Folia biologica (Kraków), vol. **54** (2006), No 3-4

Toxicity of Essential Oils Extracted from *Origanum onites* L. and *Citrus aurentium* L. against the Pine Processionary Moth, *Thaumetopoea wilkinsoni* Tams.

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Accepted June 20, 2006

CETIN H., ERLER F., YANIKOGLU A. 2006. Toxicity of essential oils extracted from *Origanum onites* L. and *Citrus aurentium* L. against the pine processionary moth, *Thaumetopoea wilkinsoni* Tams. Folia biol. (Kraków) **54**: 153-157.

The pine processionary moth (PPM), *Thaumetopoea wilkinsoni* Tams. (Lepidoptera: Thaumetopoeidae), is an important forest pest in the Mediterranean area, additionally urticating hairs of the caterpillars of this species cause strong allergic reactions on skin of humans and animals. In the present study, essential oils extracted from aerial parts of *Origanum onites* L. and fruit peels of *Citrus aurentium* L. were tested at three doses (0.1, 0.5 and 1%) against 4th and 5th instar larvae of the pest. The results showed that the activities were concentration dependent. The LD_{50} and LD_{90} values were 0.288 and 0.926% for *O. onites*, 0.530 and 2.306% for *C. aurentium*, respectively.

Key words: *Thaumetopoea wilkinsoni*, larvicidal activity, essential oil, *Origanum onites*, *Citrus aurentium*.

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Thaumetopoea wilkinsoni Tams., the Pine Processionary Moth (PPM) (Lepidoptera: Thaumetopoeidae), is very important pest of pine trees in the Near East and Turkey (MENDEL 2000). In many studies on the PPM in Turkey, this species was regarded as *Thaumetopoea pityocampa* Schiff (CARUS 2004; KALENDER *et al*. 2005; KANAT & ALMA 2004). However, recent molecular studies showed that the PPM species in Mediterranean area was *T. wilkinsoni* (GAFNI *et al*. 1996; MENDEL 1990; SALVATO 2002). Its caterpillars feed on pine needles and move through the trees in a long procession, one leading and the other following, each with its eyes half closed and its head snugly fitted against the rear extremity of the caterpillar in front. The PPM is of particular concern also due to the public health risk (contact dermatitis) associated with the urticating hairs produced by late-instar larvae. The caterpillar hairs of *Thaumetopoea* genus can cause urticarial dermatitis with intense itching, oedema with or without dermatitis, conjunctivitis and keratitis if there is eye contact, rhinitis, pharyngitis, and bronchitis as a result of penetration of the respiratory tract (BRUCHIM *et al*. 2005; SPEIGHT & WAINHOUSE 1989; VEGA *et al*. 2003).

In Turkey, the control of the pine processionary moth (PPM) is accomplished mainly by aerial spraying but increasingly there are calls for more environmentally friendly approaches to its control. Also in small areas like parks and gardens where spraying would be unacceptable, mechanical control is used. Chemical insecticides used in aerial spraying sometimes cause detrimental effects on non-target organisms in forested areas (ALTERO & MOLLER 2000; BECK *et al*. 2004).

Plants may be a source of alternative agents for control of PPM, because they are rich in bioactive chemicals, are active against a limited number of species including specific target insects, and are bio-degradable. Essentially, plant products have long been used traditionally by human communities in many parts of the world against pest species of insects (ISMAN 2000). In recent years, considerable effort has been focused on plant-derived materials for potentially useful products as commercial insecticides (ARNASON *et al*. 1989).

Researchers now are looking for natural and plant-based insecticides for control of PPM and other pests in forested areas. Therefore, the present

study was carried out to determine the larvicidal efficacy of the essential oils from two naturally occuring plants in Antalya (southwestern Turkey), *Origanum onites* L. and *Citrus aurentium* L., against PPM.

