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Larvicidal Toxicity of *Vitex agnus-castus* L. (Verbenaceae) Extracts to *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Thaumetopoeidae) Larvae

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ABSTRACT

In this study, the toxicities of acetone, petroleum ether and methanol extracts of chaste tree (*Vitex agnus-castus* L.) (Verbenaceae) plant to the pine processionary moth (PPM) (*Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Thaumetopoeidae)) larvae in the petri dishes (12x1.5cm) under laboratory conditions were investigated. Each dose was dissolved using acetone, petroleum ether and methanol, and diluted with distilled water. 1 ml of this solution was obtained and sprayed to each instar larvae. Tests were separately performed for five larvae instars and each test was repeated three times. Sterile water+ethanol as the negative control and the commercial active substance chemical Diflubenzuron (25%) as a positive control were used. At 96th h of the exposure, the extracts caused between 20 and 100% mortalities. According to LD_{25}, LD_{50} and LD_{90} values, while the most toxic values were recorded as 0.086, 0.154 and 0.001 (for L_1; L_5; L_5) mg/larvae in the petroleum ether extract, the lowest values were 3.684, 8.861 (for L_4; L_3) in the methanol extract and 754.883 (for L_1) mg/larvae in the petroleum ether extract, respectively. The results illustrated that three extracts of *V. agnus-castus* have a larvicidal potential in the control of PPM larvae in comparison with the controls.

Keywords: *Vitex agnus-castus* L., Plant extract, Pine processionary moth, Toxicity

Vitex agnus-castus L. (Verbenaceae) Ekstraktlarının *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Thaumetopoeidae) Larvalarına Karşı Larvasidal Etkileri

ÖZET

Bu çalışmada, hayıt bitkisinin (*Vitex agnus-castus* L.) (Verbenaceae) aseton, petrol eteri ve metanol extraktlarının 0,25, 0,5 ve 1 mg/mL dozlarında, 24., 48., 72. ve 96. saatlerinde çam keseböceği (*Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Thaumetopoeidae)) larvalarına karşı laboratuvar şartlarında ve petri kaplarında (12 x 1.5 cm) toksisiteleri incelenmiştir. Her bir doz; aseton, petrol eteri ve metanol kimyasalları kullanılarak çözülmüş ve saf su ile seyreltilmiştir. Bu çözeltinin 1 mL'si alınarak, Petri kaplarındaki her bir larva dönemine püskürtülmüştür. Tüm testler beş larva dönemleri için ayrı ayrı yapılmış, her bir doz için 3'er kez tekrarlanmıştır. Saf su+etanol negatif kontrol ve ticari aktif madde olan kimyasal Diflubenzuron (%25) ise pozitif kontrol olarak kullanılmıştır. Uygulamanın 96. saatinde, bu ekstraktlar %20-100 arasında ölümlere sebep olmuştur. LD_{25}, LD_{50} ve LD_{90} değerlerine göre, en yüksek toksik değer L_1 için 0.086, L_5 için 0.154 ve L_5 için 0.001 mg/larva olarak petrol eteri ekstraktında, en düşük toksik değer ise L_4 için 3.684, L_3 için 8.861 metanol ekstraktında ve L_1 için ise 754.883 mg/larva olarak petrol eteri ekstraktında belirlenmiştir. Bu sonuçlar, *V. agnus-castus*'un üç farklı ekstraktının çam keseböceği larvalarının mücadelesinde larvasidal potansiyele sahip olduklarını göstermiştir.

Anahtar Kelimeler: Hayıt, Bitki ekstraktı, Çam keseböceği, Toksisite

INTRODUCTION

Forests are very important ecological environments as they protect the natural balance of we live in. 27.6% of the surface area of Turkey is forested and more than 50 tree species adapted to these areas were determined (Oktem, 1987). Among the trees, red pine (*Pinus brutia* Tenellus) (Pinaceae) is wide spread in the timberland areas of Turkey. It is used economically in various ways such as firewood, timber with its high quality as wood, and has become the most essential plant in the forestry industry (Neyisci, 1987; Oktem, 1987). However, there are many factors causing decrease of the red pine population. Illegal and uncontrolled cuttings, opening agricultural land, forest fires, unplanned and improper zoning permits (etc.) are the most important ones. Furthermore, insects known as smokeless fire also threaten its population. Among the insects, the pine processionary moth (PPM) (*Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae) is one of the most damaging insects on the red pine trees. PPM adults are not harmful, but its larvae feed on needles of different *Pinus* species (such as *P. halepensis, P. silvestris, P. pinea, P. nigra* with *Cedrus libani* Richert (Pinaceae)) as well as red pine (*P. brutia*) and cause economic losses. When the population of PPM is extremely high, it can lead to the death of the trees (Canakcıoglu, 1993; Kanat *et al*., 2002).

