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# RESEARCH ARTICLE

## STUDY ON CARBOHYDRASES IN BEETLE *ONITIS PHILEMON* (FAB.)

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**ABSTRACT**

Characteristics of ten carbohydrases from midgut (MG) and hindgut (HG) were studied in male and females of dung beetles *Onitis philemon* (Fab). Most of the carbohydrases showed their optimum activities at pH - 7.2. Enzymes cellulase, trehalase showed optimum pH 5.6 and 6.0. The pH optima of both maltase and invertase was 6.8. Temperature optima for all the carbohydrases which were studied in both guts and sexes occurred at 45°C. The Km values of amylase in MG and HG of male dung beetles were 0.2162% and 1.1420% respectively. While the Km values of amylase in MG and HG of female dung beetles were 1.140 % and 2.660 % respectively. The Km values of invertase enzyme were $4.87x10^{-3}$ M (in male MG), $3.894x10^{-2}$ M (in male HG); $4.10x10^{-3}$ M (in female MG) and $1.168x10^{-2}$ M (in female HG). In male MG the trehalase Km valve was $7.342x10^{-4}$M while in female MG the Km value was $6.956x10^{-4}$M. The Km value of maltase in male MG was $1.884x10^{-3}$M and in female MG was $1.579x10^{-3}$M. The half life of amylase activity at 60°C was 70 min. (in male MG); 48 min. (in male HG); 80 min. (in female MG) and 53 min. (in female HG). At the same temperature half life of invertase was at 40 min. (in male MG); 35 min. (in male HG); 30 min. (in female MG) and 28 min. (in female HG). The half life of trehalase and maltase in mid guts at 60°C were 50 min (in male); 53 min. (in female); 35.5 min. (in male) and 43.5 min (in female) respectively. The digestion period of 75 min. (MG and HG amylase in both sexes); 60 min. (MG and HG invertase in both sexes and MG trehalase of male and female) and 30 min. (MG maltase of both sexes) were fitted very well within the liner part of enzymatic action. Bacteriological studies revealed the presence of symbiotic amylolytic, cellulolytic saccharolytic and trehalolytic bacteria in hind gut section of both sexes. MG was major source for carbohydrases, but HG also contributes significantly. This indicates that HG also plays a vital role in the process of digestion of coprophagus adults due to the presence of symbiotic microbiota.



## INTRODUCTION

*Onitis philemon* (Fab.) (Scarabaeidae: Scarabaeinae) is a dung beetle also known as scavenger beetle. Most of the beetles from scarabaeinae consume cattle dung as food. They are beneficial insects as they are actively involved in the biological control of dung dropping of the cattle and eventually the dung borne dipterous flies of medical and veterinary importance (Waterhouse, 1974). The cattle dung of excrement can be considered as diluted food and contains many of the same classes of chemical compounds found in the classical food (Day, 1978). Several literature reviews are published on the nutritive value of animal wastes (Smith *et al.,* 1971; Smith, 1973; Whistone *et al.,* 1974). The nutrient levels in the cattle waste on percentage basis are 8 to 16%; 0.9 to 1.3%; 15.9 to 16.7%; 2.4 to 3.0% for protein, liquid carbohydrates, solid carbohydrates and fats respectively (Jaswal, 1971; Chawala, 1984; Surbook *et al.,* 1971). As the cattle dung mostly contains structural carbohydrates like cellulose, lignin etc. the problem of such polysaccharides digestion in terms of efficient nutrient utilization from animal wastes would be worthy consideration. In insects, alimentary canal and different enzymes involved in the process of digestion show great variation, as they consume wide variety of food materials. Successful adaptations of an insects to a particular food is very essential for all physiological and biochemical processes (Slansky, 1982). The midgut is the main part of the alimentary canal, which is responsible for secretion of enzymes, digestion and absorption of digested food, intermediary metabolism and other functions in most of the insects. Insects are the most diverse and successful group in the terrestrial ecosystem. This diversity would hardly possible without symbiotic bacteria. Symbiotic bacterias are

