

Chemical composition and biological properties in *Mentha spicata* under conventional and organic fertilization

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ABSTRACT. Introduction: Spearmint (*Mentha spicata*) is widely used in the pharmaceutical and food industries, thanks to chemical properties largely influenced by genetic and environmental factors, especially soil conditions. **Objective:** To determine the effect of conventional and organic fertilization on the chemical and biological properties of *M. spicata*. **Methods:** We conducted field trials in a randomized block experimental design, with four replications, using unfertilized crops and crops fertilized with urea (0,15 t ha⁻¹) or vermicompost (5 and 10 t ha⁻¹). **Results:** All fertilization treatments increased essential oil yield, carvone content, and total polyphenol concentration with respect to the control. They also increased the antioxidant capacity and the inhibitory activity of the acetylcholinesterase, butyrylcholinesterase, α -amylase, and α -glycosidase enzymes. The effect was more marked in the crop fertilized with 5 t ha⁻¹ vermicompost. **Conclusions:** Both conventional and organic fertilization increase the yield and quality of *M. spicata* essential oils. However, organic fertilization with 5 t ha⁻¹ vermicompost yields rich total polyphenols and carvone. This improves antioxidant and medicinal properties, acting on enzymes related to Alzheimer's disease and diabetes

Keywords: carvone, polyphenols, antioxidant activity, medicinal plants, vermicompost.

RESUMEN. “Composición química y propiedades biológicas de la hierbabuena, *Mentha spicata*, con fertilización tradicional y orgánica”. **Introducción:** La hierbabuena (*Mentha spicata*) es muy usada en las industrias farmacéutica y alimentaria, gracias a sus propiedades químicas influenciadas en gran medida por factores genéticos y ambientales, especialmente las condiciones del suelo. **Objetivo:** Determinar el efecto de la fertilización convencional y orgánica sobre las propiedades químicas y biológicas de *M. spicata*. **Métodos:** Hicimos ensayos de campo en un diseño experimental de bloques al azar, con cuatro repeticiones, utilizando cultivos no fertilizados y cultivos fertilizados con urea (0,15 t ha⁻¹) o vermicompost (5 y 10 t ha⁻¹). **Resultados:** Todos los tratamientos de fertilización aumentaron el rendimiento de aceite esencial, el contenido de carvonas y la concentración total de polifenoles, con respecto al control. También aumentaron la capacidad antioxidante y la actividad inhibidora de las enzimas acetilcolinesterasa, butirilcolinesterasa, α -amilasa y α -glicosidasa. El efecto fue más marcado en el cultivo fertilizado con 5 t ha⁻¹ de vermicompost. **Conclusiones:** Tanto el abono tradicional como el orgánico aumentan el rendimiento y la calidad de los aceites esenciales de *M. spicata*. Sin embargo, la fertilización orgánica con 5 t ha⁻¹ de vermicompost produce ricos polifenoles totales y carvonas. Esto mejora las propiedades antioxidantes y medicinales, actuando sobre las enzimas relacionadas con la enfermedad de Alzheimer y la diabetes

Palabras clave: carvona, polifenoles, actividad antioxidante, plantas medicinales, vermicompost.

The *Mentha* genus consists of 19 species of the *Lamiaceae* family, including spearmint (*M. spicata*). There is much interest worldwide in the cultivation of species of the genus *Mentha*, since its essential oils are among the 10 most demanded in the market (Kumar et al., 2011). Spearmint is used in the pharmaceutical and food industries and in the production of essential oils. Insecticidal, antioxidant, and antimicrobial activities have been reported in essential oils of *M. spicata* (Scherer et al., 2013). The Food and Drug Administration (FDA) considers the regular consumption of sustainably produced spearmint to be healthy (Yi & Wetzstein, 2011).

Essential oils of spearmint consist mainly of carvone, limonene, and 1,8-cineole. Their biosynthesis depends on genotype, environmental conditions (photoperiod and temperature), crop management (fertilization and irrigation), and time of material collection (Kara, 2015). The medicinal properties of essential oils are determined by their chemical composition, an aspect that has been scarcely studied in *M. spicata* (Scherer et al., 2013).

