Yield and composition of the essential oil of *Mentha piperita* L. (Lamiaceae) grown with biosolid

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This research evaluated the effects of biosolid levels on yield and chemical composition of *Mentha piperita* L. essential oil. Mint plants were grown in a greenhouse in pots containing the equivalent to 0, 28, 56, and 112 $t.ha^{-1}$ biosolid. Three evaluations were made at 90, 110, and 120 days after planting (DAP). The oil was extracted from the dry matter of shoots by hydrodistillation, and composition was determined by GC/MS. Oil production was slightly affected by the biosolid, increasing when plants were grown with 28 $t.ha^{-1}$, a condition which did not result in quality improvement. Menthyl acetate was the component obtained at the highest percentage in all treatments. At 90 DAP, plants showed a higher percentage of menthol, the second-highest oil constituent, with a content of 42.3% in plants grown without biosolid. The presence of biosolid favored menthofuran formation. As with menthol, menthone decreased with plant development. Under these conditions, plant harvesting is recommended at 90 DAP, period in which the menthol level was higher. Since the production of biosolid is on the rise, a suitable destination must be given to it, and restrictions exist for its use in relation to the environment and plants. Thus, although cultivation with 28 $t.ha^{-1}$ is within the limits allowed by law, such a rate, which increased oil yield, did not improve oil quality. Therefore, biosolid from the Barueri Station is not recommended for cultivation of this specie.

Key words: menthol, menthyl acetate, peppermint, sewage-sludge.

Rendimento e composição do óleo essencial de *Mentha piperita* L. (Lamiaceae) cultivada com biossólido: Esse estudo avaliou os efeitos dos níveis de biossólido no rendimento e na composição química do óleo essencial de *Mentha piperita* L. Plantas de menta foram cultivadas em casa de vegetação em vasos contendo o equivalente a 0, 28, 56 e 112 $t.ha^{-1}$ de biossólido. Três avaliações foram realizadas aos 90, 110 e 120 dias após plantio (DAP). O óleo foi extraído da matéria seca da parte aérea por hidrodestilação e sua composição determinada por CG/EM. Sua produção foi afetada de modo discreto pelo biossólido, aumentando quando as plantas foram cultivadas com 28 $t.ha^{-1}$, condição que não resultou em sua melhora de qualidade. O acetato de mentila foi o componente encontrado em maior porcentagem em todos os tratamentos. As plantas apresentaram aos 90 DAP maior percentagem de mentol, segundo maior constituinte do óleo, com conteúdo de 42,3% naquelas cultivadas sem biossólido. A presença do biossólido favoreceu a formação de mentofurano. Como o mentol, a mentona diminuiu com o desenvolvimento das plantas. Nessas condições, recomenda-se sua colheita aos 90 DAP, época em que o nível de mentol foi maior. Com o aumento de produção de lodo de esgoto, há necessidade de sua adequada destinação, havendo restrições para seu uso em relação ao ambiente e à planta. Assim, apesar de o cultivo com 28 $t.ha^{-1}$ estar dentro do permitido pela legislação, essa condição, que aumentou o rendimento do óleo, não melhorou sua qualidade. Assim, o biossólido da Estação de Barueri não foi recomendável para o cultivo da espécie.

Palavras-chave: acetato de mentila, hortelã-pimenta, lodo de esgoto, mentol.

INTRODUCTION

Known as mint or peppermint, *Mentha piperita* L. is used for medicinal and food purposes (Lorenzi and Matos, 2002). Its cultivation has economic importance, due to its ability to produce and store essential oil, whose main constituent is menthol, used in oral hygiene products, pharmaceuticals, cosmetics, and foods. Menthol also has high antifungal and antibacterial potentials, thus becoming one of the most demanded substances by the scents and essences industry (Souza et al., 1991). Because of this and other reasons, peppermint essential oil ranks high in terms of total sales volume (Moraes, 2000).