In the past, different control methods (such as mechanical, biological and chemical control) have been used to control PPM larvae, but their damages were not wholly prevented and a permanent solution has not been revealed. Therefore, this trouble is still continuing on the trees in the coniferous forests of Turkey and the world. In chemical control, the excessive and random use of synthetic chemicals in agricultural and forest areas caused many adverse effects on human and environmental health (Breuer & Devkota, 1990). In particular, they negatively affect beneficial organisms that protect natural balance and non-target organisms (Guncan & Durmusoglu, 2004). Because of all these negativities and the continuing loss by PPM larvae, there is a need to develop alternative control methods that are eco-friendly and protect natural balance against this pest.

In this context, plant-derived compounds should be considered for the control of PPM larvae. Many plant species that contain phenolic compounds and essential oils with potent biological activity are used as critical natural bio-agents (Mokbel & Fumio, 2006; Batish *et al*., 2008). According to recent studies around the world, more than 200,000 flowering plant species have been found. Many of them contain different insecticidal compounds and only 1% of them have considered useful. Among these compounds, plant extracts were reported to be one of the most important plant-derived compounds that have pesticidal effects as a result of studies on many insect pests (Isman, 2006). They are natural compounds extracted from various parts of plants (e.g., flowers, seeds, leaves, fruits and husks). The plant extracts, when used against insect pests, they can help to reduce insect resistance and environmental pollution, with no residual (permanent) effect on the environment. From this perspective, natural insecticides do not pose much threat to human and environmental health (Guncan and Durmuşoğlu, 2004; Isman, 2006). Among these plants, the extracts of the naturally grown the plant species belonging to genus *Vitex* L. constitute an important place. The genus *Vitex* belongs to Verbenaceae family and comprises approximately 300 species in the worldwide. There are two species (*Vitex agnus-castus* L. and *Vitex pseudo-negundo* Haussknecht) grown in natural areas of Aegean and Mediterranean regions in Turkey (Eryigit *et al*., 2015; Tin et al., 2017). Among these species, *V. agnus-castus* was well known by ancient herbalist since the Middle Ages due to its medicinal properties, and also used as a contraceptive and antiaphrodisiac drug (Cambie and Brewis, 1997; De Kok, 2007). The species has a great importance because of its specific taste, aroma, use as its medicinal value, and containlarge amounts of essential oil. Also, it has been identified that ethanol extract of *V. agnus-castus* plant had an antibacterial effect (Karaman *et al*., 2008).

In many studies carried out in the past, the insecticidal effects of plant extracts and essential oils against PPM larvae were reported (Cetin *et al*., 2006; Kesdek *et al*., 2013; Kesdek *et al*., 2014; Germinara *et al*., 2017; Usanmaz Bozhuyuk *et al*., 2018; Yigit *et al*., 2019; Kesdek *et al*., 2020).

The major objective of the study was to investigate the larvicidal efficacy of the three different extracts obtained from chaste tree plant species (*V. agnus-castus*) growing in their natural environment the five instars larvae of PPM under laboratory conditions.

MATERIAL and METHODS

Collecting Insect from the Forest

Thaumetopoea pityocampa (Denis & Schiffermüller, 1775) (Lepidoptera: Thaumetopoeidae) larvae that used in this study were collected from Esenköy (the old name is Dont) province, Fethiye, Muğla. Pouches (nets) on branches of red pine trees were cut with the help of gloves and pruning shear sand placed into 30 × 45 × 30 cm-size card board boxes, under wrapped filter paper. The larvae were feed fresh leafy shoots cut from non-infected shoots. The larvae were removed from pouches using force sand were placed into petri dishes at 25 ± 2˚C and 65 ± 5% RH under laboratory conditions. This process was carried out separately for each larval instar (between September 2018 and April 2019).

Plant Material

The chaste tree (*Vitex agnus-castus* L.) plant species used in this study was collected at the flowering stage from Babadağ Mountain (Fethiye-Muğla) in June of 2018. The aerial parts (flower fresh leaves) of the plant were dried in shade before processing with a grinder. Dried plant herbarium samples have been stored in the Department of Environmental Protection Technologies, Fethiye A.S.M.K. Vocational High School, Mugla Sıtkı Kocman University.

