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# Larvicidal effect of *Achillea biebersteinii* Afan. (Asteraceae) essential oil against larvae of pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae)

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**Abstract:** The pine processionary moth, *Taumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae), is an important pest of coniferous trees in the forests of Turkey and throughout the world. In order to control this pest, different methods such as mechanical, biological, and chemical control have been used. However, this pest is still a major problem in Turkey, as well as globally. The study aimed to investigate the larvicidal effect of essential oil obtained from *Achillea biebersteinii* Afan. (Asteraceae) in different doses (10, 15, and 20 µL Petri$^{-1}$) over time (12, 24, 36, and 48 h) against *T. pityocampa* larvae in laboratory conditions (25 °C (± 2), 65% (± 5) RH, and 14/10 L/D). At the end of the study, the larvae mortalities were observed to range from 3.33% to 100%. While the highest mortality rates (between 40% and 100%) were recorded on first and second instar larvae, the lowest mortality rates (between 3.3% and 73.3%) were determined for third, fourth, and fifth instar larvae. The results showed that *A. biebersteinii* essential oil has a critical larvicidal effect on the first, second, third, fourth, and fifth instar larvae of *T. pityocampa* in comparison with the controls and can be used in controlling the larvae of this pest.

**Key words:** *Achillea biebersteinii*, essential oil, larvicidal effect, *Taumetopoea pityocampa*

## 1. Introduction

Forests are ecological environments which protect the natural balance of the world we live in, providing shelter, nest, hunting, feeding, and breeding (as well as making significant contributions in meeting various needs of people), continuously evolving with unique life chains (Tan, 1992; Köse, 2007). A total of 27.6% of Turkey is covered with forests and there are more than 50 tree species adapted to these areas. A total of 35% of them are wide-leaved trees, 54% are coniferous pine species, and 11% are beech, fir, juniper, cedar, spruce, alder, chestnut, hornbeam, linden, ash, and eucalyptus species. Among these forest trees, red pine (*Pinus brutia* Ten.) (Pinaceae) spreads throughout the Mediterranean, Aegean, and Marmara regions of Turkey and hosts wild life. Its high-quality wood is used economically for firewood, timber, etc., and it has become the most essential plant in the forestry industry (Öktem, 1987; Anonymous, 2003). Red pine covers approximately 47% of the forests in the Mediterranean region, 40% of the forests in the Aegean region, and 10% of the forests in the Marmara region. However, it is known that this species is also found in some areas of the western Black Sea region (Neyişçi, 1987).

There are many factors that threaten the life of red pine, which provides shelter to wildlife with its natural beauties and fills the diverse needs of people (Neyişçi, 1987; Ertuğrul, 2002; Kanat and Alma, 2004). Among these factors, the most significant are illegal and uncontrolled cutting, agricultural land clearance, forest fires, unplanned and improper zoning permits, road construction, migrations, and wars. In addition to these causes of damage, there are insects, which are referred to as smoke-free fire. One of the most important is the pine processionary moth, *Taumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae) (Özdal, 2002). The adults of *T. pityocampa* do not cause harm. However, the larvae feed on coniferous pine species such as *Pinus halepensis* Mill., *P. silvestris*, *P. pinea*, *P. nigra* with *Cedrus libani* A. Rich.

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(Pinaceae), as well as red pine (*P. brutia*) in Turkey, and occasionally cause economic losses. They cause growth retardation of between 22% and 65% in diameter in coniferous trees and reduced height. As a result, weakened trees are invaded by pests, causing more rapid transmission of diseases (Çanakcıoğlu, 1993; Ertuğrul, 2002; Kanat et al., 2002; Köse, 2007). When the population of *T. pityocampa* is large, it can cause the death of trees. Today, *T. pityocampa* larvae have a significant impact on pines, especially in Aegean and Mediterranean countries, and cause damage, together with global warming and increasingly changing environmental conditions (destruction of natural enemies by forest fires and chemical applications, deterioration of natural equilibrium, air pollution, etc.). However, tourism is crucial in these countries; therefore, the concept of protection of natural areas and environmental awareness has spread in them. Since damage to the trees is quite visible, it causes an increase in social pressure in favor of control of this pest.

