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Effect of Low Temperature on the Fatty Acid Compositions of Adult of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae)

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ABSRACT

Fatty acid composition of *Acanthoscelides obtectus* adults which were exposed to low temperature (+4°C) for 10, 20 and 30 days were investigated. *A. obtectus* were reared in condition of 28 ± 2°C and 65 ± 5% relative humidity and dark until they were adult and beans were used as feed. The fatty acid compositions of triacylglycerol that were extracted from whole body of adult *A. obtectus* were analyzed using gas chromatography. It was determined that total fatty acid compositions of adults were composed of C12:0-C21:0 fatty acids. The major components determined in samples were oleic acid (C18: 1ω9), palmitic acid (C16: 0) and linoleic acid (C18: 2ω6). It was showed that oleic acid (59.56-61.74%), palmitic acid (16.36-18.02), linoleic acid (7.21-8.38%) and stearic acid (6.29-7.34%) constituted the major part of fatty acid compositions of adult. The results obtained from fatty acid composition analyses revealed that the decrease in percentage of total saturated fatty acid (SFA) was accompanied by increase in percentage of total monounsaturated (MUFA) fatty acids at low temperature.

1. Introduction

The common bean *Phaseolus vulgaris* (L.) is one of the most commonly used vegetables in human nutrition worldwide and is of the main sources of protein, particularly in developing countries (Lopes et al., 2015). Bruchids encompass a group of approximately 1700 insect species (Johnson et al., 2004). However attack by bruchids (Coleoptera: Chrysomelidae) during storage compromises the quality and commercial value of beans. *Acanthoscelides obtectus* (Say) is one of the major insect pests affecting the common bean (Hagstrum and Subramanyam, 2009). The bean weevil causes significant damage to haricot-bean and bean. Larvae usually eat the pod contents completely, decreasing the yield by 50-60%. Partially damaged grains lose their germinating power and taste quality. The pest damages grain in both field and storehouses, becoming rather harmful. Control measures include keeping the temperature below zero in storehouses, fumigation, and insecticide treatments in fields. The most favorable conditions for insects are the temperatures 27-29^{0}C (beetles), 24-27^{0}C (larvae) and 22-26^{0}C (pupa). Higher and lower temperatures cause a decrease of fecundity. *A. obtectus* very sensitive to temperatures below zero. The bean weevil prefers high humidity, 80-88% (Anonymous, 2016), cooler climates at higher elevations and thus can be found in mountainous and subtropical regions (Cardona, 1989).

Lipid in the form of triacylglycerol is the most common energy reserve (Beenakkers et al., 1981) and its accumulation prior to dormancy is documented in many insect species (Adedokun and Denlinger, 1985).

Many poikilothermic animals adapt to changing environmental temperatures by modifying the degree of unsaturation of their lipids. At low ambient temperatures, the proportion of unsaturated, saturated fatty acids increases in phospholipids to maintain cell membrane fluidity and normal cellular functions (Hazel, 1995; Hazel and Williams, 1990). Two major metabolic compensation mechanisms exist. First, short-chain PLFAs result in higher membrane fluidity than long-chain PLFAs, leading to temperature induced changes in the ratio of C16 to C18. Second, unsaturated PLFAs result in more fluidity than saturated PLFAs, therefore the Unsaturation Index (UI) increases with lower tem-

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perature (Hazel and Williams, 1990). Also temperature, insect's age and gender, biological factors such as diet and activity, adult time affect the fatty acid composition (Cohen, 1990). Effects of temperature on fatty acids composition in different stages of insect life cycle were mostly studied, for example in *Melanogryllus deser*tus Palas (Bozkuş, 2003). It has been found that many insects have adapted by increasing the unsaturated fatty acid to saturated fatty acid ratio during cold acclimation (Bennett and Lee, 1997). According to Atapour et al. (2007) saturated fatty acids significantly decreased and conversely unsaturated fatty acids increased from August (pre-diapause) to October (initiation of diapause) for *Chilo suppressalis* (Lepidoptera: Pyralidae).

Laboratory studies has to be carried out to fight against this insect species. To develop or improve a fight against these species in laboratory conditions, physical and biological characteristics, including nutritional, reproductive and metabolic needs must be known very well. This study was conducted to investigate the effects of a warehouse at low temperatures on fatty acid composition of *A. obtectus* adults.