In recent years, the demand for organic products has increased, and food of plant origin free of agrochemicals has a higher value in the market (García-Mier et al., 2021). The management of these crops cannot include the use of chemical fertilizers, and the use of organic fertilizers that do not generate an environmental impact has become indispensable. Thus, vermicompost offers an environmentally friendly alternative to chemical fertilizers (Ramnarain et al., 2019). Vermicompost is the product obtained from the processing of organic waste in the digestive tract of earthworms. This process involves the oxidation and stabilization of organic compounds by the joint action of earthworms and microorganisms (Hanc et al., 2017).

Vermicompost is an excellent option to decrease the toxicity of urban waste (Wang et al., 2013). These organic substrates are rich in nutrients and biologically active substances, such as humic and fulvic acids (Churilova & Midmore, 2019). Moreover, they constitute a suitable medium for the development of beneficial bacteria, which promote plant growth (Rekha et al., 2018). These bacteria stimulate the production of phytohormones, especially auxins, gibberellins, and cytokinins. They also promote phosphorus solubilization and nitrogen fixation (Pathma & Sakthivel, 2012).

The aim of the present work was to determine the effect of conventional and organic fertilization on the chemical composition and biological properties of spearmint (*Mentha spicata* L.).

MATERIALS AND METHODS

Crop conditions and plant material: The trial was implemented on December 2, 2019, in the town of El Zanjón, Santiago del Estero, Argentina (27°45'S, 64°18'W). The crop was grown in 9m² plots, with 0,7m spacing between furrows. The experiments included an unfertilized control, chemical fertilization with urea (0,15t ha⁻¹), and organic fertilization with two doses of vermicompost (5 and 10t ha⁻¹). The crop was periodically irrigated to ensure adequate water supply. During the trial, the average temperature was 28,1°C, and the relative humidity was 62%. Table 1 shows the physical and chemical characteristics of the soil and vermicompost.

TABLE 1

Electrical conductivity, pH, total organic carbon, and total nitrogen in soil and vermicompost.

	Electrical conductivity (dS m ⁻¹)	pH	Total organic carbon (%)	Total nitrogen (%)
Soil	0,57	8,10	1,35	0,13
Vermicompost	0,80	7,90	5,70	0,58



On February 19, 2021, coinciding with the beginning of the flowering phenological stage, the crop was harvested. Harvesting was performed manually, at 10cm height. Plant material was dried in a forced ventilation oven at 40°C. Samples were taken from this material for chemical determinations.

Extraction of essential oils and quantification of carvone concentration: Essential oils were extracted by hydrodistillation at 60°C for 2,5h, using a Clevenger apparatus. The yield of essential oils was calculated as μl of oil per 100g dry weight of plant material, and the results were expressed as percentage. Carvone concentration was determined by Gas Chromatography-Mass Spectrometry (GC/MS) according to the methodology described by Scherer et al. (2013).

Determination of leaf manganese concentration: Plant material was ground in a Wiley-type mill and sieved through a 40-mesh grid. Subsequently, digestion was carried out with 2N HCl. Manganese concentration was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES; PSFO 2.0, Leeman Labs INC., USA), according to the technique described by Chrysargyris et al. (2017).

Quantification of total phenol concentration and radical scavenging activity: To extract total polyphenols, plant material was homogenized in methanol (50% v/v). It was then centrifuged for 15min at 4000xg and 4°C. Aliquots of 0,5ml were taken, to which 2,5ml Folin-Ciocalteu reagent was added. After incubating for 5min at room temperature, 2ml 7,5% sodium carbonate was added. The mixture was incubated for 2h, and absorbance was read at 740nm (Chrysargyris et al., 2017). The concentration of total polyphenols was expressed as milligrams of gallic acid equivalents per gram of dry weight.

