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Effects of Endosulfan on *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) Larvae

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Thaumetopoea pityocampa larvae are very harmful to pines and they also cause allergic reactions in men and animals. In this study, different concentrations of endosulfan were administered to *T. pityocampa* larvae via pine needles which were prepared by the dipping method. The data obtained were statistically evaluated using probit analysis and a $LC_{50/48hrs}$ value for *T. pityocampa* larvae found to be 1.679 mg/l. Also, 12, 24, 36 and 48 hrs after 1.679 mg/l endosulfan treatment, ultrastructural changes in the midgut epithelium of *T. pityocampa* were investigated. No pathological changes were observed after 12 hrs, swelling and vacuolization of mitochondria and dilation of endoplasmic reticulum after 24 hrs, swelling of mitochondria and breaking of mitochondrial cristae and dissolving of nucleoplasm after 36 hrs, finally large vacuoles in the midgut epithelium cells were observed after 48 hrs.

Key words: *Thaumetopoea pityocampa* larvae, insecticides, endosulfan, mortality, ultrastructural study.

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Thaumetopoea pityocampa, the pine processionary caterpillar, is the most important endemic pine pest in the Mediterranean area, not only because of its high defoliating power, but also due to the human health problems caused by the urticating hairs of the larvae (RAUSELL *et al.* 1999). In both men and animals, *T. pityocampa* larvae cause symptoms such as atopy, urticarial dermatitis, oedema, conjunctivitis, dyspnea and anaphylactic reactions (VEGA *et al.* 1999; VEGA *et al.* 2003; KALENDER *et al.* 2004a). Thaumetopoein, a protein localized on the hairs of *T. pityocampa* larvae, produces allergic reactions (REBOLLO *et al.* 2002). Although there is an increasing demand for environmentally friendly alternative methods such as viruses, fungi, pheromones, parasites, bacteria, particularly insect growth inhibitors and chemical insecticides are used to control this lepidopteran insect (RAUSELL *et al.* 1999; OGUTCU *et al.* 2005).

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9 methano-2,4,3-benzodiexathiepin-3-oxide), a broad spectrum insecticide of the organochlorine group, is a central nervous system poison. It has been classified by WHO (1986) in the category of technical products that are moderately hazardous. Endosulfan controls a wide range of sucking and chewing insect pests notably of the orders of Lepidoptera, Coleoptera, Heteroptera, Homoptera, Tysanoptera, Diptera and some species belonging to the order of Acarina. The pesticide is used on non-food crops such as cotton and tobacco and also on food crops such as vegetables, fruits, corn, cereals, tea and coffee (KULLMAN & MATSUMURA 1996).

The aim of the present study was to assess the effect of endosulfan on *T. pityocampa* larvae. The mortality of larvae was determined and $LC_{50/48hrs}$ (the concentration required to kill half a population of animals in 48 hrs) dose was calculated. After application of the $LC_{50/48hrs}$ dose, ultrastructural investigations of midguts taken from live larvae were performed.

Material and Methods

Insects

Larvae of *T. pityocampa* were collected from Kahramanmaras, Turkey. In the laboratory the larvae were fed with pine needles (*Pinus nigra*). Larvae were individually reared in the laboratory at 25°C ± 1and relative humidity of 60% ± 10 under a 12 : 12 light : dark photoperiod.

Chemical

Endosulfan, technical purity 95%, was obtained from Refik Saydam Hifzissihha Institute, Poison Research Center, Ankara, Turkey.

Toxicity Tests

Tests were done on fourth instar larvae (L4) of *T. pityocampa*. Experiments commenced at night because larvae of *T. pityocampa* are active during this period. Larvae were starved 2 days before the beginning of the experiments. Larvae were divided into control and test groups. Each group consisted of 20 specimens. Endosulfan was diluted in distilled water in nine concentrations 0.05, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/l. Fresh *Pinus nigra* needles were dipped in each concentration for approx. 30 seconds. Next, they were air dried for approx. 1 hr and 10 needles (total weight 0.5 g) were placed in plastic Petri (ϕ 10 cm) dishes. A single larva was put into each plastic dishe and their feeding was controlled. The mortality of larvae was determined after 12, 24, 36 and 48 hrs and the $LC_{50/48hrs}$ dose was calculated. In the control group only distilled water was used.

