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FATTY ACIDS OF *TROGODERMA GRANARIUM* EVERTS DIAPAUSE LARVAE IN ACCORDANCE TO LARVAL DIET AND REARING TEMPERATURE.

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ABSTRACT: Fatty acids of diapause larvae (DL) reared on three larval diets (wheat, cowpea and peanut) and kept at two constant degrees of temperatures (25 and 35°C) were examined by Gas chromatography.

Six acids accounted for approximately more than 80% of the total were named palmitic (C16:0), palmitoleic (16:1), stearic (C18:0), oleic (C18:1), linoleic (C 18:2) and linolenic (C 18:3). Results showed that C 18:3 either recorded very small relative proportions or completely disappeared. The double unsaturated fatty acid C18:2 was greatly affected by the larval diet in nondiapause larvae (NDL). The monuunsaturated fatty acid (18:1 showed relative proportions in DL reared on wheat and cowpea greater than their corresponding values in NDL. The monounsaturated fatty acid (16:1 took one trend in diapausing larvae, it recorded relative amounts in Dl greater than their corresponding values of NDL.

INTRODUCTION

Diapause in insects is characterized by a number of behavioral, physiological, biochemical, genetic and morphological characteristics (Tauber and Tauber, 1976). The incidence of diapause is influenced by temperature, food, population density and the genotypic composition of the population (Nair and Desai, 1972). The khapra beetle *Trogoderma granarium* Everts is a pest in food stores, particularly of cereals and other seeds. Diapause larvae of this insect species showed an enormous increase in the amount of fat, glycogen and protein over that present in nondiapause larvae (Karnavar and Nair, 1969). An accepted physiological characteristic of diapause is that an accumulation of lipids occur prior to the period of reduced metabolism, the lipids are then gradually utilized as an available energy supply (Tombes, 1966). The later author noticed that weevils of *Hypera postica* clearly minored the fatty acid composition of the food source-artificial diet with high linoleic and fresh alfalfa with high linolenic acid. The aim of this study is to estimate the change in fatty acids relative amounts of *T. granarium* diapause larvae reared on three larval diets and kept at two different constant degrees of temperatures.

MATERIAL AND METHODS

Insects used in the present study came from the stock culture of *T. granartum*

reared in Insect Pest Control Laboratory and maintained on three larval hosts (wheat, cowpea and peanut) for 3 years. A standard procedure was allowed to obtain the experimental larvae so as to minimize the risk of accidental loss of any of the diapause characteristics of the stock. A continual supply of diapause larvae was insured by placing patches of more than 1000 newly hatched larvae on approximately 30g of crushed food (Nair and desai, 1973). Culture and experimental larvae were bred according to Rajendran (1982).

Nondiapause Larvae (NDL):

Newly hatched larvae were reared on crushed wheat, cowpea and peanut at 34 ±1°C and 65±5 R.H. When pupation started, the nondiapaused larvae were attained the full larval growth (18-20 days). These larvae were collected and hold as control.

ii- Diapause larvae kept at 35°C:

In crowded cultures as reported by Nair and Desai, 1973, small refuges described by (Burges, 1959) were put on the surface of the food inside the jars. The last instar larvae tended to hide and cluster inside these refuges. 90 days later, diapause larvae were collected after all the nondiapause larvae had been pupated.

2 - Diapause larvae kept at 25°C:

Twenty days old larvae from the culture of each diet were released on the fresh food and kept at 25°C. Small refuges were put on the surface of larval host. The nondiapause larvae removed as pupae and the diapause larvae were collected from refuges after an incubation period of 90 days at this degree (Gothi et al., 1984).

