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Characterization of the harmful effect of olive mill wastewater on spearmint

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ABSTRACT

In this study, changes in viability, biomass production, essential oil yield and essential oil composition of *Mentha spicata* L. (spearmint) exposed to olive mill wastewater (OMW) were investigated. Spearmint cuttings were sensitive to OMW and, after 6 h of incubation in raw or diluted OMW, their viability was null. The short contact of raw OMW with mint cuttings caused an irreversible damage in rhizogenesis and shoots development. Roots were more sensitive to phytotoxicity than shoots. In a field essay, spearmint showed a good capability to recover when OMW was spread at 8 l m^{-2} at the vegetative phase of growth (45 days after plantation). At this dose, a slight increase of mostly of the mint essential oil constituents was obtained. When the dose applied was 16 l m^{-2}, phytotoxicity was manifested by a high reduction of biomass and essential oil yield. The essential oil composition was also affected and a disappearance of many of mint essential oil constituents was observed with an increase of 59% for carvone, the major compound of spearmint essential oil. As far as we know, this is the first report on the effect of field application of OMW on an aromatic plant essential oil yield and composition.

1. Introduction

Olive oil production is an important and constantly growing economic activity in Mediterranean countries responsible for 98% of the entire worldwide olive oil production. In addition to oil, this activity produces huge quantities of liquid and solid wastes during a short period of time (November–March). The liquid effluent called OMW (olive mill wastewater) is characterized by a high load of recalcitrant organic matter (COD of 45–220 g l^{-1}) and phenolic compounds (0.5–24 g l^{-1}), a low pH (3.5–5.5) and a high salinity depending essentially on olive oil extraction process [1].

Because of its special characteristics, OMW treatment and valorization are serious problems for Mediterranean countries producing annually around 30 million m^3 of OMW [2]. Even though many physicochemical and biological treatments were suggested, their practical application is generally limited because of the high cost of the treatment. Nowadays, the principal destinies of OMW are direct spreading to agricultural soils and storage in evaporation ponds. OMW spreading to agricultural soils could be a successful way of OMW valorization if spreading is done in controlled conditions with convenient doses [3–6]. Unfortunately, uncontrolled spreading of OMW is frequent in rural areas of Morocco especially in absence of a law dressing the spreading modalities. Uncontrolled spreading of OMW could be harmful by causing disequilibrium on soil nutrients [7], immobilization of soil nitrogen [6] and repression of soil fungi and nitrifiers [8]. Concerning plants, uncontrolled OMW application could inhibit germination of seeds [2,5], growth of early plants [9–11] and chlorophylls synthesis [5]. Recently, Ouzounidou et al. [12] reported that OMW application caused for tomato a significant restriction of absorption and translocation of K, Na, Fe, Ca and Mg, a decrease in the photochemical efficiency of PSII and a big decrease in photochemical quenching.

OMW phytotoxicity was essentially linked to its high phenolic compounds content [2,5,11] that are the modified forms of phenols of the olive fruit [13]. During crushing, just 2% of phenols pass into the oil, 53% pass into OMW and 45% into pomace [14]. OMW contains a very high diversity of monophenolic and polyphenolic compounds such as hydroxytyrosol, tyrosol, caffeic acid, *trans*-cinnamic acid, 4-hydroxyphenylethyl alcohol, verbascoside, 3,4-dihydroxybenzoic acid and luteolin [13,15]. The phenols content in OMW is characterized by its high variability depending on culture conditions in orchards, degree of ripeness of olive fruit, climate conditions, storage conditions, oil extraction process etc. [13,15]. Even though phenols are considered as the principal cause of OMW phytotoxicity, Capasso et al. [10] demonstrated that OMW remained phytotoxic to vegetable marrow and tomato plants even after total extraction of the polyphenols, suggesting that other chemical products contribute to the overall phytotoxicity. Pérez et al. [9] reported that deionized OMW, or OMW for which organic matter is totally

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removed, is still phytotoxic to barley and tomato germination and early plant growth. Theses results suggest that other factors contribute to the global stress caused by OMW against plants.

Studies interested in OMW phytotoxicity showed that OMW stress could disturb different functions in the plant such as germination, growth and photosynthesis [2,5,9–12]. For aromatic plants, essential oil yield and composition are other parameters reflecting the stress the plant could endure [16], such as infection by pathogens [17] and saline stress [18].

