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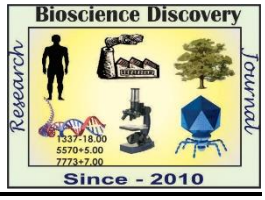

Digestive enzymes of white grubs, *Leucopholis lepidophora* Bl. and *Holotrichia fissa* Br. (Coleoptera; Scarabaeidae: Melolonthinae)

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Abstract

Leucopholis lepidophora and *Holotrichia fissa* are the common white grubs found in the sugarcane and other agricultural fields of Kolhapur district, Maharashtra, India. Partial characterization of digestive enzymes viz. amylase, invertase, trehalase cellulase, protease and lipase in mid gut (MG) and hind gut (HG) sections in both the species of white grubs was done. The bacterial microflora of proctodial dilation / fermentation chamber of hind gut of *L. lepidophora* was investigated.As scarabaed grubs, *L.lepidophora* and *H. fissa* eat roots of plants and decaying orgsanic matter from the soil containing cellulose and other soluble nutrients and whether these grubs could able to extract nutrients and energy from such diet so as to fulfill the nutritional requirement is aim of this investigation.The specific activities, optimum pH, optimum temperature and optimum time period values fo the enzymes under study were investigated.The measurement of activities of amylase,invertase, trehalase and cellulase was done as per the method of Ishaaya and Swirski(1970),protease of Ishaaya *et al*.,(1971) and lipase of Hayashi and Tappel (1970) by using colour reagent of Itaya (1977). Thermolability at 60^{0C} for MG amylase and invrtase in *L. lepidophora* and the effects of activators like NaCl and KCl and inhibitors like $MgCl_2$ and $MnCl_2$ on the mid gut amylase enzymes of *L. lepidophora* and *H. fissa* were studied. Km values of amylase in MG and HG sections of *L. lepidophora* were 0.000042% and 0.13% of starch respectively and in *H. fissa* Km values were 0.00012% and 2.6% of starch in mid and hind gut sections. The Km value of mid gut invertase in *L. lepidophora* was 11.84 mM and in *H. fissa* was 16.17 mM of sucrose. The Km values for trehalase in *L. lepidophora* were 29.55 mM and 531.38 mM of trehalose in MG and HG sections respectively and in MG of *H. fissa* Km value of trehalse was 2.58 mM of trehalose. In proctodeal dilation of hind gut section of *L. lepidophora* grubs showed the presence of symbiotic bacteria flora mostly Gram negative and some Gram-negative rods which were found to be starch, sucrose, trehalose, cellulose, olive oil and caesin hydrolysing.

INTRODUCTION:

In strict sense white grubs are the larvae of Melolonthinae (Wolcott, 1933). But this term has now wider usage embracing the larvae of Rutelinae, Cetoniinae, Dynastinae and other families of Scarabaeoidea super family (Ritcher, 1966). The family Scarabaeidae is one of the largest families of the order Coleoptera, whose larvae are predominating in grassland soil (Lavelle *et al*., 1997). Many of them are highly polyphagus pests and among the most troublesome soil insects associated with the root injuries of various agricultural crops threatening the crop production in Maharashtra and various endemic pockets many

parts of the India (Raodeo *et al.,* 1976; Pal, 1977; Yadava and Sharma, 1995; Bhawane *et al*., 2012 and Mane and Mohite, 2014). The fossorial scaraeboied grubs are saprophagus and herbivorous (Crowson, 1981) and feed on the plant roots, organic matters of the soil and feces of the cattle and other vertebrate animals and their food is diluted one having low nutritive values (McQuillan and Webb,1994; Gaikwad *et al*.,1997; Zhang and Jackson, 2008). and hence it can be an effective resource in the decomposition of varied biowaste in the future (Koyama *et al*., 2003). The white grub species understudy, are polyphagus root feeding insects of agricultural crops. *Holotrichia fissa* is having larval duration of about 6 months causing moderate damage to the roots of the crops, where as *Leucopholis lepidophora* is endemic in the sugarcane fields located along the banks of Kumbi-Kasari rivers of Kolhapur district of Maharashtra where rainfall is heavy and soil is alluvial and monoculture of sugarcane, existed as main cash crop. The larval period of this species is comparatively longer duration of 8 to 10 months and condition of soil type, soil moisture and availability of food are favorable for the multiplication and persistence of this species during last three decades and causing extra ordinary damage to the sugar cane and other crops (Patil and Hapase, 1981; Adsule and Patil, 1990; Patil *et al*., 1991; Bhawane *et al*., 2012). The alimentary canal of scarabaeoid grubs have many characteristic features and physico-chemical properties like long and specious midgut occupying the major part of the abdominal cavity, modified and expanded HG called proctodeal dilation or fermentation chamber, highly alkaline midgut with suitably stable enzymes and a specific gut symbiotic microflora (Berberet and Helms, 1972; Terra, 1990; Li and Brune, 2005; Zhang and Jackson, 2008 ;Wada *et al*.,2014; Bhawane *et al*., 2016 ;Gaikwad and Bhawane 2016 a&b). The white grub species understudy are polyphagus root feeding insects of agricultural crops and threatening the crop production. In spite of this, the basic information on their digestive physiology is lacking. Although the chemical composition of the roots of the various agricultural crops is not thoroughly studied but the information available on the chemical composition of the grass roots indicates that it contains little soluble carbohydrates and much cellulose and other structural carbohydrates (Sutherland, 1971). The utilization of soluble sugars, structural carbohydrates, proteins and lipids present in roots consumed is of great importance nutritional economy of grubs. If this is so microfiora present in the gut may play important role in the process of digestion of structural carbohydrates, proteins and lipids. This study is designed to study the digestive enzymes of xylophagus white grubs understudy.

