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Original Article

Chemical Composition and Insecticidal Activity of Myrtle (*Myrtus communis* L.) Essential Oil against Two Stored-Product Pests

Abbas Khani* and Farzaneh Basavand

Department of Plant Protection, Faculty of Agriculture, University of Zabol, P.O. Box 9861335856, Zabol, Iran

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Abstract

Essential oil extracted from the leaves of Myrtle, *Myrtus communis* L. (Myrtaceae) was tested *in vitro* for volatile toxicity against two stored-product insects (*Tribolium confusum* and *Callosobruchus maculatus*). The chemical composition of the plant oil was examined by gas chromatography-mass spectrometry (GC-MS). The major oil components were α-pinene (10.4%), α- Terpineol (10.1%), linalool (9.0%), 1, 8-cineole (7.6%), Geranyl butyrate (6.3%), Geraniol (6.2%), Caryophyllene oxide (5.3%) and Neryl acetate (5.0%). In the fumigant toxicity test, significant differences in mortality of insects to essential oil vapor were observed in different concentrations after an exposure time of 24 hours. *C. maculatus* (LC_{50}=9.5 µl/l air) was more susceptible to the plant oil than *T. confusum* (LC_{50}=260.7 µl/l air). The results suggested that essential oil of *M. communis* L. could be used as a potential biocontrol agent for stored-product insects.

Key words: Fumigant toxicity, GC-MS, *Myrtus communis* L., Stored-products, Essential oil.

Introduction

Confused flour beetle, *Tribolium confusum* (Jacquelin du Val) attacks stored grains and foods. Adults and larvae feed on broken kernels and finely ground materials in grain storages, flour mills, warehouses and wherever grain or grain products are stored. This species is probably the most serious pest of the genus *Tribolium* and one of the most economically important beetles [1].
A key pest of cowpea is the cowpea weevil, *C. maculatus* (Fab.), a bruchid that infests both pods in the field and seeds in storage. Tanzubil [2] found that this insect can damage 100% of stored seeds causing weight losses of up to 60%.
Control of storage pest insects is dependent on the use of chemical methods such as fumigation treatments. At present, phosphine and methyl bromide are the principal fumigants available for pest control in durables, perishables and in quarantine treatments [3]. However, the extended use of broad-spectrum insecticides has resulted in the development of insect resistance, environmental pollution, workers danger and destruction of non-target organisms. In recent years, interest in screening medicinal plants for insect control has increased significantly and interest in the oils was renewed with emerging demonstration of their fumigant and contact insecticidal activities to a wide range of pests in the 1990s [4]. The insecticidal activity of essential oils and plant extracts against different coleopteran stored product insects has been evaluated [5].
In fact, pesticides derived from plant essential oils do have several important benefits. Due to their volatile nature, there is a much lower level of risk to the environment than with current synthetic pesticides. Predator, parasitoid and pollinator insect populations will be less impacted because of the minimal residual activity, making essential-oil-based pesticides compatible with integrated pest management programs [6].

*Corresponding author: Department of Plant Protection, Faculty of Agriculture, University of Zabol, P.O. Box 9861335856, Zabol, Iran. Tel. and Fax: +98-542-2242501.
E-mail Address: abbkhani@yahoo.com & abbkhani@uoz.ac.ir

Myrtus communis L. (Myrtaceae), a medicinal and aromatic species, is an aromatic evergreen perennial shrub or small tree, 1.8-2.4m in height with small foliage and deep fissured bark. It is native to Southern Europe, North Africa and West Asia. It is distributed in south, north, and central parts of Iran [7]. Various biological activities have been reported for *M. communis* L., such as antibacterial [8], antifungal [9, 10] and insecticidal properties [11-14].

In the present study, the chemical constituent of essential oil from *M. communis* L. leaves was determined, and the insecticidal activity of these essential oils was tested against the adult stages of the stored-products pests, *Tribolium confusum* and *Callosobruchus maculatus*.

Materials and Methods

Insects

Callosobruchus maculatus and *Tribolium confusum* were reared according to method of khani and Asghari [15].

