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INSECTICIDAL EFFECTS OF ESSENTIAL PLANT OILS AGAINST LARVAE OF GREAT SPRUCE BARK BEETLE, *DENDROCTONUS MICANS* (KUGELANN) (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE)

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ABSTRACT

Essential oil obtained from the plant species *Artemisia spicigera* C. Koch and *Helichrysum plicatum* L. (Asteraceae), *Mentha longifolia* L., *Origanum acutidens* L., *Rosmarinus officinalis* and *Salvia multicaulis* Vahl (Lamiaceae) were tested for their toxicities against the larvae of *Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae: Scolytinae) in laboratory conditions. The results show that essential oils of *A. spicigera*, *H. plicatum*, *M. longifolia*, *O. acutidens*, *R. officinalis* and *S. multicaulis* have an insecticidal effect against the larvae of *D. micans*, in comparison with controls. The mortality of the larvae of *D. micans* increases significantly after treatment with the essential oils ($P < 0.01$). The amounts of essential oils applied were 5 and 10 μL in each, corresponding to 38.46, and 76.92 $\mu\text{L/L}$ air. The mortality rates of 1-2 larval stages of *D. micans* after 72 h of treatment with 5-10 μL doses of essential oils of *R. officinalis* (73.3%), *O. acutidens* (73.2%) and *M. longifolia* (70.8%) clearly demonstrate an insecticidal effect. *M. longifolia* (26.6%), *A. spicigera* (25.5%) and *H. plicatum* (24.4%) have the highest mortality rates for 3-4 larval stages.

KEYWORDS:

Dendroctonus micans, essential oils, insecticidal effect.

1. INTRODUCTION

One of the major causes of crop losses is herbivory by larvae and adults of some insect species [1]. Scolytinae subfamily is one of the most dangerous insect groups for forests. Bark beetles are a rich species group representing approximately 220 genera with 6,000 species in the subfamily Scolytinae [2].

They are small (1-9 mm) and usually cylindrical. The subfamily is very diverse, with >6000 species known worldwide, having a wide variety of life histories. Most species of bark beetles cause little or no economic damage, as they normally infest branches, stumps, and stems of standing dead or severely weakened trees, or downed woody material. A few species will attack and kill living, apparently healthy trees. For instance, members of the bark beetle genus *Dendroctonus* can cause severe outbreaks, resulting in high levels of tree mortality [3]. One of these insects, the great spruce bark beetle, *Dendroctonus micans* Kugelann (Coleoptera: Curculionidae, Scolytinae), has been causing significant damage to spruce stands in Europe and Asia. *D. micans* was first discovered in oriental spruce forests in the Republic of Georgia in 1957, and infested over 100,000 ha of oriental spruce stands in 1963 [4-6]. *D. micans* is established in Austria, Belgium, Czechoslovakia, Denmark, Finland, France, Germany, Netherlands, Sweden, Rumania, Turkey, Great Britain, the former USSR and former Yugoslavia [7]. The usual hosts are *Picea* spp., mainly *P. abies*, *P. sitchensis* and *P. orientalis*, but also occasionally *P. breweriana*, *P. engelmannii*, *P. glauca*, *P. jezoensis*, *P. mariana*, *P. obovata*, *P. omorika* and *P. pungens* [8]. This beetle has adapted to many climatic and ecological conditions [10]. Several countries have this significant pest and spent considerable amounts of money and effort trying to control it [9, 10].

D. micans was firstly discovered in Turkey in 1966 [11] and infested 36% of the spruce trees in the native distribution range of the spruce in the eastern Black Sea region [12]. Millions of trees were dead as a consequence of infestations in the oriental spruce forests [13]. Oriental spruce, *Picea orientalis* (L.) Link. distributes throughout the eastern Black Sea mountains in Turkey and Caucasus mountains in Georgia.

Its total area is 286,851 ha in Turkey [14]. According to Akinci *et al.* [15], the areas damaged dominantly by *D. micans* in native distribution of oriental spruce forests were in north-eastern Turkey. A total of 22.8 million m^3 stand-

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ing trees were damaged. In 11.43 million m³ standing trees, infestations were observed and 6.96 million m³ of trees were cut which occupied 120,000 ha of *D. micans* infestation areas. In pure spruce stands, 26.5% of the trees were infested during 2001 – 2007.

