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Effects of Cypermethrin on Total Body Weight, Glycogen, Protein, and Lipid Contents of *Pimpla turionellae* (L.) (Hymenoptera : Ichneumonidae)

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ABSTRACT. We investigated the changes in total body weight, glycogen, protein, and lipid contents of the endoparasitoid *Pimpla turionellae* L. (Hymenoptera : Ichneumonidae) reared on *Galleria mellonella* L. (Lepidoptera : Pyralidae) exposed to various sublethal doses of cypermethrin added to the food of host larvae. Cypermethrin affected the total body weight of larvae, pupae, and adult females but not males. Results revealed that the levels of glycogen, protein, and lipid in all stages and sexes of the wasp tended to decline with respect to controls. Females showed the most striking decrease in glycogen content whereas larvae were more susceptible to cypermethrin than pupae and adults in terms of decrease in protein and lipid contents.

KEY WORDS : *Pimpla turionellae*, cypermethrin, body weight, lipid, protein, glycogen.

INTRODUCTION

Pesticide research and development has brought a large number of chemicals in protecting the crop against insect pests. However, these chemicals have posed a grave environmental problem because of their indiscriminate usage in fields (Tillman & Mulrooney, 2000). Insecticides are also toxic to many nontarget organisms (Nath et al., 1997; Suh et al., 2000) and their usage can disrupt the balance between a host and its natural enemy, resulting in an increase of pest numbers (Van Driesche & Bellows, 1996; Tomberlin et al., 2002). Studies have reported that insecticides cause numerous sublethal effects, including increases and decreases in fecundity (Takada et al., 2001) and developmental rate (Willrich & Boethel, 2001), changes in sex ratio, diapause, and morphology by direct interaction with parasitoids and indirectly through host physiology (Croft, 1990). Pyrethroids are currently among the major insecticides used against pests (Usmani & Knowles, 2001). Although pyrethroids are effective at low rates and relatively inexpensive, it has been also shown that they are harmful to beneficial insects (Nowak et al., 2001; Xu et al., 2001). Cypermethrin ((±) α-cyano-3-phenoxybenzyl (±) cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) is a pyrethroid insecticide that is used to control insect pests (Cox, 1996). Like all pyrethroids, it kills insects by disrupting normal functioning of the nervous system (Vijverberg & Bercken, 1990).

Pimpla turionellae L. (Hymenoptera : Ichneumonidae) is a poliphagous endoparasitoid that spends its immature stages in pupae of various lepidopterous species. It serves as a potential biological control agent of many lepidopterous pests (Kansu & Uğur, 1984; Fisher, 1987). Adult wasps feed on plant nectar and host pupae in nature. Because host species of this parasitoid feed on plants during larval stages, the accumulation of insecticides in host pupae is likely to occur. Therefore, it is possible for *P. turionellae* to be exposed to insecticides by way of nutrients. Biochemical parameters seem quite promising to assess and predict the effects of toxicants on beneficial species. The assessment of the potential effects that insecticides have on the total body weight and biochemical milieu of parasitoids is of great interest for success in biological control applications. This research was aimed at showing how cypermethrin that is likely to be accumulated in the host affects total body weight, glycogen, protein, and lipid contents of parasitoid during development.

MATERIAL AND METHODS

Insect Rearing

The solitary pupal endoparasitoid *P. turionellae* were reared on the pupae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera : Pyralidae) at 25 ± 1 °C, 60 ± 5% RH and a photoperiod of 12 :12 h (L : D). Adult parasitoids were fed 30% (vol :vol) honey solution and provided with host pupae (4 pupae for 10 wasps) once every three days to meet their protein requirement (Uçkan et al., 2004). Host colony was maintained by feeding the insects with a diet described by Bronskill (1961). The diet was modified by increasing the bran ratio by 50% to decrease the humidity of the composition in the diet. A piece of honeycomb was added for egg deposition and feeding of the newly hatched larvae.

