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Research Paper

Investigation of the potential use of Euphorbia latex as an insecticide in biological control against *Dendroctonus micans*

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Abstract

The latex of the *Euphorbia* plant was used in this study to find an effective and natural new insecticide in combating *Dentroctonus micans* (Kug.), Which causes great damage to *Picea orientalis* forests. For this purpose; Euphorbia plant was collected from Erzurum and species identification was made. According to the species identification, the spurge plant was determined to be *Euphorbia virgata,* and then its latex was obtained. In this study; Different concentrations of latex (0, 25, 50 and 75%) were applied to the experimental groups prepared to have fifty *D. micans* larvae in each group, and the experiment was repeated twice. As a result of the application, the LD50 (25%, 50% and 75%) values, respectively; 0.01µl/ml, 0.196 µl/ml, 0.294 µl/ml. LD90 values were found as 0.18 µl/ml, 0.354 µl/ml, and 0.531 µl/ml. Although *E. virgata* latex has a toxic effect on the larvae at any concentration, the mortality rate increased as the concentration amount increased. GC-MS analysis was performed to determine the latex content of *E. virgata*. According to GC-MS analysis, in latex content; there are terpenes, flavonoids, alkaloids, amines, fatty acids, steroids and various alcohols. These bioactive substances in *E. virgata* latex showed toxic effects on larvae. As a result of the study, it was found that *E. virgata* latex can be used as a natural and effective insecticide in the fight against *D. micans*..

Keywords: Picea orientalis; Insecticidal; Latex; *Dentroctonus micans*; *Euphorbia virgata.*

1. Introduction

It is a valuable tree species in terms of both timber industry and landscape value. *Picea orientalis* (L.)LİNK. creates all of the forests of Black Sea Regions. The most effective pest of *Picea orientalis* of *Dentroctonus micans* (Kug.). This pest has been caused hazardous regions. This pest has caused great damage in the whole Black Sea and Europhian region [1] It is a well-adopted pest in spreading areas.

Bark beetle female spruce enters through the shell, opens a tunnel in the phloem, lays its eggs hereafter mating, and the hatched larvae continue the colonize. This reproductive cycle continues with the release of pheromones, and wire-thin a few years the tree is completely dried [2]. *Dendroctonus micans* had a mechanical and chemical fight, but its spread and harm could not be prevented [3]. Today, biological control studies have started. The insecticide is used to combat many pests. Excessive use of insecticides increases the resistance of insects. Also harms people and the environment [4]. More environmentally and effectively insecticides are sought recently [5].

It is known that plant secondary metabolites play an important role in the control of pests. Biologically secondary metabolites are easily broken down and are not toxic to humans. 250,000 plant species worldwide have been reported to contain insecticidal compounds [6]. These compounds can be obtained from the plant, flower, root, stem, leaf, shell, fruit and whole plant [7]. One of the sources containing the chemical composition of plants is plant latex. Latex, which is a sticky and endogenous liquid, is secreted immediately after the injury [8;9].

It is known that latex, which has pharmacological and insecticidal properties, contains a high rate and various secondary metabolites, produced by more than 20 thousand plant species [10]. The largest group of plants producing latex is the Euphorbiaceae family. The Euphorbiacea family is one of the largest families of angiosperms. consists of 5 subfamilies, 300 genera, and 7800 species. Latex feature of the genus Euphorbia; It

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contains biologically active terpenoids and irritation power is high. Among the chemical components of Euphorbia latex; triterpenoids, phenolic compounds, alkaloids, glycosides, and cyanines found [5; 11; 12; 13].

The purpose of this study; to determine the insecticide effect of spurge plant latex collected from Erzurum region in the fight against *D. micans*, which damages *P. orientalis* forests..

2. Material and Methods

2.1. Collection of plant material

The spurge plant was collected through Börekli, D950, Palandöken / Erzurum road with 39 ° 50'42.3 "N 41 ° 10'26.6" E position. According to Ata Herbarium in Atatürk University Department of Biology, collected plants are *Euphorbia virgata* WALDST. ET KIT.

2.2. The Hand of spurge latex

The stem of the collected Euphorbia plants was cut and transferred to the latex sterile glass bottles.

2.3. Preparation of latex extract

The mixture, which was put in sterile bottles with 10 ml of latex and 10 ml of pure water, was shaken in a shaking incubator for 24 hours at 25 ° C and then lyophilized [7; 14].

According to Singh (2012) protocol, 4 latex dilutions (0%, 25%, 50%, 75%) were prepared by adding pure water.

The test setup is as follows.

- 0 µl. (Control) latex is not added, there is 200 µl of distilled water.
- 500 µl latex and 1500 µl distilled water; used as 25%
- 1000 µl latex and 1000 µl distilled water; used as 50%
- 1500 µl ml latex 500 µl distilled water; and used as 75%.