MATERIAL AND METHODS:

Collection and maintenance of experimental animals: The third instar grubs of *L. lepidophora* collected from the infected fields situated along the banks of Kumbi river, near Kuditre, Kolhapur district Maharashtra in the month of March and the third instar grubs of *H. fissa* were collected from the fields of paddy, jowar, maize and sugarcane in and around Kolhapur city, Maharashtra, India in the month of September. The grubs were kept in earthen pots containing moist soil obtained from the same fields for 15 days under laboratory conditions. The roots of jowar, maize and potatoes were given as food to the grubs.

Enzyme Preparation: The third instar grubs of *L. lepidophora* approximately 7 grams body weight and *H. fissa* weighing approximately 4 grams obtained from the laboratory stock for the preparation of MG and HG enzyme extracts. The homogenates of pooled tissue were prepared in cold 0.9% NaCl solution which were centrifuged at 10,000 rpm. in cold centrifuge at 10^{0C} for 15 minutes. Aliquots of supernatants were used as enzyme source.

Enzyme Assays: The optimum conditions for the enzymes under study i.e. pH, temperature, time and substrate concentration were determined in series of experiments in which individual factors were varied and all other factors were kept at the constant.

Amylase, invertase, trchalase and cellulase: The measurement of activities of these enzymes were made by using 3-5 dinitrosalicyclic acid reagent (DNSA) (Bernfield, 1955) and the method of Ishaaya and Swirski, (1970) as described by Gaikwad *et al*. (1997) was followed. The enzyme activities of invertase, trchalase and cellulase were expressed as µg glucose / µg protein / min and for amylase µg maltose / µg protein / min.

Protease: For the estimation of protease activity method of Brik *et al.* (1962) as followed by Ishaaya *et al*., (1971) and Gaikwad and Bhawane (2015c) was followed. The activity was expressed in terms of µg tyrosine / µg protein / min.

Lipase: The method for the measurement of lipase activity as proposed by Hayashi and Tappel (1970)

and color reagent of Itaya (1977) as described by Gaikwad and Bhawane (2015c) was used. The activity of lipase was expressed in terms of µg plamatic acid / µg protein / min.

Effect of Activators and Inhibitors: To study the effects of activators and inhibitors on the MG amylase enzymes of both the grub's different concentration (0.01 M to 0.5 M) of NaCl, KCl, $MgCl_2$ and $MnCl_2$ in the assay mixtures and the enzyme activity is determined. The homoginates of MG were prepared in chilled glass distilled water.

Effect of high temperature exposure of MG amylase and invertase enzymes (Thermolability) in *L. lepidophora*: The MG homoginate of *L. lepidophora* was exposed to high temperature at 60^{0C} and procedure for determination of residual activity of amylase and invertase from the homoginates was as per Bhawane *et. al*. (2016).

Bacteriological Studies: For this purpose, mid HG region (Proctodeal dilation) of 3rd instar grub of *L. lepidophora* were used. The third instar grubs were cleaned with distilled water twice, blotted and surface sterilized with alcohol and dissected under aseptic condition so as to obtain HG regions. The HG taken in starile salt solutions contains sterile gut fluid, yeast extract and sterile sugars under study (Hungate, 1966) and the contents were thoroughly mixed with the help of sterile glass rods. Two sets were prepared for aerobic and anaerobic cellulolytic bacteria. For anerobic bacteria melted wax was poured over the salt solution. After 72 hours of incubation on salt solution contains HG content were used as inoculums and culture media used for isolating amylolyte, sacchrolyrtic, trchaloytic, lipolytic and proteolytic bacteria were prepared by following the standard procedures.

Determination of protein content from MG and HG homoginate: The soluble protein contents from the MG and HG homoginates were estimated by Lowery's method (Lowery et al., 1951).

RESULTS AND DISCUSSION: The characteristics of digestive enzymes of grabs of *L. lepidophora* and *H. fissa* are summarized in table No. 1.

Effect of pH: In both the gut sections of *L. lepidophora* the optimum pH for amylase and invertase was 7.2; for trehalse was 5.2; for cellulose and lipase was 5.6 and for protease was 11. In case of *H. fissa* optimal pH for amylase and invertase was 7; for trchalase was 5 and for protease it was 11.

Effect of Temperature: In both gut sections of *L. lepidophora* the maximum activity of amylase, invertase, trchalase and cellulose showed at 55^{0C} and for cellulose and lipase maximum activity occurred at $45^{0\,C}$. In case of *H. fissa* optimum temperature for amylase and invertase was 35^{0C}, for trehalase and protease was 55^{0C}.

Effect of Time: The time period for linear activity of enzymes occurred within the 60 min.for amylase, invertase, trehalase and cellulase in both the grubs in their both the gut sections where studied. For protease time duration of 20 min. both the gut sections of both the gut sections were suitable for linear activity. For lipase of *L. lepidophora* the optimum time of 12 min. was found suitable for the linear activity in both the gut sections.

Effect of substrate concentration: The relationship between substrate concentration of starch for amylase in MG and HG of both grubs; of sucrose for invertase in MG of both grubs, of trchalose the trehase in MG and HG of *L. lepidophora* and MG of *H. fissa* were studied. The Km values obtained by plotting Line weaver Burk plots are given in the table no.1.

The effect of high temperature on the stability of MG amylase and invertase of *L. lepidophora*: The theoretical duration of high temperature treatment for 50% loss of activities for these enzymes at 60^{0C} was 86 min for MG amylase and 34 min for MG invertase was observed in *L. lepidophora*.

