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STUDIES ON THE HYDROGEN - ION CONCENTRATION AND DIGESTIVE ENZYMES IN THE ADULT OF *PARAMECOPS FARINOSA* (WIED.) (COLEOPTERA : CURCULIONIDAE)

H.P. SAHA AND Y.D. PANDE
DEPARTMENT OF LIFE SCIENCES, TRIPURA UNIVERSITY, AGARTALA - 799004, INDIA.

The hydrogen ion concentration and distribution of digestive enzymes in the alimentary canal of adult *Paramecops farinosa* (Wied.) were studied. pH of the gut of the insect showed wide variations. In fed condition the foregut was acidic (5.8 ± 0.17), the midgut slightly acidic to neutral (6.4 ± 0.03) while hindgut was neutral to slightly alkaline (7.6 ± 0.09). In the gut 9 enzymes, *viz.* amylase, cellobiase, maltase, melibiase, sucrase, trehalase, lactase, lipase and protease were found.

INTRODUCTION

Studies on insect digestive physiology are important, for these are helpful in understanding ways in which stomach poisons are absorbed and assimilated in the insect body and cause mortality. As most of the contact insecticides are ineffective against coleopterous insects owing to their thick integument and, therefore, for their effective control, stomach insecticides which act through digestive system are being increasingly used. Some of the factors which are believed to govern utilization of food constituents in insects are hydrogen-ion concentration, digestive enzymes and rate of conduction of food. Information on these aspects in insects particularly Coleoptera group has been made available by a number of workers namely, Swingle, (1931), Sinha (1958), Lal & Ghai, (1958), Bharadwaj & Goel (1988) and Shukla & Upadhyaya (1990).

Calotropis gigantea (Asclepiadaceae) is a perennial herb with commercial and medicinal values (Ann., 1950). *Paramecops farinosa* (Wied.), a curculionid, was observed to cause serious damage to this herb in Tripura. No attempts seem to have been made to study the physiology of digestion of *P. farinosa*. The present study was, therefore, undertaken to investigate the physiology of digestion of adult *P. farinosa*.

MATERIALS AND METHODS

The adults were collected from their natural habitat and reared in the laboratory to ensure a regular supply for studies. Both fed and starved (18 to 48 hrs.), equal-sized adult weevils were used in the present study. Adults were quickly dissected in living condition in double distilled water. The entire digestive tract was transferred onto a clean glass slide and divided in fore-, mid- and hindgut. Each part was split open longitudinally and the extraneous materials were cleared off. These different gut regions were then processed further for the present study.

pH determination : The determination of pH was done simultaneously by two methods (Terra *et al.*, 1985; Waterhouse, 1940). After dissecting fore-, mid- and hindgut of the fed and the starved (48 hrs) insects, these were collected separately on cavity blocks and either added with 20 µl universal pH indicator (E Merck, pH 4-10) or BDH range indicator solution. The resultant coloured solution was compared with suitable standard. E Merck pH paper method was also employed. The pH of the food material was also determined by similar methods.

Qualitative analysis of enzymes : The homogenate preparation : The fore-, mid- and hind-gut of several starved (24 hrs) specimens were homogenised separately with a little ice-cold distilled water in teflon homogeniser. Homogenates thus prepared were diluted by addition of more water and centrifuged at 3000 r.p.m. for 10 minutes. Supernatants were kept at freezing temperature and used for enzyme assessment.

Enzyme assay : For qualitative analysis of different enzymes, several reaction mixtures were prepared with the gut extract, the substrate and 20 mM phosphate buffer at pH 7.0 mixed in equal

proportion (1:1:1) (Table II).

Table I : Hydrogen-ion concentration in different parts of alimentary canal of *P. farinosa.*

Region	pH	
	Fed	Starved
Foregut	5.8 ± 0.17	ɔ.0 ± 0.05
Midgut	6.4 ± 0.03	5.2 ± 0.01
Hindgut	7.6 ± 0.9	5.5 ± 0.03

A drop of Tolune was also added as antibacterial agent to each tube containing the reaction mixture. Further, the native enzyme extract denaturated in boiling water bath for about 10 minutes was also mixed with the substrate and buffer (1:1:1) to prepare a control mixture. The control and reaction mixtures were then incubated at 37 ± 1°C for 12 to 24 hrs with intermittent shaking. After that the reaction mixtures were subjected to necessary test to detect the presence or absence of the enzymatic activity as adopted by Singhal (1931), Hinman (1933), Baldwin & Bell (1967) and Thomas & Nation (1984).