Scora and Chang (1997) stated that mint is a plant that can become easily adapted to a variety of climate and soil conditions. However, Veronese et al. (2001) mentioned that mint and mint oil yield are modified by biotic and abiotic factors. This statement is supported by some agronomic studies recorded in the literature, especially those investigating the influence of fertilization on the development of this species. Piccaglia et al. (1993) grew mint plants at different nitrogen levels and observed an increase in essential oil yield. On the other hand, Praszna and Bernath (1993) verified a decrease in essential oil content when *M. piperita* L. plants were grown in the absence of potassium.

The number of studies on the use of biosolid is presently increasing; this is a solid residue resulting from sewage treatment, which is used for soil fertilization; this alternative allows minerals and organic matter present in the biosolid to be recycled (Melo and Marques, 2000). Tsutya (2000) stated that the presence of biosolid is beneficial to soil structure and fertility, which contributes toward the development and productivity of plant species cultivated in such soils.

According to Czepak (1998), the higher the dry matter yield of plants the higher their essential oil yield. Garcia et al. (2000) verified biomass increases in several herbaceous species grown in the presence of biosolid, at levels of 40 and 80 t.ha^{-1}. Zubillaga and Lavado (2002) recorded that the addition of biosolid increased lettuce biomass by 20 to 40%.

In spite of the fact that some literature records indicate biomass increases with the use of biosolid, restrictions for its agricultural use must be taken into account, because as it comes from sewage collected in metropolitan regions, it could present compounds that are toxic to plants (Bugbee, 2002), such as heavy metals, which, at high contents could be detrimental for the development of some crops (Berton, 2000). On the other hand, this material may contain several other toxic contaminants like detergents, insecticides, and pathogens, presently considered harmful to human and environmental health (Melo and Marques, 2000). In order to be used, biosolid must previously conform to rules set by CETESB (Companhia de Tecnologia de Saneamento Ambiental). Thus, it is assured that sewage will not cause damages to the environment and humans (Straus, 2000).

M. piperita L. plants grown in soil with increasing Cd concentrations of 0.12 to 6.1 mg.kg^{-1} as a consequence of successive biosolid applications did not present alterations in biomass and essential oil composition (Scora and Chang, 1997). However, Zheljazkov and Nielsen (1996) verified a reduction in fresh matter and oil yield in mint grown in soil from a mining company, with excessive amounts of Cd, Pb, Cu, Mn, and Zn. In spite of these contradictory results, the authors agree that *M. piperita* L. can be considered a phytoremediator in soils contaminated with heavy metals, due to the ability of developing without accumulating these metals in its tissues or in its essential oil.

It is important to consider the necessity for studies showing the maximum limits of tolerance of different crops to the presence of biosolid. Thus, the present study had the objective of evaluating yield and chemical composition of the essential oil of *M. piperita* L. plants grown under different biosolid levels.

MATERIAL AND METHODS

The experiment was conducted between February and June 2003. Stem fragments approximately 10 cm long of *M. piperita* L. from Departamento de Produção Vegetal of Faculdade de Ciências Agronômicas of UNESP, Botucatu Campus, SP, Brazil, were placed and maintained in a KNO_3 solution at a concentration of 0.6 g.L^{-1} during 15 days for rooting (Soares and Sacramento, 2001). Two seedlings per pot (5.5 L capacity) were then transferred and maintained in a greenhouse at a mean maximum temperature of 32.5°C and a mean minimum temperature of 13.6°C, mean relative humidity of 66%, and mean global solar radiation of 234.78 cal.cm^{-2}.day^{-1}, at the Departamento de Botânica of the Instituto de Biociências, UNESP, Botucatu Campus, São Paulo, Brazil.

A dark red dystrophic Latosol treated with lime was used to prepare the cultivation substrate. The biosolid used (table 1) in the experiment was obtained from the Barueri Sewage Treatment Station (STS), in the metropolitan region of São Paulo, SP, Brazil.

The completely randomized experimental design consisted of four treatments, which corresponded to biosolid levels, three replicates, and three harvesting times, performed at 90, 110, and 120 days after planting (DAP).