Essential oil extraction

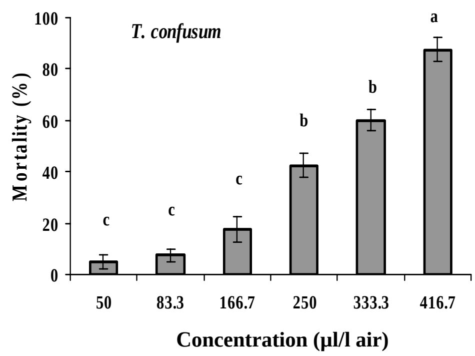
The leaves of *M. communis* L. collected from Iranshahr (27° 15' N, 60° 40' E; alt. 590 m), located in Sistan and Baluchestan province, Iran, from June to July, 2010. Essential oil extraction was done following the method described by Khani et al. [16]. The extracted oil was dehydrated with anhydrous sodium sulphate (10 min) and immediately stored in airtight glassware in refrigerator at 4 °C.

Gas chromatography-mass spectrometry

The essential oils was analyzed on a gas chromatograph mass spectrometer (GC–MS) (Shimadzu –17A–QP5050, Japan). The GC column was DB-5 (30 m × 0.25 mm i.d, 0.25 µm film thickness). The column oven temperature was set at 60 °C for 3 min, and then increased to 260 °C at a rate of 5 °C/min. Injector and detector temperatures were 230 and 245 °C, respectively. The GC mass analysis was carried out with the same characteristics as used in GC. The ionization energy was 70 ev with a scan time of 1s and mass range of 40–500 amu. Unknown essential oil was identified by comparing its GC retention time to that of known compounds and by comparison of its mass spectra, either with known compounds or published spectra.

Fumigant toxicity

To determine the fumigant toxicity of the oil, filter paper (2.0 cm diameter) was impregnated with 3, 5, 10, 15 and 20 µl of oils without using any solvent for *T. confusum* and impregnated with 0.3, 0.4, 0.5, 0.75, 1, 1.25 and 1.5 µl of oils for *C. maculatus*. Then the filter paper was attached to the under surface of the screw cap of a glass vial volume (60 ml) to generate concentrations of 50, 83.33, 166.66, 250 and 333.33 µl/l air for *T. confusum* and concentrations of 5, 6.66, 8.33, 12.5, 16.66, 20.83 and 25 µl/l air for *C. maculatus*. The cap was screwed tightly on the vial containing ten adults (1-7 days old with undefined sex) of each species of insect separately. Each concentration and control was replicated four times. All experiments were carried out in the dark in growth chamber set at 27±1 °C and 65±5% relative humidity. Mortality was determined 24 h after exposure to essential oil from *M. communis* L. leaves. When no sign of leg or antennal movement were observed, insect were considered as dead.



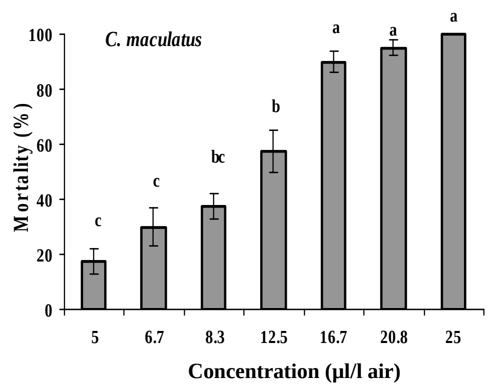


Fig 1 Lethal effect of essential oil from *Myrtus communis* L. leaves on *Tribolium confusum* and *Callosobruchus maculatus* after 24 hours.

To assessment 50% lethal doses (LC_{50}) after an exposure time of 24 hours, the concentrations of the essential oils causing 10 to 90% mortality were used. Five different concentrations (83.33, 166.67, 250 and

333.33 and 416.67 µl/l air for *T. confusum* and 5, 6.66, 8.33, 12.5 and 16.66 µl/l air for *C. maculatus*), each with four replicates and ten individuals per each replicate, were used. The mortality was determined as described in previous experiment. The treatment bottles were monitored for 48 h after recording the data and no affected insect recovered. Data obtained from each dose response bioassay were subjected to probit analysis. LC_{50} values were determined by log-probit regression using SPSS 16.0 for Windows [17].

Statistical analysis

Statistical analysis of the mortality data was performed by one-way analysis of variance (ANOVA) with a post-hoc Tukey test using SPSS version 16.00. Normality of data was tested using, Kolmogorov-Smirnov test, a non-parametric test. The results were expressed as mean± SE, and considered significantly different at $P< 0.05$.