The 1,1-diphenyl, 2-picrylhydrazyl (DPPH) scavenging effects of the extracts were detected according to the method described by Gülçin et al. (2020). We added 0,5ml 0,1mM DPPH to 1,5ml aliquots of the sample solution in ethanol ($10\text{-}50\mu\text{g ml}^{-1}$). It was incubated for 30min, and absorbance was read at 517nm. The blank sample contained ethanol, which was used as solvent for DPPH radicals and reaction medium.

The DPPH scavenging effect was calculated as follows: $\text{RSC (\%)} = (1 - A_s/A_c) \times 100$, where A_c and A_s are the absorbances of the control and samples, respectively. The half maximal scavenging of the chelating concentration (IC_{50}) was estimated by plotting the percentages against extract concentrations ($\mu\text{g ml}^{-1}$).

Enzyme inhibition assays: The inhibitory capacity of methanolic extracts on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes was quantified. For this purpose, we used the spectrophotometric technique described by Yakoubi et al. (2021). In both reactions, acetylthiocholine iodine and butyrylthiocholine chloride were respectively used as substrates; galantamine was used as standard. Absorbance readings were performed at 412nm using sodium phosphate buffer as blank solution.

The inhibitory effect of the extracts on α -glycosidase was determined according to the method described by Tao et al. (2013). Twenty μl of an enzyme solution was added to 75 μl of different concentrations of methanolic extracts in sodium phosphate buffer pH 7,4 and incubated for 10min. Subsequently, 50 μl p-nitrophenyl-D-glycopyranoside was added and incubated at 37°C for 15min. Absorbance was read at 405nm using sodium phosphate buffer as a blank solution.

The inhibitory activity of the extracts on α -amylase enzyme activity was measured according to the method proposed by Xiao et al. (2006). A solution containing 1g starch in a 100ml volume of distilled water was used. The reaction mixture contained equal volumes of starch solution, sodium



phosphate buffer pH 6,9, and different concentrations of methanolic extracts. Then, a solution of α -amylase was added and incubated at 35°C for 1h. The reaction was stopped by the addition of 0,1M HCl solution, and absorbance was read at 580nm using sodium phosphate buffer as blank solution.

All results were expressed as the concentration producing 50% inhibition in enzyme activity (IC_{50} , $\mu\text{g ml}^{-1}$).

Experimental design and statistical analysis: A randomized block experimental design with four treatments and four replicates was used. Results were analyzed with ANOVA and Tukey's test.

RESULTS

All fertilization treatments significantly increased yields of essential oils in spearmint crops (Fig. 1A). Thus, whereas the yield of essential oils in the control was 0,8%, in the urea fertilization treatment it increased to 1,2%. The highest yield (1,7%) was obtained in the organic fertilization with 5 t ha^{-1} vermicompost. On the other hand, organic fertilization with 10t ha^{-1} vermicompost produced yields of essential oils similar to those obtained in urea fertilization.

Carvone was the main component present in spearmint essential oils, exceeding 50% in all cases (Fig. 1B). The essential oils obtained from the control had 60,2% carvone; however, the content of this compound increased to 66,7% in the treatment fertilized with urea. Organic fertilization produced a significant increase in the carvone content in essential oils, which reached values close to 72% in both doses of vermicompost.

Both urea and vermicompost fertilizations increased foliar manganese concentrations (Fig. 1C). The concentration of this micronutrient increased by 13% in the leaves of the crop fertilized with urea, with respect to the unfertilized control. The greatest impact was observed in the crop fertilized with 5t ha^{-1} vermicompost, in which the leaf concentration of manganese was 46% higher than in the control. The values obtained in the crop fertilized with 10t ha^{-1} vermicompost did not differ with respect to the crop fertilized with urea.

Fertilization with urea increased the concentration of total phenols in spearmint leaves by 38%, with respect to the control (Fig. 2A). This variable had a significant response in plants fertilized with 5t ha^{-1} vermicompost, doubling in relation to the control. Plants treated with 10t ha^{-1} vermicompost had a concentration of total phenols similar to those fertilized with urea.

