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Carbohydrate digestiive enzymes in the mid gut of dung beetle *liatongus Rhadamistus* (Coleoptera: Scarabaedae: Scarabaeinae)

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Abstract
Liatongus rhadamistus is common dung beetle found in South-Western Maharashtra, India. Characteristics of some Carbohydrases were studied from mid gut of male and female of this dung beetle. Most of the Carbohydrases showed their maximum activities at pH 7.2.While some Carbohydrase were showing pH optima at 5.6 (Cellulase) and 6.0 (Trehalase) in both sexes. Temperature optima for all Carbohydrases which were studied in both sexes occurred at 45^0C. The Km value of Amylase in male was 1.143% and in female was 1.60%.The Km values of Invertase enzyme were 5.56 x 10^{-3} M in male and 1.297 $^{-2}$ M in female beetles. The 50% denaturation of Amylase activity was occurred within 46 minutes in male and 45 minutes in female at 60^0 C. At same temperature the half-life of Invertase was 21 minutes in male and 24 minutes in female. The digestion period of 60 minutes were fitted very well within the linear part of enzymatic action for both enzyme and sex. All Carbohydrases which were studied in this dung beetle shows that Amylase contribute significantly in digestion of carbohydrate in both sexes.

Keywords: *Liatongus rhadamistus* dung beetle, Characteristic of Carbohydrases, Km values

1. Introduction
Liatongus rhadamistus is a common true dung beetle found in South-Western Maharashtra. Dung beetles are coprophagous insects of the Coleopteran families such as Scarabaeidae and Geotrupidae (Halffter and Matthews 1966) [12]. Dung beetles are an important component of dung fauna. These beetles are Tunnellers and dig small tunnels in the ground just below the dung pad. These beetles play a vital role in natural ecosystems. We are presently engaging the study of digestive physiology of dung beetles. 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 of alimentary canal of adults of *Liatongus rhadamistus.*

2. Materials and Methods
2.1 Insect Collection
The adults of *Liatongus rhadamistus* beetles were collected from the dung pads (2 to 3 days old) from the grazing fields of Phaltan region, Maharashtra, India. These beetles were maintained in the laboratory under constant condition in earthen pots.

2.2 Enzyme Preparation
The adult beetles were obtained from the laboratory stock for the preparation of mid gut 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.

2.3 Assay
Amylase, Invertase, Trehalase, Cellulase, Inulinase, and Salicinase: The activities of these enzymes were determined by using 3-5 dinitrosalicylic acid (DNSA) reagent (Bernfeld, 1955) [2]. The aldehyde group formed due to enzymatic action on substrate reduces the DNSA reagent which was measured spectrophotometrically at 540 nm (Ishaaya and Swirski, 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.

2.4 Maltase, Cellobiase, 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.

2.5 Thermolability
The beetles 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 both 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.

2.6 Protein estimation

The soluble protein content of the enzyme extract was determined by Lowry *et al.* (1951) [18] 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) [6] regent was added. The optical density was read at 640 nm after 20 minutes.

3. Results

The various characteristics of different carbohydrate digesting enzymes such as Amylase, Invertase, Trehalase, Cellulase, Cellobioase, Salicinase, Inulinase, Maltase, Melibiase and Raffinase in mid gut of the adult dung beetles of the *Liatongus rhodamisyus* are concluded in the Table no.1

1. **Effect of pH:** Most of the Carbohydrases showed their maximum activities at pH 7.2. (Table no.1 and Gr.1, 2, 3 and 4).While some Carbohydrase were showing pH optima at 5.6 (Cellulase) and 6.0 (Trehalase).
2. **Effect of Temperature:** All the Carbohydrases which were studied showed maximum activities at temperature 45^0C irrespective of sexes (Table no.1 and Gr.5&6).
3. **Effect of substrate concentration:** The relationship between substrate concentration and rates of hydrolysis for mid gut of male and female amylase and Invertase are showed in Gr. 7 and 8.
4. **Effect of Time:** The Table no.1 shows the digestion period of 60 minutes for amylase and Invertase of both the sexes were fitted very well within the linear part of enzymatic action.
5. **Thermolability:** The effect of higher temperature on the stability of amylase and Invertase are shown in Table no.1and Gr.9 and 10.The half-life of amylase activity were 46 minutes (in male) and 45 minutes (in female) at 60^0 C. At some temperature the half-life of Invertase was 21.5 minutes (in male) and 24 minutes (in female).