Among NPK macronutrients in the Barueri STS biosolid, K was at the lowest concentration and was used as a reference to define treatments. The lowest biosolid level contained the amount of K recommended for conventional mint fertilization. Based on this, the treatments, characterized by different biosolid levels at which plants were grown were denominated 0 t.ha^{-1}, a control prepared based on chemical fertilization, according to specifications by Van Raij et al. (1996); 28 t.ha^{-1}, a treatment containing the equivalent to 28 t.ha^{-1} biosolid (70 g biosolid / 5 kg dirt); 56 t.ha^{-1} biosolid (140 g biosolid / 5 kg dirt), and 112 t.ha^{-1} biosolid (280 g biosolid / 5 kg dirt).

Total dry matter in plant shoots was determined at three harvesting periods after drying in a forced aeration oven, at a temperature of 35°C until constant dry matter was achieved. The dry material was then submitted to hydrodistillation in a Clevenger-type device for two hours in order to extract the oil, allowing oil yield to be calculated as mL.100 g^{-1} dry matter (DM).

The essential oil's chemical composition was analyzed in a gas chromatograph attached to a Shimazdu model QP-500 mass spectrometer (GC/MS) with a DB-5 (30 m × 0.25 mm × 0.25 μm) capillary column, using He as carrier gas (flow 1.0 mL.min^{-1}), with the injector at 240°C and the detector at 230°C in the following program: 50°C (5') – until 160°C, 3°C per minute, 1/35 split. Two mg oil were diluted into 1 mL ethyl acetate, of which 1 μL was injected. In order to identify the substances, the mass spectra were compared with those obtained from the GC/MS system database (Nist. 62 Libr.) and from the literature (McLafferty et al., 1989); Kovats retention index was determined, by comparing the data with those found in the literature (Adams, 1995).

Table 1. Composition of biosolid obtained from the Barueri Sewage Treatment Station (STS), in the metropolitan region of São Paulo, SP, Brazil, used to grow *M. piperita* L. plants.

Components (dry matter)	Contents
Total - OM (%)	54.64
Compost - OM (%)	52.52
Total N (%)	3.27
Total P (%)	3.27
Total K (%)	0.27
Organic C (%)	29.2
Ca (%)	2.52
Mg (%)	0.49
S (%)	0.66
Cu (mg kg^{-1})	570
Fe (mg kg^{-1})	39,320
Zn (mg kg^{-1})	2,381
Mn (mg kg^{-1})	194
B (mg kg^{-1})	9.0
Na (mg kg^{-1})	583
Cd (mg kg^{-1})	10.9
Pb (mg kg^{-1})	165
pH	7.3

The results were submitted to the F test by regression analysis of variance (SAS, 1996) at the 0.05 significance level.

RESULTS AND DISCUSSION

No significant interaction between biosolid level × cycle was verified in relation to essential oil yield. However, mean yield was slightly higher in plants grown with biosolid, especially at 28 t.ha^{-1} (figure 1). Similar results were observed by Scora and Chang (1997) in *M. piperita* L.; the authors did not observe oil yield differences among plants grown with biosolid-amended applications at 100 days following transplanting.

Topalov and Zhelyazkov (1991) verified higher oil yield when *M. piperita* L. plants grown without biosolid were harvested between 97 and 100 DAP. A mean oil yield of 1.00 mL.100 g^{-1}DM was observed in plants grown without biosolid. Valmorbida (2002), in an investigation where mint was grown in a complete nutritive solution under the same climatic conditions, verified a mean oil yield of 1.27 mL.100 g^{-1} DM. In the present work, the mean yields of 1.51 and 1.27 mL.100 g^{-1}DM obtained with plants grown at