Bacterialogical Results: The bacetriological studies on the hind gut proctodeal dilation of the *L. lepidophora* grubs contain amylolytic, saccharolytic, trchalolytic cellulolytic, casenolytic and lipolytic bacterial colonies. Amylolytic bacteria were mostly Gram +ve motile rods of yellowish colour and few Gram -ve rods on starch agar medium. The saccharolytic bacteria grown on sucrose agar medium were both Gram +ve and Gram -ve rod colonies. The trehalolytic bacteria were Gram +ve rods grown on trchalose agar medium. In cellulose broth containing filter paper contain many colonies Gram -ve rods and few colonies of Gram +ve rods with few colonies of cocci grown on cellulose agar plate. Many colonies of yellowish red colours were developed on skimmed milk agar plates and they are Gram -ve motile rods. The colonies isolated on olive oil agar plates contain mostly Gram +ve motile rods.

Effect of activators and inhibitors: Both activators Nacl and KCl increases the enzyme activity by 17-22% and inhibitors caused reduction in enzyme activity by 65-76% even at their 0.01 M concentration in assay mixture of MG amylase assays of the grubs.

Specific activities of the enzymes: The specific activity of amylase is highest followed by invertase, lipase, protease, trehalase and cellulase in descending order.

The white grubs under study ingest the roots of various agricultural crops and the grasses and their effects on these crops is of great economic importance. But the basic information on their digestive physiology is lacking. The present *in vitro* studies indicate that the nutrients present in such diet must be extracted with the assistance of digestive enzymes present within the mid and hind gut regions as the presence of digestive enzymes like amylase, invertase, trehalase, cellulase, lipase and protease are detected. Overall the food of these grubs seems to be diluted one because the roots likely to be contain little soluble sugars, proteins and lipids much of cellulose other structural carbohydrates and the utilization such type of food might be of great importance in the nutritional economy of the grubs (Bauchop and Clarke, 1975). Observations on the chemical composition of grass roots were made by Sutherland (1971). If this is so the symbiotic micro-organisms present in the gut of the grubs probably play important role in the digestion of structural polysaccharides and other nutrients present in the food. The presence of saccharolytic, amylolytic, trchalolytic, cellulolytic, lipolytic and proteolytic bacetrial microflora is detected in the fermentation chamber of the hind gut of *L. lepidophora*.

Recently investigations on the digestive enzymes in the gut of insects particularly among the insects having xylophagus diet as in scarabaeoid larvae and adults, has become increasingly important for the utilization of biomass resources to produce eco-friendly bio fuels for clean environment (Haung *et al*., 2010 and Wada *et al*., 2014). The digestive enzymes in various scarabaeids were investigated by earlier workers (Wiedemann, 1930; Schlottke,1945; Courtois and Chararas, 1966; Rossler, 1961; Debries *et al*., 1964; Yamane *et al*., 1965; Soo Hoo and Dudzinski, 1967; Bauchop and Clarke, 1975; Mishra and Sensarma, 1985; Bhawane and Bhanot, 1989; Cazemier et al., 1997; Gaikwad *et al*., 1997; Bhawane *et al*. 2016, Gaikwad and Bhawane, 2015a, b, and c; 2016a and b).

The pH value of the MG and HG sections in both the species is 7.5 to 8.5 i.e. neutral to weakly alkaline. Earlier workers have reported alkaline pH for the many scarbaeid larval gut (Soo Hoo and Dudzinski ,1967; Rossler, 1961; Schlott ke, 1945; Wildbolz, 1954; McGhie, *et al*., 1995; Lemke, *et al*., 2003; Egert, *et al*., 2005; Biggs and McGregor, 1996; Ricou, 1958). It is interesting to note that pH value for amylase and invertase is 7.0 to 7.2 for trehalase, cellulase and lipase was acidic between 5 to 5.6 and for protease it was highly alkaline i.e. 11. Results on the similar line was reported by earlier for amylase and invertase in various white grub species (Gaikwad, *et al*., 1997; Bhawane, *et al*., 2016; Gaikwad and Bhawane, 2015 a and b; 2016 a and b). However, in other scarabaeid grub's alkaline pH 8.5 was reported (Wada *et al*., 2014), neutral pH (Yamane, *et al*.,1968) for amylase. Schlottke (1945) reported the optimal pH range of amylase was 6.5 to 8.5 which was less alkaline than the pH value of mid gut (11 to 11.5) and he ascribe this difference in P^H due to the neutralization effect of the food ingested. But in other beetles like *Callosobruchus, Tenebrio* and *Tribolium* pH range for amylase was acidic (4.6 to 5.8) (Podoler and Applabeaum, 1971; Abblabeaum and Konijan 1905 and Bounchore et al, 1976). For the scarabaeid beetle *Adoretus* acidic pH of 6 was reported by Bhardwaj (1986) for amylase and invertase. In other insect's acidic pH, (5.5 to 6.5), for invertase was recorded by earlier workers (Krishna, 1958; Evans and Payane, 1964; Nishide and Kusano, 1976).

The optimal pH range of gut trehalase and cellulase in the white grub species under study was acidic. Observations on the similar line were made in other scarabaeid larvae and other insects by earlier workers (Wharton *et al*., 1965; Huber and Lefebee, 1971; Dahalman, 1971; Potts and Hewitt, 1972; Ishayya and Swirski, 1976; Gaikwad *et al*., 1997; Gaikwad and Bhawane, 2015 a, 2016 a and b and Bhawane *et al*., 2016). The optimal pH of lipase in *L. lepidophora* grub was acidic (5.6). However for other white grubs and in other insects alkaline pH was reported by earlier workers (Gilbert *et al*.,1965; Gerring and Freyvogel, 1975; Male and Storay, 1981; Thomas and Niton, 1984; Teo and Woodring,1988;Gaikwad *et al*., 1997; Gaikwad and Bhawane, 2015c). Mostly the protease in majority of the insects showed alkaline pH and the same is true for both the species of white grubs under study which is higher than the pH of the gut (Ishaaya et al., 1971; Eguchi and Iwamato, 1976; Eguchi *et al*., 1982; Christeller *et al*.,1989; Gaikwad *et al*., 1997; Gaikwad and Bhawane 2015c and Zhang and Brune, 2004). All the enzymes under study excepting invertase in *H. fissa* (35 ^{OC}) showed temperature optima between 45 ^{OC} to55^{OC}. Observations on the similar line made by earlier workers in different

insects including white grubs (Day and Powing, 1949; Terra *et al*., 1977; Kusano and Tanabe 1986; Teo and Heng,1987; Ishaaya and Swirski, 1970; Gaikwad *et al*., 1997 and Gaikwad and Bhawane, 2015c Bhawane *et al*.,2016).

