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LARVICIDAL EFFECT OF SOME PLANT EXTRACTS ON THE PINE PROCESSIONARY MOTH, *Thaumetopoea pityocampa* (Denis & Schiffermuller) IN LABORATORY CONDITIONS

Memis Kesdek[1], Saban Kordali[2], Kerziban Coban[1], Ayse Usanmaz[3], Sezai Ercisli[2]

[1]Muğla Sıtkı Koçman University

[2]Atatürk University

[3]Iğdır University

Abstract. The pine processionary moth, *Taumetopoea pityocampa* (Denis and Schiffermüller) is one of the most widespread defoliator insects found in the forest areas of Turkey. Although different methods have been used to control this major forest pest up to now, the problem is still going on largely unsolved in the forest areas of Turkey. The objective of this study was to determine larvicidal effects of extracts obtained from six different plant species, *Achillea wilhelmsii* C. Koch, *Nepeta meyeri* Benth., *Satureja hortensis* L., *Origanum onites* L., *O. rotundifolium* Boiss., *Tanacetum argyrophyllum* (C. Koch) and on the 2nd, 3rd, and 4th instar larvae of *T. pityo*campa in laboratory conditions.Test the toxicity of six plant extracts against to the 2nd, 3rd, and 4th instar larvae of *T. pityocampa*, 10 larvae of this insect with 15 gr amounts fresh needles (1 year old) of *Pinus brutia* were placed to Petri dishes (9 × 1.5 cm deep). Each dose was dissolved in acetone and 0.25, 0.5 and 1 mg of the plant extracts found in 1 ml solution were sprayed on the 2nd, 3rd, and 4th instar larvae of *T. pityocampa* in the Petri dishes, corresponding to 2.08, 4.16 and 8.33 $mg \cdot l^{-1}$ air concentrations. Petri dishes were covered with a lid. All tests carried out at 26ºC (±2), 60% (±5) relative humidity and 14/10 h light/dark photoperiod in laboratory conditions. When exposure, mortality of the larvae was after the determined at 24, 48 and 96 h. Petri dish applied with sterile water and acetone were used as control group. All the tests were made in triplicate. The results showed that six plant exracts have a larvicidal effect on the 2nd, 3rd, and 4th instar larvae of *T. pityocampa* in comparison with controls. Therefore, these naturally occurring plant extracts could be useful for managing the larvae populations of *T. pityocampa*.

Key words: plant extracts, *Taumetopoea pityocampa*, larvicidal effect, pine, mortality percentage

Corresponding author: Sezai Ercisli, Faculty of Agriculture, Ataturk University, 25240 Erzurum, Turkey, e-mail: sercisli@gmail.com

INTRODUCTION

The pine processionary moth, *Thaumetopoea pityocampa* (Den. and Schiff.) (Lep. Thaumetopoeidae) is one of the most important forest pest especially in Mediterranean, Aegean and Marmara regions of Turkey. This pest was firstly recorded in İstanbul provinces in 1929. Later on, reported this pest was in various regions of Turkey from 1937 to 1939 [Hovas 1929, Turkmen and Oner 2004, Cebeci at al. 2010a]. The larvae of this pest feeds on *Pinus* L. species (*Pinus brutia* Ten., *P. nigra* Arnold, *P. pinaster* Aiton, *P. pinea* L., *P. sylvestris* L.) and, also *Larix decidua* Mill., *Cedrus atlantica* Endl. and *C. libani* Rich. into a forest area of over 1.5 million hectare [Demolin 1969, Atakan 1991]. These larvae cause serious economic and ecological losses on the host trees. As feeding of the larvae, the annual diameter increments of host trees decrease. The diameter decreases have been reported to be from 12 to 65% [Babur 2002, Hodar et al. 2002, Carus 2004, Kanat et al. 2005]. These trees can become highly prone to the incidence of secondary insects. Furthermore, these insects can cause to tree mortality in the next times [Akkuzu and Selmi 2002, Avcı and Oğurlu 2002]. Therefore, the protection of coniferous forests requires regular application of various control methods against pine processionary moth. These control methods involve in mechanical, physical, chemical, bio-technical and biological measures for this pest management. In the past, some chemicals such as Endosulfan, Dimilin and Malathion had been used efficiently to control this pest in many countries. But, these chemicals have a negative effect on the environment and specifically on many beneficial organisms. Therefore, environmentally friendly methods must prefered to control of pine processionary moth [Roessler 1989]. Many studies have reported to control pine processionary moth in Turkey up to now [Acatay 1953, Özkazanc 1987, Avcı 2000, Kanat and Sivrikaya 2004, Özcankaya and Can 2004, Er et al. 2007, Kanat and Mol 2008, Cebeci et al. 2010b]. But, the problem is still going on largely unsolved in the forest areas of Turkey.

The forest plants are extremely important in the life of people and other animals throughout the world as they provide basic needs such as hunting, food, reproducing, clothing, shelter and health care. Among them *Pinus* species (Pine trees) is one of the economically important plants. In addition, these trees are very important for the forest villagers as wood supply and building material in Turkey, too. *Pinus* species are usually spread along the coastal regions of Turkey, especially in the Mediterranean, Marmara, Aegean and Western Black Sea regions. Besides, Pine trees can grow in height from sea level to 2000 meters of Turkey.

Plant extracts are natural plant products that contain natural flavours and fragrances grouped as monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes (hydrocarbons and oxygenated derivatives) and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acids and alcohols) that provide characteristic odours [Kordali et al. 2007]. Actually, plant products have long been used traditionally human communities in many parts of the world against pest insect species. In addition, recent studies on many plant extracts showed that plant extracts have an effect on insects and mites [Ho et al. 1994, Huang et al. 1998, Isman 2000]. There are more than 2.000 species of plants are known to possess some insecticidal proportion [Klocke 1989]. However, plant products of a large number of plant species have been found to have toxic and/or repellent effects

against different insects and pests [Regnault-Roger 1997]. Recent investigations in several countries confirm that some plant products not only repel insects, but also have contact and fumigant insecticidal actions against species pests, and fungicidal actions against some important plant pathogens [Isman 2000]. In the recent years, there has been a growing interest in research concerning the alternative pesticides and antimicrobial active compounds, including the plant extracts and essential oils that are relatively less damaging to the mammalian health and environment [Misra and Pavlostathis 1997, Roy and Dureja 1998, Isman 2000, Çakır et al. 2004, Kordali et al. 2005]. Insecticidal activity of many plant products against various insect pests has been demonstrated by many researchers [Isman 2000, Yıldırım et al. 2005, Kordali et al. 2007, Kumar et al. 2012]. Also, the deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behaviour and reduction of fecundity and fertility [Jbilou et al. 2006].