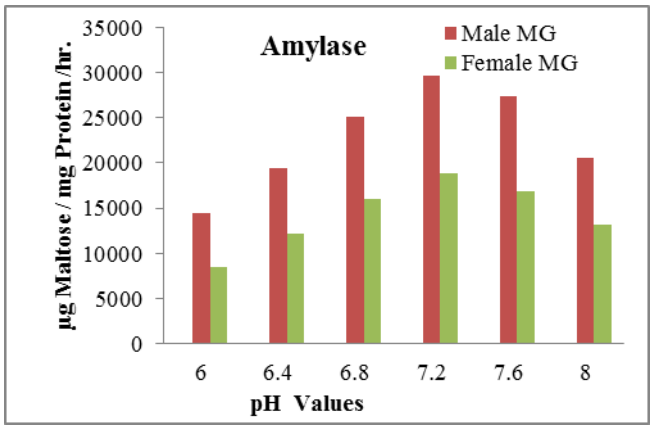


Fig 1: Effect of pH

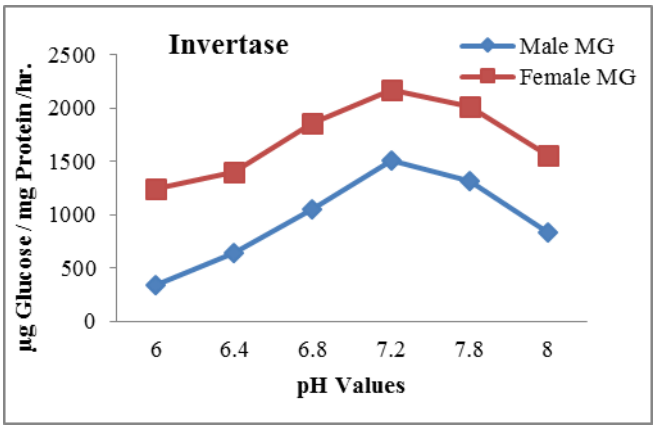
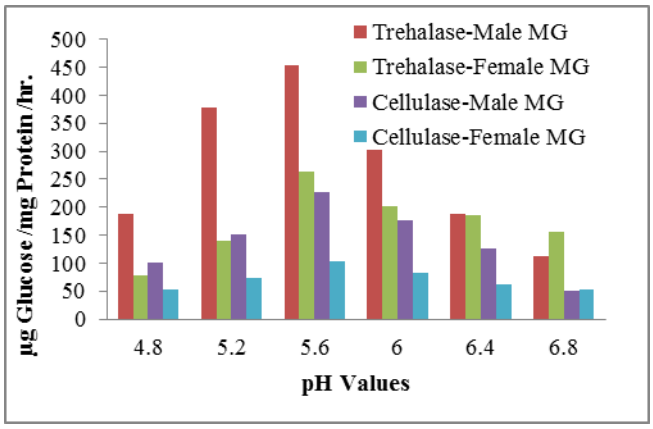


