Gas Liquid Chromatographic Analysis of Treated Biocontrol Agents Mint Oil

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ABSTRACT

Gas liquid chromatographic analysis of spearmint oil revealed that the oil of this species has 13 different derivatives. The untreated control plants expressed all of them except phellandrene, which present in the bioagents treatment; the later showed the absence oflimonene and menthone. The least concentration value was noticed with caryophellene (1.072 %). Gas liquid chromatographic analysis of peppermint oil expressed different 13 derivatives. ß-purine was detected in this species but not in spearmint one. Menthol showed the highest concentrations were 10.333 and 10.255 respectively. The least values were recorded with Limonene (1.804 and 0.325 %) for the control and bioagent treated plants respectively.

Keywords: Gas Liquid Chromatographic**,** spearmint oil, peppermint oil.

INTRODUCTION

Medicinally, the various mints have been used worldwide for centuries as a cure or relief for numerous ailments from flatulence and digestive complaints to fevers (**Bove, 1996 and Hoffman, 1996)**. Peppermint is taken internally as a tea, tincture, oil or extract and applied externally as a rub or liniment. Herbalists consider peppermint as astringent, antiseptic, antipruritic, antispasmodic, antiemetic, carminative, diaphoretic, mild bitter, analgesic, anticatarrhal

Antimicrobial, rubefacient, stimulant and emmenagogue (Bove, 1996 and Hoffman, 1996). It has traditionally been used to treat a variety of digestive complaints such as colic in infants, flatulence, diarrhea, indigestion, nausea and vomiting, morning sickness and anorexia and as a spasmolytic to reduce gas and cramping. Also, it is currently used to treat irritable bowel syndrome, Crohn's disease, ulcerative colitis, gallbladder and biliary tract disorders and liver complains (**Blumenthal, 1998 and Fleming, 1998**). Peppermint oil is used to relieve menstrual cramps, and externally for neuralgia, myalgia, headaches, migraines and chicken pox **(Bove 1996** and **Blumenthal, 1998**).

Essential oils from some Mentha species were obtained by Drying hydro distillation and analysed by GC–MS: numerous compounds were identified. The most abundant were menthone, isomenthone,

menthol, carvone, piperitone oxide, D-limonene and eucalyptol. Chemometric similarity measures and principal component analysis were calculated, allowing comparisons based on secondary metabolite content (**Hawryłet. al., 2015).**

The study is aimed to in Vitro studies to evaluate the efficacy of different concentrations of mint oil as a fungicide in Petri dishes. Extraction of the mint oil and comparison between the oil components using gas chromatography (GC) technique.

EXPERIMENTAL

Separation of mint oil

The fresh mint plants were cut into small pieces, which transferred into a mint tube. Mint tubes were then hauled from the field, two at a time, back to the mint distillery where the oil will be extracted. Steam works its way through the mint plants vaporizing the oil from leaves and taking it to the top of the tube. The oil and water vapor then exits the tub and flows to the condenser tanks, which are full of cool water. The vapor runs through a series of tubes called the condenser and is eventually turned back into a liquid. The liquid comes out bottom of the condenser and into a tank called the separator. In the separator the oil floats to the top because it is lighter than the water maker comparison between the oil compounds was achieved using gas chromatography (GC) technique.

Separation conditions of essential oil instrument:

Gas Liquid Chromato-graphy/ Unicam PRO- GC.

COLUMN: 3% OV-17 (Methyl phenyl Silicone) on ChromosorbWHP

Mesh: 100-120; Dimensions: 1.5×4mm; Temperature Programing (InitialTemp. 70ºC; Initial Time. 5min; Rate 8ºC/min; Final Temp. 200ºC; Final Time 40min; Injector 250 ºC; Detector 300 ºC. Gases Flow Rate($N_2$30 ml/ min; H_2 33 ml/ min; Air 330 ml/ min).

Effect of mint oil on the growth of pathogenic fungi

Different concentrations of mint oil i.e., 1.5%, 3% and 4.5 % were prepared in PDA medium either *Fusarium oxysporum* or *Rhizocotinia solani*(B) (6mm in diameter) was put in the middle of each Petri dish. Control dishes included PDA medium only and three dishes were used as replicates for each treatment. All dishes were incubated at 25ºC until the control dish was full with each fungal growth.