The genus *Achillea* L. is one of the most important genera of the Asteraceae family and comprises about 85 species, widespread throughout the world. There are about 42 species of this genus in Turkish flora and about 20 of them are endemic [Davis 1982, Baytop 1999]. The species of *Achillea* genus are known as 'Civanperçemi', 'Pireotu' and 'Yılan çiçeği' in Anatolia. *Achillea wilhelmsii* is known as 'Serviotu', 'Kardeş kınası', and also 'Ayvadene' (Konya), 'Kardeşkanı' (Sivas), 'Kılıç otu' (Malatya), 'Paspanos', 'Pasvana', 'Pesvana' (Erzurum).

The genus *Origanum* L. (oregano) is an important genus of the Lamiaceae family and comprises about 900 species, widespread throughout the world. This genus has 24 species, and 27 taxa are available in the flora of Turkey and the East Aegean Islands, 16 of them are endemic [Guner et al. 2000]. *Origanum* species have traditionally been used as a spicy additive for food instead of thyme. This genus is rich in essential oils and bitter substances [Baytop 1999, Esen et al. 2007]. The species of *Origanum* genus are known in Anatolia as 'Yalancı kekik', 'Kekik', 'İstanbul kekiği' and 'Keklik otu'. *Origanum* species are traditionally used as sedative, diuretic, degasifier, sweater antiseptic and also in the treatment of gastrointestinal diseases and constipation [Baytop 1999]. *Origanum onites* is known as 'Bilyalı kekik', 'Taş kekiği', 'İzmir kekiği', 'Güve kekiği' and 'Peynir kekik' [Oflaz et al. 2004]. However, *O. rotundifolium* Boiss. is known as 'Yuvarlak yapraklı kekik'.

The genus *Satureja* L. (savory), which is one of the most important genera belonging to Lamiacaeae family, is frequently used as tea or additives in commercial spice mixtures of many foods to offer aroma and flavour. They are well known as aromatic and medicinal plants, and also distributed in northern Anatolia [Davis 1982, Baytop 1999]. *Satureja hortensis* (summer savory) is well known as aromatic and medicinal plant, which is widely distributed in the Eastern Anatolia region of Turkey, and locally named as 'Koç otu' [Şahin et al. 2003].

The genus *Tanacetum* L., which is an important member of the Asteraceae family, is widespread in Europe and western Asia and consists of about 150–200 species. These species have traditionally been used as a spicy additive for food, in cosmetics and as herbal remedies due to their biologically active compounds [Rohloff et al. 2004]. This genus, represented in Turkish flora by 44 species and altogether 59 taxa, is rich in es-

sential oils, bitter substances and sesquiterpene lactones [Davis 1982, Baytop 1999]. *Tanacetum* species are known in Anatolia as 'Pire otu' and their essential oils are used as repellent against insects [Baytop 1999, Baser et al. 2001].

The multiregional genus *Nepeta* L. is belonging to the Lamiaceae (Labiatae) family and has approximately 250 species, widespread in South-West and Central Asia, Europe, Africa and North America. There are about 40 species of this genus in Turkey. These species have traditionally been used as diuretic, diaphoretic, antitussive, antispasmodic, antiasthmatic, febrifuge, emmenagogue and sedative agents. *Nepeta* species are known in Anatolia as 'Kedi nanesi' [Baytop 1999, Dirmenci et al. 2004, Topcu and Ulubelen 2007, Kaya et al. 2007].

Turkey flora is characterized by the abundance of aromatic plants among its components. The feature differentiating these plants from all others, in spite of the fact that they belong to many different families, is the production of chemically related secondary compounds, the low molecular weight and volatile isoprenoids. This remarkable presence of aromatic species is important in determining the insectidal potential within this ecosystem. Thus, the aim of this study is to evaluate possible toxicity of the extracts obtained from six plants (*Achillea wilhelmsii* C. Koch, *Nepeta meyeri* Benth., *Satureja hortensis* L., *Origanum onites* L., *O. rotundifolium* Boiss. and *Tanacetum argyrophyllum* (C. Koch) in different localities of Turkey, against to the 2nd, 3rd, and 4th instar larvae of *T. Pityocampa* in laboratory conditions.

MATERIALS AND METHODS

Biological material. This study was conducted between the years 2011 and 2012. The 2nd, 3rd, and 4th instar larvae of *T. pityocampa* were collected from infected *Pinus brutia* trees in the forest areas (Esenköy/Fethiye/Muğla) in South Aegean Region of Turkey. Altitude of these forest areas is 170–250 m. Tests are also carried out in under the same condition and the same laboratory.

Plant material. *Achillea wilhelmsii* C. Koch*, Nepeta meyeri* Benth*., Satureja hortensis* L., *Origanum onites* L., *O. rotundifolium* Boiss. and *Tanacetum argyrophyllum* (C. Koch) were collected from different localities of Turkey between August 2011 and August 2012. Voucher specimens have been deposited in the herbarium of Ataturk University, Faculty of Agriculture, the Department of Plant Protection, Erzurum, Turkey. Aerial parts of the plants were dried in shade and ground in a grinder.

