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Composition of Lipids in Flight Muscles of *Leucopholis lepidophora*

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Abstract: ***The neutral lipids (NL) and phospholipids (PL) with their constituents were studied in the flight muscles of male and female adults of Leucopholis lepidophora , by employing thin layer chromatography (TLC) and bioassay technique. The quantity of neutral lipids in male and female flight muscles were measured 39.192 and 40.039 mg/gm wet weight of tissues respectively. The main component of neutral lipids was triacylglycerol. The phospholipid values in male and female were 23.163 and22.999 mg/gm wet weights of tissues respectively. The NL: PL ratio in male and female adults was recorded to 2:1. The neutral lipids found in six forms. Triacylglycerol was the main component, it exhibits 29.60 and 32.08 mg/gm wet weight of tissues in male and female flight muscles respectively. monoacylglycerol, diacylglycerol, cholesterol, were moderate and cholesterol ester and free fatty acids low in quantity. Phospholipids exhibited seven constituents; phosphatidyl-choline and phosphatidyl-ethanolamine in high concentration. I male and female adults it constitutes 376.1, 345.3 and 345.1, 370.5 mg/gm wet weight of tissues respectively. Lysophosphatidyl-choline and sphingomyelin moderate in concentration, and phosphatidyl-inositol , phosphatidyl-serine and phosphatidic acid low in quantity.***

Keywords: lipids, Male and female, flight muscles, Thin layer chromatography, and *Leucopholis lepidophora*.

1. Introduction

The white grub larvae of *L. lepidophora* are important pest of sugar cane in general and south west Maharashtra in particular [2] 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. The white grub have become known polyhagus and found in a particular type of agro ecosystem. In India Lefray [25] Ghosh [19] studied the white grubs of sugarcane. White grubs are most destructive insect pest all over the world. In many insects such as *Manduca sexta*, lipids are major fuel for flight which is mobilized as diacylglycerol (DG) into the circulation [3], [8]. DG is derived from triacylglycerol (TG) stores in the fat body and constitutes the main form in which fatty acids are mobilized to the site of utilization, for example, flight muscle. The mobilization of triglyceride stores during flight is controlled by adipokinetic hormone [18], [40] which increases the rate of triglyceride hydrolysis and promotes a concomitant release of diacylglycerol into the hemolymph. Increases in lipase activity of fat body homogenates after initiation of flight or AKH injection have been observed in several insects [5], [6]. 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. The role of sterol in insect development and metamorphosis was described by Madariaga, *et al*; [29] and Dwivedy [16],[17] The significance of phospholipid with PC and PE was explained by Turunen [38], [27], [32] The laboratory of the Department of zoology, Shivaji University Kolhapur is actively engaged in research on the white grubs. Bhanot [10] studied the biology of the white grub in Kolhapur region; while Patil [34] 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 fat bodies of (which is the polyphagus pest) of *L.lepidophora.* Lipids are bio-chemically important components of insect. Lipid performs a variety of functions in insect physiology. Triacylglycerol is utilized for biological energy [14] The significance of phospholipids with PC and PE was explained by Locke and Krishnan [27]. Flight is important for insect dispersal. In the jargon of the aeronautics industry, flight pushes the envelope of organism design [12]. The main insect muscles are the leg muscles. Flight muscle is one of the most widely studied light-related tissues in insects mainly because of the variation in the metabolic activity of the different types of muscle [36], [39], [30]. The present report describes the lipid profile in flight muscles of *L. lepidophora.*

2. Methods and Materials

The male and female adults of *Leucopholis lepidophora* were collected from sugarcane field of Sangrul village, 18 k.m. away from Kolhapur city (M.S.). Adults were used for flight muscle lipid extraction.

A) Extraction of Lipids

The flight muscles of male and female adults were weighed and 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 Floch *et.al;* [21] and evaporated in vacuum at 40^0c. The lipid samples were weighed and preserved at -20^0c until further use. The total lipid in the sample was determined gravimetrically.

B) Separation of Neutral Lipids and Phospholipid

The neutral lipids and phospholipids were separated by thin layer chromatography (TLC) using silica gel G and about 200 mesh containing CaSo4, as a binder, (E Merck Germany). The TLC plates (20 X 20 cm) were prepared according to Wagner *et.al* [43]. 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. $65\text{-}70^0$c) diethyl ether-acetic acid (85:15:2 v/v) as

recommended by Gloster and Flecter [20]. The phospholipid plates were developed in chloroform-methanol-ammonia (115:45:5 v/v) as recommended by Barwal and Kalra [7] .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.

