Chemical composition of Rhododendron luteum essential oil and its larvacidal effectiveness on Diprion pini larvae

Temel GOKTURK (temel.gokturk@gmail.com)
Artvin Çoruh University
Medea BURJANADZE
Agricultural University of Georgia

Research Article

Keywords: Pine sawfly, yellow azalea, biochemical components, toxicity

Posted Date: April 27th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1568215/v1

Abstract

Diprion pini is known as a significant forest pest that damages pine needles in many countries of the world today. Due to the risk of chemical contamination of synthetic pesticides applied against insects, essential oil use is becoming more popular. In this research, the chemical composition of *Rhododendron luteum* essential oil was analyzed by using GC-MS, and its larvicidal effects on six *D. pini* larval stages were investigated. The research was conducted in the Artvin Coruh University Central Laboratory under laboratory conditions settings 25±1ºC temperature, 65±5% relative humidity, and a photoperiod of 14:10 h (L:D) in 2019-2020. β-caryophyllene (15,39%), benzyl benzoate (11,05%), δ-cadinene (7,06%), benzyl salicylate (6,08%), and α-pinene (4,14%) were found as the primary components of *R. luteum* oil. It is observed that the applications of *R. luteum* essential oil at the doses of 10, 15, and 20 µL/Petri and the controls at 24h, 48h, 72h and 96h induced fatalities at varied rates (20,1-100 %) on six *D. pini* larval stages (L_1, L_2, L_3, L_4, L_5, L_6). The highest toxicity was identified at L_1 (0,72 µL/larva) and the lowest toxicity was determined at L_6 (11,92 µL/larva) according to LD_{50} and LD_{90} values. To sum it up, *R.luteum* essential oil showed high toxicity against L_1 and L_2 larval stages of *D. pini*.

Introduction

Forests are valuable assets to be preserved in order to hand down to future generations. Forests, which we can also refer to as the "Green Homeland," are, the country's prestige, dignity and safety. When comparing the continents of the world, Europe has the most forest area with 46 percent. North and Central America comes in second with 25,7%, followed by Africa with 21,8%. Russia, Brazil, America, Canada, and China are the countries with the most forests in the world. The world's total forest area is 4 billion hectares, with 22,6 million hectares in Turkey (Gokturk, 2021).

It is vital to recognize the elements that endanger forests in order to assure their survival. Environmental pollution, fire, concretization, poor planning, disease, and insect damage are just a few of the threats for forest sustainability. In particular, harmful insects are among the variables that have the greatest destructive impact on natural forests (Gokturk, 2021).

The pine sawfly (*Diprion pini* L. (Hymenoptera: Diprionidae)) is a pest of *Pinus sylvestris* in the United States, Canada, Russia, England, North Africa and Europe, and it damages pine species and *Pinus silvestris* (Augustaitis, 2007). While the adults of this species do not damage the Scotch pine trees, its larvae eats the needle leaves of the trees during the six stages of larvae stage, beginning with the first larval stage, causing the trees to stop development and become 80–94 percent smaller in diameter and height with growth retardation. As a result, secondary pests and diseases spread more quickly in weakened trees, causing them to dry out completely (Augustaitis, 2007; Meshkova et al., 2019). *D. pini* can be found practically everywhere in Turkey where Scots pine is available, and in some regions, it can cause considerable economic damage. *D. pini* also caused harm to Scotch pine woods in Artvin province (Eastern Black Sea Region of Turkey) (Gokturk &Tozlu, 2019).

In the control of *D. pini*, chemical insecticides are frequently applied (Linstedt et al., 2006; Wojciechowska et al., 2016). The environmental damage caused by chemical applications, as well as the insecticide resistance of hazardous insects, necessitates the search for other control materials as alternative control methods (Khani & Asghari, 2012). Studies on silvicultural studies, the usefulness of pheromone traps (Anderbrant et al., 2005), and the use of entomopathogens have all been published studies on control methods (Dadasoglu et al., 2016; Simsek & Kondur, 2017).

Current research on the use of essential oils from medicinal and aromatic plants applied as insecticides is becoming increasingly popular. The utilization of secondary chemicals and essential oils in plants as pesticides has grown more significant as an alternative to synthetic pesticides (Isman, 2006). Many research have shown that certain herbal essential oils and extracts are excellent pesticides in the control of pests and diseases (Usanmaz Bozhuyuk et al., 2018; Kesdek et al., 2020). Essential oils, extracts, and chemicals obtained from plants have been found to have ovicidal, larvicidal, insecticidal, repellant, attractant, and antifeedant properties in various efficiency studies on harmful insects (Kordali et al., 2008).