Obtaining of Plant Extracts

Obtaining of *V. agnus-castus* plant extracts was performed as described by Kesdek et al. (2014) and Kucukaydın et al. (2020). The aerial parts of the plant were dried in shaded environmental and milledina grinder. Then, in order to prepare the acetone, petroleum ether and methanol extracts, the dried and powdered the aerial parts of *V. agnus-castus* (each one 100 g) were extracted with acetone, petroleum ether and methanol (for each one 200 mL × 3) 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.). Residue of *V. agnus-castus* plant species was diluted with sufficient HPLC grade acetone (Sigma-Aldrich, Milwaukee, WI, U.S.A.) and sterile water to give100% (w/w) stock solutions. The extracts were stored in a freezer at 4ºC for use further tests.

Bioassay of the Larvicidal Effectiveness of the Plant Extracts

In this present study, each dose was dissolved in acetone, petroleum ether and methanol (100 mg/mL) concentration. Then, 0.25 mg, 0.5 mg, and 1 mg/mL of the plant extracts were hand sprayed (Manual Potter Spray Tower-Burkard Scientific Limited, Uxbridge, UK) on the 1st, 2nd, 3rd, 4th and 5th instar larvae of *T. pityocampa* in the Petri dishes (12 x 1.5 cm) and parafilm was wrapped around the Petri dishes. All toxicity tests carried out at 25ºC (±2), 65% (±5) relative humidity and 14/10 h light/dark photoperiodin laboratory conditions. The plant extracts have applied to larvae and were recorded dead or alive after 24., 48., 72. and 96. hrs. Sterile water+ethanol was used as the negative control while the commercial active substance chemical Diflubenzuron (25%) was used as a positive control. All tests were separately performed for five larvae instars and each dose replicated three times. The larvicidal activity of the plant extracts was expressed as % mean mortality of the larvae.

Statistical Analysis

The differences among the contact toxicities of *V. agnus-castus* plant extracts were determined according to analysis of variance (ANOVA) test by using SPSS 17.0 software package. Mortalities were expressed as mean (percentage) ± standard error. Differences between means were tested through Duncan test and values with $P<0.05$ were considered significantly different. LD_{25}, LD_{50} and LD_{90} values at 96 h were calculated with regression analysis by probit using SPSS (Finney, 1971). 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

Larvicidal Effect of the Plant Extracts

In the present study, the applications at three different doses (0.25, 0.5 and 1mg/mL) acetone, petroleum ether and methanol extracts of *V. agnus-castus* caused mortalities at different rates against *T. pityocampa* larvae compared to controls. The three extracts exhibited various toxicities against the five instar larvae of *T. pityocampa*, depending on the exposure time and concentration of tested samples. The results were summarized in Table 1. The mortality rates were recorded between 20.0 and 100% for five larval periods after 96 h of the treatments. It was determined that depending on the application times of the extracts, the mortality rates increased. When the mortality rates

of the three extracts were compared for 24, 48, 72 and 96 hrs exposure, it was found that there were statistical differences between the treatments for each larval period.

In general, the most effective dose for L_1, L_2, L_3, L_4 and L_5 instars was established to be 1 mg/mL. While the lowest mortality rate was recorded to be 20.0% for L_3 instar in the 0.25 mg/mL dose of methanol extract after 96 h, the highest mortality rate was found as 100% for L_2 instar (at 1 mg/mL dose of petroleum ether and methanol extracts), for L_3 instar (at 0.25, 0.5 and 1 mg/mL doses of petroleum ether extract) and for L_4 instar (at 0.5 and 1 mg/mL doses of methanol extract), respectively (Table 1; $P<0.05$).

At the 24th h of the treatment, the lowest larvicidal toxicity was recorded in the 0.25 mg/mL acetone and methanol extracts of *V. agnus-castus* to be 3.3% for the L_5; and for the L_2 and L_4 instars of *T. pityocampa*, respectively. Similarly, in the same treatment time, while the lowest larvicidal toxicity was recorded in the 0.5 mg/mL of methanol extract to be 3.3% for the L_3 and L_4 instars, the same toxicity rate (3.3%) was observed in the 1 mg/mL of acetone and methanol extracts for the L_3 instar. However, the highest larvicidal toxicity was recorded in the 1 mg/mL of the petroleum ether extract to be 80.0% for the L_2 instar. But, there was no mortality in the 0.25 (acetone and methanol) and 0.5 (acetone) mg/mL of two extracts of *V. agnus-castus* for the L_3 instar (Table1; $P<0.05$).

