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Effects of the pyrethroid insecticide deltamethrin on the hemocytes of *Galleria mellonella*

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Abstract: In the present study, the effects of deltamethrin on cell-mediated immune response and genotoxic damage of *Galleria mellonella* (Lepidoptera: Pyralidae) were investigated. Different concentrations of deltamethrin (5, 20, 50, 100, and 150 µg) were administered to *G. mellonella* at 24, 48, 72, and 96 h. Five types of hemocytes were identified in *G. mellonella*: prohemocytes, plasmatocytes, granulocytes, spherulocytes, and oenocytes. Total hemocyte counts (THCs) were significantly affected by deltamethrin. At 72 h, THCs of the treated group had significantly decreased compared with the control groups at high deltamethrin concentrations (50, 100, and 150 µg); however, at 96 h, THCs had significantly increased at deltamethrin concentrations of 100 and 150 µg. Micronucleus formation was significantly affected by deltamethrin; in particular, high concentrations of deltamethrin (100 and 150 µg) caused an increase in micronucleus formation at all time points. We conclude that deltamethrin affected THCs and caused the induction of micronucleus formation via genotoxic damage in *G. mellonella*.

Key words: Deltamethrin, *Galleria mellonella*, hemocyte, micronuclei

1. Introduction

Insect immune responses are divided into humoral and cellular responses. Humoral immunity includes the production of antimicrobial peptides, reactive oxygen and nitrogen derivatives, and coagulation and melanization of hemolymph (Bogdan et al., 2000; Lowenberger, 2001; Büyükgüzel et al. 2007), whereas cellular immunity comprises hemocyte-mediated reactions such as phagocytosis, nodule formation, and encapsulation (Lavine and Strand, 2002; Durmuş et al., 2008). Both these systems are influenced by environmental conditions such as starvation, thermal stress, and pesticides (Sharma et al., 2003; Girón-Pérez, 2010; Ghasemi et al., 2014).

Immune responses of insects show great similarity to the innate immune response of mammals (Kavanagh and Reeves, 2007). Jones (1964) suggested that the morphology, embryonic origin, amoeboid movement, and phagocytic activity of insect hemocytes are comparable to those of the white blood cells of mammals. Therefore, some insect species are used as model organisms in pathogenic, genotoxic, and biochemical studies (Cook and Arthur, 2013; Emre et al., 2013). *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) is a favored model organism for such studies because of its short life cycle, larval size, ease of rearing, and ability to show results within days (Emre et al., 2013). The hemocytes of *G. mellonella* were studied by Ashhurst and Richards (1964). They identified five different types of hemocytes: prohemocytes, plasmatocytes, adipohemocytes, oenocytes, and spherulocytes.

Environmental pollutants (such as insecticides and heavy metals) can alter the number of and/or induce structural abnormalities in hemocytes. These changes can be used to characterize the genotoxic, physiological, and biochemical effects of pollutants. Therefore, hemocytes (via changes in cell number and development of structural abnormalities) are frequently used to demonstrate the cytogenetic damage caused by toxic chemicals (Yeh et al., 2005). Genotoxicity biomarkers are widely measured in ecotoxicology as molecular toxic effects of major environmental pollutants (Wessel et al., 2007). The micronucleus is a biomarker widely used in biomonitoring studies performed to evaluate the effects of pesticide exposure (Siu et al., 2004; Bolognesi et al., 2011).

Synthetic pyrethroids, such as deltamethrin, have become some of the most widely used pesticides owing to their selective action, low mammalian toxicity, and low accumulative capability in vegetables and soil compared with other pesticide types such as organophosphates and organochlorines (Erstfeld, 1999). Therefore, their possible effects on living organisms must be evaluated.

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The aim of this study was to evaluate the effects of the synthetic pyrethroid deltamethrin on total hemocyte count (THC) and micronucleus formation in the model organism *G. mellonella*.