2.4. Toxicity test

D. micans larvae, which are spruce pests, were designed as 50 larvae in each group. Experiment groups; control group (0%) and 25% latex group, 50% latex group and 75% latex group. Larvae were feed by bark and dust of *P. orientalis*. The latexes were smeared on the spruce pieces placed on the larvae and placed in a place that does not receive direct sun at room temperature and the number of dead was counted daily [15]. Our experiment was made in 2 repetitions.

2.5. Statistical analysis

All experiments were performed 2 times and the average of values was presented. The data were analyzed by analysis of variance, and means were compared by using Duncan's Multiple Range Test at $p < 0.05$ significance level.

3. Results

3.1. Latex effect on larvae mortality

In our study, the toxic effect of *E. virgata* latex on *D. micans* larvae was determined. As a result of the application; latex showed toxic effect after 48 hours, LD50 and LD90 values as shown in Table 1.

Table 1. Latex toxicity on D.micans larvae (LD50 and LD90) after 48 hours

Exposure Time	Number of larvae	Concentration	LD50	LD90
	100	Control		
48 hours	100	%25 latex	0,01 µl/ml*	0,18 µl/ml*
	100	%50 latex	0.196 µl/ml*	0.354 µl/ml*
	100	%75 latex	0,294 µl/ml*	0,531 µl/ml*

* indicates significant differences between latex groups studied at different concentrations and control (p<0.05).

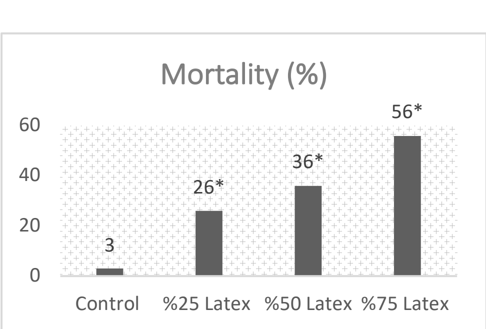


Figure 1. Concentration-related mortality rate
* indicates significant differences between latex groups studied at different concentrations and control (p<0.05).

When *D.micans* larvae were exposed to *E.vigrata* latex for 48 hours; ıt was observed that the higher the amount of latex concentration, the higher the mortality rate.

This situation can be explained by the increase in the numbers of the substance of the property pesticides in the latex content within its concentration. Mortality at each concentration is shown in Figure 1.

The biological activity of latex was calculated using probit analysis. According to the results of this analysis, it was determined that the biological activity of latex was present at every concentration used in the study, and the results were shown in Figure 2.

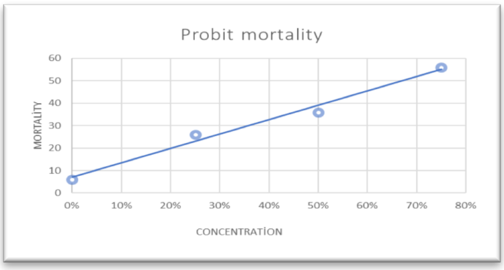


Figure 2. Probit analysis result

After determining the lethal effect of *E. vigrata* latex on *D. micans* larvae, its content was determined by GC-MS (Figure 3). According to the analysis results; terpenes, phenolic substances, amides, alkaloids, acids, aromatic compounds and alcohols were detected in latex. With the increase in latex concentration, the amount of this substance increased and had a lethal effect on *D. micans* larvae. Also these substances have antimicrobial, genotoxic and carcinogenic properties (Tables 2 and 3).

3.2. GC-MS analysis results of latex content

GC-MS results were shown in Figure 3. The substances found in the GC-MS analysis, their percentages and the chemical group in which they are found were shown in Table 2. In Table 3, the properties of chemical substances determined by GC-MS and their usage in various fields are given.

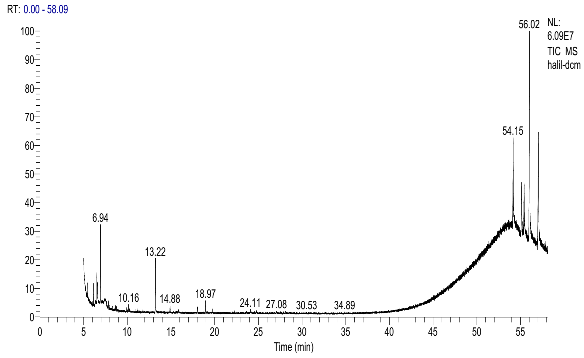
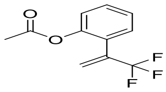
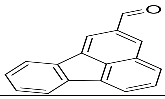
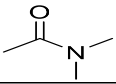
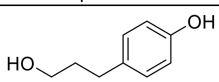
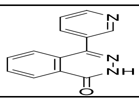
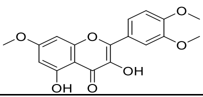
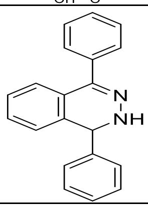
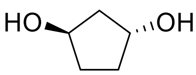
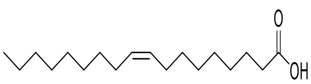


Figure 3. GC-MS analysis results of latex content.

