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Original Research Article

Olive Mill Pomace Impact on the Phytochemical Content and Antioxidant Activity of *Rosmarinus officinalis L*

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ABSTRACT

Sustainable methods for enhancing the quality of medicinal herbs are required to provide high amounts of antioxidants widely used in the food, pharmaceutical, and cosmetic industry. In this study, the impact of olive pomace (OMP) addition on total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of two selected *Rosmarinus officinalis* at different proportions 0 and 25% of OMP was examined. The experiments were done in a shade house at the National Agency of Medicinal and Aromatic Plants in Taounate, Morocco. The various parameters TPC, TFC, antioxidant activity (DPPH), pH, CEC (meq.100g^{-1}), EC (µS.cm^{-1}), macro- and micronutrient content (mg.kg^{-1}), and nutrient uptake (g. plant^{-1}) were measured after the samples were extracted by sonication. The results indicated that rosemary leaves treated with 25% OMP amendment exhibited the highest TPC (68.48 mg GAE g^{-1} DW), TFC (6.13 ± 6.103 mg GAE g^{-1} DW), and antioxidant activity (1.279± 0.26 mg/mL DW). Additionally, the addition of OMP to the soil increased the level of macro- and micronutrients, mainly P, K, and Ca, as well as nutrient absorption factors including P, K, Ca, Cu, and Zn in the rosemary leaves, all of which were increased slightly but significantly. Notably, there was a positive association between DPPH, TPC, TFC, and soil organic matter. These results show that OMP addition has a great impact on the nutritional quality of rosemary leaves and directly increases TPC, TFC, and antioxidant activity. This study emphasizes the positive impacts of OMP on the bioactive qualities and overall nutrient profile of rosemary plants.

Keywords: *Rosemarinus officinalis*, phenolic content, Antioxidant activity, olive pomace.

Introduction

The use of precise fertilizer application techniques has raised yield production levels as the world's demand for food rises. As a result, extensive research was done to identify practical soil management techniques that maintain ideal levels of soil organic matter content when utilizing organic fertilizers.[1] Low organic matter soils can lower yields and crop productivity even when inorganic fertilizers offer enough nutrients.[2]
Understanding nutrient interactions and strategically combining mineral and organic fertilizers are essential for increasing crop yield while using sustainable farming techniques.[3]

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Organic additions improve soil quality by altering its physicochemical characteristics and decreasing bulk density, which raises the water content, microbial diversity, and general permeability of the soil.[4] These additions may improve the fertility and health of the soil. [5] It favorably affects how well plants use nutrients and engage in photosynthetic responses. to increase plant productivity and metabolism, organic fertilizers are a vital supply of the macronutrients N, P, K, Ca, Mg, S, and micronutrients Fe, Mn, Cu, and Zn. [6–8] The increase in crop yields containing abundant biologically active compounds holds great potential for their utilization in cosmetics and pharmaceutical products due to their remarkable antioxidant activity.[9–11] To maintain the therapeutic value of herbs while ensuring the long-term profitability of herb production, it is also crucial to grow medicinal plants in minimally-input sustainable farming systems.[12] Rosemary (*Rosmarinus officinalis*), a perennial herb of the Lamiaceae family, which grows natural in the Mediterranean region. [13] The plant grows under different pedoclimatic conditions. [14,15]
In recent years, rosemary cultivation was successfully attributed in both arid and semi-arid regions with critical climate conditions. [16] This adaptable herb has several use in food, medication, spices, and cosmetics. [17] In the food sector, rosemary is appreciated as a natural preservative in addition to its culinary uses. [18] Rosemary has a long medicinal history as natural medication for multiple diseases, including asthma, [19] digestive disorder, [20] inflammation, [21] dyslipidemia, [22] cancer, [23] as well as showcasing antimicrobial and antiviral properties. [24–26] The broad range action of its phytoconstituents of *Rosmarinus officinalis*

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includes polyphenols, flavonoids, and tannins. [27,28] Bioactive compound accumulation is highly affected by several environmental factors such as growth place, light, temperature, radiation, soil dryness and salinity, diseases, and herbivore attack.[29]

Since there has been no report on the impact of organic olive pomace addition on phenolic accumulation of *Rosamarinus officinalis*. Therefore, this article was undertaken to evaluate the impact of olive mill pomace (OMP) on bioactive compound accumulation using different OMP concentrations (0 and 25%).

