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RESEARCH ARTICLE

STUDY OF CARBOHYDRASES IN GRUB OF *ONTHOPHAGUS CATTA* (COLEOPTERA: SCARABAEIDAE: SCARABAEINAE)

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ABSTRACT

Onthophagus catta is dung beetle species feeding on cattle dung. The characteristics of some Carbohydrases from mid gut (MG) were studied in the third instar grub of ***Onthophagus catta***. Most of the Carbohydrases showed their maximum activities at pH 7.2.While some Carbohydrases were showing pH optima at 5.6 (Cellulase), 6.0 (Trehalase) and 6.8(Raffinase). Temperature optima for all Carbohydrases which were studied in mid gut occurred at 45^0C. The Km value of Amylase was 0.222% of starch. The Km values of Invertase enzyme was 5.56 X 10^{-3} M and to that of Trehalase was $7.048X10^{-3}$M.The half life of enzyme activities were 22.30 minutes (for Amylase), 25.0 minutes(for Invertase) and 27.15 minutes(for Trehalase)at 60^0 C. The digestion period of 60 minutes were fitted very well within the linear part of enzymatic action for Invertase and Trehalase enzymes, while Amylase showed digestion period of 30 minutes was fitted very well within the linear part of enzymatic action.

INTRODUCTION

In insects epithelial cells of mid gut generally secretes different types of digestive enzymes (Reeck *et al*., 1999; Terra and Ferreira, 1994; Terra *et al*., 1996). These enzymes enable them to digest a different variety of consumed food (Applebaum, 1985) including polymeric molecules. In mid gut some Carbohydrases and Proteases can break down the complex molecules into simple absorbable elements (Terra, 1990). *Onthophagus catta* is a common dung beetle abundantly found in South-Western Maharashtra. These beetles are coprophagous insects which feed on cattle dung. Dung beetles are an important component of dung fauna. According to their habitat, dung beetles are divided into three groups as Rollers, Tunnellers and Dwellers. *Onthophagus catta* are the Tunnellers and they dig tunnels under ground at the depth ranging between 6 to 22 cm and construction brood balls (Gaikwad and Bhawane, 2015a). The dung beetles play a vital role in natural ecosystems. The adult beetles and grubs feed on the liquid and colloidal content of dung. The alimentary canal is adapted for coprophagy. The activity of most digestive enzymes is reflected with degree of adaptation to food components. Therefore, we presently have worked on carbohydrates digesting enzymes in the mid gut of grub of *Onthophagus catta.*

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MATERIALS AND METHODS

Insect collection

Collection of dung beetles: The adult dung beetles are most abundant in South-western Maharashtra. As these dung beetles are powerfully attracted towards fresh cattle dung, therefore, they are easily collected from the dung pads. One to three days old dung pads were selected for the collection purpose.

Laboratory Maintenance and Rearing: The adult dung beetles were maintained under laboratory conditions in convenient sized earthen pots. Rearing method proposed by Blume and Aga (1975) and Hunter (1991, 1996) was followed. The beetles in earthen pots maintained at laboratory temperature of 22 to 26 °C. After a week time brood ball formation in rearing pots was checked by overall pouring the content from rearing pots. The brood balls after recovering from the rearing pots were maintained in earthen pots by covering with moist sand- soil mixture.

Enzyme Preparation

The III[rd] instar grubs were obtained from the laboratory stocked brood balls for the preparation of mid gut (MG) enzyme extracts. Homogenates of the pooled tissues were prepared in 0.9% chilled NaCl, which were cold, centrifuged for 15 minutes at 10000 rpm. Aliquots of supernatants were

used as enzyme source. Homogenates were stored in freezer until used.

Assay

Amylase, Invertase, Trehalase, Cellulase, Inulinase, and Salicinase: The 3-5 dinitrosalicylic acid (DNSA) reagent (Bernfeld, 1955) was used to determine the activities of these enzymes. The aldehyde group formed due to enzymatic action on substrate reduces the DNSA reagent which was measured spectrophotometrically at 540 nm (Ishaaya and Swirksi, 1970). The assay mixture for enzymes consists of 1 ml appropriate substrate, 1 ml 0.1 M buffer of appropriate pH and 0.5 ml supernatant. The test-tubes were incubated at appropriate temperature and period of time. The reactions were terminated by adding 2 ml of DNSA followed 2 ml of distilled water. The test –tubes were heated in boiling water bath exactly for 5 minutes. Then tubes were cooled immediately. The activities for Invertase, Trehalase, Cellulase, Inulinase and Salicinase are expressed as µg glucose / mg protein / hr. and for Amylase as µg maltose / mg protein / hr.