Mentha spicata L., also called spearmint, soft mint or Moroccan mint, is an aromatic plant widely cultivated in Morocco and potentially exposed to an OMW spreading. Spearmint essential oil is principally concentrated in leaves [18,19] and its composition is widely affecting spearmint value for pharmaceutical and fragrance industries.

The purposes of this work are to test the degree of sensibility of spearmint to OMW characterized by a high load of phenols and a high salinity, and to investigate the expression of OMW phytotoxicity against spearmint biomass and essential oil yields and composition in a field experiment.

2. Material and methods

2.1. OMW characterization

OMW was obtained from a semi-modern press process (3 phase unit) of olive oil production (Abbou mill, industrial district of Dokkarat, Fez, Morocco). OMW physicochemical characterization is showed in Table 1. COD (chemical oxygen demand) was determined according to standard micromethod with a COD meter HACH [20] and BOD_5 (biological oxygen demand), with the manometric method using a respirometer OXI TOP IS6 [20]. Total phenols were determined by using the colorimetric method with Folin–Ciocalteau reagent [21]. Total nitrogen, total phosphorus and fats were determined according to Rodier [20].

2.2. Effect of OMW on spearmint viability and biomass production

Flasks of 250 ml containing 100 ml of OMW in different dilutions [1/4, 1/2, 3/4, ND (OMW not diluted)] were prepared. Contents of phenols in these solutions are 1.82 g l^{-1}, 3.65 g l^{-1}, 5.47 g l^{-1} and 7.30 g l^{-1} respectively. Control assay was distilled water. Fresh defoliated basal spearmint cuttings of 12 cm were soaked basipedly to nearly the half of their height in flasks as 210 cuttings for each OMW dilution. At different times since their soaking (1/2 h, 1 h, 2 h, 3 h, 4 h, 6 h and 8 h), 30 cuttings were taken from each OMW dilution and from the control (distilled water), rinsed with water and planted in crates of (5 cm × 3 cm × 4 cm) full of soil as one cutting per crate. Soil added to fill the crates was previously air dried and sieved through 4 mm. Soil pH is of 7.82 ± 0.04 (at 25 °C), its conductivity of 0.21 ± 0.01 (mS cm^{-1} at 25 °C), and its humification degree of 10.68 ± 2.96%. The soil humification degree was determined using Tiurin method [22]. The soil is a lime made of 51.16 ± 5.88% of sand, 32.56 ± 3.92% of silt and 16.25 ± 1.96% of clay. After cuttings plantation, crates have received equally suitable water irrigation. After 15 days and 30 days from plantation, 5 spearmint plants were taken from each lot to observe roots development; also the percentage of viability of spearmint planted cuttings and dry weight of developed roots and shoots were determined.

Table 1
OMW physicochemical characterization.

Parameter	Average value
pH (25 °C)	4.70
Conductivity (25 °C) (mS cm^{-1})	14.90
COD (gO_2 l^{-1}).	120.00
BOD_5 (gO_2 l^{-1})	67.50
Total phenols (g l^{-1})	7.30
Total N (g l^{-1})	0.30
Total P (g l^{-1})	0.07
Fats (%)	2.60

2.3. OMW spreading to spearmint (field experiment)

The study area consisted in a field of Saïs valley which is a site containing 42% of Moroccan mills. The field position is 4°54′W and 34°6′N. The weather is typical Mediterranean, semiarid to arid, with an average rainfall of 400 mm $year^{-1}$ and an average annual temperature of 18–20 °C.

The field was divided into plots of 2 m^2. OMW was spread on the plots without any treatment. Plots C′, P'_1 and P'_2 were amended in April 2004 with 0 l m^{-2}, 8 l m^{-2}, and 16 l m^{-2} of OMW respectively, and were planted with non-rooted spearmint cuttings. OMW is spread in a homogenous way just before cuttings plantation. Then, all plots received water irrigation equally. Plots C, P_1 and P_2 were amended with raw OMW at 0 l m^{-2}, 8 l m^{-2}, and 16 l m^{-2} respectively, and were planted with propagated cuttings. Cuttings propagation was done for 45 days in pots containing 1 kg of a mixture 1:1 (v/v) of soil and substrate for plant cultivation (peat substrate; 25% of organic matter, pH 6.2). After 45 days, propagated cuttings roots (roots dry weight = 167.66 ± 83.01 mg) were washed with water to eliminate cultivation substrate, and they were then planted in the experimental field that freshly received OMW, as it was for non-propagated cuttings. Each plot received 45 cuttings in total, arranged in groups of three cuttings planted as bunches spaced by nearly 40 cm. After cuttings development, just one cutting is left in each planting location.