Rhododendron L. family Ericaceae is a genus of woody, evergreen shrubs belonging to the Ericaceae family. With more than 850 species, it is distributed in temperate regions of North America, Europe, and Asia. There are five species distributed in northern Turkey, especially in the Eastern Black Sea coastal region; *R. caucasicum* Pallas, *R. luteum* Sweet, *R. ponticum* L., *R. smirnowii* Trautv, *R. ungernii* Trautv (Bahadir et al., 2018). Among these species, *R. luteum* and *R. ponticum* include andromedotoxin which is a poisonous substance in their leaves and blossoms (Baytop, 1999). Sheeps and goats that feed on the blooms and young shoots of these plants get poisoned (Puschner et al., 2001). Honey, which is made from pollen collected by bees from their flowers, is poisonous due to andromedotoxin and is called to as "mad honey" by the public (Sutlupinar et al., 1993). Tannin, essential oil, erycholine, arbutin, and andromedol are all found in the leaves of *R. luteum*. It is utilized by the locals as a diuretic, cold, edema, and analgesic in rheumatic pains due to its toxic properties (Baytop, 1999). *Rhododendron luteum* is a thickly branching shrub that can reach a height of 4 meters and is deciduous throughout the winter. The leaves are spirally organized in numerous rows on the stalks, ovate, wide inverted lanceolate, ciliated and finely serrated on the palm's edge, and hairy on both sides. The yellow flowers, which bloom before leafing, have a sharp smell and are 5 cm in diameter. Flower boards in the form of compound inflorescences are located at the tips of the shoots. It has spread over Central and Eastern Europe, Turkey, and the Caucasus region. It can be grown in parks and gardens due to its flowers and leaves that turn red in autumn (Eminagaoglu et al., 2015).

There is limited research on obtaining essential oil from the plant of *R. luteum*. Benzyl benzoate, benzyl salicylate, limonene, p-cymene, β-caryophyllene, methyl benzoate, (E)-β-ocimene, α-pinene, α-terpineol, cadinol, cadinene and α-muurolene are the chemical major components of the oil derived from *R. luteum* flowers, according to studies (Tasdemir et al., 2003; Alan et al., 2010; Usta et al., 2011). There hasn't been any research carried out on the effectiveness of essential oils on *D. pini* so yet. However, the antibacterial

activity of *R. luteum* essential oil on bacteria and fungi was determined in a study, and it was found to be effective against bacteria (Usta et al., 2012).

In this research, the chemical major components of essential oil obtained from *R. luteum* from natural regions, as well as its larvicidal activity against six larval stages of *D. pini* were investigated.

Materials And Methods

Test insects

In July-August (2020), 1st, 2nd, 3rd, 4th, 5th and 6th instar larvae of *D. pini* were collected from Scots pine forest areas of Borcka district (Artvin, Turkey). They were fed with fresh yellow pine leaves under laboratory conditions (25 ºC (± 2), 65% (± 5) RH, and 14/10 L/D) until the experiments.

Plant Material And Isolation Of Essential Oils

The *R. luteum* flowers used in the study were harvested in June 2019–2020 from Artvin Genya Mountain (Turkey). The collected flowers were dried in the shade at room temperature until they reached constant weight (11–13 percent humidity). For the Steam Distillation process, Neo-Clevenger (Wise Therm™) equipment was employed. Dry samples were ground first, were ground, weighed as 50 grams, transferred into 500 ml balloons, added onto 500 ml of water (1:10). and then placed in the Neo-Clevenger device. The distillation process of the essential oil was processed for 3 h. The essential oil obtained was stored in the refrigerator at 4°C in 10 mL glass tubes with Teflon caps until used in the experiment.

Chemical Composition

The Hewlett Packard system, HP-Agilent 5973 N GC-FID, and GC-MS (Gas Chromatography-Mass Spectrometer) 6890 GC system were used to determine chemical composition and quantity. The essential oils obtained from distillation were diluted with acetone at a ratio of 1:10 (v/v) and 0,1 µL of oil was injected into the HP-Innowax FSC column (30m*0,25mm*0,25µm film) with a glass syringe.

At flow rate of 1 mL/min, helium was used as the carrier gas in the system. In the study, the injection block temperature was 230ºC and the detector temperature was 250ºC. The temperature of the FID column was begun at 50^{o}C and gradually raised by 3^{o}C every minute until it reached 230^{o}C. The samples were kept at the starting temperature for 3 minutes and the ending temperature for 15 minutes.