Materials and Methods

Chemicals and reagents

All reagents (Folin-Ciocalteu reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl radical), gallic acid, and quercetin) and solvents (ethanol and methanol) employed were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the other reagents and solvents used were of analytical grade.

Experimental design

The used fresh OMP was collected from a cold three-phase continuous system olive oil mill (Pieralisi Extraction Systems-Italy) in Taounate city, Morocco (Latitude 34° 32' 5.39" North, longitude -4° 38' 14.39" West, altitude above sea level: 566 m). It was dried at 40°C in an oven (Memmert BE600 incubator/oven with natural convection at 70 °C, Germany) for three days before being ground and sieved to obtain a sample with a diameter of 1 mm to ensure their best incorporation in the soil matrix.[30]

Different proportions (0%, 25%, 50%, 75%, and 100%) of OMP were used in plastic pots (Tall Drainage Pot, 9 x 9 x 16cm, Excellent Drainage-South Africa) for *R. officinalis L.* cultivation to study the olive mill pomace addition on soil physicochemical characteristics and phytochemical content (total phenolic and flavonoid content), and antioxidant activity of two selected *R. officinalis L.* at 0 and 25% OMP amendment.

The soil sample was taken in the summer of 2020 from five agricultural plots located in the National Agency of Medicinal and Aromatic Plants (ANPMA), Taounate. 5 samples were taken randomly from a depth of 0–40 cm and mixed to obtain a composite sample (2 kg) after removing the litter between 0 and 5 cm. To reduce the sample weight, a quartering method was used to obtain a sample of 200g. The composite samples will be ground to 1 mm for certain physicochemical analyses.[31] The physicochemical properties of soil and OMP under study are shown in Table 1.

The test lasted six months 'from March 2021 to August 2021' in a shade house at the National Agency of Medicinal and Aromatic Plants (ANPMA), Taounate, Morocco, at variable temperatures and humidity depending on outdoor climatic conditions.

The viability of the germinated samples was assessed after 180 days. Only the mixtures containing 0%, 25%, and 50% of OMP remained alive. While, the 50% batch exhibited a notable impact on the growth and development of the plant samples, resulting in a reduced mass when compared to the 0% and 25% batches. The leaf dry weight of the plants treated with two different proportions of OMP (0% and 25%) is presented in Table 2.

The soil contained in the pots was kept at 4°C for physicochemical property analysis. And the plant collected from the pots with 0% and 25% of OMP was dried in an oven at 40°C (Memmert BE600 incubator/oven with natural convection at 70 °C, Germany), and the leaves were ground, and extracted with the sonication method for the TPC, TFC, and antioxidant activity determination.

Extraction procedure

To initiate the extraction process, precisely 50 mg of the powdered sample was mixed with 1 mL extraction solvents: methanol (≥99.9%), ethanol (≥99.8%), and distilled water (using the water distillation unit 19580, DELTALAB, Spain). To ensure optimal extraction efficiency, the mixture was sonicated for 15 minutes three times with 10-minute intervals between each round in an ultrasonic bath (Transonic T460, Germany). The resulting extracts were filtered at membrane filter PTFE 0.45 µM before being stored in glass tubes (at 4°C).

Total phenolic content quantification (TPC)

The quantification of total phenolic was determined spectrophotometrically by the colorimetric method using the Folin-Ciocalteu reagent. [32] Briefly, 100 µL of each extract was blended with 0.5 mL of a 10-fold dilution of the Folin-Ciocalteu reagent and 400 µL of a 7.5% sodium carbonate solution. The absorbance of the resulting solution was measured at 765 nm using a UV-Vis spectrophotometer (Jenway 6305, Fisher Scientific, UK). The results are expressed in milligrams of gallic acid equivalent per gram (mg GAE/g).

Total flavonoid content quantification

The TFC was measured using the aluminum method as previously described. [33] 400 µL of the extract, the standard solution, and the distilled water used as a control were added to 120 µL of a 5% $NaNO_2$ solution. After 5 minutes, 120 µL of 10% $AlCl_3$ were added and stirred vigorously. And 6 minutes after, 800 µL of 1 M NaOH was added and stirred again. The absorbance was immediately measured at 510 nm, compared to the control. All results are expressed in milligrams of quercetin per gram (mg QE/g).

Antioxidant activity

The radical reduction activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined according to the protocol described by Kim et al. [34] 50 µL of each extract and the control were blended with 1 µL of freshly prepared DPPH solution. The absorbance was then measured at 517 nm after 30 min of incubation.

