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HYDROGEN-ION CONCENTRATION AND THE DIGESTIVE ENZYMES IN THE DIGESTIVE TRACT OF *ODOIPORUS LONGICOLLIS* (OILV.) (COLEOPTERA : CURCULIONIDAE)

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The present study on the pH and digestive enzymes of the banana pseudostem weevil *Odoiporus longicollis* (Oliv.) is one of the scientific approach because this insect is a serious insect pest of banana plantation in the state and North East India .The digestive tract is divisible into foregut (stomodaeum), midgut (mesenteron) and hindgut (protodaeum). The different parts of digestive tract of fully grown larvae showed p^H ranging from 6.0-6.4 in the stomodaeum, 6.8-6.9 in the mesenteron and 6.1-6.4 in the proctodaeum, respectively. Whereas the different parts of digestive tract of adult revealed p^H ranging from 6.1-6.4 in the stomodaeum, 6.8-7.0 in the mesenteron and 6.3-6.6 in the proctodaeum. The haemolymph of the larvae had p^H ranging from 6.6-6.8. The p^H of the healthy pseudostem was recorded ranging from 5.4-5.8 and that of infected pseudostem weevil was recorded from 6.5-7.5. The different parts of the alimentary canal of fully grown larvae showed the presence of 7 p^H digestive enzymes in the mesenteron, 3 p^H in the proctodaeum and nil in the stomodaeum. The presence of 7 p^H digestive enzymes in the digestive tracts of both the larval and adult stages except the enzyme lipase which is absent in the digestive tract of adult weevil have been observed.

Key words : pH, digestive enzymes, digestive tract, haemolymph.

INTRODUCTION

The banana pseudostem weevil *Odoiporus longicollis*(Oliv.)is a serious insect pest of banana plantation in North East India and other remaining portion of Indian Union and abroad (Lall 1950, Isahaque, 1978; Prasad & Singh, 1987). The larval instars have done the maximum damage to the crop before and during the fruiting. There are 5 different larval instars among which 3rd, 4th and 5th instars have shown the maximum destruction by boring and cutting with their cutting and chewing type of mouth parts in the inner soft portion of banana pseudostem during June to September. Starting from the 3rd instars, the feeding nature is voracious and the maximum consumption of cut part has been observed during the 4th & 5th instars. Once an individual pest infected a banana pseudostem of a community of banana, it will be propagated as possible as earlier to all the neighbour healthy banana plants before fruiting causing an unexpected damage during fruiting and at the time of harvest. On this account, every body needs how to control this insect pest at low cost because banana is very important fruit of mankind since time immemorable. The hydrogen ion concentration and digestive enzymes in the alimentary tract of fully grown larvae and the adult *Odoiporus longicollis (Oliv.)* are needed to know in detail. So that this present studies have been attempted because no work on the topic had been reported by any worker so far. But in order to control the pest systematically the hydrogen ion concentration (p^H) and digestive enzymes of coleopteran have been studied by Swingle (1930a), Krishna (1958), Lal & Ghai (1958), Chattoraj & Mall (1967) and Awasthi (1968).

MATERIALS AND METHODS

The required materials were procured from the laboratory. The specimens both 5th instar larvae and adult were starved for 6 - 24 hours before taking up the experiments. For the determination of the Hydrogen ion concentration of the different regions of the digestive tract in this insect, various techniques adopted by many workers like Swingle (1930), Waterhouse (1940), Lall & Ghai (1958) and Srivastava & Srivastava (1961). The gut was too small so that the measurements of p^H by p^H meter and have been used the natural diet saturated with suitable indicators also not possible. Therefore, the "Indicator Techniques" adopted by David (1927) and Prasad & Shukla (1975) have been found suitable for the determination of p^H. In addition to this, electronic p^H meter was also used for confirmation of the p^H ranges.

Electronic pH indicator: **:** The starved individuals both the fully grown larvae and adult were dissected as quickly as possible in double distilled water to take out the required alimentary canals with the aid of stereoscopic binocular microscope. The alimentary canals were thus taken out and freed from the extraneous tissues. The taken out canals were washed in double distilled water till the parts were cleared and absorbed the adhering water from the parts with the help of absorbent paper. The different parts of the gut were systematically punctured in order to test the p^H with the cleared dissecting scissors. The solution of oozing fluids were and doubled distilled water of appropriate proportion was prepared immediately and emerged end point of the electronic p^H meter in the solution. After this the numerical indications in the box of electronic p^H meter were recorded . This experiment was repeated 10 times.