Maltase, Cellobioase, Melibiase, Lactase, and Raffinase

The activity of these enzymes were determined by using GOD-POD reagent (Span diagnostics, Pvt. Ltd. Surat, INDIA) The reaction mixture for these enzymes consists of 1 ml 0.1 M buffer of appropriate pH and 0.5 ml homogenate. The maltose, cellobiose, melibiose, lactose and raffinose were used as substrate for above respective enzymes. The test-tubes were incubated at appropriate temperature and period of time. The reactions were terminated by keeping tubes in boiling water bath for 5 minutes. Then tubes were brought to room temperature and 1.5 ml of GOD-POD reagent was added. The colours developed in reaction mixtures were read at 510 nm.

Protein estimation: The soluble protein content of the enzyme extract was determined by Lowry (1951) method using bovine serum albumin as standard. Assay mixture consisted of 0.5 ml of homogenate, made to 1 ml with double distilled water, to this added 5 ml of Lowry's 'C'solution. Then after 10 to 15 minutes, 0.5 ml of Folin-Ciocalteus (1927) regent was added. The optical density was read at 640 nm after 20 minutes.

RESULTS

The characteristic of various carbohydrate digesting enzymes (such as Amylase, Invertase, Trehalase, Cellulase, Cellobioase, Salicinase, Inulinase, Maltase, Melibiase, Raffinase and Lactase) in mid gut of grub of the *Onthophagus catta* are concluded in the Table 1.

Effect of pH. : Most of the Carbohydrases showed their maximum activities at pH 7.2.While some Carbohydrase were showing pH optima at 5.6 (Cellulase), 6.0 (Trehalase) and 6.8 (Raffinase) (Table 1 and Figures 1, 2, 3 and 4).

Effect of Temperature: All the Carbohydrases which were studied showed maximum activities at temperature 45^0C mid gut section (Table 1 and Figures 5, 6 and 7).

Effect of substrate concentration: The relationship between substrate concentration and rates of hydrolysis for MG Amylase, Invertase and Trehalase were studied. The Km values of enzymes were calculated from Line weaver –Burk's plots (Table 1 and Figures 10, 11 and12).

Effect of Time: The table 1 shows the digestion period of 30 minutes for amylase and 60 minutes for Invertase and Trehalase were fitted very well within the linear part of enzymatic action.

Table 1. Characteristic of Carbohydrases activity in Grub mid gut of *Onthophagus catta*

S.No.	Enzymes	p H Optima	Tempt. Optima0^C	50% Inactivation	Linear Time in minutes	Specific Activity µg-----/mg protein/Hr.	Km
1	Amylase	7.2	45	22.30 minutes	30	3000.0 µg Maltose	0.222% of starch
2	Invertase	7.2	45	25.0 minutes	60	338.25µg Glucose	5.56 X 10^{-3} M
3	Trehalase	6.0	45	27.15minutes	60	300.00 µg Glucose	7.048X10^{-3}M
4	Cellulase	5.6	45	-------	------	144.37 µg Glucose	--------
5	Cellobioase	7.2	45	--------	--------	156.18 µg Glucose	--------
6	Salicinase	7.2	45	--------	--------	222.75 µg Glucose	--------
7	Inulinase	7.2	45	--------	--------	247.50 µg Glucose	--------
8	Maltase	7.2	45	--------	--------	133.87 µg Glucose	--------
9	Melibiase	7.2	45	--------	--------	105.18 µg Glucose	--------
10	Raffinase	6.8	45	--------	--------	63.75 µg Glucose	--------
11	Lactase	7.2	45	--------	--------	47.81 µg Glucose	--------

Thermolability

The grubs were dissected in 0.9% saline and their mid guts were taken out for the enzyme extract preparation. A portion of enzyme extract was immediately stored in refrigerator for control purpose. The remaining portion of enzyme extract was then subjected to high temperature treatment by keeping the test-tubes containing enzyme in water bath maintained at 60^0C for different period time. The various heat treated enzyme extracts were stored in the refrigerator, until they were used for experiment. The activities of residual enzymes left after heat treatments were determined by the procedures as described earlier for respective enzymes.