Table 2. According to GC-MS analysis, substances of found presence, percentages, and chemical groups.

Arrival Time (min)	Formula of Matter	Name	%	CAS No	Type
6,15		2-(3,3,3-trifluoroprop-1-en-2-yl)phenyl acetate	1,34	136476-27-2	Phenolic
6,15		fluoranthene-2-carboxaldehyde	1,34	98677-82-8	Aromatic
6,53		N,N-Dimethylacetamide	4,81	127-19-5	Amid
6,94		4-(3-hydroxypropyl)phenol	5,10		Phenolic
6,94		4-(3-Pyridyl)-1(2H)-phthalazinone	5,10		Alkaloids
7,44		Quercetin 7,3',4'-trimethyl ether	0,50	6068-80-0	Phenolic
7,55		1,2-Dihydro-1,4-diphenylphthalazine	0,97	106200-50-4	Alkaloids
7,87		Trans-1,3-Cyclopentanediol	0,61	16326-98-0	Alcohol
7,87		(Z)-9-Octadecenoic acid	0,61	112-80-1	Acid

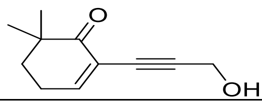
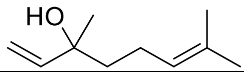
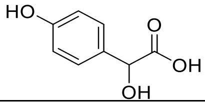
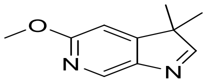
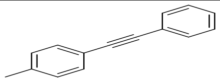
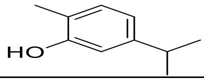
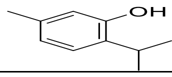
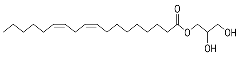
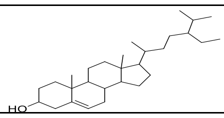
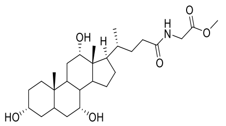
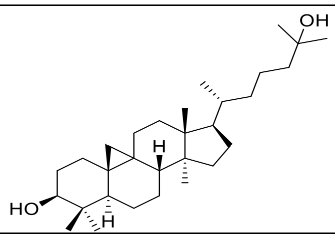
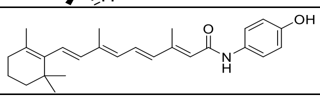
10,16		2-(3-hydroxyprop-1-yn-1-yl)-6,6-dimethylcyclohex-2-en-1-one	0,56		Terpene
13,22		Linalool	4,18	78-70-6	Terpene
14,89		4-Hydroxymandelic acid	0,64		Phenolic
18,03		3,3-Dimethyl-5-methoxy-6-azaindole	0,73		Alkaloids
18,03		1-(p-Tolyl)-2-phenylacetylene	0,73		Aromatic
18,97		Carvacrol	1,12	499-75-2	Terpene phenolic
18.97		Thymol	1,12	89-83-8	Phenolic terpen
54,15		1-Monolinolein	11,53	54284-45-6	Fatty acid
54,15		B-Sitosterol	11,53	2625-46-9	Steroid
55,41		N-(24-Oxo-3α,7α,12α-trihydroxy-5β-cholan-24-yl)-glycine methyl ester	8,46	57326-16-6	Steroid
56,02		3β-cycloartane-3,25-diol	30,67	26525-84-8	Steroid
57,03		N-(4-Hydroxyphenyl) retinamide	18,60	65646-68-6	Terpene

Table 3. Biotechnological importance of latex content

Latex content	Properties	Reference
2-(3,3,3-trifluoroprop-1-en-2-yl)phenyl acetate	They are phenolic compounds with antiviral, antibacterial, anti-inflammatory, antiangiogenic and antimitotic properties and are included in the bezofuran group..	[20]
Fluoranthene-2-carboxaldehyde	Fluonthen is a member of the non-external polycyclic aromatic hydrocarbon (PAH) class, containing a five-membered ring condensed with naphthalene and benzene rings. It is genotoxic and carcinogenic.	[21]
N,N-Dimethylacetamide	May be toxic with skin absorption. May irritate eyes and skin.	[30]