Different methods of pest control—mechanical, biological, and chemical—were used to control the larvae of *T. pityocampa* in the past, but the damage has not been wholly prevented, and a permanent solution has not been revealed. This pest species presents an on going significant problem in coniferous forests of Turkey and the world. Following the discovery of the dangers of DDT (dichloro-diphenyl-trichlorethane) during the Green Revolution (1960s), many different synthetic chemicals were produced. Use of these synthetic pesticides in agricultural and forest areas caused many adverse effects such as the polluting of the surrounding water, air, and soil, the extinction of some species, growth and developmental disorders, increase in cancer cases, human birth defects, and environmental health issues. In chemical control, between 1975 and 2006, active substances such as dimiline, triflumuron, deltamethrin, beta-cyfluthrin, DDT, parathion-methyl, and azinphos-methyl were used (Yelekci et al., 1980; Breuer and Devkota, 1990). However, excessive and inconsistent use of these chemicals hurt other organisms and caused decreases in biodiversity. In particular, they adversely affected the beneficial organisms that protect the natural balance. In addition, they caused decreases or complete extinction of many populations of beneficial organisms (Güncan and Durmuşoğlu, 2004).

Similarly, the chemicals led to the deaths of other useful and neutral insect populations such as honey bees, along with birds, reptiles, etc. They caused many lethal diseases in humans through the food chain (Peter, 1984; Ecevit, 1988). Because of all of these negative effects and the ongoing loss of trees to *T. pityocampa* larvae, there is a need to develop alternative control methods that are ecofriendly and protect the natural balance against this pest.

From this point of view, plant-origin compounds (essential oil and extracts) come to the fore in the effort to control the *T. pityocampa* pest. Plants used as critical natural bio-agents contain phenolic compounds and essential oils with potent biological activity (Mokbel and Fumio, 2006; Batish et al., 2008). According to recent studies, more than 200,000 plant species have been identified as possessing pesticide-containing compounds, but only 1% of them have been found useful. Among these compounds, pyrethrum, rotenon, nicotine, and neem have been reported to be the most important plant-derived compounds that show pesticidal effects in studies on many insect pests (Isman, 2006). Essential oils are volatile and fragrant natural compounds obtained from different parts of plants (flowers, seeds, leaves, fruits, shells, etc.), which are usually in liquid form at room temperature, quickly crystallize, and are colorless or light yellow. Essential oils, extracts, and compounds obtained from different plants have been reported to have insecticidal, ovicidal, repellent, attractant, and antifeedant effects in many studies on harmful insects (Regnault-Roger et al., 1993; Shaaya et al., 1993; Yıldırım et al., 2005; Kordali et al., 2006; Kordali et al., 2008; Kesdek et al., 2015; Güdek and Çetin, 2017; Usanmaz Bozhüyük et al., 2017, Üstüner et al., 2018). Plant-derived compounds, especially essential oils, can help to reduce insect resistance and environmental pollution when used against pests in the agricultural field with no residual (permanent) effect on the environment. From this perspective, natural insecticides do not pose much threat to human and environmental health (Aksoy, 1982; Lüleyap, 1996; Turanlı et al., 2006).

Genus *Achillea* L. is in the family Asteraceae (Compositae), it is reported that there are approximately 115 species in the world in the genus, mostly in Europe and Asia (Benedek et al., 2008). In Turkey, a total of 42 species are distributed, 23 of which are endemic (Duman, 2000). These species are of great importance because of their insecticidal, antifungal, antibacterial, and allelopathic effects along with their specific taste, aroma, use as spice, and their medicinal value, and contain large amounts of essential oil. Among the *Achillea* species in Turkey, *A. biebersteinii* is grown widely in natural areas (Polat et al., 2013). In this study, the larvicidal effect on *T. pityocampa* larvae of essential oil obtained from *A. biebersteinii* belonging to the genus *Achillea* growing in natural areas was investigated.

## 2. Materials and methods

### 2.1. Biological material

Pine processionary moth (*T. pityocampa*) larvae used in the study were collected from Esenköy, Fethiye. Pouches (nets) on branches of red pine trees were cut with the help of gloves and pruning shears and placed into 30 × 45 × 30 cm size cardboard boxes under wrapped filter paper. The larvae were fed with fresh leafy shoots cut from uninfected

shoots. The larvae were removed from the pouches using forceps and were placed into Petri dishes at 25 ± 2 °C and 65 ± 5% humidity under laboratory conditions. This process was carried out separately for each larval instar (between October 2016 and March 2017).