For histological investigations a separate test was arranged by application of the $LC_{50/48hrs}$ dose, for 20 larvae in each group. After 12, 24, 36 and 48 hrs midguts from live larvae were dissected and prepared for EM.

Data analysis

48 hrs after feeding the total number of dead larvae were counted. The effect of endosulfan on *T. pityocampa* was calculated using the probit analysis LC_{50} (lethal concentration) determination method. The LC_{50} value was calculated by the LC_{50} software program, version 1.00 computer program developed by EPA (US EPA 1999).

Electron microscopy

For ultrastructural examination of midguts, primer fixation was made in 3% glutaraldehyde (Agar Sci. Ltd., Essex, England) in sodium phosphate buffer (200 mM, pH 7.4) (Merck, Alfred Paluka Co., Turkey) for 3 hr at 4°C. Material was washed with the same buffer, postfixed in 1% osmium tetroxide (Agar Sci. Ltd., Essex, England) and in sodium phosphate buffer pH 7.4 for 1hr at 4°C. Tissue samples were washed with the same buffer for 3 hr at 4°C, dehydrated in ethanol series (Agar Sci. Ltd., Essex, England), and embedded in Araldite (Agar Sci. Ltd., Essex, England). Thin sections were cut with Leica EM UC6 (Leica Co., Austria) ultramicrotome. Midguts were stained with 2% uranyl acetate and lead citrate. The sections were viewed and photographed on a Jeol 100 CX II transmission electron microscope (Jeol Ltd., Japan) at 80 kV.

Table 1

The relationship between endosulfan concentrations and the mortality rate of *T. pityocampa* larvae

Concentrations (mg/l)	*Mortality of larvae (%)				Total mortality rate (%)
	12 hrs	24 hrs	36 hrs	48 hrs	
0.05	–	–	–	–	–
0.5	–	–	5	5	10
1.0	–	–	10	15	25
1.5	–	5	10	20	35
2.0	5	15	15	20	55
2.5	5	15	20	25	65
3.0	5	15	25	30	75
3.5	10	15	25	35	85
4.0	15	20	25	40	100

*Each experimental group consisted of 20 larvae.

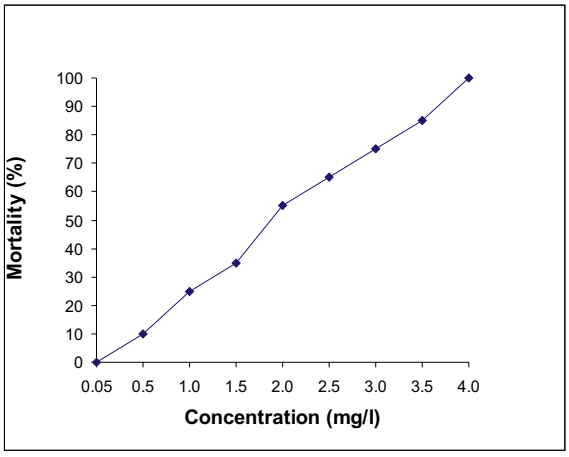

Fig. 1. The relationship between endosulfan concentration and the mortality rate of *T. pityocampa* larvae throughout the 48 hrs period.