2. Material and methods

2.1. Preparation of experimental insects and nutrients

The insects were reared in our laboratory at 28 ± 2 °C and 70 ± 5% relative humidity in the dark. Larvae were fed an artificial diet developed by Bronskill (1961); the diet was supplemented with deltamethrin {[(S)-cyano-(3-phenoxyphenyl)-methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate} at concentrations of 5, 20, 50, and 100 µL per 100 g of nutrient. Control insects were fed a deltamethrin-free diet. Dimethyl sulfoxide (DMSO) was used as a solvent for deltamethrin. We accordingly prepared a solvent control group containing DMSO. The control and deltamethrin-supplemented diets were placed in 500-mL jars and last instars (averaging 150–200 mg) of *G. mellonella* larvae (N: 20) were placed in each jar. After 24, 48, 72, and 96 h, larvae were collected from the jar, and THCs and micronucleated cells were evaluated.

2.2. Micronucleus assay

For analysis of micronuclei in hemocytes, larvae were pierced on the first hind leg with a sterile needle, and one drop of hemolymph was spread on a glass slide and fixed with methanol for 5 min. The slides were then stained with 10% Giemsa in Sorensen's buffer for 10 min. A total of 1000 hemocytes were scored for each slide at a magnification of 1000×. Particles that were separate from the main nucleus, painted in the same color and smaller than the main nucleus, were recorded as micronuclei (Venier et al., 1997), and micronucleated cells were counted.

2.3.Hemocyte types

For analysis of hemocyte types, one drop of hemolymph was spread on a glass slide and fixed with methanol for 5 min. The slides were then stained with 10% Giemsa in Sorensen's buffer for 10 min. Each slide was scanned under a light microscope at a magnification of 1000×, and hemocytes were classified according to the method described by Jones (1967).

2.4. Total hemocyte counts

For analysis of THCs of *G. mellonella*, larvae were pierced on the first hind leg with a sterile needle. Hemolymph was diluted (1:10) with Tauber–Yeager solution (NaCl, 4.65 g; KCl, 0.15 g; $CaCl_2$, 0.11 g; gentian violet, 0.005 g; and 0.125 mL of acetic acid/100 mL) (Tauber and Yeager, 1936). The diluted hemolymph was withdrawn with a micropipette (10 µL) and applied to a Neubauer hemocytometer. The number of circulating hemocytes per cubic millimeter was calculated using the formula suggested by Jones (1962).

2.5. Statistical analysis

Each experiment was repeated at least three times. All parameters were expressed as mean ± SE. Equality of variances was tested using Levene's test. Statistical differences between the treated and control groups were determined by analysis of variance followed by the Student–Newman–Keuls test using SPSS 13.0. The level of significance was set at 0.05.

3. Results

Five types of hemocytes were identified in *G. mellonella* larvae: prohemocytes, plasmatocytes, granulocytes, spherulocytes, and oenocytes (Figure 1).

Prohemocytes are small, round, oval, or elliptical variably sized cells. The plasma membrane is generally smooth, and the nucleus is large and centrally located and almost fills the cell (Figure 1A). Plasmatocytes are polymorphic and of variable sizes. The plasma membrane is irregular, and the nucleus is located at the center of the cell (Figure 1B). Granulocytes are of variable sizes, spherical or oval. They have a characteristic granular cytoplasm, and the nucleus is relatively small and centrally located (Figure 1C). Spherulocytes are ovoid or round cells of variable sizes with a characteristic spherulitic cytoplasm; the nucleus is generally small and centric or eccentric (Figure 1D). Oenocytes are oval, thick, spherical cells. The plasma membrane is generally smooth and sessile, and the nucleus is generally eccentrically located and small (Figure 1E).

THCs of the control groups were determined as 227.33 × 10^4 cells/mL. In the solvent control group (DMSO), THCs were decreased compared with the control group, and the

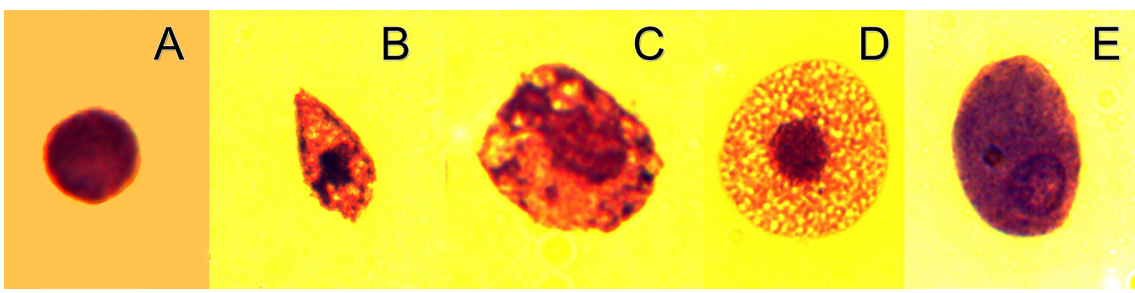

Figure 1. A- Prohemocyte, B- plasmatocyte, C- spherulocyte, D- oenocyte, and E- granulocyte in the last instar of *G. mellonella*.