**2.2. Plant material**

*A. biebersteinii* (Asteraceae) was collected at the flowering stage from natural areas of Atatürk University Campus and the Pasinler district of Erzurum Province between June and September in 2015–2016. Dried plant samples were diagnosed by Dr. Yusuf Kaya in Department of Biology, Atatürk University. Herbarium samples were stored in the Herbarium of the Department of Plant Protection, Faculty of Agriculture, Atatürk University.

**2.3. Obtaining essential oil**

*A. biebersteinii* essential oil was obtained as described by Çakır et al. (2016). Collected plant materials were dried in darkness and ground in a grinder. The dried plant samples (500 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. Hydrodistillation of *A. biebersteinii* yielded 0.63% (w/w) essential oil. The yields were based on dry materials of plant samples. Essential oil was stored in the refrigerator at 4 °C for use in trials.

**2.4. GC and GC/MS analysis**

GC and GC/MS analyses of essential oil were made by taking one-to-one from the previous study (Çakır et al., 2016).

**2.5. Bioassay of the larvicidal effect of essential oil**

In order to determine the larvicidal effect of the essential oil obtained from *A. biebersteinii* against *T. pityocampa* larvae, the essential oil was dissolved in 1:2 volatile oil/ethanol and the final concentration was prepared as 10, 15, and 20 µL Petri$^{-1}$ and 2.5, 3.75, and 5 µL Petri$^{-1}$ doses. Two layers of sterilized filter paper were then placed under the Petri dishes (120 mL volume, 9 cm width × 1.5 cm depth). A total of 10 larvae were placed into each Petri dish and 1 mL from the essential oil solutions prepared as the stock was sprayed to contact the larvae. Ethanol was evaporated under atmospheric conditions for 5 min.

In order to feed the larvae, uncontaminated fresh pine leaves were added in sufficient amounts (according to larval instar; 6–10 g by increasing 1 g each larval period) and parafilm was wrapped around the Petri dishes. Previously prepared essential oil solutions were mixed using a vortex 1 min before application. Pure water + ethanol was used as the negative control, while the commercial chemical Dimylene @ 25 WP (25% diflubenzuron) was used as the positive control. The experiments were carried out at 25 ± 2 °C temperature, 65 ± 5% relative humidity, and 14/10 h light/dark laboratory conditions; each trial was done with 3 replications for each larval period. Dead larvae were counted at 12, 24, 36, and 48 h after the application.

**2.6. Data analysis**

Mortality rates on 5 larval instars of *T. pityocampa* of the essential oil obtained from *A. biebersteinii* plant species were determined, and % mortality tables were created for each larval instar at the end of 12, 24, 36, and 48 h.

To determine whether there was a statistically significant difference between the obtained results, variance analysis (ANOVA) was applied using the SPSS (Statistical Package for Social Sciences 17.0) software package. Differences between means were tested with the Duncan test. $LD_{50}$ and $LD_{90}$ (median lethal dose) values were calculated using Finney's method (Finney, 1971), and the EPA Probit analysis program was used to determine $LD_{50}$ and $LD_{90}$ values at 95% confidence limits of each application.

## 3. Results

**3.1. Chemical compositions of the essential oil**

Chemical compositions of the essential oil from aerial parts of *A. biebersteinii* are given in Table 1. In the essential oil of *A. biebersteinii*, 38 components were identified representing 99.3% of the total composition. The main components were 1,8-cineole (38.1%), camphor (23.6%), borneol (5.9%), and α-terpineol (5.2%).

*GC and GC/MS analyses of essential oil were given by taking one-to-one from the previous study (Çakır et al., 2016).

**3.2. Larvicidal effect of the essential oil**

In this study, the larvicidal effect of essential oil obtained from *A. biebersteinii* plant species against 5 larval instars of major forest pest *T. pityocampa* were investigated. As a result of the experiments, it was observed that the applications at 10, 15, and 20 µL Petri$^{-1}$ doses of essential oil caused deaths at different rates on 5 larval instars of *T. pityocampa* compared to the controls.

In the application of the 15 µL Petri$^{-1}$ dose of *A. biebersteinii* essential oil on the $L_1$ instar larvae, the lowest mortality rate was found to be 40.0% after 12 h, and the rate recorded was 83.3% after 48 h (Table 2; $P < 0.05$). Similarly, the mortality rates at 24 and 36 h for 10, 15, and 20 µL Petri$^{-1}$ doses of *A. biebersteinii* essential oil against the $L_1$ instar larvae were 70.0%, 76.6%, 63.3%, 80.0%, 100%, and 100%, respectively.