Extraction. In order to prepare the acetone extracts, the dried and powdered flowers of, *Achillea* wilhelmsii C. Koch, *Nepeta meyeri* Benth*., Satureja hortensis* L., *Origanum onites* L., *O. rotundifolium* Boiss. and *Tanacetum argyrophyllum* (C. Koch) (each one 200g) were extracted with acetone (750 ml × 4) for 48 h at room temperature. The extracts were filtered using Whatman filter paper (No. 1) and then concentrated under reduced pressure at 40ºC using a rotary evaporator (RV 05 Basic 1B IKA Group, Wilmington, NC, U.S.A.). Residues of each plant species were diluted with sufficient HPLC grade acetone (Sigma-Aldrich, Milwaukee, WI, U.S.A.) and sterile water to give 100% (w/w) stock solutions. The extracts (yields 13.6, 12.7, 16.66, 15.6%, 14.94 and 19.8% respectively) were stored in a freezer at 4ºC until further tests.

Bioassays. In order to test the toxicity of six plant extracts against to the 2nd, 3rd, and 4th instar larvae of *T. pityocampa*, 10 larvae of this insect with 15 g amounts fresh needles (1 year old) of *Pinus brutia* were placed to Petri dishes (Glass Petri dishes 9 cm wide × 1.5 cm deep, corresponding to 120 ml volume). In this present study each dose was dissolved in acetone (100 $mg \cdot ml^{-1}$) concentration. 0.25 mg, 0.5 mg, and 1 mg of the plant extracts were sprayed on the 2nd, 3rd, and 4th instar larvae of *T. pityocampa* in the Petri dishes, corresponding to 2.08, 4.16 and 8.33 $mg \cdot l^{-1}$ air concentrations. All tests carried out at 26ºC (±2), 65% (±5) relative humidity and 14/10 h light/dark photoperiod in laboratory conditions. When exposure, mortality of the larvae was determined after the at 24, 48 and 96 h. Petri dish applied with **sterile** water and acetone were used as control group. Three replicates were used for each dose and exposure time combination and larvicidal activity of the plant extracts were expressed as % mean mortality of the larvae.

Statistical analysis. The differences among the contact toxicity of six plant extracts were determined according to analysis of variance (ANOVA) test by using SPSS 17.0 software package. Mortality was expressed as mean (percentage) ± standard error. Differences between means were tested through Duncan test and values with $p < 0.01$ were considered significantly different. LD_{25}, LD_{50} and LD_{90} values at 96 h were calculated with regression analysis by probit using SPSS. Probit analysis of dose-mortality data was conducted to estimate the LD_{25}, LD_{50} and LD_{90} values and associated 95% confidence limits for each treatment.

RESULTS AND DISCUSSION

The plantal extracts obtained from the various plant leaves, fruits, roots, seeds, flowers and barks in their crude form have been used as conventional insecticides for centuries. The toxicity effects of extracts obtained from *Achillea wilhelmsii*, *Nepeta meyeri*, *Satureja hortensis*, *Origanum onites*, *O. rotundifolium* and *Tanacetum argyrophyllum* on the 2nd, 3rd, and 4th instar larvae of *Taumetopoea pityocampa* are summarized in Tables 1, 2 and 3; Figures 1a, b, c, 2a, b, c, and 3a, b, c). The results show that extracts of *Achillea wilhelmsii*, *Nepeta meyeri*, *Satureja hortensis*, *Origanum onites*, *O. rotundifolium* and *Tanacetum argyrophyllum* have a larvicidal effect on the 2nd, 3rd, and 4th instar larvae of *T. pityocampa* in comparison with controls.

In the present study, the minimum mortality rate (20%) after 24 h of treatment with the 0.5 mg and 0.25 mg in doses of extracs of *O. onites* and *S. hortensis* was determined for the 2nd instar larvae of *T. pityocampa.* However, the highest mortality rate (73.33%) after 24 h of treatment with 1 mg has been found for *T. argyrophyllum* and *N. meyeri* against the 2nd instar larvae of *T. pityocampa* (tab. 1; fig. 1a). Although the lowest mortality rate (26.6%) after 48 h of treatment with the 0.25 mg dose against the 2nd instar larvae of *T. pityocampa* was fixed for *O. onites* and *S. hortensis* extracts, the highest mortality rate (83.3%) was found in the 1 mg dose of *N. meyeri* extract (tab. 1; fig. 1b). Similarly, the lowest mortality rate (36.6%) was established after 96 h in the 0.25 mg dose for *O. onites* extract. In addition to, the highest mortality rate (100%) was observed in the 1 mg dose of *N. meyeri* extract after 96 h against the 2nd instar larvae of *T. pityo-*

campa (tab. 1; fig. 1c). In general, the most mortality rates on the 2nd instar larvae of *T. pityocampa* were fixed in all times (24, 48, and 96 h) and all doses (0.25, 0.5, and 1 mg) for extract of *N. meyeri*. Besides, it was established that there was mortality in all doses and times for extracts of six plants on the 2nd instar larvae of *T. pityocampa* (tab. 1; fig. 1a, b, c). However, there was no mortality in the control groups during the test period (tab. 1; fig. 1a, b, c).

Table 1. Percentages of the 2nd instar larvae mortality of *Taumetopoea pityocampa*

Treatment extracts (acetone)	Dose (mg·ml^{-1})	Mortality (%) exposure time (h)		
		24	48	96
A. wilhelmsii	0.25	40.0 ±5.77 bcde	46.6 ±3.33 bcdef	66.6 ±3.33 bcde
	0.5	53.3 ±3.33 bcde	70.0 ±5.77 defg	80.0 ±5.77 cde
	1	26.6 ±3.33 abc	30.0 ±0.0 ab	63.3 ±6.66 bcde
N. meyeri	0.25	66.6 ±6.66 de	76.6 ±3.33 fg	96.6 ±3.33 e
	0.5	63.3 ±16.6 cde	73.3 ±12.0 efg	90.0 ±10.0 de
	1	73.3 ±17.6 e	83.3 ±12.0 g	100 ±0.0 e
S. hortensis	0.25	20.0 ±10.0 ab	26.6 ±8.81 ab	43.3 ±16.6 bc
	0.5	33.3 ±8.81 abcd	40.0 ±5.77 bcd	80.0 ±11.5 cde
	1	33.3 ±6.66 abcd	50.0 ±5.77 bcdef	80.0 ±11.5 cde
O. onites	0.25	23.3 ±3.33 ab	26.6 ±3.33 ab	36.6 ±6.66 b
	0.5	20.0 ±10.0 ab	33.3 ±13.3 bc	46.6 ±17.6 bc
	1	36.6 ±6.66 abcd	40.0 ±10.0 bcd	56.6 ±14.5 bcd
O. rotundifolium	0.25	23.3 ±14.5 ab	43.3 ±8.81 bcde	66.6 ±8.81 bcde
	0.5	30.0 ±17.3 abcd	33.3 ±20.2 bc	43.3 ±23.3 bc
	1	53.3 ±23.3 bcde	66.6 ±24.0 cdefg	80.0 ±20.0 cde
T. argyrophyllum	0.25	26.6 ±14.5 abc	36.6 ±12.0 bc	66.6 ±6.66 bcde
	0.5	33.3 ±17.6 abcd	40.0 ±15.2 bcd	63.3 ±27.2 bcde
	1	73.3 ± 6.6 e	76.6 ±23.3 fg	76.6 ±23.3 cde
Kontrol (sterilewater + acetone)	–	0.0 ±0.0 a	0.0 ±0.0 a	0.0 ±0.0 a