After 48 h from the treatment, the lowest larvicidal toxicity was determined in the 1 mg/mL petroleum ether and methanol extracts of *V. agnus-castus* to be 6.67% for the L_5 instar of *T. pityocampa*. In the same treatment time, the lowest larvicidal toxicity was established in the 0.5 mg/mL of methanol and petroleum ether extracts to be 13.3% for the L_4; and L_5 instars, respectively. The same toxicity rate (13.3%) was observed in the 0.25 mg/mL acetone extract of *V. agnus-castus* for the L_4 and L_5 instars. Contrary to all these, the highest larvicidal toxicity rates were determined in the 0.25, 0.5 and 1 mg/mL of petroleum ether extract to be 46.6 (for the L_4 instar), 90.0 and 100% (for the L_3 instar), respectively. In addition, after 48 h from treatment, different mortalities observed in five larval periods at three doses of the extracts (Table1; $P<0.05$).

At the 72nd h of the treatment, it was found that acetone, methanol and petroleum ether extracts caused mortality from 13.3 to 100% in five larvae instars. The lowest mortality rates were observed in the 0.25, 0.5 and 1 mg/mL of three extracts to be 16.6% (acetone and methanol extracts) for the L_3 and L_4 instars, 16.6% (methanol extract) for the L_5 instar, and 13.3% (methanol and petroleum ether extracts) for the L_5 instar, respectively. However, the highest toxicities were determined in the 0.25, 0.5 and 1 mg/mL of three extracts to be 66.6% (petroleum ether extract) for the L_4 instar, 96.6% (petroleum ether extract) for the L_3 instar, and 100% (methanol and petroleum ether extracts) for the L_2, L_3 and L_4 instars, respectively. In general, while the most mortality was established in the petroleum ether extract for L_4 instar after 72 h, the lowest mortality was recorded in the methanol extract for L_5 instar (Table1; $P<0.05$).

After 96 h from the treatment, it was recorded that three extracts caused mortality from 20.0 to 100% in L_1, L_2, L_3, L_4 and L_5 instars. The lowest mortality rate was found in the 0.25 mg/mL of methanol extract for the L_3 instar. However, the highest toxicity rate in the 0.25, 0.5 and 1 mg/mL was determined to be 100% for the L_3 instar (petroleum ether extract), for the L_3 and L_4 instars (petroleum ether extract), and for the L_2, L_3 and L_4 instars (methanol and petroleum ether extracts), respectively. In general, while the most mortality was recorded in the petroleum ether extract for L_3 instar after 96 h, the lowest mortality was recorded in the methanol extract for L_5 instar (Table 1). Morever, the most effective dose was detected to be 1mg/mL (Table1; $P<0.05$). Diflubenzuron (25%) is a widely used insecticide among commercial chemicals for *T. pityocampa* larvae. In the present study, 100% toxicity was determined after 96 h with all doses of Diflubenzuron (25%) (0.25, 0.5 and 1 mg/mL) (Table1; $P<0.05$).

On the other hand, the LD values (LD_{25}, LD_{50} and LD_{90}) of the study was summarized in Table 2. When LD values after 96 h treatment of *V. agnus-castus* three extracts were compared for their effects on the five larval periods of *T. pityocampa*, the most toxicity effects based on the LD_{25}, LD_{50} and LD_{90} values were found to be 0.086 (for L_1 instar), 0.154 (for L_5 instar) and 0.001 (for L_5 instar) mg/larvae in the petroleum ether extract of *V. agnus-castus*, the lowest toxicity values were 3.684 (for L_4 instar), 8.861 (for L_3 instar) in the methanol extract and 754.883 (for L_1 instar) mg/larvae in the petroleum ether extract, respectively. Similarly, LD_{25} values after 96 h were recorded to be 0.246, 0.086 and 0.255 (for L_1 instar); 0.630, 0.488 and 0.865 (for L_2 instar); 1.005, 0.747 and 1.184 (for L_3 instar); 0.965, 0.728 and 3.684 (for L_4 instar); 0.929, 2.737 and 2.347 (for L_5 instar) mg/larvae in the acetone, petroleum ether and methanol extracts of *V. agnus-castus*, respectively (Table 2). In addition to this, the LD_{50} and LD_{90} values of the extracts used in the study were given for each larval

periods separately in Table 2. The results of the study showed that the larvicidal toxicity increased with increasing exposure time. The three extracts of *V. agnus-castus* led to meaningful toxicities to the five larvae instars of *T. pityocampa* (Tables 1, 2). As a result, after 96 h from treatment, when mortality rates of the L_1, L_2, L_3, L_4 and L_5 instars of *T. pityocampa* were compared, L_5 instar of *T. pityocampa* was the most resistant against *V. agnus-castus* extracts, While, L_3 instar was the most susceptible against *V. agnus-castus* extracts (Tables 1, 2).

