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Histochemical detection of protein concentration in the gut lining of larval instars (Ist- IVth) of *Callosobruchus chinensis* Linn. propagating on moong.

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Abstract- This study was carried out to assess the localization of protein in the gut of larval instars of *Callosobruchus chinensis* Linn. propagating in Moong. Histochemical analysis of the gut lining of the larval instars in *Callosobruchus chinensis* showed glycogen. However, the protein content was not the same in foregut, midgut and hindgut of larval instars.

Key words: Protein, Gut Lining, Larval instars, *C.chinensis.*

INTRODUCTION

The aim of the current study was to analyze histochemical characterization and the determination of protein in the gut lining of larval instars for better understanding for the presence of Protein. The gastrointestinal system is one of the most metabolically active system and prominent at all life stages due to energy utilization and perform digestion of the food, nutrient absorption and expelling waste products. Many compounds act mainly on the midgut of the insects interfering with larval development, even at no - lethal concentration. In this view, compounds can affect energy reserve (Sugar, Glycogen, Protein and Lipids) altering the development of the larvae and the adults.

In the larval phase, insect use carbohydrates and protein as the main source of energy for their development.[1] The gut sections of the larval instars subjected to HgBB histochemical staining technique have however displayed a concentric lining of protein deposition.

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MATERIALS & METHODS

I. Maintenance of laboratory culture of the pest:

Fresh adult pest assorted from the host specific sampling jars were released in pairs of both male and female in small size sterile multiplication jars of 200ml size containing 50gms of uninfected fresh seeds of Moong to facilitate their fresh reproduction and perpetuation. The multiplication jars were tag labeled as MPC (Moong Pest Culture) in order to get the accurate observation and results. Five replications of each culture were simultaneously maintained having the jar tags MPC I-IV respectively. In each unit jar of host specific culture of the pest beetle atleast 50gms of fresh uninfected and properly cleaned host grains was kept as the host food resources on which atleast 5 pairs of male and female adult beetles were release for propagation.

II. Collection of larval instars for histochemical studies:

As a matter of fact, the eggs laid on the outer seed coat of Moong were noticeable under hand lens or dissecting binocular microscope but the larval instars growing inside the cotyledons seed kernel of the grains were not easily observed for recording the emergence of

first larval instar and its successive growth through different instars totaling for a number in all the host varieties. The infected grains were soaked in lukewarm water and cut open with the help of scalpel. The larval instar growing inside the seed kernel making zigzag tunnel with the help of their strong mandibular teeth were carefully taken out from the tunnels with the help of sharp pointed needle and brush.

On the basis of the size and shape larval instars collected from the infected seed kernel were assorted into host wise plastic tube marked ML-I to ML-IV. These larvae procured from two different host culture jars, served as the essential raw material for the histochemical studies of the gut for localization of tissue proteins in relation to different host of leguminous grains.

Fixative Solution

1. Carnoy's Fixative

Rec. for carbohydrate and protein histochemistry; Fixation -3-6 hr.

Absolute alcohol ..... 60.00 ml
Chloroform ..... 30.00 ml
Glacial acetic acid ..... 10.00 ml
Wash 2-3 hrs. in absolute alcohol

Chapman's Mercury Bromophenol Blue Technique for Proteins.

Fixative - Carnoy's Fixative.

Procedure:

- The slides were deparaffinized in xylene for 10 min.
- The sections were hydrated by passing through absolute, 90%, 70%, 50%, 30% alcohol and distilled water: 5-10 min each or dips.
- The slides were stained in 1% $HgCl_2$ + 0.05% sodium bromophenol blue + 2% aqueous acetic acid for 10 - 15 min.
- The slides were transferred to 0.05% acetic acid for 2 changes of 5min.
- The slides were transferred to tertiary butyl alcohol for 2 changes of 30 min.
- The slides were transferred to 0.5ml n-butylamine in 100ml xylene until sections turn blue completely.
- The slides were transferred to xylene for 2 changes of 10 min.
- The slides were mounted in DPX.

OBSERVATION

HgBB histochemical staining for host specific detection of protein in the Foregut sections of larval instars (I^{st}-IV^{th}) of *C.chinensis* L. feeding on Moong (Table 1).

The unstained section of the foregut of all the three larval instars developing on Moong host, when treated with the histochemical stains HgBB for locating the protein deposition have also given variegated degrees of colour reactions as follows. The second and third larval instars foregut wall display trace to moderate reaction in the form of light blue coloration in all the layers of the gut wall, more intense in the cuticular intima (Table-1).

On the contrary the IInd instar larval foregut derived from Moong host as given more prominent blue colour reaction which also shows (+++) higher amount of protein present in these sections, unfortunately the sections of the IV^{th} instar could not give desired colour reaction in the larvae developing on Moong host except in the cuticular intima (+++) gives intense degree reaction due to absence of requisite protein (Table-1).

HgBB histochemical staining for host specific detection of protein in the Midgut sections of larval instars (I^{st}-IV^{th}) of *C.chinensis* L. feeding on Moong (Table 2).