Fig 2: Effect of pH



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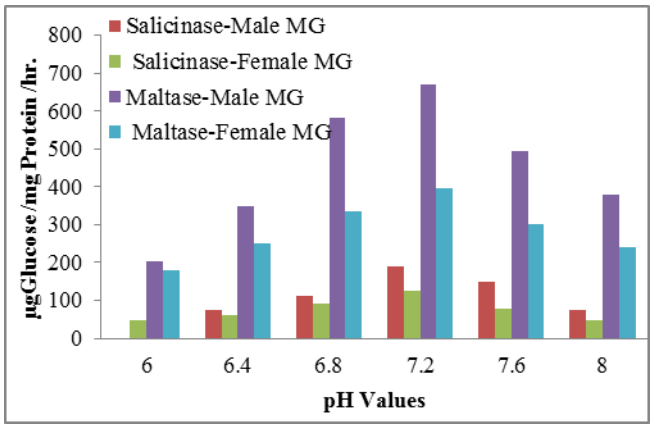


Fig 4: Effect of pH

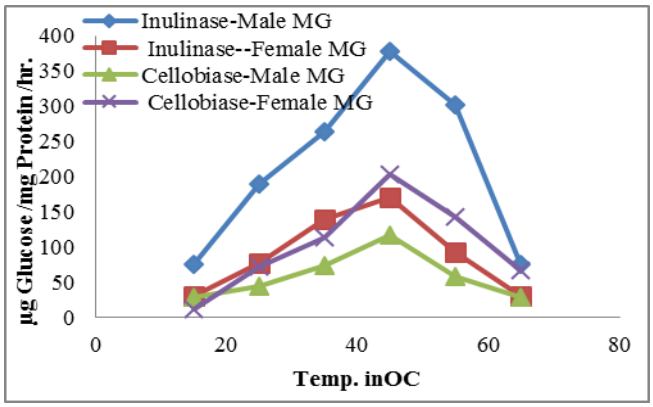


Fig 5: Effect of Temperatures

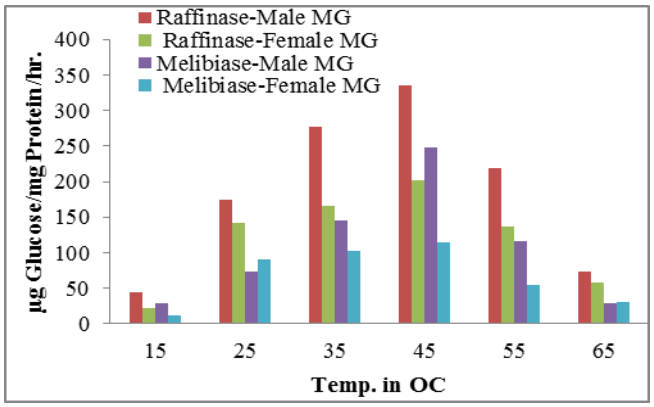


Fig 6: Effect of Temperatures

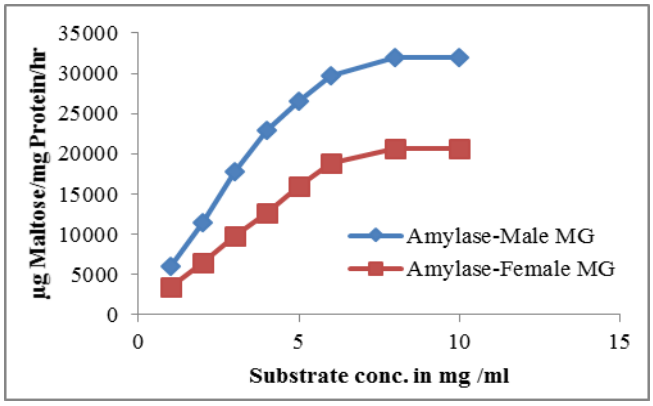


Fig 7: Effect of Substrate conc.