It was observed that the mortality rates increased with the increase of application doses and times (Table 2). The lowest mortality rate was 43.3%, while the highest mortality rate was 83.3% at 12, 24, 36, and 48 h for 10, 15, and 20 µL Petri$^{-1}$ against the $L_2$ instar of *T. pityocampa*. The most effective dose was 20 µL Petri$^{-1}$ for all exposure times against the $L_2$ instar (Table 3; $P < 0.05$). The minimum and maximum mortality rates were 20.0% and 73.3% at 12, 24, 36, and 48 h of application of *A. biebersteinii* essential oil (10, 15, and 20 µL Petri$^{-1}$) against the $L_3$ instar. However,

**Table 1.** Chemical composition (%) of the oil of aerial parts of *A. biebersteinii*.

| RI[a] | Components | Oil (%) | Identification methods |
|---|---|---|---|
| 938 | α-Pinene | 1.0 | GC, MS, RI |
| 957 | Camphene | 2.4 | GC, MS, RI |
| 983 | β-Pinene | 1.1 | GC, MS, RI |
| 1023 | α-Terpinene | 0.6 | GC, MS, RI |
| 1034 | *p*-Cymene | 1.7 | GC, MS, RI |
| 1042 | 1,8-Cineole | 38.1 | GC, MS, RI |
| 1067 | γ-Terpinene | 1.1 | GC, MS, RI |
| 1106 | Linalool | 0.7 | GC, MS, RI |
| 1130 | *Cis-p*-Menth-2-en-1-ol | t | GC, MS, RI |
| 1134 | α-Campholenal | 1.6 | MS, RI |
| 1153 | Camphor | 23.6 | GC, MS, RI |
| 1162 | Sabina ketone | 1.5 | MS, RI |
| 1172 | Borneol | 5.9 | GC, MS, RI |
| 1178 | Terpinen-4-ol | 3.3 | GC, MS, RI |
| 1185 | *p*-Cymen-8-ol | t | GC, MS, RI |
| 1190 | α-Terpineol | 5.2 | GC, MS, RI |
| 1211 | trans-Carveol | t | GC, MS, RI |
| 1242 | Cuminaldehyde | 0.5 | GC, MS, RI |
| 1254 | Piperitone | 0.4 | GC, MS, RI |
| 1278 | Bornyl acetate | 1.4 | GC, MS, RI |
| 1289 | Thymol | 1.0 | GC, MS, RI |
| 1296 | Carvacrol | t | GC, MS, RI |
| 1332 | *p*-Mentha-1,4-dien-7-ol | 0.8 | MS, RI |
| 1357 | Eugenol | t | GC, MS, RI |
| 1399 | (Z)-Jasmone | 0.3 | GC, MS, RI |
| 1419 | β-Caryophyllene | 0.4 | MS, RI |
| 1474 | *γ*-Gurjunune | t | GC, MS, RI |
| 1476 | *B-Chamigrene* | 0.3 | MS, RI |
| 1486 | Germacrene D | 3.0 | GC, MS, RI |
| 1574 | Spathulenol | 0.3 | MS, RI |
| 1579 | Caryophyllene oxide | 0.3 | GC, MS, RI |
| 1631 | γ-Eudesmol | 0.5 | MS, RI |
| 1651 | β-Eudesmol | 1.0 | MS, RI |
| 1844 | (*Z,Z*)-Farnesyl acetone | 0.6 | GC, MS, RI |
| 1923 | *n*-Hexadecanoic acid | 0.3 | GC, MS, RI |
| 1955 | Methyl linoleate | 0.3 | GC, MS, RI |
| 2000 | *n*-Eicosane | 0.2 | GC, MS, RI |
| 2100 | n-Heneicosane | 0.2 | GC, MS, RI |
| Grouped components (%) | | | |
| Monoterpene hydrocarbons | | 7.9 | |
| Oxygenated monoterpenes | | 84.0 | |

**Table 1.** (Continued).

| | | |
|---|---|---|
| Sesquiterpene hydrocarbons | 3.7 | |
| Oxygenated sesquiterpenes | 2.7 | |
| Others | 1.0 | |
| Total | 99.3 | |

[a]Calculated retention index to n-alkanes ($C_8$-$C_{28}$) on SGE-BPX5 capillary column. GC: coinjection with standards; MS: tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data (Adams, 2007); RI: identification based on comparison of retention index with those published data (Adams, 2007); t: trace (less than 0.1%).

