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LIPID CHANGES IN THE LARVAE OF *Leucopholis lepidophora (BL)*

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ABSTRACT

The neutral lipid (NL) and phospholipids (PL) with their constituents were studied in third instars larvae of white grubs of *Leucopholis lepidophora* by employing thin layer chromatography (TLC) and bioassay technique. The neutral lipid increased about 38% from early to late third larval instars. The percentage of triacylglycerol in early to late third third larval instar was 87.76 and 86.79 respectively. The ratio of NL:PL in early and late third larval instars was 2:1. The neutral lipid is dominated over the phospholipids. Phospholipids gradually increased from early to late third larval instars.

Neutral lipids consist of six components. Among the neutral lipids triacylglycerol (TG) as the major component, monoacylglycerol (MG) and diacylglycerol (DG) in moderate concentration, whereas; cholesterol (CHO), cholesterol ester (CE) and free fatty acids (FFA) in low quantity. Among the phospholipids; Phosphatidyl-choline (PC) and phosphatidyl-ethanolamine (PE) were main phospholipid constituents; lysophosphatidyl- lysophosphatidyl-choline (LPC), sphingomyelin were modrate in concentration . Phosphatidyl-inositol (PI), phosphatidyl-serine (PS), and phosphatidiec acid (PA) were low in quantity. The physiological significance with their constituents were discussed in relation with larval development.

Keywords: Neutral Lipids, Phospholipids, TLC, Larvae and *Leucopholis lepidophora*

INTRODUCTION

The white grub larvae of *L. lepidophora* are important pest of sugar cane in general and south west Maharashtra in particular, **[1]**. The Larval development persists of 225-295 days. It damages the roots of different crops. So it becomes a problem of sugarcane cultivators, especially in Maharashtra.

Lipid is bio-chemically important component of the insect. Recently it has been observed that, many of the pesticides and the insecticides were accumulated in lipid. Lipid performs a variety of functions in insect physiology; such as, the TG is utilized for biological energy **[2, 3]**. The role of sterol in insect development and metamorphosis was described by [**4-6]**. The significance of phospholipid with PC and PE was explained by **[7-9]**.

The laboratory of the Department of zoology, Shivaji University Kolhapur is actively engaged in research on the white grubs. **[10]** studied the biology of the white grub in Kolhapur region; while **[11]** investigated biochemical aspects like proteins, carbohydrates and enzymes. The literature survey indicated that, lipid of this species have not been studied. Hence in the present study the lipid were investigated in the third larval instars (which is the polyphagus pest) of *L.lepidophora.*

MATERIALS AND METHODS

The third larval instar of *L. lepidophora* was collected from sugarcane field. They kept in laboratory for acclimatization. After removing the fecal matter, larvae washed with distilled water for twice. Then blot the water with blotting paper. Larvae were accurately weighed and used for lipid extraction.

In the present study a starvation experiment was performed on late third larval instars of *L. lepidophora.* The normal larvae were selected and put in a cylindrical jar containing river water. The mouth of jar kept open for aeration. No food was supplied to the larvae for last nine days (216 hrs). The observation were made on every day which indicate that, the larvae contract the body and remain at the bottom of jar without any movement. After nine days the larvae were removed from the water, and maintain at laboratory condition till they exhibit normal activities and then used for lipid study. The larvae maintain in earthen pots with normal food, treated as control.

EXTRACTION OF LIPIDS

All solvents were of analar grade obtained from E, Merck and Co. Germany, BDH, England. Unless otherwise indicated solvents were re-distilled in the laboratory under anhydrous condition before use.

The early and late third larval instars homogenized with 20 ml. of chloroform-methanol (2:1, v/v) at room temperature. The homogenates were allowed to stand for 2-3 hours at 4^0C and filtered. The filtrate was washed according to **[12]** and evaporated in vacuuo at 40°C. The lipid samples were weighed and preserved at -20^0C until further use. The total lipids in the sample determined gravimetrically.