C) 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 [42] . The cholesterol and its ester were estimated according to Abell *et.al* [1]. The rest of the neutral lipid components were assayed titrometrically by the method of Skipski *et.al* [35]. The phospholipid was determined by the method of Marinetti [28].

3. Results

I) Neutral Lipids
The TLC 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 components. The neutral lipids in male and female flight muscle were measured 39.192 and 40.039 mg/gm.wet weight of tissues respectively. The neutral lipids consists of six components; of these triacylglycerol (TG) being the major component. Monoacylglycerol (MG), diacylglycerol (DG) and cholesterol (CHO) were found moderate in concentration; whereas free fatty acids (FFA) and cholesterol ester (CE) were occurred low in quantities. The TG concentration in male and female flight muscles was about 29.60 and 32.08 mg/gm wet weight of tissues respectively.

II) Phospholipids
The phospholipids are illustrated in plate No.1, Fig B and Table 2. The phospholipids in male and female flight muscles were measured 23.163 and 22.999 mg/gm.wet weight of tissues respectively. The TLC separation of phospholipids included following constituents; phosphatidyl-choline (PC), ,phosphatidyl-ethanolamine (PE), Lysophosphatidyl-choline (LPC), sphingomyelin (SPG), phosphatidyl-inositol (PI), phosphatidyl-serine (PS) and phosphatidic acid (PA). Among the phospholipids PC and PE were predominant. In male and female flight muscle they measure about 376.1, 345.3 µg –P / gm and 345.1, 370.5 µg –P/ gm wet weight of tissues respectively. The LPC and SPG were estimated in moderate concentration, whereas PI, PS and PA less in amount.

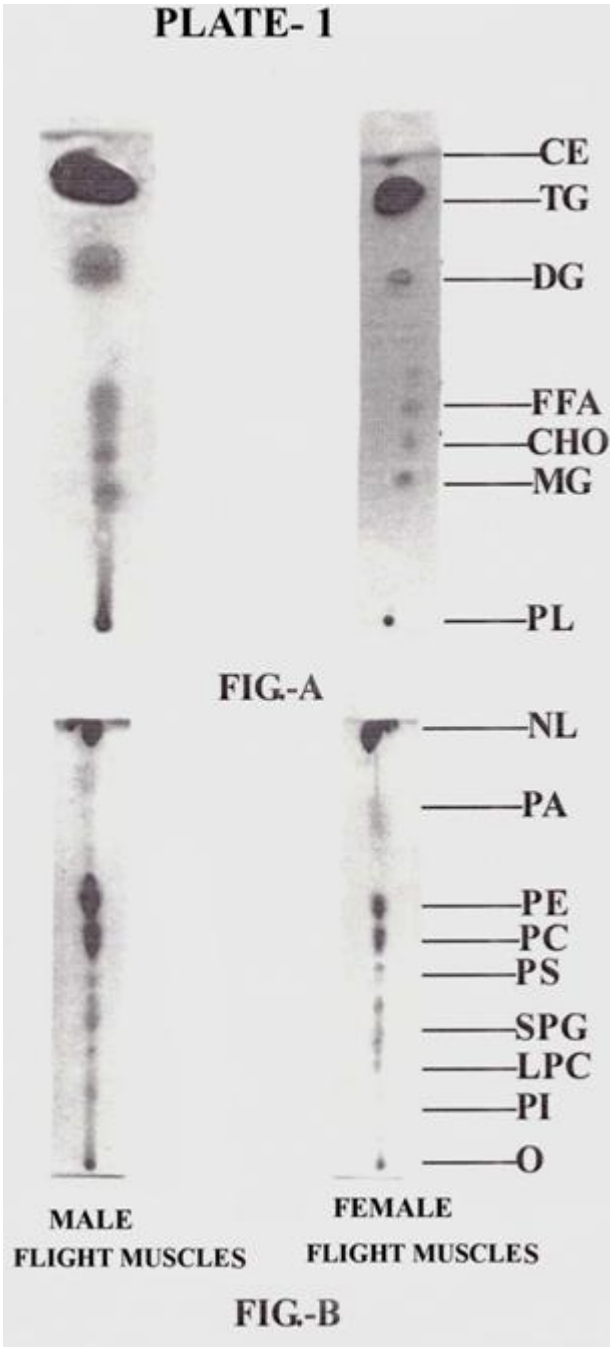

Table 1: Alterations in total lipids, neutral lipids and its components in the male and female Flight muscles of *L. lepidophora.*