Material and Methods

Plant collection and extraction of essential oils

The plant material was collected by the authors from natural habitats of the species in Antalya. Dried aerial parts of *O. onites* and fresh fruit peels of *C. aurantium* were used for extraction of essential oils by hydrodistillation using the Clevenger apparatus as described by SARAC and TUNC (1995) and TUNC and SAHINKAYA (1998). The oils were stored in dark glass tubes in a refrigerator at 4°C until evaluation.

Thaumetopoea wilkinsoni strain

Larvae of *T. wilkinsoni* were collected from pine trees (*Pinus brutia* Ten.) in the Campus of Akdeniz University, Antalya, Turkey. First, branchlets of trees carrying natural nests were cut by cutting poles (Fig. 1). Then the nests were carefully split apart, larvae (Fig. 2) were separated gen-

Fig. 1. The winter nest of *T. wilkinsoni.*

Fig. 2. The third-fourth instar larvae of pest in its winter nest.

Table 1

Larvicidal toxicity of *O. onites* and *C. aurentium* essential oils against 4th and 5th instar larvae of PPM (%Mortality±SE)

Plant species	Control	0.1%	0.5%	1%	%LD_{50} (LCL-UCL)[x]	LD_{90} (LCL-UCL)
Origanum onites L.	0.0±0.0 a[y]A[z]	20.0±9.1 aA	70.0±9.1 bA	97.5±2.5 cA	0.288 (0.242-0.338)	0.926 (0.754-1.209)
Citrus aurenticum L.	0.0±0.0 aA	7.5±2.5 aB	45.0±13.2 bB	72.5±4.7 cB	0.530 (0.440-0.645)	2.306 (1.653-3.798)

[x] 95% fiducial limits; LCL, lower limit; UCL, upper limit

[y] Means within a line followed by the same lower case letter are not significantly different (DMRT, P≤0.05)

[z] Means within a row followed by the same capital case letter are not significantly different (DMRT, P≤0.05)

tly from the nests by tweezers, transferred into jars (each 5 l) and transported to the Pesticide Toxicology Laboratory Insectary, Department of Biology, Faculty of Arts and Science, Akdeniz University for testing.

Preparation of essential oil doses and larvicidal assays

A one millilitre of each essential oil was dissolved in 100 ml of distilled water (stock solution) using Tween 80 (0.3%). From this stock solution (1%), doses of 0.5 and 0.1% were prepared. These dose levels were based on preliminary studies showing that they yield between 10 and 90% larval mortalities.

Larvicidal assays were carried out according to the method described by SEMIZ *et al*. (2006) with minor modifications. All tests were run at 26°C (±2), 40% (±10) relative humidity (RH) and 14/10 h light/dark photoperiod in the laboratory. Fourth-fifth instar larvae were used in the assays. Ten larvae were placed in glass jars (each 250 ml) and, for feeding purposes, the jars were supplied with small *P. brutia* branches with 1 year old needles. Four replicates were used for each dose level. One control group was set up using distilled water containing 0.3% Tween 80. Each dose of essential oil (0.1 ml per larva) was topically applied only once by Pasteur pipette, 1 ml of solution for each glass jar.

Phytotoxicity tests

The phytotoxicity of the oils was evaluated by using pine shoots dipped into plastic pet bottles including 0.5 l tap water to maintain the turgor of the needles. The shoots were sprayed with the highest concentration (1%) of both essential oils until run-off and observed during a 7 day period. There were five replicates for each oil, and also five replicates for control with distilled water using Tween 80 (0.3%). Compared to healthy controls, any sign concerning possible oil phytotoxicity in the treated shoots was considered as an indication of phytotoxicity.

Data collection and analysis

Larval mortality was assessed based on observations after 24 h of exposure. Mortalities were determined by observing those individuals that were neither moving nor feeding in the test media. Mortality values were expressed as a percentage of initial numbers in each jar.

Duncan's multiple range test (DMRT) was used to test for differences among means and controls. LD_{50}, LD_{90} and the 95% confidence limit of lower confidence limit (LCL) and upper confidence limit (UCL) were calculated using a probit analysis program (US Environmental Protection Agency 1999).