2. Materials and Methods

Acanthoscelides obtectus were reared in condition of 28 ± 2°C and 65 ± 5% relative humidity and dark until they were adult and beans were used as feed. Newly hatched adults of *A. obtectus* were carefully separated and four groups were set for experimental trials. Each group was represented by 10 individuals of *A. obtectus*. The trials were carried out as three sets in parallel for each time-period tested. Newly hatched adults in control group were placed into tubes with chloroform/methanol (2:1, v/v) and then they were stored in a freezer until the analysis day. As mentioned earlier, for each time period, a group was formed with 10 individual adults and three identical sets of each group were prepared. These groups were kept at 4°C for 10, 20 and 30 days to reveal effect of temperature on fatty acid composition of *A. obtectus.*

Experimental groups were placed into plastic cups whose bottom was covered with onionskin paper and lids were covered with one layer of cheesecloth. Then they were kept at 4°C cooler. In this way, these groups were subjected to low temperature for 10, 20 and 30 days, respectively. At the end of each experimental group of low temperature applications was taken from deep milling separately and were put in vials, and then chloroform / methanol mixture was added. This group was stored in a freezer until the analysis day. At the beginning of each analysis, the samples were allowed to equilibrate to room temperature and homogenized in chloroform/methanol mixture (2/1, v/v). Samples of species were extracted by a method suggested by Folch et al., (1957) and they were transesterified with BF_3-methanol (Moss et al., 1974). Methyl esters of fatty acids were analyzed by using a HP Agilent 6890N model gas chromatograph, equipped with a flame ionization detector and fitted with DB-23 capillary column (60 m, 0,25 mm i.d. and 0,25µm). Injector and detector temperatures were kept at 270 and 280°C, respectively. Column temperature program was 190°C for 35 min and then increased at a ratio of 30^0C/min up to 220°C where it was maintained for 5 min. Identification of normal fatty acids was carried out by comparing sample FAME peak relative retention times with those obtained for Alltech standards. Results were expressed as relative percentages of FID response area. Each reported result was presented as the average of three results obtained by GC analysis. The results were presented as means±SD.

3. Results and Discussion

The fatty acid compositions of the *A. obtectus* adults subjected to low temperature were determined by a gas chromatographic method. Fatty acid compositions of the experimental group and control group are given in Table 1.

Based on analysis, fatty acid composition varied from C12:0 to C21:0.The results showed that oleic acid (C18: 1ω9), palmitic acid (C16: 0) and linoleic acid (C18: 2ω6) were highly detected fatty acids in experimental groups. Studies conducted with the various orders of insects have been reported that the overall fatty acid structure is composed of 10-20 carbon fatty acids (Thompson, 1973). In this study, the fatty acid composition ranging from C12:0 to C21:0 was determined for *A. obtectus.*

In total, 16 fatty acids were determined in fatty acid composition. Oleic fatty acid (C18:1) and palmitic acid (C16:0) were determined as dominant fatty acid, in both experimental group and control group. Oleic acid is energetically more favorable (to manufacture) than linoleic acid (one less double bond).Hence insects that upregulate oleic acid rather than linoleic acid for adaptation to low temperatures may be preserving finite energy reserves while still gaining the benefit of a wide window of fluidity (Çakmak, 2010). Other fatty acids have been identified and determined as percent and they were less than percentage of dominant ones. These were myristic acid (C14:0), stearic acid (C18:0), palmitoleic acid (C16:1), and linolenic acids (C18:3).

In the control group total percentage of monounsaturated fatty acids (MUFA) was determined as 61,60%, on the other hand for 10, 20 and 30-day experimental groups the values, were found as 62,16%, 61,95% and 63,00%. For the total amount of polyunsaturated fatty acids (PUFA), the largest proportion (14,06%) was detected for the 20-day experimental group at low temperature, while the lowest ratio (12,61%) was determined for 30-day experimental group. The 20-day experimental group (14,06%) exhibited the highest increase in the percentage of total pol-

yunsaturated fatty acids in overall experimental groups. The fatty acid composition of insects varies depending on temperature changes. The results have shown the relationship between the temperature and degree of saturation of fatty acid. According to our results, the low temperature led to increase in the level of unsaturated fatty acid. This result is consistent with literature (House et al., 1958).

Table 1. Fatty acid composition of *Acanthoscelides obtectus* (Say) adults at different times exposed to 4 °C

