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SHORT COMMUNICATION

Evaluation of *Mentha arvensis* essential oil and its major constituents for fungitoxicity

Manpreet Kaur Makkar[1] · Sunita Sharma[2] · Harleen Kaur[2]

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Abstract Essential oil and major constituents of menthe were evaluated for fungicidal activities. Gas chromatography–mass spectrometry (GC–MS) of essential oil from leaves of *Mentha arvensis* cv. CIM-Saryu revealed that menthol was found in highest amount (77.94%) followed by isomenthone (5.24%), neomenthyl acetate (5.18%) and menthone (5.00%). Menthol and menthone were extracted from the essential oil by column chromatography. Essential oil, menthol and menthone were screened for their fungicidal activity against *Rhizoctonia solani* and *Fusarium moniliforme*. Menthol was highly effective as compared to essential oil as well as menthone. All of them exhibited less activity than standard bavistin at all the tested concentrations.

Keywords *Mentha* · Essential oil · Menthol · Menthone · Fungitoxicity and ED_{50}

Introduction

The cultivation of aromatic plants to obtain essential oils has been greatly increased mainly due to the increasing demand generated by food, cosmetics and pharmaceutical industries (Lubbe and Verpoorte 2011). Among many aromatic species, production of menthol mint has a great economic importance because of its large number of applications in different sectors of pharmaceutical industry (Souza et al. 2014).

Mints comprise a group of species of the genus *Mentha* belonging to the family Lamiaceae. *Mentha* species are generally found in circumboreal distribution and are native to the temperate regions of western and central Asia, Europe and North America (Aflatuni et al. 2005). The world market for menthol mint essential oils is 20,000 tons per year. In India, about 145,000 hectares of menthol mint are now cultivated (Srivastava et al. 2002).

There are several methods by which constituents of *Mentha* could be extracted. On distillation, the aerial parts of this plant yield essential oil having a variety of compounds in varying amounts such as menthol, menthone, menthyl acetate, menthofuran, carvone, linalool, linalyl acetate and piperitone oxide which are used in pharmaceutical, flavor, food, cosmetic, beverage and allied industries (Verma et al. 2009). In hydro distillation method the material is completely immersed in water and then boiled. In this procedure the surrounding water act as a barrier in preventing the material from overheating. Upon cooling, the water and the essential oil separate in the collector (Gill et al. 2014).

The pathogenic fungi are microorganisms that cause economic damage directly in the agricultural production system, besides indirectly cause ecological problems, due to the use of pesticides for its control (Kimati et al. 1997). Use of synthetic fungicides to control the disease causes several adverse effects i.e. development of resistance in the pathogen, residual toxicity, pollution in the environment, high cost etc. Therefore, it has become necessary to adopt ecofriendly approaches for better crop health and for yield. Plant essential oils are potential source of antimicrobial compound of natural origin. *Mentha* oil is also reported to possesss a wide spectrum of herbicidal (Vaughn and

✉ Sunita Sharma
sunita_sharma@pau.edu

[1] Deparment of Chemistry, Punjab Agricultural University, Ludhiana 141004, India

[2] Deparment of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141004, India

Spencer 1993), antifungal (Samber et al. 2014), antibacterial (Padmini et al. 2010), antioxidant (Derwich et al. 2011), antimicrobial (Iscan et al. 2002) and insecticidal activities (Bosly 2013).

Hence, the objective of this work was to evaluate the essential oil production and quality extracted by hydrodistillation of leaves as well as to evaluate the antifungal potential of menthol mint essential oil and some isolated substances of this oil i.e. menthol and menthone, on the fungi *Fusarium moniliforme* and *Rhizoctonia solani*, searching for alternatives to synthetic fungicides in the treatment of commercial seeds.

Materials and methods

Plant material

The plant material was grown in the Herbal Garden, Department of Agronomy, Punjab Agricultural University, Ludhiana. The crop was raised by following Package of Practices, Punjab Agricultural University, Ludhiana in triplicates using Randomised Block Design. The samples grown were harvested on June 25, 2015. The stems and leaves were separated, dried in ambient conditions and then in hot air oven at 40 °C to obtain constant weight and were stored in air tight glass containers for further analysis.

Essential oil extraction

The leaves and stems were ground in electric grinder and were used for the extraction of essential oil by hydro distillation method. The yields (%) of extracted essential oil of stems and leaves were compared.