2.4. Spearmint yield determination

For each plot, weight and size of shoots, essential oil yield and weight, size and number of developed stolons were determined 118 days after cuttings plantation. Essential oil composition was then determined by GC/MS. Essential oil was produced from spearmint leaves previously dried in dark and finely crushed for hydrodistillation. The distillation apparatus consisted of a heating cap, a 1.5 l extraction flask, a cooling system and a receiver for hydro distillate. Thirty grams of dried plant leaves and 800 ml of water were used and the distillation was carried out for 3 h after the mixture reached boiling at 100 °C. Hydrodistillation repetitions were done at least in duplicate depending on the spearmint leaves availability. Essential oil was characterized using a gas chromatograph Trace GC Ultra equipped with autoinjector (Triplus) directly interfaced with a mass spectrophotometer with flame ionization detector (Pdains Q). Capillary column was VB-5 (5% of diphenyl and 95% of dimethypolysiloxane), 30 m length, 0.25 mm i.d. and 0.25 mm thickness. Separation conditions were: 25 °C for 2 min, 25–180 °C at 4 °C/min and 180–300 °C at 40 °C/min. Temperature of the injector was 220 °C. The volume injected was 1 μL. The carrier gas was helium with a flow rate of 1.4 ml min^{-1}. The oil constituents were identified by comparison of their retention indices and their mass spectra with those of authentic samples. Quantitative analysis (in percent) was performed by peak area measurement.

2.5. Effect of OMW spreading on Mentha piperita leave phenols

Cuttings (20 cm) of peppermint were planted in pots of 2 kg as three cuttings spaced by 10 cm per pot. Seven repetitions (pots) were prepared for each essay. OMW was spread to soil in pots as 9 ml 100 g^{-1} (corresponding to 2 l m^{-2}), 22.5 ml 100 g^{-1} (corresponding to 5 l m^{-2}) and 54 ml 100 g^{-1} (corresponding to 12 l m^{-2}) at the vegetative phase of growth of *Mentha piperita* L. (38 days after

Table 2
Percentage of viability of mint cuttings planted on soil after their incubation in OMW at different dilutions and for different durations ($n = 30$).

	Control	D 1/4	D 1/2	D 3/4	ND
0 h			96.66		
1/2 h	96.66	43.33	36.66	30.00	16.66
1 h	100.00	26.66	16.66	6.66	6.66
2 h	96.33	36.66	6.66	0.00	0.00
3 h	93.33	3.33	13.33	3.33	0.00
4 h	96.66	23.33	3.33	0.00	0.00
6 h	100.00	0.00	0.00	0.00	0.00
8 h	96.66	0.00	0.00	0.00	0.00

D 1/4: OMW diluted to 1/4 with distilled water; D 1/2: OMW diluted to 1/2 with distilled water; D 3/4: OMW diluted to 3/4 with distilled water; ND: not diluted OMW.

cuttings plantation). The spreading at 54 ml 100 g^{-1} caused plants death. 110 days after peppermint cuttings plantation, phenols were determined in leaves according to Shobana and Akhilender Naidu [23]. One gram of fresh leaves was finely crushed while cooled with 5 ml of ethanol/distilled water (1:1) in a mortar. The crush was centrifuged at 7.500 × *g* during 15 min. Phenols were determined in the supernatant according to Box et al. [21].

2.6. Statistical analyses

Statistical analyses were performed using software Graph pad prism, version 4. Student test was used to compare means of two samples. Statistical test was performed at 0.05 significance level.

3. Results and discussion

3.1. Effect of OMW on spearmint viability and biomass production

Incubation of spearmint cuttings in OMW at different dilutions and for different durations was toxic for spearmint. As shown in Table 2, spearmint cuttings viability was reduced as the time of incubation increased, and also as OMW was more concentrated. These results are in agreement with findings of several authors. Indeed, Della Greca et al. [11] demonstrated that OMW phytotoxicity to algae depended on the time of contact between algae and OMW. A long contact with OMW would allow phytotoxic substances existing in OMW such as phenols, organic acids and fats [10,24] to affect the structure of the membrane and to modify its functions including metabolic efficiency and stability [25,26]. Diluted OMW was less phytotoxic to spearmint cuttings viability. These findings are in agreement with data which showed that OMW dilution could be considered as a treatment that allowed a reduction of OMW phytotoxicity [2,5,12].