Results and Discussion

Chemical composition of essential oils

The chemical composition of essential oils of *M. communis* L. leaves is presented in the Table 1. The major components were α-pinene (10.4%), α-terpineol (10.1%), linalool (9.0%), 1,8-cineole (7.6%), geranyl butyrate (6.3%), geraniol (6.2%), caryophyllene oxide (5.3%) and neryl acetate (5.0%). Furthermore, lesser amounts of the other important insecticidal components include caryophyllene (2.9%), methyl eugenol (2.8%), β-pinene (2.4%), α-cadinol (1.5%) and Trans carveol (1.7%) were existed in essential oil of this plant.

Literature revealed the qualitative and quantitative analysis of different chemotypes of *M. communis* L. essential oils from different countries. The oil in leaves of *M. communis* L. growing in Turkey contained 1,8 cineole, linalool, myrtenyl acetate and myrtenol as major components [18]. α- pinene (29.4%), limonene (21.2%), 1,8 cineole (18%), linalool (10.6%), linalyl acetate (4.6%) and α-terpineole (3.1%) were reported to be the major components of myrtle leaves [19]. The essential oil of *M. communis* L. in France contained 14 compounds, α-pinene and 1,8-cineol (Eucalyptol) together representing around 86% [9]. The oil in leaves of natural populations of *M. communis* L. growing in Tunisia contains α-pinene (19.2%), 1,8-cineole (16%), linalool (7.7%), α-terpineol (7.5%) and limonene (5.7%) [20].

The oil obtained from leaves of *M. communis* L. in Italy (Sardinian) were characterized by high contents of α-pinene, limonene and 1,8-cineole [21].

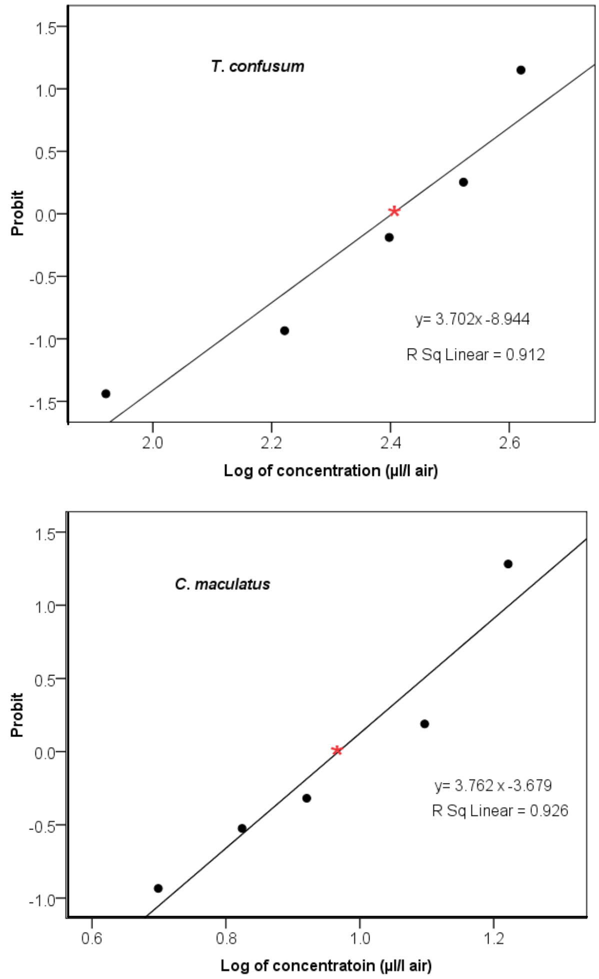


Fig 2 Mortality probit line for *Tribolium confusum* and *Callosobruchus maculatus* 24 hours after exposure to essential oil from *Myrtus communis* L. leaves. LC_{50} point is shown by the red star.