Values followed by different letters in the same column differ significantly at $P \leq 0.05$ according to Duncan Multiple test
a mean ±SH of three replicates, each set up with 10 larvae

However, there was no mortality after 24 h in the 0.25 mg dose extracts of *O. onites* and *N. meyeri*, and also in the 1 mg dose extracts of *T. argyrophyllum*, *O. onites* and *O. rotundifolium* on the 3rd instar larvae of *T. pityocampa* in this study. Although the minimum mortality rate (3.33%) after 24 h was recorded in the 0.5 mg dose of extracts of *O. onites* and *N. meyeri*, the highest mortality rate (20%) was found in the 0.25 mg dose extract of *A. wilhelmsii* on the 3rd instar larvae of *T. pityocampa* (tab. 2; fig. 2a). Similarly, there was no mortality after 48 h treatment with 1 mg dose extracts of *O. onites* and *O. rotundifolium* on the 3rd instar larvae of *T. pityocampa*. But, the highest

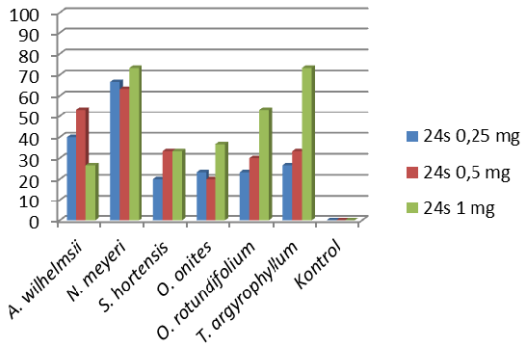

Fig. 1a. Mortality of 2nd larvae instar of *Taumetopoea pityocampa* (Denis & Schiffermüller), in relation to exposure 24 h of six plant extracts in the 0.25, 0.5 and 1 mg·ml^{-1} doses

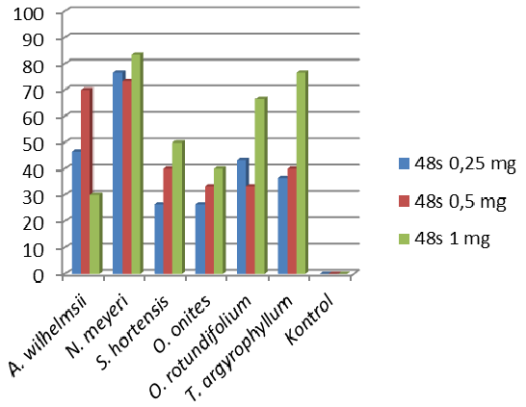

Fig. 1b. Mortality of 2nd larvae instar of *Taumetopoea pityocampa* (Denis & Schiffermüller), in relation to exposure 48 h of six plant extracts in the 0.25, 0.5 and 1 mg·ml^{-1} doses

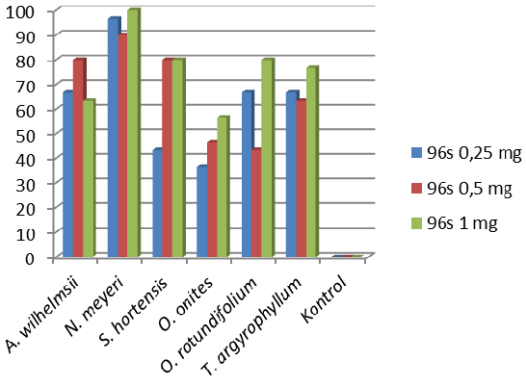

Fig. 1c. Mortality of 2nd larvae instar of *Taumetopoea pityocampa* (Denis & Schiffermüller), in relation to exposure 96 h of six plant extracts in the 0.25, 0.5 and 1 mg·ml^{-1} doses

mortality rate (23.33%) was found in the 0.25 mg dose extract of *A. wilhelmsii* after 48 h. Besides, the lowest mortality rate (3.33%) after 48 h of treatment was ebtablished for extracts of *O. onites* and *N. meyeri* (in the 0.25 mg dose), extracts of *O. onites* and *T. argyrophyllum* (in the 0.5 mg and in the 1 mg doses, respectively) (tab. 2; fig. 2b). On the other hand, the mortality wasn't found after 96 h in the 1 mg extract of *O. onites* for the 3rd instar larvae of *T. pityocampa*. However, the lowest rate (3.33%) was recorded after 96 h of treatment with the dose 0.25 mg of *O. onites* and *N. meyeri* extracts. On the contrary, the highest mortality rate (43.3%) after 96 h was established for extract (in the 0.25 mg dose) of *A. wilhelmsii* against the 3rd instar larvae of *T. pityocampa* (tab. 2; fig. 2c).