Extraction of Lipids:

Lipids were extracted from insect tissues according to the method adopted by Folch et al. (1957) and modified by Hamilton et al. (1992) as follows: One gram of larvae were homogenized for one minute with10 ml methanol. Twenty ml of chloroform were added, homogenization was continued for a further two minutes. The homogenate was filtered through a suitable filter paper into glass stoppered bottle and the solid residue was suspended in 30 ml of chloroform : methanol (1:1 v/v). The resultant was homogenized for a further 3 minutes and filtered once more. The solid residue was washed on the filter paper with 20 ml of chloroform and the solid residue was further washed with 10 ml of methanol. The filtrates were combined in a measuring cylinder for the above extractions and aqueous solution containing 0.88% KCl whose volume corresponds to one-quarter that of the combined filtrates was added. The mixture was shaked throughly and allowed to settle. The upper layer, which is the aqueous layer, was removed by aspiration. Water methanol (1:1 v/v) was then added to the lower layer (the volume of this water : methanol solution is one quarter of the lower layer). This mixture was shaked throughly and allowed to settle, the upper layer was removed by aspiration. The lipids were removed from the lower layer which consists of chloroform : water by evaporation of the solvent in a rotary film evaporator. The lipids were redissolved in a small volume of chloroform and stored at -20°C.

Derivative formation:

Fatty acid methyl esters were prepared according to Hartman and Lago (1973). Sodium hydroxide/sulforic acid was used as reagent. 200 ml of lipids were boiled with 5 ml 0.5N NaOH in MeOH for 3-5 mm. 15 ml of the estrification mixture were added, refluxed

for 3 min. transferred to a separating funnel with 25 ml petroleum ether and 50 ml H_2O, The organic phase was washed with 2x25 ml H_2O and analyzed by GC. (The estrification mixture was prepared as follows : NH_4C1/H_2SO_4/MeOH solution was prepared by adding 2g NH_4C1 to 60 ml MeOH followed by 3 ml conc. H_2SO_4 and refluxed for 15 min.)

Gas chromatography analysis:

Fatty acid methyl esters were analyzed by using hp 6890 gas chromatograph instrument equipped with innowax-Crosslinked polyethylene glycol column 30 ml i.d., 0.32 mm; 0.5 μm film thickness.

- Oven temperature programmed 150°C for one minute, then elevated to 235°C with a rate of 17°C/min., then raised to 245°C with a rate of 1°C/min and hold at 245°C for 5 min.
- Carrier gas Nitrogen 1.3 ml/min.
- Detector: FID, 275°C.
- Injection temperature 260°C.

RESULTS AND DISCUSSION

Tables (1,2 and 3) summarize the relative prportions of fatty acids detected in diapause and non-diapause larvae (DL and NDL) of *T. Granarium* reared on three diets; wheat, cowpea and peanut, which kept at 25°C and 35°C From the obtained data, it was observed that six major fatty acids namely palmitic (C16:0), palmitoleic (Cl6:1), stearic (C18:0), oleic (C18:1), lenoleic (C18:2) and linolenic (Cl8:3) in DL and NDL were detected in DL and DL, representing more than 80% of the total constituents.

In NDL, the total relative proportions of these six fatty acids measured in DL were greater than their the corresponding values measured in NDL, except those appeared in DL reared on the oily diet (peanut) kept at 35°C (Tables 1,2 and 3).

The least relative amounts of these six major compounds was recorded in those reared

Table (1) : Fatty acids (%) of *T. granarium* diapause larvae reared on wheat and kept at 25 and 35°C.

Major fatty acids	Nondiapause larvae	Diapause larvae kept at	
		25°C	35°C
Palmitic (16 :0)	12.76	15.21	12.13
Palmitoleic (16:1)	17.40	17.79	22.32
Stearic (18:0)	0.86	0.89	1.05
Oleic (18:1)	45.03	47.41	51.84
Linoleic (18:2)	12.87	11.28	5.42
Lenolenic (18:3)	0.31	ND	ND
Total	89.23	92.58	92.76
Saturated	13.62	16.10	13.18
Unsaturated	75.70	76.48	79.58
Unsat/sat	5.56	4.75	6.04

ND : Not detected

Table (2) : Fatty acids (%) of *T. granarium* diapause larvae reared on cowpea and kept at 25 and 35°C.