**Table 2.** Larvicidal effect of *Achillea biebersteinii* essential oil on the $L_1$ instar larvae of *Taumetopoea pityocampa*.

| $L_1$ Instar Larvae | | | | | |
|---|---|---|---|---|---|
| Essential oil | Dose (µL Petri$^{-1}$) | Death (%) ± SE (standard error) | | | |
| | | Exposure time (hour) | | | |
| *Achillea biebersteinii* | | 12 | 24 | 36 | 48 |
| | 10 | 50.0 ± 5.77 b | 70.0 ± 5.77 b | 76.6 ± 3.33c | 83.3 ± 3.33 c |
| | 15 | 40.0 ± 11.5 b | 63.3 ± 3.33 c | 80.0 ± 5.77 b | 93.3 ± 6.66 b |
| | 20 | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a |
| Positive control (Dimylene @ 25 WP) | 15 | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a |
| Negative control (Pure water + ethanol) | - | 0.0 ± 0.0 c | 0.0 ± 0.0 d | 0.0 ± 0.0 d | 0.0 ± 0.0 d |

The values indicated by the different letters in the same column are significantly different ($P \leq 0.05$) according to the Duncan test.

**Table 3.** Larvicidal effect of *Achillea biebersteinii* essential oil on the $L_2$ instar larvae of *Taumetopoea pityocampa*.

| $L_2$ Instar Larvae | | | | | |
|---|---|---|---|---|---|
| Essential oil | Dose (µL Petri$^{-1}$) | Death (%) ± SE | | | |
| | | Exposure time (hour) | | | |
| *Achillea biebersteinii* | | 12 | 24 | 36 | 48 |
| | 10 | 43.3 ± 8.81 c | 66.6 ± 8.81 b | 73.3 ± 3.33 bc | 73.3 ± 3.33 b |
| | 15 | 56.6 ± 6.66 b | 60.0 ±5.77 b | 66.6 ± 3.33 c | 73.3 ± 3.33 b |
| | 20 | 63.3 ± 6.66 b | 70.0 ± 0.0 b | 80.0 ± 5.77 b | 83.3 ± 8.81 b |
| Positive control (Dimylene @ 25 WP) | 15 | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a |
| Negative control (Pure water + ethanol) | - | 0.0 ± 0.0 d | 0.0 ± 0.0 d | 0.0 ± 0.0 d | 0.0 ± 0.0 c |

The values indicated by the different letters in the same column are significantly different ($P \leq 0.05$) according to the Duncan test.

after 48 h, mortality rates were 43.3% and 50.0% for 10 and 15 µL Petri$^{-1}$, respectively (Table 4; $P < 0.05$).

The lowest and highest mortality rates were 13.3% and 53.3% at 12, 24, 36, and 48 h for 10, 15, and 20 µL Petri$^{-1}$ doses of *A. biebersteinii* essential oil against the $L_4$ instar of *T. pityocampa*. However, differences between mortality rates were not significant at all application times for 10 and 15 µL Petri$^{-1}$ doses, and the results were determined to be close to each other. The most effective dose was 20 µL Petri$^{-1}$ (Table 4; $P < 0.05$).

The lowest and highest mortality rates were 3.33% and 56.6%, at 12, 24, 36, and 48 h for 10, 15, and 20 µL Petri$^{-1}$

**Table 4.** Larvicidal effect of *Achillea biebersteinii* essential oil on the $L_3$ instar larvae of *Taumetopoea pityocampa*.

| $L_3$ Instar Larvae | | | | | |
|---|---|---|---|---|---|
| Essential oil | Dose (µL Petri$^{-1}$) | Death (%) ± SE | | | |
| | | Exposure time (hour) | | | |
| *Achillea biebersteinii* | | 12 | 24 | 36 | 48 |
| | 10 | 20.0 ± 0.0 d | 40.0 ± 5.77 c | 43.3 ± 6.66 c | 43.3 ± 6.66 c |
| | 15 | 30.0 ± 0.0 c | 36.6 ±3.33 c | 46.6 ± 3.33 c | 50.0 ± 5.77 c |
| | 20 | 43.3 ± 8.81 b | 63.3 ± 8.81 b | 70.0 ± 5.77 b | 73.3 ± 3.33 b |
| Positive control (Dimylene @ 25 WP) | 15 | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a |
| Negative control (Pure water + ethanol) | - | 0.0 ± 0.0 e | 0.0 ± 0.0 d | 0.0 ± 0.0 d | 0.0 ± 0.0 d |

