Effects of Cadmium on Total Lipid Content and Fatty Acids of the Greater Wax Moth, *Galleria mellonella*

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ABSTRACT: The beekeeping pest insect *Galleria mellonella* larvae (greater wax moth) were reared on an artificial diet contaminated indipendently with cadmium chloride ($CdCl_2$) at different concentrations(1.25, 2.50, 5.00, 10.0 20.0, 40.0 μg/g food fresh weight). Results of these studies suggested that Cd exposure of *G. mellonella* may influence its whole body lipid contents. We decided, therefore, to analyze lipid content of G. *mellonella* exposed to different concentrations of Cd. Lipid concentrations were measured photometrically by phosphovanillin method. Significant decrease in the total lipid content was found in Cd-contaminated larvae and pupae. In this study, six kinds of lipids were separated, and they were phospholipid, monoglyceride, diglyceride, sterol, fatty acids, triglyceride. And fatty acids were proved to consist of palmitic acid, stearic acid, oleic acid and linolenic acid. Palmitic acid, linoleic acid and oleic acid were found high rate in all groups, but stearic acid was low. In conclusion, lipid contents decreased in Cd-contaminated groups in comparison to the control group.

Key words: Cadmium, Fatty acids, *Galleria mellonella*, Gas liquid chromatography, Lipid content.

INTRODUCTION

Metal accumulated in animals are metal, species and tissue specific, depending on the biology and physiology of a species (Rittner 1991). Investigations on various lepidopteran species have shown developmental retardation and growth reduction as well as a depression or inhibition of reproduction due to metal stress (Zelenayora 1986). On the other hand, heavy metals are accumulated the surface of earth or dissolved in water and can influence insects.

Heavy metals had effect on insects with an insecticide. Heavy metals interfere with metabolism, longevity and oxygen consumption. Gintenreiter *et al.* (1993) reported that *Lymantria dispar* responded to metal-contaminated diets with the prolongation of developmental stages. Ortel (1995) demonstrated that heavy metal effected on the total lipid content in *L. dispar*, and its hemolymph. Besides, effects of insecticides and metals have been investigated on the adults of some parasitic species (Powell *et al.* 1986, Ortel and Vogel 1989), and any effects host metal stress may have on a parasitoid, especially in insect-insect host-parasite relationships, although plant-insect relationships are better documented (Quimby *et al.* 1979). Parasitic Hymeno-ptera are considered to be more sensitive to anthropogenic toxicants than herbivorous hosts (Führer, 1985). Ortel (1991) also demonstrated in the wax moth, *Galleria mellonella* pupal parasitoid, *Pimpla turionellae* that cadmium effected on chemical composition and total water content. There were some other reports that temperature and pH effects on Cd bioaccumulation by nymphs of the burrowing mayfly, *Hexagenia rigida* from water column (Odin *et al.* 1996), effects of temperature on Cd uptake by the midge larvae, *Chironomus riparius*(Bervoets *et al.*, 1996) and effects of heavy metals during the embryonic development of Acridid grasshoppers (Devkota and Schmidt 1999).

Chemical analyses of the whole body may reveal sublethal impairment of insect larvae due to metal exposure. Consequently in this study the effects of cadmium on total lipid content and fatty acids of the greater wax moth, *G. mellonella* were described.

MATERIALS AND METHODS

Galleria mellonella from a laboratory culture fed on an artificial diet(oat meal, sucrose, glycerol, vitamin, water) modified according to Beck (1960). *G. melllonella* were maintained at 27 ± 1℃, 70% humidity, and a 0L : 24D photoperiodic regime. *G. melllo-nella* were contaminated via diet throughout larval development. Cadmium ($CdCl_2$) were added to the diets to the following concentrations: 1.25, 2.50, 5.00, 20.00, 40.00 μg/g based on nutrient medium dry weight. 4th instar larvae, last instar larvae and two days old pupae were taken for lipid analyses according to expose Cd.

Lipid extraction were conducted according to Folch *et al.* (1957). Animals were dried at 80℃, and homogenized in chloroform : methanol (2:1, v/v). Lower layer of mixtures by Folch's method were used sample for TLC.

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Total lipid concentrations were measured by the sulfo-phospho-vanillin method (Frings and Dunn, 1970) with spectrophotometer (Shimadzu UV-2100, Japan) at 540nm. The standard curve was performed with 1% olive oil.