Major fatty Acids	Nondiapause larvae	Diapause larvae kept at	
		25°C	35°C
Palmitic (16 :0)	25.31	15.54	17.56
Palmitoleic (16:1)	2.46	14.90	13.74
Stearic (18:0)	3.57	1.19	1.73
Oleic (18:1)	42.77	48.07	46.41
Linoleic (18:2)	5.17	8.87	7.91
Lenolenic (18:3)	1.52	1.17	0.83
Total	80.80	89.75	88.18
Saturated	28.88	16.73	19.29
Unsaturated	51.92	73.02	68.89
Unsat/sat	1.80	4.36	3.57

Table (3) : Fatty acids (%) of *T. granarium* diapause larvae reared on peanut and kept at 25 and 35°C.

Major fatty Acids	Nondiapause larvae	Diapause larvae kept at	
		25°C	35°C
Palmitic (16 :0)	10.17	11.59	11.58
Palmitoleic (16:1)	8.25	10.59	8.10
Stearic (18:0)	1.79	1.85	1.31
Oleic (18:1)	45.00	44.29	39.19
Linoleic (18:2)	21.36	21.73	24.30
Lenolenic (18:3)	ND	ND	ND
Total	86.57	89.96	84.48
Saturated	11.96	13.44	12.89
Unsaturated	74.61	76.61	71.59
Unsat/sat	6.24	5.71	5.56

on cowpea (80.8%) (Table 2) and the greatest was, however, recorded in those reared on wheat (89.23%) (Table 1). In DL insects reared on peanut recorded the lowest relative amount of the major six fatty acids (84.48%) (Table 3) while, the least one was recorded in larvae reared on cowpea (Table2).

In the available literature, *Lambremont* and Blum (1963) and Lambremont et al. (1964) found eight major constituents representing 98% of the total fatty acids found in diapausing boll weevil adults *Anthonomus grandis* (Boheman) adults. Their relative composition was found to be the same in both total body fat and in the isolator triglyceride fraction. Of these eight fatty acids, six were found in the present study. Tombes (1966) found that seven fatty acids accounted for approximately 98% of the total fatty acids of *Hyperapostica* aestivated adults. Among of these seven, six fatty acids detected in the present study were found.

The polyunsaturated fatty acid linolenic acid (C18:3) either appeared in very small relative proportions in NDL (0.31 and 1.52% in larvae reared on wheat and cowpea, respective-

ly) (Tables 1 and 2) or completely disappeared as in those reared on peanut as oily diet (Table 3). The same trend was noticed in DL, where (C18:3) recorded in small relative amounts as in larvae reared on cowpea and kept at 25°C (1.17%) and 35°C (0.83%) (Table 2), or completely disappeared as in those reared on wheat and kept at 25°C or 35°C peanut (Tables 1 and 3). These results came in contrary with the results obtained by Tombes (1966) on *Hyperapostica* during adult aestivation and Labremont et al. (1964) on the diapausing adults of boll weevil *Anthonomus grandis*, who reported that polyunsaturated linolenic acid (C18 :3) increased in the relative percent of the total fatty acids during aestivation or diapause.

The double unsaturated fatty acid (C 18:2) was greatly affected by the larval diet, the greatest relative proportion was recorded in NDL reared on the oily diet (peanut) (21.36%) (Table 3), and the least one was, however, obtained in those reared on cowpea (5.17%) (Table2).

The monounsaturated fatty acids (C18:1 and C16:1) also affected by larval diet, but C 18:1 showed little variation in relative proportions. They were 45.03, 42.77 and 45.00% in NDL reared on wheat, cowpea and peanut, respectively, (Tables 1,2 and 3). Cl6:1 showed values of 17.4, 2.46 and 8.25% relative proportions in NDL reared on wheat, cowpea and peanut, respectively. As in other animals, the fatty acid content in insects is influenced by diet (Oilmour, 1961). Lambremont et al. (1964) reported that the important factors controlling the type of fatty acid incorporated in the triglyceride fraction were adult diet and physiological state.