Foliar methanolic extracts from plants fertilized with urea or vermicompost had higher radical scavenging activity than those from the control, and therefore lower IC_{50} values (Fig. 2B). Coinciding with the behavior of total phenols, the highest antioxidant capacity was observed in methanolic extracts from plants fertilized with 5t ha^{-1} vermicompost.

Methanolic extracts of spearmint leaves inhibited the activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. This biological property was accentuated by chemical and organic fertilizations, with a concomitant decrease in IC_{50} values (Fig. 3A, B). The effect was similar on the activity of both enzymes, and the highest inhibitory activity was observed in foliar extracts from plants fertilized with vermicompost. There were no significant differences between the vermicompost doses studied, which presented IC_{50} values of approximately 37 and 45 $\mu\text{g ml}^{-1}$ for acetylcholinesterase and butyrylcholinesterase, respectively.

Foliar extracts also inhibited the activities of α -amylase and α -glycosidase enzymes (Fig. 3 C,D). Although all fertilization treatments enhanced this biological property, the effect was most marked in the crop fertilized with 5t ha^{-1} vermicompost. In that treatment, foliar extracts had IC_{50} values of approximately 20 and 18 $\mu\text{g ml}^{-1}$ for α -amylase and α -glycosidase, respectively. The highest dose of organic fertilizer had a similar response to urea.

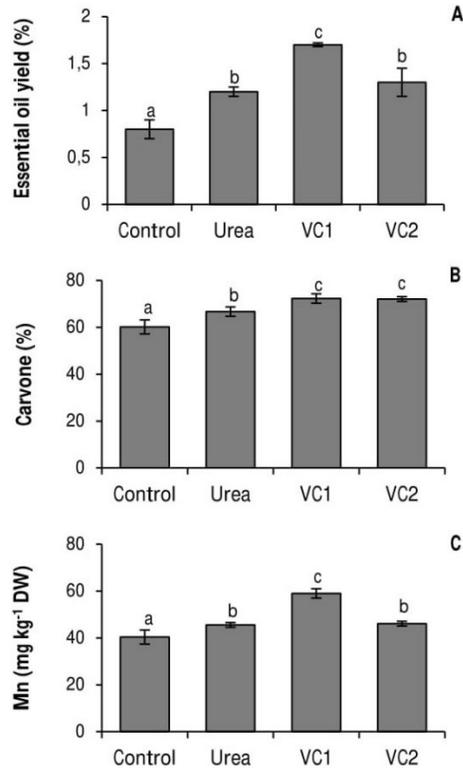


Fig. 1. Essential oil yield (A), carvone content (B), and leaf manganese concentration (C) in spearmint crops without fertilization (control) or fertilized with 0,15 t ha⁻¹ urea (urea), 5 t ha⁻¹ vermicompost (VC1), or 10 t ha⁻¹ vermicompost (VC2). Values represent means \pm SD of four replicates; when labeled with different letters, they are significantly different (Tukey's test; $P < 0,05$).

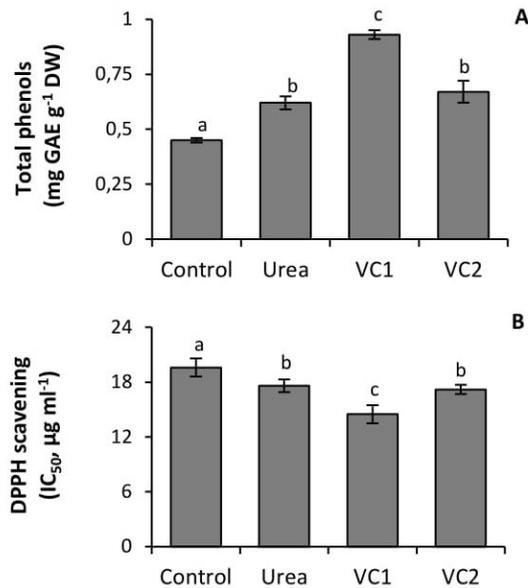


Fig. 2. Concentration of total phenols (A) and radical scavenging activity (B) in spearmint crops without fertilization (control) or fertilized with 0,15 t ha⁻¹ urea (urea), 5 t ha⁻¹ vermicompost (VC1), or 10 t ha⁻¹ vermicompost (VC2). Values represent the means \pm SD of four replicates; when labeled with different letters, they are significantly different (Tukey test; $P < 0,05$).