The digestion period of 60 minutes found to be fit within the linear part of enzyme activities of amylase, invertase, trehalase and cellulase in the white grubs under study but the protease and lipase showed period of 20 minutes and 12 minutes respectively for their linear activities. Observations on the similar line made in the scarabaeid grubs by earlier workers (Gaikwad *et al*., 1997; Gaikwad and Bhawane 2015 a,b andc Bhawane *et al*., 2016).

The Km value for amylase in both the gut sections of both the grubs, for invertase of MG section of both the grubs and trehalase in both MG and HG of *L. lepidophora* and MG in *H. fissa* were calculated by drawing the Line Weaver Burk's plots (table No.1). The Km values of MG amylase were comparitively less than the values of HG amylase in both the grubs indicating MG amylase is more efficient than the HG amylase. Earlier workers reported the Km values for amylase in various insects including scarabaeid grubs (Teerra *et al*, 1977; Baker, 1983; Bounciore *et al*., 1976; Poddler and Applebaum, 1971; Gaikwad *et al*., 1997; Gaikwad and Bhawane, 2015 a and b;2016 a and b and Bhawane *et al*., 2016). The Km values of MG invertase in both the grub species were determined which indicates the invertase of *L. lepidophora* is more efficient than the invertase of *H. fissa* earlier workers have reported the Km values for invertase in other scarabaeidae (Gaikwad and Bhawane ,2015 a and b; 2016 a and b and Bhawane *et al*., 2016). The Km values for the trehalase for the present grubs (table No. 1), the trehalase of MG *H. fissa* is more efficient than the trehalase of MG and HG in *L. lepidophora*. In other scarabaeids Km values of trehalase were reported by earlier workers (Bhawane *et al*., 1991; Bhawane and Mandalik, 1992; Gaikwad *et al*., 1997; Gaikwad and Bhawane 2015a and Bhawane *et al*., 2016).

Homoginates of MG in both the grubs when exposed to higher temperature of 60^{0C} for different time periods. MG amylase of *L. lepidophora* was found to be more heat stable than the MG and amylase of *H. fissa* (table No. 1). The observations on the heat stable amylase in other scarabaeids and other insects were reported by earlier workers (Gaikwad *et al*., 1997; Bhawane *et al*., 2016; Gaikwad and Bhawane 2105 a and b, 2016 a and b, Teo, 1973; Ishaaya *et al*., 1971).

From the data presented in the table No. 1 it is very much clear that the mid gut is the major source digestive enzymes in the white grubs under study excepting the HG of *L. lepidophora* where cellulase activity is 3 times higher than the MG. The general view is that the MG is the main site of digestion of food in insects (Dadd, 1970; Law *et al*., 1977; Engelmann and Geraerts, 1980). But in the HG also there is significant activities for most of digestive enzymes studied indicating HG also play important role in the process of digestion of food. Observations on the similar line were made by earlier workers in some insects including scarabaeids (Bhanot and Bhawane, 1989; Bhawane *et al*., 1989 and 2016; Gaikwad *et al*.,1997; Gaikwad and Bhawane, 2015 a, b and c; 2016 a; Thomas and Nitan, 1984). The presence of digestive enzymes in the HG sections of xylophagus and saprophagus grubs under study can be assigned to the bacterial micro-flora in the fermentation chamber The existence of digestion of food and the presence of micro-flora in the HG was reported by earlier workers in scarabaeid gut (Ricou, 1958; Soo Hoo and Dudzinski, 1967; Bhawane and Bhanot, 1989; Gaikwad *et al*., 1997; Bhawane *et al*., 2016). Earlier studies on the occurrence cellulose digesting microbial organism in the fermentation chamber of hind gut existed in scaraba grubs like *Potisia cuprea, Oryetes nasicornis, Costelytra zealandiaca, Pachnoda ephibbiate, P. marginate, Melolontha, melolontha, Holotrichia parellela, Onitis philemon, Seriscesthis germinata* and others (Ricou, 1958; Soo Hoo and Dudzenski 1967; Werner, 1926; Bayon and Mathelin 1980; Cazemier *et al*., 1997 ; Lekme *et al*., 2003; Egret *et al*.,2003& 2005; Zhang and Jackson, 2008; Huang *et al*., 2010; Gaikwad *et al*., 1997). The cellulase activity in the hind gut of *L. lepidophora* occurred in acidic pH of 5.6 and the pH of the gut is neutral to slightly alkaline. However, in the hind gut cellulose activity is reported and it is suspected that proctodeal content might be responsible for maintaining required acidic pH for the digestion of cellulose present in roots ingested by the grubs. The cellulase activity in the hind gut of the scarabaeid grub of Japanase horned beetle, *Trypoxylus dichotomous* by Wada et al. (2014) occurred at neutral pH of 7. The digestive tracts of white grub species under study equipped with digestive enzymes for digesting starch, sucrose, trehalose, cellulose, lipids and proteins and microbial analysis

of HG showed the structural carbohydrates like cellulose and proteins, lipids and other soluble sugars present in the food of the grubs must be efficiently digested and utilized by the grubs and thus helps in nutritional economy.