Incubation of spearmint cuttings in diluted OMW, with a concentration of phenols below 5.47 g l^{-1} (OMW diluted to 1/2, 1/4 and 3/4), did not causes a significant decrease in weight of roots and shoots of surviving plants whatever the time incubation (Tables 3 and 4). When spearmint cuttings were incubated in raw OMW, a net decrease of roots and shoots weights were observed for 1 h of incubation. However, after 30 min of incubation, a decrease of roots weight was observed. Theses results showed that spearmint roots were more sensitive to raw OMW than shoots that are in concordance with findings of Ouzounidou et al. [12] about effect of OMW on tomato. It can be concluded from the present survey, that a short contact of spearmint cuttings with OMW could cause an irreversible damage in rhizogenesis and shoots development.

3.2. Effect of OMW on spearmint yield (field experiment)

OMW spreading at doses of 8 l m^{-2} (plot P'_1) and 16 l m^{-2} (plot P'_2), just before implantation of spearmint non-propagated cuttings, caused cuttings wilting and death. However, control plants survived. The death of cuttings in plots receiving OMW should be a consequence of the inherent toxicity of OMW (Table 2) which could be due to phenols principally [2,10].

Spearmint showed a good capability to recover when OMW is applied at the same doses (8 l m^{-2} and 16 l m^{-2}) but at the vegetative phase of growth (45 days after cuttings propagation). At harvest, for 8 l m^{-2}, no significant differences were observed between shoots weight and height (Fig. 1a and b), essential oil yield (Fig. 1c), weight, size and number of stolons (Fig. 1d–f) and the control assay. These results are in agreement with those of Rinaldi et al. [4] who suggested that the OMW spreading at the vegetative phase of plants growth could be during the winter months, especially in semi-arid environments like Morocco. The same authors reported that wheat tolerates better spreading at the early growing

Table 3
Dry weight of roots developed after 45 days from incubation in OMW in different dilutions and for different durations ($n = 20$).

	Control	D 1/4	D 1/2	D 3/4	ND
0 h			0.013 ± 0.010		
1/2 h	0.014 ± 0.014	0.016 ± 0.015	0.037 ± 0.029	0.017 ± 0.022	0.003 ± 0.003
1 h	0.016 ± 0.013	0.008 ± 0.005	0.016 ± 0.011	0.028 ± 0.025	0.002 ± 0.001
2 h	0.012 ± 0.007	0.014 ± 0.012	0.044	0.000	0.000
3 h	0.011 ± 0.007	–	0.018 ± 0.009	0.011	0.000
4 h	0.011 ± 0.006	0.014 ± 0.006	–	0.000	0.000
6 h	0.013 ± 0.011	0.000	0.000	0.000	0.000
8 h	0.012 ± 0.008	0.000	0.000	0.000	0.000

D 1/4: OMW diluted to 1/4 with distilled water; D 1/2: OMW diluted to 1/2 with distilled water; D 3/4: OMW diluted to 3/4 with distilled water; ND: not diluted OMW.

Table 4
Dry weight of shoots developed after 45 days from incubation in OMW in different dilutions and for different durations ($n = 20$).

	Control	D 1/4	D 1/2	D 3/4	ND
0 h			0.104 ± 0.055		
1/2 h	0.094 ± 0.044	0.082 ± 0.049	0.075 ± 0.065	0.060 ± 0.036	0.041 ± 0.026
1 h	0.128 ± 0.070	0.017 ± 0.063	0.068 ± 0.051	0.000	0.009 ± 0.004
2 h	0.124 ± 0.063	0.070 ± 0.059	0.051	0.023	0.000
3 h	0.100 ± 0.038	–	0.066 ± 0.041	0.000	0.000
4 h	0.096 ± 0.047	0.079 ± 0.049	–	0.000	0.000
6 h	0.114 ± 0.061	0.000	0.000	0.000	0.000
8 h	0.092 ± 0.046	0.000	0.000	0.000	0.000

D 1/4: OMW diluted to 1/4 with distilled water; D 1/2: OMW diluted to 1/2 with distilled water; D 3/4: OMW diluted to 3/4 with distilled water; ND: not diluted OMW.