DISCUSSION

In the past, some studies were reported by different researchers to detect the toxicities of essential oils and plant extracts to *T. pityocampa* larvae. Cetin et al. (2006) determined that O*riganum onites* L. (Lamiaceae) and *Citrus aurentium* L. (Rutaceae) essential oils led to mortalities from 72.5 to 97.5% against L_4 and L_5 instars of *T. pityocampa* after 24 h of the treatment. Varcin and Kesdek (2020) stated that mortality rates were found in the 250, 500 and 1000 µL/L doses between 11.1 and 30.0% against L_4 and L_5 instars of *T. pityocampa* for *V. agnus-castus* oil after 24 h of the treatment. In another study, after 24 h of the treatment, the essential oil obtained from *Achillea gypsicola* Huber-Morath (Asteraceae) caused the lowest mortalities on L_2, L_3 and L_4 instars (80%, at 20 µL/Petri; 60%, at 10 µl/Petri; and 46.6%, at 10 µl/Petri, respectively) (Kesdek *et al*., 2013). In addition, in another study, it was explained that *V. agnus-castus* essential oil caused the lowest mortality rates (6.67% for L_2, 500 µl/L; 10.0% for L_2 and L_3, 250 µL/L and 26.6% for L_2 and L_4, 1000 µL/L) on L_2, L_3 and L_4 instars at three different doses (Varcin and Kesdek, 2020). In the present study, we determined that the acetone, petroleum ether extract and methanol extracts of *V. agnus-castus* caused different mortality rates (from 3.33 to 80%) in the 0.25, 0.5 and 1 mg/mL against L_2, L_3, L_4 and L_5 instars after 24 h from treatment (Table 1).

In another study, it was stated that the mortality rates in the treatment with the 10, 15 and 20 µL/Petri doses of *Achillea biebersteinii* Afan. (Asteraceae) oil against L_2, L_3 and L_4 instars after 48 h were detected as 73.3%, 73.3% and 83.3% for L_2 instar, 43.3%, 50.0% and 73.3% for L_3 period, 36.6%, 43.3% and 53.0% for L_4 instar, respectively (Kesdek *et al*., 2020). Usanmaz Bozhuyuk et al. (2018) found that the essential oils of *Seriphidium santanicum* (L.) Sojákand, *Artemisia absinthium* L. (Asteraceae) caused the lowest mortality with 6.66% and the highest mortality with 100% deaths after 48 h from the application of three different doses (10, 15 and 20 µL/Petri) against the five instars of *T. pityocampa*. Varcin and Kesdek (2020) determined that *V. agnus-castus* essential oil caused the mortality rates in three different doses (250, 500 and 1000 µL/L) on the L_2, L_3 and L_4 instars (10.0%, 6.67% and 26.6% for L_2 instar; 10.0%, 33.3% and 46.6% for L_3 instar; 16.6%, 30.0% and 26.6% for L_4 instar after 48 h from the treatment, respectively. In addition, they recorded that *V. agnus-castus* oil lead to the lowest mortality with 10.0% and the highest mortality with 76.6% rates after 48 h from the application in the doses against the five instars of *T. pityocampa* larvae. In our study, we established that the extracts of *V. agnus-castus* caused different mortalities (from 13.3 to 100%) in the 0.25, 0.5 and 1 mg/mL doses against L_2, L_3 and L_4 instars after 48 h from treatment (Table 1).

Kesdek *et al*. (2013) determined that *Origanum acutidens* (Hand.-Mazz.) Letswaart, *Origanum onites* L., *Origanum rotundifolium* Boissier, *Satureja hortensis* L., *Satureja spicigera* (C. Koch) Boissier, *Thymus sipyleus* Boissier (Lamiaceae), *Tanacetum argyrophyllum* (C. Koch) Tvzel and *Artemisia gypsicola* (Asteraceae) plant essential oils caused the mortalities from 73.3 to 100% 72h post treatment, using 10 and 20 µL/Petri doses on the L_2, L_3, and L_4 instars of *T. pityocampa*. Varcin and Kesdek (2020) recorded that *V. agnus-castus* essential oil caused the mortality rates in three different doses (250, 500 and 1000 µL/L) on the L_2, L_3 and L_4 instars (between 13.3 and 80.0%) after 72 h from the treatment. In the present study, it was determined that the extracts of *V. agnus-castus* caused different mortality rates (from 13.3 to 100%) in the 0.25, 0.5 and 1 mg/mL doses against L_2, L_3 and L_4 instars after 48 h from treatment (Table 1).