The common methods for controlling these types of insects are chemical, biological and physical control (or a combination of these methods). Natural products are well-known to have a range of useful biological properties against insect pests. The search for natural compounds having pest-suppressing potential continues in an attempt to find alternatives, which pose fewer risks to humans and the environment than existing conventional pesticides [16].

Turkey has the flora that is rich in indigenous aromatic and medicinal plant species. *Rosmarinus officinalis* L., *Artemisia spicigera* C. Koch and *Helichrysum plicatum* L. (Asteraceae), *Mentha longifolia* L., *Origanum acutidens* L. and *Salvia multicaulis* Vahl (Lamiaceae) are widely grown in Turkey since antiquity, and are known for their medicinal and aromatic properties.

The genus *Helichrysum*, belonging to the family Asteraceae is represented by approximately 300 species in the world. This genus is represented in Turkish flora by 18 taxa, of which 9 are endemic. The plants of this genus are notable for their involucre, made up of a large number of bracts located in several rows, which are yellow and shiny [17].

Rosmarinus officinalis L. is an evergreen shrub of the Lamiaceae family, native to most Mediterranean countries, which grows to a height of 1.5 m. Its leaves are 2.5 cm and it has small, light blue flowers. Leaves and flowers yield highly aromatic essential oils. The genus *Rosmarinus* contains several species with a range of varieties and forms [18]. *Rosmarinus* oil is usually isolated through steam distillation of fresh or slightly wilted leaves and flowers. The most abundant constituents documented for this oil are α -pinene, 1,8-cineole, verbenone and camphor [19].

The genus *Artemisia*, small herbs and shrubs, is one of the largest and most widely distributed genera of the Compositae family. Members of this genus have a characteristic scent or taste, have botanical and pharmaceutical interest, and are used in the liqueur-making industry. Among them, *A. spicigera* are found growing naturally in large areas of south-eastern Anatolia region of Turkey [20]. The essential oil of *A. spicigera* contains camphor (34.9%), 1,8-cineole (9.5%), borneol (5.1%), spathulenol (3.7%), piperitone (2.6%), caryophyllene oxide (1.8%) and α -terpineol (1.6%) [21].

The genus *Origanum* L. (Tribe Mentheae, Family Labiales) is represented in Turkey by 22 species or 32 taxa, 21 being endemic to Turkey. The rate of endemism among the Turkish *Origanum* species is 63%. Out of 52 known taxa of *Origanum*, 32 are distributed in Anatolia, meaning 60% of all *Origanum* taxa are recorded to grow in Turkey. This high rate is suggestive that the gene centre of *Origanum* is Turkey [22]. Essential oils of *Origanum acutidens*

tidens are mainly composed of carvacrol (87.0%), p-cymene (2.0%), linalool acetate (1.7%), borneol (1.6%) and β -caryophyllene (1.3%) [23].

The genus *Mentha* L. is well-known due to high aromatic species having economic importance. The *Mentha* species constitute one of the most popular essential oil crops. The essential oil of *Mentha longifolia* contains menthol (32.5%) menthone (20.7%), pulegone (17.8%) 1,8-cineole (5.6%), terpineol-4 (4.8%) and piperitone (2.2%) [24].

The genus *Salvia* belongs to the Lamiaceae family (subfamily Nepetoideae), which is one of the most widely distributed around the world and comprises herbs and shrubs growing in the temperate and warmer zones of the world. The main compounds of *Salvia multicaulis* were 1,8-cineole (17.0%), camphor (13.2%), α -pinene (9.3%), valeranone (8.5%) and α -eudesmol (5.7%) [25].

Essential oils obtained from plants are under particular investigation for their broad spectrum of pest control properties. They have been shown to possess insecticidal activity as vapors [26-29].

In Turkey, aromatic plants are widely distributed and there is a very rich and diversified flora, famous for its nutritional and medicinal characteristics. They are used for the production of cosmetics, perfumes, detergents, as well as in pharmacology and food flavoring. In addition to these rapidly evolving traditional sectors, a new industrial application may develop in the field of plant protection.