The antioxidant activity was calculated according to the following equation:

$$\% \text{ Inhibition} = [((A0-As))/A0]*100.$$

The assay was performed in triplicate, and IC_{50} values were expressed as mean S, with A0 as the absorbance of the negative control and As as the absorbance of the test sample after 30 minutes.

Physico-chemical analyses of soil mixture samples

The pH, electrical conductivity (EC) and cation exchange capacity (CEC) were measured using a pH meter (Hannah Instruments, Hungary), conductivity meter (HANNA instruments, Hungary) and the $BaCl_2$ compulsive exchange method, respectively. [35,36] The organic matter content was calculated by calcining the soil mixture in a muffle furnace (Tabletop Muffle Furnace, Thermo Fisher Scientific, USA) at 500 °C for 1 hour. [37]

Macro- and micronutrient analyses

Soil mixture macro- and micronutrients were analyzed using ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy, ULTIMA 2, UK). The nutrients present in the samples were estimated by digesting 1 g of sample in a diacid mixture (9 mL of a freshly prepared acid mixture containing 65 % HNO_3 and 37% HCl) using standard analytical procedures. [38]

Plant leaf analysis

Plant leaf sample total mineralization with nitric acid and hydrogen peroxide was carried out. 0.4 g of the finely ground (<2 mm) plant sample is placed in a Sabilex tube containing 2.5 mL of 67% nitric acid. This tube is placed in a heating block at 60 °C for 1 hour and 30 minutes. Then 2.5 mL of 30% H_2O_2 is added, and the tubes are placed back on the heating block at 50 °C for 25 minutes and heated at 60 °C for 3 hours. [39]

All samples are then filtered through a 0.45 µm membrane PTFE filter. The eluate was poured into polyethylene bottles and diluted with 20 mL of distilled water. Then samples were stored at 4°C before ICP-AES plant leaf nutrient analysis, while a standard and a blank sample were prepared for each mineralization series. [40] The plant nutrient uptake was calculated using the following formula: [41]

$$\text{Nutrient uptake (g. plant-1)} = \text{leaf dry weight (g)} \times \text{plant nutrient content (\%)}$$

Statistical analysis
The statistical analysis was carried out using SPSS Version 21 to determine Pearson correlation coefficient (*r*) at significance level of 95% (*p*<0.05).

Results and Discussion

Total phenolic content (TPC)
Figure 1 describes the obtained results of quantification of the total phenolic content of different extracts of *R. officinalis* treated with two different proportions of OMP (0% and 25%). The finding analysis revealed that treatment of plants with 25% OMP significantly increased phenolic accumulation, which was translated by the highest recovery using different extractor solvents. The highest amounts of TPC were registered for ethanol and methanol extract of *R. officinalis* treated with 25% OMP with values of 69.97±0.92 mg GAE/g and 67.44±0.77 mg GAE/g, respectively. While, the lowest values of TPC were registered for the aqueous extract of a plant treated with two studied proportions of OMP (0% and 25%) with amounts of 33.92±1.72 mg GAE/g and 39.45±3.28 mg GAE/g, respectively. The outcomes agree with those reported by the previous report. [1] Several factors have been found to be able to affect antioxidant recovery, such as particle size, temperature, extraction duration, solid/solvent ratio, and solvent polarity. [43] Olive mill wastewater was found to increase the plant biomass and essential oil yield. [44] Olive pomace is an organic matter used to enrich arid and semi-arid soils and is also used as a biofertilizer in Morocco. [44,45] Organic biofertilizer application ameliorates the phenolic profile of plants, such as *Labisia pumila benth*. [46] The advantage of fertilizers made from OMP is the absence of pathogenic microbes and the availability of different nutritional ingredients for plants, which could boost plant productivity. [47]