Kesdek *et al*. (2014) found that extracts of six different plant species (*Nepeta meyeri* Benth, *Satureja hortensis* L., *Origanum onites* L., *O. rotundifolium* (Lamiaceae), *Achillea santolinoides* (C. Koch) Lag. and *Tanacetum argyrophyllum* (C. Koch) (Asteraceae)) had a larvicidal effect (between 3.33 and 100%) on the L_2, L_3 and L_4 instars of *T. pityocampa* after 96 h from the treatment. In another study, five different commercial volatile oils (thyme, sage, poppy, garlic and rosemary) were established to be 70-100% effective on the *T. pityocampa* larvae (Yigit *et al*., 2019). In the present study, three extracts of *V. agnus-castus* caused different mortalities (between 20.0 and 100%) in the 0.25, 0.5 and 1 mg/mL doses against L_2, L_3 and L_4 instars after 96 h from treatment (Table 1). When these studies were compared, it was seen that they supported each other.

As the result of this study, the acetone, petroleum ether and methanol extracts of *V. agnus-castus* led to considerable mortalities after 96 h from the application of three different doses against the L_1, L_2, L_3, L_4 and L_5 instars of *T. pityocampa* (Table 1).

Table 1. The larvicidal efficay of some extracts of *V. agnus-castus* plant on the five instars larvae of *T. pityocampa*

Extract	Dose (mg/mL)	Mortality (%) Exposure Time (h) 24	48	72	96
L_1 INSTAR LARVAE					
***Vitex agnus-castus* (Acetone)**	**0.25**	10.0 ± 10.0 ab	26.6 ± 8.8 bcd	46.6 ± 12.0 bcd	63.3 ± 8.8 cde
	0.5	23.3 ± 14.5 bcde	43.3 ± 8.8 defg	53.3 ± 8.8 bcde	60.0 ± 11.5 bcde
	1	66.6 ± 8.8 h	70.0 ± 5.8 g	80.0 ± 5.8 fghı	96.6 ± 3.3 h
***Vitex agnus-castus* (Petroleum ether)**	**0.25**	13.3 ± 6.7abc	23.3 ± 3.3 bc	33.3 ± 6.7 bc	46.6 ± 3.3 bc
	0.5	10.0 ± 5.8 ab	20.0 ± 5.8 b	30.0 ± 0.0 b	43.3 ± 3.3 bc
	1	36.6 ± 8.8 def	36.6 ± 8.8 def	50.0 ± 16.3 bcde	60.0 ± 8.16 bcde
***Vitex agnus-castus* (Methanol)**	**0.25**	13.3 ± 6.7abc	23.3 ± 12.0 bc	36.6 ± 17.6 bcd	40.0 ±15.2 b
	0.5	16.6 ± 6.7 abcd	26.6 ± 3.3 bcd	33.3 ± 8.8 bc	46.6 ± 6.7 bc
	1	20.0 ± 10.0 abcd	26.6 ±14.5 bcd	46.6 ± 20.2 bcd	53.3 ± 17.6 bc
Positive Control (Kormilin)	**0.25**	100 ± 0.0 ı	100 ± 0.0 h	100 ± 0.0 ı	100 ± 0.0 h
	0.5	100 ± 0.0 ı	100 ± 0.0 h	100 ± 0.0 ı	100 ± 0.0 h
	1	100 ± 0.0 ı	100 ± 0.0 h	100 ± 0.0 ı	100 ± 0.0 h
Negative Control (S. water + ethanol)	**-**	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
L_2 INSTAR LARVAE					
Extract	**Dose (mg/mL)**	**Mortality (%) Exposure Time (h) 24**	**48**	**72**	**96**
***Vitex agnus-castus* (Acetone)**	**0.25**	20.0 ± 10.0 bcd	36.6 ± 6.7 def	50.0 ± 0.0 cde	53.3 ± 3.3 c
	0.5	10.0 ± 10.0 ab	40.0 ± 10.0 defg	53.3 ± 8.8 de	60.0 ± 10.0 c
	1	53.3 ± 6.7 f	73.3 ± 3.3 h	93.3 ± 3.3 hı	96.6 ± 3.3 e
***Vitex agnus-castus* (Petroleum ether)**	**0.25**	16.6 ± 3.3 abc	20.0 ± 0.0 bc	36.6 ± 6.7 bc	56.6 ± 6.7 c
	0.5	20.0 ± 5.8 bcd	43.3 ± 3.3 defg	50.0 ± 0.0 cde	56.6 ± 6.7 c
	1	80.0 ± 11.5 g	90.0 ± 0.0 ı	100 ± 0.0 ı	100 ± 0.0 e
***Vitex agnus-castus* (Methanol)**	**0.25**	3.3 ± 3.3 ab	16.6 ± 6.7 b	30.0 ± 10.0 b	40.0 ± 11.5 b
	0.5	13.3 ±6.7 abc	33.3 ± 8.8 cde	43.3 ± 6.7 bcd	53.3 ± 3.3 c
	1	76.6 ± 6.7 g	86.6 ± 3.3 ı	100 ± 0.0	100 ± 0.0 e
Positive Control (Kormilin)	**0.25**	100 ± 0.0 h	100 ± 0.0 ı	100 ± 0.0 ı	100 ± 0.0 e
	0.5	100 ± 0.0 h	100 ± 0.0 ı	100 ± 0.0 ı	100 ± 0.0 e
	1	100 ± 0.0 h	100 ± 0.0 ı	100 ± 0.0 ı	100 ± 0.0 e
Negative Control (S. water + ethanol)	**-**	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
L_3 INSTAR LARVAE					
Extract	**Dose (mg/mL)**	**Mortality (%) Exposure Time (h) 24**	**48**	**72**	**96**
***Vitex agnus-castus* (Acetone)**	**0.25**	0.0 ± 0.0 a	16.6 ± 6.7 abc	26.6 ±3.3 b	26.6 ± 3.3 bc
	0.5	0.0 ± 0.0 a	16.6 ± 3.3 abc	30.0 ± 10.0 bc	36.6 ± 8.8 bcd
	1	3.3 ± 3.3 a	33.3 ± 20.7 bcde	36.6 ± 17.6 bcd	40.0 ± 15.2 bcd
***Vitex agnus-castus* (Petroleum ether)**	**0.25**	20.0 ± 5.8 c	43.3 ± 8.8 de	63.3 ± 14.5 e	100 ± 0.0 g
	0.5	36.6 ± 3.3 d	90.0 ± 10.0 f	96.6 ± 3.3 fg	100 ± 0.0 g
	1	53.3 ± 3.3 e	100 ± 0.0 f	100 ± 0.0 g	100 ± 0.0 g
***Vitex agnus-castus* (Methanol)**	**0.25**	0.0 ± 0.0 a	16.6 ± 8.8 abc	16.6 ± 8.8 ab	20.0 ± 5.8 ab
	0.5	3.3 ± 3.3 a	33.3 ± 16.6 bcde	36.6 ± 13.3 bcd	40.0 ± 10.0 bcd
	1	3.3± 3.3 a	10.0 ± 0.0 ab	23.3 ± 3.3 ab	30.0 ± 5.8 bcd
Positive Control (Kormilin)	**0.25**	100 ± 0.0 f	100 ± 0.0 f	100 ± 0.0 g	100 ± 0.0 g
	0.5	100 ± 0.0 f	100 ± 0.0 f	100 ± 0.0 g	100 ± 0.0 g
	1	100 ± 0.0 f	100 ± 0.0 f	100 ± 0.0 g	100 ± 0.0 g
Negative Control (S. water + ethanol)	**-**	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	0.5	33.3 ± 3.3 e	80.0 ± 0.0 e	93.3 ± 6.7 fg	100 ± 0.0 f