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closely associated with about 20-50% of insects (Bourtzis and Miller, 2003; Feldhaar and Gross, 2009; Kikuchi, 2009). Taking into account, the beneficial services of dung beetles to human being, it is aimed to study their digestive physiology. No literature is available on the digestive enzymes of coprophagus species of scarabaeinae excepting the account on the grubs of this species by Gaikwad *et al.,* (1997). The food of grub and adult dung beetle is basically the same, but it is surprising that there are important morphological and anatomical differences between larvae and adults alimentary canal. The adult dung beetles feed on the liquid and colloidal content of dung and the larvae feed mainly on the solid content of dung and their gut is adapted for coprophagy. The long alimentary canal of adults is adapted for microphagy, necrophagy and saprophagy as vast amount of diluted food is to be consumed to extract the required nutrients. This indicates beetles utilizing the animal waste show digestive system, which is completely adapted for that food. Since the activity of the digestive enzymes is mostly reflect as degree of the adaptation to food components. Therefore, in this communication carbohydrates digesting enzymes of adult *O. philemon* are studied.

## MATERIALS AND METHODS

### Collection and maintenance of experimental animals

The adult dung beetles are powerfully attracted to fresh dung pads. The beetles were collected from two to three days old buffalo dung pads and were maintained at laboratory conditions. For maintenance the earthen pots containing loose moist mixture of soil and sand in equal parts were used as per the method proposed by Blume and Aga (1975). Fresh dung from the same field was given as a food to the beetles. The earthen pots were covered with another inverted earthen pot.

### Enzymology

The enzyme activities were determined in both mid gut (MG) and hind gut (HG) sections of alimentary canal. Enzymes like amylase, invertase, trehalase, maltase, lactase, salicinase, raffinase, etc. were studied. The enzyme activities were determined as follows.

### Enzyme preparation

Adults of similar size were obtained from the laboratory stock for the preparation of MG and HG enzyme sources. The pooled tissues along with the contents were used to prepare homogenate in 0.8% chilled NaCl and centrifuged in cooling centrifuge for 15 min. at 1000 g at 10$^{o}$C. Aliquots of supernatants were used as enzyme source. The characteristics of the enzymes like pH optima, temperature optima, effect of time and substrate concentration were determined with the help of series of assays in which individual factor varied and all other factors were kept at the optimal level.

### Amylase, Invertase, Trehalase, Cellulase, Inulinase and Salicinase

The activities of these enzymes were determined by estimating the reducing sugars concentration of the digestion end products due to these enzymes with 3-5, dinitrosalicylic acid (Bernfield, 1955). The detailed enzyme assay is described elsewhere (Gaikwad *et al.,* 1997).

### Maltase, Cellubiase, Lactase and Raffinase

The activities of these enzymes were determined by estimating the glucose concentration of the digestion end products of these enzymes action by GOD-POD reagent (Spandiagnostics, Pvt. Ltd. Surat, India).

### Thermolability (Amylase, Invertase, Trehalase, Maltase)

For the study of thermolability, the enzyme extracts were subjected to high temperature at 60$^{o}$C for different period of times, treated enzyme extracts were stored in the refrigerator until they were used for estimation of activities of residual enzymes left after heat treatments.

### Effects of inhibitors and activators

It is determined by using different concentrations (0.01M to 0.2M) for $CuSO_4$, $ZnSO_4$, NaCl and KCl and (0.002M to 0.05M) for $HgCl_2$. The homogenates of enzyme extracts prepared in chilled distill water were used in assays.

### Effect of Starvation

To study the effect of starvation on male, adult *O. philemon* were collected from field as mentioned earlier. These beetles were segregated and kept individually in small plastic containers. These containers were maintained in moist earthen pots for ten days. Parallel control was arranged which provided with dung as food. After ten days they were utilized for the study of enzymes amylase, invertase, trehalase and maltase as descried earlier.

### Estimation of protein

The soluble protein content of the enzyme source was determined by Lowry *et al.,* (1951).