REFERENCES

Adsule VN and Patil SM, 1990. *Leucopholis lepidophora* Bl. a new white grub pest of groundnut in western Maharashtra. *Groundnut News*. Pp. 2- 7.

Applebaum SW and Konijan AM, 1905. The utilization of starch by the larvae of flour beetle. *Tribolium castaneum. Journal of Nurition.,* **85**:272-282.

Baker JE, 1983. Properties of amylases of mid gut in larvaeof *Sitophilus zeamais* and *Sitophilus granrius. Insect Biochem.* **13**: 421-428.

Bauchop T and Clarke RTJ, 1975. Gut microbiology and carbohydratedigestion in the larva of Costelytva zealandica (Coleoptera: Scarabaeidae), *N. Z. Journal of Zoology.,* **2**: 237-243.

Bayon C and Mathelin J, 1980. Carbohydrate fermentationand by-product absorption studied with labeled cellulose in *Oryctes nasicornis* larvae (Coleoptera: Scarabaeidae). *J. Insect Physiol*., **2**:833–840.

Berberet RC and Helms TC, 1972. Comparative anatomy and histology of selected systems in larval and adult *Phyllophaga anxia* (Coleoptera: Scarabaeidae). *Ann. Entomol. Soc. America.,* **65**(5): 1026-1053.

Bernfeld P, 1955. Amylase a and b. *In Colowick S. P Ed. Method of enzymology*, Academic Press. NewYork., 1:149-150.

Bhardwaj AC, 1986, Digestive enzymes levels in mdgut of *Adoretus lasiopygus* Burmeister (Scarabaeidae: Coleoptea). *Proc.Indian Acad. Sci.* (Anim. Sci.) **95** (2):205-214.

Bhawane GP, Gaikwad YB, Mamlayya AB and Gaikwad AR, 2016. Study on carbohydrases in beetle, *Onitis philemon* (Fab.). *Asian journal of Science and Technology*, **7**(6): 3044-3055.

Bhawane GP and Mandlik DB, 1992. Gut trehalase in adult *Holotrichia serrata* Fab (Coleoptera: Scarabaeidae). *J.Curr.Biosci*, **9**(3): 114-120.

Bhawane GP, Patil SB, Gaikwad AR and Bhanot RK., 1998. Induced effects of plant extractives on the digestive enzymes of the grubs of *Leucopholis lepidophora* Bl. (Coleoptera: Scarabaeidae). *Recent Advances in Ecobiological Research.* Vol-II (Edited by M.P. Sinha) published by A.P.H. Publishing Corporation. New Delhi. pp 249 - 256.

Bhawane G P, Mamlayya AB, Wagh SR and Chaugule AK, 2012. Diversity of white grub beetlesand their host range from Northern Western Ghats, Kolhapur district, (MS) India. *The Bioscan,* **7** (4): 589 -596.

Bhawane GP and Bhanot RK, 1989. Digestive physiology in third instar Scarabaeoid larvae of Holotrichia serrata Fab.J. *Zool. Soc. India*., **40**(1&2):37-44.

Bhawane GP, Bhanot RK and Pawar BK, 1991. Trehalase activity in *Leucopholis lepidophora* Bl. Adult. *Indian J. Comp. Anim. Physiol*., **2**(2): 94-101.

Biggs DR and McGregor PG, 1996. Gut pH and amylaseand protease activity in larvae of the New Zealand grass grub(*Costelytra zealandica*; Coleoptera: Scarabaeidae) as a basis for selecting inhibitors. *Insect Biochem. and Mol.Biol.,* **26:**69–75.

Birk Y, Harpaz I, Ishaaya I and Bondi A, 1962. Studies of the proteolysis activity of the beetle *Tenebrio* and *Tribollium*. *J. Insect Physiol*., **8**:417-429

Bounciore V, Poerio E, Silano V and Tomasi M, 1976. Physical and catalytic properties of amylase from *Tenebrio molitor* L. larvae. *J. Biochem.*,153: 621-625.

Cazemier AE, Hackstein JHP, Op den Camp HJM, Rosen J and van der Drift C, 1997. Bacteria in the intestinal tract of different species of arthropods. *Microbial Ecology,* **33**:189–197.

Cazemier AE, Verdoes JC, Reubsaet FAG, Hackstein JHP, van der Drift C and Op den Camp HJ, 2003. *Promicromonospora pachnodae* sp. nov., a member of the(hemi)cellulolytic hindgut flora of larvae of the scarab beetle*Pachnoda marginata*. *Antonie van Leeuwenhoek*, **83**:135–148.

Christeller JT, Shaw BD, Gardiner SE and Dymock J, 1989. Partial purification and characterization of the major midgut proteinases of grass grub larvae (*Costelytra zealandica*, Coleoptera: Scarabaeidae). *Insect Biochem.*, **19**:221–231.

Courtois JE, Chararas C, 1966. Les enzymes hydrolysant les glucides (hydrates de carbone) chez les insectes xylophages parasites des conifères et de quelques autres arbres forestiers. *Beih. Mater.Org*., **1:**127–150.

Crowson RA, 1981. *The Biology of the Coleoptera*, Academic Press,London, UK.Pp 1-802.

Dadd RH, 1970. Digestion in Insects,*Chemical Zoology Arthropoda, Florkin M. Scheer B.T. eds,*Academic press.New York, Pp.117-145.