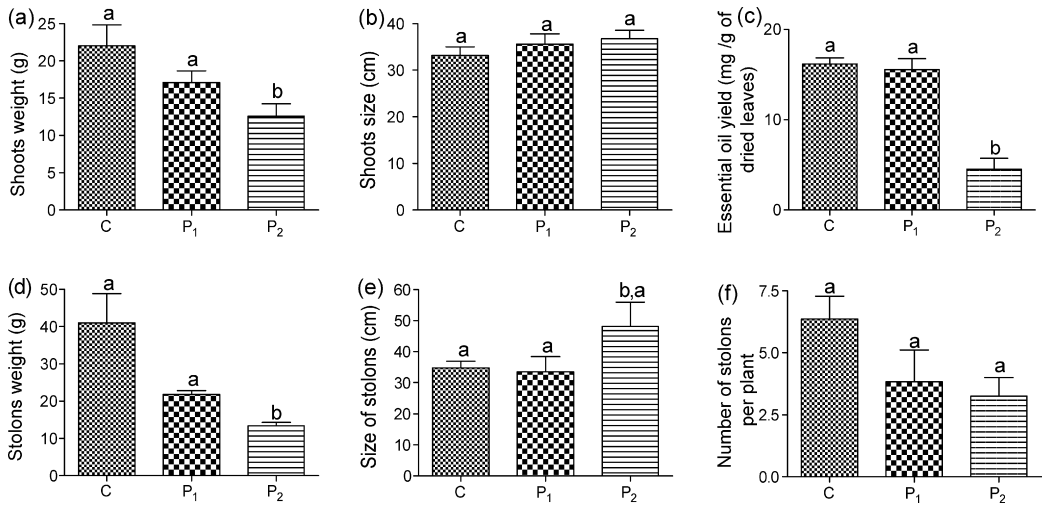

Fig. 1. Effect of OMW spreading at doses 8 l m^{-2} (P_1) (n = 14) and 16 l m^{-2} (P_2) (n = 7) on spearmint shoots weight (a), height (b), essential oil yield (c), stolons weight (d), stolons size (e) and number of stolons per plant (f) in comparison with control (n = 11). Small letters refer to significant differences (P < 0.05) among data.

stages (stage 4 leaves) than in the stage 6 leaves after which leaf formation had finished. However, for spearmint the production of leaves lasts throughout all the vegetative phase of growth as new leaves grow and old leaves drop. This means that spearmint would be indifferent to the timing of OMW implication in the vegetative phase of growth if the roots system could endure the spreading.

OMW spreading at 16 l m^{-2} (P_2) caused a decrease in shoots weight (Fig. 1a) without decrease in their height (Fig. 1b), essential oil yield was deeply reduced (Fig. 1c), the number of stolons did not change (Fig. 1f) but their weight decreased (Fig. 1d) and their size increased (Fig. 1e). Even if spearmint plants were 45 days at OMW spreading, application of the high dose of 16 l m^{-2} caused an important decrease in spearmint yields. Mekki et al. [5] reported that OMW applied at 40 l m^{-2} at the vegetative phase of growth of tomato (*Lycopersicon esculentum*), chickpea (*Cicer arietinum*), bean (*Vicia faba*), wheat (*Triticum durum*) and barley (*Hordeum vulgare*) caused a significant decrease in biomass yield, chlorophylls a and b, and total proteins. Yields reduction obtained for the spreading of 16 l m^{-2} should be partly due to soil compactness, causing impairment in soil aeration and limiting roots development [5], immobilization of soil nitrogen [6] and imbalances on soil micronutrients [7,12] limiting biomass and essential oil yield of mint crops [27]. OMW characterized by a high salinity [[24], Table 1] could reduce yields of biomass and essential oil of spearmint that have a fairly low tolerance level to salinity [16] probably by inhibiting supply of cytokinin from roots to shoots, and thus it could alter the ratio between leaf of cytokinin and abscisic acid [16]. The stress the plant could endure, caused in our case by OMW spreading, increases some enzymes in the plant, like the guaiacol peroxidase, which contributes to the restriction in cell expansion and causes growth limitation [28]. Fiorelli et al. [29] showed that OMW is a favorable medium for auxin production by *Azotobacter vinelandii* that should explain the elongation of stolons obtained for the spreading of 16 l m^{-2}.