Table 2. Percentages of the 3rd instar larvae mortality of *Taumetopoea pityocampa*

Treatmentex-tracts(acetone)	Dose ($mg \cdot ml^{-1}$)	Mortality (%)		
		exposure time (h)		
		24	48	96
A. wilhelmsii	0.25	20.0 ±5.77 c	23.3 ±3.33 b	43.3 ±14.5 d
	0.5	6.66 ±3.33 ab	16.6 ±3.33 ab	26.6 ±3.33 abcd
	1	6.66 ±6.66 ab	13.3 ±8.81 ab	40.0 ±10.0 cd
N. meyeri	0.25	0.0 ±0.0 a	3.33 ±3.33 a	3.33 ±3.33 a
	0.5	3.33 ±3.33 ab	6.66 ±6.66 ab	13.3 ±3.33 abc
	1	6.66 ±3.33 ab	13.3 ±8.81 ab	16.6 ±8.81 abcd
S. hortensis	0.25	13.3 ±8.81 bc	16.6 ±12.0 ab	26.6 ±16.6 abcd
	0.5	6.66 ±6.66 ab	10.0 ±10.0 ab	26.6 ±21.8 abcd
	1	10.0 ±5.77 abc	13.3 ±8.81 ab	36.6 ±14.5 bcd
O. onites	0.25	0.0 ±0.0 a	3.33 ±3.33 a	3.33 ±3.33 a
	0.5	3.33 ±3.33 ab	3.33 ±3.33 a	6.66 ±6.66 a
	1	0.0 ±0.0 a	0.0 ±0.0 a	0.0 ±0.0 a
O. rotundifolium	0.25	6.66 ±6.66 ab	10.0 ±10.0 ab	13.3 ±13.3 abc
	0.5	6.66 ±3.33 ab	6.66 ±3.33 ab	10.0 ±0.0 ab
	1	0.0 ±0.0 a	0.0 ±0.0 a	10.0 ±10.0 ab
T. argyrophyllum	0.25	6.66 ± 3.33 ab	10.0 ±0.0 ab	10.0 ±0.0 ab
	0.5	6.66 ±6.66 ab	10.0 ±10.0 ab	23.3 ±18.5 abcd
	1	0.0 ±0.0 a	3.33 ±3.33 a	10.0 ±0.0 ab
Kontrol (sterilewater + acetone)	–	0.0 ±0.0 a	0.0 ±0.0 ab	0.0 ±0.0 ab

Values followed by different letters in the same column differ significantly at $P \leq 0.05$ according to Duncan Multiple test
a mean ±SH of three replicates, each set up with 10 larvae

In comparison with the toxicities of six plant extracts, the highest mortality rates on the 3rd instar larvae of *T. pityocampa* were fixed for extract of *A. wilhelmsii* in all times (24, 48, and 96 h) and doses (0.25, 0.5 and 1 mg). But, the lowest mortality found for the extract of *O. onites* on the 3rd instar larvae of *T. pityocampa.* However, there was no mortality in the control groups during the test period (tab. 2; fig. 2a, b, c).

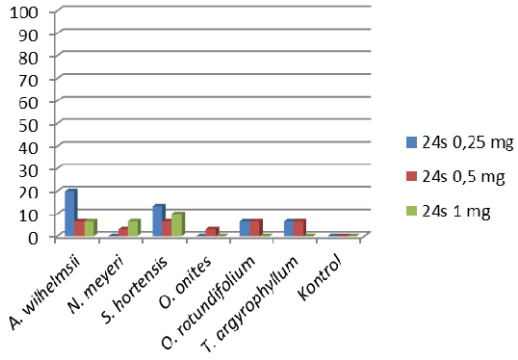

Fig. 2a. Mortality of 3rd larvae instar of *Taumetopoea pityocampa* (Denis & Schiffermüller), in relation to exposure 24 h of six plant extracts in the 0.25, 0.5 and 1 mg·ml^{-1} doses

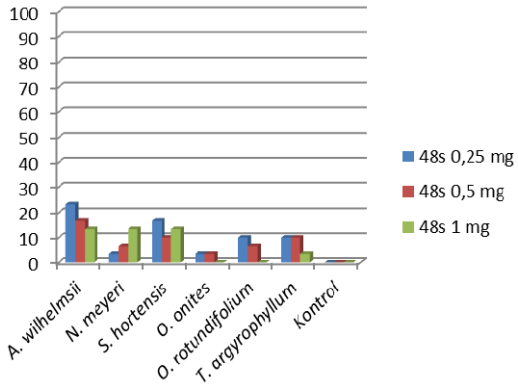

Fig. 2b. Mortality of 3rd larvae instar of *Taumetopoea pityocampa* (Denis & Schiffermüller), in relation to exposure 48 h of six plant extracts in the 0.25, 0.5 and 1 mg·ml^{-1} doses

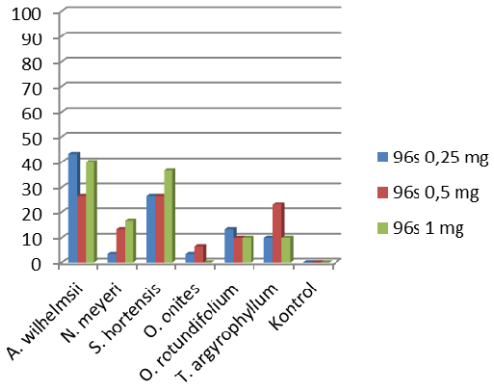

Fig. 2c. Mortality of 3rd larvae instar of *Taumetopoea pityocampa* (Denis & Schiffermüller), in relation to exposure 96 h of six plant extracts in the 0.25, 0.5 and 1 mg·ml^{-1} doses