The major compounds in the essential oils of *M. communis* L. leaves were α pinene (30.0%), 1,8 cineole (28.8%), and limonene (17.5%) in Italy [21]. In other study, the major compounds reported were α-pinene (19.2%), 1,8-cineole (16.0%), linalool (7.7%), α-terpineol (7.5%) and limonene (5.7%) [22]. Mahboubi and Ghazian [23] reported the presence of 1,8-cineole (36.1%), α-pinene (22.5%), linalool (8.4%), bornyl acetate (5.2%), α-terpineol (4.4%), linalyl acetate (4.2%) and limonene (3.8%) as major components of leaves of *M. communis* L. in Iran. The essential oil of *M. communis* L. in Elba Island contained α-pinene (51.8%), 1,8-cineol (24.6%) and limonene (7.6%) as major components [12]. The main components found in *M. communis* L. in Southern Turkey were linalool (31.3%), linalyl acetate (17.8%), 1,8-cineol (14.7%), geranyl acetate (9.1%), α–pinene (8.4%) and α-terpineol (6.5%) [14]. Twenty one different components were identified in the essential oils of *M. communis* leaves collected from Cham Moord area in Lorestan province, Iran, that the main components were limonene (18.5%), α pinene (13.3%) and 1,8 cineole (10.6%) [24].

In our study the composition of essential oils of *M. communis* L was relatively similar to the previous reports of other parts of world, which the major components were α-pinene, linalool and 1,8 cineole. Compared to other researches, in our study, limonene, linalyl acetate, bornyl acetate, geranyl acetate and myrtenyl acetate were absent and myrtenol (1.36%) was at low amount. On the other hand, geranyl butyrate (6.3%), geraniol (6.2%), caryophyllene oxide (5.3%) and neryl acetate (5.0%), α- terpinyl acetate (4.1%) were at relatively high amounts.

Fumigant toxicity

In all cases, considerable differences in mortality of insect to essential oil vapor were observed in different concentrations. The mortality increased significantly with rising concentrations (Fig 1, 2) ($F_{5,18}$= 64.6; P=0 for *T. confusum* and $F_{6,21}$= 45.7; P=0 for *C. maculatus*). Results indicated that the oil of tested plant was significantly more toxic against *C. maculatus* than *T. confusum*, as inferred by the confidence intervals of LC_{50} (Table 2). Based on LC_{50}, the oil of *M. communis* displayed strong insecticidal activity against *C. maculatus* (LC_{50}=9.50 µl/l air), but revealed fewer activity against *T. confusum* (LC_{50}=260.7 µl/l air) (Table 2). Similarly, a difference response of the insect species to the essential oils has previously been reported for these stored product insects [15,16]. The difference of sensitivity may be attributed mainly to the phenotypic resistance (modifications in the target site), metabolic resistance (ability to detoxify insecticides) or behavioral modification. Behavioral changes that minimize contact between insect and insecticide may cause a severe impact in the insecticide application efficacy, especially if resistance is selected by physiological features [25]. *T. confusum* had a tendency to be less active (behavioral resistance mechanism) and had a limited movement (our observations) compared to *C. maculatus* that may reduce breathing and insecticide penetration via the insect spiracle.

Based on our knowledge, no study has been previously reported on the insecticidal activity of *M. communis* oil against *C. maculatus* and *T. confusum*. However, the insecticidal efficacy of *M. communis* oil has been reported against other insects.

The LC_{50} values of essential oils of *M. communis* L. against *Tetranychus urticae* Koch (Acari: Tetranychidae) was 53.2 µl /l air [26]. At 24 h-exposure period, LC_{50} for *M. communis* L. leaves essential oil on eggs, larvae, pupae and adults of khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), were 54.7, 307.4, 328.6 and 68.4 µl/l air, respectively [27]. Toxicity of *M. communis* L. essential oil on some mosquito was reported. *M. communis* L. essential oil induced only a 36.7% larval mortality on the mosquito, *Aedes albopictus* (Diptera: Culicidae) at a dosage of 300 ppm [12]. Amer and Mehlhorn [13] tested the larvicidal effect of essential oils extracted from 41 plants against third instar larvae of three different mosquitoes and showed that *M. communis* L. essential oil caused 100% larval mortality at low dosage (50 ppm) against *A. aegypti*. Probit analysis showed that LC_{50} values of *M. communis* L. essential oil against *Ephestia kuehniella* (Lepidoptera: Pyralidae), *Plodia interpunctella* (Lepidoptera: Pyralidae) and *Acanthoscelides obtectus* (Coleoptera: Bruchidae) were 12.7, 22.6 and 49.6 µl/l air, respectively [14]. Methanol extracts of *M. communis* L. revealed insecticidal activity on *Callosobruchus chinensis* [28]. The extracts from leaves and flowers of *M. communis* L. were found to be toxic against fourth-instar larvae of *Culex pipiens* molestus, and thymol, carvacrol, and α-pinene were the most toxic pure components [11].