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Table No. 1 : Characteristics of digestive enzymes in the white grubs *L. lepidophora* and *H. fissa*

Sr. No.	Enzymes	White grub species	Tissue*	Optimum pH	Optimum temperature 0^{oc}	Optimum time in min.	50% in activation at high temperature, at 60^{0C}.	Specific activity	Km values
1.	Amylase [1]	*L. lepidophora*	MG HG	7.2 7.2	55 55	60 60	at 80 min	763000 138600	0.000042% of starch 0.13% of starch
		H. fiisa	MG HG	7.0 7.0	35 35	60 60		389000 180000	0.000127 of starch 2.06% of starch
2.	Invertase [2]	*L. lepidophora*	MG HG	7.2 7.2	55 55	60 60	at 354 min	1180000 23000	11.84 mM sucrose ---
		H. fissa	MG HG	7.0 7.0	35 35	60 60		91200 42300	16.17 mM sucrose ---
3.	Trchalase[2]	*L. lepidophora*	MG HG	5.2 5.2	55 55	60 60		1190 452	29.45 mM of Trchalose 531.38 mM of Trchalose
		H. fissa	MG HG	5.0 5.0	55 55	60 60		2540 426	
4.	Celluose [2]	*L. lepidophora*	MG HG	5.6 5.6	45 45	60 60		157 410	
5.	Protease [3]	*L. lepidophora*	MG HG	11.0 11.0	55 55	20 20		12000 1300	
		H. fissa	MG HG	11.0 11.0	55 55	20 20		1100 2510	
6.	Lipase [4]	*L. lepidophora*	MG HG	5.6 5.6	45 45	12 12		13800 2280	

* Tissue MG = mid gut and HG = Hind gut 1) Activity of amylase = µg maltose / mg protein / hr, 2) Activity of invertase, trchalase and cellulose = µg glucose /mg protein/hr 3) Activity of protease = µg tyrosine / mg protein / hr and 4) Activity of lipase = µg palmatic acid / mg protein / hr.

Table No. 2 : Effect of Activities and Inhibitors on the MG amylase of *L. Lepidophora* and *H. fissa*

Sr. No.	White Grub Species	Activators/ Inhibitors	Activators/ Inhibitors in assay mixture				Activity of control enzyme*
			0.5 M	0.1 M	0.05 M	0.01 M	
1.	*L. Lepidophora*	Nacl Activator	5860	5280	4379	3585 (+22%)	2920
	H. fissa		3284	2597	2146	1416 (+22%)	1159
2.	*L. Lepidophora*	KCl Activator	4683	4164	31821	3511 (+20%)	2920
	H. fissa		2511	2103	1438	1359 (+17%)	1159
3.	*L. Lepidophora*	$MnCl_2$ Inhibitor	--	140	525	837 (- 70%)	2920
	H. fissa		--	236	494	635 (- 65%)	1159
4.	*L. Lepidophora*	$MgCl_2$ Inhibitor	--	1219	340	796 (-73%)	2920
	H. fissa		--	90	150	280 (- 76%)	1159

* Activity of amylase of MG *L. lepidophora* µg maltose / mg protein / hr.
Figures in parenthesis indicate % increase or decrease of amylase activity due activators and inhibitors.

Dahalman DL, 1971. Purification and properties of trehalase from tobacoo hornworm larvae. *J. Insect Physiol.,* **17**: 1677-1687.

Day MZ and Powing RF, 1949. A study of the processes of digestion in certain insects. *Australian Journal Scientific Research.* **2**: 175-215.

Debris MM, Chararas C and Courtois JE, 1964. Répartition des enzymes hydrolysant les polyssacharideschez quelques insectes parasites des peupliers et un xylophage du cèdre. *C. R. Séances Soc. Biol.Filiales.,* **158**: 1241–1243.

Egert M, Stingl U, Bruun DL, Wagner B, Brune A, and Friedrich MW, 2005. Structure and topology of microbialcommunities in the major gut compartments of *Melolontha melolontha* larvae (Coleoptera: Scarabaeidae). *Applied and Environ, Microbiol.*, **71:**4556–4566.

Egert M, Wagner B, Lemke T, Brune A and Friedrich MW, 2003. Microbial community structure in midgut andhindgut of the humus-feeding larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). *Applied and Environ. Microbiol.*, **69:**6659–6668.

Eguchi M and Iwamoto A, 1976. Alkaline proteases in the mid gut tissue and digestive fluid of the silkworm, *Bombyx mori*. *Insect Biochem*., **6**: 491-496.

Eguchi M, Iwamoto A and Yamauchi K, 1982. Inter- relation of proteases from the midgut lumen, epithelia and peritrophic membrane of the silkworm, Bombyx *mori* L. *Comp. Biochem. Physiol.,* **72A:**359-363.

Engelmann F and Geraerts WPM, 1980. The protease and the protease inhibitor in the midgut of Leucophaea madarae. *J. Insect Physiol*., **26**: 703-710.

Evans WAL and Payne DW, 1964. Carbohydrases of the alimentary tract of the desert locust *Schistocerca gregaria* Forsk. *J. Insect Physiol*., **10**: 657–674.

Gaikwad A R and Bhawane GP, 2016a. Study of carbohydrases in grub of *Onthophagus catta* (Coleoptera: Scarabaeidae: Scarabaeinae). *Asian Journal of Science and Technology*,7 (3): 2618-2625.

Gaikwad A R and Bhawane GP, 2016b. Carbohydrate digestive enzymes in the midgut of dung beetle *Liatongus rhadamistus* (Coleoptera: Scarabaeidae: Scarabaeinae). *International Journal of Entomological Research.,* **1**(6): 37-41.

Gaikwad AR and Bhawane GP, 2015a. Study of carbohydrases in grub of *Liatongus rhadamistus* (Coleoptera: Scarabaedae: Scarabaeinae). *Review of Research,* **4** (10): 1-7.