In this study, the lowest mortality rate (3.33%) for the 4th instar larvae of *T. pityocampa* was recorded after 24 h of treatment with the doses of the extracts of *O. onites* (0.25 mg), *A. wilhelmsii* (1 mg) and *N. meyeri* (0.25 and 0.5 mg). The highest mortality rate (60%) was found after 24 h of treatment with 1 mg dose of the extract of *O. rotundifolium* on the 4th instar larvae of *T. pityocampa*. However, there was no mortality in the 0.25 mg dose of the extract of *S. hortensis* for the 4th instar larvae of *T. pityocampa* after 24 h treatment. In addition to these, it was determined the same mortality rate (33.33%) in the 0.25 and 0.5 mg doses of *A. wilhelmsii* and *T. argyrophyllum* extracts, and in the 1 mg dose of *S. hortensis* extract on the 4th instar larvae of *T. pityocampa* after 24 h treatment (tab. 3; fig. 3a). Although the minimum mortality rate (3.33%) after 48 h was fixed in the 0.25 and 0.5 mg doses of extract of *N. meyeri*, the maximum mortality rate (80%) was found in the 1 mg dose of the extract of *O. rotundifolium* on the 4th instar larvae of *T. pityocampa*. However, there was no mortality in the 0.25 mg dose of *S. hortensis* extract after 48 h (tab. 3; fig. 3b). It was determined to the mortality for all the plant extracts and in all doses against the 4th instar larvae of *T. pityocampa* after

Table 3. Percentages of the 4rd instar larvae mortality of *Taumetopoea pityocampa*

Treatmentextracts (acetone)	Dose ($mg \cdot ml^{-1}$)	Mortality (%)		
		exposure time (h)		
		24	48	96
O. onites	0.25	3.33 ±3.33 ab	20.0 ±0.0 abc	20.0 ±0.0 ab
	0.5	16.6 ±3.33 abc	23.3 ±3.33 abc	26.6 ±6.66 bc
	1	50.0 ±5.77 de	73.3 ±8.81 fg	86.6 ±6.66 gh
A. wilhelmsii	0.25	33.3 ±6.66 cd	53.3 ±3.33 def	63.3 ±3.33 defg
	0.5	33.3 ±3.33 cd	76.6 ±6.66 g	90.0 ±5.77 h
	1	3.33 ±3.33 ab	16.6 ±3.33 ab	26.6 ±3.33 bc
S. hortensis	0.25	0.0 ±0.0 a	0.0 ±0.0 a	6.66 ±3.33 ab
	0.5	20.0 ±5.77 bc	33.3 ±8.81 bcd	50.0 ±10.0 cde
	1	33.3 ±12.0 cd	43.3 ±12.0 cde	56.6 ±8.81 de
T. argyrophyllum	0.25	33.3 ±3.33 cd	63.3 ±3.33 efg	73.3 ±8.81 efgh
	0.5	33.3 ±6.66 cd	50.0 ±10.0 de	60.0 ±15.2 def
	1	40.0 ±11.5 d	53.3 ±8.81 def	63.3 ±8.81 defg
N. meyeri	0.25	3.33 ±3.33 ab	3.33 ±3.33 a	13.3 ±8.81 ab
	0.5	3.33 ±3.33 ab	3.33 ±3.33 a	10.0 ±5.77 ab
	1	20.0 ±10.0 bc	40.0 ±20.8 bcde	46.6 ±21.8 cd
O. rotundifolium	0.25	16.6 ±8.81 abc	36.6 ±14.5 bcd	53.3 ±8.81 de
	0.5	10.0 ±5.77 ab	20.0 ±11.5 abc	26.6 ±6.66 bc
	1	60.0 ±11.5 e	80.0 ±11.5 g	83.3 ±12.0 fgh
Kontrol (sterilewater + acetone)	–	0.0 ±0.0 a	0.0 ±0.0 a	0.0 ±0.0 ab

Values followed by different letters in the same column differ significantly at $P \leq 0.05$ according to Duncan Multiple test
a mean ±SH of three replicates, each set up with 10 larvae

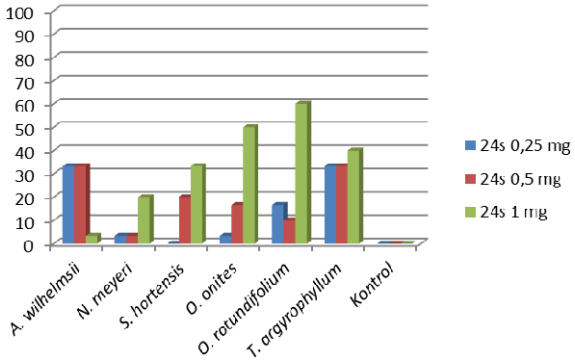

Fig. 3a. Mortality of 4rd larvae instar of *Taumetopoea pityocampa* (Denis & Schiffermüller), in relation to exposure 24 h of six plant extracts in the 0.25, 0.5 and 1 mg·ml^{-1} doses

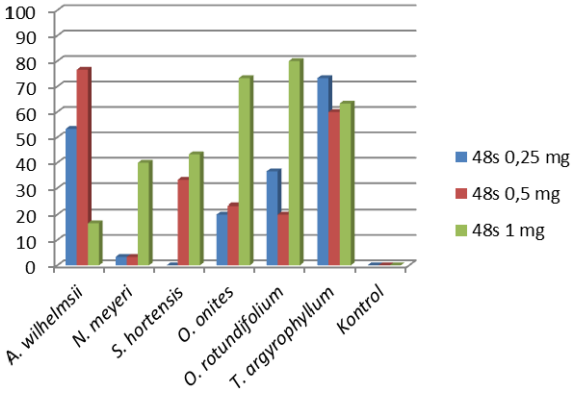

Fig. 3b. Mortality of 4rd larvae instar of *Taumetopoea pityocampa* (Denis & Schiffermüller), in relation to exposure 48 h of six plant extracts in the 0.25, 0.5 and 1 mg·ml^{-1} doses

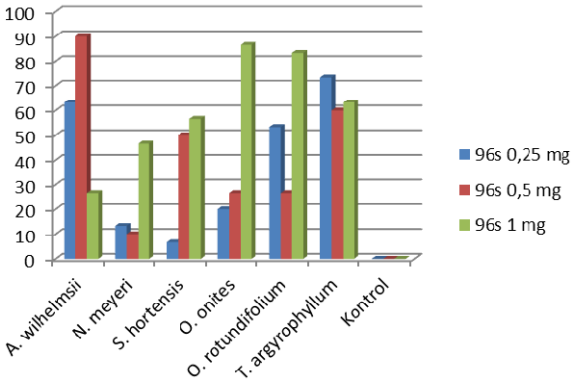

Fig. 3c. Mortality of 4rd larvae instar of *Taumetopoea pityocampa* (Denis & Schiffermüller), in relation to exposure 96 h of six plant extracts in the 0.25, 0.5 and 1 mg·ml^{-1} doses