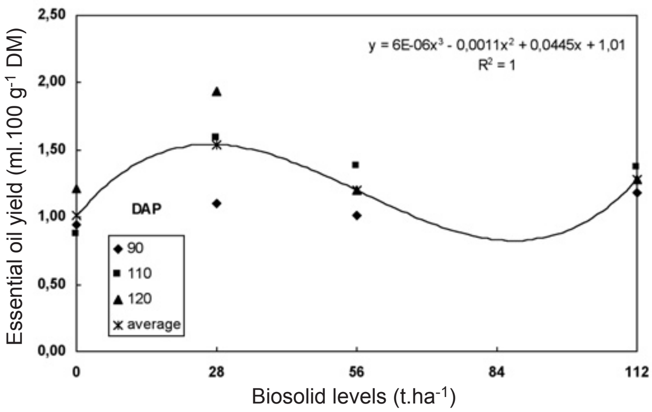

Figure 1. Essential oil yield in *M. piperita* L. plants grown under different biosolid levels. Means of three harvesting times. At 90, 110, and 120 DAP, there was no significant interaction between biosolid level x cycle – without adjustment.

28 and 112 t.ha^{-1} biosolid could be considered satisfactory. However, results may have been discrepant due to different experimental conditions to which plants were submitted, which interfere with mint essential oil yield and composition (Reitsma et al., 1961; Marotti et al., 1993).

During essential oil synthesis in *M. piperita* L. (figure 2), the isoprenoid pathway leads to the formation of geranil pyrophosphate, from which limonene is originated, next forming piperitenone which, in turn, forms pulegone, which can form menthone and/or menthofuran (Dey and Harbone, 1997; Croteau et al., 2000). Menthone forms neomenthol and menthol, which may undergo esterification and thus be converted into menthyl acetate (Murray, 1972). Based on this, it is understood that in the present research the conditions to which mint plants were submitted favored the formation of menthyl acetate.

No significant interaction between biosolid level x cycle was verified in relation to menthyl acetate content. However, the average menthyl acetate value decreased as biosolid level increased (figure 3). Nevertheless, the component with the highest content found in plants was menthyl acetate, consisting of 40.55% of total oil content, on average. Scora and Chang (1997) reported menthone as a major component in *M. piperita* L. plants grown in soil with high Cd levels as a result of biosolid application. Zheljazkov and Margina (1996), studying the application of increasing levels of N, P, and K fertilizers in *M. piperita* L., verified that the second-highest essential oil component, after menthol, was menthyl acetate in all cultivars studied, with a mean percentage of 27.3%. According to these authors, increasing fertilizer levels increased menthyl acetate content, without changing menthol content. Thus, in the present study, the recommended fertilization might have overestimated nutrients required by the mint plants; as the biosolid was rehydrated, elements were probably made available at levels above the necessary, thus justifying menthyl acetate as a major component.

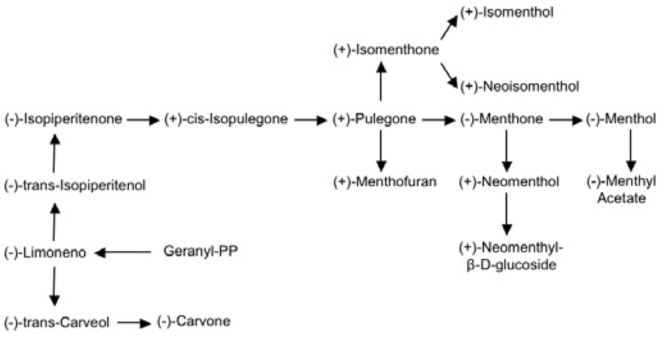

Figure 2. Essential oil components biosynthesis pathway.

Marotti et al. (1993) reported that menthyl acetate content increased from 1.6 to 17.0% up to the end of the development cycle of *M. piperita* L. plants. However, the same authors explained that determining harvest time accurately is important for best yield and oil quality. According to Marotti et al. (1993), yield and oil composition depend on pedoclimatic conditions and on the ontogenic stage of the plant, and a long photoperiod is essential for plant development and yield. These researchers reported that cultivation season and day length interfere with oil composition, because mint is a long-day plant. This statement may justify variations in essential oil yield and composition under different cultivation conditions, including the higher menthyl acetate levels obtained in the present research. Rabak (1917) investigated *M. piperita* L. in the USA and obtained the highest oil yield when plants reached maximum flowering, as affected by soil and climate conditions. The author also suggested that oil yield reductions may occur if plants are dried before distillation, due to changes that favor the formation of esters and the production of free acids. He also concluded that oil yield in mint plants decreases with maturation, increasing the percentage of esters, while the content of menthol, an important oil component, is related to esters content.