Hydro distillation method

Extraction of essential oil was done by hydro distillation using Clevenger-type apparatus. Leaves or stems (100 gm) of *Mentha arvensis* were taken in 1 l round bottomed flask and 700 ml of water was added to it. The contents were thoroughly mixed and flask was kept overnight at room temperature. The flask was placed in heating mantle and was fitted with Clevenger-type apparatus. The contents were refluxed for 10 h (until no more oil was obtained). The essential oil (upper layer) containing little water was collected separately in conical flask. Same procedure was repeated to process 20 batches to get essential oil in high yields. Essential oil layers were collected in a conical flask.

Partitioning of essential oil layer

Essential oil layer obtained by hydro distillation was transferred into 250 ml separatory funnel. Essential oil was partitioned thrice using diethyl ether (100, 50 and 50 ml). The diethyl layer (upper layer) containing oil was passed over anhydrous sodium sulphate to remove traces of water present if any. Diethyl ether was distilled off and the oil was stored at 4 °C in dark glass bottles for further analysis. The yield (%) of essential oil obtained was calculated as:

$$\text{Yield}(\%) = \frac{\text{Weight of essential oil extracted(g)}}{\text{Weight of sample taken(g)}} \times 100$$

Gas chromatography–mass spectrometry of essential oil of *Mentha* leaf

Extracted essential oil of *Mentha* leaves was analyzed using gas chromatography–mass spectrometry (GC–MS) (QP2010 Plus, Shimadzu, Japan), equipped with an Rtx-5 MS capillary column (30.0 m × 0.20.25 mm i.d., 0.25 µm film thickness) for the separation of the components. The injector was maintained at 260 °C and operated in split injection mode with the split valve closed for 1 min. Helium gas was used as the carrier gas at a constant pressure of 69 kPa. The column oven was initially maintained at 50 °C for 2 min, raised to 210 °C at 3 °C/min, then to 280 °C at 10 °C/min. The interface temperature was 270 °C and the ionization mode was electron impact (70 eV). The mass selective detector was operated in the scan mode between 40 and 600 m/z. Data acquisition was started 3.0 min after injection. MS parameters used were; ionization voltage (EI) 70 eV, peak width 2 s, mass range 40–650 amu and detector voltage 1.5 V. The percentage of components in the essential oil was calculated from the relative area of each peak analyzed by flame ionization detector. Peak identification was carried out by comparison of the mass spectra with mass spectra data available of NIST08, WILEY8, Perfumery and Flavor and Fragrance libraries. The composition of *Mentha* oil is tabulated in Table 1.

Isolation of menthol and menthone from essential oil by column chromatography

Essential oil from leaves of *M. arvensis* (10 g) was subjected to column chromatography to extract menthol and menthone. Column was packed with silica gel (600 g) for column chromatography with 60–120 mesh size activated at 110 °C for 1 h. Essential oil was dissolved in *n*-hexane (boiling point—68 °C) and then adsorbed on silica gel for 30 min. Column was eluted with hexane and polarity was increased using dichloromethane. Each fraction was

Table 1 GC–MS data of *Mentha* leaf essential oil

Sr. No.	Name	Retention time	Percent area
1	3-Hexen-1-ol	6.095	0.08
2	2-Propen-1-one	7.405	0.02
3	β-Pinene	10.570	0.04
4	Isopulegol	11.452	0.23
5	Limonene	12.869	0.28
6	Eucalyptol	12.958	0.05
7	1-Phenyloxirane	13.524	0.05
8	2-Furanmethanol	14.893	0.03
9	Linalool oxide	15.627	0.03
10	Linalool	16.221	0.11
11	Mentha-2,8-dien-1-ol	17.189	0.06
12	Isopulegol	18.376	0.82
13	Isomenthone	18.826	5.24
14	Menthone	19.293	5.00
15	Menthol	20.533	77.94
16	α-terpeniol	20.864	0.37
17	Cyclopentanone	21.245	0.10
18	Hexanoic acid	21.740	0.08
19	Pulegone	22.842	0.59
20	Piperitone	23.547	1.32
21	Menthyl acetate	24.448	0.10
22	Cyclopentanone	25.003	0.05
23	Neomenthyl acetate	25.375	5.18
24	Isomenthyl acetate	25.930	0.12
25	Isopulegol acetate	26.118	0.09
26	Eugenol	28.093	0.18
27	β-Bourbonene	29.271	0.14
28	Caryophyllene	30.756	0.52
29	Germacrene	33.338	0.72
30	Bicyclogermacrene	33.965	0.14
31	Cadinene	35.045	0.11
32	Nerolidol	36.622	0.07
33	Spathulenol	37.208	0.11
34	Cadin-4-en-10-ol	40.157	0.04

monitored using thin layer chromatography and isolation of pure products was confirmed by IR and ^{1}H NMR analysis.