Thermolability: The effect of higher temperature on the stability of Amylase, Invertase and Trehalase are shown in Table 1and in Figures 8 and 9.The half life activities of these enzymes were22.30 minutes (of Amylase), 25.0 minutes (of Invertase) and 27.15minutes (of Trehalase).

DISCUSSION

The optimal pH of the most of the Carbohydrases, except Cellulase (at5.6), Trehalase (at 6.0) and Raffinase (at 6.8) in mid gut of present dung beetle grub falls within the pH range of haemolymph and alimentary canal.

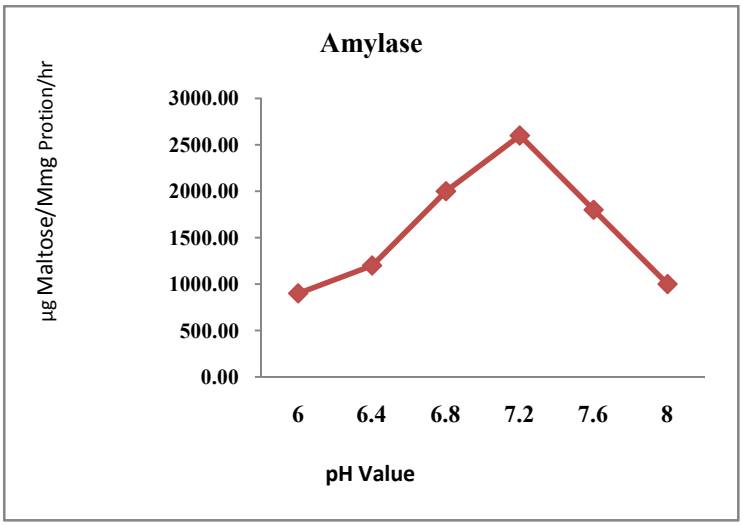

Figure 1. Effect of pH

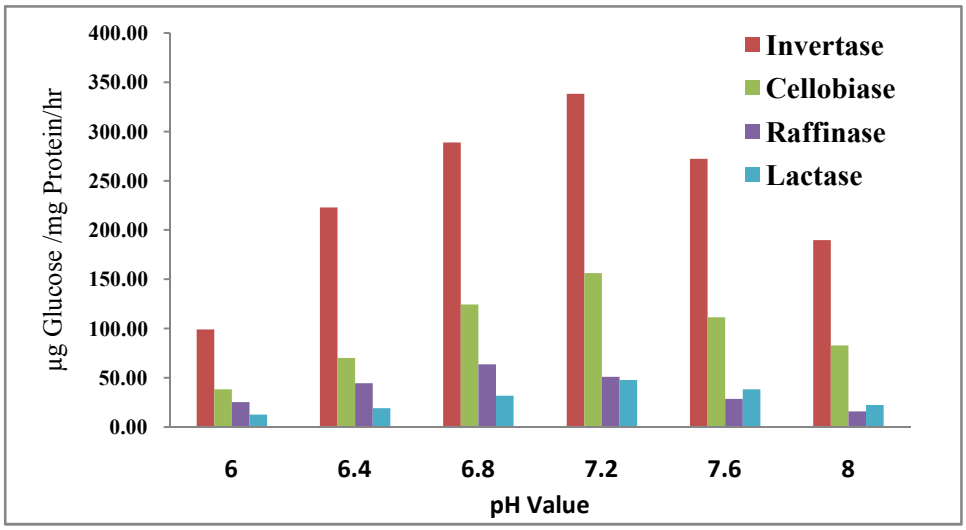

Figure 2. Effect of pH

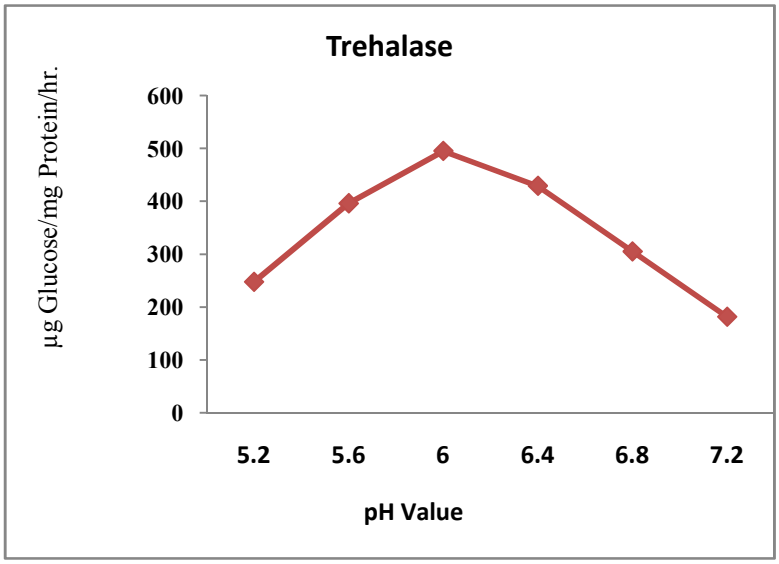

Figure 3. Effect of pH

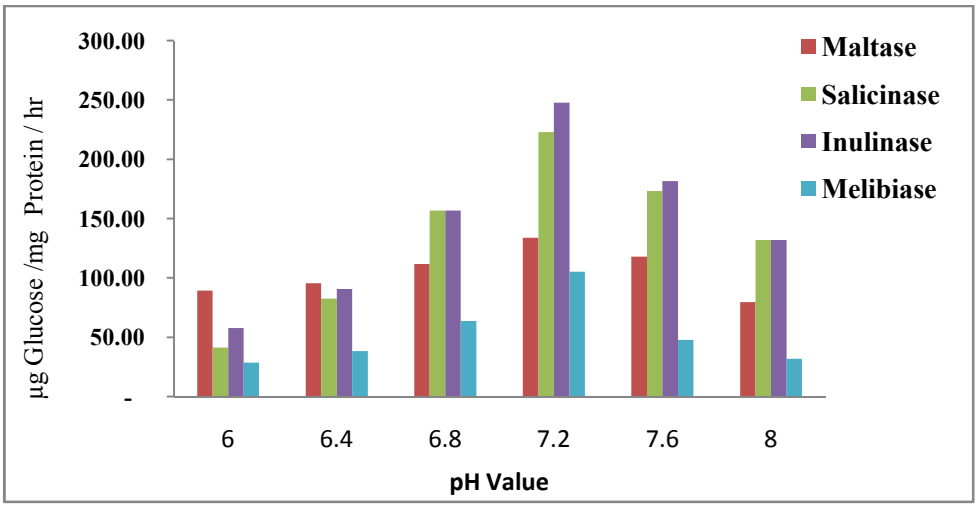

Figure 4. Effect of pH

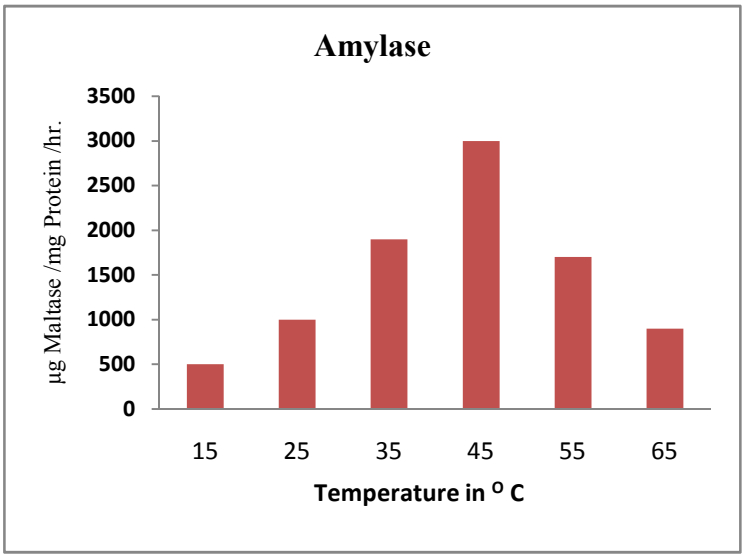

Figure 5. Effect of temperature

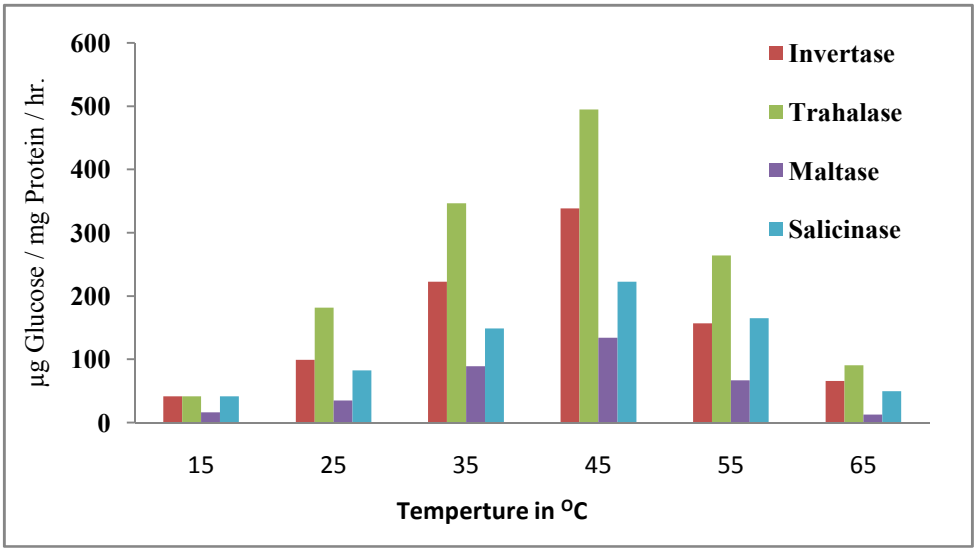

Figure 6. Effect of temperature

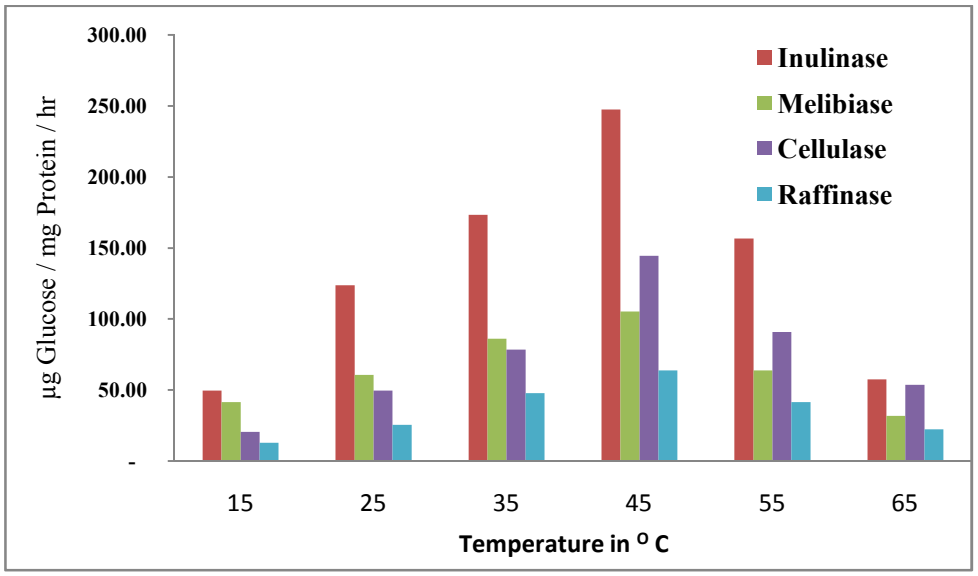