In mature leaves from flowering peppermint grown under long-day conditions, the menthyl acetate content of the volatile oil may exceed 10%, thus altering the flavor character of the essence distilled from such plants. While the reduction of menthone to menthol and the subsequent synthesis and accumulation of menthyl acetate would appear

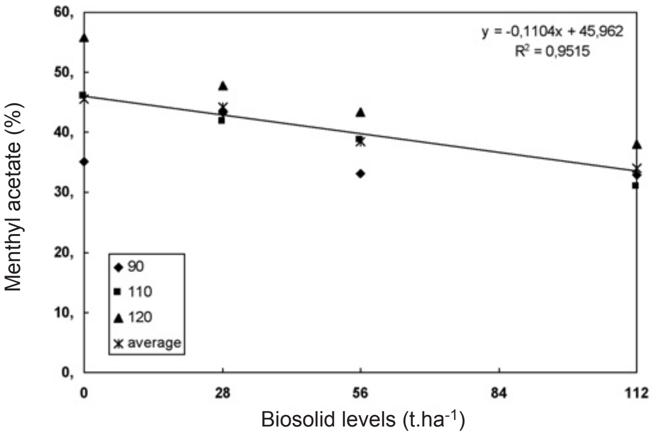

Figure 3. Menthyl acetate content in *Mentha piperita* L. plants grown under different biosolid levels. Means of three harvesting times. At 90, 110, and 120 DAP, there was no significant interaction between biosolid level x cycle – without adjustment.

to be enzymatic processes associated with maturation and the onset of reproductive growth in mint, no direct experimental evidence bearing on this possible metabolic relationship is presently available, and almost nothing is known about the enzymes and mechanisms involved in either the reduction or esterification step (Reitsma et al., 1961). In the present research, *M. piperita* L. plants did not bloom. This suggests that inadequate photoperiod and temperature conditions occurred for this species. This fact may have been responsible for the higher level of menthyl acetate verified in the plants.

Menthol content (figure 4) showed a significant interaction between biosolid level × cycle, and plants showed a higher percentage of menthol in the first harvest (90 DAP), especially those grown in the absence of biosolid with a 42.3% content (table 2). In this harvest, menthol content varied according to a quadratic regression. At 110 DAP, plants did not show differences in their menthol levels, while at 120 DAP a linear increase in menthol content was observed as biosolid levels increased. However, plant age led to a reduction in menthol content; at 90 DAP, menthol was the second-highest oil constituent.

Valmorbida (2002) grew *M. piperita* L. plants in nutritive solution with varying K levels, and observed a mean menthol content of 27.8%. Zheljazkov and Margina (1996) evaluated *M. piperita* L. essential oil in field-grown plants under increasing fertilizer levels, and verified that cultivar Zephir showed 60.9% menthol when grown without fertilizer and 58.1% when supplied with NPK. Again, it is suggested that elements made available at levels above those required were responsible for the results obtained in the present study. In contrast, Zheljazkov and Margina (1996) and Valmorbida (2002) did not observe decreases in menthol content during the development cycle.

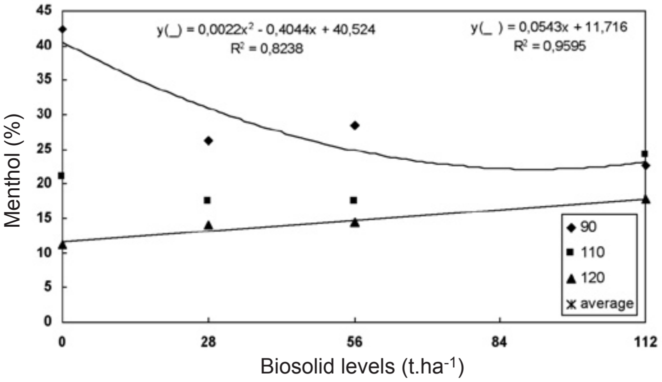

Figure 4. Menthol content in *M. piperita* L. plants grown under different biosolid levels, at three harvesting times, at 90, 110, and 120 days after planting (DAP). Means of three replicates. At 110 DAP, there were no biosolid level differences in menthol content, at 0.05 significance - without adjustment.