Separation of Neutral Lipids and Phospholipids

The neutral lipid and phospholipids were separated by thin layer chromatography (TLC) using silica gel G. (about 200 mesh containing $CaSo_4$, as a binder, E Merck Germany). The TLC plates (20 X 20 cm.) were prepared according to **[13]**. The known quantities of samples dissolved in chloroform were applied with Hamiltons microsyringe (No. 8206-B) on activated plates. For neutral lipid the plates were developed in hexane (b.p. 65-70^{o}C) diethyl ether-acetic acid (85:15:2, v/v) as recommended by **[14]**. The phospholipid plates were developed in chloroform-methanol-ammonia (115:45:5 v/v) as recommended by **[15]**. The standards of neutral lipids and phospholipids (Sigma, U.S.A.) were co-chromatographed in each respective run and then plates were kept in iodine chamber for identification of individual spots of lipids.

The iodine was allowed to evaporate and the silica gel from the individual spots of glycerides was scraped and eluted in 1 ml of diethyl-ether and assayed according to **[16]**. The cholesterol and its ester were estimated according to **[17]**. The rest of the neutral lipid components were assayed titrometrically by **[18]**. The Phospholipids were determined by the method of **[19]**.

RESULTS

Neutral Lipids

The TLC separation and qualitative changes in the individual components of neutral lipid are illustrated in **Plate No.1, Figure A**, whereas, **Table 1** exhibits the quantitative alterations in the neutral lipid. It shows MG, DG, TG, CHO, CE and FFA. Quantitatively TG occurred high in concentration MG and DG were moderate in quantity; whereas, CHO, CE & FFA were low in amount.

Neutral lipid in early and late third larval instars was 35.27 and 43.25 mg/gm. wet wt. of tissues respectively. Triacylglycerol in early and late third larval instars was 30.40 and 35.95 mg/gm. respectively. Similarly the values of MG and DG in early and late third larval instars were 1.749, 2.094 and 1.822, 2.840 mg/gm. wet wt. of tissues respectively. The value of cholesterol in early third larval instar was 0.429 and in late third larval instars was 0.629 mg/gm. wet wt. of tissues. In controlled third larval instars the neutral lipid was 74.96 mg/gm. whereas, in starved third larval instars, the NL was 13.44 mg/gm. The values of MG, DG and TG in controlled third larval instars were 4.160, 8.32 and 60.00 mg/gm. respectively. The same decreased values in starved third larval instars were 1.088, 1.632 and 9.248 mg/gm. respectively. The quantity of FFA in controlled third larval

instars was 0.513 mg/gm. increased to 1.212 mg/gm. In starved third larval instars.

Phospholipids

The TLC separation and quantitative changes in the individual constituents of PL are shown in **Plate No.1 Figure B**; whereas, **Table 2** exhibits the quantitative alterations in the PL constituents. The PL reveals following constituents such as, Phosphatidyl – inositol (PI) Lysophosphatidyl – choline (LPC), Sphingomyelin (SPG), Photsphatidyl-Serine (PS), Phosphatidyl Choline (PC), Phosphatidyl-inositol (PI), and Phosphatidic acid (PA). The PC and PE were higher in quantity, LPC and SPG were moderate in quantity, whereas, PI, PS and PA occurred in low quantity.

Phospholipids in early third larval instars were 16.54 mg/gm. increased to 21.63 mg/gm. in late third larval instars. The PC and PE values in early third larval instars were 288.2and 273.5 µg-p/gm. wet wt. of tissues, increased to 359.5 and 386.7 in late third larval instars respectively.

In controlled third larval instars the PL was 5.601 mg/gm. decreased to 4.205 mg/gm. in starved third larval instars. The PC and PE values in controlled third larval instars were 85.99 and 90.75µg-p/gm. In starved third larval instars the PC and PE values was 62.74 and 78.92 µg-p/gm. respectively.

DISCUSSION

Neutral lipid was increased from early third to late third larval instars. The late third larval instars is voracious feeders and excess amount of lipid coming from diet is accumulated in the body. The accumulated lipid is further used for development and growth **[20]**. This finding is in good agreement with the findings of **[21-23]**. The TG also accumulated from early to late third larval instars. The TG accumulated was 86% of the neutral lipid and further used for energy during development. This is in good agreement with the finding of **[3, 24-29]**. Sucrose concentration affected the ability of larvae to accumulate lipid reserves **[30]**. The sterols are very important in insect physiology, cannot be synthesized by the insects. Therefore the diet is only source for cholesterol. The influence of CHO was also observed on TG and FFA. Difference in the distribution of the PUFA (Poly Unsaturated Fatty Acids) between both lipid classes was enhanced by presence of CHO in diet **[4]**.

