Lipid Profile of Adult Oryctes rhinoceros (Linn) (order: coleoptera, subfamily- fearabaeoidea)

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ABSTRACT:

The neutral lipids (NL) and phospholipids (PL) with their constituents were studied in the male and female adults of Oryctes rhinoceros, by employing thin layer chromatography (TLC) and bioassay technique. The quantity of neutral lipids in male and female were measured 52.70 and 57.03 mg/gm wet weight of tissues respectively. The main component of neutral lipids was triacylglycerol. The phospholipid values in male and female were 17.26 and 16.85 mg/gm wet weight of tissues respectively. The NL: PL ratio in male and female adults was recorded to 3:1. The neutral lipids found in six forms. Triacylglycerol was the main component, 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, Lysophosphatidyl-choline and sphingomyelin in moderate concentration, and phosphatidyl-inositol, phosphatidyl-serine and phosphatidic acid low in quantity.

KEY WORDS: lipids, Male and female adults, Thin layer chromatography, and Oryctes rhinoceros.

INTRODUCTION

Oryctes rhinoceros is the pest of coconut trees. Nirula17 made a thorough inspection of one million coconut palms heavily infected by the rhinoceros beetle and estimated the annual loss as of $ 6, 00,000 to the coconut planters. Recent report of the damage caused by this beetle in Assam and Andaman Islands are very alarming. According to government of Assam, rhinoceros beetle presents a collossal problem to the coconut industry attacking both young and adult trees. Lipids are bio-chemically important components of insect. Lipid performs a variety of functions in insect Physiology. Triacylglycerol is utilized for biological energy (Downer[5]). The role of sterol in insect development and metamorphosis was described by Madariaga *et al*; [16] and Dwivedy [7, 8]. The significance of phospholipids with PC and PE

was explained by Locke and Krishnan[15], The present report describes the lipid profile of adult *O rhinoceros.*

MATERIALS AND METHODS

The late third instars larvae and prepupae were collected from dunghill of Agriculture College, near the Shivaji University, campus Kolhapur, (M.S.) they were reared at laboratory and adults were used for lipid extraction.

A) Extraction of Lipids

The male and female adults (removing wings and elytra) 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;* [10] 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 Phospholipids

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;* [28] 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 [13]. 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.

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 [26]. 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 [24]. The phospholipid was determined by the method of Marinetti[17].

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 were measured 52.17 and 57.03 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 adults was about 44.40 and 50.05 mg/gm wet weight of tissues respectively.

II) Phospholipids

The phospholipids are illustrated in Fig B and Table 2. The phospholipids in male and female were measured 17.26 and 16.85 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 they measure about 280.1, 260.3 µg –P / gm and 279.1, 275.5 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.

DISCUSSION

The female adults of Oryctes rhinoceros exhibited high concentration of lipids than the male. These observations support the finding made by Pagani et al; [20] in Ceratitis capitata. Barwal and Kalra [2] reported that, the NL percentage in Tribolium castanum was 90. The NL: PL ratio in male and female adults was 3:1, indicated that the neutral lipids was dominated over the phospholipid. Among the neutral lipids TG constitute the major component than the remaining ones. These observations support earlier findings on fruit moth Vitulla edmandsae serratilinella by Miller and Blankenship [18]. The TG percentage of the neutral lipids in male and female adults was 87.57 and 90.95, respectively. These findings agreed with the findings on Periplaneta Americana (Reisser and Bollade[21]) and T, castaneum (Barwal and Kalra2). Gilbert [12] reported that, TG represents major lipid component in insects during all stages of insect development Further Walker et al., [27] Chippendale and Cripps *et al* [4]; reported that, TG mobilize during the pharate adult stage and might be utilized for energy, whereas in pharate adults of *Galeria mellonella* and during first day of imaginal life the diglycerides are utilized [22] . Chang-Hao Chien et.al [5] and Estela et al [9] reported that, lipids acts as an energy source during insect development. In the present study, TG exhibits high concentration in male and

female adults and for utilization as energy source. The ratio of CHO: CE in male and female adults of *O. rhinoceros* was 1:1 and 2:1, respectively. These observations support the findings on *Dacus oleae* by Viogue Maza[25]. Phospholipids are expressed as mg/gm wet weight of tissues and their values in male and female adults were 17.26 and 16.85 mg/gm, respectively. In the present investigation male adults adults exhibited high phosphplipid contents than female adults and agreed with the findings on *Pucnoscelus straiatus* by Shehata[22]. Among the phospholipids the PC and PE were major constituents. Similar results were reported by Geer *et a*l [11.] in *Gromphadorhina protentosa.* The PC: PE ratio was 1:1 which indicated that the PC and PE are equal in their values. Similar observations were made on *Sitophilus zeamais* by Yadav and Musgrave[29], on *Gerris remigis* by Lee *et al*., [14] and on *T. castaneum* by Barwal Kalra[2].

