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Research article

Phytochemical composition and insecticidal effect of essential oil from *Origanum vulgare* L.

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Abstract

Origanum genus belonging to Lamiaceae family has been used extensively throughout the world. Air-dried aerial part of *Origanum vulgare* L. was subjected to steam distillation to yield the essential oil of which the compounds were elucidated by GC-MS analysis. The main constituents of essential oil were determined as thymol (40.35%), *p*-cymene (17.32%), *γ*-terpinene (15.66%), and carvacrol (12.15%). The insecticidal activity of essential oil was executed against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) by fumigation method. It was presented that the essential oil was highly effective against both species. After treatment of essential oil on *S. oryzae*, 72% and 88% mortality were observed at 48 hours (h) and 72 h respectively. The mortality of *R. dominica* was determined as 94% and 96% at 48 h and 72 h respectively. Median lethal concentration (LC_{50}) values were found for *S. oryzae* as 0.086 µL /mL and 0.057 µL /mL at 24 h and 48 h respectively. Moreover, these values (LC_{50}) were presented as 0.11 µL /mL and 0.065 µL /mL at 24 h and 48 h respectively for *R. dominica.* Hence, the essential oil of *O. vulgare* could be useful for the managing field populations of *S. oryzae* and *R. dominica*.

Keywords: *Origanum vulgare*; essential oil; insecticidal activity

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Introduction

Plant-derived materials have been applied extensively for the treatment of various diseases since ancient times. Due to the variety of bioactive compounds, they play a significant role in drug development process (Elmastas et al., 2004; Demirtas et al., 2013; Aksit et al., 2014; Elmastas et al., 2016; Erenler, Sen, Yaglioglu, et al., 2016; Erenler, Sen, Yildiz, et al., 2016; Erenler et al., 2014; Karan et al., 2017).

Origanum genus (Lamiaceae family) distributed through the Mediterranean region, Europe and West and Central Asia has been represented nearly 50 species in the worldwide, fourteen of which are endemic for Turkey (Davis, 1988). Since *Origanum* species have been used in food, cosmetic and pharmaceutical industry, they have been cultivated in many European and Asian African countries. *Origanum* species have been cultivated in Turkey extensively and exportation of these species have increased steadily lately (Ozkan et al., 2010). *Origanum* species are well known with their essential oils that display a great deal of biological activity (Pezzani et al., 2017). *Origanum* species have been commercially traded and widely

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consumed worldwide as common herbs (Prerna et al., 2015). It was reported that *Origanum* species consisted of bioactive compounds being responsible for biological activities (Oke-Altuntas et al., 2018). *Origanum* species have been reported to reveal a large number of biological activities such as antimicrobial (Alboofetileh et al., 2018), antioxidant, anticancer (Erenler, Sen, Aksit, et al., 2016), herbicide (Fouad et al., 2015), antifungal (Waller et al., 2018), antimutagenic (Mossa et al., 2013) and anti-adipocyte differentiation (Lin et al., 2013). *Origanum* tea has been widely used for traditional medicine in the treatment of ailments such as stomach upset, bronchial disorders, as digestive and antiseptic, sudorific, diarrhoea, infection diseases, antiseptic and stimulant (Joshi et al., 2015).

The crop production has been threatened by the insect pests throughout the world. Synthetic insecticides such as methyl bromide and phosphine fumigants have been commonly applied to wipe out stored pets and to protect stored food and agricultural materials (Athanassiou et al., 2015). On the other hand, usage of synthetic chemical resulted in the development resistance in stored-product insects. Furthermore, due to the damaging to the ozone layer, chemical residue, carcinogenic effect, some synthetic insecticides have been banned (Jagadeesan et al., 2015). It is important to find novel fumigant that should be harmless to the ecosystem, and non-target organism to combat stored product insects. EOs have gained the great interest to replace synthetic products in fighting pets (Isman, 2006). The rice weevil, *Sitophilus oryzae* and the bean weevil, *Rhyzopertha dominica* have been known as the widespread and destructive insect pests of stored grains and stored products (Aref et al., 2015).

Essential oils (EOs) have been extensively used in food and pharmaceutical purposes for years all over the world. EOs have been generated from plant materials. Due to their insecticidal and herbicidal effect, EOs have been employed in agricultural industry lately (Dhakad et al., 2018).