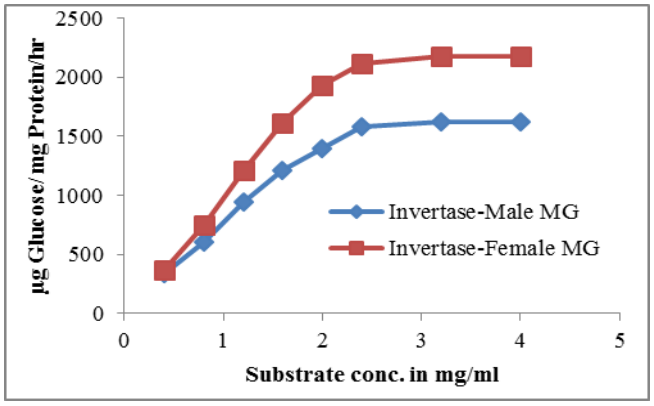


Fig 8: Effect of Substrate conc.

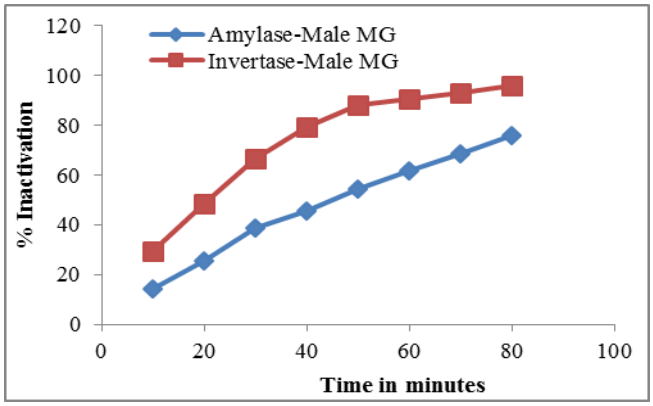


Fig 9: Thermolability

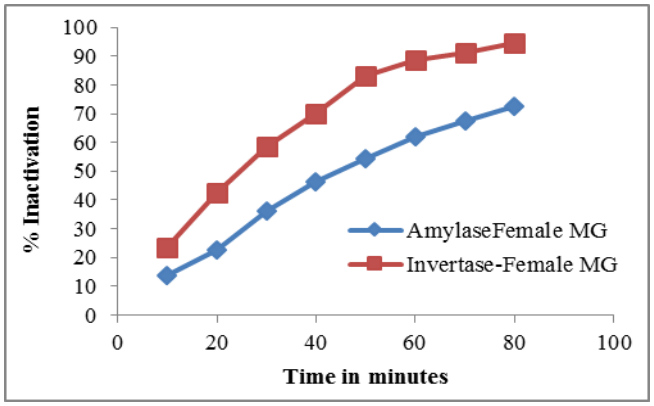
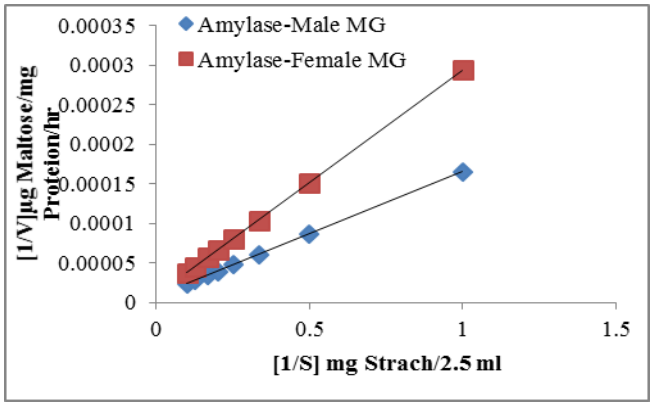
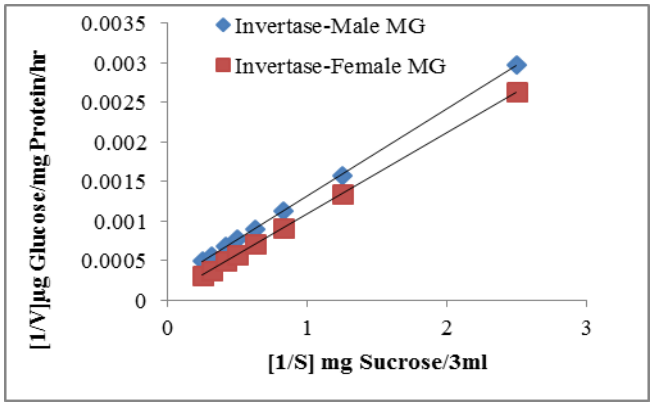


