

Determining the Toxicity of Some Thyme Essential Oils Against the Pine Processionary [*Thaumetopoea pityocampa* (Lepidoptera: Notodontidae)]

Şeyma YİĞİT¹ İzzet AKÇA^{1*} Erol BAYHAN² Selime BAYHAN² Fethullah TEKİN³
İslam SARUHAN¹

¹Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, Samsun, Turkey

²Dicle University, Faculty of Agriculture, Department of Plant Protection, Diyarbakır, Turkey

³GAP International Agricultural Research and Training Center, Diyarbakır, Turkey

(*Corresponding author e-mail: iakca@omu.edu.tr)

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ABSTRACT: One of the major forest pests in Turkey, *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae), causes serious damage in pine trees. Mechanical and chemical control tactics are applied for management of this pest in forest areas where the chemical control is mostly preferred. However, due to its adverse effects of chemicals on the environment and human health, alternative control methods have gained importance. In this study, three different doses (0,1%, 0,5% and 1%) of each of five different thyme essential oils (*Origanum majorana*, *O. multiflorum*, *O. saccatum*, *Thymus cilicicus*, *T. spicata*) were applied to the larvae of the pine processionary in four replications. When the 1% dose of all oils were assessed, the highest mortality rate was observed in *T. cilicicus* with 95%, followed by *O. saccatum* (90%), *T. spicata* and *O. multiflorum* (80%) and *O. majorana* (75%). As a result of the study, it was seen that the application of essential oils is effective even at low doses, and the effect increases along with the respective doses. This study revealed that thyme oil could be used as a bio insecticide in the control of *T. pityocampa* in forests.

Keywords: Thyme species, Pine, Toxicity, Some essential oils

Bazı Kekik Türlerine Ait Uçucu Yağların Çam Kese Tırtılı [*Thaumetopoea pityocampa* (Lepidoptera: Notodontidae)]'na Karşı Toksisitesinin Belirlenmesi

ÖZ: Türkiye'de orman alanlarında önemli orman zararlılarından biri olan *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae) özellikle çam ağaçlarında ciddi zararlara neden olmaktadır. Ülkemizde orman alanlarında bu zararlının mücadelesinde mekanik ve kimyasal mücadele yapılmaktadır. Son yıllarda bu zararlıya karşı kimyasal mücadele en çok tercih edilen yöntemdir. Fakat kimyasal mücadelenin çevre ve insan sağlığına olan zararlı etkilerinden dolayı günümüzde alternative mücadele yöntemleri ön plana çıkmaktadır. Tarımsal alanlarında zararlılara karşı bitkisel yağların kullanımı ile ilgili birçok çalışma bulunmaktadır. Ancak çam kese böceğine karşı bitkisel yağların etkisi üzerine yapılmış çalışmalar oldukça sınırlıdır. Bu çalışmada beş farklı kekik türüne ait uçucu yağların (*Origanum majorana*, *O. multiflorum*, *O. saccatum*, *Thymus cillilus*, *T. spicata*) üç farklı dozu (%0,1, %0,5 ve %1) dört tekrerrülü olarak çam kese tırtılına uygulanmıştır. Denemeler %70 nem ve 20±2°C laboratuvar şartlarında yürütülmüştür. Çalışma beş gün takip edilmiş ve sonuçlar yüzde ölüm oranı üzerinden değerlendirilmiştir. Tüm yağların %1 dozu değerlendirildiği zaman en yüksek ölüm oranı *T. cilicicus*'da %95 belirlenmiştir. Diğer türlerde ise sırasıyla *O. saccatum* (%90), *T. spicata* ve *O. multiflorum* (%80) ve *O. majorana* (%75) olarak bulunmuştur.

Anahtar Kelimeler: Kekik türleri, Çam, Zehirlilik, Bazı uçucu yağlar

INTRODUCTION

Thaumetopoea pityocampa (Lepidoptera: Notodontidae) is one of the most important forest pests in Turkey (Baroni, 1997; Bilgili, 2002). This pest feeds on pine trees and spins webs on these trees (Androic, 1956). This pest was seen in Southern Germany, Southern France, Northern Europe, the Near East, and Turkey. *T. pityocampa*, which is common in Turkey especially in the Mediterranean region (Vega et al.; 2003) was also detected in the Central Black Sea Region in 2007 (Ince et al.; 2007). This pest has an annual generation. Overall, it is estimated that 1,500,000 hectares of forest areas of this country are infested with this pest. Infestation rate was reported as 47%, 40% and 10% for the Mediterranean Region, the Marmara Region and the Aegean Region, respectively (Kanat et al.; 2002). Some researchers stated that the pine processionary is

the cause of the loss of up to 60% of our forest areas (Anonymous, 1995; Kanat, 2002; Semiz et al.; 2006). Also, the weak trees that the pest feeds on become vulnerable to secondary pests like Scolytus. In addition to all these damages, the fuzz on the larva cause allergic reactions on humans and animals, causing respiratory failure, conjunctivitis and asthma (Kanat, 2002; Ekerbiçer et al.; 2002). Tree mortality is inevitable if control methods are not applied (Akkuzu and Selmi, 2002; Avcı and Ogurlu, 2002).