Table 1 (Continue): The larvicidal efficay of some extracts of *V. agnus-castus* plant on the five instars larvae of *T. pityocampa*

L_5 INSTAR LARVAE					
Extract	**Dose (mg/mL)**	**Mortality (%) Exposure Time (h)**			
		24	**48**	**72**	**96**
***Vitex agnus-castus* (Acetone)**	**0.25**	3.3 ± 3.3 ab	13.3 ± 3.3 a	23.3 ± 3.3 bcd	26.6 ± 6.7 abcd
	0.5	6.7 ± 3.3 abc	16.6 ± 3.3 a	30.0 ± 0.0 bcd	33.3 ±3.3 bcd
	1	6.7 ± 3.3 abc	16.6 ± 3.3 a	26.6 ± 3.3 bcd	50.0 ± 5.8 d
***Vitex agnus-castus* (Petroleum ether)**	**0.25**	6.7 ± 3.3 abc	16.6 ± 3.3 a	30.0 ± 5.8 bcd	33.3 ± 8.81 bcd
	0.5	6.7 ± 3.33 abc	13.3 ± 3.3 a	26.6 ± 3.3 bcd	26.6 ± 3.3 abcd
	1	5.0 ± 4.08 a	6.7 ± 3.3 abc	13.3 ± 0.0 abc	25.0 ± 4.1 abcd
***Vitex agnus-castus* (Methanol)**	**0.25**	6.7 ± 3.33 abc	36.6 ± 21.8 b	40.0 ± 25.1 d	46.6 ± 27.2 cd
	0.5	6.7 ± 3.33 abc	13.3 ± 3.3 a	16.6 ± 3.3 abc	23.3 ± 3.3 abcd
	1	6.7 ± 6.66 abc	6.7 ± 6.7 a	13.3 ± 3.3 abc	23.3 ± 3.3 abcd
Positive Control (Kormilin)	**0.25**	100 ± 0.0 f	100 ± 0.0 d	100 ± 0.0 f	100 ± 0.0 e
	0.5	100 ± 0.0 f	100 ± 0.0 d	100 ± 0.0 f	100 ± 0.0 e
	1	100 ± 0.0 f	100 ± 0.0 d	100 ± 0.0 f	100 ± 0.0 e
Negative Control (S. water + ethanol)	**-**	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a