4-(3-hydroxypropyl)phenol	It is used in medicine as antidiabetic, anti-inflammatory and analgesic substance.	[30]
4-(3-Pyridyl)-1(2H)-phthalazinone	It is an antimicrobial, antitumor, antidiabetic and antiallergic phthalazinoin used in the pharmaceutical industry..	[22]
Quercetin 7,3',4'-trimethyl ether	Quercitin, a plant metabolite, is a flavonoid with antimicrobial effect.	[23]
1,2-Dihydro-1,4-diphenylphthalazine	It is an alkaloid used in medicine.	[24]
Trans-1,3-Cyclopentanediol	It contains a cyclopentane ring and has an antiviral effect. It is also used in the treatment of cardiovascular diseases.	[30]
(Z)-9-Octadecenoic acid	It is the most common and abundant unsaturated fatty acid in nature. It is used commercially in the preparation of lotions and as a pharmaceutical solvent. It also has biofuel properties..	[25]
Linalool	Linalool used in the pharmaceutical industry; is a monoterpenic compound used in the treatment of chronic and various diseases.	[26]
12. 4-Hydroxymandelic acid	It is widely used in the production of aromatic drugs and flavors. It is also widely used in the food industry and cosmetics industry.	[30]
Carvacrol	It has analgesic, anti-inflammatory, anticancer, antibacterial, antifungal, accelerating wound healing and cell proliferation.	[30]
Thymol	It is a phenolic terpene used in traditional medicine as a sweetener and preservative in the food industry and in mosquito medicines.	[30]
B-Sitosterol	B sterol, which is a cholesterol-like physterol, is anti-Alzheimer's.	[30]
N-(24-Oxo-3α,7α,12α-trihydroxy-5β-cholan-24-yl)-glycine methyl ester(Cholic acid)	It is used in the treatment of Zellweger syndrome.	[27]
Cycloartane(3β-cycloartane-3,25-diol)	It is a steroid group compound that has a cytotoxic effect..	[28]
N-(4-Hydroxyphenyl)retinamide(Fenretinid,)	Fenretinide is an orally active synthetic phenylretinamide analog of retinol (vitamin A) with potential antineoplastic and chemopreventive activities. It also inhibits the growth of tumor cells.	[29]

4. Discussion and Conclusion

Latex of *Euphorbia* sp. genus belongs to plants contains quite rich compounds. Many of these are triterpene and diterpene. It also contains phenolic substances, alkaloids, amines, fatty acids, alcohols and aromatic compounds. So, they have been used antimicrobial, antiviral and anticancer effects. Also; In the literature, it has been stated that *Euphorbia* latex has a toxic effect on many animals [5;7;9;11; 16].

The toxicity of *E. roylated* latex, which has a toxic effect on mice and fish, increases with increasing exposure time and concentration [7]. The same effect has been found to apply to *Anopheles mosquitoes* [17]. In many studies, the insecticidal effect of *Euphorbia* sp. latex has been studied. The LD50 of *E. antiquorum* latex for *Aedes aegypti* larvae is 10.7 ml / dl [18].

It has been stated that the toxic effect of *E. bupleuorides* latex exists on german cockroaches (*Blatella germenica*), which are resistant to many insecticides, and the mortality rate increases with increasing concentration [5]. The larvacidal effect of use *E. splendens* latex in *Megaselia scalaris* larvae has been studied and has been reported to stop embryonic development [9;19]. The insecticide effect of *E. fischeriana* latex against *Helicoverpa armigeral*, which damages plants such as tomatoes and peppers, was investigated. As results show that, our study very correspondence with other studies. We can say that *E. virgata* latex as a natural and effective insecticide and latex can be used due to both eats contains and bioactive ingredients. In our study, the insecticidal effect of *E. virgata* latex collected from Erzurum against *D. micans*, which damages the *P. orientalis* plant and even causes the destruction of spruce forests, was investigated. for this purpose, *E. vigrata* latex was applied to *D. micans* larvae in different concentrations. It was found that each concentration had a toxic effect on the larvae, but mortality values increased with increasing concentration. This wise overlaps with literature information (Table1). The toxic effect *of E. virgata* latex on *D. micans* was observed after 48 hours. The mortality rate increased as the exposure time and dose increased (Figures 1 and 2). According to the GC-MS analysis; *E. virgata* latex content was found to contain terpenes, flavonoids, alkaloids, steroids and various alcohols (Table2). GC-MS analysis results were compatible with previous studies [5; 9;11].

Bioactive substances in *E. virgata* latex content have had a toxic effect on *D. micans* larvae, *E. virgata l*atex can be used as a natural insecticidal for *D. micans* pest and spruce forests can be protected from this pest1

Conflict of Interest

The authors declare no conflict of interest.

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