Techniques for detection of digestive enzymes

***Preparation of* extract :** For the preparation of extracts for detection of enzymes, the methods adopted by Krishna (1955), Verma & Prasad (1972) were used in the present experiment. In order to prepare the extract, the starved individuals, in case of larvae for 1-2 days and 3-4 days in case of adult were dissected and taken out the alimentary canals. After removing the extra tissues and adhering water, different parts of the alimentary canals like foregut, midgut and hindgut were cut and transferred separately to watch glasses containing 0.1ml of doubled distilled water. In this way, foregut, midgut and hindgut from 15 larval and adult individuals were separately collected in the separate watch glasses where the materials were homogenized with a little amount of glycerin. Then the solutions were centrifuged at 5000-10000 rpm for 15 minutes each. The supernatant thus obtained were incubated with suitable substrate and buffers at 37-38°C. A few drops of toluene were also added to the solution to prevent the bacterial growth. The incubation duration ranged from 24-96 hours. After incubation, the mixtures were subjected to suitable tests to detect the products of digestion. All the tests were accompanied by controls using boiled extract as well as a mixture of substrate buffers and toluene in boiling water for about half an hour in order to destroy the enzyme action.

***Chemical tests* :** After incubation, the mixtures were analyzed by different chemical tests in order to confirm the presence and absence of various enzymes. The methods adopted for the detection of enzymes in the present experiments were those of Swingle (1931), Krishna (1955, 1958), Mall & Chattoraj (1968) and Shukla & Upadhyay (1978).

Table 1 : Tests Employed.

Enzymes	% Substrate used	Tests employed	Results	Phosphate buffer (p^H)
Amylase	0.5 boiled starch	1. KI-iodine test. 2. Fehlings test	Absence of blue colour	6.5
Maltase	3% maltose soln.	1. Fehlings soln.test 2. Osazone test.	Reduction of the reagent	6.0
Lactase	5%lactose soln.	1. Benedicts soln.test 2. Osazone test.	Reduction of the reagent	6.0
Invertase	5% sucrose soln.	1. Fehlings soln.test. 2. Osazone test.	Reduction of the reagent	6.5
Lipase	Condensed milk	B.T.B. emulsion test	Change of colour pink to yellow	
Protease	1.Hens egg albumen 2.1% soln.of Alkaline casein.	1% acetic acid	Absence of precipitate	7.0
Esterase	An emulsion of ethyl acetate + 1% Na-carbonate soln + a drop of Phenol red		Change of colour pink to yellow	

RESULTS AND DISCUSSION

P^H of the digestive tract : It is evident from the Table I that the p^H of the stomodaeum, mesenteron and proctodaeum of the digestive tract of both fully grown larvae and adult were acidic in nature ranging from 6.0-7.0. The different parts of digestive tract of fully grown larvae showed p^H ranging from 6.0-6.4 in the stomodaeum, 6.8-6.9 in the mesenteron and 6.1-6.4 in the proctodaeum respectively. The haemolymph of the larvae had p^H ranging from 6.6-6.8. Whereas the different parts of digestive tract of adult revealed p^H ranging from 6.1-6.4 in the stomodaeum, 6.8-7.0 in the mesenteron and 6.3-6.6 in the proctodaeum. The haemolymph of the adult showed the similar p^H with that of larvae. In both the cases, the only mesenteron had weakly acidic. These observations were made after starving the specimens for about 24 hours. It was interesting to observe that there were no much variations of p^H in the well-fed and starved individuals of this insect

p^H of sap of the host pseudostem : The host pseudostem was divided into healthy and infected at the time of observations. The p^H of the healthy ones was ranging from 5.4-5.8 and that of infected pseudostem was recorded from 6.5-7.5(shown in the Table 1).It was therefore, concluded that the healthy host pseudostem had acidic but it was found to change into weakly alkaline in the infested portions of the host pseudostem.

Table II : pH of the different parts of the digestive tract of *O. longicollis*.

No. of Experiment	pH									
	Larval Alimentary tract				Adult Alimentary tract				Host plant Pseudostem	
	STM	MES	PRO	HAE	STM	MES	PRO	HAE	Healt -hy	Infec- ted
1st	6.0	6.8	6.4	6.6	6.1	6.8	6.5	6.8	5.5	6.5
2nd	6.4	6.8	6.4	6.8	6.4	6.8	6.5	6.6	5.5	7.0
3rd	6.4	6.8	6.4	6.8	6.3	6.8	6.5	6.6	5.6	7.5
4th	6.3	6.8	6.2	6.6	6.4	7.0	6.5	6.6	5.5	7.5
5th	6.4	6.9	6.1	6.6	6.4	6.8	6.6	6.6	5.4	7.5
6th	6.3	6.9	6.4	6.6	6.4	6.8	6.3	6.6	5.6	7.0
7th	6.3	6.9	6.4	6.6	6.4	6.8	6.3	6.6	5.8	7.0
8th	6.0	6.9	6.4	6.7	6.4	7.0	6.5	6.8	5.8	7.5
9th	6.4	6.9	6.4	6.6	6.4	7.0	6.5	6.8	5.5	7.5
10th	6.4	6.9	6.1	6.8	6.4	7.0	6.6	6.8	5.5	6.5
Ranges	6.0-6.4	6.8-6.9	6.1-6.4	6.6-6.8	6.1-6.4	6.8-7.0	6.3-6.6	6.6-6.8	5.4-5.8	6.5-7.5
Mean	6.2	6.9	6.3	6.7	6.3	6.9	6.5	6.7	5.6	7.0

STM : Stomodaeum ; MES : Mesenteron; PRO : Prctodaeum; Haemolymph (HAE).

