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Lipid alteration in Testes and Male Accessory Glands of *Leucopholis lepidophora*

Suvarna B. More

Department of Zoology ,P. V. P. Mahavidyalaya, Kavathe Mahankal, Dist –Sangli. India(MS),416405

Abstract: ***The neutral lipids (NL) and phospholipids (PL) with their constituents were studied in the testes and male accessory glands of Leucopholis lepidophora by employing thin layer chromatography (TLC) and bioassay technique. The quantity of neutral lipids and phospholipids in testes and male accessory glands were 39.51, 36.39, and 12.25, 5.39 mg/gm. wet weight of tissues respectively. Neutral lipids consisted of triacylglycerol (TG) as the major component, monoacylglycerol (MG) and diacylglycerol (DG) inmoderate in concentration, whereas, cholesterol (CHO), cholesterol ester (CE) and free fatty acids (FFA) low in concentration. Phosphatidyl-choline (PC) and phosphatidyl-ethanolamine (PE) were major phospholipid constituents. Phosphatidyl-inositol (PI), lysophosphatidyl- choline (LPC), sphingomyelin (SPG) phosphatidyl-serine (PS), and phosphatidic acid (PA) low in amount. The physiological significance with their constituents was discussed in relation with testes and male accessory glands of Leucopholis lepidophora.***

Keywords: Neutral Lipids, Phospholipids, TLC, testes and male accessory glands , *Leucopholis lepidophora.*

1. Introduction

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 Triacylglycerol is utilized for biological energy [4]. The role of sterol in insect development and metamorphosis was described by Madariaga, *et.al;* and Dwividy [12,5 ,6]. The significane of phospholipids with PC and PE was explained by Locke and Krishnan and Niemierko *et.al;* [11,15]. Lipophorin (Lp) in the hemolymph of insects is known to selectively deliver lipids from sites of absorption or synthesis to sites of storage and utilization, such as the fat body, ovary and testis; Eun-Suk Jung and Hwa-Kyung Yun [7]. The laboratory of Department of Zoology, Shivaji University Kolhapur is actively engaged in research on white grubs. Bhanot studied the biology of the white grubs in Kolhapur region[3], while Patil investigated biochemical aspects like proteins, carbohydrates and enzyme[16]. The literature survey indicated that, lipid of this species have not been studied. Hence in the present study, the lipids were investigated in the testes and male accessory glands of *Leucopholis lepidophora.*

2. Materials and Methods

The female adults of *L. lepidophora* were collected from sugarcane field of Sangrul village. The testes and male accessory glands were dissected out and used for lipid extraction.

I) Extraction of Lipids

The testes and male accessory glands were weighed accurately and homogenized with 20 ml. of chloroform-methanol (2:1, v/v) at room temperature. The homogenate were allowed to stand for 2-3 hours at 4^0c and filtered. The filtrate was washed according to Folch *et.al* and evaporated in vacuum at 40^0c [8] The lipid samples were weighed and preserved at 20^0c until further use. The total lipid in the sample was determined gravimetrically.

II) 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 Wagner *et.al*; [21*]*. The known quantities of samples dissolved in chloroform were applied with Hamilton's micro syringe (No. 8206-B) on activated plates. For neutral lipid the plates were developed in hexane (b.p.65-70^0c) diethyl ether-acetic acid (85:15:2, v/v) as recommended by Gloster and Flecter [10]. The phospholipid plates were developed in chloroform-methanol-ammonia (115:45:5 v/v) as recommended by Barwal and Kalra [2]. 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.

III) Estimation of Neutral Lipids and Phospholipids

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 Viogue and Holman[20] The cholesterol and its ester were estimated according to Abell *et.al;* [1]. The rest of the neutral lipid components were assayed titrometrically by Skipski *et.al;* [17]. The Phospholipid was determined by the method of Marinette [14]

3. Results and Discussion

i) Neutral Lipids

Thin layer chromatography separation of various neutral lipid components are illustrated in Plate No.1, Fig. A whereas, Table No.1 exhibits quantitative variations in the neutral lipid and its components. The neutral lipids were expressed as mg/gm. wet wt. of tissues; and their values in the testes and male accessory glands were 39.51 and 36.39 mg/gm. wet wt. of tissues respectively. The TLC separation of neutral lipids consists of six components; of these

triacylglycerol being major component; monocyglycerol and diacyglycerol were moderate in concentration; whereas, cholesterol, cholesterol ester and free fatty acids were less in quantities. The concentration of TG in The testes and male accessory glands were about 24.66 ± 1.23 and 24.43 ± 1.22 mg/gm wet weight of tissues respectively.

ii) Phospholipids

The thin layer chromatography separation and qualitative changes in the individual constituents of phospholipids are illustrated in Plate No.1 Fig., B; whereas, Table No.2 exhibits the quantitative variations in the phospholipids constituent. The phospholipid values are expressed in mg/gm. wet. weight of tissues and their values in the testes and male accessory glands of *L. lepidophora* were 12.25 and 5.385 mg/gm. wet. weight of tissues respectively. The thin layer chromatography separation of phospholipids indicated the following constituents, PI, LPC, SPG, PS, PC, PE and PA. In the phospholipid constituents PC and PE were predominant and their values in the testes and male accessory glands were, 203.3 and 215.3 µg-P/gm. and 80.14, 75.58 µg-P/gm. wet wt. of tissues respectively. The LPC and SPG were moderate in their values, whereas PI, PS and PA occurred less in amount.