Without correction factor, the proportional values of the components were calculated as a percentage according to the FID area. The components were separated using an electron ionization device with a 70 eV ionization energy. Essential Oil Components Library, Wiley GC/MS Library, Adams Library, MassFinder Library were applied to identify components of *R. luteum* essential oil and were also confirmed by

comparing their retention indices. In order to calculate relative retention indices (RRI), alkanes were employed as reference points.

Bioassays Using Essential Oils

In order to determine the larvicidal effect of the essential oil obtained from the *R. luteum* plant against *D. pini* larvae in the laboratory, the essential oil was dissolved with 1:2 (essential oil/ethanol) and stock solutions were prepared with final concentrations of 5, 10 and 20 µL/Petri and doses of 1,25 – 2,50 and 5 µL/Petri correspondings to these concentrations were applied. Two layers of sterilized blotting paper were placed under the glass Petri dishes (9 cm wide × 1,5 cm deep, based on 120 mL volume).

Twenty larvae were placed in each petri dish and 1 mL of the essential oil solutions prepared as the stock was sprayed on the larvae, and the ethanol was evaporated by keeping it under atmospheric conditions for 5 minutes. To feed the larvae, 10 g of fresh yellow pine leaves were added, and the Petri dishes were coated with parafilm. Before usage, pre-prepared essential oil solutions were mixed for 1 minute using a vortex device. Pure water + ethanol was used as negative control and commercial chemical NeemAzal® EC (Neem-Oil-Based Botanical Insecticide – 0,03% Azadirachtin) was used as the positive control. Experiments were conducted at 25 ± 2ºC temperature, 65 ± 5% relative humidity and 14:10 (L:D) h photoperiod in the laboratory conditions, and each experiment was carried out with 3 replications. At the 24th, 48th, 72nd, and 96th hours after the application, dead larvae were counted.

Analysis And Evaluation Of Data

The death rates in the *D.pini* larval stage of the essential oil derived from the *R.luteum* plant were calculated, and mortality tables for the larval stage were established at the end of the 24th, 48th, 72nd, and 96th hours. Analysis of variance (ANOVA) was used with the SPSS (Statistical Package for Social Sciences 17.0) software package to assess whether there is a statistically significant difference between the data obtained, and the Duncan test was used to evaluate the differences between the means. the Finney (1971) method was applied to calculate the LD_{50} and LD_{90} values and the EPA Probit Analysis Program was used to determine the LD_{50} and LD_{90} values at the 95% confidence limits of each application.

Results

Fresh flowers of *Rhododendron luteum* were analyzed by gas chromatography/mass spectrometry (GC-MS). A total of 47 compounds were identified, accounting for 98,45% of the essential oil extracted from *R. luteum* flowers. The primary constituents determined were β-caryophyllene (15,39%), benzyl benzoate (11,05%), δ-cadinene (7,06%), benzyl salicylate (6,08%), and α-pinene (4,14%) (Table 1).

As a result of the application of essential oil obtained from the *R. luteum* plant against *D. pini* larvae, it was observed that all essential oils had a significant toxic effect on *D. pini* larvae compared to the controls. However, it was shown that as the application dose and duration increase, the fatality rates of *D. pini* larvae applied with essential oil obtained from the *R. luteum* plant, increased dramatically. (Table 2). When compared to controls, it was shown that essential oil treatments at concentrations of 5, 10, and 20 µL/Petri resulted in mortality rates ranging from 20,1–100% in L_1-L_6 larvae of *D. pini* (Table 2).

Table 1
Chemical components percent of the oil obtained from flowers of *R. luteum*