RESULTS

The fungicidal effect of mint oil.

Mint oil at the concentrations of 1.5, 3 and 4.5 % was tested against either *Fusarium oxysporum or Rhizoctonia solani(B)* on PDA medium. Results in

Table (1) and Figures (1&2) clear that mint oil at all tested concentrations significantly reduced the growth of both pathogens. Growth reduction of *F. oxysporum* was increased from 51% to 86 % respectively at 1.5 and 4.5% mint oil concentrations. Reduction of *R. solani(B)* growth ranged from 63 and 83%

Gas liquid chromatographic analysis of spearmint oil. Table (2) clear that the oil of this species has 13 different derivatives. The untreated control plants

expressed all of them except Phellandrene; which present in the biofertilized treatment. The later showed the absence of Limonene and Menthone. Both Menthone and Isomentone expressed the highest concentrations (12.132 and 10.962 %) of spearmint control oil. Menthol, Limonene and 1.8– cineol respectively resulted 9.353, 9.263 and 9.115 % concentrations. The least concentration values were noticed with Caryophellene (1.072 %) and Pulegone (1.809 %).

Effect of bioagent on oil content.

Biofertilizer treatment resulted so high Menthol (40.906 %) and Carvone (20.145 %). The least component values (0.210 and 0.935 %) were respectively recorded with Caryophellene and Pulegone derivatives.

On the other hand; peppermint oil expressed different 13 derivatives Table (3). ά-Pinene and ß. pinene were detected in all species. Menthol showed the highest concentrations (20.087 and 15.368 %) respectively for control and biofertilized peppermint plants. This was followed by 1.8cineole (9.724 and 11.782 %), in the same respect.

Phellandrene concentrations were 10.333 and 10.255 % respectively. The least values were recorded with Limonene (1.804 and 0.325 %) for the control and biofertilized plants respectively. These percentages were 3.853 and 2.481 % for Caryophyllene, in the same respect.

Table (1): Effect of different concentrations of mint oil on the mycelial growth diameter (cm) of *Fusarium oxysporum* and *Rhizoctonia solani(B)* (cm).

Mint oil %	***Fusarium oxysporum***	**R%**	***Rhizocotinia solani (B)***	***R%**
1.5	4.25	51	3.22	63
3.0	2.98	66	2.97	66
4.5	1.18	86	1.43	83
Control	8.67		8.63	
L.S.D at 5%	0.24		0.35	
L.S.D at 1%	0.35		0.51	

Colony reduction % compared with control.% :R*

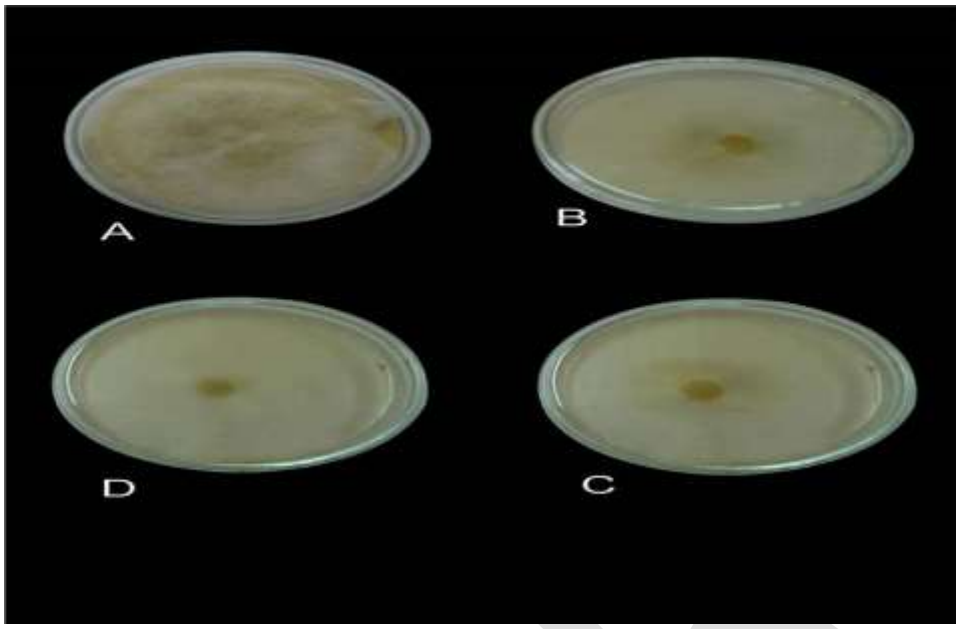

Figure (1): Effect of different concentrations of mint oil in PDA medium on the growth of *Fusarium oxysporm*