Gaikwad AR and Bhawane GP, 2015b. Study of Amylase and Invertase Activity in Adults of *Chironitis Arrowi* (Janssens) (Coleoptera: Scarabaeidae: Scarabaeinae). *European academic researcH,* III, **4**:786-4795.

Gaikwad AR and Bhawane GP, 2015c. The Digestive Lipase and Protease in the Dung Beetle, *Chironitis Arrowi* (Janssens). *International journal of science and research,* **4**(11): 1778 -1782.

Gaikwad AR, Bhawane GP, Patil S B and Disale SD, 1997. Digestive enzymes of *Onitis philemon* (Fab.) grubs (Coleoptera: Scarabaeidae: Scarabaeinae). *Recent Advances in Ecobiological Research. Vol-I (Edited by M.P. Sinha),* A.P.H. Publishing Corporation. New Delhi. Pp 335 – 357.

Geering K, Freyvogal TA, 1975. Lipase activity and stimulation mechanism of esterases in mid gut of female *Aedes aegypti*. *J. Insect Physiol*., **21**:1257-1266.

Gilbert LI, Chino H and Domroese KA, 1965. Lipolytic activity in insect tissue and its significance in lipid transport. *J. Insect Physiol*., **11**:1050-1070.

Hayashi K and Tappel A, 1970. Specificity and other properties of lysosomal lipase of ratliver. *J. Biol. Chem.,* **245**:169-175.

Huang SW, Zhang HY, Marshal S and Jackson TA, 2010. The scarab gut: A potential bioreactor for bio-fuel production. *Insect Science*, **17**:175–183.

Huber R E, Lefebee YA, 1971. The purification of some properties of soluble trehalose and sucrose form *Drosophila melanogaster*. *Canadian J. Biochem.,* **49**:1155-1164.

Hungate RE, 1966. *The Rumen and its Microbes.* AcademicPress, New York. Pp1-434.

Ishaaya I and Swirksi E, 1970. Invertase and amylase activity in the armoured scales, *Chrysomphalus aonidum* and *Aonidiella auranti*. *J.Insect.Physiol*., **16**:1599-1606.

Ishaaya I and Swirski E, 1976. Trehalase, invertase and amylase activities in the black scale *Saissetia oleal* and their relation to host adaptability. *J. Insect Physiol*., **22**: 1025-1029.

Ishaaya I, Moore I and Joseph D, 1971. Protease and amylaseactivity in larvae of the Egyptain cotton worm. *Spodoptera littoralis.J. Insect Physiol.,* **17**:945-953.

Itaya K, 1977. A more sensitive and stable colorimetric determination of free fatty acids in blood. *J Lipid Res.*,**18**: 663-665.

Koyama M, Iwata R, Yamane A, Katase T, and Ueda S, 2003. Nutrient intake in the third instar larvae of *Anomala cuprea* and *Protaetia orientalis* submarmorea (Coleoptera: Scarabaeidae) from a mixture of cow dung and wood chips: Results from stable isotope analyses of nitrogen and carbon. *Applied Entomology and Zoology*, **38**:305–311.

Krishna SS, 1958. Further studies on digestion of food in the gut of *Trogoderma* larva. I. Digestive enzymes carbohydrases. *Physiol. Zoology*., **31**: 316-323.

Kusano T and Tanabe S, 1986. Enzymatic properties of the midgut amylase activity and its changes during development in the cabbage armyworm, *Mammestra brassicae* L. *Kontyu (Tokyo)*,54:12-24.

Law JH, Dunn P and Krame KJ, 1977. Insect protease and peptidase. *Advances in Enzymology (Meister, A. Ex.) Vol. 5*, John Wily, New York, Pp 1-335.

Lemke T, Stingl U, Egert M, Friedrich MW and Brune A, 2003. Physicochemical conditions and microbial activities in the highly alkaline gut of the humus-feeding larva of *Pachonda ephippiata* (Coleoptera: Scarabaeidae). *Appl. Environ. Microbiol*., **3**(69): 6650–6658.

Li XZ and Brune A, 2005. Selective digestion of the peptideand polysaccharide components of synthetic humic acids bythe humivorous larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). *Soil Biology & Biochemistry*, **37**: 1476–1483.

Lowry OH, Rosebrough NI, Farr AL and Randall RJ, 1951. Protien measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**:267-275.

Male KB and Story KB, 1981. Enzyme activities and isozyme composition of triglyceride, diglyceride and monoglyceride lipases in *Periplaneta americana, Locusta migratoria* and *Polia adjuncta*. *Insect Biochem.*, **11**: 25-31.

Mane PB and Mohite PB, 2014. Efficacy of newer molecules of insecticides against white grub in sugarcane. *Asian J. Bio. Sci.*, **9** (2):173-177

McGhie TK, Christeller JT, Ford R and Allsopp PG, 1995. Characterization of midgut proteinase activities of white grubs: *Lepidiota noxia, Lepidiota negatoria,* and *Antitrogu consanguineous* (Scarabaeidae, Melolonthini). *Arch. Insect Biochem. Physiol*., **28**:351–363.

McQuillan PB and Webb WR, 1994. Selective soil organic matter consumption by larvae of *Adoryphorus couloni* (Burmeister) (Coleoptera: Scarabaeidae). *Journal of the Australian Entomological Society*, **33**:49–50.

Mishra SC and Sen-Sarma PK, 1985. Carbohydrases in xylophagous coleopterous larvae (Cerambycidae and Scarabaeidae) and their evolutionary significance. *Mater. Org*., **20:**221–230.

Nishide K and Kusano T, 1976. Carbohydrases of digestivetract of the larvae of cabbage butterfly, Pieris rapae Boisduval. *J. Facul. Agric. Tolttori. Univ*., **11**:12-22.