There are various different control methods of this pest including use of different forms of chemicals, (Bescelli, 1969; Mol and Küçükosmanoğlu, 2002; Isman, 2001), pheromone traps (Küçükosmanoğlu and Arslangündoğdu, 2002), essential oils (Kanat and Alma, 2004), parasites and predators (Bescelli, 1969; Er et al.; 2007; Cebeci,

2010; Avcı, 2004; Çanakçıoğlu and Mol, 1998), microbial agents (Bescelli, 1969; Mol and Küçükosmanoğlu, 2002; Can and Düzbastılar, 2002). Chemical control methods have been mostly preferred for management of this pest in forest areas. In recent years, the use of alternative control methods has gained importance due to the adverse effects of synthetic chemicals on the environment and human health. Because of the various disadvantages of chemical insecticides, scientists have begun to investigate the insecticidal properties of plant extracts, essential oils and secondary metabolites. These substances are known to have fumigant, repellent, contact, egg production reducing and nourishment inhibiting properties effect on the insects (Koul et al.; 1990; Yano and Kamimura, 1993; Vahitha et al.; 2002; Tunaz et al; 2009).

Alternative methods used in the control of pine processionary are rather limited. There are many studies on the use of essential oils against many pests in agricultural areas (Özcan et al. 2009; Upadhya, 2010; Polatoğlu et al.; 2011, Erler et al.; 2006, Keita et al.; 2001; Papachristos, 2002).

Essential oils are preferred in pest control because of releasing no toxic substances to the nature, being decomposed in a short time without any adverse effect to soil and water, leaving no residues that threaten to human health. Since essential oils have a broad biological spectrum for pests, they can

also be effective in the development and reproduction of new insecticides and repellents (Isman, 2000; Saraç and Tunç, 1995; Petrakis and Panos, 2005; Isman et al.; 2008).

Particularly, the low toxicity of essential oils in mammals and without known harmful effects to the environment make them to be strong alternative to insecticides (Isman, 2000; Rabenhorst, 1996; Misra and Pavlostathis, 1997). Studies on the insecticide effect of essential oils on the pine processionary is very limited (Kanat and Alma, 2004; Çetin and Yanıkoğlu, 2006; Kesdek et al.; 2014).

In this study, the effect of 3 different doses of each of 5 different thyme essential oils against *T. pityocampa* larvae were investigated.

MATERIAL AND METHOD

This study was conducted under controlled conditions (25°C temperature and 65% relative humidity) in the Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayıs University, Samsun. Different thyme varieties (*Origanum majorana*, *O. multiflorum*, *O. saccatum*, *Thymus cillilus*, *Thymbra spicata*) grown in Diyarbakir's ecological conditions were used in the research. Essential oils were obtained from these plants via water vapor-distillation method using a Neo-Clevenger apparatus (Linskens, 1997). Information on the plants used in the study are given in Table 1.

Table 1. Analyzed thyme essential oil components

Essential Oil Type of Thyme	Contents
<i>Thymbraspicata</i> var. <i>spicata</i> L.	Carvacrol %60, gamma-terpinene %21, Cymene %5.
<i>Origanum majarona</i> (<i>Origanumonites X Origanum syriacum</i> var. <i>bevanii</i>)	terpinen-4-ol %25, Carvacrol %23, gamma-terpinene %17
<i>Origanum saccatum</i> P. H. DAVIS	Cymene % 47, gamma-terpinene % 27, Carvacrol %17
<i>Origanum minutiflorum</i> O. Schwarz et P. H. davis	Carvacrol%56, gamma-terpinene %13, trans-sabinene hydrate %6
<i>Thymus cilicicus</i> Boiss. et Bal.	Carvacrol %72, gamma-terpinene %5, Cymene %4.

Thaumetopoea pityocampa larvae were collected from pine trees of the Ondokuz Mayıs University campus. The larvae used in the study were selected from a single colony. The experiment was conducted against the larvae, with different doses of each essential oil in 4 replications. 5 gr of pine needles were placed on drying papers moistened with pure water in 10x10 cm plastic containers. Then, 10 larvae were placed in each plastic box.

Preparation of essential oil doses: One milliliter 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.

The plastic boxes containing larvae was sprayed with 5 ml of the prepared doses (0.1, 0.5 and 1.0%). The control boxes were sprayed with deionized water. Mortality rates were determined by counting dead larvae in each box on 1st, 3rd, and 5th day after application. The data were analyzed using IBM SPSS Statistics for Windows Version 22.0.