Bioassays

Cypermethrin (Imperator, 250 g/liter EC, Zeneca Ltd., İzmir, Turkey) was used in all bioassays as water source

and prepared in distilled water as parts per million of active ingredient. Nine serial dilutions of cypermethrin (5; 50; 100; 150; 200; 300; 400; 500; and 1000 ppm) were used in determining PD_{50} (the median pupation dose) of *G. mellonella* larvae. Different doses of cypermethrin were added in 10 g of the diet in each 80 ml jar. Last instars of *G. mellonella* (n = 10; average weight = 0,16 ± 0,01 g) were exposed to selected doses of cypermethrin for 7 days. The jars were maintained in another laboratory under the same conditions mentioned for the stock cultures. Larvae were removed from the jars and the pupation rate for each dose was observed for 30 days. Experiments were repeated four times. Mortality data were derived from pupation rates, and PD_{50} with 95% confidence limits was calculated by using probit analysis (PriProbit, PriProbitNM (C) 1998-2000 Masayuki Sakuma, Kyoto University, Kyoto, Japan) after Abbott's correction (ABBOTT, 1925) for natural mortality. The 30-d PD_{50} (95% CI) of cypermethrin was 207,3 (181,7 – 235,1) ppm. Therefore, we applied doses less than PD_{50} value to host instars to evaluate the dose-dependent effect of cypermethrin on total body weight, glycogen, protein, and lipid contents of *P. turionellae* stages/sexes (defining larvae, pupae, adult males and females).

G. mellonella larvae were exposed to four different doses (20; 50; 100; and 150 ppm) below PD_{50} value to evaluate the effects of the insecticide on total body weight, glycogen, protein, and lipid contents of *P. turionellae* last instars, pupae, and adults. Batches of 50 host larvae (0,16 ± 0,01 g) were exposed to 50 g of the diet including the selected doses of the cypermethrin for 7 days. Larvae were removed from the diet and those pupated were parasitized by *P. turionellae* females. Parasitoid larvae, pupae, and 0 to 24-h-old adult males and females maintained from host pupae were used in analyses. Groups of 10 insects were sampled at each stage/sex and fresh-weighed. The method developed by ROE et al. (1961) was conducted for glycogen extraction. An anthrone test developed by CARROL et al. (1956) was used for the detection of glycogen obtained from larvae, pupae, and adults using glucose (Merck, Darmstadt, Germany) as standard. Protein extraction was also made with the method developed by ROE et al. (1961) and total protein content of the same samples was estimated by the method of LOWRY et al. (1951) using bovine serum albumin (BSA) (Merck) as standard. The lipid fractions in larvae, pupae, and adults were extracted and total lipid contents were determined using the method described by FOLCH et al. (1957). Total wet weight of each group was calculated, and insects were kept in 5 ml chloroform – methanol (2 :1 vol :vol) at –20 °C until extractions. Samples taken in chloroform – methanol solution were homogenized at 26.000 *X g* for 5 min. After filtering through Whattman paper No : 41, the extracts were placed in hexane solution and washed through distilled water 5 times. Total lipid content as a percentage of wet weight was calculated graviometrically. Groups of 10 individuals of each stage/sex were repeated three times for glycogen-protein and lipid analyses. The effect of cypermethrin on total body weight of *P. turionellae* was determined by pooling the results of mean weight estimations of insects used in glycogen-protein and lipid analyses (6 replicates with 10 individuals per replicate). The amount of average total glycogen, protein, and lipid was estimated as a percentage of wet weight of insects.

Statistics

Data for total body weight and glycogen, protein, and lipid contents were subjected to two-way analysis of variance (ANOVA) (SPSS, 1999) to determine the main effects of cypermethrin doses and stage/sex on total weight and glycogen, protein, and lipid contents, respectively. Differences due to cypermethrin doses in total weight of each stage/sex were inferred using one-way ANOVA (SPSS, 1999). The relationship between cypermethrin doses and percent amount of glycogen, protein, and lipid contents was also compared with one-way ANOVA for each stage/sex. Means were separated using the Tukey's HSD posthoc test (SPSS, 1999). An arcsine square-root transformation was performed on percentage values of glycogen, protein, and lipid contents before analyses. Results were considered statistically significant when $P<0,05$.