The values indicated by the different letters in the same column are significantly different ($P \leq 0.05$) according to the Duncan test.

doses of *A. biebersteinii* essential oil against the $L_5$ instar of *T. pityocampa*. In addition, the mortality rate was 36.6% for 10 µL Petri$^{-1}$, while it was 40% for 15 µL Petri$^{-1}$ 48 h after application. According to these data, the larvicidal effect was determined to be lower for all doses (except 20 µL Petri$^{-1}$) of *A. biebersteinii* essential oil against the $L_5$ instar of *T. pityocampa* 48 h after application (Table 6; $P < 0.05$).

As a result, according to the data, when the mortality rates of 5 larval instars were compared, the $L_5$ larval instar of *T. pityocampa* was the most resistant against *A. biebersteinii* essential oil, while $L_1$ and $L_2$ larval instars were the most susceptible (in rates varying between 73.3% and 100%) against *A. biebersteinii* essential oil (Tables 2, 3, 4, 5, and 6).

## 4. Discussion

The main components of *A. biebersteinii* essential oil were 1,8-cineole (38.1%) camphor (23.6%), borneol (5.9%), and α-terpineol (5.2%). Numerous studies on the essential oil compositions of *A. biebersteinii* have been published by various authors. The major compounds of *A. biebersteinii* were 1,8-cineole (32.82%), carvacrol (10.85%), and piperitone (7.34%) (Ghani et al., 2008; Başer, 2016; Polatoğlu et al., 2013). The composition of *A. biebersteinii* essential oil from Turkey was determined. Of the components of *A. biebersteinii* oil, piperitone was richest (49.90%) in plants from Ankara, while 1,8 cineole (29.93%) and camphor (17.35%) were the main constituents in plants of Erzurum origin (Küsmenoğlu et al., 2011). In another study, the richest compounds were 1,8 cineole (46.1%) and camphor (17.6%) among 47 compounds found *A. biebersteinii* oil (Chialva et al, 1993). Therefore, constituents of *A. biebersteinii* essential oil in our study and previous studies were similar to each other. Rates of components were different because of soil and climatic conditions.

Some studies have been performed by a few researchers to determine the larvicidal effect of essential oils against larvae of *T. pityocampa*. Mortality rates of essential oils obtained from *Origanum onites* L. and *Citrus aurantium* L. plants against $L_4$ and $L_5$ instars of *T. pityocampa* were between 72.5% and 97.5% after 24 h of 1% doses (Çetin et al., 2006).

In this study, mortality rates were determined to be 13.3%, 33.3%, and 33.3% against the $L_4$ instar of *T. pityocampa* for *A. biebersteinii* essential oil at 10, 15, and 20 µL Petri$^{-1}$ doses after 24 h, respectively. These 2 studies support each other. In another study, after 24 h on $L_2$, $L_3$, and $L_4$ instars, among the essential oils obtained from *Achillea gypsicola* Hub.-Mor. and *Satureja spicigera* (C. Koch) Boiss. plant species, the lowest mortality was 46.6% for the $L_4$ instar and 60% for the $L_3$ instar at 10 µL Petri$^{-1}$ doses, 80% for the $L_2$ instar at 20 µL Petri$^{-1}$ doses of *A. gypsicola* essential oil (Kesdek et al., 2013). In this study, after 24 h at 10, 15, and 20 µL Petri$^{-1}$ doses against $L_2$, $L_3$, and $L_4$ instars of *A. biebersteinii* essential oil, the lowest mortality rates recorded were 60.0% (for 15 µL Petri$^{-1}$), 36.6% (for 15 µL Petri$^{-1}$), and 20.0% (for 10 µL Petri$^{-1}$) (Tables 3, 4, and 5). When these studies were compared, it was determined that they support each other.