Total flavonoid content (TFC)
Figure 2 displays the results of the flavonoid content dosage using the chloride aluminum method. The application of OMP at a dose of 25% significantly increased the TFC amounts of different extracts carried out using solvents with different polarities (*p*<0.05). The ethanol extract was found to have the greatest TFC value (6.13±0.1 mg QE/g) followed by methanol extract (4.58±0.5 mg QE/g) for the plant treated with OMP at a dose of 25%. Meanwhile, the lowest TFC quantities were established in all extracts of the plant treated with OMP at a concentration of 0% (Figure 2). It was discovered that TFC extractor solvent effects are comparable to those on TPC. The ranking of decreasing TFC was ethanol extract > methanol extract> aqueous extract for both treatments (*p*<0.05). The finding agrees with those of a previous report. [42] This could be explained by the polarity characteristics of flavonoid components that are more extracted with less polar extractor solvents. [48,49] The amelioration of bioactive accumulation and biomass production of medicinal plants constitute the main objective for the food and pharmaceutical industries. [50] To achieve this objective, the use of biofertilizers offers significant advantages for the synthesis of secondary metabolites as well as biomass. [50,51] According to statistical analysis, the use of OMP at a dose of 25% was correlated positively with total flavonoid content (*r*=0.918, *p*<0.05). El Hassani et al. reported that the application of olive mill wastewater during the vegetative phase of growth decreased the plant yield and partially or totally the germination of seeds of several plants. [44]

Antioxidant activity
The antioxidant potential of different extracts examined by DPPH assays allowed us to obtain the results presented in Figure 3. Findings revealed that the extracts exhibited an interesting antioxidant ability with significant differences between all extracts of different plants treated with both doses of OMP (*p*<0.05). Furthermore, the antioxidant activity varies significantly with the kind of extractor solvent (*p*<0.05). The antioxidant results were in accordance with the outcomes of Kasparavičienė et al. who proclaimed that ethanol extract exhibited an interesting antioxidant activity dose-dependent. [52] Kabubii et al. found that the agroecological zones affected the phytochemical content and antioxidant activity of *Rosmarinus officinalis*. [53] Statistical differences were observed comparing different extracts, which can be explained by the ability of each extractor solvent to recuperate antioxidant compounds from a vegetal matrix (p<0.05). On the other hand, pedoclimatic conditions considerably affected the phytochemical profile of plants and consequently their antioxidant activity. The application of organic compost has been found to increase the antioxidant activity of Strawberries. [54] Higher antioxidant levels are typically correlated with the best conditions for plant growth. [55] In fact, the correlation analysis revealed a positive correlation between antioxidant activity and 25% OMP treatment (*r*=0.453, *p*<0.05) (Table 3). Within this framework, the application of olive mill pomace could have a great impact on crop culture as an alternative sustainable biofertilizer.

Impact of OMP treatment on Rosamarinus officinalis mineral profile
Table 4 describes the mineral profile of *R. officinalis* under two different treatments with OMP at two doses 0% and 25%. The quantification of eleven minerals allowed us to register different concentrations according to the type of treatment. The highest amounts were registered for K (49030.01 mg.kg^{-1}), Ca (74798.745 mg.kg^{-1}), and Na (15208.6 mg.kg^{-1}) for samples treated with OMP 0%.

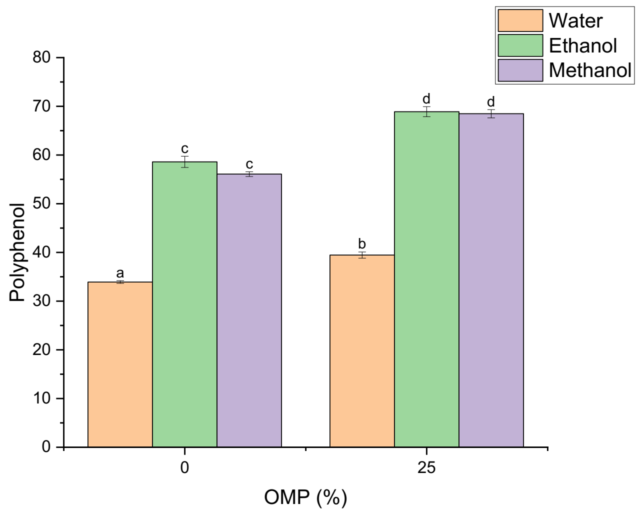

Figure 1: Total phenolic content of samples treated with different proportions of olive mill pomace. Values with different letters are significantly different at; p ≤ 0.05.