RESULTS

The percentage of pupation did not differ from that of the control group when *G. mellonella* larvae exposed to 5 ppm of cypermethrin and the rate was 100% in both cases. The decrease in pupation rate was dose-wise in the order; 92,5; 80; 72,5; 57,5; 35; 20; and 5% when larvae exposed to increasing doses from 50 to 500 ppm. Finally, none of the larvae pupated at 1000 ppm. The effect of cypermethrin on total body weight was dose ($P<0,001$) and stage/sex ($P<0,001$) dependent, and the relationship between doses and weight was significantly influenced by stage/sex ($P<0,05$) (Table 1). Changes in the total weight of cypermethrin treated stages/sexes are presented in Table 2. Total body weights of larvae, pupae and adult females were considerably affected by cypermethrin treatment. However, males did not exhibit a significant weight loss due to cypermethrin treatment (larva : $F= 4,62$; $df= 4, 25$; $P<0,01$, pupa : $F= 7,47$; $df= 4, 25$; $P<0,001$, male : $F= 1,62$; $df= 4, 25$; $P>0,05$ and female : $F= 5,50$; $df= 4, 25$; $P<0,01$). Cypermethrin treatment at 20 ppm induced a slight increase in weight for pupae and adult females (Table 2).

Two-way ANOVAs indicated that the effects of cypermethrin doses and stages/sexes on total glycogen, protein, and lipid contents of *P. turionellae* were significant. Cypermethrin dose – stage/sex interactions were not significant ($P>0,05$) for glycogen and protein, indicating that variation as a result of dose was consistent among stages/sexes. However, stage/sex significantly influenced the relationship between cypermethrin dose and total lipid content (Table 1). Glycogen level of all stages/sexes tended to decrease on exposure to cypermethrin, but did not differ significantly among experimental groups (Fig. 1). The content did not also differ significantly between controls and cypermethrin treated groups in larvae ($F= 0,11$; $df= 4$; 10; $P>0,05$), pupae ($F= 3,18$; $df= 4$; 10; $P>0,05$), and males ($F= 0,10$; $df= 4$; 10; $P>0,05$). However, there was a significant decrease in experimental groups with respect to control group in terms of glycogen level of females ($F= 20,42$; $df= 4$; 10; $P<0,001$). Total

protein in each stage/sex also tended to decrease on exposure to different doses of cypermethrin (Fig. 2). Protein content of cypermethrin treated groups was significantly lower than that of the control group for larvae (F= 12,92; df= 4; 10; P<0,001). There was also a decrease in total protein for pupae (F= 6,46; df= 4; 10; P<0,01) with respect to the control group, but this decline was only significant at 20 ppm. Cypermethrin treated adults had also lower percentage of protein than the control group. However, the decline was not significant (males : F= 1,52; df= 4; 10; P>0,05, females : F= 0,45; df= 4; 10; P>0,05). The effect of cypermethrin on lipid content was not dose dependent and did not decrease all the time with increasing cypermethrin doses (Fig. 3). The difference in decrease between control and experimental groups was only significant in larvae (F= 8,52; df= 4; 10; P<0,01). Lipid contents of cypermethrin treated pupae (F= 2,78; df= 4; 10; P>0,05), males (F= 3,47; df= 4; 10; P>0,05), and females (F= 2,63; df= 4; 10; P>0,05) were not significantly different from those of their controls.

TABLE 1

ANOVAs of the effects of cypermethrin dose, stage/sex, and their interactions on total body weight and glycogen, protein, and lipid contents of *P. turionellae*.

