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Different Responses of the Quality Parameters of *Coriandrum sativum* to Organic Substrate Mixtures and Fertilization

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Abstract: In order to standardize the quality of agricultural products, it is necessary to control the factors affecting plant development, such as plant nutrition. The best results in terms of homogeneity of the quality of vegetable crops were achieved using inert substrates and application of nutrients; however, production costs are high due to the cost of irrigation systems and substrate management and importation. This work aims to evaluate the effect of the local substrate mix and the amount of organic fertilizer on different quality parameters of coriander. To evaluate the quality of coriander, we considered different parameters such as size, biomass, antioxidant capacity and aroma (evaluated by volatile compounds detection with gas chromatography). The results show that the culture system differentially affects each parameter, and the compounds associated with the aroma of coriander and the diameter of plants are sensitive to the culture system, while the length of plants, number of leaves and antioxidant activity are not affected by the concentration of fertilizer. Moreover, organic farming conditions do not reduce quality parameters of the crops when using adequate fertilization. Additionally, local substrates would be practical substitutes for expensive importations.

Keywords: *Coriandrum sativum*; quality; organic culture

1. Introduction

Coriander is among the most highly demanded horticultural products in the market for aromatic herbs; the highest demand, worldwide, is for use in the food industry. However, it is important to consider that current demand in the food industry is oriented towards the consumption of high quality products and value added services, which provide greater benefits. In this context, organic agricultural products have grown in consumer demand.

Organic agriculture is a method that consists in reducing the use of agricultural inputs such as synthetic fertilizers and pesticides, as well as genetically modified seeds and species. Instead, it relies on practices that are compatible with the environment and that aim to maintain or increase soil fertility in the long term. Although organic agriculture is still a small industry (1%–2% of global food sales), its importance is growing worldwide. In Mexico, the success of organic products should be attributed to the export market, which represents an important source of income for small producers [1].

In general, organic products are sold at higher prices than conventional products because consumers are willing to pay more for products that satisfy certain ideological and health needs; however, they demand guarantees on the quality of organic products [2]. Due to the minimal use of agrochemicals, organic products can give the impression of lower quality [2], considering that quality, or the degree of excellence of a product, is given by the attributes or properties that characterize it; in the case of coriander, the attributes that reflect its quality are size, number of leaves per plant, color, presence of pathogenic damage, weight and homogeneity between plants [3].

Among the factors influencing plant quality and development, plant nutrition and accessibility of water are directly related to the size of plants [4]; thus, to increase the quality and homogeneity of the product, it is necessary to apply an adequate amount of water and an adequate concentration of micro and macro nutrients. Conducting an annual evaluation of the concentrations of these nutrients in different soils to ensure the quality and quantity of the crops is not effective because the concentration varies depending on the pH of irrigation water, fertilization, time of year, number of crop cycles and geographic region. The best results in terms of homogeneity and quality of vegetable crops have been achieved using substrates as growth medium and applying nutrients; such growing systems allow for controlling pH, electrical conductivity and the concentration of minerals available to the plants [5]. The use of substrates to optimize crops is documented in scientific articles and patents; however, as has been reported in some studies, not any substrate can be used for any crop; it is necessary to evaluate the physical and chemical characteristics of each substrate to determine if they are appropriate for a specific type of crop [6,7].

Usually, when a crop is established, different substrates are combined to ensure that water retention, nutrient supplementation (if any), aeration, electrical conductivity, pH buffering and type of growth media are adequate for that crop. In addition to commercial substrates, producers can use readily available materials as substrates, and even reuse them in different crop cycles, such is the case of the fallen leaves of *Gymnopodium floribundum*, known locally as “dzidzilché leaf” [8] and the fallen leaves of Tahonal (*Viguiera dentata*).

In this work, we characterized three substrate mixtures (dzidzilché leaf, coconut fiber and tezontle gravel) and evaluated their effect on different physical and biochemical parameters (fresh weight, dry weight, length, stem diameter, volatile compounds and antioxidant capacity) of coriander plants fertilized with two concentrations of organic fertilizer and grown in protected organic systems, with the aim of establishing a system of protected organic farming using local materials as a source of substrate.

2. Results and Discussion

2.1. Physicochemical Characterization of Substrate Mixtures

With the aim of determining the mixture of substrates suitable to the organic cultivation of coriander, we evaluated two different mixtures of three substrates (dzidzilché leaf, coconut fiber and tezontle gravel): Mixture 1 (M1) 1:1:1 and Mixture 2 (M2) 4:3:3. We considered the individual chemical and physical properties of each substrate and evaluated the same properties on both mixtures: effective porosity (PT), real density, distribution of particle size, density, pH and conductivity.