Increases in menthyl acetate content correspond to a reduction in menthol percentage. The best time to harvest mint plants would be at 90 DAP, since menthol is economically more important than other oil components. Therefore, in the present research, biosolid favored menthyl acetate synthesis in relation to menthol. Though having little economic importance, menthyl acetate is used in perfumery, emphasizing floral notes, especially of roses, and is used in toilet waters, having a lavender odor. It has been used to flavor caraway extracts or for mint flavors (The Merck Index, 1996). However, biosolid was harmful to menthol and menthyl acetate yield.

A significant interaction between biosolid level × cycle in relation to menthofuran content is shown in figure 5. The highest levels were obtained as biosolid level increased, except in plants grown with 112 $t.ha^{-1}$ at 110 DAP. In addition, menthofuran percentage was lower at 90 DAP, especially in plants grown without biosolid, at which time they showed higher menthol contents. According to figure 2, the formation of menthofuran resulted in decreased menthol content. Thus, the presence of biosolid in a mint crop favored the formation of menthofuran. Grahle and Holtzel (1963), cited by Piccaglia et al. (1993), stated that mint plants resprout under short days during the fall, causing an increase in menthofuran content. It is important to point out that in the present study, harvests to evaluate essential oil began in May; therefore, photoperiod conditions, in addition to the presence of biosolid could perhaps justify the menthofuran contents observed. This component showed a tendency to increase as plants progressed in age, in agreement with results observed by Charles et al. (1990), who studied the influence of osmotic stress on mint essential oil composition. It must be considered that although experimental conditions were different, both may have caused stress to the plants (figure 5).

Valmorbida (2002) grew *M. piperita* L. plants in nutritive solution with varying levels of K, and observed a mean menthofuran content of 11.79%. Gershenszon et al. (2000) studied monoterpene regulation in *M. piperita* L. leaves infected by pathogens, and verified a maximum menthofuran content of 4.9%.

Scora and Chang (1997) grew mint plants in soil containing increasing levels of Cd varying from 0.12 to 6.1 $mg.kg^{-1}$, resulting from biosolid application, and observed that menthofuran decreased from 6% in the control treatment containing 0.12 $mg.kg^{-1}$ Cd to 3.4% on average in the

Table 2. Essential oil composition (%) in *Mentha piperita* L. plants grown under different biosolid levels, at three harvesting times.

Components (%)	Biosolid levels (t.ha^{-1})											
	0			28			56			112		
	Days after planting			Days after planting			Days after planting			Days after planting		
	90	110	120	90	110	120	90	110	120	90	110	120
Menthyl acetate	35.01	46.08	55.68	43.26	41.78	47.87	33.11	38.63	43.40	32.90	31.00	37.93
Menthol	42.32	20.91	11.28	26.16	17.44	14.00	28.46	17.36	14.48	22.60	24.91	17.74
Menthofuran	4.56	17.25	17.45	9.44	26.11	25.84	13.79	28.38	28.94	25.33	24.08	30.21
Menthone	4.05	1.82	1.79	8.18	2.75	1.13	11.48	3.69	1.85	4.93	7.85	3.69
1.8-Cineol	5.56	2.93	3.24	3.10	2.23	2.15	3.71	2.73	2.25	2.41	2.80	1.94
α-Limonene	0.00	2.03	2.10	0.73	1.94	1.58	1.17	2.12	1.95	2.18	2.22	1.99