Figure 7. Effect of temperature

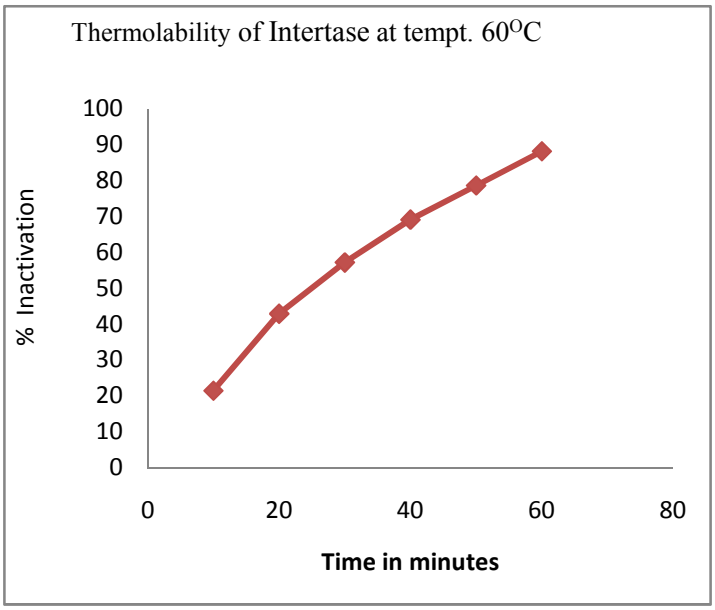

Figure 8. Thermolability

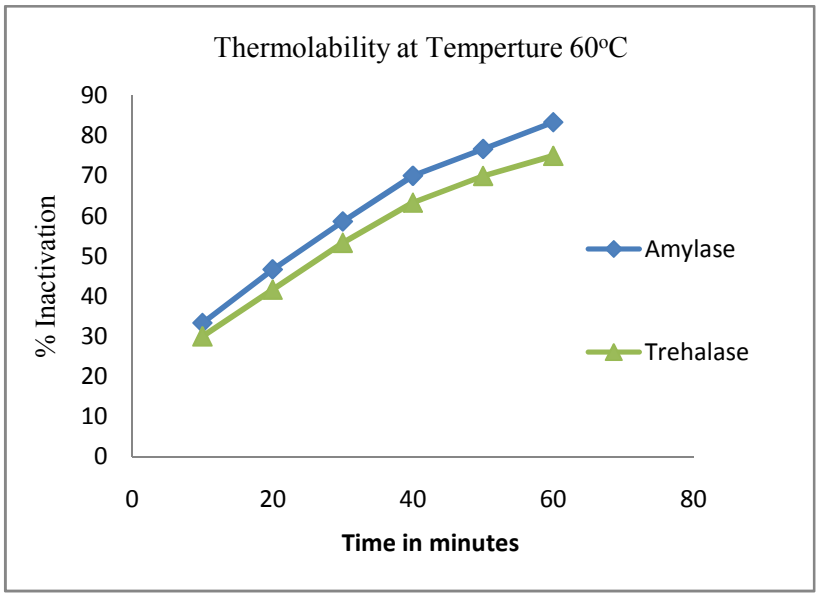

Figure 9. Thermolability

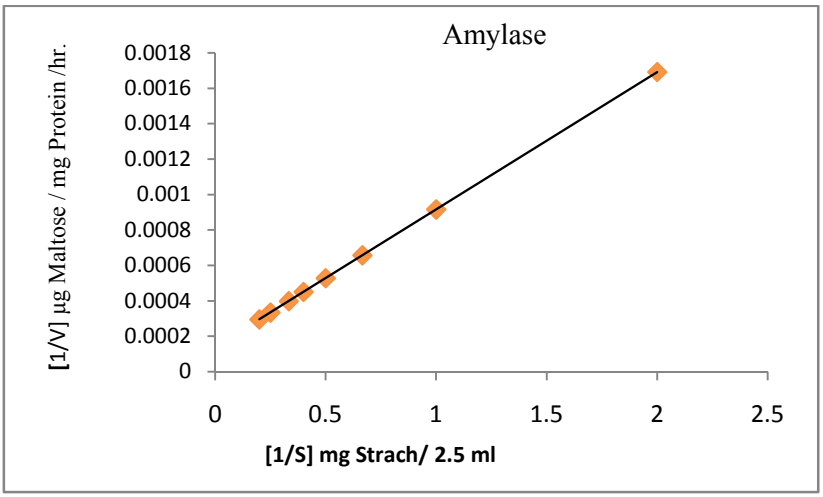

Figure 10. Y=0.0001406+0.0007754X

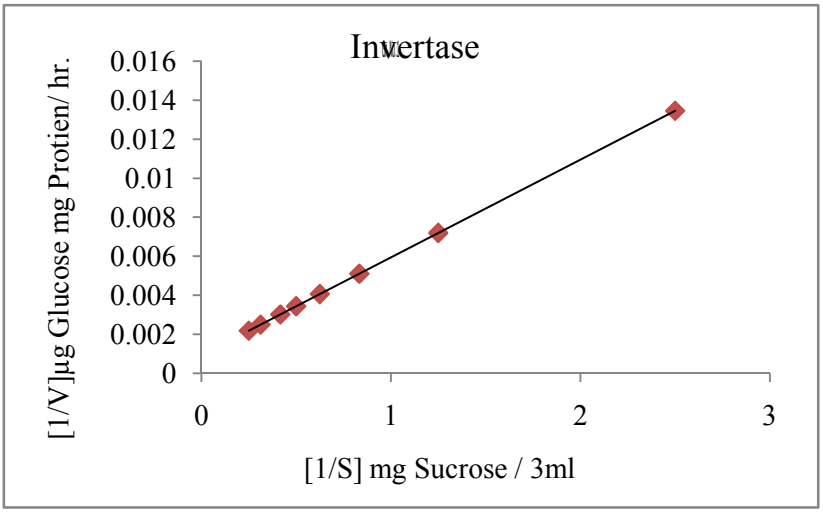

Figure 11. Y=0.000922+0.005016X

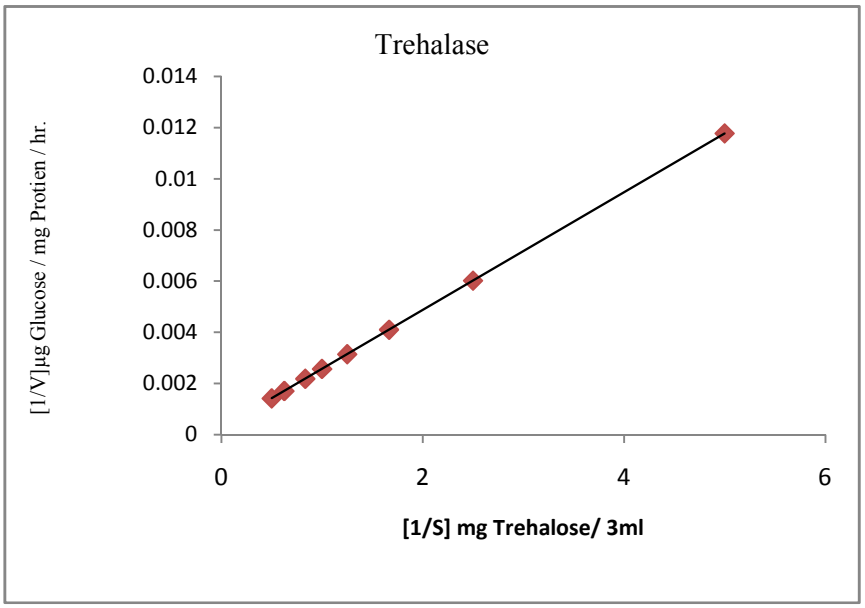

Figure 12. Y =0.00027+0.002301X

Except above three enzymes other Carbohydrases (Amylase, Invertase, Maltase, Inulinase, Salicinase, Melibiase, Lactase and Cellobioase) showed pH optima at 7.2. The range of maximum activity of these enzymes lies in between pH 6.8 to 7.4. This range of maximum activity of these enzymes is very close to the pH of the gut content indicating that pH conditions in alimentary canal are suitable for these Carbohydrases activity. Similar range of pH for these enzymes was observed for larvae and adults of *Holotrichia, Leucopholois, Onthophagus, Chironitis, Onitis, and Liatongus* (Bhanot, 1992; Patil, 1996; Gaikwad *et al*., 1997; Gaikwad, 1998; Gaikwad and Bhawane, 2015b). However in other insects like pulse beetle, *Tribollium* and *Tenebrio*, acidic pH range for amylase (4.6 to 5.8) was reported (Podoler and Applebaum, 1971; Applebaum and Conigan, 1965; Buonocore *et al*., 1976), for Invertase (5.0 to 6.5) was reported in the insects like cockroach, cabbage butterfly, khapra beetle and locust (Wigglesworth, 1972; Nishide and Kusano, 1976; Krishna, 1958; Evans and Payne 1964).