After application in a study to test larvicidal effect at the lowest dose (1 mg ml$^{-1}$) of *Achillea wilhelmsii* C. Koch plant extract on $L_2$, $L_3$, and $L_4$ instars of *T. pityocampa*, the mortality rates were 26.6% for the $L_2$ instar, 6.66% for the $L_3$ instar, and 3.33% for the $L_4$ instar after 24 h (Kesdek et al., 2014). The same investigators recorded mortality rates as 30.0%, 13.3%, and 16.6% in the $L_2$, $L_3$, and $L_4$ periods 48 h after the application.

In another study, larvicidal activities of 9 species (*Laurus nobilis* L., *Liquidambar orientalis* Miller, *Juniperus communis* subsp. nana Willd, *Cupressus sempervirens* L., *Lavandula stoacheas* L., *Lavandula angustifolia* Miller, *Eucalyptus camaldulensis* Dehnh, and *Thymus vulgaris* L.) were tested against $L_4$ and $L_5$ instar larvae of *T. pityocampa*

**Table 5.** Larvicidal effect of *Achillea biebersteinii* essential oil on the $L_4$ instar larvae of *Taumetopoea pityocampa*.

| **$L_4$ Instar Larvae** | | | | | |
|---|---|---|---|---|---|
| Essential oil | Dose (µL Petri$^{-1}$) | Death (%) ± SE | | | |
| | | Exposure time (hour) | | | |
| *Achillea biebersteinii* | | 12 | 24 | 36 | 48 |
| | 10 | 13.3 ± 3.33 c | 20.0 ± 0.0 c | 23.3 ± 3.33 c | 36.6 ± 3.33 c |
| | 15 | 20.0 ± 5.77 c | 23.3 ±3.33 c | 26.6 ± 3.33 c | 43.3 ± 3.33 c |
| | 20 | 33.3 ± 8.81 b | 40.0 ± 5.77 b | 43.3 ± 6.66 b | 53.3 ± 8.81 b |
| Positive control (Dimylene @ 25 WP) | 15 | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a |
| Negative control (Pure water + ethanol) | - | 0.0 ± 0.0 d | 0.0 ± 0.0 d | 0.0 ± 0.0 d | 0.0 ± 0.0 d |

The values indicated by the different letters in the same column are significantly different (P ≤ 0.05) according to the Duncan test.

**Table 6.** Larvicidal effect of *Achillea biebersteinii* essential oil on the $L_5$ instar larvae of *Taumetopoea pityocampa*.

| **$L_5$ Instar Larvae** | | | | | |
|---|---|---|---|---|---|
| Essential oil | Dose (µL Petri$^{-1}$) | Death (%) ± SE | | | |
| | | Exposure time (hour) | | | |
| *Achillea biebersteinii* | | 12 | 24 | 36 | 48 |
| | 10 | 3.33 ± 3.33 c | 13.3 ±6.66 c | 26.6 ± 8.81 c | 36.6 ± 6.66 c |
| | 15 | 20.0 ± 5.77 b | 33.3 ± 8.81 b | 36.6 ± 6.66 bc | 40.0 ± 10.0 c |
| | 20 | 20.0 ± 5.77 b | 33.3 ± 3.33 b | 43.3 ± 3.33 b | 56.6 ± 3.33 b |
| Positive control (Dimylene @ 25 WP) | 15 | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a | 100 ± 0.0 a |
| Negative control (Pure water + ethanol) | - | 0.0 ± 0.0 d | 0.0 ± 0.0 d | 0.0 ± 0.0 d | 0.0 ± 0.0 d |

The values indicated by the different letters in the same column are significantly different (P ≤ 0.05) according to the Duncan test.

on *Pinus brutia* Ten. at different concentration levels (25%, 50%, and 100%) in order to determine mean mortality time. Wood turpentine (0.51 min for 50%) was recorded as the most effective essential oil, followed by thyme herb oil (0.60 min), juniper berry oil (0.65 min), laurel leaf oil (0.59 min), cypress berry oil (1.37 min), essential oil of styrax (2.42 min), and sulfate turpentine (10.31 min) in terms of mean mortality time (Kanat and Alma, 2004).