Peak	Compound Name	RT (min)	RI	Content (%)	Identification Methods
1	2-Hexenal, (E)	3,50	854	0,37	GC/MS/RI
2	*α*-Pinene	5,04	939	4,14	GC/MS/RI
3	β-Pinene	6,92	980	1,15	GC/MS/RI
4	*trans*-2-(2-Pentenyl) Furan	7,12	1001	0,49	GC/MS/RI
5	Limonene	8,27	1031	2,37	GC/MS/RI
6	Benzene Acetaldehyde	9,09	1050	0,72	GC/MS/RI
7	Linalool	11,43	1098	2,05	GC/MS/RI
8	Borneol	13,38	1165	0.39	GC/MS/RI
9	*α*-terpineol	15,07	1188	3,18	GC/MS/RI
10	Myrtenol	16,52	1192	1,41	GC/MS/RI
11	Decanal	17,11	1201	0,22	GC/MS/RI
12	β-Cyclocitral	18,23	1222	0,17	GC/MS/RI
13	Isobornylacetate	20,52	1286	0,18	GC/MS/RI
14	β-Elemene	21,44	1391	0,43	GC/MS/RI
15	Methyleugenol	22,01	1401	0,55	GC/MS/RI
16	Longifolene	23,35	1403	0,26	GC/MS/RI
17	*α*-Gurjunene	24,49	1411	1,62	GC/MS/RI
18	β-Caryophyllene	25,31	1418	15,39	GC/MS/RI
19	(E)-Caryophyllene	26,28	1419	2,83	GC/MS/RI
20	Calarene	27,03	1432	0,43	GC/MS/RI
21	*α-cis*-Bergamotene	28,57	1440	2,11	GC/MS/RI
22	Aromadendrene	29,19	1441	0,36	GC/MS/RI
23	Humulene	30,04	1454	1,31	GC/MS/RI
24	γ-Gurjunene	31,35	1470	0,39	GC/MS/RI
25	γ-Muurolene	32,22	1477	0,46	GC/MS/RI
26	Germacrene D	33,11	1480	2,32	GC/MS/RI
27	β-Ionone	34,52	1488	1,15	GC/MS/RI

Peak	Compound Name	RT (min)	RI	Content (%)	Identification Methods
28	γ-Amorphene	35,06	1498	2,25	GC/MS/RI
29	*α*-Muurolene	34,11	1500	3,37	GC/MS/RI
30	Epizonarene	36,42	1501	1,24	GC/MS/RI
31	γ-Cadinene	37,24	1513	0,52	GC/MS/RI
32	δ-Amorphene	38,21	1514	3,06	GC/MS/RI
33	δ-Cadinene	39,32	1524	7,06	GC/MS/RI
34	*α*-Calacorene	42,57	1542	0,14	GC/MS/RI
35	Elemol	43,04	1549	2,14	GC/MS/RI
36	Germacrene B	43,51	1556	1,01	GC/MS/RI
37	1,6-Germacradien-5β-ol	47,01	1585	3,08	GC/MS/RI
38	Caryophyllene Oxide	48,23	1581	1,32	GC/MS/RI
39	Vıridiflorol	50,32	1594	2,04	GC/MS/RI
40	Isoaromadendrene epoxide	51,45	1612	1,09	GC/MS/RI
41	γ-Eudesmol	52,43	1630	2,21	GC/MS/RI
42	Benzyl Benzoate	53,55	1770	11,05	GC/MS/RI
43	α-Oxobisabolene	54,27	1755	1,17	GC/MS/RI
44	Hexahydro farnesylacetone	55,49	1842	1,04	GC/MS/RI
45	Benzyl Salicylate	56,04	1867	6,08	GC/MS/RI
46	Phenylethyl Salicylate	57,23	1987	1,06	GC/MS/RI
47	Thymol	58,45	2195	1,07	GC/MS/RI
	Total identified			**98,45**	

[a] GC: co-injection with standards, [b] MS: previously identified based on computer coordination of the mass spectra of peaks with Wiley 7N and TRLIB libraries and according to Adams (2007), [c] RI: Retention index, [d] RT: Retention time

Table 2
The effectiveness of *R. luteum* plant essential oil on the four instar larvae of *D. pini*

Essential Oil	Dose (µL/Petri)	Mortality (%)			
		Exposure Time (h)			
L_1 Instar Larvae					
R. luteum		24	48	72	96
	10	80,5 ± 4,42 c	83,1 ± 3,48 c	91,2 ± 1,28 b	99,2 ± 1,17 a
	15	85,2 ± 3,14 b	95,2 ± 4,32 b	98,7 ± 2,31 a	100 ± 0,0 a
	20	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a
Positive Control (*NeemAzal*)	10	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a
	15	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a
	20	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a
Control (Steril Water + Etanol)		0,0 ± 0,0 d	0,0 ± 0,0 d	0,0 ± 0,0 c	0,0 ± 0,0 b
L_2 Instar Larvae					
R. luteum	10	76,1 ± 2,14 d	81,7 ± 1,62 c	86,4 ± 4,17 c	90,2 ± 2,41 c
	15	81,7 ± 1,22 c	87,3 ± 2,21 b	90,8 ± 1,54 b	96,3 ± 3,22 b
	20	92,4 ± 2,62 b	97,8 ± 1,17 a	98,9 ± 2,07 a	100 ± 0,0 a
Positive Control (*NeemAzal*)	10	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a
	15	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a
	20	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a
Control (Steril Water + Etanol)		0,0 ± 0,0 e	0,0 ± 0,0 d	0,0 ± 0,0 d	0,0 ± 0,0 d
L_3 Instar Larvae					
R. luteum	10	65,3 ± 1,54 e	67,2 ± 4,38 f	72,5 ± 2,21 e	75,1 ± 1,74 e
	15	72,2 ± 3,42 d	72,8 ± 3,41 e	77,1 ± 3,14 d	79,9 ± 1,35 d
	20	75,6 ± 2,48 d	78,4 ± 3,49 d	83,1 ± 1,25 c	85,4 ± 1,07 c
Positive Control (*NeemAzal*)	10	80,1 ± 2,11 c	82,4 ± 2,14 c	87,5 ± 3,18 b	90,2 ± 2,33 b
	15	87,0 ± 3,38 b	90,7 ± 3,14 b	93,5 ± 1,11 a	98,7 ± 1,92 a
	20	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a	100 ± 0,0 a
Control (Steril Water + Etanol)		0,0 ± 0,0 f	0,0 ± 0,0 g	0,0 ± 0,0 f	0,0 ± 0,0 f