### Bacteriological studies

The bacteriological studies were confined to the hind gut of 3$^{rd}$ instar larvae of *O. philemon*. The 3$^{rd}$ instar larvae were dissected for alimentary canal under aseptic conditions. The hind guts were taken in sterile saline solution to make an innoculum. The innoculum was divided in to two sets. One set was used for study of aerobic organisms and other set for study of anerobic organisms. The melted was poured over innoculum. Both sets of innoculum were incubated at 28$^{o}$C for 24 hrs and used for further study. Determination of amylolytic, saccharolytic, and cellulolytic organisms were done. For amylolytic bacteria starch-agar medium, for saccharolytic bacteria, sucrose agar medium and for cellulolytic bacteria cellulose broth were used.

## RESULTS

The Characteristics of various digestive enzymes in dung beetle *O. philemon* are summarized in the Table 1. 3.1 Effect of pH: - In both sexes the optimal pH for enzymes such as amylase, cellubiase, melibiase, lactase, raffinase, inulinase,

salicinase in MG. and HG was 7.2, while invertase and maltase (pH optima 6.8), cellulase (pH optima 5.6) and trehalase (pH optima 6.0).

**Effect of Temperature:** All the carbohydrases which were studied showed maximum activities at temperature 45$^{o}$C irrespective of gut sections and sexes.

**Effect of substrate concentration:** The relationship between substrate concentration and rates of hydrolysis for MG and HG amylase, invertase and trehalase in both sexes and MG maltase of both sexes are observed. The Km values were obtained by plotting Lineweaver-Burk plot are shown in the table 1.

**Effect of Time:** Effect of time on amylase, invertase, trehalase and maltase is observed. The digestion period of 75 min (for amylase in both guts and sexes and for trehalase in MG of both sexes) and 30 min (for maltase in MG of both sexes) were fitted very well within the linear part of enzymatic action.

**Thermolability:** The effect of high temperature on the stability of amylase, invertase, trehalase and maltase enzymes are observed. The theoretical duration of high temperature treatment for 50 % loss of activities for these enzymes was at 60$^{o}$C as shown in the table 1. 3.6

**Bacteriological studies:** The bacteriological study in hind gut section of both sexes showed presence of amylolytic, cellulolytic, saccharolytic and trehalolytic bacteria. The amylolytic Gram +ve, non motile bacteria formed yellowish colonies on starch-agar medium. The saccharolytic bacteria grown on sucrose-agar medium were Gram +ve, motile and slender rods. The trehalolytic bacteria were Gram +ve motile rods and formed yellow colonies on trehalose-agar medium. In the cellulose-broth Gram +ve motile cellulolytic bacteria were grown.

**Effect of Activities and inhibitors:** The effect of activators and inhibitors on MG amylase and invertase activity of male *O. philemon* (Table - 2). NaCl and KCl solutions even at 0.01M concentrations showed significantly increase in the activity of amylase and invertase on the other hand inhibitors viz. $CuSO_4$ and $ZnSO_4$ at 0.01M and $HgCl_2$ at 0.005 M bring out considerable reduction in the enzyme activity of the amylase and invertase.

**Effect of Starvation:** In case of the starved beetle there is considerable reduction of these enzyme activities in both MG and HG of male *O. philemon* (Table - 3). Generally the activity of these enzymes much more decreased in HG than MG.