count was 219.67 ′ 10^4 cells/mL (Table 1). Exposure of *G. mellonella* larvae to deltamethrin resulted in a significant decrease in the number of hemocytes at all concentrations compared with the control and solvent control groups at 24 h. This decrease was parallel to the increase in deltamethrin concentrations. At 48 h, THCs decreased at concentrations of 5, 50, 100, and 150 µg compared with the control groups. Furthermore, at 72 h, THCs decreased significantly at all deltamethrin concentrations compared with the control groups, and at 96 h, THCs increased significantly at deltamethrin concentrations of 100 and 150 µg.

The effects of deltamethrin on micronucleus formation in *G. mellonella* larvae are presented in Table 2. The micronucleated cell number of the control groups was determined as 1.67/1000 cells at 24 h. This number was significantly affected by deltamethrin and was increased at concentrations of 20 µg and higher. Furthermore, DMSO increased micronucleus formation in the solvent control group (3.67/1000 cells). At 48 h, higher concentrations of deltamethrin (50, 100, and 150 µg) increased micronucleus formation. The micronucleated cell number was 1.00/1000 cells in the control group at 72 h. All deltamethrin concentrations and DMSO had increased micronucleus formation by this time. The highest concentrations of deltamethrin (100 and 150 µg) increased micronucleus formation compared with the control groups and other concentrations at 96 h.

4. Discussion

We identified five different types of hemocyte in *G. mellonella.* Studies have shown that the hemocytes

Table 1. Effects of different deltamethrin concentrations on THC of *G. mellonella*.

	THC × 10^4			
	24 h (mean ± SE)	48 h (mean ± SE)	72 h (mean ± SE)	96 h (mean ± SE)
Control	227.33 ± 3.71a	230.67 ± 2.03a	237.67 ± 3.38a	249.33 ± 2.40bc
Control+DMSO	219.67 ± 1.76b	228.67 ± 5.17a	234.67 ± 2.40a	252.67 ± 3.48b
5 µg	209.00 ± 1.53c	205.67 ± 2.96c	224.67 ± 2.85b	240.67 ± 2.40c
20 µg	207.33 ± 3.71c	219.67 ± 3.28ab	224.00 ± 4.04b	228.33 ± 3.38d
50 µg	198.33 ± 0.88d	214.00 ± 1.73bc	213.67 ± 2.02c	229.67 ± 4.05d
100 µg	194.33 ± 1.20d	214.67 ± 3.76bc	207.00 ± 2.31c	281.67 ± 1.76a
150 µg	142.33 ± 2.96e	203.67 ± 1.76c	207.67 ± 0.67c	286.00 ± 3.78a

Data are expressed as mean ± SE of three replicates. Different letters (a, b, c, d, and e) indicate statistical differences between groups at $P < 0.05$.

Table 2. Effects of different deltamethrin concentrations on micronucleus formation of *G. mellonella*.

	Micronucleated cell number/1000			
	24 h (mean ± SE)	48 h (mean ± SE)	72 h (mean ± SE)	96 h (mean ± SE)
Control	1.67 ± 0.33b	2.33 ± 0.33c	1.00 ± 0.00c	2.00 ± 0.00b
Control+DMSO	3.67 ± 0.33a	3.33 ± 0.33bc	3.33 ± 0.33b	2.67 ± 0.33b
5 µg	2.67 ± 0.33ab	3.33 ± 0.33bc	3.33 ± 0.33b	2.67 ± 0.33b
20 µg	3.67 ± 0.33a	4.67 ± 0.33bc	3.33 ± 0.33b	3.00 ± 0.58b
50 µg	3.67 ± 0.67a	5.00 ± 0.58a	4.67 ± 0.33b	3.33 ± 0.33b
100 µg	4.00 ± 0.00a	4.33 ± 0.33ab	6.00 ± 0.58a	5.67 ± 0.33a
150 µg	4.00 ± 0.00a	5.33 ± 0.33a	6.67 ± 0.33a	6.00 ± 0.00a