Essential Oil	Dose (µL/Petri)	Mortality (%)			
		Exposure Time (h)			
L_4 Instar Larvae					
R. luteum	10	56,1 ± 1,85 e	60,7 ± 3,32 e	63,2 ± 1,42 e	67,2 ± 2,17 e
	15	62,6 ± 3,42 d	65,2 ± 2,48 d	67,9 ± 4,04 d	72,4 ± 2,55 d
	20	65,3 ± 2,48 c	71,1 ± 1,97 c	75,8 ± 1,19 c	79,2 ± 2,11 c
Positive Control (*NeemAzal*)	10	69,4 ± 1,81 c	74,4 ± 3,41 b	76,6 ± 1,14 c	82,4 ± 2,35 bc
	15	71,0 ± 2,31 b	78,1 ± 1,73 a	81,2 ± 2,17 b	85,1 ± 3,14 b
	20	76,4 ± 2,18 a	80,9 ± 2,35 a	85,2 ± 1,08 a	100 ± 0,0 a
Control (Steril Water + Etanol)		0,0 ± 0,0 f	0,0 ± 0,0 f	0,0 ± 0,0 f	0,0 ± 0,0 f
L_5 Instar Larvae					
R. luteum	10	45,3 ± 3,55 d	47,2 ± 1,57 e	54,5 ± 2,27 e	62,2 ± 3,77 e
	15	47,9 ± 2,22 d	52,4 ± 1,85 d	57,1 ± 2,48 d	66,4 ± 1,75 d
	20	55,1 ± 3,19 c	57,2 ± 2,81 d	62,2 ± 2,44 c	70,5 ± 1,10 c
Positive Control (*NeemAzal*)	10	60,1 ± 1,42 b	65,5 ± 1,44 c	70,9 ± 2,33 b	78,8 ± 1,38 b
	15	66,8 ± 1,33 a	70,0 ± 2,57 b	74,8 ± 1,92 a	80,1 ± 3,23 b
	20	67,5 ± 2,87 a	73,8 ± 1,59 a	80,5 ± 2,15 a	94,2 ± 2,34 a
Control (Steril Water + Etanol)		0,0 ± 0,0 f	0,0 ± 0,0 e	0,0 ± 0,0 f	0,0 ± 0,0 f
L_6 Instar Larvae					
R. luteum	10	20,1 ± 1,58 e	24,7 ± 2,44 e	30,7 ± 3,11 d	35,8 ± 1,55 e
	15	26,2 ± 1,29 d	30,1 ± 1,42 d	33,3 ± 1,41 d	40,1 ± 2,17 d
	20	29,8 ± 2,11 c	36,2 ± 3,14 c	39,3 ± 2,14 c	42,8 ± 2,48 d
Positive Control (*NeemAzal*)	10	51,1 ± 1,42 b	54,1 ± 2,14 b	59,2 ± 1,31 b	56,2 ± 1,44 c
	15	57,9 ± 3,13 a	59,8 ± 2,57 a	62,5 ± 2,22 a	68,7 ± 2,24 b
	20	59,4 ± 1,71 a	61,5 ± 1,59 a	64,3 ± 2,15 a	87,2 ± 2,19 a
Control (Steril Water + Etanol)		0,0 ± 0,0 f	0,0 ± 0,0 f	0,0 ± 0,0 f	0,0 ± 0,0 e

[a] Values followed by various letters in the same column differ crucially at $P \leq 0,05$ according to Duncan Multiple test. [b] Mean ± SE of three.replicates, each.set up with 20 larvae

Different mortality rates on six *D. pini* larval stages (L_1, L_2, L_3, L_4, L_5, L_6) was observed. In larvae, higher mortality was observed at the highest dose (20 µL/Petri) of essential oil obtained from the *R. luteum* plant. It was discovered that increasing the application dosage and exposure periods of the tested essential oil obtained from the *R. luteum* plant, resulted in an increase in larval death rates.