4. Discussion

The testes are made up of a series of tubular follicles varying greatly in number and arrangement in different insects. Each follicle consists at first a layer of epithelium resting on a basement membrane; later the entire group of follicles is enclosed in a connective sheath to form the organ. The phospholipid fatty acids from exocrine and reproductive tissue of male *Periplanata americana* was studied by Stanley- Samuflson and Pipa [18]. They reported that, phospholipid of testes contained higher proportion of palmitic acid than the exocrine tissues. In the present investigation the neutral lipids in testis and male accessory glands consists of, MG, CHO, FFA, DG, TG and CE. Comparatively triacyiglycerol was a major component. MG, DG and cholesterol were observed moderate in quantity, whereas CE and FFA low in quantity. The percent values of TG in testes and male accessory glands were 82.66 and 74.33 mg/gm wet weight of tissues respectively. The role of TG in male accessory glands was probably to provide nutrients to sperms. The phospholipids are expressed as mg/gm. wet weight of tissues. Their values in testes were 12.25 mg/gm decreases to 5.385 mg/gm wet weight of tissues in male accessory glands. This clearly indicated that, the phospholipids in testes were three fold more than male accessory glands. Among the phospholipids PC and PE were major constituents. The values of PC and PE in testes and male accessory glands were 203.3, ± 10.26, 215.3 ± 10.76 and 80.14 ± 475.58 ± 3.62 µg-P/gm. wet weight of tissues respectively. The PC and PE are the membrane constituents; the mature testes exhibit sperms, increases membrane quantities, concomitantly increases the PC and PE. The male accessory glands varied in number and forms and discharge their content into the ducts. In addition in some insects part of the ducts themselves may be glandular in function. The NL: PL ratio in testes and male accessory glands were 3:1 and 8:1 respectively, indicating the dominance of neutral lipids over the phospholipids in testes and male accessory glands. This is an agreement with the findings by Geer on *Drosophila hydal* [9]. and Tierno and Brenner on *Triatoma infestans*[19].

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Table 1: Alterations in total lipids, neutral lipids and its components in testes and male accessory glands of *L.lepidophora*

Lipid type	Testes	Male Accessory Glands
Total Lipids	51.77 ± 5.59	41.77 ± 2.03
Neutral Lipids	39.51 ± 1.97	36.39 ± 1.77
MG	5.035 ± 0.25	4.071± 0.20
CHO	1.039 ± 0.05	0.389± 0.01
FFA	0.232 ± 0.01	1.089 ± 0.05
DG	8.072 ± 0.40	6.107 ± 0.30
TG	24.66 ± 1.93	24.43 ± 1.22
CE	0.472 ± 0.02	0.303 ± 0.01

The values for total lipids, neutral lipids and its components are expressed as mg/gm. Wet weight of tissues.

Table 2: Alterations in phospholipids and its constituents in of testes and male accessory glands of *L .lepidophora*.

Lipid type	Testes	Male accessory glands
Phospholipids	12.25± 0.52	5.385± 0.25
PI	10.52± 0.50	7.010± 0.25
LPC	18.90± 0.96	16.25± 0.92
SPG	20.52± 0.99	19.03± 0.96
PS	9.312± 1.02	8.178± 0.40
PC	203.3±10.26	80.14± 4.00
PE	215.3±10.76	75.58± 3.62
PA	9.320± 0.46	9.218± 0.46

The values for 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.

PLATE - 1

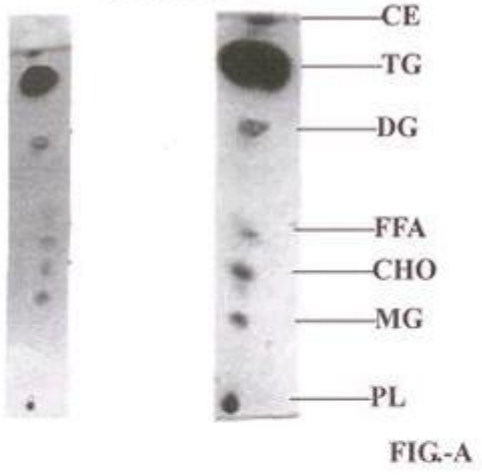

FIG.-A

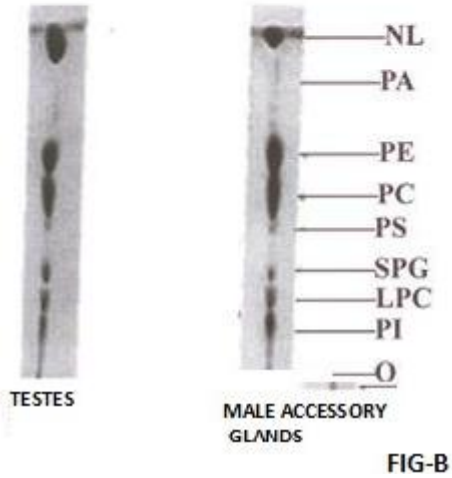

FIG-B