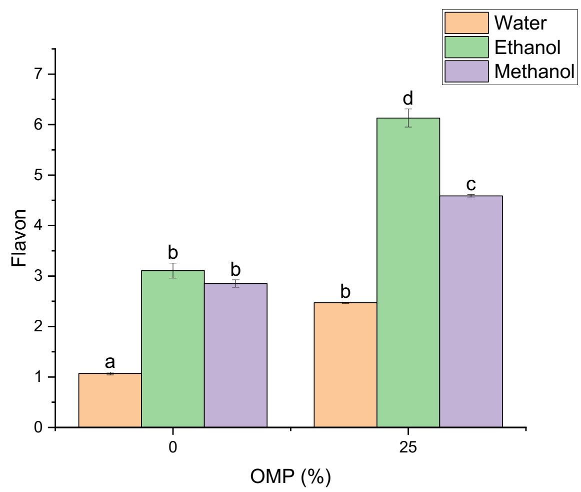

Figure 2: Total flavonoid content of samples treated with different proportions of olive mill pomace. Values with different letters are significantly different at; p ≤ 0.05.

Table 1: Physicochemical properties of experimental Soil and Olive mill pomace used in this study

Parameters	Soil	Olive mill pomace (OMP)
Total sand	29.05	-
Total Silt	14.15	-
Clay	26.81	-
pH (H_2O)	7.80	5.21
pH (KCl)	7.17	5.08
EC (mS cm^{-1})	1.185	0.07
CEC (meq $100g^{-1}$)	1215	-
Organic matter (%)	1,8	91.48
P ($mg.Kg^{-1}$ DW)	2094.21	16379.1
K ($mg.Kg^{-1}$ DW)	28173.6	197559
Zn ($mg.Kg^{-1}$ DW)	1189.98	481.5
Mn ($mg.Kg^{-1}$ DW)	9455.4	137.88
Cu ($mg.Kg^{-1}$ DW)	328.95	207

The values obtained on dry weight represent the average of three repetitions

Table 2: The leaf dry weight (g) of the plant

	Leaf dry weight (g)
0% OMP	9.27
25 % OMP	13.17

Table 3: correlation matrix between total phenolic compounds (TPC), total flavonoid content (TFC) and antioxidant determination (DPPH) of two selected Rosemary ethanol extract at 0 and 25% Of OMP amendment.

	TPC	TFC	DPPH
0 % OMP	1	-0.082	0.547**
25% OMP	1	0.918***	0.453*

*** Correlation is significant at the 0.001 level. ** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

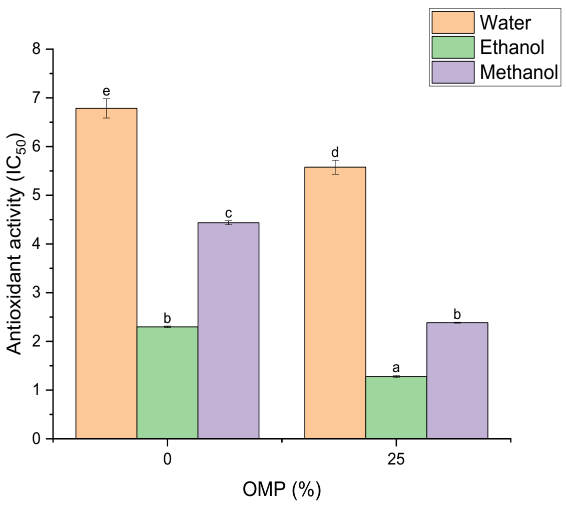

Figure 3: Antioxidant activity of different samples treated with different proportions of olive mill pomace. Values with different letters are significantly different at; $p \leq 0.05$.