[5, 6] studied the larvae of *M. domestica* and reported that, larvae will not grow on the diet which exclude CHO. Observing the finding of CHO on various insects reported by many workers and our present data, it is conclude that, the CHO is not synthesized by insects; whatever CHO reported in present investigation might be coming from the diet. The probable functions of CHO in

the larvae coupled with Phospholipids. The CHO-PL synergistically maintained fluidity of membrane and membrane performs various biological functions. In controlled third larval instars neutral lipid was 74.96 mg/gm. was decreased by five fold in starved third larval instars. This decrease was mainly due to TG utilization. In the present study TG was decreased by six fold as comparison between control and starved larvae. TG might be hydrolyzed to DG, MG and FFA for energy. This is good in agreement with the finding of **[2, 3, 23-25, 31, 32]**.

In the present study FFA in controlled larvae was 0.513 mg/gm increased to 1.212 mg in starved larvae; indicates more than double amount of FFA in starved larvae. This might be due to hydrolysis of lipases of TG and convert into FFA, hence the FFA quantity in starved larvae is double which is utilized for energy during development **[31]**. The Phospholipids was increased from early to late third larval instars. The size of the late third larval instars was larger than the size of early third larval instars, which increases the membrane quantity concomitantly phospholipids, PC and PE increases; because PC and PE are membrane constituents. Our results are similar to those of **[7-9, 33]**.

CONCLUSION

While concluding the present study the following are significant findings.

1. The larval development period of *L. lepidophora* is of 225-295 days with three instars
2. Increase in both neutral lipid and phospholipids from early to late third larval instars.
3. In this species neutral lipid is 58.38% and Phospholipid is 42.82% of the total lipids.
4. Cholesterol played important role in larval growth.
5. Monoacylglycerol increased by three folds from early to late third larval instars.
6. Tricylaglycerol is present 86% of the neutral lipid and utilized for energy. This is clearly observed in controlled and starved larvae.
7. In starved larvae FFA is increased more than double as compared with the control, which is utilized for energy.
8. Phosphatidyl-choline and phosphaticlyl-ethanolamine are the main constituents of the phospholipid.
9. Thus in the present study observed that, the TG is hydrolyzed to DG to MG and FFA which utilized for energy for late third larval instars, as

well as controlled and starved larval instars.