Fig 10: Thermolability



Male Y=0.0000078 +0.0001575 X
Female Y=0.0000093 + 0.0002842 X

Fig 11: Line weaver Burk's plots



Male Y=0.0002278 + 0.001084 X
Female Y = 0.0000636 + 0.001028 X

Fig 12: Line weaver Burk's plot

Table 1: Characteristic of Carbohydrases activity in mid gut of Adult dung beetle *Liatongus rhadamistus*

S. No	Enzymes	Sex	p H Optima	Tempt. Optima 0^C	50% Inactivation	Linear Time in minutes	Specific Activity µg-----/mg protein/Hr.	Km
1	Amylase	Male	7.2	45	46 minutes	60	29714.28 µg Maltose	1.143%
		Female	7.2	45	45 minutes	60	18823.52µg Maltose	1.60%
2	Invertase	Male	7.2	45	21.5 minutes	60	1583.99 µg Glucose	5.56×10^{-3} M
			7.2	45	24 minutes	60	2174.12 µg Glucose	1.297×10^{-2} M
3	Trehalase	Male	6.0	45	------	--------	452.57 µg Glucose	--------
		Female	6.0	45	--------	--------	278.52 µg Glucose	-------
4	Cellulase	Male	5.6	45	-------	------	226.28 µg Glucose	--------
		Female	5.6	45	---------	---------	103.52 µg Glucose	----------
5	Cellobioase	Male	7.2	45	--------	--------	116.57 µg Glucose	--------
		Female	7.2	45	--------	--------	174.00 µg Glucose	--------
6	Salicinase	Male	7.2	45	--------	--------	188.57 µg Glucose	--------
		Female	7.2	45	--------	--------	124.23 µg Glucose	--------
7	Inulinase	Male	7.2	45	--------	--------	377.14 µg Glucose	--------
		Female	7.2	45	--------	--------	170.82 µg Glucose	--------
8	Maltase	Male	7.2	45	--------	--------	670.25 µg Glucose	--------
		Female	7.2	45	--------	--------	396.00 µg Glucose	--------
9	Melibiase	Male	7.2	45	--------	--------	247.72 µg Glucose	--------
		Female	7.2	45	--------	--------	114.00 µg Glucose	--------
10	Raffinase	Male	7.2	45	--------	--------	335.15 µg Glucose	--------
		Female	7.2	45	--------	--------	102.00 µg Glucose	--------

4. Discussion

Most of the Carbohydrases (Amylase, Invertase, Maltase, Inulinase, Salicinase, Melibiase, Lactase and Cellobiase) studied in mid guts of this dung beetle showed maximum activities at pH 7.2 except Cellulase (at pH 5.6) and Trehalase (at pH 6.0).The range of maximum activity of these enzymes lies in between pH 6.8 to 7.4.This range is very close to the pH of the mid gut content indicating that pH conditions in alimentary canal are suitable for these Carbohydrases activity. In some other Scarabaeid beetles similar range of pH for these enzymes were observed for larvae and adults of *Holotrichia* (Bhanot, 1992) [3]; *Leucopholois* (Patil, 1996) [21]; *Onthophagus, Chironitis, Onitis, Liatongus* (Gaikwad et.al., 1997; Gaikwad, 1998 [7]; Gaikwad and Bhawane 2015 a, b, and 2016). Earlier works however reported acidic range of pH for amylase (4.6 to 5.8) in insects like pulse beetle, *Tribollium* and *Tenebrio* by Poddler and Applebaum (1971) [22]; Applebaum and Conigan (1965) [1] and Bounocor *et al.*, (1976),Such acidic range 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 and Evans and Payne 1964) [29, 20, 14].