In present studies mid gut sections of grub showed pH optima at 6.0 for Trehalase. Such acidic pH optima for this enzyme also observed in other Scarabaeids (Bhanot, 1992; Patil, 1996; Gaikwad, 1998; Gaikwad and Bhawane, 2015b; Kumbhar, 1996). Activity of Cellulase was maximum at pH 5.6 in mid gut. This enzyme is mainly active in acidic pH which was reported in other insects (Patil, 1996; Gaikwad, 1998; Gaikwad and Bhawane, 2015b; Lasker, 1959; Mc Bee, 1959; Mc Bee, 1959; Wharton *et al*., 1965; Potts and Hewitt, 1973). The Cellulase activity might have been resulted due to gut micro-organisms (Ricau, 1958; Soo Hoo and Dudzinski, 1967). Temperature optima for all these Carbohydrases in mid guts sections of grub showed at 45^0C.Similar higher temperature optima also recorded in some insects (Terra *et al*., 1996; Gaikwad, 1998; Gaikwad and Bhawane, 2015b; Kusano and Tanabe, 1986; Teo and Heng, 1987). The Km values of Amylase, Invertase and Trehalase were calculated from Line weaver –Burk's plots. In present dung beetle grub the Km values of Amylase was 0.222% of starch. Similar Km values were recorded in other dung beetle grub (Gaikwad *et al*., 1997; Gaikwad, 1998; Gaikwad and Bhawane, 2015b). The Km values of Invertase were 5.56 X 10^{-3} M. Only in few insects the Km values for gut Invertase was determined (Bhanot, 1992; Patil, 1996; Gaikwad *et al*., 1997; Gaikwad, 1998; Gaikwad and Bhawane, 2015b). The digestion period of 30 minutes for Amylase and of 60 minutes for Invertase and Trehalase were fitted very well within linear part of enzymatic action. While in grub of Liatongus *rhadamistus* the digestion period of 60 minutes were fitted very well within the linear part of enzymatic action for both amylase and Invertase in both gut sections (Gaikwad and Bhawane, 2015b). Similarly Gaikwad and Bhawane (2015c) reported in *Chironitis arrowi* digestion periods of 60 minutes (for amylase in both sexes and guts) and 80 minutes (for Invertase in both sexes and guts) were fitted very well within the linear part of enzymatic action.

The effect of higher temperature on the stability of Amylase, Invertase and Trehalase were studied in this grub. The half life activities of these enzymes were 22.30 minutes (for Amylase); 25.00 minutes (for Invertase) and 27.15 minutes (for Trehalase) at 60^0 C. Gaikwad and Bhawane (2015c) reported in *Chironitis arrowi* the half life of Amylase were occurred at 60^OC within 23 minutes (MG) and 40 minutes (HG) in male and 29 minutes (MG) and 41.30 minutes (HG) in female. The 50% inhibition of Invertase at 60^OC were occurred within 16 minutes (MG) and 20 minutes (HG) in male and 10 minutes (MG) and 17.75 minutes (HG) in female beetle. While in grub of *Liatongus rhadamistus* (18) the half life of amylase activity were32 minutes (MG) and 23 minutes (HG) at 60^0 C. At some temperature the half lives of Invertase were 35.2 minutes (MG) and 28 minutes (HG). The result indicates mid gut enzymes are more heat stable than hind gut enzymes. Raffinase activity was detected in mid gut sections of this dung beetle grub.

Conclusion

The data in Table 1 shows that the mid gut secretes various carbohydrate digestive enzymes. These results agree with the general view that the mid gut is chief site of digestive enzyme secretion (Dadd, 1970; Law *et al*., 1977; Engelmann and Geraerts, 1980). Out of 11 Carbohydrates studied in this beetle Amylase, Invertase and Trehalase were most active and efficient enzymes. These enzymes showed their maximum activities at temperature 45^OC and at slightly acidic and slightly alkaline pH. The half life activities of Amylase, Invertase and Trehalase were showed some sort of similar periods at 60^OC.

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