The interaction of roots with water and air spaces, which are necessary for plant growth, is described by the properties of PT, real density and distribution of particle size. The substrates with a PT below 50% are not recommended because the lack of air space would cause anoxia to root cells. Table 1 shows the PT of the substrate mixtures; both samples had values above 50%; however, the mixture M2 had a porosity close to 80%, which has been described as optimal for substrates of organic origin since these substrates degrade and reduce their porosity during the growing cycle [7].

Table 1. Physicochemical characterization of the two substrate mixtures.

	PT (%)	Dr (g/cm^3)	C (dS·m^{-1})	pH	Moisture (%)	Water Absorbance Capacity (Times Their Weight)	Distribution of Particle Size		
							>2 mm	2–1 mm	<1 mm
M1	50.15	0.61	0.73	6.3	7.26	1.0	28.58	17.66	53.76
M2	71.21	0.46	0.99	5.1	11.00	1.0	40.80	15.02	45.26

M1: 1:1:1; dzidzilché leaf, coconut fiber and tezontle gravel. M2: 4:3:3; dzidzilché leaf, coconut fiber and tezontle gravel. PT: Effective porosity; Dr: real density; C: conductance.

The measurement of real density was obtained to predict the behavior of the substrate mixtures within the gutters in terms of oxygenation, remaining in place and not being carried by wind and water. For M1, real density was 0.61 g/cm^3, the highest recommended value for the cultivation of vegetables in greenhouses; it is preferable to use substrates with densities lower than 0.5 g/cm^3 [7], as in the case of M2, which had a density of 0.46 g/cm^3.

Generally, substrates consist of particles of different sizes [9]; the physical properties of a substrate tend to vary considerably depending on the percentage distribution of the size ranges into which the particles are classified. Since these particles are not spherical and have different sizes, in practice, the porosity of the substrate increases with average particle size and vice versa. The size of the external pores formed by interparticle spaces depends on the size of the particles; this is why granulometry has been related to porosity and to moisture holding capacity (Table 1). Considering this, it is recommended that the substrates contain approximately equal proportions of different size particles, discarding the substrates in which more than 80% of the particles are smaller than 1 mm, since this would cause the substrate to be too compact, adversely affecting plant growth. In this regard, the distribution of particle size in the two mixtures under study was appropriate for coriander cultivation.

The conductance of a substrate is associated with its salinity; thus, salinity represents a constraint when considering the use of a material as a substrate, since the salinity of the culture medium may be toxic and negatively interfere with the assimilation and exchange of minerals. The plant response to salinity depends on the plant genotype, but it is generally recommended that the conductance of agricultural soils does not exceed 2.0 $dS \cdot m^{-1}$. The substrate mixtures under study did not exceed the range of 2 $dS \cdot m^{-1}$ and can thus be considered suitable for use in crops (Table 1).

Regarding the pH of the substrates, it must be considered that plants can survive in a wide range of soil pH without presenting physiological disorders; however, when soil pH is extreme (<5.0 and >7.5), a constant administration of nutrients in assimilable form is necessary, resulting in increased production costs or reduced crop yield. Both substrate mixtures, M1 and M2, had a pH between 6.3 and 5.1, respectively (Table 1), which are acceptable ranges; however, it is important to consider that the pH of organic substrates decreases over time with the production of H^+ as a direct result of the degradation of organic material.

2.2. Evaluation of the Size Parameters of Coriander Cultivated on Two Substrate Mixtures with Two Different Concentrations of Fertilizer

To determine the effect of nutrition of the physical-chemical parameters of the substrate mixtures, and of the possible interactions between these two factors on the quality of coriander, we used a 2 × 2 factorial experimental design with two levels of mixtures (M1 and M2) and two of fertilizer (50 and 100), with a total of four treatments for coriander cultivation. Sixty days after germination, we evaluated the length, diameter and number of leaves per plant cultivated under each of the treatments. It is important to note that crop quality must be homogenous; therefore, to ensure the reliability of our data, we estimated the sample size (for all crops) according to the size of the population and with a confidence level of 95%.

The plants cultivated under the four treatments reached a length between 14 and 16.07 cm (Table 2), which is an attractive size for the market, especially considering that we used an organic growing system;in contrast, the length of plants from seeds that have been genetically improved and/or that are cultivated with the application of agrochemicals to stimulate growth ranges from 8 to 30 cm. Statistical analysis of the length of coriander plants showed no significant difference between substrate mixtures or between fertilizer concentrations (Table 3). Similar results were obtained by comparing the effect of different organic fertilizers on the height of coriander plants; only the comparison with inorganic fertilizers showed significant differences [10].