A. 0 (control) B. 1.5%

C. 3% D. 4.5%

Notice: there is no growth at all at the concentration of 4.5% mint oil.

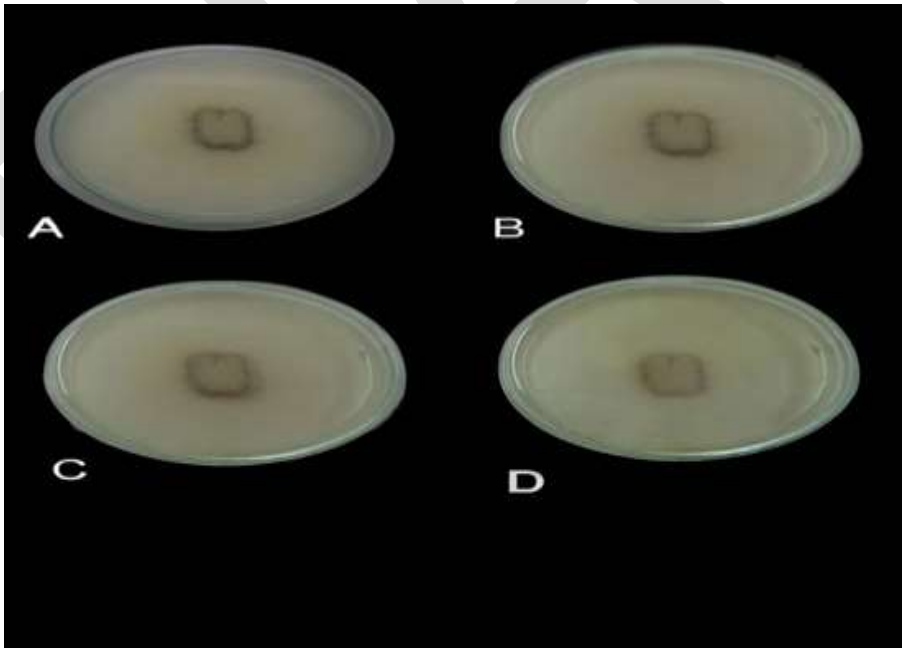

Figure (2): Effect of different concentrations of mint oil in PDA medium on the growth of *Rhizocotonia solani (B).*

A. 0 (control) B. 1.5%

C. 3% D. 4.5%

Notice: that there is no growth at all at the concentration of 4.5% mint oil

Table (2): Gas chromatographic analysis of spearmint oil extracted from the untreated and plants treated with bioagents (*Trichoderma harzianum* + *Bacillus subtalus11* against *Rhizocotonia solani (B)*).

Component	Untreated control			Biologically fertilized		
	RT(min.)	Height	Conc.%	RT(min.)	Height	Conc.%
ά- Pinene	4.283	34.069	1.314	4.050	4.263	0.783
β - Pinene	5.033	4.567	0.216	5.933	8.123	2.020
1.8- Cineole	8.400	107.015	9.115	8.650	37.712	7.728
Limonene	9.017	107.032	9.263	--	--	--
Pulegone	10.300	41.599	1.809	10.300	4.323	0.935
Menthol	13.283	107.155	9.353	13.183	107.319	40.906
Iso menthol	15.333	98.979	6.458	14.267	3.453	1.158
Menthone	14.400	107.188	12.132	--	--	--
Isomenthone	16.550	107.250	10.962	15.117	4.801	1.141
Menthylacetate	15.800	107.228	4.958	15.600	51.569	8.131 20.145
Carvone	17.633	102.868	5.569	15.950	95.058	0.210
Caryophellene	18.717	28.446	1.072	19.067	1.505	5.811
Phellandrene	--	--	--	7.933	28.149	

Table (3): Gas chromatographic analysis of peppermint oil extracted from the untreated and plants treated with bioagents (*Trichoderma harzianum +Bacillus subtalus11* against *Rhizocotonia solani (B)*).