The status of staining reaction in the midgut section of the II^{nd} larval instar feeding on Moong host has given greenish blue indicating the qualitative & quantitative differences of protein present in epithelial layers as well as layers of muscle fibres. Similar colour reaction has been also observed in the III^{rd} larval instar midgut sections with prominent cryptic cells coloured blackish blue and light blue in the larval growing in Moong respectively(Table 2).

The IV^{th} larval instar midgut section has almost given similar blue colour reaction without black or green tinse developing on Moong host (Table-2). It might be indicative of the presence of identical type of protein in these layers.

HgBB histochemical staining for host specific detection of protein in the Hindgut sections of larval instars (I^{st}-IV^{th}) of *C.chinensis* L. feeding on Moong (Table 3).

The condition of histochemical reaction appears hindgut section of the larvae II, III & IV thriving on Moong host have provided more intense blue and green colour reaction in II^{nd} and IV^{th} instar respectively. The staining status of the III^{rd} larval instar hindgut section on Moong host is disproportionate - some part of the epithelial follicle staining very light almost negative while other staining deep blue or highly positive (Table-3).

Table 1- HgBB histochemical staining reaction of the different layers of foregut of larval instars (Ist – IVth) of *C.chinensis* L. propagating on host Moong.

Host specific layer reaction.	Layers of Foregut of Larval Instar from Moong host					
Larval Instar	Peritonium	Muscles		Basement Membrane	Epithelium	Intima
		CM	LM			
Instar I	No result was achieved.					
Instar II	+	+++	+++	++	++	+++
Instar III	++	+	+	++	++	+++
Instar IV	++	+	+	++	++	+++

Legend: - = No reaction, + = Trace reaction, ++ = Moderate reaction, +++ = High reaction.

Table 2- HgBB Histochemical staining reaction of the different layers of Midgut of larval instars(Ist – IVth) of *C.chinensis* L. propagating on host Moong.

Host specific layer reaction.	Layers of Midgut of Larval Instar from Moong host					
Larval Instar	Peritonium	Muscles		Basement Membrane	Epithelium	Intima
		CM	LM			
Instar I	No result was achieved.					
Instar II	+	+	++	++	++	+++
Instar III	+	+++	+++	+++	++	+++
Instar IV	+	++	++	++	++	+++

Legend: - = No reaction, + = Trace reaction, ++ = Moderate reaction, +++ = High reaction.

Table 3- HgBB Histochemical staining reaction of the different layers of Hindgut of larval instars(Ist – IVth) of *C.chinensis* L. propagating on host Moong.

Host specific layer reaction.	Layers of Hindgut of Larval Instar from Moong host					
Larval Instar	Peritonium	Muscles		Basement Membrane	Epithelium	Intima
		CM	LM			
Instar I	No result was achieved.					
Instar II	+ +	+	++	-	+	+
Instar III	++	++	++	-	+	+
Instar IV	++	++	++	-	+	+

Legend: - = No reaction, + = Trace reaction, ++ = Moderate reaction, +++ = High reaction.

RESULT & DISCUSSION

The alimentary canal of insects is subdivided in Foregut, Midgut and Hindgut. The midgut is a region of absorption of nutrients and of synthesis and secretion of enzymes and hormones. This absorption is fundamental to the survival. The species, as any changes in this region may modify the growth and development of the insects.[2]

Histological sections were stained with Mercury Bromophenol blue for total protein, with the objective of targeting the differential deposition of protein in bound as well as free stage in the layers of the gut wall of larval instars II, III, & IV actively feeding on Moong, the histochemical staining technique Mercury bromophenol blue adopted in the present research work provided a very informative picture in respect to the host specific propagation of the pest. The presence polysaccharides by PAS indicates that the total protein (Bromophenol blue) of the mucus gland secretion in B., morio in glycoprotein.[3,4]

The Mercury Bromophenol blue for total protein showed that peritoneum layer of all the larval instar fore, mid- & hindgut region has given trace reaction (+) irrespective of the host varieties, nevertheless, the foregut & hindgut peritoneum of all the larval instar feeding on Moong host has given moderate reaction (++) Table 1,2,3. The intima layer in the foregut region of all the larval instar propagating on Moong host as well as the peritrophic membrane in the midgut region in place of the intima has given moderate to strong reaction indicative of high degree of protein deposition in these layers (Table 1,2)[5]. The high degree of protein deposition is observed in Moong host.

The histochemical findings showing better deposition of proteins in the foregut and midgut muscle layers in the larvae of *C.chinensis* propagating on Moong has given a clear indication of host specificity towards Moong seeds. Similar investigation in terms of characterization of midgut trypsin like protein, enzyme three trypsinogen factors from the lesser grain borer, has been found in the structure of *Rhizopertha dominica*.[6,7]

However, the histochemical result showed that the layers of hindgut in IInd, IIIrd and IVth instar feeding on Moong host has given moderate (++) reaction in peritoneum and muscular layer, almost negative (-) reaction in basement membrane except the epithelium and intima layer which has given trace (+) reaction in all the three instars. Microsection of the same gut area of the larvae derived from Moong has clearly given moderate to high reaction in the layers of peritoneal muscles and intima (Table 1,2,3). Lashman Sah & Srivastava have investigated the histochemical and histopathological changes in protein deposition in hindgut of *C.chinensis* treated with insecticide Gamma-BHC, fenitrothion and Endosulfan.

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