Table 2. Average length, number of leaves and stem diameter of coriander plants for each of the treatments.

Treatment No.	Substrate Mixture	Fertilizer Concentration (%)	Length of Plants (cm)	Number of Leaves	Stem Diameter (mm)
1	1	50	15.07	3.30	1.62
2	2	50	14.68	3.57	1.56
3	1	100	16.07	3.13	1.95*
4	2	100	14.00	3.33	1.43

The asterisk represents a statistically significant difference (Least Significant Difference (LSD) $p < 0.003$).

Table 3. Average percentage of 2,2-diphenyl-1-picrylhydrazyl (DPPH) degradation in coriander plants per treatment.

Treatment	% of DPPH Degradation (Compared to Control)
M1 50	43.71
M2 50	42.31
M1 100	48.95
M2 100	51.87

Another important characteristic is the number of leaves per plant; this characteristic not only makes plants more visually attractive, but is also related to the characteristic aroma of coriander. The number of leaves of coriander plants cultivated under the different treatments showed no statistically significant difference (Table 2), with an average of three leaves per plant, which is expected for this variety of coriander.

The stem diameter of coriander plants is associated with plant vigor, which depends on the quality of substrate and nutrients during cultivation. The experimental analysis of the association between stem diameter variability and fertilizer concentration found statistically significant differences. However, the analysis showed there was no interaction between the two factors (Table 2); the effect of each fertilizer concentration on stem diameter was independent of the other. The best treatment was substrate Mixture 1 with a fertilizer concentration of 100%.

Figure 1 shows the effect on dry weight—there were significant differences between treatments, but principal effect analyses do not show significant differences between fertilizer concentrations or by the interaction of the factors. The best treatment was M2 100, After knowing that no fertilization concentration had any effect, we suggest that biomass production decreases in response to physic properties of the substrate mix.

In contrast to what was observed, Figure 2 shows that fertilizer concentration had a significant effect on the fresh weight of coriander plants, while the mixture of substrates and the interaction between factors had no effect. The best treatment was a fertilizer concentration of 100%, which could be explained by the need and capacity of plants to absorb water in the presence of the concentration of minerals in the growth medium.

2.3. Evaluation of Antioxidant Capacity

Reactive oxygen species (ROS) are produced by metabolism in response to different types of stress; they are responsible for cellular alterations such as mutations in the DNA sequence, protein denaturation, peroxidation of cell membranes and cell death [11,12]; at the organism level, ROS can cause premature aging, cardiovascular and degenerative diseases [13], and even some cancers. Antioxidant compounds such as flavonoids, tocopherols, catechins, carotenoids, *etc.*, are able to degrade ROS, which is known as antioxidant capacity [14,15]. Antioxidant compounds are present in plants [16] and are important protective agents for human health; thus, the presence of these compounds provides an added value to food products and is one of the characteristics sought in functional foods.

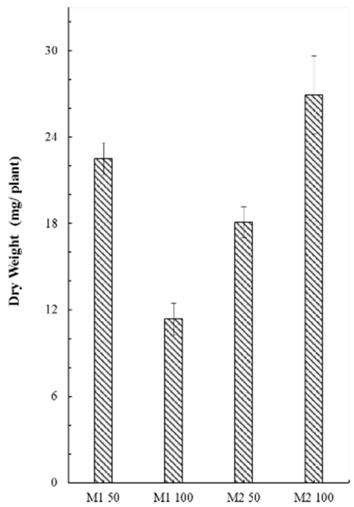

Figure 1. Graph of the substrate mixture (M1 and M2)—fertilizer concentration (50 and 100) effecton dry weight of coriander plants.

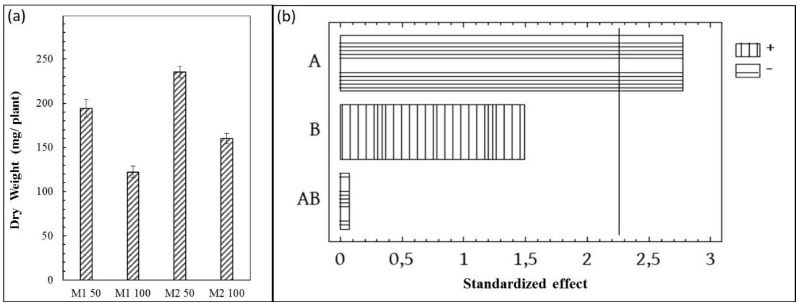

Figure 2. Effect of substrate mixture and fertilization on fresh weight. (**a**) relation between dry weight and substrate (M1 or M2) and fertilizer concentration (50 or 100); (**b**) Pareto graph of the fresh weight of coriander plants. A: Factor A (fertilizer concentration) effect; B: Factor B (Substrate mix) effect; AB: Effect of factor interaction.