Lipid compositions were separated by HPTLC. A solvent system for HPTLC were petroleum ether : diethyl ether : acetic acid (80 : 20 : 1, v/v). The lipid bands were detected with 0.5% ethanolic phosphomolybdic acid.

Fatty acids were analyzed by Gas liquid chromatography(HP-6890, Column; DB^{TM}-225 capillary column, Carrier gas ;Helium at 40cm/sec, Detector; FID, 300℃)

RESULTS

In all Cd-contaminated larvae and pupae total lipid contents were lower than control groups (Table 1). Total lipid contents were showed the 4th instar larvae(32.5~7.4%), last instar larvae(30.1~52.4%) and two day old pupae (44.1~83.7%)(Table 1). Therefore, lipid contents decreased in Cd-contaminated groups in comparison to the control group.

Lipids composition was determined by HPTLC and separated phospholipid, mono-, di-, triacylglycerol, sterol, and fatty acids (Table 2).

Qualitative and quantitative fatty acid analysis was performed GLC analysis of the fatty acid methyl esters (FAMES), in conjunction with analysis of appropriate standard FAMEs (palmitic acid, stearic acid, oleic acid and linoleic acid). In this results fatty acid content was not detected different patterns of it, but stearic acid was found the lowest level in all groups and stages (Table 3).

DISCUSSIONS

Despite of the homogenous rearing conditions, even control groups showed certain variabilities in developmental periods,

Table 1. Total lipid contents (mg/g dw) in developmental stages of the greater wax moth, *Galleria mellonella* exposed to different concentrations of cadmium

Cadmium conc.*	Stages		
	4th instar larvae	Last instar larvae	Pupae
Control	42.55 ± 1.05**	172.80 ± 9.38	92.04 ± 6.98
1.25	20.07 ± 0.95	84.56 ± 7.93	40.64 ± 9.82
2.50	14.81 ± 1.00	92.69 ± 4.46	43.69 ± 9.65
5.00	23.60 ± 0.10	94.76 ± 18.30	73.63 ± 18.33
10.00	21.18 ± 0.98	54.41 ± 10.66	68.85 ± 10.80
20.00	26.16 ± 1.12	80.96 ± 9.56	76.98 ± 11.47
40.00	22.74 ± 0.47	78.62 ± 3.56	65.21 ± 10.41

* Cadmium concentrations in artificial diet(μg/g).

** Unit : mg/g dry weight.

growth and physiological difference. There are several factors which contribute to this variability; effects of heavy metal were also out of them. The changes in chemical composition caused by heavy metal contamination seems to depend on its concentration and on the species itself (Radhakrishnaiah and Busappa 1986). After 5μg Cd per individual were injected into the bug *Chrysochoris stolli* levels of proteins, lipids and carbohydrates were analyzed in hemolymph, fat body and ovaries, which revealed tissue-dependent reactions on this acute Cd-stress(Islam and Roy 1983). There are also some reports dealing with effects of heavy metals on the lipid content, fatty acid oxidation and fatbody, which is the energy source in insects (Martoja *et al.* 1983, Bodar *et al.* 1988). Fatbody-cells failed to differentiate if 2μg Cd/g fresh weight were injected *Locusta migratoria*, which impaired egg production in females (Martoja *et al.* 1983). *Pimpla turionellae* showed a striking decrease in lipid content, when exposed to Cd (Ortel 1991). In this study, lipid contents of *G. mellonella* exposed to different concentrations of Cd decreased in comparison to the control group (Table 1), which could indicate a similar mechanism as mentioned above and in agreement with Ortel (1991). Ortel (1995) demonstrated that whole body lipid concentration of day-3 4th instar larvae *L. dispar* were significantly reduced in Cd-contaminated. And lipid content proved to be dose-dependent for Cd applied. That is , lipid content was lower in high Cd level than in low it.