other treatments containing higher Cd levels. Based on the composition of the biosolid from the Barueri Station (table 1), Cd levels in the treatments containing the equivalent to 28, 56, and 112 t.ha^{-1} were equal to 0.15, 0.31, and 0.61 mg.kg^{-1}, respectively, which were lower than levels studied by Scora and Chang (1997). When plants were grown with biosolid from Barueri, menthofuran content increased. The different environmental conditions to which both researches were submitted, including the higher Cd levels used by Scora and Chang (1997), may have been responsible for the different results observed. It is therefore likely that in the present research menthofuran alterations were due to the presence of biosolid, and to growing season and different Cd levels present in different treatments. It must be considered, however, that at 110 DAP the Cd effect may have appeared just as it did in the Scora and Chang (1997) study, since in the treatment with 112 t.ha^{-1}, containing 0.61 mg.kg^{-1} Cd, menthofuran showed a tendency to decrease. The levels of Cu (7.98, 15.96, and 31.92 mg.kg^{-1}), Pb (2.31, 4.62, and 9.24 mg.kg^{-1}), and Zn (33.33, 66.67, and 133.34 mg.kg^{-1}) in the three treatments evaluated in the present research were lower than the levels of Cu (23-182 mg.kg^{-1}), Pb (17-173 mg.kg^{-1}), and Zn (93-601 mg.kg^{-1}) evaluated by Scora and Chang (1997), and thus may justify the different results observed. Zheljazkov and Margina (1996), cultivating mint under increasing levels of fertilizers, did not detect menthofuran.

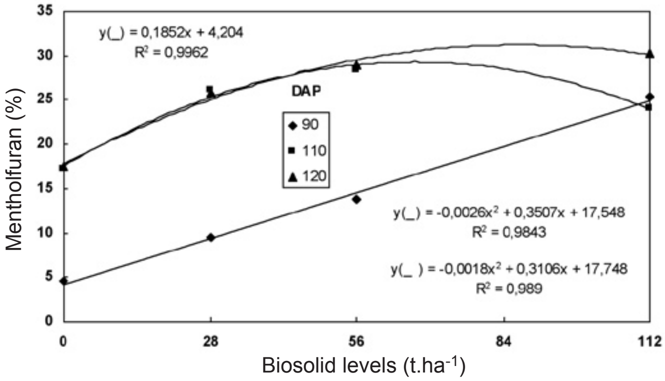

Figure 5. Menthofuran content in *Mentha piperita* L. plants grown under different biosolid levels, at three harvesting times, at 90, 110, and 120 days after planting (DAP). Means of three replicates.

The significant interaction between biosolid level × cycle in relation to menthone content (figure 6) allows the observation to be made that this component peaked at 90 DAP in plants grown with 28 and 56 t.ha^{-1} biosolid, and at 110 DAP in plants submitted to 112 t.ha^{-1}. Like menthol, the percentage of menthone decreased as mint plants developed. This was expected, since menthone is a menthol precursor in the essential oil biosynthesis pathway (figure 2).

A significant interaction between biosolid level × cycle was shown for 1,8-cineol and α-limonene contents. The percentage of 1,8-cineol was higher in plants grown without biosolid, at 90 DAP (figure 7), while α-limonene increased with biosolid levels (figure 8) during that period. In both cases, no significant difference was verified between biosolid levels at 110 and 120 DAP.

At 90 days, in the absence of biosolid, similar productions of menthone and menthofuran were observed; after that, there was a reduction in the production of menthone and an increase in the production of menthofuran (table 2). Since menthone production decreased, there was a decrease in the production of menthol and an increased esterification of the latter into menthyl acetate (figure 2). In the presence of biosolid, greater menthofuran production takes place, as compared to menthone, with a resulting decline in menthol production. From a qualitative point of view (figure 2), increases in monoterpene alcohols such as menthol and decreases in monoterpene ketones and oxides (i.e. menthone

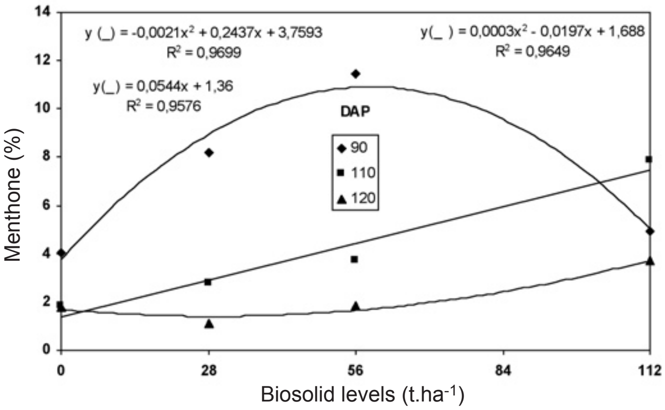

Figure 6. Menthone content in *Mentha piperita* L. plants grown under different biosolid levels, at three harvesting times, at 90, 110, and 120 days after planting (DAP). Means of three replicates.