Data are expressed as mean ± SE of three replicates. Different letters (a, b, and c) indicate statistical differences between groups at $P < 0.05$.

of Lepidoptera can be classified as prohemocytes, plasmatocytes, granulocytes, spherulocytes, and oenocytes on the basis of their morphological, histochemical, and functional characteristics (Ashhurst and Richards, 1964; Neuwirth, 1973; Lavine and Strand, 2002; Salem et al., 2014). Our results are consistent with the results of these previous studies.

In the present study, THCs of *G. mellonella* were significantly affected by deltamethrin. At 72 h, THCs of the treated group showed a tendency to decrease compared with the control groups. Similar results were observed in *Litopenaeus vannamei* (Boone) (Mello et al., 2011), *Schistocerca gregaria* (Forskal) (Halawa et al., 2007), *Rhynocoris kumarii* (Ambrose and Livingstone) (George and Ambrose, 2004), and *Papilio demoleus* (Linnaeus) (Pandey et al., 2012) when exposed to insecticides. This reduction may result from the formation of nodules, encapsulation, apoptosis, and inhibition of endocrine glands (Sharma et al., 2003; Pandey et al., 2007) and also from the inhibition of larval hematopoietic function and cell proliferation (Zhu et al., 2012). In our study, at the end of 96 h, an increase in THCs was observed at high deltamethrin concentrations (100 and 150 µg). These results are consistent with those of previous studies in which THCs increased after treatment with deltamethrin and lambdacyalothrin in *S. gregaria* (Al-Hariri and Anjum, 2001); atrazine in *Lymnaea palustris* (Muller) (Russo and Lagadic, 2000); hexaflumuron in *Spodoptera litura* (Fabricius) (Zhu et al., 2012); and monocrotophos, dimethoate, methylparathion, and quinalphos in *R. kumarii* (George and Ambrose, 2004). In addition, George and Ambrose (2004) interpreted the increase as a defensive action against the hemocyte detoxification of pesticides. We propose that the increase in THCs stems from the increase in hematopoiesis.

DMSO, used as a solvent for deltamethrin, is an antioxidant compound. However, owing to its sulfhydryl content, it also shows prooxidant properties (Sanmartin-Suarez et al., 2011). At 24 h, DMSO had decreased THCs compared with the control groups, possibly as a result of its prooxidant property.

Pesticides are more or less toxic to living organisms. Using these chemicals in high doses causes deletions and anomalies in chromosomes, mutations, and micronucleus formation (Tosun et al., 2001). Increased numbers of micronuclei are important as indirect indicators of toxic, mutagenic, and carcinogenic substances (Rencuzogullari and Topaktas, 2000; Şekeroğlu and Şekeroğlu, 2011). Studies have shown that different types of pesticides induce micronucleus formation in living organisms (Rencuzogullari and Topaktas, 2000; Çelik et al., 2005; Uckan and Sak, 2010). In our study, micronucleated cell numbers in the treated group increased significantly compared with the control groups, particularly at high concentrations of deltamethrin (100 and 150 µg). Similar effects were observed in *Pimpla turionellae* (Linnaeus) exposed to cypermethrin (Uckan and Sak, 2010) and *Pardosa astrigera* (Koch) exposed to acetamiprid and chlorpyrifos (Li et al., 2011). Although DMSO is known as a radical scavenger, in our study, DMSO induced micronucleus formation at 24 and 72 h compared with the control group. Some researchers have reported the genotoxic potential of DMSO (Rencuzogullari et al., 2002; Diaz et al., 2007). In addition, Diaz et al. (2007) showed that DMSO alone increases micronucleus formation. We propose that this increase results from the genotoxic potential of DMSO.

In our study, micronucleus formation also occurred in the control group. This may have resulted from the contamination of natural nutrients (honeycomb and bran) with toxicants.

In summary, our study showed that deltamethrin affects the immune system of *G. mellonella*. Because of the similarities of the innate immune system between mammals and lepidopteran insects, these results provide indirect information regarding the possible effects (genotoxic and immunotoxic) of deltamethrin in mammalian cells.

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