	Source	df	MS	F	P	r^2
Total body weight	Cypermethrin dose	4	240,037	13,191	0,000	0,80
	Stage/sex	3	1894,876	104,131	0,000	
	Cypermethrin* Stage/sex	12	42,675	2,345	0,011	
	Error	100	18,197			
Glycogen	Cypermethrin dose	4	$2,091 \times 10^{-4}$	5,906	0,001	0,85
	Stage/sex	3	$2,130 \times 10^{-3}$	60,15	0,000	
	Cypermethrin* Stage/sex	12	$6,859 \times 10^{-5}$	1,937	0,059	
	Error	40	$3,541 \times 10^{-5}$			
Protein	Cypermethrin dose	4	$1,419 \times 10^{-3}$	7,519	0,000	0,55
	Stage/sex	3	$8,602 \times 10^{-4}$	4,559	0,008	
	Cypermethrin* Stage/sex	12	$9,468 \times 10^{-5}$	0,502	0,901	
	Error	40	$1,887 \times 10^{-4}$			
Lipid	Cypermethrin dose	4	$3,336 \times 10^{-3}$	8,658	0,000	0,93
	Stage/sex	3	$5,913 \times 10^{-2}$	153,486	0,000	
	Cypermethrin* Stage/sex	12	$1,545 \times 10^{-3}$	4,011	0,000	
	Error	40	$3,853 \times 10^{-4}$			

TABLE 2

Cypermethrin-related changes in total fresh weight (mg) of *P. turionellae*.[a]

	CYP	Last Instars $\bar{x}$ ± SE	Pupae $\bar{x}$ ± SE	Males $\bar{x}$ ± SE	Females $\bar{x}$ ± SE
Weight*	C	45,29 ± 3,77a	35,39 ± 1,57ab	20,04 ± 0,52a	26,13 ± 0,97ab
	20	40,99 ± 1,66ab	37,94 ± 2,06a	18,69 ± 0,66a	28,39 ± 1,10a
	50	33,75 ± 3,87ab	29,48 ± 1,26bc	18,97 ± 1,37a	25,48 ± 0,89ab
	100	32,21 ± 2,39b	27,64 ± 0,97c	18,40 ± 0,79a	23,39 ± 0,86b
	150	31,62 ± 1,45b	30,13 ± 1,85bc	16,76 ± 1,08a	23,47 ± 0,50b

a. Numbers in column followed by the same letter are not significantly different (P>0,05; Tukey's HSD test).
CYP : Cypermethrin doses (ppm), C : Control group.
* Six replicates with 10 individuals per replicate.

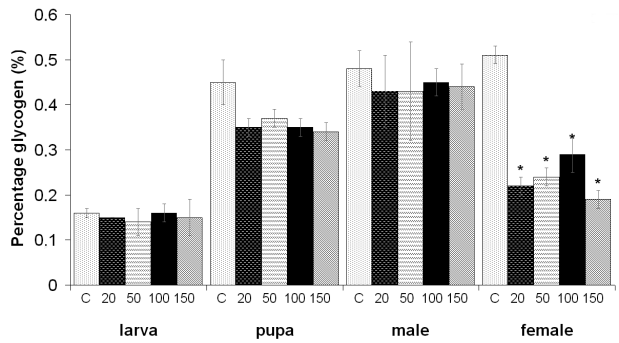

Fig. 1. – Glycogen levels (% of fresh weight) in successive developmental stages of *P. turionellae* exposed to different doses of cypermethrin.
Each bar represents mean ± SE of three replicates with 10 individuals per replicate.
Significant differences are indicated by * (P<0,05).

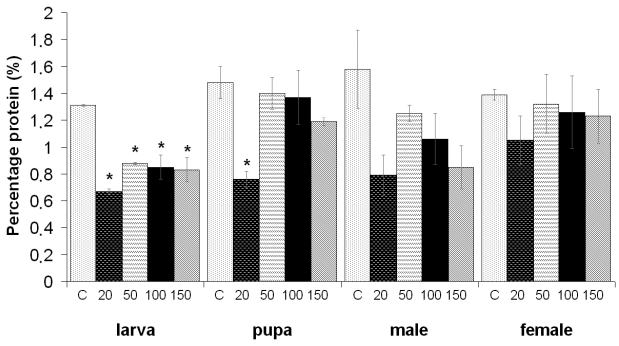

Fig. 2. – Protein levels (% of fresh weight) in successive developmental stages of *P. turionellae* exposed to different doses of cypermethrin.
Each bar represents mean ± SE of three replicates with 10 individuals per replicate.
Significant differences are indicated by * (P<0,05).