3.3. Effect of OMW on spearmint essential oil composition

OMW spreading caused a dose–response change in spearmint essential oil composition (Appendix A and Table 5). OMW spread at 8 l m^{-2} caused an increase in almost all the spearmint essential oil constituents, in comparison with the control assay (Table 5). The increase in comparison with the control was of 20% of limonene, 38% of carvyl acetate, 34% of carveol acetate and 16% of dihydrocarvyl acetate. However, a decrease of 13% of carvone and 81% of carveol was obtained.

When the amount spread was 16 l m^{-2}, a general decrease and even a disappearance of many of the essential oil constituents was observed (Appendix A and Table 5). We registered a decrease of 12% of limonene, 26% of dihydrocarvone, 94% of dihydrocarvyl acetate and 90% of carveol acetate. Compounds as camphene, p-menth-8-en-2-ol, carveol, pulegone and carvyl acetate have totally disappeared. However, an increase of 59% was obtained for carvone, the major compound of the spearmint essential oil. Some compounds such as 1-octen-3-ol, terpinene and sabinene hydrate

Table 5
Effect of OMW spreading on the percentage of major volatile oil content of fresh leaves of *Mentha spicata*. C: control (plot not receiving any OMW), P_1: plot receiving OMW at 8 l m^{-2}, and P_2: plot receiving OMW at 16 l m^{-2}.

Compound	Retention time	C	P_1	P_2
α-Pinene	8.90	0.47	0.67	0.23
Camphene	9.39	0.07	0.16	–
β-Phellandrene	10.31	0.39	0.52	0.34
1-Octen-3-ol	10.56	–	–	0.30
β-Pinene	10.99	1.37	2.10	0.29
Limonene	12.30	8.42	10.11	7.37
1,8-Cineole	12.36	5.95	5.48	7.77
Cis-Ocimene	12.71	0.09	0.17	–
Ocimene y	13.07	0.06	0.07	–
Terpinene	13.42	–	–	0.15
Sabinene hydrate acetate	13.69	–	–	0.50
1-Octen-3-ol acetate	15.42	0.12	0.14	0.16
3-Octanol acetate	15.85	0.28	0.34	–
Borneol	17.23	0.41	0.32	0.71
Sabinene hydrate	17.66	–	–	0.43
p-Menth-8-en-2-ol	18.28	6.52	6.88	–
Dihydrocarvone	18.32	3.44	3.40	2.53
Carveol	19.12	2.27	0.42	–
p-Mentha-6,8-dienol	19.53	0.20	0.12	–
Pulegone	19.80	0.19	0.15	–
Carvone	19.98	44.94	38.61	70.01
Bornyl acetate	21.45	0.33	0.34	–
iso-Limonene	22.18	0.70	0.75	–
Dihydrocarvyl acetate	22.87	15.40	17.93	0.87
Carvyl acetate	23.17	0.57	0.79	–
Carveol acetate	23.99	2.73	3.68	0.27
Bourbonene	24.75	0.58	0.88	1.49
Caryophyllene	25.83	1.07	1.70	0.75
Humulene	26.88	0.11	0.17	–
Germacrene D	27.74	1.08	1.70	2.36
Calamenene	28.96	0.14	0.22	0.29

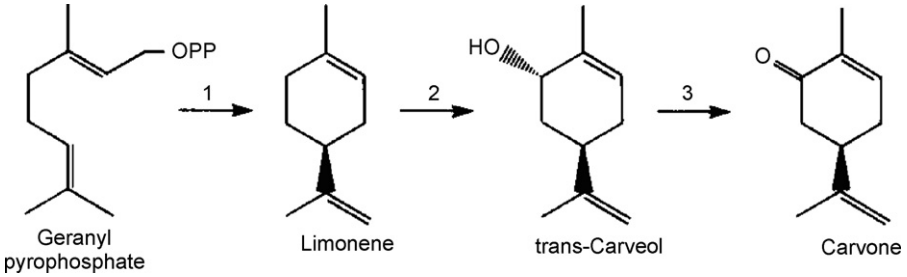

Fig. 2. Pathway for the biosynthesis of carvone from geranyl pyrophosphate in spearmint according to Gershenzon et al. [19]. The enzymes involved are geranyl pyrophosphate: limonene cyclase (1), limonene hydroxylase (2), and trans-carveol dehydrogenase (3).