**Table 1. Characteristics of digestive carbohydrases in *O. philemon***

| Sr. No | Enzymes | Sex* | Organ** | Characteristic of enzymes | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | PH optima | Temp. Optima | 50% inactivation at 60$^{o}$C | Linear activity duration in minutes | Specific activity (µg/µgprotein/hr) | Km values |
| 1 | Amylase | M | MG | 7.2 | 45$^{o}$C | 70 | 75 min | 23272 | 0.2162 % |
| | | | HG | 7.2 | 45$^{o}$C | 48 | 75 min | 5600 | 1.1420 % |
| | | F | MG | 7.2 | 45$^{o}$C | 80 | 75 min | 28000 | 0.1400 % |
| | | | HG | 7.2 | 45$^{o}$C | 53 | 75 min | 9000 | 2.6600 % |
| 2 | Invertase | M | MG | 6.8 | 45$^{o}$C | 40 | 60 min | 527.28 | $4.87 \times 10^{-3}$ M |
| | | | HG | 6.8 | 45$^{o}$C | 35 | 60 min | 612 | $3.89 \times 10^{-2}$ M |
| | | F | MG | 6.8 | 45$^{o}$C | 30 | 60 min | 432 | $4.10 \times 10^{-3}$ M |
| | | | HG | 6.8 | 45$^{o}$C | 28 | 60 min | 892.5 | $1.168 \times 10^{-2}$ M |
| 3 | Trehalase | M | MG | 6 | 45$^{o}$C | 50 | 60 min | 119.58 | $7.342 \times 10^{-4}$ M |
| | | | HG | 6 | 45$^{o}$C | --- | --- | 234.6 | -- |
| | | F | MG | 6 | 45$^{o}$C | 53 | 60 min | 196.71 | $6.956 \times 10^{-4}$ M |
| | | | HG | 6 | 45$^{o}$C | --- | --- | 280.5 | --- |
| 4 | Maltase | M | MG | 6.8 | 45$^{o}$C | 5.5 | 30 min | 338.1 | $1.884 \times 10^{-3}$ M |
| | | | HG | 6.8 | 45$^{o}$C | -- | -- | 357 | -- |
| | | F | MG | 6.8 | 45$^{o}$C | 43.5 | 30 min | 306.64 | $1.579 \times 10^{-3}$ M |
| | | | HG | 6.8 | 45$^{o}$C | --- | --- | 306 | --- |
| 5 | Cellulase | M | MG | 5.6 | 45$^{o}$C | | | 68.44 | |
| | | | HG | 5.6 | 45$^{o}$C | --- | --- | 105.6 | --- |
| | | F | MG | 5.6 | 45$^{o}$C | | | 32.99 | |
| | | | HG | 5.6 | 45$^{o}$C | --- | --- | 132 | --- |
| 6 | Inulinase | M | MG | 7.2 | 45$^{o}$C | | | 339.42 | |
| | | | HG | 7.2 | 45$^{o}$C | --- | --- | 303.6 | --- |
| | | F | MG | 7.2 | 45$^{o}$C | | | 456 | |
| | | | HG | 7.2 | 45$^{o}$C | --- | --- | 660 | --- |
| 7 | Salicinase | M | MG | 7.2 | 45$^{o}$C | | | 188.53 | |
| | | | HG | 7.2 | 45$^{o}$C | --- | --- | 211.2 | --- |
| | | F | MG | 7.2 | 45$^{o}$C | | | 216 | |
| | | | HG | 7.2 | 45$^{o}$C | --- | --- | 308 | --- |
| 8 | Melibiase | M | MG | 7.2 | 45$^{o}$C | | | 127.49 | |
| | | | HG | 7.2 | 45$^{o}$C | --- | --- | 99.45 | --- |
| | | F | MG | 7.2 | 45$^{o}$C | | | 190.09 | |
| | | | HG | 7.2 | 45$^{o}$C | --- | --- | 153 | --- |
| 9 | Cellobiase | M | MG | 7.2 | 45$^{o}$C | | | 160.28 | |
| | | | HG | 7.2 | 45$^{o}$C | --- | --- | 122.4 | --- |
| | | F | MG | 7.2 | 45$^{o}$C | | | 256.36 | |
| | | | HG | 7.2 | 45$^{o}$C | | | 221 | --- |
| 10 | Raffinase | M | MG | 7.2 | 45$^{o}$C | | | 3642 | |
| | | | HG | 7.2 | 45$^{o}$C | --- | --- | 15.3 | --- |
| | | F | MG | 7.2 | 45$^{o}$C | | | 37.09 | |
| | | | HG | 7.2 | 45$^{o}$C | --- | --- | 21.25 | --- |