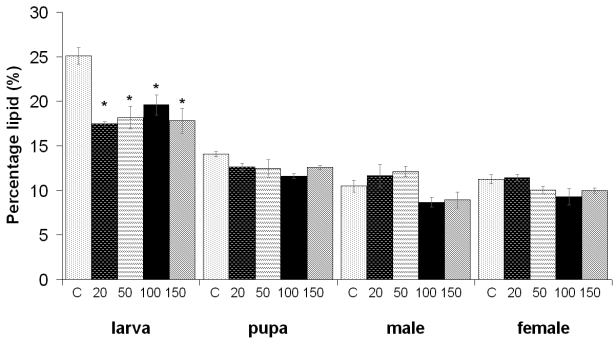

Fig. 3. – Lipid levels (% of fresh weight) in successive developmental stages of *P. turionellae* exposed to different doses of cypermethrin.
Each bar represents mean ± SE of three replicates with 10 individuals per replicate.
Significant differences are indicated by * (P<0,05).

DISCUSSION

Insects tended to lose weight in all cypermethrin treated groups except for pupae and females at 20 ppm. It has been reported that low dosages of toxicants may have beneficial effects on organisms (ORTEL, 1996). Thus, it can be speculated that the increase at 20 ppm relative to control group might also prove this assumption. However, the effect at 20 ppm was not significant in both cases, therefore from the statistical point of view; this can not be demonstrated to be the same hormesis effect reported by TOMBERLIN et al. (2002). Significant decreases were mostly apparent at higher doses of cypermethrin except for males. Studies on *G. mellonella* (MATHOVA, 1990) and *Lymantria dispar* L. (Lepidoptera : Lymantriidae) (ORTEL, 1996) larvae feeding on heavy metal contaminated food revealed that larvae lose weight at only high doses of heavy metals. The weight loss of *P. turionellae* may be attributed to the interference of sufficient food supply from host due to the antifeeding effect of cypermethrin on host.

Data on glycogen decrease were not significant for larvae, pupae and males. Only female glycogen level decreased significantly with respect to controls, but the reduction did not seem to be dose-dependent; thus suggesting that the effect is probably related to damage of reproductive system. This situation may also be of importance for the continuity of the generation, because the effect of cypermethrin is much likely to be transferred to the eggs. The eggs may have received less glycogen during oogenesis due to cypermethrin exposure. Accordingly, the glycogen amount in eggs laid by *P. turionellae* females exposed to 2,4-D and maleic hydrazine displayed significant decrease (ÖZKAN & YANIKOĞLU, 1999). It appeared that the adverse effect of cypermethrin application on glycogen content of *P. turionellae* increased through development. There was a sharp increase in glycogen level at larva to pupa transition in control and cypermethrin treated groups. The sharp increase in glycogen reserve at pupal stage was also noted during metamorphosis of *Ceratitis capitata* Wiedman (Diptera : Tephritidae) (TOLMASKY et al., 2001; NESTEL et al., 2003). However, the ratio of glycogen increase in our cypermethrin treated groups was lower than that of control groups at larva to pupa transition. This result might be associated with the increased effect of cypermethrin on pupae relative to larvae. There was no difference in glycogen level between male and female wasps in controls. However, glycogen levels of males in all cypermethrin treated groups were higher than those of females. In other studies, sexual difference in susceptibility to pesticides has also been noted in some parasitoids with males being generally more susceptible than females (SCHOONEES & GILLIOMEE, 1982; SCOTT & RUTZ, 1988; RATHMAN et al., 1992) or vice versa (SPOLLEN & HOY, 1992). The differences may be partly related to variation in size and physiology between sexes (BAKER et al., 1995; CROFT, 1990). Glycogen depletion in *P. turionellae* may also appear as a result of cypermethrin-induced effects on glycolytic pathway.