2. MATERIALS AND METHODS

2.1. Plant material and isolation of essential oil

The aerial parts of *Rosmarinus officinalis* L. were purchased at vegetative stage from a local market. *Artemisia spicigera* C. Koch and *Helichrysum plicatum* L. (Asteraceae), *Mentha longifolia* L., *Origanum acutidens* L. and *Salvia multicaulis* Vahl (Lamiaceae) were collected at the flowering stage in August 2009-2010 in Turkey. Collected plant materials were dried in the shadow and ground in a grinder. The dried plant samples (500 g) were subjected to hydrodistillation (plant material in boiling water) using a Clevenger-type apparatus for 4 hours. Hydrodistillation of *A. spicigera*, *H. plicatum*, *M. longifolia*, *O. acutidens*, *R. officinalis* and *S. multicaulis* yielded 0.25, 0.82, 1.38, 0.60 1.46 and 0.80 % (w/w) of essential oils, respectively. The yields were based on dry materials of plant samples.

2.2. Insects

The larvae of *D. micans* were collected from spruce forests in Artvin-Hatila National Park (41° 51' N, 41° 06' E) at the Black Sea region of Turkey. Within the study area, there are mixed stands made up of spruce, hornbeam, fir, beach and oak, but at altitudes of 1700-2100 m forest formation types are almost pure oriental spruce trees. Bioassays were carried out under the same conditions and in the same laboratory.

2.3. Bioassays

In order to test the toxicity of the oil against to larvae, 25 larvae of the insects with 25 g of sawdust (wood particles) were placed in Petri dishes (9 cm). Aliquots (5 and 10 µl) of the oils were applied with a variable pipette on Whatman No. 1 filter papers (2 cm × 2 cm) attached to the upside of the Petri dish, corresponding to 38.46 and 76.92 µl/L air concentrations. After exposure, mortality of the larvae was determined at 24, 48 and 72 h. Petri dish applied with only sterile water was used as control group. Three replicates were used for each dose and exposure time combination, and insecticidal activity of the oil was expressed as % mean mortality of the larvae (1-2; 3-4).

2.4. Data analysis

In order to calculate significant differences in toxicity between doses and exposure times, two-way analysis of variance (ANOVA) using the SPSS 10.0 software package at $P < 0.01$ and $P < 0.05$ levels was carried out.

3. RESULTS AND DISCUSSION

These plant essential oils possessed very strong insecticidal activity. *A. spicigera*, *H. plicatum*, *M. longifolia*, *O. acutidens*, *R. officinalis* and *S. multicaulis* have potential for development as new effective insect control agents

against *D. micans* larvae, one of the most important pests in Turkey.

The toxicities of 5 and 10 µl/Petri dish doses of the oils on spruce forest *D. micans* were determined (Tables 1 and 2). The mortality increased with increase in exposure time. The oils caused 71.6–96.6% mortality of *D. micans* larvae 1-2 and 8.6–40.8% mortality of *D. micans* larvae 3-4, respectively, at 10 µl/Petri dish after 72-h exposure. *D. micans* 1-2 larval stage, at the end of 24 h at doses of 5-10 µl of the most effective essential oil of *R. officinalis* evidenced 51.0% mortality, and 80.0-96.6% within 48 and 72 h with the volatile oil of *O. acutidens*.

Overall, the essential oil extracted from *R. officinalis* was found to be the most effective with a rate of 73.3%, followed by the oils extracted from *O. acutidens* and *M. longifolia* with 73.2 and 70.8%, respectively (Table 1; Fig. 1). In addition, the essential oil of *A. spicigera* was the most effective one with 19.1% at the end of 24 h after doses of 5-10 ml were applied on *D. micans*' third and fourth period larvae. However, it was determined that the essential oil of *M. longifolia* was the most effective at the end of both 48 and 72 h, with rates of 32.5 and 40.8%, respectively. At the end of 72 hours, usually against third- and fourth-period larvae, the most effective essential oils were those of *M. longifolia* (26.6%), *H. plicatum* (25.5%) and *A. spicigera* (24.4%), respectively (Table 2).

TABLE 1- The results of multiple comparison with mean (M) and std. error (SE) of exposure time and dose of essential oil of six plant species on *D. micans* (1 and 2 larval stages).