RESULTS AND DISCUSSION

All thyme treatments used in the study were effective against the pine processionary larvae in at

certain rates (Table 2). However, the most effective thyme was determined to be *T. cillius*. When thyme types were evaluated separately, it was observed that the mortality ratio increases as the treatment doses increased. When all doses were evaluated altogether, the upper doses were in a different group statistically than the other doses. When we look at the top dose of each oil, the highest mortality rate was 95% in *T.*

cillius. The mortality rates of other types, were found to be 90% for *O. saccatum*, 80% for *T. spicata* and *O. multiflorum*, and 75% for *O. majorana*. LD 90 value of *T. spicata*, *O. majorana*, *O. multiflorum*, *O. saccatum* and *T. cillius* were 1.358, 1.212, 1,080, 0.898 and 0.825, respectively. When LD 90 values are taken into account, it is also found that *T. cillius* was the lowest and therefore the most effective one.

Table 2. Effect of different thyme oils on the larvae of pine processionary

Type of Tyhme	Dozes	% mortality rate	LD ₅₀	LD ₉₀
<i>Origanum majarona</i>	0	5,00±5,00 B* 1**	0,576	1,212
	0,1	20,00±11,5B h1		
	0,5	55,00±5,00A fg		
	1	75,00±5,00A abcde		
<i>Origanum multiflorum</i>	0	5,00±5,00 C1	0,365	1,080
	0,1	55,00±12,58 B ef		
	0,5	60,00±8,16 AB def		
	1	80,00±9,57 A abc		
<i>Origanum saccatum</i>	0	5,00±5,00 C 1	0,341	0,898
	0,1	45,00±5,00 B g		
	0,5	70,00±5,77A bcde		
	1	90,00±10,00A ab		
<i>Thymus cillilus</i>	0	5,00±5,00D 1	0,382	0,825
	0,1	30,0±5,77C gh		
	0,5	65,00±5,00B bcdef		
	1	95,00±5,00A a		
<i>Thymbra spicata</i>	0	5,00±5,00 C 1	0,668	1,358
	0,1	20,00±8,16 BC gh		
	0,5	30,00±5,77B h1		
	1	80,00±8,16 A abcd		

*Upper case letters in the same column indicate intra-type dose comparison,

**Lower case letters in the same column indicate all doses comparison.

Çetin et al. (2006) applied 3 different doses (0.1%; 0.5; and 1) of essential oils of *Origanum onites* L. and *Citrus aurentium* L. to pine processionary larvae and found that thyme oil was effective. In the same study, they calculated LD 50 and LD 90 values for *O. onites* as 0.288 and 0.926, respectively. The LD 50 and LD 90 values of thyme oils used in our study are found to be close to those obtained in their study.

In the study by Kanat and Alma (2004), nine different types of essential oils (*P. brutia* Ten., *Laurus nobilis* L., *Liquidambar orientalis* Miller, *Juniperus communis* subsp. *nana* Syme., *Cupressus sempervirens* L., *Lavandula stoechas*, *Lavandula angustifolia*, *Eucalyptus camadulensis* and *Thymus vulgaris*) were applied to pine processionary larvae in 3 different concentrations (25, 50 and 100%). All the essential oils showed an insecticidal activity against the larvae in all three concentrations applied. The most effective essential oil against the larvae was the steam-distilled wood turpentine, followed by thyme herb oil, juniper berry oil, laurel leaf oil, lavender

flower oil, eucalyptus leaf oil, lavender leaf oil, cypress berry oil, essential oil of styrax and sulfate turpentine, respectively, in order of MMT values (Kanat and Alma, 2004).

There are studies on the effect of thyme types on other various pests. Saraç and Tunç (1995) applied essential oils including *Thymbra spicata* var. *spicata* against the adults of *T. confusum* and *S. oryzae* and against the larvae of *Ephestia kuehniella* and found that thyme has a repellent effect against these pests. Erler et al. (2006) tested the effect of carvacrol on *Tribolium confusum* adults and eggs, and Mediterranean flour moths (*Ephestia kuehniella*) larvae and eggs. More than 90% mortality rate was obtained in all periods of the insects. Yıldırım et al. (2005) found that thyme types such as *Origanum acutidens*, *Satureja hortensis*, and *Thymus vulgaris* have a fumigant effect on *Sitophilus granarius* adults and *Ephestia kuehniella* larvae.

CONCLUSION

The mortality effect of essential oils obtained from different plant types on pine processionary was determined in many studies (Kanat and Alma, 2004; Çetin and Yanikoglu, 2006; Kesdek et al., 2014).

In our study, we found that the application of essential oils derived from different types of thyme on larvae of pine processionary caused a 70% to 90% mortality, and as a result, thyme types were found to be very effective.

As a result of the study, it was seen that the application of essential oils is effective even at low doses, and the effect increases along with the respective doses. This study revealed that thyme oil could be used as a bio insecticide in the control of *T. pityocampa* which are important pests of forests. This data obtained from the study will shed light for upcoming studies in this arena.

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