*Sex: M = male; F = female, **Organ: MG = midgut; HG= hindgut

**Table 2. Activator/Inhibitor study in male midgut amylase and invertase of *O. philemon***

| Inhibitor/activator | Enzyme | Molarity | | | | | Control |
|---|---|---|---|---|---|---|---|
| | | 0.2 M | 0.1 M | 0.05 M | 0.02 M | 0.01 M | |
| NaCl | Amylase | 19088.10 | 19088.10 | 18710.48 | 18710.48 | 12831.49 | 7627.85 |
| | Invertase | 470.99 | 470 | 470.90 | 430.97 | 359.82 | 259.85 |
| KCl | Amylase | 13621 | 13621 | 12940.1 | 11577.98 | 10807.23 | 7627.85 |
| | Invertase | 415.76 | 415.76 | 415.76 | 394.18 | 341.1 | 259.85 |
| CuSO4 | Amylase | 272.42 | 817.26 | 2043.17 | 3813.92 | 5448.46 | 7627.85 |
| | Invertase | --- | --- | 20.78 | 62.36 | 103.94 | 259.85 |
| ZnSO4 | Amylase | --- | --- | 408.63 | 1634.54 | 4086.35 | 7627.85 |
| | Invertase | --- | --- | --- | 31.18 | 51.97 | 259.85 |
| HgCl2 | | 0.05M | 0.02M | 0.01 M | 0.005 M | 0.0025 M | |
| | Amylase | --- | --- | --- | 272.42 | 1089.69 | 7627.85 |
| | Invertase | --- | --- | --- | --- | 41.57 | 259.85 |

Activity of amylase in µg maltose/mg protein/hr and invertase in µg glucose/mg protein/hr.

**Table 3. Effect of starvation on amylase invertase, trehalase and maltase in male mid gut and hind gut regions of *O. philemon***

| Enzyme | Region | Activity in control | Activity after starvation | % change |
|---|---|---|---|---|
| Amylase | MG | 17587 | 12487 | -29.00 |
| (µg maltose/mg protein/hr.) | HG | 5400 | 2781 | -48.50 |
| Invertase | MG | 498.75 | 236.9 | -52.50 |
| (µg glucose/mg protein/hr.) | HG | 567.28 | 255.5 | -54.96 |
| Trehalase | MG | 107.58 | 36.04 | -66.50 |
| (µg glucose/mg protein/hr.) | HG | 213.6 | 58.95 | -72.40 |
| Maltase | MG | 330.1 | 222.65 | -32.55 |
| (µg glucose/mg protein/hr.) | HG | 380 | 220.52 | -41.97 |

## DISCUSSION

The pH optima of most of the carbohydrases, excepting cellulase and trehalase in both MG and HG of the dung beetle under study fall within the pH range of haemolymph and alimentary tract. The pH of the haemolymph is 7.0 and that of alimentary canal content is 7.0 to 7.5 (determined by pH paper method). It is interesting that the adult dung beetles and both gut sections amylase showed optimal pH value 7.2. The range of maximum activity of this enzyme lies in between pH 6.8 to 7.4. This range of maximum activity of amylase is very close to the pH of the gut content indicating that pH conditions in alimentary canal are suitable for amylase activity. Similar range of pH was observed for the larvae and adults of *Holotrichia*, *Leucopholis*, *Onthophagus*, *Chironitis*, and grubs of *O. philemon* (Bhanot, 1992; Patil, 1996; Gaikwad, 1998; Gaikwad *et al.,* 1997). But in other insects like pulse beetle *Tribolium* and *Tenebrio* acidic pH range for amylase (4.6 to 5.8) was reported (Poddler and Applebaum, 1971; Applebaum and Konijan, 1905 and Bounciore *et al.,* 1976). The maximum activity of invertase was recorded at pH 7.2 in the adults of *O. philemon*. This result indicates that maximum invertase activity takes place at neutral pH. In some other larvae and adult dung beetles such as *Chronitis arrowi*, *Onthophagus catta* and grubs of *O. philemon* shows similar range of pH optima (Gaikwad *et al.,* 1997; Gaikwad, 1998). As in majority of insects such as harvester termite, black scale insect, fruit flies, *Manduca sexta* and *Heliothis zea* the pH optima for midgut trehalase is acidic (Potts and Hewitt, 1972; Ishaaya and Swirski, 1976; Huber and Lefebee, 1971; Dahlman, 1971; Burton, 1975). Optimum pH value of the trehalase in the present dung beetle is in accordance with the earlier Scarabidae studied by Bhanot, (1992); Patil, (1996); Kumbhar, (1996); Gaikwad *et al.,* (1997). In present investigation both gut of adult *O. philemon* showed pH optima at 6.8 for maltase enzymes. Similar pH optimum of maltase is present in some Scarabaeid larvae and adults (Kumbhar, 1996; Gaikwad *et al.,* 1997; Patil, 1996; Gaikwad, 1998).