Lipid Type	*Male Flight muscles*	*Female Flight muscles*
Total Lipids	62.355 ± 2.44	63.038 ± 2.30
Neutral Lipids	39.192 ± 4.15	40.039 ± 1.23
MG	2.458 ± 0.20	1.465 ± 0.07
CHO	1.626 ± 0.18	1.868 ± 0.09
FFA	0.890 ± 0.08	1.324 ± 0.03
DG	3.346 ± 0.22	2.334 ± 0.10
TG	29.60 ± 2.79	32.08 ± 1.10
CE	1.272 ± 0.05	0.968 ± 0.05

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 male and female flight muscles of *L. lepidophora.*

Lipid Type	Male Flight Muscles	Female Flight Muscles
Phospholipids	23.163 ± 0.32	22.999 ± 0.93
PI	15.70 ± 0.75	20.70 ± 0.68
LPC	66.22 ± 2.79	55.47 ± 3.12
SPG	78.36 ± 2.89	70.22 ± 2.73
PS	23.87 ± 0.79	30.28 ± 0.99
PC	376.1 ± 12.57	345.1 ± 14.9
PE	345.3 ± 14.01	370.5 ± 14.7
PA	20.97 ± 0.68	27.70 ± 2.03

The values of phospholipids are expressed as mg/gm. wet weight of tissues; whereas, values of individual constituents are expressed as µg-P/gm. wet weight of tissues.

4. Discussion

The male flight muscles of *Leucopholis lepidophora* exhibited high concentration of lipids than the female. The NL: PL ratio in male and female adults was 2:1, indicated that the neutral lipids was dominated over the phospholipid. Among the neutral lipids TG constitute the major component. The TG of the neutral lipids in male and female flight muscles was 29.60 and 32.08 respectively. These findings agreed with the findings on *T, castaneum* [7] Domroese and Gilbert [15] studied some aspects of lipid catabolism in moth *H. ceropia*. He reported that, the utilization of lipids as a fuel for flight. Lipid is the available substrate as well as the preferred substrate in flight-muscle metabolism in male moths. TG exhibits high concentration in male and female flight muscle and for utilization as energy source [22], [13]. The ratio of CHO: CE in male and female flight muscles *O. rhinoceros* was 1:1 and 2:1, respectively. Phospholipids are expressed as mg/gm wet weight of tissues and their values in male and female flight muscles of *Leucopholis lepidophora* were 23.163 and 22.999 mg/gm, respectively. In the present investigation male flight muscles exhibited high phosphplipid contents than female flight muscles. Among the phospholipids the PC and PE were major constituents. The PC: PE ratio was 1:1 which indicated that the PC and PE are equal in their values. Kallapur *et .al* [23] studied effect of environmental temperature on lipid composition of flight muscle mitochondria in *S. gregaria* , and observed that elevated temperature resulted in depressed level of PC and increased level of PE. Beenkkers [9] studied transport of fatty acids in *L. igratoria* during sustained flight. He reported that, during flight a considerable increase in fatty acid content was indication of an efficient transport of various species of insects by many investigators, such as Van Handel [41] Locust *migratoria migratorioides* , Mwangi and Gondsworthy [31] *Nilaparrata lugens*, Chi [11] and *Rhapalosiphium maides* Liquido and Irwan, [26] Cheng – Jijiang [12] *G. firmus.*, Zera *et.al.* [44] Zhang *et.al* [45] and Zaho and Zera [46] In insects flight activity usually depends on lipids. 13 Insect flight muscles do not directly store lipids and require an efficient system to transport hydrophobic fatty acids to the muscle cell which in locusts is mediated by apolipoproteins . Lipid utilization during sustained flight has been studied on lepidopteran and orthopteran species by Norbert [33] He reported that, in migrating lepidopteran and orthopteran insects, lipid is the preferred fuel for sustained flight activity. Diacylglycerol is delivered by lipophorin to the flight muscle and hydrolyzed to free fatty acid and glycerol. Lipid is the available substrate as well as the preferred substrate in flight-muscle metabolism in male moth [15] Active fatty acid activating enzymes is present in flight muscle, and fatty acid oxidation in *H. cecropia* is discussed in relation to vertebrate and other invertebrate systems. [17]. Phospholipid synthesis during flight muscle development in american silkmoth *H. cecropia* was studied by Thomas and Gilbert [37]. They found that the major phospholipid fraction of flight muscles and sarcosomes were PC and PE. Atkins studied lipid loss of flight muscle in the beelte *Dendroctomus pseudosugae* and reported that the flown beetles contained significantly less lipids [4].

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