When the mortality rates of *D. pini* larvae exposed to *R. luteum* essential oil at doses of 5, 10, and 20 µL/Petri and at the 24th, 48th, 72nd, and 96th hours were compared, it was determined that the mortality rates between the doses were different depending on time (Table 2). When the mortality rates on *D. pini* larvae caused by *R. luteum* essential oil were compared over time, the greatest mortality rates were reported as 100% in the L_1 stage larve of *D. pini* after 96 hours, and the lowest mortality rates were observed as 20.1% in the L_6 stage after 24 hours as the lowest mortality rate. The mortality rates of *D. pini* larvae are observed as 80,5-100%, 76,1-100%, 65,3–85,4%, 56,1–79,2%, 45,3–70,5% and 20,1–42,8% in L_1, L_2, L_3, L_4, L_5 and L_6 stages respectively at 24h, 48h, 72h, and 96h. In conclusion, based on these results, the larval mortality rates of *D. pini* larvae exposed to *R. luteum* essential oil in different larval stages of *D. pini* are observed as higher at 1st and 2nd larval stage, and less mortality rates at 3rd, 4th, 5th, and 6th larval stage (Table 2).

NeemAzal® EC is an insecticide that is applied as control to *D. pini* larvae and it is utilized for many agricultural and forest problem insects. In this study, all dosages of NeemAzal delivered at the L_1, L_2 periods resulted in 100% mortality at 24, 48, 72, and 96 hours. In this study, 100% mortality was observed after 24, 48, 72 and 96 hours at all doses of NeemAzal when administered to the L1, L2 of *D. pini* larval stages. When the larval period is considered in terms of hours and doses for effect of NeemAzal applications on *D. pini* larval stages, mortality rates were observed between 51,1-100%. In the negative controls, no fatalities were found (Table 2).

When the lethal dose (LD_{50} and LD_{90}) toxicities are examined for 96 hours after the application of *R. luteum* essential oil on *D. pini* larvae, differences are given (Table 3).

Table 3
LD_{50} and LD_{90} values of *R. luteum* essential oil on the four instars larvae of *D. Pini*

Treatment Essential oils	LD_{50} (Limits)[a]	LD_{90} (Limits)[b]	X^{2} [c]	Slope ± SE (Limits)[d]
L_1 Instar Larvae	0,72 (0,06 – 0,92)	3,28 (0,84 – 3,42)	6,27	9,24 ± 0,92 (0,53 – 7,62)
L_2 Instar Larvae	0,85 (0,82 – 2,18)	4,56 (1,71 – 2,14)	7,95	7,55 ± 0,87 (0,83 – 4,28)
L_3 Instar Larvae	2,35 (0,45 – 2,82)	6,41 (1,92 – 2,82)	15,54	5,29 ± 0,89 (0,83 – 2,14)
L_4 Instar Larvae	2,47 (0,36 – 2,27)	7,28 (2,15 – 5,16)	17,22	4,18 ± 0,87 (0,62 – 2,55)
L_5 Instar Larvae	3,22 (0,25 – 2,12)	7,84 (2,42 – 3,51)	17,22	3,47 ± 0,87 (0,83 – 2,98)
L_6 Instar Larvae	4,68 (0,24 – 1,29)	11,92 (2,69 – 14,17)	10,21	3,14 ± 0,65 (0,45 – 2,66)

[a] The lethal dose causing 50% mortality after 96 h, [b] The lethal dose causing 90% mortality after 96 h, [c] Chi-square value $P \leq 0,01$, [d] Slope of the concentration-mortality regression line ± standard error.

LD_{50} values were resulted as 0,72 – 0,85 – 2,35 – 2,47 – 3,22 and 4,68 µL/larva for L_1, L_2, L_3,L_4, L_5 and L_6 instar larvae, respectively; LD_{90} values were recorded as 3,28 – 4,56 – 6,41 – 7,28 – 7,84 and 11,92 µL/larva for L_1, L_2, L_3,L_4, L_5 and L_6; and highest toxicity according to LD_{50} and LD_{90} values for L_1 (0,72 µL/larva), lowest toxicity for L_6 (11,92 µL/larva) were observed. The LD_{50} and LD_{90} values for other larval stages are resulted as 0,85 and 4,56 µL / larva for L_2, as 2,35 and 6,41 µL / larva for L_3, as 2,47 and 7,28 µL/ larva for L_4, as 3,22 and 7,82 µL/ larva for L_5 (Table 3).