The activity of cellulase was determined in both gut sections of adult beetle and it showed maximum activity at pH 5.6. This enzyme is mostly active in acidic pH which was reported in other insects (Lasker, 1959; Mc Bee, 1959; Wharton *et al.,* 1965; Potts and Hewitt, 1973; Patil, 1996; Gaikwad *et al.,* 1997; Gaikwad, 1998). The Cellulase activity might have been resulted due to gut micro-organisms. In both gut sections of adult beetle pH optima for cellobiase were 7.2. This enzyme is a very little investigated in insects. The data on qualitative estimation of this enzyme is only available for some insects which implies similar optimum pH for this enzyme (Mishra and Sensarma, 1985; Patil, 1996; Kumbhar, 1996; Gaikwad, 1998). In present dung beetle both gut sections showed maximum activity of melibiase and lactase at about neutral pH (7.2). Only few reports are available on activity of these enzymes but that too on the qualitative estimation (Krishna, 1958; Burton, 1975; Mishra and Sensarma, 1985; Patil, 1996). Gaikwad *et al.,* (1997) and Gaikwad, (1998) investigated the pH optima of these enzymes at pH 7.2. The raffinase, inulinase and salicinase enzymes in present dung beetle showed maximum activities at pH 7.2. Similar pH optima were also reported for these enzymes in *Holotricha serrata* by Patil (1996); in *O. philemon* grubs by Gaikwad *et al.,* 1997; in *Chironitis arrowi* and *Onthophagus catta* by Gaikwad (1998). Qualitative estimation of these enzymes were made by Krishna (1958), Retief and Hewitt (1973), Burton (1975) and Mishra and Sensarma (1985). Temperature optima for all the carbohydrases studied in both gut sections of adult showed at 45$^{o}$C. Similar higher temperature range for *Richnocera americana, Biattella germonica, Chironitis arrowi* and *Onthophagus catta* (Terra *et al.,* 1977; Day and Powing, 1949; Gaikwad, 1998) and also recorded. For temperate insect species the lower optimum temperature values were reported by Ishaaya and Swirski (1970) and Burton (1975). The Km values of different carbohydrases (amylase, invertase, trehalase and maltase) were calculated from the Lineweaver – Burk's plots. In other insects reported Km values for midgut