Pal SK, 1977. White Grubs and their Management.Monograph No.5., Director, *Central Arid Zone Research Institute, Jodhpur, (Rajasthan) India*, Pp. 1-42.

Patil, A.S. and Hapase, DG, 1981. Research on sugarcane borers in Maharashtra State. *Proceedings of National Symposium on stalk borer.* Pp.165-175.

Patil SM, Adsule VM and Khaire VM, 1991. Efficacy of some insecticides against the white grub infesting chillies. *J. Maharashtra Agric. Univ.,* **16** (2):276 -277.

Poddler H, Applebaum SW, 1971. The α-amylase of thebeetle *Callosaobruchus chinensis* I. Purification and action pattern. *J. Biochem.,* **121**: 317-320.

Potts RC, Hewitt PH, 1972. Some properties of an aryl-bglucosidase from the hervester termite. *Trinevervitermer trinervoides. Insect Biochem.,* **2**: 400-408.

Raodeo AK, Deshpande SV, Deshpande, AD, Puri SN and Biiapate GG, 1976. A large scale compaign for the control of white grubs *(Holotrichia serrata* F.) in Maharashtra State. *PANS,* **22** (2):223-228.

Ricou MG, 1958. Les diastases du tube digestif de *Melolontha melolontha* L. *Rev. Pathol. Vég. d'Entomol. Agric. Fr*., **37**:249–253.

Ritcher PO, 1966.*White grubs and Their Allies.Number Four*, Oregon State University Press,USA,Pp.1-200.

Rössler ME, 1961. Ernährungsphysiologische Untersuchungen an Scarabaeidenlarven (*Oryctes nasicornis* L., *Melolontha melolontha* L.). *J. Inst. Physiol*., **6** :62–80.

Schlottke E, 1945. Über die Verdauungsfermente im Holz fressender Käferlarven. *Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere*., 61: 88–140.

Soo Hoo CF and Dudzinski A, 1967. Digestion by the larvaof the pruinose scarab, *Sericesthis geminate*. *Entomologia Experimentalis et Applicata*, **10**:7–15.

Sutherland ORW, 1971. Feeding behaviour of the grass grub *Costelytrazealandica* (White) (Coleoptera: Melolonthinae) - 1. The influence of carbohydrates. *N.Z. Journal of Science* **14***:* 18-24.

Teo LH and Heng, SK 1987. The trehalase of the grasshopper *Valanga nigricornis. Comp. Biochem. and Physiol.*, **87**(B)(2):373-378.

Teo LH, and Woodring JP, 1988. The digestive protease and lipase in the house cricket *Acheta domesticus*. *Insect Biochem*., **18**: 363-367.

Teo LH, 1973. Comparison of the quantitative distribution and thermo stability of the digestive enzymes of *Valanga migricornt* (Acrididae). *Nanyang University J*., **7**:78-88.

Terra WR, Ferreira C, De Biavichi AG, 1977. Action pattern kinetic properties and electrophoretical studies of and a-amylase present in midgut homogenates from *Rhynchosceara americana*. Diptera larvae. *Comparative Biochemical Physiology*, **56B**:201-209.

Terra WR, 1990. Evolution of digestive systems of insects. *Annual Review of Entomology*, 35: 181–200.

Thomas K K and Niton JKL, 1984. Protease, amylase and lipase activities in the mid gut and hind gut of the cricket, *Gryllus rubens* and mole cricket, *Scapteriscus acletus*. *Comp. Biochem. Physil*., **2**:297-304.

Wada N, Sunairi M, Anzai H, Iwata R, Yamane A and Nakajima M, 2014. Glycolytic Activities in the Larval Digestive Tract of *Trypoxylus dichotomus* (Coleoptera: Scarabaeidae), *Insects*, **5**: 351-363.

Werner E, 1926. Der Erreger der Zelluloseverdauung beider Rosenkaferlarve (*Potosia cuprea* Fbr.) *Bacillus cellulosam fermentans* n. sp. *Zentrallblatt Bakteriologie* II, **67**: 297–330.

Wharton DR A, Wharton ML, Lola JE, 1965. Celloulase in the cockroach with special reference to *Periplaneta Americana*. *J. Insect Physiol.*, **11**:947-959.

Wiedemann JF, 1930. Die Zelluloseverdauung bei Lamellicornierlarven. *Z. Morph. Ökol. Tiere*., **19**: 228–258.

Wildbolz T, 1958. Beitrag zur Anatomie, Histologie und Physiologie des Darmkanals der Larve von *Mololontha melolontha* L. *Mitt. Schweiz. Entomol. Ges*., **27**: 193–239.

Wolcott GN, 1933. *An Economic Entomology of West Indies*, Porto Rico Entomological Society.Pp.1-688.

Yadava CPS and Sharma GK, 1995. Indian white grubs and their management, *Indian Council of Agricultural Research, New Delhi. Tech. Bull. No.2 All India Coordinated Research Project on white grubs*. Pp.1- 26.

Yamane A, Nitto M and Shibamoto T, 1965. Food habit of forest insects (V). Comparison of carbohydrate-hydrolyzing enzymes from the larvae of wood-boring beetles, Japanese horned scarabaeid, and pine eggar (in Japanese). *Trans. 76th Meet. Jpn. For. Soc*., **1**: 393–395.

Zhang HY and Brune A, 2004. Characterization and partial purification of proteinases from the highly alkalinemid gut of the humivorous larvae of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). *Soil Biology & Biochemistry*, **36**:435–442.

Zhang HY and Jackson TA, 2008. Autochthonous bacterial flora indicated by PCR-DGGE of 16S rRNA gene fragments from the alimentary tract of *Costelytra zealandic* (Coleoptera: Scarabaeidae). *Journal of Applied Microbiology*, **105**: 1277–1285.

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