Time (h)	Dose (µl)	<i>H. plicatum</i>	<i>M. longifolia</i>	<i>A. spicigera</i>	<i>O. acutidens</i>	<i>S. multicaulis</i>	<i>R. officinalis</i>	Control	Grand M ± SE
		M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	
24	5	40.0±	50.0±	45.0±	39.3	8.6	38.0	4.0	32.1±
	10	38.3±	45.0±	38.3±	46.6	16.6	64.0	4.0	36.1±
	Mean ± SE	39.1±2.3c	47.5±3.4b	41.6±1.7c	43.0±1.5c	12.6±1.4c	51.0±1.4c	4.0±0.8c	34.1±0.5c
48	5	65.0±	73.3±	61.6±	82.6	68.6	71.3	16.6	62.7±
	10	53.3±	81.6±	61.6±	77.3	68.6	82.0	16.6	63.0±
	Mean ± SE	59.1±2.3b	77.5±3.4a	61.6±1.7b	80.0±1.5b	68.6±1.4b	76.6±1.4b	16.6±0.8b	62.9±0.5b
72	5	76.6±	85.0±	68.3±	94.6	89.3	87.3	20.6	74.5±
	10	66.6±	90.0±	76.6±	98.6	90.6	97.3	20.6	77.2±
	Mean ± SE	71.6±2.3a	87.5±3.4a	72.5±1.7a	96.6±1.5a	90.0±1.4a	92.3±1.4a	20.6±0.8a	75.9±0.5a
Grand M±SE		56.6±1.2b	70.8±1.2a	58.6±1.2b	73.2±1.2a	57.1±1.2b	73.3±1.2a	13.7±1.2c	57.6±0.4

Values followed by different lower-case letters in the same column differ significantly at $P < 0.01$ (**).

TABLE 2 - The results of multiple comparison with means and std. errors of exposure time and dose of essential oil of six plant species on *D. micans* (3 and 4 larval stages)

Time(h)	Dose (µl)	<i>H. plicatum</i>	<i>M. longifolia</i>	<i>A. spicigera</i>	<i>O. acutidens</i>	<i>S. multicaulis</i>	<i>R. officinalis</i>	Control	Grand M ± SE
		M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	
24	5	13.3±	5.0±	21.6±	0.6	4.6	2.0	0.6	6.8±
	10	11.6±	8.3±	16.6±	0.6	2.0	0.6	0.6	5.8±
	Mean ± SE	12.5±2.3c	6.6±3.4b	19.1±1.7b	0.6±1.5b	3.3±1.4b	1.3±1.4b	0.6±0.8a	6.3±0.6c
48	5	28.3±	33.3±	28.3±	2.6	7.3	0.6	0.6	14.4±
	10	21.6±	31.6±	25.0±	3.3	2.0	3.3	0.6	12.5±
	Mean ± SE	25.0±2.3b	32.5±3.4a	26.6±1.7a	3.0±1.5b	4.6±1.4b	2.0±1.4b	0.6±0.8a	13.5±0.6b
72	5	43.3±	45.0±	31.6±	18.0	30.0	11.3	1.3	25.8±
	10	28.3±	36.6±	30.0±	32.6	25.3	6.0	1.3	22.9±
	Mean ± SE	35.8±2.3a	40.8±3.4a	30.8±1.7a	25.3±1.5a	27.6±1.4a	8.6±1.4a	1.3±0.8a	24.3±0.6a
Grand M±SE		24.4±1.0a	26.6±1.0a	25.5±1.0a	9.6±1.0b	11.8±1.0b	4.0±1.0c	0.8±1.0d	14.7±0.3

Values followed by different lower-case letters in the same column differ significantly at $P < 0.01$ (**).