Antifungal activity

Essential oil, menthol and menthone were analysed for their antifungal activity. Two fungi were selected for bioassay viz. *Fusarium moniliforme* and *R. solani.* A culture of the test fungi was grown on potato dextrose agar (PDA) medium for certain period (generally 7 days) at ambient temperature (25 ± 1 °C) for growth. Stock solution (1000 µg/ml) of test compounds was prepared in dimethyl sulphoxide (DMSO) and further dilutions were done (500, 250, 100 and 50 µg/ml) and stored at 4 °C for further use. Potato Dextrose Agar media, containing specific concentration of the test compound was poured on to the Petri plates. After solidification, small disc (0.5 cm dia.) of the fungus culture was cut with a sterile cork borer and transferred aseptically upside down in the centre of Petri plate. Petri plates were incubated in BOD incubator at 25 ± 1 °C. Growth of fungal colony was measured after every 24 h till the fungus in the control plates (containing dimethyl sulfoxide) completely occupied it. Three replications were maintained for each treatment. The percentage growth inhibition over control was calculated as

$$I = \frac{100(C - T)}{C}$$

where I = inhibition percentage, C = growth in control, T = growth in treatment.

Results and discussion

Essential oil yields

Essential oil was yellow in color with a very strong and persistent odour of mint. Our results showed that in leaves essential oil content was 3.02% whereas it was absent in stems. The yield of essential oils reported in the literature of some species of the genus *Mentha* varied from 0.05 to 1.60% (Bhat et al. 2002).

GC–MS analysis

Gas chromatography–mass spectrometry (GC–MS) of oil exhibited the presence of 34 compounds accounting for 100.0% of oil. Menthol (77.94%) was the major compound and the other minor compounds present were isomenthone (5.24%), neomenthyl acetate (5.18%), menthone (5.00%) and piperitone (1.32%). Table 1 tabulates the retention time and percent area of compounds present in *Mentha* essential oil. Souza et al. (2014) reported menthol (70.00%) as the main component of menthol mint essential oil followed by menthyl acetate (7.00–12.00%) which is an indicator of maturity. A total of 12 compounds were reported including menthol, pulegone and piperitone. Padalia et al. (2013) analysed the oil from 9 cultivars of *M. arvensis* and reported 33 constituents were menthol (73.70–85.80%), menthone (1.50–11.00%), menthyl acetate (0.50–5.30%), isomenthone (2.10–3.90%), limonene (1.20–3.30%) and neomenthol (1.90–2.50%). All the cultivars were rich in menthol and the highest menthol content was found in MAS-1 (85.50%) followed by Kosi (78.70%),

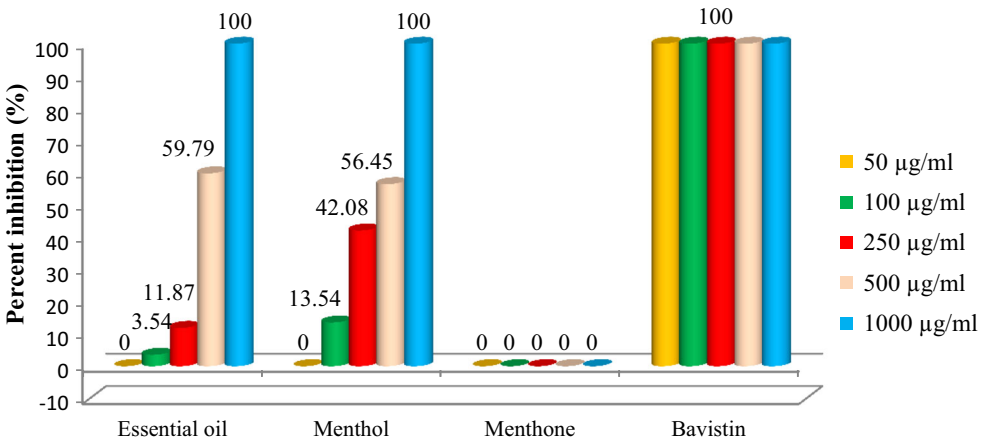

Fig. 1 Comparison of antifungal activity of essential oil and its major compounds against *Fusarium moniliforme*