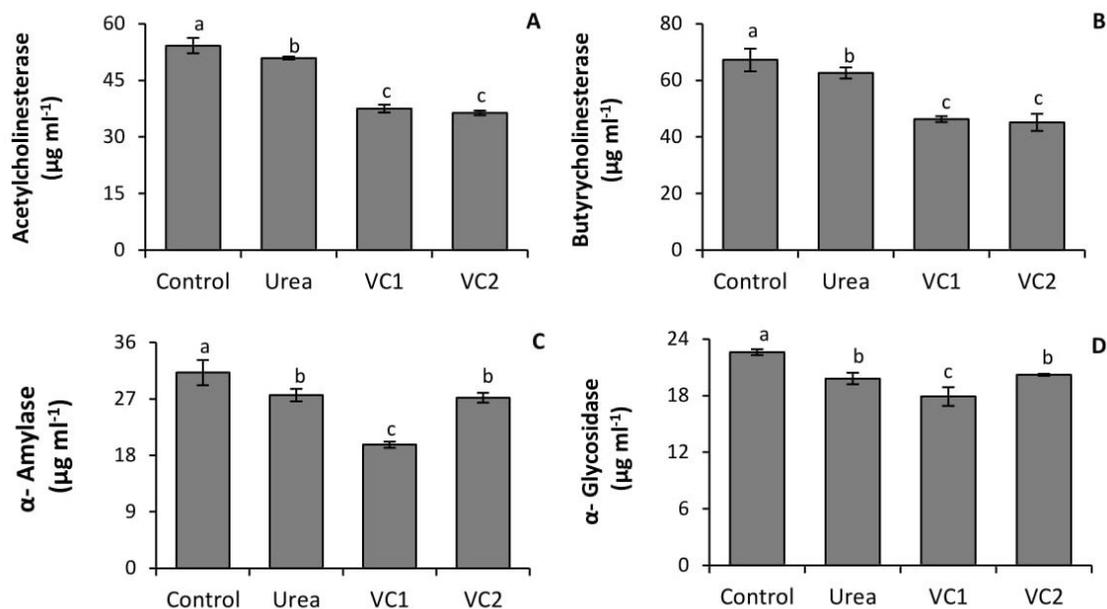


Fig. 3. Inhibitory capacity of the acetylcholinesterase (A), butyrylcholinesterase (B), α -amylase (C), and α -glycosidase (D) enzymes in methanolic extracts of spearmint leaves obtained from crops without fertilization (control) or fertilized with 0,15 t ha⁻¹ urea (urea), 5 t ha⁻¹ vermicompost (VC1), or 10 t ha⁻¹ vermicompost (VC2). Values represent means \pm SD of four replicates; when labeled with different letters, they are significantly different (Tukey's test; $P < 0,05$).

DISCUSSION

All fertilization treatments increased the yield of essential oils and their quality, given by the carvone content (Fig. 1 A, B). Fertilization with 5t ha⁻¹ vermicompost produced the highest yield of essential oils (1,7%) and carvone content (72%) in relation to the control and urea fertilization. The dose of 10 t ha⁻¹ vermicompost reduced the yield of essential oils in comparison with the treatment of 5t ha⁻¹ vermicompost, although their quality was maintained. On the other hand, the yield values of essential oils in the different treatments followed the same trend as the foliar concentration of manganese (Fig. 1C).