Discussion

Despite the chemical components being different in the few studies on *R. luteum* essential oil, the main components were generally found to be compatible with the results that we obtained, as β-caryophyllene, benzyl benzoate, δ-cadinene, benzyl salicylate, and α-pinene (Tasdemir et al., 2003; Alan et al., 2010; Usta et al., 2012). The number of main components obtained in our research was 47, and the main component ratios were detected as β-caryophyllene (15,39%), benzyl benzoate (11,05%), δ-cadinene (7,06%), benzyl salicylate (6,08%), and α-pinene (4,14%). Tasdemir et al. (2003) found 32 different chemical components in the essential oil obtained from the flowers of *R. luteum* and these components were limonene (14,6%), benzyl alcohol (16,3%), p-cymene (8,4%), α-fenchone (5,4). %), phenylethyl alcohol (4,3%) have determined that they are the most abundant.

Tasdemir et al. (2003) determined 32 different chemical components in the essential oil extracted from the flowers of *R. luteum* and these components were limonene (14,6%), benzyl alcohol (16,3%), p-cymene (8,4%), α-fenchone (5,4%), phenyl ethyl alcohol (4,3%) have determined that they are the most abundant.

In another study, it was stated that the 34 components obtained from the analysis were β-caryophyllene (34,0%), methyl benzoate (11,7%), (E)-β-ocimene (10,4%), and α-pinene (10,0%) (Alan et al. 2010). In another research, α-cadinol (8,9%), δ-cadinene (7,6%), α-terpineol (7,2%), benzyl salicylate (6,2%), α-muurolene (4,1%) and 1.6- germacradien-5 β-ol (3,4%) were found to be the primary components (Usta et al., 2012). Many factors might affect the results, including volatile matter environmental conditions, oil volume and composition, storage term, harvest time, plant age, development period, climate factor, geographical region, and the number of leaves (Yosr et al., 2012).

Although there are a limited number of studies concerning chemical compositions of *R. luteum* essential oil, there has been no research to investigate the effect of *R. luteum* essential oil on *D. pini* larvae. Since an essential oil of *R. luteum* was applied on *D. pini* larvae for the first time in this research, that's why the results obtained could not be compared with any research.

Conclusion

According to the results of the study, it has concluded that the death rate increased gradually as the application dose and time of essential oils increased. When the mortality rates were evaluated according to the application dosages, the highest dosage (20 µL/Petri) resulted in the greatest deaths (100%). The 6th stage larvae showed the least death rates. However, when the 10, 15, and 20 µL/Petri doses of the applied essential oil and the mortality rates at the 24th, 48th, 76th, and 96th hours were examined, it was resulted that the mortality rates between the doses were different from each other. In comparison to controls, maximum toxicity rates on *D. pini* L_1, L_2, L_3, L_4, L_5, and L_6 instar larvae were reported at higher concentrations and longer exposure durations. According to the results of the analysis, the lowest mortality rates were found in L_6 instar larvae, whereas the greatest mortality rates were found in L_1 instar larvae.

According to these results, the tested *R. luteum* oil has a high content and it is suggested that it can be utilized as an insecticidal control agent against L_1 and L_2 instar larvae of *D. pini*.

Furthermore, the effects of *R. luteum* essential oil should be evaluated in the field and the results should be compared to laboratory data. Finally, it is anticipated that this study would serve as a starting point for future research.

Declarations

Author contributions Participated in the design of the study, carried out field study, collected the data, and write the manuscript – Temel Gokturk

Analyzed the data, contributed to the interpretation of data and supported field study, write the manuscript – Medea Burjanadze

Read and approved the final manuscript – Temel Gokturk, Medea Burjanadze

Funding Not applicable

Declaration of Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statements All data generated or analysed during this study are included in this published article.