Table 2. LD_{25}, LD_{50} and LD_{90} values of some extracts of *V. agnus-castus* plant on the five instars larvae of *T. pityocampa*

L_1 INSTAR LARVAE					
	LD_{25}	**LD_{50}**	**LD_{90}**	**X^2**	**Slope ± SE**
***Vitex agnus-castus* (Acetone)**	0.246	0.724	5.619	17.408	1.440 ± 0.683
***Vitex agnus-castus* (Petroleum ether)**	0.086	1.974	754.883	2.298	0.496 ± 0.731
***Vitex agnus-castus* (Methanol)**	0.255	2.408	171.796	14.433	0.691 ± 0.676
L_2 INSTAR LARVAE					
	LD_{25}	**LD_{50}**	**LD_{90}**	**X^2**	**Slope ± SE**
***Vitex agnus-castus* (Acetone)**	0.630	1.080	3.005	13.384	2.884 ± 0.738
***Vitex agnus-castus* (Petroleum ether)**	0.488	1.003	3.941	11.351	2.157 ± 0.816
***Vitex agnus-castus* (Methanol)**	0.865	1.342	3.090	14.272	3.538 ± 0.792
L_3 INSTAR LARVAE					
	LD_{25}	**LD_{50}**	**LD_{90}**	**X^2**	**Slope ± SE**
***Vitex agnus-castus* (Acetone)**	1.005	2.725	18.137	19.178	1.557 ± 0.665
***Vitex agnus-castus* (Petroleum ether)**	0.747	0.869	1.160	9.549	10.238 ± 15.443
***Vitex agnus-castus* (Methanol)**	1.184	8.861	405.957	6.586	0.772 ± 0.724
L_4 INSTAR LARVAE					
	LD_{25}	**LD_{50}**	**LD_{90}**	**X^2**	**Slope ± SE**
***Vitex agnus-castus* (Acetone)**	0.965	1.923	7.139	44.930	2.250 ± 0.671
***Vitex agnus-castus* (Petroleum ether)**	0.728	0.848	1.133	2.611	10.182 ± 18.002
***Vitex agnus-castus* (Methanol)**	3.684	1.159	0.129	10.370	1.342 ± 0.690
L_5 INSTAR LARVAE					
	LD_{25}	**LD_{50}**	**LD_{90}**	**X^2**	**Slope ± SE**
***Vitex agnus-castus* (Acetone)**	0.929	5.187	136.036	5.367	0.903 ± 0.665
***Vitex agnus-castus* (Petroleum ether)**	2.737	0.154	0.001	2.730	0.540 ± 0.772
***Vitex agnus-castus* (Methanol)**	2.347	0.812	0.108	19.391	1.463 ± 0.706

[a]The lethal concentration causing 25% mortality after 96 hour.
[b]The lethal concentration causing 50% mortality after 96 hour.
[c]The lethal concentration causing 90% mortality after 96 hour.
[d]Chi square value.

CONCLUSION

In conclusion, many studies by different researchers show that chemicals used against diseases and pests in the forest and agricultural areas are critical damages to human and environmental health hand also ecological balance. Therefore, there is a need for alternative methods to protect the environment and human health and natural balance. For these reasons, components derived from plants come into prominence. In this study, larvicidal toxicities of *V. agnus-castus* extracts (acetone, petroleum ether and methanol) *T. pityocampa* larvae were investigated. In general, according to the results of our study, we determined that as the application doses and time of *V. agnus-castus* extracts increased, larvicidal toxicities also increased. The highest deaths (100%) were determined at the 1 mg/mL dose of Methanol and Petroleum ether extracts for L_2; 0.25, 0.5 and 1 mg/mL doses of petroleum ether extract for L_3; 0.5 and 1 mg/mL doses of petroleum ether extract for L_4 instars. The lowest deaths were determined in L_5 instar larvae. In the light of these data, when the larvicidal toxicity of the acetone, petroleum ether and methanol extracts

of *V. agnus-castus* to the five larval instars of *T. pityocampa* was carefully examined, it is seen that the three extracts could be an alternative in the control of *T. pityocampa* which is one of the most consequential damages for coniferous trees. Finally, we surely hope that this study will be a good resource for further studies.

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