Concerning the sample treated with OMP 25%, the highest amounts were registered for Ca (94480.75 $mg.kg^{-1}$), K (68388.9 $mg.kg^{-1}$), Cu (10923.1575 $mg.kg^{-1}$), and P (6264.952 $mg.kg^{-1}$). As it can clearly be observed, the OMP 25% has a direct impact on mineral profile of plant under study by increasing the quantity of mineral, accounting Cu (4469.875 $mg.kg^{-1}$ to 10923.1575 $mg.kg^{-1}$), P (4719.03 $mg.kg^{-1}$to 6264.4925 $mg.kg^{-1}$), K (49030.01 $mg.kg^{-1}$ to 68388.9 $mg.kg^{-1}$), Ca (74798.745 $mg.kg^{-1}$ to 94480.75 $mg.kg^{-1}$), Zn (197.663 $mg.kg^{-1}$ to 233.543 $mg.kg^{-1}$), and Cr (19.90 $mg.kg^{-1}$ to 35.38 $mg.kg^{-1}$). In contrary, the same OMP dose decreased significantly the quantity of the following minerals: Al (1806.79 $mg.kg^{-1}$ to 718.575 $mg.kg^{-1}$), Na (15208.6 $mg.kg^{-1}$ to 10760.6375 $mg.kg^{-1}$), Mn (115.2 $mg.kg^{-1}$ to <0.01 $mg.kg^{-1}$), Mg (15766.495 $mg.kg^{-1}$ to 9783.27 $mg.kg^{-1}$), and Fe (4249.775 $mg.kg^{-1}$ to 2207.7275 $mg.kg^{-1}$). The obtained results agree with those reported by Paulauskiene et al. [56]
The mineral status of soil constitutes a key factor for culture development. In fact, the application of organic fertilizers such as humic substances fertilizer, complex fertilizer, and compost significantly increased the mineral composition of six pumpkins cultivars by elevating the content of calcium, iron, manganese, sodium, and zinc in fruit and reducing the quantity of copper.[56] *Rosmarinus officinalis* needs a significant amount of accessible macro and micro-elements, which are provided in remarkable amounts by the application of olive mill pomace. The bioavailability of these minerals is important in the photosynthesis process, which confirms the elevation of bioactive amounts quantified in *Rosmarinus officinalis* after treatment with OMP at a dose of 25% (Figure 1 and Figure 2). Humic fertilizer enhanced mineral absorption and ameliorated the nutrient accumulation that plays an important role in herb development. [57] Olive mill pomace has a low pH, high organic matter, and a considerable quantity of bioactive compounds that enrich the soil with humus, mineral elements, and organic matter used by the plants to ensure their optimal development. [44]

Impact of olive mill pomace on plant nutrient uptake
Table 5 displays the results of the determination of nutrient uptake of plants cultivated under different treatments. It has been clearly seen that the treatment of plants with OMP 25% significantly increased the amounts of nutrient uptake, including Ca (69.35667738 $g.plant^{-1}$ to 124.521443 $g.plant^{-1}$), K (45.4777761 $g.plant^{-1}$ to 90.10944295 $g.plant^{-1}$), P (4.375426209 $g.plant^{-1}$ to 8.256195179 $g.plant^{-1}$), Zn (0.183272686 $g.plant^{-1}$ to 0.294583673 $g.plant^{-1}$), and Cu (4.143491733 $g.plant^{-1}$ to 14.39582031 $g.plant^{-1}$). The nutrient uptake increased the bioavailability of minerals in plants, which enhanced plant performance and development. On the contrary, the application of olive mill pomace at a dose of 25% significantly decreased the uptake of accessible minerals from the soil through their roots, such as Mg, Mn, Al, and Fe. The results agree with those reported by Yang et al. concerning K and P. [58] Indeed, organic fertilizers have been found to improve soil nutrient status and soil microbial community, which furnishes the accessible and necessary plant elements. [59] The incorporation of organic manure markedly ameliorated morphophysiological root systems of Rice (*Oryza sativa* L.) and nutrient uptake resulted in a significant increase in the grain yields. [58] In the same context, Sharma et al. proclaimed that the organic manure application improved the fruit quality in terms of soluble solid content, nutrient status, and soil physicochemical parameters. [60] The improvement of nutrient availability and soil physicochemical properties boosts the physiological response of plants, improving metabolic activity and increasing secondary metabolite production. [60,61] Olive mill pomace could be of great importance to improve the agricultural traits and yield of *Rosamarinus officinalis* and play a considerable role in soil quality improvement.

Impact of olive mill pomace on leaf dry weight of plant
Table 2 describes the leaf dry weight of the plants treated with two different proportions of OMP (0% and 25%). It is clearly seen that the highest value was registered for leaves of plants treated with OMP 25% with a value of 13.17 g. While the leaf dry weight of plants treated with OMP 0% did not exceed 9.27 g (Table 2)

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Table 4: Macro- and micronutrient levels in a plant grown in two different soils (mg.kg^{-1})

	Cu	Al	P	Na	Mn	Mg	K	Fe	Ca	Zn	Cr
% OMP	4469.87^{a}	1806.79^{a}	4719.03^{a}	15208.6^{b}	115.2^{b}	15766.49^{a}	49030.01^{a}	4249.77^{b}	74798.74^{a}	197.66^{a}	19.90^{a}
25 % OMP	10923.15^{b}	718.57^{b}	6264.49^{b}	10760.6375^{a}	< 0.01^{a}	9783.27^{b}	68388.9^{b}	2207.72^{a}	94480.75^{b}	233.54^{b}	35.38^{b}