Table 2

48 hrs toxicity results of the endosulfan bioassay on *T. pityocampa* larvae

Point	Concentration mg/l	95% Confidences limits	Intercept ±SE**	Slope ±SE
*LC 1.00	0.303	0.133-0.478	4.2962±0.1819	3.1284±0.4764
LC 5.00	0.500	0.269-0.709		
LC 10.00	0.654	0.392-0.877		
LC 15.00	0.783	0.503-1.014		
LC 50.00	1.679	1.379-1.976		
LC 85.00	3.600	2.945-4.941		
LC 90.00	4.311	3.425-6.313		
LC 95.00	5.633	4.259-9.133		
LC 99.00	9.302	6.344-18.440		

Note: Each experimental group consisted of 20 larvae. *LC: Lethal concentration, **SE: Standard error

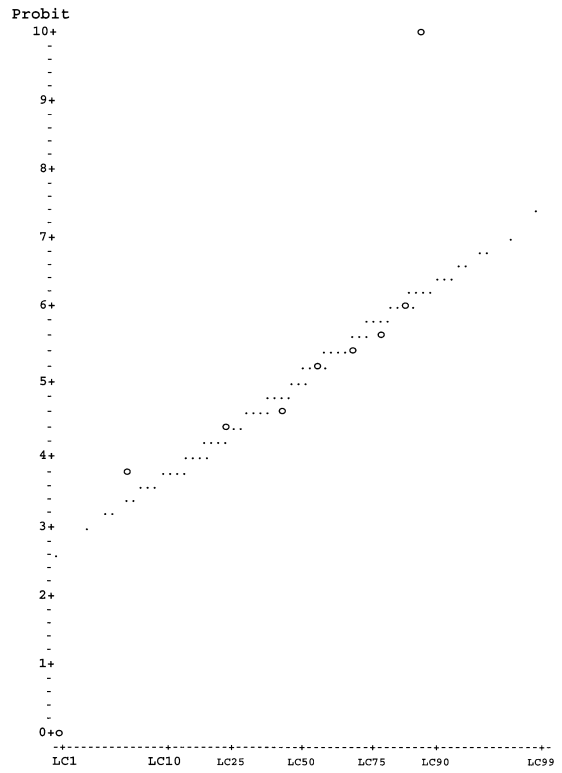

Fig. 2. Plot of adjusted probits and predicted regression line of endosulfan treatment to *T. pityocampa* larvae

Results

In the present study, nine distinct concentrations of endosulfan were administrated to *T. pityocampa* larvae. The mortality rate of larvae was calculated as a percentage after 12, 24, 36 and 48 hrs of endosulfan treatment (Table 1). No mortality was observed in the control group and at a 0.05 mg/l concentration during 48 hrs; 100% mortality occurred at 4.0 mg/l (Table 1). The first dead larvae were observed in concentrations of 0.5 mg/l and 1.0 mg/l after 36 hrs, and in a concentration of 1.5 mg/l after 24 hrs. Mortality was observed already after 12 hrs at 2.0, 2.5, 3.0, 3.5 and 4.0 mg/l concentrations of endosulfan. The dose-response graph plotting the relation between the mortality rate and endosulfan concentrations is given using a linear scale (Fig. 1) – the mortality of *T. pityocampa* larvae increased depending on the dose of endosulfan. In addition, approximately 4-6 hrs before death, the movement of larvae increased and their colour became darker.

Data obtained from the toxicity tests were evaluated using the probit analysis method. The $LC_{50/48}$ value for the pine processionary caterpillar was found to be 1.679 mg/l (Table 2). 95% confidence limits were between 1.379-1.976 mg/l (Table 2). The probit regression line properly predicted the LC values (Fig. 2).

Electron microscope examination of the midgut of control larvae revealed that it is lined by columnar epithelial cells. The apical surface of epithelial cells has abundant and long microvilli. Midgut cells contain numerous mitochondria and rough endoplasmic reticulum (Fig. 3).

EM examination of midgut cells showed no pathological changes 12 hrs after treatment at 1.679 mg/l (Fig. 4). Swelling and vacuolization in mitochondria and dilation of endoplasmic reticulum of midgut epithelial cells appeared approx. 24 hrs after endosulfan treatment (Fig. 5). The swelling of mitochondria and breaking of mitochondrial cristae were observed 36 hrs after treatment. Additionally, the dissolution of nucleoplasm occurred (Fig. 6). Besides the swelling of mitochondria and dilation of the endoplasmic reticulum, vacuoles of large size were observed 48 hrs after endosulfan treatment (Fig.7).