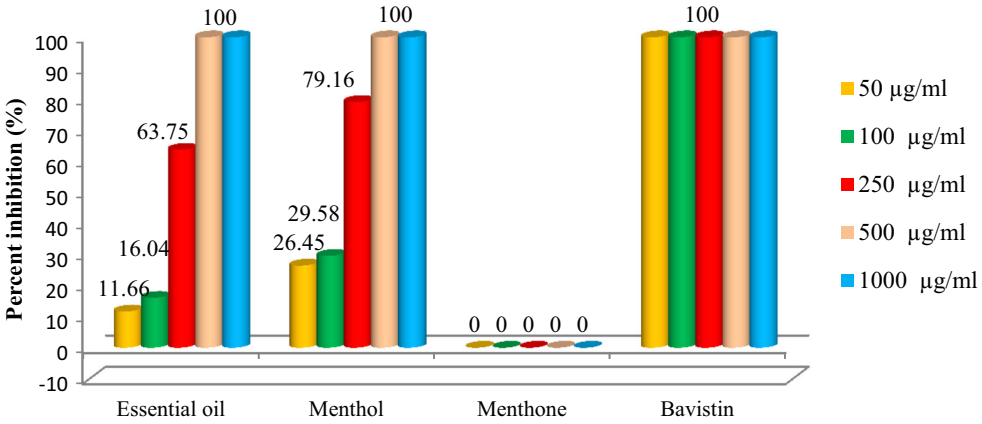

Fig. 2 Comparison of antifungal activity of essential oil and its major compounds against *Rhizoctonia solani*

Table 2 ED_{50} (µg/ml) of essential oil, menthol and menthone along with the standard bavistin

	ED_{50} (µg/ml)	
	Fusarium moniliforme	*Rhizoctonia solani*
Essential oil	495	331
Menthol	462	189
Menthone	> 1000	> 1000
Bavistin	8	5

Shivalik (78.10%) and Damroo (78.00%). The menthone content varied between 1.50 and 11.00%.

Isolation of menthol and menthone

Essential oil from leaves of *M. arvensis* (10 g) was subjected to column chromatography to extract menthol and menthone. Column was packed with silica gel (600 g) eluted with hexane and polarity was increased using dichloromethane. The hexane fraction showed two spots on TLC. On increasing the polarity with dichloromethane, hexane: dichloromethane (4:1), a pale yellowish liquid (0.51 g), showing single spot on TLC having boiling point 220 °C, refractive index 1.453 and density of 0.892 g/cm^3 was obtained and identified as menthone. Further its confirmation was done from its IR and ^{1}H NMR data.

On further increasing the polarity with dichloromethane, hexane: dichloromethane (1:1), a white crystalline compound (6.80 g), having melting point 42–43 °C which showed single spot on TLC (R_f value 0.54) was obtained. IR and ^{1}H NMR data proved this compound to be menthol.

Antifungal activity

Essential oil, menthol and menthone were tested for their in vitro fungicidal activity against *F. moniliforme* and *R. solani*. The percent inhibition of the compounds against *F. moniliforme* and *R. solani* is presented in Figs. 1 and 2 respectively along with ED_{50} values in Table 2. Bavistin was used as standard and Dimethylsulfoxide as control against both the test fungi. The result showed that menthol was found most promising against both the pathogenic fungi and showed better activity as compared to the oil.

Essential oil showed 100% inhibition of both the fungal species at concentration of 1000 μg/ml with ED_{50} of 495 μg/ml and 331 μg/ml for *F. moniliforme* and *R. solani* respectively. Menthol was 100% effective against *R. solani* at 500 μg/ml and 1000 μg/ml with ED_{50} value of 189 μg/ml whereas it showed 100% inhibition of *F. moniliforme* at 1000 μg/ml only with ED_{50} value of 462 μg/ml. Menthone was found to be least effective against both the fungal species. ED_{50} value for menthone was more than 1000 μg/ml hence not reported. However, none of the compounds showed better control than standard bavistin against both the tested fungi.

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