Component	Untreated control			Biologically fertilized		
	RT(min.)	Height	Conc.%	RT(min.)	Height	Conc.%
ά- Pinene	3.950	103.840	4.750	3.950	64.793	2.866
β - Pinene	5.900	102.775	8.175	5.850	105.651	6.944
Phellandrene	8.017	100.528	10.333	7.983	106.036	10.255
1.8- Cineole	8.667	99.838	9.724	8.650	106.156	11.782
Limonene	9.000	39.048	1.804	9.583	8.471	0.325 5.866
Pulegone	10.050	98.370	6.627	9.967	106.393	15.368
Menthol	13.650	88.060	20.087	13.433	107.019	4.287
Menthone	13.883	85.270	4.214	13.833	53.148	5.270
Iso menthol	14.933	79.650	4.363	14.833	68.697	4.573 5.969
Menthylacetate	15.633	72.416	5.962	15.567	107.404	3.557
Isomenthone	15.917	69.488	3.587	15.867	107.458	2.481
Carvone	17.367	98.730	3.961	17.317	80.605	
Caryophellene	18.733	102.951	3.853	18.733	60.294	

DISCUSSION

The highest antioxidant properties of essential oils might be related to its phenolic contents like phenolic acids, rosmarinic acid and polyphenols as reported in a previous study (**Mimica-Dukic et al., 1998**). Therefore, the reason of the poor activity of these essential oils, probably, is due to its lack or low amount of phenolic contents; synergistic or antagonistic effect of its components (**Candan et al., 2003**). It has earlier been reported that plant phenols can behave as ROS (Reactive Oxygen Species) scavengers, metal chelators and enzyme modulators and prevent lipid peroxidation **(Rodrigo and Bosco, 2006**). The results show a difference in the contents of the essential oil of two mint species, out of polyphenol, flavonoides and tannins. M. piperita is richest in these compounds and shows stronger antioxidant activity with respect to M. spicata. The present study confirmed the antioxidant activity of two mint species.

Mint oil at the concentrations of 1.5, 3.0 and 4.5 % of PDA medium significantly reduced the growth of *F. oxysporum* and *Rhizoctoniasolani(B)*; as compared with control. The fungicidal effect of mint oil was also reported by **Imai *et al.,* (2001)**. Application of suitable concentration of mint oil to the seeds or seedlings could be useful and safe method to control soil borne pathogens as reported by **Pereira *et al.,* (2006)**.

Gas liquid chromatographic analysis proved that spearmint and peppermint oils had 11 and 12 different derivatives. β-pinene was detected in peppermint oil but not of the other species. On the other hand; application of the biocontrol agents to the soil of spearmint species led to the presence of Phellandrene and absence of both Limonene and Menthone these treatments showed so high Mentol and Carvone in spearmint oil. Menthol also showed the highest concentration derivative of peppermint oil; both for the untreated and bioagent treated samples.

Gas liquid chromatographic analysis of peppermint oil expressed different 13 derivatives. ß-pinene was detected in this species but not in spearmint one. Menthol showed the highest concentration (20.087 and 15.368) respectively for control and biofertilized peppermint plants which followed by 1.8 cineole (9.724 and 11.782 %) in the same respect and Phellandrene concentrations were 10.333 and 10.255 respectively. The least values were recorded with Limonene (1.804 and 0.325 %) for the control and bioagent treated plants respectively

CONCLUSION

Mint species are used widely throughout the world as an important medicinal plant. Their oils are one of the most popular and widely used essential oils, mostly because of its main components such as menthol and carvone. Menthol showed the highest concentration (20 . 087 and 15 . 368) respectively for control and biofertilized peppermint plants which followed by 1.8 cineole (9.724 and 11.782 %) in the same respect and Phellandrene concentrations were 10.333 and 10.255 respectively . The least values were recorded with Limonene (1.804 and 0.325 %) for the control and bioagent treated plants respectively.

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