amylases were 0.14% of Starch for *Richnocera americana*, 0.077% of starch for *Sitophilus granarius*, 0.13% of starch for *Sitophilus zeamize* (Terra *et al.,* 1977; Baker, 1983), 0.4% of starch for midgut of *O. philemon* and 0.188% in hingut of *O. philemon* (Gaikwad *et al.,* 1997). In *Tenebrio molitor* and *Calasobruchus chinensis* 0.18% and 0.23% of starch as Km values were observed by Bounciore et al., 1976 and Poddler and Applebaum (1971). In the present dung beetle male MG Km value (0.2162%) is lowest than HG and both gut sections of female. Again Km values of amylase in male MG and HG are lower than female MG and HG. This indicates that amylase enzyme in male dung beetle is more efficient over amylase in female. From the Km values it is also clear that amylase in MG is more efficient over amylase in hindguts in both sexes. Km values for male MG and HG invertase are 4.87 $X10^{-3}$ M and 1.168 X $10^{-3}$ M sucrose respectively and for female MG and HG are 4.10 X $10^{-3}$ M and 1.168 X $10^{-2}$ M sucrose respectively. This indicates that MG invertase in female gut sections is slightly efficient than invertase in male gut sections. Only in few insects the km values for gut invertase are determined. These are 2.337 X $10^{-3}$ M in larval midgut and 7.789 X $10^{-3}$ M in male midgut of *Holotrichia serrata* (Patil, 1996); 2.43 X $10^{-3}$ M and 7.12 X $10^{-4}$ M for *C. orientalis* (Kumbhar, 1996) and 5.56 X $10^{-3}$ M (larvae) 8.65 X $10^{-3}$ M (male) and 6.49 X $10^{-3}$ m (female) for *O. catta* (Gaikwad, 1998).

The Km values of midgut trehalase are 7.342 X $10^{-4}$ M (in male) and 6.956 X $10^{-4}$ M (in female) of trehalose. Earlier workers had reported different km values for trehalase in different insect species viz. in *Holotricha serrata* 2.11 X $10^{-3}$ M; 4.332 X $10^{-4}$M in midgut of *O. philemon* and *O. Catta* larvae 7.048 X $10^{-3}$ (Bhawane and Mandlik, 1992; Gaikwad et al.; Gaikwad, 1998). The Km values of the present dung beetle are small and similar to other dung beetles, than Km values given above for other insects, indicating that this enzyme is very efficient present insect than other insect. The Km values for midgut maltase were 1.88 X $10^{-3}$ M in males and 1.578 X $10^{-3}$ M of maltose in female. Gaikwad (1998) obtained Km values for maltase in midguts of *C. arrowi* is 5.337 X $10^{-3}$ M (male) 4.95 X $10^{-4}$ M (female). Patil (1996) obtained Km values for larval MG and HG maltase of *H. Serrata* are 9.25 X $10^{-3}$ M and 1.38 X $10^{-3}$ M of maltose. The Km values of present insect are similar other dung beetles. Amylase enzyme showed digestion period of 75 min. which is found to be fit within the linear parts of enzymatic activity curves Gaikwad (1998) reported 30 min. (in *O. catta*) and 60 min (in *C. arrowi*) which were found to be fit within the linear part of enzymatic activity. Similarly Patil (1996) was reported the linear activity period for this enzyme in *H. serrata* upto 16 min, Gaikwad *et al.,* found 60 minutes in grub of *O. philemon* while Kumbhar (1996) found this period up to 20 min in *C. orientalis*. This indicated that present dung beetle showed longer period of linear parts of enzymatic activity. Invertase and trehalase showed digestion period of 60 minutes which is found to be fit within the linear parts of enzymatic activity curve. Similar linear activity periods of these enzymes also recorded by Patil (1996), in *H. serrata* and by Kumbhar (1996) in *C. orientalis*, by Gaikwad *et al.,* (1997) in MG of *O. philemon* grub. In *C. arrowi* trehalase enzyme showed linear part digestion period of 80 min. (Gaikwad, 1998). The midgut maltase showed linear part digestion period up to 30 min. in present insect similar linear digestion period for maltase were record by Kumbhar (1996) in *C. orientalis*, Patil (1996) in *H. serrata* and by Gaikwad (1998) in *C. arrowi*. The effect of high temperature on the stability of amylase invertase, trehalase and maltase were studied in present dung beetle. Gut extracts containing carbohydrases were exposed to higher temperature of 60$^{o}$C. The comparative studies on carbohydrases showed amylase is more heat stable than others and requires more time for its 50% therotical degradation. Amylase is more heat stable in female while invertase is more heat stable in male. Trehalase and maltase shows that they are more heat stable in female. Generally carbohydrases in midgut are more heat stable than in hindgut. Amylase of *Valanga* (Teo, 1973) is very heat stable and lost its 50% activity due to treatment at 60$^{o}$C after 93 min. Similarly amylase of *Spodoptera* only lost its activity above 65$^{o}$C which shows that the amylase is rather heat stable (Ishaaya *et al.,* 1971) Invertase of the *Valanga* requires time of 29 min. for 50% denaturation at 60$^{o}$C (Teo 1973) but in present dung beetle 28 to 40 min are required for the similar denaturation Trehalase was very unstable in tobacco horn worm *Manduca sexta* at temparature above 57$^{o}$C (Dahlman, 1971). The trehalase in female midgut even at 60$^{o}$C, the 50% activity exist upto 53 min exposures. Comparable results were obtained for trehalase in *L. lepidophora* (Bhawane *et al.,* 1989); in *Holotrichia* (Bhawane and Mandalik, 1992); in *C. orientalis* (Kumbhar, 1996); *H. serrata* (Patil, 1996) and in *C. arrowi* (Gaikwad, 1998). The midgut is the measure source of the carbohydeases in present insect This result agree with the general view that midgut is the chief site of digestive enzyme secretion (Dadd, 1970; Engelmann and Gerarets, 1980). However hindgut also contributes significantly in the secretion of digestive enzymes in this insect. This indicates that hindgut also plays important role in the process of digestion of coprophagus insects. Similar results were also obtained by Thomas and Nation (1984) in hindgut of *G. rubens* and *S. acletus*; by Bhawane and Bhanot (1989) in hindgut of *H. serrata* by Bhanot (1992), by Patil (1996) in larvae and adults of *H. serrata* by Kumbhar (1996) in larvae of *C. orientalis* Determination of origin of various enzymes in the hind gut of present dung beetle is because of three reasons 1) It cannot be doubted that the digestive enzymes come from the midgut with the food. 2) Potential source is the microorganisms 3). It may be from the epithelial cells of hindgut (Martoja, 1966; Bhawane and Bhanot, 1989).