The results obtained in the present work coincide with those reported by Ram, Ram, and Singh (1995), who demonstrated that doses of 0,16t N ha⁻¹ increased the production of biomass and the yield of essential oils in *M. arvensis* and *M. citrata*. They also partially agree with those reported by Chrysargyris, Nikolaidou, Stamatakis, & Tzortzakis (2017). These authors observed that fertilization with nitrogen increased the yield of essential oils in hydroponically grown spearmint, reaching maximum values of 2,5%. Nevertheless, carvone concentration decreased, which was associated with a reduction in manganese concentration.

Singh et al. (2001) studied the effect of manganese deficiency on the yield of essential oils and their composition in three cultivars of *M. spicata* (MSS-5, Arka, and Neera). In all three cultivars, manganese deficiency significantly reduced the yield of essential oils. The effect on the chemical composition of essential oils depended on the cultivar. Thus, manganese deficiency did not affect carvone concentration in cultivar MSS-5, reduced it in cultivar Neera, and increased it in cultivar Arka.

The crop response to organic fertilization is of particular interest, since it allowed increasing the yield of essential oils and their quality through an environmentally friendly technology. Some reports indicate a similar crop response in different species of the genus *Mentha*. In Iran, fertilization

with a combination of 25% urea (23,75kg N ha⁻¹) and 75% vermicompost (10,1t ha⁻¹) significantly increased the number of leaves and the yield of essential oils in *M. arvensis* and *M. piperitha* (Keshavarz et al., 2018). Loera-Muro et al. (2021) proposed the substitution of inorganic fertilizers by vermicompost leachates in hydroponic crops of *Mentha spicata* and *Rosmarinus officinalis*. Their study demonstrated that such organic fertilizer doubled root biomass production in both species.

According to Carvalho and Fonseca (2006), essential oils of *M. spicata* must have at least 51% carvone content to preserve their biological and medicinal properties. Essential oils from all treatments exceeded this content, demonstrating their high quality. Fertilization with vermicompost significantly increased the quality of essential oils, reaching 72% carvone content values, which makes them a highly valued product in the international market. There are projects worldwide aimed at increasing carvone content through different biotechnological strategies. For example, in India, cultivars MSS-1, Punjab Spearmint-1, MSS-5, and IIM(J)26 were developed with 60, 68, 70, and 76.6% carvone content, respectively (Chauhan et al., 2009). In the Brazilian state of Espírito Santo, *M. spicata* essential oils yielded 67% carvone content (Scherer et al., 2013). *M. spicata* essential oils obtained in Greece presented 68,4% carvone content (Kokkini et al., 1995), and those in Turkey showed 48,4% carvone content (Şarer et al., 2011). This variation in chemical composition has been attributed to temperature, humidity, solar radiation, and edaphic characteristics.

Fertilization also increased the leaf concentration of total phenols, which was the highest in the crop fertilized with 5t ha⁻¹ vermicompost (Fig. 2A). In agreement with this result, methanolic extracts of spearmint leaves had a high antioxidant capacity, particularly in that organic fertilization treatment (Fig. 2B). This increase in total polyphenol concentration and antioxidant activity contributes to improve the quality of the products obtained, since the food industry demands natural antioxidants. These products inhibit lipid peroxidation and do not have the adverse effects of synthetic substances. Among the harmful effects of synthetic antioxidants, cardiovascular diseases, cancer, diabetes, cataracts, cognitive disorders, and neurological diseases have been reported (Scherer et al., 2013).

The antioxidant capacity of phenols is due to redox reactions, in which these compounds act as reducing agents, singlet oxygen quenchers, or metal chelators. This antioxidant mechanism is relevant, as it prevents lipid oxidation in animal and plant tissues (Ćavar Zeljković et al., 2021). Thus, plant phenols are appreciated because they preserve the quality of food without producing adverse effects (Riachi & De Maria, 2015).