96 h. The highest mortality rate (90%) was found in the 0.5 mg dose of *A. wilhelmsii* the extract after 96 h for the 4th instar larvae. But, the lowest mortality rate (6.66%) after 96 h was determined in the 0.25 mg dose of *S. hortensis* extract against the 4th instar larvae of *T. pityocampa* (tab. 3; fig. 3c). In comparison with toxcities of six plant extracts, the highest mortality rates on the 4th instar larvae of *T. pityocampa* were determined for extract of *T. argyrophyllum* in all times (24, 48 and 96 h) and doses (0.25, 0.5 and 1 mg). But, the lowest mortality was found for the extract of *S. hortensis* on the 4th instar larvae of *T. pityocampa*. However, there was no mortality in the control groups during the test period (tab. 3; fig. 3a, b, c).

Table 4. The LD values of extracts obtained from six plants against 2nd instar larvae of *Taumetopoea pityocampa* (Denis & Schiffermüller)

	The second larvae				
Treatments	LD_{25}[a]	LD_{50}[b]	LD_{90}[c]	(X^2)[d]	Slope ±SE
A. wilhelmsii	*	*	0.000	4.652	0.156 ±0.478
N. meyeri	0.000	0.001	0.110	19.671	0.671 ±1.926
S. hortensis	0.107	0.259	1.388	19.223	1.758 ±1.031
O. onites	0.100	0.651	20.765	13.809	0.845 ±0.545
O. rotundifolium	0.010	0.135	17.421	35.385	0.607 ±0.528
T. argyrophyllum	0.002	0.046	22.127	39.653	0.477 ±0.640

*Very high values

Previous authors reported that the essential oil extracted from aerial parts of *O. onites* had toxic at different doses on 4th and 5th instar larvae of *Thaumetopoea wilkinsoni* [Çetin et al. 2006]. The same researchers found that the plant products of *O. onites* had toxic effect on the 4th and 5th instar larvae of *T. wilkinsoni.* In this study, we have found that the extract of *O. onites* has a larvicidal effect (between 3.33 and 86.6%) on 2nd, 3rd and 4th instar larvae of *T. pityocampa* (tab. 1, 2 and 3; fig. 1a, b, c, 2a, b, c and 3a, b, c).

It was stated that the essential oil of *O. rotundifolium* had an insecticidal effect on adults of *S. granarius* [Yıldırım et al. 2011]. In this study, we have determined that the extract of *O. rotundifolium* has a larvicidal effect in all the exposure times (24, 48 and 96 h) and treatment doses (0.25, 0.5 and 1 mg) with mortality rates (between 6.66 and 83.30%) on the 2nd, 3rd, and 4th instar larvae of *T. pityocampa* (tab. 1, 2 and 3; fig. 1a, b, c, 2a, b, c and 3a, b, c).

In an previous study, it was found that the essential oil of *S. hortensis* had an insecticidal effect on *Bruchus dentipes* [Tozlu et al. 2011]. However, it was determined that the essential oil of *S. hortensis* had insecticidal activities (fumigant, repellent and contact toxicity) on *T. castaneum*, *E. kuehniella* and *P. interpunctella* [Maedeh et al. 2011]. In addition to, it was stated that the essential oil extracted from *S. hortensis* had insecticidal effect against the male and female adults of *Callosobruchus maculatus* [Heydarzade and Moravvej 2012]. In the current study, we have found that the extract of

S. hortensis has a larvicidal effect in all the exposure times and treatment doses between 6.66 and 80% of the mortality rates on all instar larvae of *T. pityocampa* (extcept in the 0.25 mg dose after 24 and 48 h on the 4th instar larvae) (tab. 1, 2, 3; fig. 3a, b, c).

Table 5. The LD values of extracts obtained from six plants against 3rd instar larvae of *Taumetopoea pityocampa* (Denis & Schiffermüller)

	The third larvae				
Treatments	LD_{25}[a]	LD_{50}[b]	LD_{90}[c]	(X^2)[d]	Slope ±SE
A. wilhelmsii	96.628	0.002	0.000	10.218	0.145 ±0.385
N. meyeri	1.495	5.063	51.385	5.865	1.273 ±0.897
S. hortensis	0.246	6.403	3122.884	28.336	0.477 ±0.384
O. onites	0.019	0.003	0.000	12.396	0.849 ±2.128
O. rotundifolium	0.006	0.000	0.000	11.940	0.291 ±1.311
T. argyrophyllum	0.000	0.000	0.000	19.600	0.000 ±1.061

The essential oil obtained from *T. argyrophyllum* has an insecticidal effect against *S. granarius* adults [Kordali et al. 2012]. In this study, it was determined that the extract of *T. argyrophyllum* has an important larvicidal effect in all exposure times and treatment doses between 3.33 and 76.60% of the mortality rates (except after 24 h, 1 mg dose 3rd instar larvae) on all instar larvae of *T. pityocampa* (tab. 1, 2, 3; fig. 1a, b, c; 2a, b, c and 3a, b, c).

The essential oil isoleted from *A. wilhelmsii* had a strong insecticidal activity on *Tribolium castaneum* [Khani and Asghari 2012]. In the present study, we found that the extract of *A. wilhelmsii* has a larvicidal effect in all exposure times (24, 48 and 96 h) and all treatment doses (0.25 mg, 0.5 mg, and 1 mg) from 3.33 to 90% of the mortality rates on the 2nd, 3rd and 4th instar larvae of *T. pityocampa.* In our study, the highest mortality rates in the 0.5 mg dose were determined as 53.3% after 24 h on the 2nd instar larvae, and 76.6% after 48 h and 90% after 96 h on the 4th instar larvae of *T. pityocampa*, respectively (tab. 1, 3; fig. 1a; fig. 3b, c).