The unsaturated fatty acid (C 18:2) recorded relative amounts of 11.28 and 5.42% in DL reared on wheat kept at 25°C and 35°C, respectively as compared with the corresponding value (12.87%) in NDL (Table1). The case was inverted in DL reared on cowpea and peanut. This may be due to the effect of larval diet.

The monounsaturated fatty acid (18:1) showed relative greater amounts in DL reared on wheat and cowpea than their corresponding values in NDL (Tables 1 and 2). The case was inverted for larvae reared on the oily diet *peanut*) as 44.29 and 39.19% were detected in DL kept at 25°C and 35°C, respectively, compared with 45% in DL (Table3). The same trend could be applied for C 16:1 in DL, where relative greater amounts were recorded in DL than without regarding to larval diet or rearing temperature. This agrees with the results of Lambremont and Blum (1963) and Lambremont et al. (1964) on the diapausing adults of boll weevil, where C 18:1 was the only fatty acid that changed in relative proportions with the onset of diapause increased during inactivity and was reduced during the reproductive diapause period. Tombes (1966) on his study on aestivated alfalfa weevils reported than oleic acid concentration was relatively high in the preaestivating weevils and was reduced in the postaestivator. He added that monounsaturated oleic (C18:1) and palmitoleic (C16:1) in alfalfa weevils declined during aestivation. Lambremont et al. (1964) noted that oleic acid (C18:1) was the major fatty acid of the boll weevil and it was slightly higher in diapausing than in nondiapausing insects. Valder et al. (1969) on his work on diapausing adults of *Musca autumnalis* reported that the major unsaturated C 16:1 and C18: 1 increased by 10% in reproducing and 30% in diapausing flies during the initial 15 days.

To show the effect of diapause state and the rearing temperature on fatty acid composition of DL, it was noticed that monounsaturated fatty acids C16:1 and C18:1 recorded relative amounts in larvae kept at 25°C was greater than those kept at 35°C in larvae reared on peanut or cowpea (Tables 3 and 2). The reverse was true in the case of DL reared on wheat (Table 1). This finding needs further studies.

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الأحماض الدهنية ليرقات خنفساء الصعيد الساكنة طبقا لغذاء اليرقة ودرجة حرارة التربية

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درست نسب الأحماض الدهنية الموجودة فى يرقات خنفساء الصعيد الساكنة والمرباة على ثلاثة أنواع من العوائل (القمح ، اللوبيا والفول السودانى) ظهر من النتائج ما يلى :

- ظهرت ستة أحماض ذهنية تمثل أكثر من ٨٠٪ من مجموع الأحماض الدهنية المكتشفة وهي :ك ١٦،ك ١٦ : ١، ك ١٨، ١٨ : ١، ك ١٨ :٢، ك ١٨:٣

- أظهر الحامض الدهنى غير المشبع ك ١٨ : ٣ نسبا مئوية قليلة جدا أو اختفى تماما فى بعض الحالات .

- تأثر الحامض الدهنى غير المشبع ك ١٨ : ٢ كثيرا بنوع العائل الغذائى فى اليرقات غير الساكنة ، حيث سجلت أعلى نسبة له فى اليرقات المرباة على العائل الزيتى (الفول السودانى) وأقل نسبة لظهوره كانت فى اليرقات المرباة على القمح .

- سجل الحامض الدهنى غير المشبع ك ١٨ : ١ قيما نسبية فى اليرقات الساكنة المرباة على القمح واللوبيا أعلى من مثيلاتها غير الساكنه .

- أخذ الحمض الدهنى غيرالمشبع ١:١٦ اتجاها واحدا فى اليرقات الساكنة حيث سجل قيما نسبية أعلى من مثيلاتها في اليرقات غير الساكنة بصرف النظر عن نوع المادة الغذائية أو درجة حرارة التربية .