Digestive enzymes in larval gut : The different parts of the alimentary canal of fully grown larvae showed the presence of 7 digestive enzymes in the mesenteron (MES), 3 in the proctodaeum (PRO) and nill in the stomodaeum (STO) (Table II). The detected enzymes were amylase, maltase, lactase, invertase, lipase, protease and esterase. Enzymes like amylase, invertase and protease have been observed to present in both mesenteron and protodaeum but absence of these enzymes have been recorded from the stomodaeum. Maltase, lactase, lipase and esterase could also be detected from the mesenteron but absent in both stomodaeum and protodaeum. The presence of these enzymes revealed that the larva was capable of digesting various food components like starch, sucrose, lactose, maltose, proteins and fats. It is therefore, concluded that the most of the digestion was taken place in the mesenteron and there was no digestion in the stomodaeum due to the lack of digestive enzymes.

Digestive enzymes in the adult gut : It is evident from the Table III that the adult digestive tract possessed 6 enzymes in the tract. Most of these enzymes could be detected from the mesenteron while the stomodaeum and proctodaeum seemed to be devoid of any enzymes except amylase which was detected in proctodaeum. The detected enzymes were amylase, maltase, lactase, invertase, protease and esterase. The enzyme lipase was not found in the digestive tract of the adult. It is cleared that the adult weevil was able to digest foods comprising of starch, maltose, lactose, proteins with less amount of fat.

The activities of these enzymes were very much depends on the concentration of pH. For example, amylase was active at 6.0-7.0 pH and protease was active at 7.0-7.5 pH. Beyond these pH, the activities of the enzymes were very much reduced (Krishna, 1958).

Table III : Digestive enzymes in the different parts of digestive tract of *O. longicollis* (both fully grown larva and adult).

S. No.	Enzymes	Fully grown Larvae			Adult		
		STO	MES	PRO	STO	MES	PRO
1.	Amylase	-	+	+		+	+
2.	Maltase	-	+	-	-	+	-
3.	Lactase	-	+	-	-	+	-
4.	Invertase		+	+		+	
5.	Lipase		+				
6.	Protease	-	+	+	-	+	-
7.	Esterase	-	+	-	-	+	-

Indications : + = Presence; - = Absence.

It is evident from the Table-II that the p^H in the digestive tract was ranging from acidic to neutral in both the digestive tracts of *O. longicolli*. In case of haemolymph p^Hs for both the stages were also ranging from 6.6-6.9. Such observations were coincided with those of David (1927), Marshall (1939) and Awasthi (1968). The p^H in the divisions of digestive tract of fully grown larvae were 6.0-6.4 in the stomodaem, 6.8-6.9 in the mesenteron and 6.1-6.4 in the proctodaeum, respectively while that of adult digestive tract were 6.0-6.4 in the stomodaeum, 6.8-7.0 in the mesenteron and 6.3-6.6 in the proctodaeum. This shows that these digestive tracts are possessing weakly acidic to almost neutral. And, a gradual increase of the p^H from stomodaeum to the mesenteron and again decrease from mesenteron to proctodaeum is in conformity with those of Swingle (1931), Srivastava & Srivastava (1961). The p^H in the stomodaeum and proctodaeum of both the tracts have been found to be acidic. The p^H of the mesenteron is ranging from 6.8-7.0 in both the cases. This observation is agreed with that of Waterhouse (1949).

The pseudostem of the host has p^H ranging from 5.4-7.5. The healthy pseudostem possesses p^H from 5.4-5.8 while the infected pseudostem has p^H ranging from 6.5-7.5. These ranges in the p^H of the host pseudostem are related with the feeding of the pest. This observation is in conformity with that of Rastogi & Datta Gupta (1962).

The Table III has revealed the presence of seven digestive enzymes in the digestive tracts of both the larval and adult stages except the enzyme lipase which is absent in the digestive tract of adult weevil. The presence of such enzymes indicate that the insect can digest various food components like starch, maltose, sucrose, lactose along with protein and fat.(Mall & Chatoraj,1968). The correlation of enzymes, their substrate and pH is clearly seen in Table I. This observation is in conformity with that of Fraenkel (1940). The presence of maximum number of enzymes in the mesenteron and absence of enzymes in the stomodaeum of both the larval and adult stages shows that the mesenteron is the chief digestive and absorptive part of the insect gut and it is further, suggesting that no digestion occurs in the stomodaeum while a partial digestion may occur in the proctodaeum. Such observations are in conformity with those of Krishna (1955) and Goel & Bhardwaj (1981).

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