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Histochemical localization of lipids in the larval instar fore-gut and hind-gut peritrophic membrane of gram beetle *Callosobruchus chinensis* Linn.

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Abstract : Very little literature is available on the histochemical detection and localization of lipids in the gut of insects particularly those leading a pest life. Although lipids are biochemically high energy structural molecules which prefer to organized membranes, organelles and other important cellular structures in combination with protein yet the amount of lipid deposition in cells has been found to be very poor. Therefore a concentric blue black layer has been observed by Sudan B staining technique diagnostic of bound lipids in the peritrophic membrane of larval instars particularly in the section of foregut and hindgut. The larvae were cultured on the dry legume seeds of White peas and Rajma and reflected gradiant of variation in the lipid deposition.

Keywords : Lipid,Sudan B,Peritrophic membrane,Larval instars,*C. chinensis* Linn.

INTRODUCTION

The gut of insects is relatively simple and delicate with ectodermal cuticular lining on the inner wall of fore and hind gut especially known as peritrophic membrane.[7]

The biochemistry of peritrophic membrane has revealed that it is made up of large chunk of integral protein called peritrophins[6] and small fraction of lipids to provide hydrophobic elasticity[5]. The gut sections of the larval instars subjected to Sudan B histochemical staining technique have however, displayed a concentric lining of the black layer suggestive of significant lipid deposition.

MATERIALS AND METHODS

Since the larval instars of the pest, *C.chinensis* cultured in the lab. On common white peas(*pisum sativum*) and rajma(*vigna radiata*) were very tiny in size, the largest being the IVth instar and measuring upto 0.5 to 0.7 mm. It was very difficult to take out the gut of these larvae hence the entire larval body first transferred to 0.825% sodium chloride solution isotonic for the insect body were fixed in the Zenker's fixative which proved to better over the Carnoy's fixative.

Composition of Zenker's fixative prepared in the lab. was as follow:

A.Potassium dichromate – 250 gms.

B.Mercuric Chloride – 4 to 5 gms.

C.Distilled water – 100 ml.

D.Glacial acetic acid – 0.5 ml.

The entire larvae were transferred to the fixative from 0.825 to normal saline and kept in the fixative from 4 to 24 hrs. depending upon the advancement of larval instar. The entire larvae were shifted to the paraffin boats for preparation and sectioning the body along with the gut. The microtomic serial sections of the larvae (instar wise) were subjected to Sudan B histochemical technique as per following protocol.

Berenbaum's Acetone – Sudan Black B technique for Bound lipids.

Procedures:

A. Deparaffinization of the microtomic sections in xylene:20-30 min.

B. Hydration of the sections by passing through descending alcoholic grades: Absolute,90%,70%,50% 30%

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alcohol and distelled water -5-10 min each.

C. Rinsing in absolute acetone.

D. Staining in 2% Sudan Black B in acetone for 1hrs or mpre at 370c.

E. Transferring to acetone for 10 min.

F. De-staining in xylene : 10 min.

G. Clearing in xylene: 10 min.

H. Mounting in DPX.

OBSERVATION

When the serial sections of all the four larval instars fore-gut and hind-gut (at 6 microns) were subjected to the Sudan B staining technique protocols (Both descending and ascending alcoholic grade treatments) variable degree of blue black colour rings in form of concentric layer in almost middle part of the peritrophic membrane were observed (**Table I**).The variation in the colour intensity of blue black concentric ring signifying the variable degree of deposition and localization of lipids specially of phospholipid category was more prominent in two different host white pea (*pisum sativum*) and rajma (*vigna radiata*) dependent larval instars fore-gut and hind-gut sections. The table I represents mild (+), moderate (++), good (+++), and excellent (++++) orders of colour intensity signifying the related degree of fat deposition.

Table 1:Histochemical detection of lipids in the larval instar gut wall of gram bettle,Callosobruchus chinensis Linn.Propagating of White pea and Rajma legume seeds in storage conditions (1 year storage,moisture content 8.75% in Peas and 5.22% in Rajma)

Larval Instars	Colour intensity by Sudan B* stain for lipids in fore-gut and hind-gut sections** of larval instars going on White peas and Rajma host.			
	White pea dependent		Rajma dependent	
	Fore-gut sections	Hind-gut sections	Fore-gut sections	Hind-gut sections
I	+	++	+	+
II	++	+++	+	+
III	++	+++	++	++
IV	+++	+++	++	++

*= Berenbaum's Acetone-Sudan Black B technique for Bound lipids.

**=Frozen sections cut at 6µ thickness in cryostat.

Key : +=Mild,++=Moderate,+++=Good,++++=Excellent.

RESULTS & DISCUSSION

Sections of the Ist larval instar fore-gut and hind-gut possessing the internal peritrophic membrane protective lining propagating on the White peas have mild (+) and moderate (++) where as those propagating on Rajma host have only mild (+) lipid deposition as reflected by the colour intensity respectively the staining reaction of successive larval instar fore and hind gut derived from the two host further prognose significant variation in the lipid deposition. The fore-gut section of the IInd larval instar from White peas and Rajma display moderate (++) and mild (+) colour reaction where as the hind-gut sections of the same larval instar from the two host display good (+++) and mild (+) colour reactions respectively suggestive of related degree of lipid impregnation in the formation of peritrophic membrane.[4] Similarly the colour reaction of the IIIrd larval instar fore-gut and hind-gut sections display moderate (++) to good (+++) intensity of lipids localization on white peas based larvae but less in rajma based larval instars. The best result have been found in the peas dependent larvae again displayed only moderate colour intensity in the sections of both fore and hind-gut.

The variable degree of the colour intensity of Sudan B stain found in the peritrophic region is related with the possible variation of lipid constituent of the two different host. However with the advancement of larval instar growth and development better colour intensity has been observed in the IVth larval instar gut sections of the pest, *Callosobruchus chinensis* Linn. propagating on the white peas.[2] The scattered picture of histochemical localization of lipids by Sudan B in the peritrophic membrane of the pest can also be co-related with the site specific metabolic role of non cellular lipids deposited as buffer stack for mobilization in the catabolic process directed towards energy production[3].

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