The Km obtained was 0.77724% for larval amylase and 0.1156% for adult amylase.

The digestive enzymes of some wood boring beetle larvae were studied by Parkin [16]. Enzymes shows highest activity in their optimal pH and a small change in pH alter the catalytic metabolism of the biochemical reaction [17]. Optimal pH values for amylases in the larvae of several coleopterans were 4.0- 5.8[18]. Similarly in the present investigation, the midgut amylase had the optimal pH 5.2 in larva and 5.4 in adult. More or less similar results were obtained by Applebaum and Konjin [19] in *Callosobranchus chinensis* (pH 5.2-5.4) and Podoler and Applebaum [20] in *Tribolium castaneum* (pH 4.6-5.2). Ivanovi and Marinkovic [21] reported pH range 5.38 to 5.5 in cerambycid beetle *Morimus funereus.* The pH optima for midgut isolated amylase (AMF-3) in *M. funereus* was 5.2[22]. Khorram *et al.* [23] and Shabarari *et al.* [24] reported pH optima 6.4 in *Leptinotarsa decemlineata* and 4 in *Plagiodera versicolora* respectively. The amylase activity increase as temperature was raised from 20 °C to 35 °C in larva and from 20 °C to 40 °C in adult. There was significant drop in enzymatic activity when temperature was further raised to 55 °C. It indicates that the temperature optimum for amylase in larva is 35 °C and in adult it is 40 °C. More or less similar observations were made for α-amylase activity by Dojnov *et al.* [22] in *M. funereus* (45 °C), Mehrabadi and Bandani[25] in *Eurygaster maura* (30 °C – 40 °C), Khorram *et al.* [23] in *Leptinotarsa decemlineata* (37 °C) and Shabarari *et al.* [24] in *Plagiodera versicolora* (35 °C). The midgut amylase in larva and adult of *A. holocericea* showed linear digestion period of 15 min. Sarwade and Bhawane [26] reported linear degeneration period of 10 min. in *Platynotus belli*. The thermal inactivation of amylase indicates that 50% inactivation of amylase require very less period i.e. 15 min for larval amylase and 4 min for adult amylase when exposed to 60° temperature. Such observations suggest that the amylase in *A. holosericea* is not heat stable. This aspect is very little investigated in beetles. The half-life period of midgut amylase in *P. belli* is 6 min at 60 °C [26]. According to Applebaum and Konijn [19] generally insect amylase are capable of hydrolyzing starch, amylopectin and solubilized amylase at similar rate with similar Km values. The Km value obtained for midgut amylase in larva and adult of *A. holosericea* is 0.777% and 0.115% of starch respectively. Earlier workers reported different Km values in other coleopteran insects like Podoler and Applebaum [20] in *Callosobruchus chinensis* (Km 2.3 mg/ml), Buonocore *et al.* [27] in *Tenebrio molitor* (Km 1.8mg/ml), Dojnov *et al.* [22] in *M. funereus* (Km 0.43 mg/ml) and Sarwade and Bhawane [26] in *P. belli* (Km 4.4%). Therefore, the Km value 0.115% of adult is indication of high enzyme efficiency than the larval amylase (Km 0.777%) of *A. holosericea.*





5. Conclusion

Results of the present study may lead to understand digestive processes within the stem borers including *A. holosericea* and will help to formulate management strategies for the pest control.

6. Acknowledgements
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