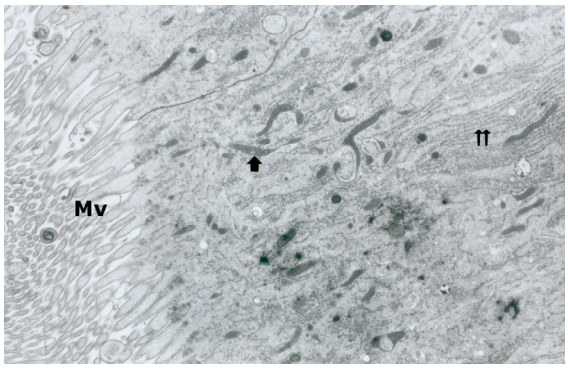

Fig. 3. Electron micrograph of midgut epithelium of control larvae of *T. pityocampa*. Mv: microvilli, endoplasmic reticulum (double arrow), mitochondria (thick arrow), ×7000.

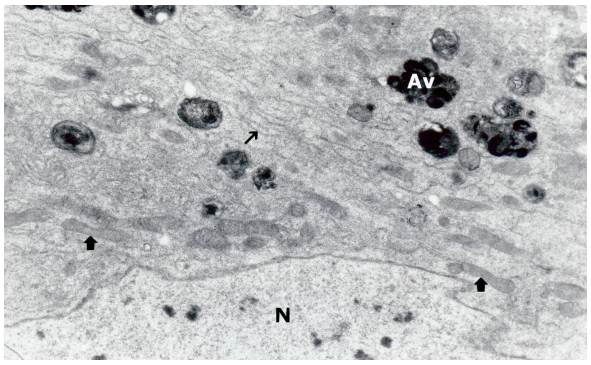

Fig. 4. Midgut epithelial cell of *T. pityocampa* larvae after 12 hrs of endosulfan treatment at 1.679 mg/l dose. N: nucleus, Av: autophagic vacuole, endoplasmic reticulum (thin arrow), mitochondria (thick arrows), ×10000.

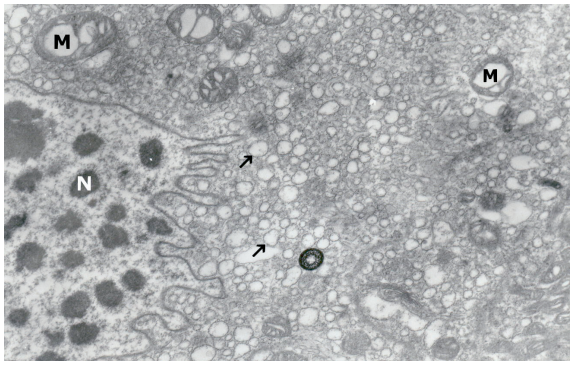

Fig. 5. Swelling of mitochondria (M) and dilation of endoplasmic reticulum (arrows) in a midgut epithelial cell of *T. pityocampa* larvae after 24 hrs endosulfan treatment at 1.679 mg/l dose. N: nucleus, ×12000.

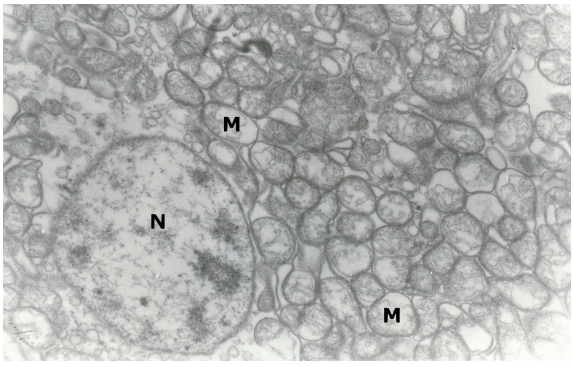

Fig. 6. Swelling and vacuolization of mitochondria (M) and dissolution of nucleoplasm in a midgut epithelial cell of *T. pityocampa* larvae after 36 hrs endosulfan treatment at 1.679 mg/l dose. N: nucleus, ×12000.