P. turionellae larvae were more susceptible to cypermethrin treatment than pupae and adults in terms of decrease in protein level. This result suggests that insecticide application affected the larval stage more than pupal and adult stages. It has been reported that organophosphorous insecticides cause a significant depletion in total protein content in the haemolymph and fat body of the silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae) (NATH et al., 1997). USMANI & KNOWLES (2001) also stated that larvae of different species were more susceptible to insecticides than adults. Our results revealed that larvae had the lowest level of protein both in cypermethrin treated and control groups. Protein level increased at larva to pupa transition. However, the increase in total protein level at larva to pupa transition at 50, 100, and 150 ppm was much more than the increase at 20 ppm and in control group. This may be partially based on the lower protein level of larvae in cypermethrin treated groups compared to controls. It has been reported that total protein increases at larva to pupa transition, but decreases in adults when larvae of *Spodoptera litura* Fabr. (Lepidoptera : Noctuidae) exposed to various insecticides (VIJAYARAGHAVAN & CHITRA, 2002). The protein level of males was higher than females in control group, but it was lower in cypermethrin treated groups. This situation indicates that cypermethrin affects the protein level of males much more than females. A hidden damage that would further affect population density might have occurred when insects were exposed to sublethal doses of insecticides at larval stage (DAVIS et al., 1988).

P. turionellae larvae displayed a striking decrease in lipid content when exposed to cypermethrin whereas no significant alterations were observed in the lipid content of pupae and adults. Similar to the protein results, insecticide treatment mostly affected the larval stages in all experimental groups. Glycogen content of *P. turionellae* larvae was not affected by cypermethrin application, but lipid content declined drastically. This could point to a shift in energy metabolism to lipid catabolism due to insecticidal stress. Adults had the lowest and larvae had the highest level of lipid in all cypermethrin treatments and controls. LOHAR & WRIGHT (1993) also verified lipid depletion in haemolymph, fat body, and oocyctes in *Tenebrio molitor* L. (Coleoptera : Tenebrionidae) females exposed to malathion. They stated that depletion of lipid

might have been due to the effect of insecticide on the adipokinetic hormone that controls lipid metabolism. Lipid content displayed a drastic decrease at larva to pupa transition in control and experimental groups. Lipids have usually been thought of as the predominant source of energy during this nonfeeding stage of insects (BEENAKKERS et al., 1981). It has been assumed that a large proportion of carbohydrates digested during larval development are converted to lipids (CANDY, 1985). The accumulation of lipids during larval stage and the main consumption of lipids during the adult stage suggest that lipids may have been secured for this last highly energy-demanding phase of metamorphosis. However, lipid depletion in our cypermethrin treated groups was lower than that of in controls during larva to pupa transition. This indicates that lipid content of larvae is more susceptible to insecticide treatment than that of pupae.

Our results may provide an overall picture of glycogen, protein, and lipid metabolism during metamorphosis under insecticidal stress. Depletion of glycogen and lipid content may be due to utilization of these reserves for energy generation as a result of insecticide-induced stress (SANCHO et al., 1998; RAMBABU & RAO, 1994). The decrease in protein content may also indicate a physiological adaptability to compensate for insecticidal stress (RIBEIRO et al., 2001). Animals require high energy under stress conditions and the energy demand may have led to the stimulation of protein catabolism. The decrease in protein content might also be due to a mechanism of lipoprotein formation, which will be used to repair damaged cell and tissue organelles (SANCHO et al., 1998; RAMBABU & RAO, 1994). Protein depletion in tissues may also constitute a physiological mechanism by retaining free amino acid content in haemolymph, to compensate for osmoregulatory problems encountered due to the leakage of ions and other essential molecules during the insecticidal stress (SRIVINAS, 1986). In the present study, it is clearly evident that cypermethrin has a toxic impact on parasitoid metabolic pathway. Whatever the reasons are, the decrease in the level of glycogen, protein, and lipid contents will adversely affect the growth, development, and reproduction of parasitoid species. This fact, in turn, may disrupt the effectiveness of parasitoid species in biological control programs. The assessment of potential effects that insecticides have on natural enemies of pests will also contribute to success in IPM programs. The authors are currently evaluating the effect of insecticide on the biological parameters of *P. turionellae*.

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