Fatty acids	**Fatty acid composition (%)**			
	Control	**The exposure time for adults at 4 °C (day)**		
		10	**20**	**30**
C12:0	0.09±0.00	0.27± 0.21	0.09±0.06	0.07±0.03
C14:0	0.27 ±0.01	0.96±0.80	0.42±0.12	0.36±0.09
C15:0	0.07±0.01	0.09±0.06	0.08±0.03	0.04±0.01
C16:0	17.47±1.36	18.02±1.46	16.52±0.22	16.36±0.63
C17:0	0.70±0.51	0.11±0.05	0.08±0.03	0.08±0.02
C18:0	6.29±0.68	6.94±1.19	6.57±0.71	7.34±0.64
C21:0	0.05±0.00	0.02±0.01	0.03±0.02	0.03±0.02
ΣSFA*	**24.94±2.42**	**24.20±1.31**	**23.99±0.94**	**24.38±1.14**
C14:1ω5	0.04±0.03	0.08±0.06	0.04±0.03	0.05±0.04
C15:1 ω5	0.03±0.02	0.12±0.11	0.08±0.07	0.14±0.11
C16:1 ω7	0.68±0.27	0.89±0.08	0.68±0.07	0.66±0.08
C17:1 ω8	0.18±0.03	0.05±0.01	0.06±0.02	0.05±0.01
C18:1 ω9	60.66±1.77	59.56±2.38	61.01±0.61	61.74±1.60
ΣMUFA*	**61.60±1.64**	**62.16±0.68**	**61.95±0.71**	**63.00±2.00**
C18:2 ω6	7,79±0.88	7.21±0.84	8.38±0.61	7.39±0.66
C18:3 ω6	0.02±0.01	0.01±0.01	0.01±0.00	0.02±0.01
C18:3ω3	4.35±0.15	5.62±0.57	5.74±0.40	5.24±0.38
C20:2 ω6	1.30±1.02	0.05±0.02	0.21±0.19	0.45±0.53
ΣPUFA*	**13.47±0.17**	**13.69±0.68**	**14.06±0.30**	**12.61±0.88**
Σω3	4.35	5.62	5.74	5.24
Σω6	9.11	7.27	8.60	7.86
Σ ω3/6	1.61	0.99	0.99	1.61

[a]Average of three lots analysed.

[b]Values reported are means±SD.

Control group and experimental groups at low temperature formed same highest percent fatty acids in fatty acid composition. Saturated (SFA), unsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in experimental groups were not demonstrated a noticeable difference among them. But, for 30-day-group kept at low temperature, the lower percentage of polyunsaturated fatty acids was obtained compared to other groups. The percentage of saturated fatty acids decreased relative to the control group, and the percentage of unsaturated fatty acids was increased in all test groups. The highest percentages of unsaturated fatty acids were detected only in fatty acid composition of 30-day group. The total percentages of polyunsaturated fatty acids were detected as highest for 20-day low temperature group. Time-dependent increase was observed in other experimental groups.

We also found that the composition of SFAs and MUFAs in *A. obtectus* adult was significantly affected by lower temperature. It has been demonstrated that increasing of PUFAs in total contents and their components will have some important impact in insect physiology at low temperatures. The increase of UFAs is related to maintenance of an appropriate fluidity of depot lipids to make them available as energy resources (Joanisse and Storey, 1996). It has been found that many insects have adapted by increasing the unsaturated fatty acid to saturated fatty acid ratio during cold acclimation (Bennett and Lee, 1997). According to Atapour et al. (2007) saturated fatty acids significantly decreased and conversely unsaturated fatty acids

increased from August (pre-diapause) to October (initiation of diapause) for *Chilo suppressalis* (Lepidoptera: Pyralidae).

Many poikilothermal animals adapt to changes in environmental temperatures by modifying the degree of unsaturation of their lipids. At low ambient temperature, the proportion of unsaturated fatty acid is changed Saturated fatty acids increase in phospholipids to maintain cell membrane fluidity and normal cellular functions (Hazel and Williams, 1990). This study has shown that the composition of saturated and unsaturated fatty acids in lipids of *A. obtectus* was influenced by storage temperature of 4°C.

The accumulation of lipids, particularly the unsaturated fatty acids (UFAs), is believed as one of the major contributing factors to cold-hardiness. In general, the content of UFAs in organism increases whereas those of saturated fatty acids (SFAs) decreases with decline in temperature and it is closely associated with their cold-resistance. This phenomenon had been reported in organisms, such as some bacteria, algae, protozoa, plants and fishes (Thompson, 1989; Murata et al., 1992; Zou et al., 2010)

Low temperature acclimation also caused a decrease in the amounts of palmitic acid (16:0) (20 and 30 day application) and increase in the amounts of oleic (18:1) (61.01% for 20 day, 61.74% for 30 day) and linolenic acid (18:3ω 3) 5.62% for 10 day, 5.74% for 20 day, 5.24% for 30 day) in TG fraction. Increases in monounsaturated fatty acids, caused by storing at low temperature (4°C) were attributable to a significant increase in oleic acid in the lipids fraction. The increase in PUFA at reduced temperatures was attributable to significantly greater percentages of linoleic and linolenic acid in total lipids.

Many studies that examine changes in phospholipids due to cold acclimation or diapause in insects report that 18:2ω-6 is the FA that increases for winter climates (Kostal et al., 2003). In our study it was seen that 20-day low-temperature applications of these fatty acids (8.38%). Linoleic acid is the precursor for eicosanoids, which play an important role in insect physiology. They influence reproduction, mediate cellular immune response and are involved in temperature regulation (Stanley-Samuelson and Nelson, 1993). In addition, the amount of linoleic acid may be altered to maintain membrane fludity at different environmental temperatures. Our study showed that proportion of unsaturated fatty acids were notably changed during low temperature and they were associated with increase in freeze tolerance capacity.

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