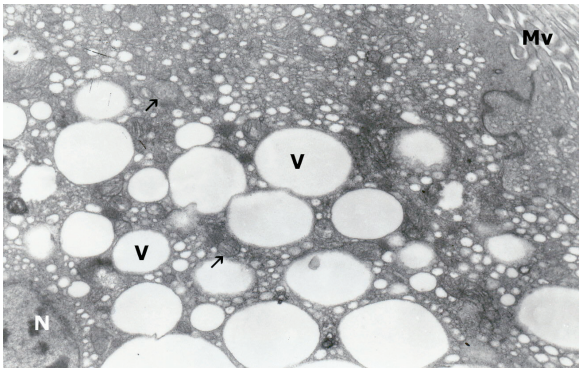

Fig. 7. Numerous vacuoles (V) in the cytoplasm of midgut epithelial cells of *T. pityocampa* larvae after 48 hrs of endosulfan treatment at 1.679 mg/l dose. Mitochondria (arrows), N: nucleus, Mv: microvilli, ×10000.

Discussion

Thaumetopoea pityocampa is one of the most important causes of forest damage in Mediterranean countries, Central Europe, the Middle East and North Africa (VEGA *et al.* 1999), bringing about environmental destruction and economic loss. For this reason this insect should be controlled. In the present study, the effect of endosulfan on *T. pityocampa* larvae, including ultrastructural changes in the cells of midgut epithelium, were investigated. It is known that the L3-L4 larval stages are mainly responsible for pine forest defoliation (BATTISTI *et al.* 1998), as well as erucism and allergies in humans and animals (VEGA *et al.* 2000). Therefore, in this study the L4 larval stage of *T. pityocampa* was used.

Endosulfan is an organochlorine pesticide used for insect control (KRANTHI *et al.* 2002; RIBEIRO *et al.* 2001; SYMINGTON 2003). The mechanism behind the toxicity of organochlorine pesticide compounds acts mainly by blocking acetylcholinesterase, an enzyme which decomposes acethylcholine. Immobilization of this enzyme results in an accumulation of excessive amounts of acetylcholine in nervous tissue and muscular motor plates, as well as in symptoms of endogenic poisoning by this neurohormone. These pesticides also cause disturbances in the permeability of cellular membranes by the inhibition of enzymes which regulate membrane flow (KALENDER *et al.* 2004b). Such pesticides also affect ion channels causing the death of insects.

In the present study insect behaviour increased extremely 4-6 hrs after endosulfan treatment. This

is an indicator of the inhibition of acethylcholine esterase. This study is based on determination of the $LC_{50/48hrs}$ dose value. The result obtained was used in a probite analysis using the EPA program. The mean $LC_{50/48hrs}$ value of endosulfan was found to be 1.679mg/l. 95% lower and upper confidence limits were 1.379 and 1.976 mg/l, respectively. Half of the population could be destroyed in the 95% confidence limits.

For ultrastructural investigations, the calculated dose of $LC_{50/48hrs,}$ 1.679 mg/l, was used. At this dose endosulfan caused pathological changes in midgut epithelium, such as the swelling of mitochondria at 24 hrs, dissolution of nucleoplasm at 36 hrs and the appearance of large vacuoles in the cytoplasm 48 hrs after endosulfan treatment. A decrease of mitochondrial enzyme activity and calcium level may be predicted according to these ultrastructural data. MOUSSA and HAFEZ (1995) have shown that pesticides inhibited mitochondrial enzyme activity. According to these findings, endosulfan not only affects the nervous system, it also affects other tissues and systems such as the muscular and endocrine systems under the control of the nervous system.

Summing up the present results, the endosulfan $LC_{50/48\ hrs}$ dose was calculated as 1.679 mg/l and this concentrations can be reliably used for the control of *T. pityocampa* larvae. However, it should be stressed that endosulfan also has toxic effects on mammals including man.

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