Table 5: Nutrient uptake (g.plant^{-1}) levels in a post-harvest plant

	Ca	Fe	K	Mg	Mn	Na	P	Al	Cu	Zn	Cr
0% OMP	69.35^{a}	3.94	45.47^{a}	14.61	0.10	14.09	4.37	1.67	4.14^{a}	0.18	0.01
25 % OMP	124.52^{b}	2.90	90.10^{b}	12.89	< 0.01	14.18	8.2	0.94	14.39^{b}	0.29	0.0

Values with different letters are significantly different at; $p \leq 0.05$.

Table 6: Impact of OMP on soil physicochemical properties

	pH (H_2O)	CEC (meq.100g^{-1})	electrical conductivity (EC) (µS.cm^{-1})	Organic Matter OM (%)
OMP 0%	8.35	12.78	2.49	5.85
OMP 25%	7.49	12.80	146.07	18.44

Impact of olive mill pomace on physicochemical properties of soil

Table 6 displays the results of the physicochemical properties of soil after treatment of *Rosmarinus officinalis* with both doses of OMP. The treatment of the obtained results showed that the application of OMP at a dose of 25% neutralized the soil pH (8.355 to 7.4975), electrical conductivity (82.49 µS.cm^{-1} to 146.075 µS.cm^{-1}), and organic matter (5.85 % to 18.44%). OMP treatment did not induce a significant change in the cation-exchange capacity (Table 6). A high negative correlation was observed between pH and organic matter and CEC (r=-0.994, r= 0.778, $p<0.05$, respectively) (Table 7). The outcomes are consistent with those reported by earlier studies. [62,63] The treatment of soil with different proportions of olive oil solid waste (2%, 4%, and 8%) sustained for 2 months decreased soil pH, increased soil electrical conductivity, and significantly increased soil nitrogen content. [63] The same findings are evoked by Amziane et al. using olive pomace at different concentrations (25%, 50%, 75%, and 100%). [62] Soil pH is a determinant factor for the absorption of macro and microelements essential for normal plant growth and development. [64] The enrichment of soil with biodegradable organic manure enhanced the ammonia release, which is implicated in pH reduction. [62] Sirisuntornlak et al. in their study proclaimed that the pH ranging between 6.3 and 7.4 was the most appropriate range for maize growth, yield, and nutrient uptake. [65] It has been found that the low pH is accompanied by low uptake of calcium and magnesium, while zinc, manganese, and iron are absorbed in high proportions. [66] Therefore, soil quality plays an important role in nutrient recycling and herb nutrition.

Effect of soil physicochemical properties on polyphenols, flavonoids, antioxidant activity

The correlations between the soil physicochemical parameters and polyphenols, flavonoids, and antioxidant activity are presented in a correlation matrix (Table 7). Where, a negative correlation was found between CEC, Soil Organic Matter, TPC, TFC, and DPPH. In addition, soil organic matter, TPC, TFC, and DPPH show a positive correlation.

A correlation between soil physicochemical properties and bioactivity of medicinal and aromatic plant extracts has been investigated, yielding insightful results. This is because the content of secondary plant compounds in plants varies according to soil composition, water-holding capacity, and photosynthetic conditions. [67]The production of these compounds in plant species is regulated by genetic factors and metabolic pathways but is also significantly influenced by growth and environmental conditions. For example, in a recent study, *mulberry strawberries* grown on volcanic soils had the highest total phenolic content. In contrast, in *Tudor strawberries*, the total phenolic content was reduced or not affected by soil composition. [68]These results underline the importance of considering soil composition and soil physicochemical parameters when growing medicinal and aromatic plants, as soil organic matter, can significantly impact the extracts' biological and antioxidant activity. [69]

Conclusion

In conclusion, the olive mill pomace application at doses of 0% and 25% affected the bioactive compounds content and mineral profile of *Rosamarinus officinalis*, as well as soil physicochemical properties. Furthermore, the sample treated with 25% OMP had the highest concentration of bioactive chemicals extracted using ethanol. The findings of this study are preliminary, and more investigations are required to determine the precise methods by which OMP physicochemical characteristics affect the polyphenol content, flavonoid content, and antioxidant activity of rosemary. This study might create fresh ways to improve rosemary's nutritional value.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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