Results and Discussion

The results of the larvicidal activity of *O. onites* and *C. aurantium* essential oils are presented in Table 1. Based on the results of the toxicity assays, both oils exhibited different larvicidal activities. The essential oil of *O. onites* was more toxic against 4th and 5th instar larvae of the pest than that of *C. aurantium*. The larvicidal activity of the oils was proportional to dosage. While *O. onites* and *C. aurantium* oils did not produce more than 20% mortality after 24 h of exposure at a lower dose (0.1%), they achieved 97.5 and 72.5% mortality at the highest dose (1%), respectively. Estimated

LD_{50} and LD90 values were 0.288 and 0.926% for *O. onites*, and 0.530 and 2.306% for *C. aurantium*, respectively. No mortality was observed in the control group during the test period.

When compared to controls, no phytotoxicity was observed on any shoot treated with test essential oils.

Different chemical and biological control methods with several insect growth regulators (diflubenzuron, triflumuron), pyrethroits (cypermethrin, cylfuthrin) and a microbial insecticide *Bacillus thuringiensis* have been applied in Turkey since 1997. Small instars (1^{st}-3^{rd}) are especially susceptible to these applications, but late instars (4^{th}-5^{th}) need higher application doses. However, higher doses of chemical insecticides are harmful for many predators and non-target organisms (ALTERO & MOLLER 2000; BECK *et al*. 2004).

Insecticidal activity of essential oils extracted from different plant species against *T. pityocampa* have been determined by other authors. This species is considered as an ecotype of the pest studied in the present study (DEMOLIN & FREROT 1993). KANAT and ALMA (2004) showed that steam-distilled wood turpentine was highly effective on *T. pityocampa*. Also, TIBERI *et al*. (1999) reported that monoterpene composition in some *T. pityocampa* spp. was effective on feeding and host selection of PPM.

Several recent studies have also indicated that essential oils extracted from *Citrus* and *Origanum* species were effective against different insect species. For example, EZEONU *et al*. (2001) reported that *C. sinensis* and *C. aurantifolia* volatile peel extracts had insecticidal activity against mosquitoes, houseflies and cockroaches. Another study with two *Origanum* species showed that *O. onites* and *O. minutiflorum* essential oils were highly effective against the house mosquito, *Culex pipiens* L. (Diptera: Culicidae) (CETIN & YANIKOGLU 2006).

In the present study, the larvae exposed to the essential oils succumbed rapidly. The rapid insecticidal action of the oils is intriguing and exciting. Studies with various essential oils suggest that they contain fast-acting ingredients that apparently toxify insect nerves or other cells and disrupt vital insect systems (LAHLOU 2004). In a previous study conducted to determine the insecticidal activity and mechanism of action of three essential oil constituents (eugenol, alpha-terpineol and cinnamic alcohol) and an equal part mixture (3-blend) against American cockroaches (*Periplaneta americana* (L.) (Orth.: Blattodea), it was reported that exposed insects demonstrated hyperactivity followed by hyperextension of the legs and abdomen, then fast knockdown or quick immobilization followed by death. Also, one of the most remarkable observations in this study was the increased frequency of heartbeats of American cockroaches in response to topical application of the constituents. Blockage of octopamine receptor binding sites was also illustrated at various concentrations of the test materials as judged by the decreased binding activity of (3H) octopamine to its receptors. In conclusion, it was indicated that the oil components showed activity as neuro-insecticides (ENAN 2001). However, further studies are required to gain more insight into the mode of insecticidal action of the essential oils.

In conclusion, the plant oils examined in this study offer great potential as new materials against PPM. The results obtained from the study could be useful in the search for new botanical larvicides. The use of the botanical derivatives in PPM control instead of synthetic insecticides could reduce environment effects.

Acknowledgements

The authors are grateful to an anonymous reviewer from Michigan State University, USA for his invaluable comments and suggestions. This study was supported by the Scientific Research Projects Administration Unit of Akdeniz University.

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