Table 1 Chemical composition of essential oil from *Myrtus communis* L. leaves

Compounds	Concentration (%)	Kovats index
Propanoic acid	0.46	670
α- pinene	10.40	936
β-pinene	2.37	978
β-Myrcene	1.46	990
1,8-cineole	7.60	1032
Linalool	8.96	1100
Terpinen-4-ol	0.96	1180
α- Terpineol	10.12	1190
Myrtenol	1.36	1195
Trans carveol	1.7	1217
Geraniol	6.15	1255
Isobornyl_acetate	0.41	1286
α- Terpinyl Acetate	4.13	1347
Neryl acetate	4.97	1363
β- Elemene	1.47	1390
Methyl eugenol	2.78	1402
Caryophyllene, (E)	2.95	1420
Curcumol	2.75	1433
α- Bergamoten	2.17	1436
Alloaromadendrene	0.35	1460
Germacrene D	1.69	1481
Nerolidol, (E)	3.50	1560
Geranyl butyrate	6.28	1563
Caryophyllene oxide	5.31	1580
α- Humulene oxide	1.49	1601
α- Cadinol	1.52	1651
Cedran-diol	1.22	1885
Phthalic acid	1.42	1985
Isopropyl palmitate	1.46	2023
Other compounds	2.60	-

Table 2 Efficiency of essential oil extracted from *Myrtus communis* L. leaves against *Tribolium confusum* and *Callosobruchus maculatus* adults

Insects	LC_{50}	95% CL	χ^2(df)	Probability	Slope ± SE
T. confusum	260.69	(184.6-390.2)	6.17 (3)	0.10	3.70±0.51
C. maculatus	9.50	(8.45-10.80)	4.03 (3)	0.26	3.76±0.55

* Ten individuals per replicate, four replicates per concentration, five concentrations per assay; LC: lethal concentration (µl/l air), CL: confidence limits (µl/l air)

In our study, both thymol and carvacrol were absent in *M. communis* L. essential oil, while α- pinene was the most abundant component.

Monoterpenes in general have been well documented to be active as fumigants, repellents or insecticides toward stored products insects [29]. The insecticidal activity of the essential oils investigated in the present study may be attributed to their major constituents of monoterpenes. Insecticidal activity of major constitutes of the tested oil were before reported.

1,8-cineole, terpineol, and linalool have been evaluated as fumigants against *Tribolium castaneum*. [30]. Essential oils rich in 1,8-cineole and carveol were effective against adults of German cockroach, *Blattella germanica* [31]. 1,8-cineole isolated from *Artemisia annua* was also a potential insecticidal allelochemical that could reduce the growth rate, food consumption and food utilization in some post harvest pests and house hold insects [32]. 1,8-cineole exhibited both contact and fumigant toxicity when tested against *T. castaneum* [33].

IC_{50} values (inhibitor concentration yielding 50% inhibition) of α-pinene against female acetylcholine esterase of *Blattella germanica* was 0.28 mg/ml [31]. Topical application of geraniol on female *Pediculus humanus capitis* De Geer (Phthiraptera: Pediculidae) demonstrated its insecticidal activity with LD_{50} value of 12.7 µg/insect [34].

The LC_{50} values of linalool against *Callosobruchus maculatus* and *Rhyzopertha dominica* were 23.6 and 31 µl/l air, respectively [35]. Linalool and α-terpineol significantly increased the nymphal duration in German cockroach, *Blattella germanica* (Linnaeus) when fed through artificial diet [36]. Similar effects against *Ostrinia nubilalis* (reared from 1st instars on diet) have been recorded with carveol, geraniol, linalool, α-pinene, α-terpineol, in the concentration range of 0.02–20.0 mg/g diet [37].

Caryophyllene oxide, isolated from *Uvaria* sp., turned out to be insecticidal and antifeedant [38]. Monzote *et at.* [39] observed that caryophyllene oxide inhibited the mitochondrial electron transport chain in rat liver. Substituted phenol, methyl eugenol, showed contact toxicity to *Sitophilus zeamais* and *T. castaneum* [40].

The essential oil from *M. communis* L. could become an interesting alternative to conventional chemical control strategies. However further studies need to be conducted to evaluate the safety of these oils before practical use in stored product insect control.

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