Cellulase enzyme in this beetle showed maximum activity at pH 5.6 in both sexes. Similar acidic range of pH optima of this enzyme was reported in other insects by Lasker, 1959 [17]; Mc Bee 1959 [19]; Wharton *et al.*, 1965; Potts & Hewitt 1973 [23]; Patil, 1996 [21]; Gaikwad, 1998 [7]; Gaikwad and Bhawane, 2015a, and 2016) [8, 10]. Ricau (1958) [24] and Soo-Hoo and Dudzinski (1967) have reported the Cellulase activity in insect as result of gut micro-organisms.

In this beetle Trehalase showed maximum activity at pH 6.0.Such acidic pH optima for this enzyme also observed in other Scarabaeids by Bhanot (1992) [3]; Patil (1996) [21]; Kumbhar (1996) [15]; Gaikwad (1998) [7] and Gaikwad and Bhawane (2015a, and 2016) [8, 10].

All the carbohydrases studied in this beetle showed temperature optima at 45^0C in both sexes. Such higher temperature optima also recorded by Terra *et al.* (1977) [27] Kusano and Tanabe (1986) [16]; Teo and Heng (1987) [26]; Gaikwad (1998) [7] and Gaikwad and Bhawane (2015a, and 2016) [8, 10].

The Michaelis constant (Km value) is related to the affinity of the enzyme for the substrate. With the help of Line weaver – Burk's plots, the Km values for Amylase and Invertase were calculated in both sexes. The Km value for Amylase in male was 1.143% of starch and in female was 1.60% of starch. This indicates amylase in male mid gut is more efficient having greater affinity towards the substrate. Similar Km values were recorded in other dung beetle adults and grubs (Gaikwad *et. al.*, 1997; Gaikwad 1998; Gaikwad and Bhawane, 2015 a, b, and 2016) [11, 7, 8, 10].

The Km values of Invertase were 5.56×10^{-3} M in (male) and 1.297×10^{-2}M in (female).These values indicates that male mid gut Invertase is much more efficient than Invertase of female mid gut. Only in few insects the Km values for gut Invertase were determined. (Bhanot, 1992; Patil, 1996; Gaikwad *et al.*, 1997; Gaikwad, 1998; Gaikwad and Bhawane, 2015 a, b and 2016) [3, 21, 11, 7, 8, 10,].

The digestion period of 60 minutes were fitted very well within the linear part of enzymatic action for both Amylase and Invertase in both sexes. Gaikwad (1998) [7] Gaikwad and Bhawane (2015a, b and 2016) [8, 10] reported 30 minutes (in *Onthophagus catta* for Amylase); 60 minutes (*in Chironitis arrowi* for Amylase and Liatongus rhadamistus grub for Amylase and Invertase) and 80 minutes (*in Chironitis arrowi* for Invertase) which were found to be fit within the linear part of enzymatic activity.

The effect of higher temperature on the stability of Amylase and Invertase were studied in this beetle. The 50% denaturation of Amylase activity was occurred within 46 minutes in male and 45 minutes in female at 60^0 C. At same temperature the half-life of Invertase was 21.5 minutes in male and 24 minutes in female. The result indicates that Amylase and Invertase enzymes in male mid gut are slightly more heat stable than in female mid gut.

The data in Table no.1 shows that the activities of other Carbohydrases such Cellobioase; Salicinase; Inulinase; Maltase; Melibiase and Raffinase were detected in mid gut of

this dung beetle. Such Carbohydrases activity also detected in other Scarabaeid insects by (Bhanot, 1992; Patil, 1996; Gaikwad *et al.*, 1997; Gaikwad, 1998; Gaikwad and Bhawane, 2015 and Gaikwad and Bhawane, 2016) [3, 21, 11, 7, 8, 10].

5. Acknowledgement
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