Table II : Digestive enzymes in the gut of adult *P. farinosa.*

E.C Number	Enzymes tested	Test substrate	Activity in the gut		
			Foregut	Midgut	Hindgut
3.2..1.21	Cellobiase	5% Cellobiose	-	+	-
3.2.1.20	Maltase	5% Maltose	-	=	-
3.2.1.22	Melibiase	5% Melibiose	-	+	-
3.2.1.20, 26	Sucrose	5% Sucrose	-	+	-
3.2.1.28	Trehalase	5% Trehalose	-	+	-
3.2.1.23	Lactase	5% Lactose	-	+	-
3.2.1.1, 2, 3	Amylase	2% Starch	+	+	-
3.1.1.3	Lipase	Olive Oil	-	+	-
3.4.21.3	Protease	2% Casein	-	+	-

+ = Present; - = Absent

Rate of passage of food : In order to study the time taken in the passage of food from mouth to outside in the form of excreta, insects were starved for two days and then given fresh leaves of *C. gigantea.* Time between the first feeding and the first discharge of the excreta was recorded.

RESULTS AND DISCUSSION

The different parts of the alimentary canal of *P. farinosa* were found to be neither strongly alkaline nor strongly acidic. In the fed condition, the foregut was acidic (5.8 ± 0.17), the midgut slightly acidic to neutral (6.4 ± 0.03) while hindgut was neutral to slightly alkaline (7.6 ± 0.9) (Table I).

In insects, in general, the pH of the various parts of the gut is never strongly alkaline or strongly acidic. However, some exceptional cases which are deviations from the general rule are available. For example, in larva and adult of blowflies (Hobson, 1931; Waterhouse, 1940), in aphids (Bramstedt, 1948) and in the adult mosquitoes (MacGregror, 1931) the gut was reported to be strongly acidic. Whereas in Lepidopterous larvae (Shinoda, 1930a & b) the gut was found to range from slightly to strongly alkaline. The pH of the midgut of *P. farinosa* was slightly acidic to neutral (6.4 ± 0.03) and supports the findings of Waterhouse (1949) who pointed out that "in most insects the midgut digestive juices vary only slightly from neutrality. Goel & Bharadwaj (1981) recorded faintly acidic midgut in case of *Tanymecus sciures* Oliv. In the present study, anterior part of the gut was found to be acidic in nature and grandually decreased towards the posterior region. This is in general agreement with the findings of Krishna & Saxena (1962). Exceptional range of pH from neutrality to alkalinity in stored grain beetle (Sinha, 1958) is negated in the weevil under study. The present study has shown that the pH of food plant of *P. farinosa* was moderately acidic (5.4 ± 0.1), whereas that of the gut of the adult weevil varied from moderately acidic to slightly alkaline (5.8 to 7.6). Singhal (1931) found that the pH of food did not influence the pH of the gut and the present study is in general agreement with this view. Appreciable variation in the gut pH in starved condition may be attributed to the fact

that in this condition as there is no stimulus to cause secretion of the enzyme, the pH remains static, maintaining an acidic medium. This is in agreement with the findings of Bhattacharya *et al.* (1987).

Further, in the present study, in the gut of *P. farinosa* 9 enzymes, *viz.* amylase, cellobiase, maltase, melibiase, sucrase, trehalase, lactase, lipase and protease were found (Table II). Presence of these enzymes points out that this weevil has the power of digesting the various components of its food like different carbohydrates alongwith protein and fat. Studies of Wigglesworth (1927 & 1953) and Day & Waterhouse (1953) indicated that the kind of enzymes present in an insect gut was generally correlated with chemical nature of its diet. The presence of lactase, a milk sugar digesting enzyme, in *P. farinosa* supports the remark: mere presence of an enzyme does not ascertain the digestion of the specific substrate (Fraenkel, 1940). Krishna & Saxena (1962) and Verma & Prasad (1972 & 1973) have reported its presence in the phytophagous insects, although lactase is a mammary gland product (Shukla & Upadhyay, 1990).

The enzyme distribution pattern in the present study showed that the enzyme, except amylase, were confined to midgut only. This indicates that the midgut is the chief digestive and absorptive part of this insect. It is in conformity with those of Krishna (1955) in *Trogoderma* larva; Lal & Ghai (1958) in *Aulacophora foveicollis*; Verma & Prased (1972) in *Mylabris pustulata*, and Goel & Bharadwaj (1981) in *Tanymecus sciures*. Since the salivary glands were absent in the insect, presence of amylase in the foregut seems to be strange. In this regard two hypotheses could be put forward. First, the enzyme in question might have passed in the foregut from the labial and mandibular glands. This phenomenon has been reported in certain insects by Soo Hoo & Dudzinski (1967) and Dixit & Mall (1977). Second, it could also be possible that the enzyme present in the midgut finds its way to the foregut by regurgitating movements which might have occurred at the time of dissecting the insect. Regarding the distribution of the enzymes, it may be pointed out that the carbohydrases, proteases and lipases are abundant in the midgut region where pH is slightly acidic. It may be reasonable to believe, therefore, that the pH optima of these enzyme lie in the slightly acidic region.

The average rate of passage of food from mouth to outside was one hour and thirty-five minutes.

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