In the previous work, we presented the insecticidal activity of *Origanum syriacum* essential oil against *S. oryzae* and *R. dominica* (Karan et al., 2018). In this work, essential oil was obtained by steam distillation and the compounds were elucidated by GC-MS analysis. In addition, the insecticidal activity tests of essential oil were executed using adult *S. oryzae* and *R. dominica*.

Materials and Methods

Plant material

Origanum vulgare was cultivated in Aromatic and Medicinal Plant Field of Gaziosmanpasa University. The plant materials were harvested in July 2017.

Isolation of essential oil

The plant material (60 g) was diluted with distilled water (300 mL) then subjected to hydrodistillation for 3 h by Clevenger apparatus. The oil was kept in fridge (+4 °C) until further usage.

GC-MS analysis

GC analysis was carried out on a Perkin–Elmer Clarus 500 Series, in divided mode (50:1), equipped with a flame ionization detector (FID) (Perkin-Elmer Clarus 500) and a mass spectrometer-equipped (Perkin-Elmer Clarus 500) BPX-5 capillary column (30 m × 0.25 mm, 0.25 um i.d., SGE Analytical Science-Trajan Scientific and Medicinal). The injection temperature was constant and FID was executed at 250°C. Helium gas was used as a carrier with a rate of 1.0 ml/min. The oven temperature was started at 50°C then was raised to 220°C with a rate of 8°C/min. Ionization energy was 70 eV and the temperature of transfer line was at 250°C of the mass spectrometer. The standard components were used for the majority of the essential oil constituents and Kovats retention indices (RIs) were determined for all the sample components.

Insects

S. oryzae and *R. dominica* cultures were supplied from the Department of Plant Protection, Bozok University. The wheat was filled into the glassware (5 mL, 1/3 was filled) and then adult males and females were added to lay eggs. Hence, single aged population was obtained. Adults were removed and cultures were incubated at 27 ± 2°C in a dark climate chamber with 60 ± 5 rh (relative humidity) for 48 h. The new adults came into view by 45 days and 4-week-old adults were applied in trials (Abay et al., 2012).

Dose-response Bioassay

Fumigation method was used for insecticidal effect of essential oil at the rate of 5, 10, 15 and 20% (v/v) (essential oil/acetone) concentrations. The paper discs were cut and then fixed to the cover of the glasses. Adults specimens (20) were placed in each glass. The essential oil mixture (10 µl) was applied to the filter paper by pipette. After evaporation of acetone for 5 min, the glasses were covered by caps and they were incubated at 25°C for 24 , 48 and 72 h. The dead adults were recorded for each 24 h. The randomized block design was used for the tests. All trials were repeated 3 times and each repetition included 3 replications (Polatoğlu et al., 2017).

Statistical analysis

The statistical analysis was carried out by ANOVA using the SPSS software (SPSS Inc., version 20). Tests of significance were carried out using Duncan's multiple range tests. Between groups statistical differences for parametric data were analyzed using Duncan's Multiple-Range Test. P<0.05 was considered significant for all tests.

Results and Discussion

Origanum species, aromatic and medicinal plants are well known with their rich essential oil content and they are consumed as herbal tea in many countries. Essential oil was obtained from *O. vulgare* and the compounds were determined by GC-MS analysis. Sixteen compounds were identified. Thymol (40.35%), ρ-cymene (17.32%), γ-terpinene (15.66%), carvacrol (12.15%) were detected as the major products (Table 1).

The toxicity of EO was investigated against *S. oryzae* and *R. dominica* by fumigation method. EO revealed the outstanding effect on both insects. The treatment of EO on *S. oryzae* resulted in the mortality of 72% and 88% for 48 h and 72 h respectively. Median lethal concentration (LC_{50}) for this adult was found as 0.057 µL /mL and 0.033 µL /mL at 48 h and 72 h respectively. After treatment of EO on *R. dominica*, the mortality was found as 94% and 96% at 48 h and 72 h respectively. However, the mortality was detected as 42% for 24 h (Table 2).

Table 1: Chemical composition of *O. vulgare* essential oil analyzed by GC-MS.