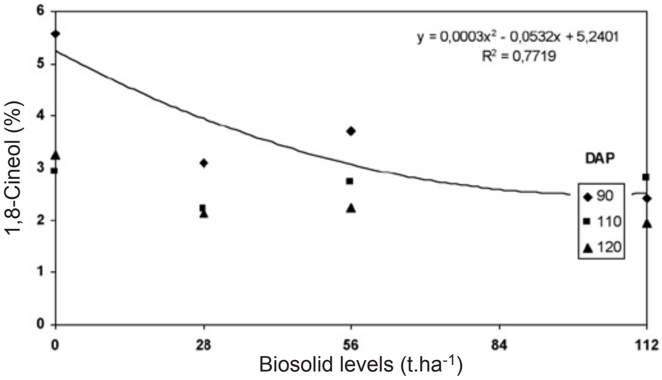

Figure 7. 1,8-Cineol content in *M. piperita* L. plants grown under different biosolid levels, at three harvesting times, at 90, 110, and 120 days after planting (DAP). Means of three replicates. At 110 and 120 DAP, there were no biosolid level differences in 1,8-cineol content, at 0.05 significance – without adjustment.

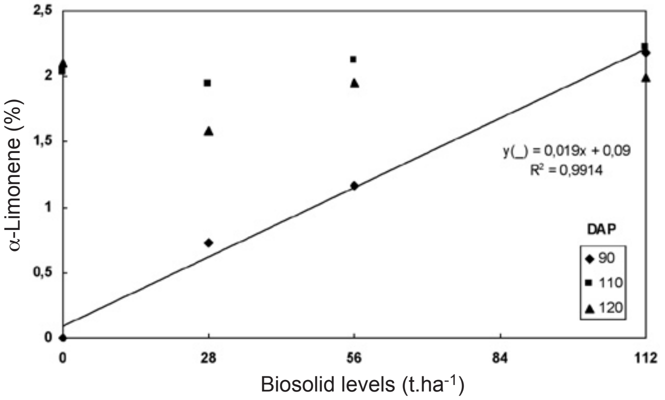

Figure 8. α-Limonene content in *Mentha piperita* L. plants grown under different biosolid levels, at three harvesting times, at 90, 110, and 120 days after planting (DAP). Means of three replicates. At 110 and 120 DAP, there were no biosolid level differences in α-limonene content, at 0.05 significance – without adjustment.

and menthofuran) suggest that the increased activity of reducing enzymes may be at least partially responsible for the progressive transformation of monoterpene ketones to alcohols (Maffei and Codignola, 1990).

The fact that the contents of substances identified in the present study and those cited in the literature are different is in agreement with Maia (1998), who proposed that plants developed under different conditions contain oils with different characteristics.

Thus, essential oil yield increased with biosolid levels and was higher when plants were grown with 28 t.ha^{-1}; this condition did not result in better oil quality. Under the conditions of the present research, the metabolic pathway of monoterpenes favored menthofuran and menthyl acetate synthesis. However, under the conditions of the experiment, plant harvesting is recommended at 90 DAP, when menthol content was higher.

Mint plants survived at all biosolid levels. However, biosolid was detrimental to oil quality. Though biosolids must be disposed of, their use is regulated by restrictions, both in relation to the environment and to plants. Although mint cultivation with 28 t.ha^{-1} is within the limits allowed by the laws that regulate biosolid application in agriculture, such a rate, which increased oil yield, did not improve oil quality. Therefore, the biosolid from the Barueri Station is not recommended for cultivation of this species.

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