In this study, the mortality rates in the treatment of *A. biebersteinii* essential oil at 10, 15, and 20 µL Petri$^{-1}$ doses against the $L_2$, $L_3$, and $L_4$ instars of *T. pityocampa* were 66.6%, 60.0% and 70.0% for the $L_2$ instar, 40%, 36.6%, and 63.3% for the $L_3$ instar, and 20.0%, 23.3%, and 40.0% for the $L_4$ instar, respectively. Again, in this study, mortality rates in $L_2$, $L_3$, and $L_4$ instars after 48 h were 73.3%, 73.3%, and 83.3% for the $L_2$ instar, 43.3%, 50.0%, and 73.3% for the $L_3$ instar, and 36.6%, 43.3%, and 53.0% for the $L_4$ instar, respectively. These two studies support each other when compared. In another study, it was determined that essential oils obtained from *Artemisia santanicum* L. and *A. absinthium* plants caused the lowest (6.66%) and the highest deaths (100%) after 48 h from application of 10, 15, and 20 µL Petri$^{-1}$ doses against $L_1$, $L_2$, $L_3$, $L_4$, and $L_5$ instars of *T. pityocampa* (Usanmaz Bozhüyük et al., 2017).

In the present study, the essential oil obtained from the *A. biebersteinii* plant showed the lowest (3.33%) and highest (100%) mortality rates after 48 h from the application of 10, 15, and 20 µL Petri$^{-1}$ doses against $L_1$, $L_2$, $L_3$, $L_4$, and $L_5$ instars of *T. pityocampa*. In this respect, both studies overlap (Tables 2, 3, 4, 5, and 6). In light of these data, if the larvicidal effect of the essential oil obtained from the *A. biebersteinii* plant on the 5 larval instars of *T. pityocampa* is carefully examined, it is seen that this could be an alternative in control against *T. pityocampa*.

Considering the lethal dose (LD) toxicities 48 h after application of *A. biebersteinii* essential oil against larvae of *T. pityocampa*, $LD_{50}$ values were recorded as 0.112, 0.116, 1.455, 2.659, and 2.532 for the $L_1$, $L_2$, $L_3$, $L_4$, and $L_5$ instar

**Table 7.** $LD_{50}$ and $LD_{90}$ values of *Achillea biebersteinii* essential oil on the $L_5$ instar larvae of *Taumetopoea pityocampa*.

| Applied Instars | $LD_{50}$ | $LD_{90}$ | $X^{\%}$ | Slope ± SE |
|---|---|---|---|---|
| 1st instar larvae | 0.112 | 1.360 | 8.070 | 2.695 ± 1.188 |
| 2nd instar larvae | 0.116 | 14.231 | 4.196 | 0.613 ± 0.741 |
| 3rd instar larvae | 1.455 | 10.271 | 3.555 | 1.510 ± 0.698 |
| 4th instar larvae | 2.659 | 82.955 | 2.539 | 0.858 ± 0.680 |
| 5th instar larvae | 2.532 | 50.200 | 4.526 | 0.988 ± 0.681 |

larvae, respectively. The most toxic effect was determined for the $L_1$ instar at 0.112 µL $Larva^{-1}$ dose and the least toxic effect was found for the $L_4$ instar at 2.659 µL $Larva^{-1}$ dose. $LD_{90}$ values were 1.360, 14.231, 10.271, 82.955, and 50.200, respectively; the most toxic effect was recorded for the $L_1$ instar at 1.360 µL $Larva^{-1}$ dose, and the lowest toxicity was recorded at 82.955 µL $Larva^{-1}$ dose for $L_4$ instar (Table 7).

Studies have revealed that chemicals used against diseases and pests in the forest and agricultural areas cause serious damage to human and environmental health and ecological balance. Therefore, there is a need for alternative methods to protect the environment, humans, and natural balance. For this purpose, plant-derived compounds have come to the fore. Essential oils have been used against many diseases and pests; positive results have been obtained. In this study, the larvicidal effect of *A. biebersteinii* essential oil against pine processionary moth (*T. pityocampa*) larvae was investigated. According to the results of the study, it was determined that as the application doses and time of *A. biebersteinii* essential oil increased, mortality rates also increased. When mortality rates were compared according to the application doses, the highest deaths (100%) were recorded at the highest dose of this essential oil (20 µL $Petri^{-1}$). The lowest deaths were determined in $L_5$ instar larvae. Based on these results, the obtained data indicated that the essential oil obtained from the *A. biebersteinii* plant could be used in the control of the larvae of the pine processionary moth (*T. pityocampa*), which is one of the most consequential pests for our forests. It is hoped that this study will be a resource for further studies.

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