No	Compounds	RI	Percent (%)
1	α-Thujene	935	1.26
2	α-Pinene	939	2.34
3	Camphene	957	0.92
4	Sabinene	992	0.12
5	β-Pinene	995	1.18
6	β-Myrcene	1003	1.35
7	α-Phellendrene	1023	0.66
8	α-Terpinene	1037	4.12
9	*p*-Cymene	1052	17.32
10	γ-Terpinene	1067	15.66
11	α-Terpinolene	1106	0.15
12	Borneol	1171	0.11
13	α-Terpineol	1198	0.72
14	Thymol	1291	40.35
15	Carvacrol	1305	12.15
16	β-Caryophyllene	1418	1.58
Total			99.99

SGE BPX5 column was used. RI: Retention indices calculated against n-alkanes, % calculated from FID data.

Table 2: Single dose of essential oil on insects for fumigant effect.

Treatment		Mortality (%)		
	Insect	24 h	48 h	72 h
Control	*Sitophilus oryzae*	1.11^{e}	11.10^{de}	11.10^{de}
	Rhyzopertha dominica	12.22^{d}	13.30^{d}	15.55^{d}
Origanum vulgare	*Sitophilus oryzae*	45.55^{c}	72.22^{b}	87.77^{a}
	Rhyzopertha dominica	42.22^{c}	94.44^{a}	95.55^{a}

The different letters in the same column indicates the significant difference at P < 0.05.

Table 3: Fumigant effect of *O. vulgare* essential oil on *R. dominica* and *S. oryzae*

Insect	hours	LC_{50} (µL/mL)	LC_{90} (µL/mL)
S. oryzae	24	0.086	0.146
	48	0.057	0.127
	72	0.033	0.091
R.dominica	24	0.111	0.184
	48	0.065	0.107
	72	0.060	0.110

Hence, effectiveness of EO increased with the time-dependence. LC_{50} values were found as 0.065 µL /mL and 0.060 µL /mL for 48 h and 72 h respectively. In comparison of LC_{90} values, significant differences were observed between the adults. LC_{90} values of *S. oryzae* and *R. dominica* were found as 0.146 µl/ml and 0.184 µl/ml for 24 h respectively. Moreover, LC_{90} values of *S. oryzae* and *R. dominica* were found as 0.127 µl/ml and 0.107 µl/ml for 48 h respectively. However, comparing for 72 h of *S. oryzae* and *R. dominica*, LC_{90} values were calculated as 0.091 µl/ml and 0.110µl/ml (Table 3). The mortality increased significantly for time dependence for *S. oryzae*. On the other hand, significant mortality was not detected between 48 and 72 h for *R. dominica*.

The chemical constituents and quantity of a plant can be varied due to some conditions such as environment, climate, altitude, location, part of the plant and harvest period (Khalfi et al., 2008). *Origanum vulgare* ssp. *vulgare* collected from Erzurum-Turkey consisted of caryophyllene (14.4%), staphulenol (11.6%), germacrene-D (8.1%), α-terpineol (7.5%) as chief products. The essential oil obtained from *Origanum vulgare* (Iran) included the thymol (18.36%), carvacrol (18%), terpinolene (17%) as main constituents that revealed the strong antibacterial activity (Kazemi et al., 2012). In addition, combination of *Origanum vulgare* essential oil and lactic acid inhibited the *Staphylococcus aureus* in meat broth and meat model (Barros et al., 2012). *Origanum vulgare* collected from Mendoza-Argentina contained the γ-terpinene (32.10%), α-terpinene (15.10%), thymol (8%), ρ-cymene (8%), β-phellandrene (7.10%), and carvacrol (4%) as the chief compounds that exhibited the strong antioxidant and anti-lipase activity (Quiroga et al., 2013). Recent work indicated that EOs exhibited the considerable insecticidal activities. Due to the increase in environmental and health trouble, the effect of usage of synthetic pesticides randomly, and increasing demand for safer food and products by consumer, acceptance of EOs has become more attractive in agriculture.

Conclusion

Due to the considerable insecticidal activity of *Origanum vulgare* essential oil, it could be used in agricultural industry to combat insects. The thymol which was the chief product of *O. vulgare* has been used in pharmaceutical process. Moreover, it has a potency to be used in stored product control programs as well.

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