TABLE No. 1

Alterations in total lipids, neutral lipids and its components in the male and female adults of *O. rhinoceros.*

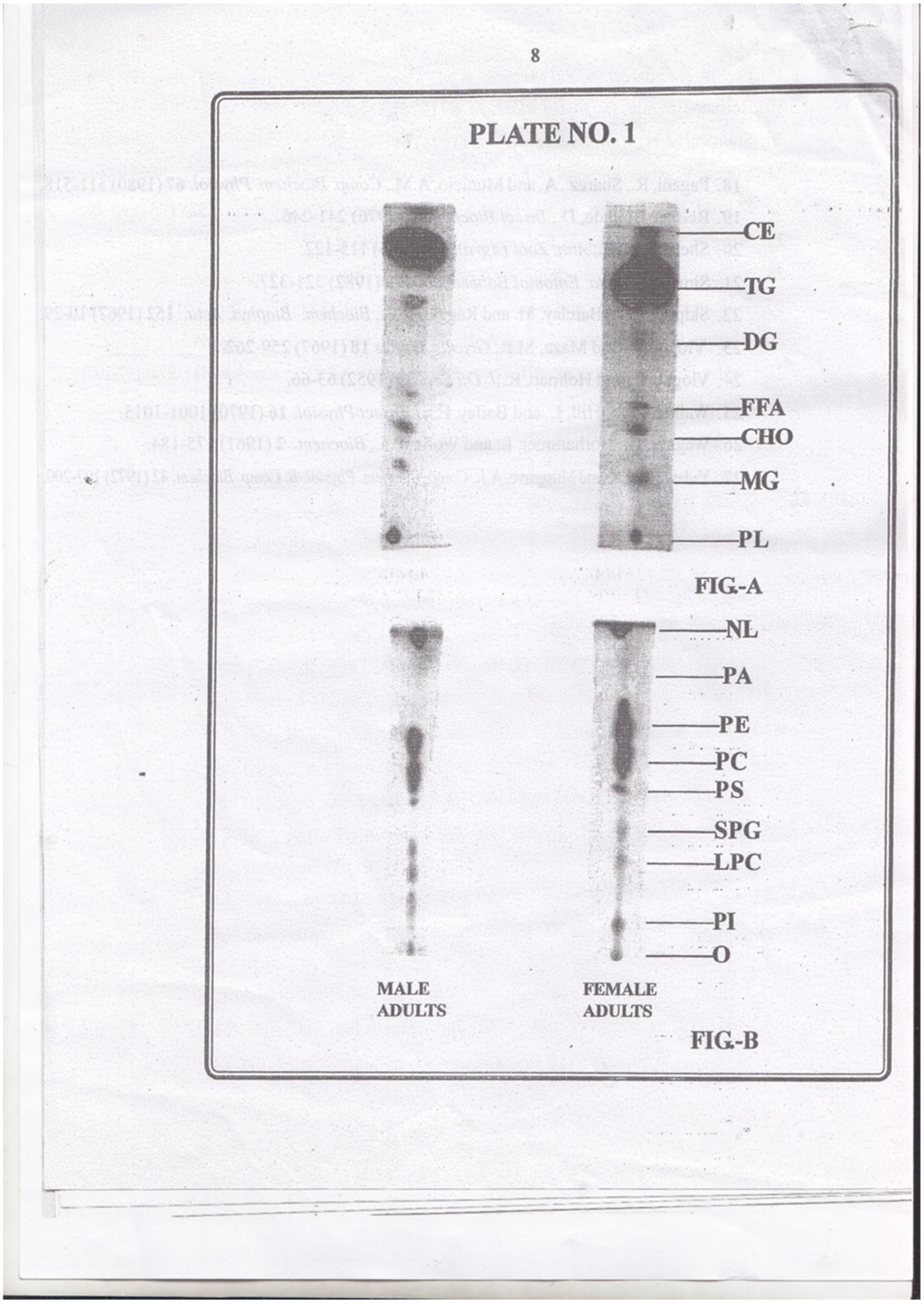
Lipid Type	Male Adult	Female Adult
Total Lipids	69.96 ± 2.74	73.88 ± 2.60
Neutral Lipids	52.70 ± 4.15	57..03 ± 2..23
MG	1.614 ± 0.15	1.343 ± 0.06
CHO	1.826 ± 0.09	0.868 ± 0.06
FFA	0.591 ± 0.04	1..629 ± 0.04
DG	1.867 ± 0.08	1.934 ± 0.06
TG	44.40 ± 2.832	50..05 ± 2.30
CE	1.102 ± 0.05	0.168 ± 0.01

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

TABLE No. 2 Alterations in phospholipids and its constituents in male and female adults of O. *rhinoceros.*

Lipid Type	Male Adult	Female Adult
Phospholipids	17 .26 ± 0.77	16.85 ± 0.83
PI	15.70 ± 0.75	13.70 ± 0.62
LPC	55.21 ± 2.76	40.47 ± 2.12
SPG	51.31 ± 2.56	35.44 ± 1.73
PS	15.16 ± 0.75	10.24 ± 0.51
PC	280.1 ± 4.57	279.1 ± 13.9
PE	260.3 ± 13.01	275.5 ± 13.7
PA	12.87 ± 0.64	20.70 ± 1.03

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.



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