The results obtained in the present work agree with those reported by García-Mier et al. (2021) in *Mentha piperita*. In that species, organic cultivation exceeded the concentration of total phenols by 62%, compared to chemical fertilization. Antioxidant activity by the DPPH test increased 572% in organic crops. Higher concentrations of total phenols were also reported in marionberries, strawberries, and corn grown under organic conditions when compared to those grown under conventional agricultural practices (Asami et al., 2003).

All methanol extracts from spearmint leaves inhibited AChE and BChE activities. This property was more noticeable in material from crops fertilized with 5 and 10t ha⁻¹ vermicompost (Fig. 3 A, B). The inhibition of these enzymes has been proposed in the treatment of Alzheimer's disease. This disease causes degeneration of brain tissue, as a consequence of a deficiency in acetylcholine and butyrylcholine (Yigit et al., 2019). For this reason, clinical trials have been conducted using natural inhibitors of AChE and BChE in the treatment of this pathology.

Yakoubi et al. (2021) reported cholinesterase activity in *Mentha pulegium* essential oils with IC₅₀ values of 115,74 and 8,19µg ml⁻¹ for AChE and BChE inhibition, respectively. Benabdallah et al. (2018) reported anticholinesterase activity in essential oils extracted from leaves of *M. aquatica*, *M.*



arvensis, *M. x piperita*, *M. pulegium*, *M. rotundifolia*, and *M. villosa*. In these essential oils, the IC₅₀ values for AChE inhibition were 32,58; 27,5; 63,9; 108,7; 52,5, and 137,5 μg ml⁻¹, respectively.

The anticholinesterase activity of aromatic species has been attributed to monoterpenes present in their essential oils (Aissi et al., 2016). Such compounds, like carvone, possess high hydrophobicity, which allows them to interact with the active site of AChE and BChE. This property has also been attributed to phenols (Rezai et al., 2018).

Spearmint extracts inhibited the activities of α-amylase and α-glycosidase enzymes (Fig. 3C, D). These enzymes hydrolyze starch and oligosaccharides, generating monosaccharide units. Therefore, digestive enzyme inhibitors are significant in the control of diabetes mellitus and hyperglycemia (Gülçin et al., 2018). The highest inhibitory activity occurred in the material from the crop fertilized with 5t ha⁻¹ vermicompost, which also had the highest concentration of total polyphenols. The results obtained agree with those reported by Gülçin et al. (2020), who showed that methanolic extracts of *Mentha pulegium* inhibited the activities of α-glycosidase and α-amylase enzymes. These authors reported IC₅₀ values of 20,38 and 23,11 μg ml⁻¹ for α-glycosidase and α-amylase, respectively. They attributed these responses to the high concentrations of total phenols present in the samples, which is also in agreement with our results. The inhibition of both enzymes involved in carbohydrate digestion could contribute to lower blood glucose concentration.

The medicinal properties present in spearmint leaf extracts suggest their use in the development of dietary supplements for the treatment of Alzheimer's disease or diabetes. In addition to these properties, the pleasant taste of the product stimulates its consumption by patients.

In conclusion, both conventional and organic fertilizations increase the yield and quality of essential oils in *M. spicata*. However, organic fertilization with 5t ha⁻¹ vermicompost yields plant material particularly rich in total polyphenols and carvone. As a consequence, the product improves its antioxidant and medicinal properties, acting on enzymes related to Alzheimer's disease and diabetes.

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ETHICAL, CONFLICT OF INTEREST AND FINANCIAL STATEMENTS

The authors declare that they have fully complied with all pertinent ethical and legal requirements, both during the study and in the production of the manuscript; that there are no conflicts of interest of any kind; that all financial sources are fully and clearly stated in the Acknowledgments section; and that they fully agree with the final edited version of the article. A signed document has been filed in the journal archives.

The declaration of the contribution of each author to the manuscript is as follows: D.A.M.: chemical analysis, data processing, result interpretation, and manuscript writing. J.A.B.S.: chemical analysis and manuscript writing. A.B.: chemical analysis and manuscript writing. J.A.L: field work. R.E.B: field work and manuscript writing.

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