The insecticidal effect of the extract obtained from *N. meyeri* could not be found in the previous studies. But, in our study, it was found that the extract of *N. meyeri* has a larvicidal effect in all the exposure times and treatment doses (except in the 0.25 mg, after 24 h, on the 3th instar larvae) between 3.33 and 100% of the mortality rates on all instar larvae of *T. pityocampa*. The highest mortality rate after 96 h of treatment with 1 mg dose was determined as 100% against the 2nd instar larvae of *T. pityocampa* (tab. 1, 2, 3; fig. 1a, b, c; 2a, b, c and 3a, b, c).

On the other hand, according to LD values, the lowest toxic effects (LD_{25} and LD_{50} values, very high values) were found for the extract of *A. wilhemlsii* on the 2nd instar larvae of *T. pityocampa*, whereas the most toxicity effects for the extracts of *A. wilhemlsii* and *N. meyeri* were determined as 0.000 (LD_{90} and LD_{25}, respectively). However, the lowest toxic effect was fixed for the extract of *S. hortensis* as 3122.884 in the LD_{90} value on the 3rd instar larvae of *T. pityocampa*. But, the highest toxicity effect was

Table 6. The LD values of extracts obtained from six plants against 4th instar larvae of *Taumetopoea pityocampa* (Denis & Schiffermüller)

	The fourth larvae				
Treatments	LD_{25}[a]	LD_{50}[b]	LD_{90}[c]	(X^2)[d]	Slope ±SE
A. wilhelmsii	1.941	0.733	0.115	18.433	1.595 ±0.215
N. meyeri	0.585	1.334	6.380	22.622	1.885 ±0.236
S. hortensis	0.380	0.714	2.367	9.939	2.461 ±0.361
O. onites	0.347	0.563	1.407	8.582	3.220 ±0.804
T. argyrophyllum	114.605	3.817	0.006	11.046	0.457 ±0.266
O. rotundifolium	0.123	0.403	3.860	21.882	1.306 ±0.516

[a] – the lethal concentration causing 25% mortality after 96 h
[b] – the lethal concentration causing 50% mortality after 96 h
[c] – the lethal concentration causing 90% mortality after 96 h
[d] – chi square value
[e] – slope of the concentration-mortality regression line ±standard error
* – for this extracts no LD values are computed because the ratios of response counts to subject counts are the same, i.e. the slope is zero

determined in the LD_{25}, LD_{50} and LD_{90} values using the extracts of *T. argyrophyllum*, in the LD_{90} of *O. onites* and LD_{50} and LD_{90} of *O. rotundifolium* as 0.000 (tab. 4, 5 and 6). Similarly, the lowest toxic effect was found for the extract of *T. argyrophyllum* as 114.605 in the LD_{25} value on the 4th instar larvae of *T. pityocampa*. Whereas, the highest toxicity effect was determined as 0.006 in the LD_{90} value using the extract of *T. argyrophyllum* on the 4th instar larvae of *T. pityocampa* (tab. 6).

CONCLUSION

The results of present the study demonstrated that extracs obtained from six different plant species, especially that of *T. argyrophyllum* had the larvicidal effect on *T. pityocampa*. Therefore, these plant extracts can be considered as potential alternatives to control against the instar larvae of *T. pityocampa*. Moreover, from the above findings it can be concluded that the incorporation of plant products such as extracts of plants could provide a suitable and cheaper alternative for management of *T. pityocampa* and such method of insect management can also applied in field studies also. However, further research is needed in order to prevent its damage on the forest trees.

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LARWOBÓJCZY WPŁYW NIEKTÓRYCH WYCIĄGÓW ROŚLINNYCH NA *Thaumetopoea pityocampa* (Denis & Schiffermuller) W WARUNKACH LABORATORYJNYCH

Streszczenie. Korowódka pniówka *Taumetopoea pityocampa* (Denis i Schiffermüller) jest jednym z najbardziej rozpowszechnionych insektów, które niszczą igłydrzew w le-

śnych rejonach Turcji. Chociaż stosowano różne metody zwalczania tego szkodnika lasów, to problem jest wciąż w znacznej mierze nierozwiązany. Celem niniejszego badania jest określenie larwobójczego wpływu wyciągów otrzymanych z sześciu różnych gatunków roślin, mianowicie *Achillea wilhelmsii* C. Koch, *Nepeta meyeri* Benth., *Satureja hortensis* L., *Origanum onites* L., *O. rotundifolium* Boiss., *Tanacetum argyrophyllum* (C. Koch) na 2., 3. i 4. stadium larw *T. pityocampa* w warunkach laboratoryjnych. Przetestowano toksyczność sześciu wyciągów roślinnych wobec 2., 3. i 4. stadium larwalnego *T. pityocampa.* Zastosowano 10 larw tego owada z 15 g świeżych (rocznych) igieł *Pinus brutia* umieszczonych na szalkach Petriego (głębokości 9 × 1,5 cm). Każdą dawkę rozpuszczono w acetonie. 0,25, 0,5 oraz 1 mg wyciągów roślinnych w 1 ml roztworu rozpylono na 2., 3., oraz 4. stadium larwalnym *T. pityocampa* na szalkach Petriego, co odpowiada 2,08, 4,16 i 8,33 $mg \cdot l^{-1}$ stężeń w powietrzu. Szalki przykryto pokrywką. Wszystkie testy przeprowadzono w temperaturze 26ºC (±2), przy wilgotności względnej 60% (±5) oraz przy 14/10 godz. fotoperiodzie światła dziennego/zaciemnienia. Śmiertelność larw określono po 24, 48 oraz 96 godzinach. Szalki Petriego ze sterylną wodą i acetonem były użyte jako kontrola. Wszystkie testy wykonano trzykrotnie. Wykazano, że sześć wyciągów roślinnych ma wpływ larwobójczy na 2., 3., oraz 4. stadium larwalne *T. pityocampa* w porównaniu z kontrolą. Te naturalnie występujące wyciągi roślinne mogą być użyteczne w zwalczaniu populacji larw.

Słowa kluczowe: wyciągi roślinne, *Taumetopoea pityocampa*, wpływ larwobójczy, sosna, procent śmiertelności

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