Thus hindgut is the highly modified structure according to the nature of food with the specialized physiology of digestion. Presence of cellulase in gut sections of this dung beetle is of special interest because of the presence of cellulolytic bacteria in the sections of gut. Similarly cellulolytic activity has been ascribed to gut microorganism by Potts and Hewitt (1973) in harvester termites and by Delalibera *et al.,* (2005) in wood borer *Saperda vestita* and the bark beetles, *Ips pini* and *Dendroctonus frontalis*. The effects of various activities and inhibitors on the midgut amylase and invertase of male dung beetle were observed and the data shows that $CuSO_4$, $ZnSO_4$ and $HgCl_2$ have inhibitory effect even at the minimum concentration. NaCl and KCl causes the activation of these enyzmes even at 0.001 M concentration. In insects this enzyme is activated by chloride ion concentration (Doane, 1969; Terra *et al.,* 1977; Baker, 1983). But amylase in *Callasobruchus chinensis* was inhibited by chloride ions (Poddler and Applebaum, 1971). Invertase enzyme is also activated by chloride ions in present dung beetle; comparatively it is less

activated than amylase. The invertase is more sensitive to inhibitor than amylase in present insect. Starvation is one of the types of environmental stress that this insect is likely to expose during their growth and development. Adults of *O. philemon* emerge from the brood balls after the monsoon showers do not get dung pad which is the natural food of the beetles. This is because grazing cattles may not visit the pastures unless and until it is properly grown. As part of study on biology of this beetle, work on effects of starvation on digestive enzymes was considered. In present dung beetle considerable reduction in the activity of amylase, invertase, trehalase and maltase enzymes due to starvation for ten days was reported. The hindgut carbohydrases are most affected due to starvation. It seems that the ingested food serves as stimulus to the enzyme secretion in this beetle as it occurs in several other insects (Engelmann, 1969; Baker, 1983). The presence of symbiotic microorganisms in hindgut of *O. philemon* improves its ability to live on suboptimal diet like dung.

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