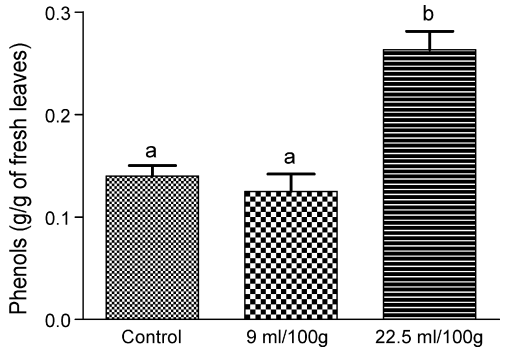

Fig. 3. Effect of OMW spreading on *Mentha piperita* L. leaves phenols.

acetate appeared in the chromatograph of the essential oil when the amount spread was 16 l m^{-2}.

Carvone is the major compound of spearmint essential oil. It is widely decisive for the value of the essential oil, for fragrance and pharmaceutical industries. Carvone is an oxygenated monocyclic ketone highly odoriferous and valuable, accumulated in spearmint glandular trichomes specially [18]. An increase of carvone quantity in the essential oil as obtained for the dose 16 l m^{-2} is considered benefit. Carvone is biosynthesized by a three-step pathway (Fig. 2) where limonene is considered as its precursor [16,18]. OMW spreading caused a differential conversion of limonene to carvone. Limonene/carvone ratio values were of 0.187, 0.261 and 0.105 for C, P_1 and P_2, respectively. OMW spreading as 8 l m^{-2} reduced limonene conversion to carvone while spreading as 16 l m^{-2} oriented the pathway to carvone synthesis. OMW spreading that causes an increase in phenols loads in leaves (Fig. 3), probably due to the absorption of OMW phenols by roots [30], would affect oxygen activity in leaves, therefore it affects enzymatic reactions, undergoing in essential oil synthesis especially by modifying precursors [31] and genes expression [32]. Some enzymes are activated while plants are on stress, like lipoxygenases [33], and undergoing in synthesis of essential oil terpenes [34] should also be effective.

OMW spreading at 8 l m^{-2} caused a more intensive conversion of carvone into its acetate derivatives (carvyl acetate, dihydrocarvylacetate, carveol acetate) and a decrease of reduction to its derivatives carveol and dihydrocarvone (Table 5) while OMW spreading at the dose of 16 l m^{-2} caused an opposite action by a reduction of reduced forms of carvone and an increase in its acetate forms.

4. Conclusion

Results from this investigation showed that cuttings plantations should be the most sensitive to OMW phytotoxicity in comparison with seeds, young plants or trees as reported in previous studies [4,5,35]. OMW phytotoxicity to spearmint was dependent on time of contact, OMW dilution, age of the plant and applied OMW dose. The harmful effect of spreading a high dose of OMW was expressed by a deep reduction of essential oil yield and a reduction or disappearance of many of essential oil constituents. For spearmint fields, farmers should avoid any direct contact of OMW with spearmint cuttings because a very short time of contact can cause damage. They also have to avoid any spreading of OMW to newly planted non-propagated cuttings. OMW could be implicated at the vegetative phase of plants aged of more than 45 days and with a spread dose lower than 8 l m^{-2}, since an OMW spreading at high doses could affect negatively spearmint biomass and essential oil yields.