References

1. Adams, R.P. (2007). Identification of essential oil components by gas chromatography /quadrupole mass spectroscopy. Allured Publishing Corporation. Carol Stream, IL., USA
2. Alan, S., Kurkcuoglu, M., Goger, F. & Base, K.H.C. (2010). Morphological, chemical and indumentum characteristics of *Rhododendron luteum* Sweet (Ericaceae). Pakistan Journal of Botany *42*(6), 3729–3737
3. Augustaitis, A. (2007). Pine sawfly (*Diprion pini* L.) related changes in Scots pine crown defoliation and possibilities of recovery. Polish Journal of Environmental *16* (3), 363–369
4. Bahadır, A., Eminagaoglu, O. & Unal, A. (2018). Rhododendron L. (Rhododendrons). (Ed. Ü. Akkemik) Exotic trees and shrubs of Turkey. General Directorate of Forestry Publications, Ankara.
5. Baytop, T. (1999). Herbal therapy in Turkey. Nobel Medicine Bookstores, Istanbul
6. Dadasoglu, F., Tozlu, G., Kotan, R., Gokturk, T. & Karagoz, K. (2016). Biological control of Pine Sawfly (*Diprion pini* L.) and molecular characterization of effective Strains. Romanian Biotechnological Letters *21*(2), 11272–11280
7. Eminagaoglu, O., Akyıldırım, H. & Aksu, G. (2015). Artvin's Natural Plants. Promat,.Istanbul.
8. Finney, D.J. (1971). Probit Analysis. Cambridge University Press (3rd Edition). London, UK.
9. Gokturk, T. (2021). Harmful bark beetles of Artvin forests. Night Publishing, Research and Evaluations in Agriculture. Forestry and Aquaculture *1*(6), 121–152
10. Gokturk, T. & Tozlu, G. (2019). The effect of Pyrethrum and *Bacillus thuringiensis* biopesticides on *Diprion pini* L.and *Neodiprion sertifer* (Geoffr.) (Hymenoptera: Diprionidae) larvae. Pakistan J Zool *51*(3), 989–994
11. Isman, M.B. (2006). Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annual Review Entomology *51*, 45–66
12. Kesdek, M., Kordali, S., Usanmaz Bozhuyuk A. & Gudek, M. (2020). Larvicidal effect of *Achillea biebersteinii* Afan. (Asteraceae) essential oil against larvae of pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae). *Turkish Journal of Agriculture and Forestry 44*, 451–460
13. Khani, A. & Asghari, J. (2012). Insecticide activity of essential oils of *Mentha longifolia, Pulicaria gnaphalodes* and *Achiilea wilhelmsii* against two stored product pests, the flour beetle, *Tribolium*

castaneum and the cowpea weevil, *Callosobruchus maculatus*. Journal of Insect Science *12*(73), 1–10

14. Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M.& Mete, E. (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. Bioresource Technology *99*, 8788–8795
15. Lindstedt, C., Mappes, J., Päivinen, J. & Varama, M. (2006). Effects of group size and pine defense chemicals on *Diprionid* sawfly survival against ant predation. Oecologia *150*, 519–526
16. Meshkova, V., Nazarenko, S. & Kolienkina, M. (2019). *Diprion pini* L. (Hymenoptera, Symphyta, Diprionidae) population dynamics in the Low Dnieper region. *Folia Forestalia Polonica Series A – Forestry 61*(1), 22–29
17. Puschner, B., Holstege, D.M. & Lamberski, N. (2002). Grayanotoxin poisoning in three goats. Journal of the American Veterinary Medical Association *218*(4), 573–578
18. Simsek, Z. & Kondur, Y. (2017). Determination of Flight Period of *Diprion pini* L. (Hymenoptera: Diprionidae) in Çankırı Scots Pine Forests via Pheromone Traps. Kastamonu University Journal of Forestry Faculty *17*(1), 194–208
19. Sutlupınar, N. & Mat, A. (1993). Poisoning by toxic honey in Turkey. *Archives of Toxicology 67*, 148 – 50
20. Tasdemir, D., Demirci, B., Demirci, F., Donmez, A., Baser, K.H.C. & Ruedi, P. (2003). Analysis of the volatile components of five Turkish *Rhododendron* species by headspace solid-phase microextraction and GC-MS (HS-SPME-GC-MS). *Z. Naturforsch. 58c*, 797–803
21. Usanmaz Bozhoyuk, A. & Kordali, S. (2019). Investigation of the toxicity of ethanol extracts obtained from six different *Satureja* L. species on Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say, 1824), (Coleoptera: Chrysomelidae). Anatolian Journal of Botany *3*, 69–79
22. Usta, A., Yayli, B., Kahrinman, N., Karaoglu, S.A. & Yayli, N. (2012). Composition and antimicrobial activity of essential oil from the flower of *Rhododendron luteum* Sweet. Asian Journal of Chemistry *24*(5), 1927–1930
23. Yosr, Z., Hnia, C., Rim, T. & Mohamed, B. (2013). Changes in essential oil composition and phenolic fraction in *Rosmarinus officinalis* L. var. *typicus* Batt. organs during growth and incidence on the antioxidant activity. Industrial Crops and Products *43*(1),412–419