Modifications in fatty acid composition of lepidopteran larvae can be subjected to rearing conditions, the diet consumed and to the developmental stages (Chang and Friedman 1971, Grau and Terriere 1971). The changes in the quantitative compositions of lipids and fatty acid in the whole body may also be produced by metal stress. Therefore, a detail analysis of lipid classes in insect should be conducted. In this study, six kinds of lipid classes-phospholipid, mono-, di-, triglycerid, sterol and fatty acid - were observed in control and Cd-exposed group, but diglycerid was no detected at the 4th and last instar larvae (Table 2). On the other hand, four kinds of fatty acid in the wax moth, *G. mellonella* was detected, and they were palmitic, stearic, oleic and linoleic acid (Table 3). Insect hemolymph composition is known to change with developmental stage and within one stage (Chang and Friedman 1971). Moreover, *L. dispar* responded to metal-contaminated diets with prolongation of developmental stages (Gintenreiter *et al.* 1993). Hence, the interpretation of lipid and fatty acid composition in the wax moth may be difference, since larvae and pupae could have been in different developmental phases (within stadium) at time of sampling, and also they were described the percentage to the total lipid content. In spite of that, this study lends further support to the observation that *G. mellonella* is sensitive to Cd and these results suggest that the whole body or hemolymph lipid concentration are affected directly by Cd exposure rather than indirectly by developmental retardation.

Table 2. Composition of lipid during developmental stages of *Galleria mellonella* in control and cadmium exposed group

Lipid	Stage and cadmium concentration*																				
	4th instar larvae							Last instar larvae							Pupae						
	c	1.25	2.5	5	10	20	40	c	1.25	2.5	5	10	20	40	c	1.25	2.5	5	10	20	40
PL**	21.13	29.26	32.84	29.99	38.48	25.21	29.72	12.58	13.76	12.21	10.56	11.74	12.48	11.92	8.21	6.55	9.00	9.24	8.73	8.51	8.08
MG	4.17	0.431	1.81	0.44	10.15	3.65	1.88	1.81	2.12	0.73	3.200	11.14	6.71	0.52	2.10	1.22	2.03	2.95	2.46	2.39	1.26
ST	12.04	7.21	14.58	6.47	19.45	9.41	8.87	8.48	4.46	4.38	8.85	12.66	13.01	10.17	5.06	3.17	2.18	2.04	2.82	2.55	1.58
FA	30.08	22.29	26.41	19.23	3.079	26.52	25.01	0.48	4.21	9.39	6.49	3.98	6.77	8.38	4.98	2.62	5.92	7.95	6.98	5.48	4.40
TG	25.78	40.33	16.37	38.3	23.45	27.84	29.93	71.03	71.00	67.44	67.15	55.95	57.33	64.83	66.91	71.37	70.79	64.02	68.04	71.10	71.42

* Cadmium concentraion in artificial diet (μg/g), ** PL : phospholipid, MG : monoglyceride, DG : diglyceride, ST : sterol, FA : fatty acid, TG :triglyceride, UK : unknown, - : race

Table 3. Compositon of fatty acid during developmental stages of *Galleria mellonella* in control and cadmium exposed group

Fatty acid	Stage and cadmium concentration*																				
	4th instar larvae							Last instar larvae							Pupae						
	c	1.25	2.5	5	10	20	40	c	1.25	2.5	5	10	20	40	c	1.25	2.5	5	10	20	40
P**	22.53	28.00	20.95	25.89	22.11	24.30	24.59	35.89	33.28	30.76	32.37	29.77	34.85	35.08	29.63	24.39	27.03	28.95	31.18	32.26	31.03
S	1.935	1.656	2.653	1.544	2.382	2.036	2.061	5.209	6.661	6.752	5.585	3.978	5.494	4.177	4.084	3.921	4.173	4.475	3.336	3.102	3.890
O	28.55	33.67	26.33	32.51	28.12	30.82	26.90	36.79	34.25	34.62	35.27	33.21	35.72	35.74	32.06	30.80	31.84	32.47	36.08	35.09	30.08
L	26.97	22.46	28.58	23.87	29.11	27.80	28.95	15.67	19.62	22.08	18.90	18.43	17.62	18.57	20.20	21.07	20.71	21.50	19.14	20.38	17.82
UK	20.02	14.22	21.48	16.18	18.28	15.05	17.49	6.447	6.190	5.786	7.868	14.61	6.328	6.436	14.03	19.81	16.25	12.61	10.27	9.162	17.18

* Cadmium concentraion in artificial diet (μg/g), ** P : palmitic acid, S : stearic acid, O : oleic acid, L : linolenic acid, UK : unkown

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