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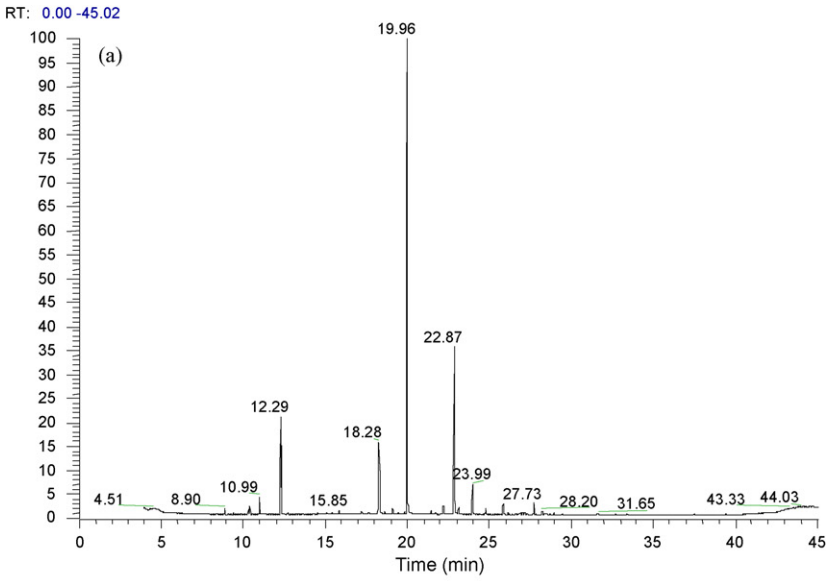
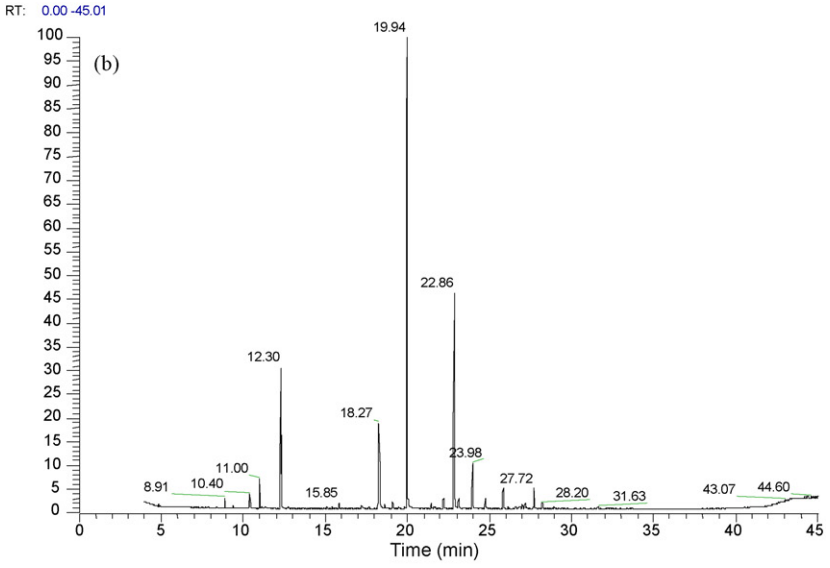
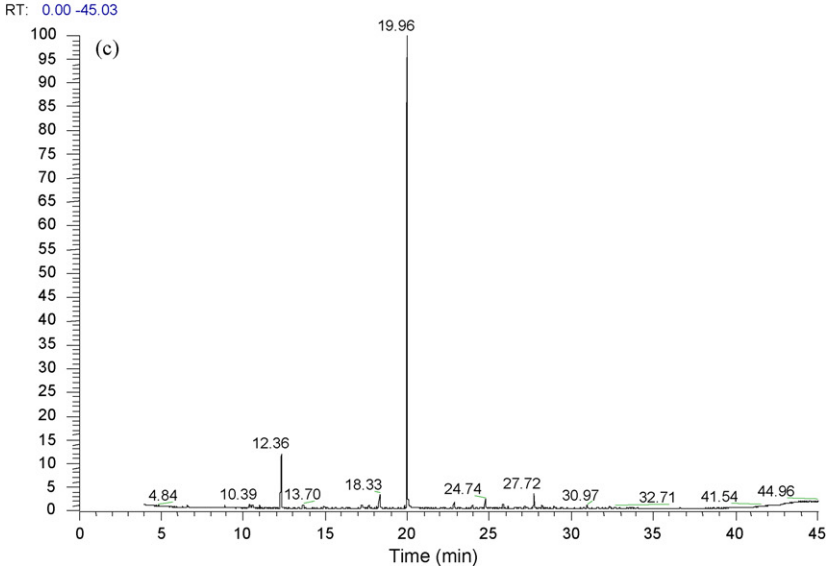
Appendix A.

Comparison of GC/MS profiles of the spearmint essential oil obtained in control assay (a), field receiving OMW at 8 l m^{-2} (b), and field receiving OMW at 16 l m^{-2} (c). RT = retention time.


RT: 0.00 -45.02
(a)
19.96
22.87
12.29
18.28
23.99
4.51
8.90
10.99
15.85
27.73
28.20
31.65
43.33
44.03
Time (min)


RT: 0.00 -45.01
(b)
19.94
22.86
12.30
18.27
23.98
8.91
10.40
11.00
15.85
27.72
28.20
31.63
43.07
44.60
Time (min)


RT: 0.00 -45.03
(c)
19.96
12.36
4.84
10.39
13.70
18.33
24.74
27.72
30.97
32.71
41.54
44.96
Time (min)

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