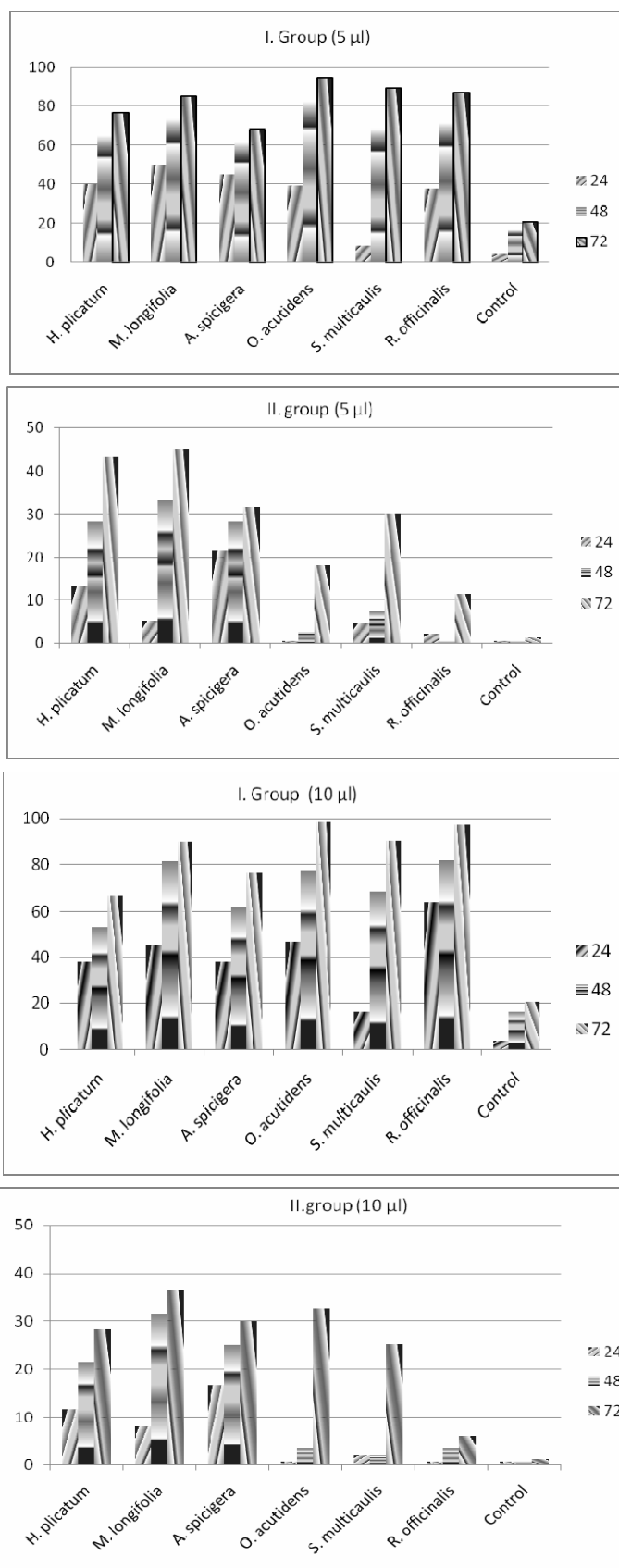


FIGURE 1 - Mortality of *Dendroctonus micans* (1 and 2 larval stages) at different doses of the test essential oil vapors and exposure times.

A. spicigera essential oils caused significant mortality (about 80–90%) at 9 µl/L air dose after 48-h exposure against *Sitophilus granarius* adults [25]. Toxicity of the essential oil from *Mentha longifolia* (Lamiaceae) leaves was evaluated against *Sitophilus zeamais*, an insect that is the major cause of deterioration in stored grains, including maize. The essential oil at 0.50 µl/g of maize grains in the contact bioassay caused 100 % mortality of *S. zeamais* [30]. *O. acutidens* oil showed 68.3 and 36.7% mortality against *Sitophilus granarius* and *Tribolium confusum* adults, respectively [31].

R. officinalis essential oils were tested in their vapor form against *Acanthoscelides obtectus*. Choice and non-choice tests revealed that most of them had a repellent action, reduced fecundity, decreased egg hatchability, increased neonate larval mortality, and adversely influenced offspring emergence [32]. These results suggested that the essential oils isolated from different plant species might have different toxicity, which can be attributed to their different chemical composition and different major and/or minor components. *A. spicigera*, *H. plicatum*, *M. longifolia*, *O. acutidens*, *R. officinalis* and *S. multicaulis* have potential for development as new effective insect control agents against *D. micans* larvae. The development of natural or biological insecticides will help to decrease the negative effects (residues, resistance and environmental pollution) of synthetic insecticides. In this respect, bio-insecticides may be also effective, selective, bio-degradable and associated with little or no-resistance of the pest, being less toxic to environment. In the present study, *R. officinalis*, *O. acutidens* and *M. longifolia* essential oils were the most toxic against *D. micans* larvae under laboratory conditions.

As a result, we suggest that the effects of these essential oils must be field-tested in the natural oriental spruce forests at all circumstances, and the results must be compared with those obtained in the laboratory.

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