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PLATE NO. 1

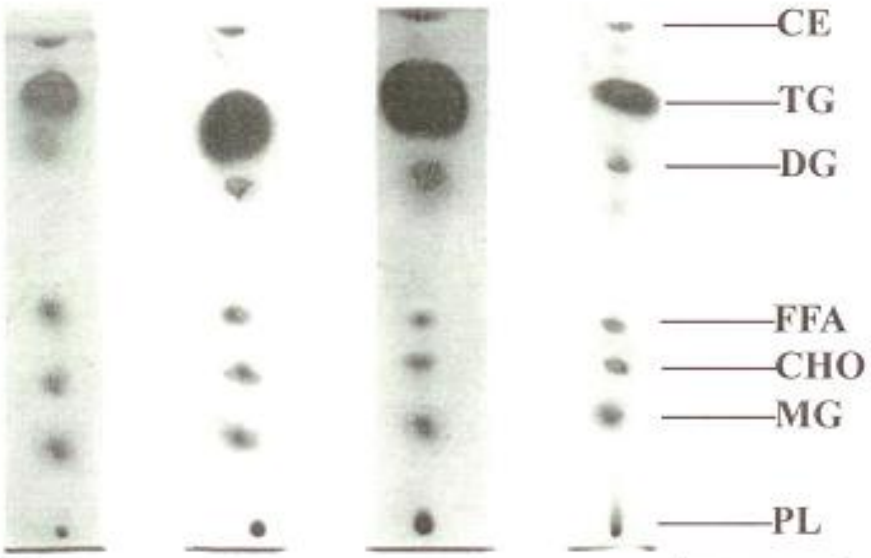

FIG.-A

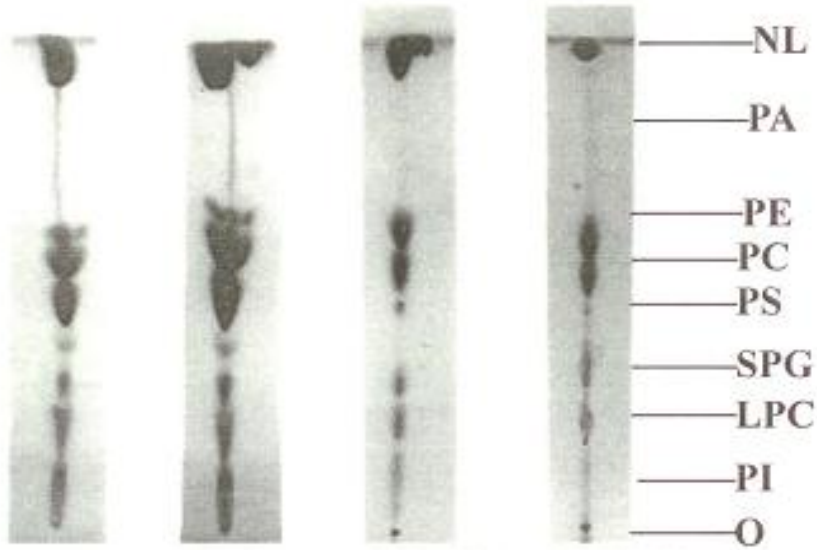

FIG.-B

Table 1: Changes in the Total Lipids, Neutral Lipids and its Components in the Early and Late Third Instar Larvae Control Larvae and Starved Larvae of *L. lepidophora*

	Third Larval Instar		Control Larvae	Starved Larvae
	Early	Late		
Total lipids	51.81± 190	63.88± 2.09	80.56± 9.12	27.645± 13.28
Neutral Lipids	35.27±2.12	43.25± 1.90	74.96± 3.74	13.44± 0.60
MG	1.749± 0.07	3.094± 0.20	4.160± 0.20	1.088± 0.05
CHO	0.429± 0.02	0.629± 0.03	0.958± 0.04	0.127± 0.01
FFA	0.621± 0.03	0.381± 0.01	0.513± 0.04	1.212± 0.10
DG	1.822± 0.08	2.840± 0.08	8.620± 0.36	1.632± 0.05
TG	30.40± 0.1	35.95± 1.70	60.00± 2.98	9.248± 0.46
CE	0.249± 0.01	0.357± 0.01	0.916± 0.45	0.136± 0.01

NOTE: The Values for Total Lipids, Neutral Lipids and its Components are Expressed as mg/gm.wet Weight of Tissues

Table 2: Changes in Phospholipids and its Constituents in Early and Late Third Instars Larvae, Control Larvae and Starved Larvae of *L. lepidophora*

	Third Larval Instar		control Larvae	Starved Larvae
	Early	Late		
Phospholipids	16.54 ± 0.83	21.63± 1.44	5.601± 0..27	4.205± 0.21
PI	20.71± 1.03	21.47± 1.10	5.530± 0..16	2.674± 0.13
LPC	30.75± 1.52	35.00± 1.75	14.24±0..51	9.224± 0.46
SPG	25.47± 2.99	40.16± 2.00	15.05± 0..75	12.55± 0.62
PS	10.24± 0.51	14.58± 1.04	8.330± 0.61	3.348± 0.16
PC	288.2± 14.4	359.5± 17.9	85.99± 4.29	62.74± 3.13
PE	273.5± 13.6	386.7± 19.3	90.75± 4..53	78.92± 3.94
PA	12.70± 1.52	7.843± 0.39	4.332± 0.21	1.402± 0.07

Note: The Values of Phospholipids are Expressed as, mg/gm.wet Weight of Tissues; Whereas, Values of Individual Constituents are Expressed in μg-P/gm.wet Weight of Tissues