To determine if the mixture of substrates or the concentration of fertilizer used in coriander cultivation has a significant effect on its antioxidant capacity, the plants grown under the different treatments were lyophilized and macerated at 30 days of culture to prepare them for methanol extraction. The antioxidant capacity of the extracts of plants grown under the different treatments was evaluated through the degradation of diphenyl-1-picrylhydrazyl (DPPH). DPPH is a stable free radical that can be neutralized by the presence of antioxidants; its decay (scavenging) can be measured by absorbance, and the antioxidant content of a sample is reported as the percentage of DPPH degradation [17]. The results obtained from the extracts of each treatment showed antioxidant activity similar to that reported previously [17]. However, the separate analysis of each effect showed no statistically significant difference between each factor when comparing mean square errors with estimated experimental errors (Table 3). If we considered that the abiotic or biotic stress can change the antioxidant activity [18,19], our results suggest that there is no difference between stress levels presented in coriander plants grown in different treatments.

2.4. Determination of the Variation of Volatile Compounds Responsible for Aroma in Coriander Plants Cultivated in Different Substrate Mixtures and Two Fertilizer Concentrations

Given the importance of aroma in consumer preferences [20], we evaluated the effect of growing conditions on the presence and concentration of 2-Octenal, 2-Dodecenal, limonene and linalool, which are considered the main volatile compounds responsible for aroma [21]. The results indicated that the extracted sample had a total of 28 peaks; the peaks with the largest percentage area corresponded to the compound eluted at 13.36 min, identified as 2-Octenal (E); then came the compound eluted at 11.69 min, identified as (E)2-dodecenal, and then the peaks identified as decanal. According to the literature, the main compounds found in mature coriander are (E)-2-decenal, Decanal, (E)-2-undecenal and (E)-2-dodecenal; linalool and limonene are found at lower concentrations (close to zero for the latter) [22,23]. Generally, the elution profiles of compounds obtained from the samples by GC were very similar; the peaks with the largest percentage area were the same and linalool and limonene were absent in all cases, which is consistent with the reports of seedling samples, even though most reports are of seeds [22]. Table 4 shows the proportion of the compounds identified in coriander samples grown under different treatments. We used variance analysis (ANOVA) to determine whether significant differences existed in the percentage of the areas of the peaks identified in each of the treatments. Table 4 shows the homogeneity of the groups, which indicates that they are all different from each other; the compound with the largest percentage area, 2-Octenal (E), was found in greater proportion in the treatment M2 100.

Table 4. Percentage of each compound with respect to the total of detected compounds.

Treatments	2-Octenal (E)	(E)–2 Dodecenal	Decanal
M1 50	42.26^{c}	11.035^{c}	5.085^{d}
M1 100	41.125^{b}	10.985^{b}	4.885^{c}
M2 50	38.03^{a}	9.6^{a}	3.37^{a}
M2 100	43.03^{d}	11.075^{c}	4.395^{b}

Different letters indicate significant statistical differences (LSD $p < 0.0000$).

3. Experimental Section

3.1. Establishment of the Experimental Design and Determination of Sample Size to Ensure Statistical Reliability

We used a 2 × 2 factorial experimental design; two factors on two categorical levels (non-continuous). Factor A: fertilizer concentration (50% and 100%); factor B: Mixture 1 (1:1:1; dzidzilché leaf, coconut fiber and tezontle gravel) and Mixture 2 (4:3:3; dzidzilché leaf, coconut fiber and tezontle gravel); as well as the possible interaction between factors (Table 5).

Table 5. Experimental design for evaluating substrate mixtures and fertilizer concentrations.

No. of Treatment	Coded Factor		Decoded Factor	
	Factor A	Factor B	Substrate Mixture	Fertilizer Concentration (%)
1	–	–	1	50
2	+	–	2	50
3	–	+	1	100
4	+	+	2	100

The experimental strategy consisted of establishing 16 cultivation boxes in gutters with a depth of 5 cm; each box had a sowing area of 0.8 m^2. The gutters were disinfected with Anibac Plus© (according to the recommendations of the fertilizer's manufacturer) and filled with the substrate mixture corresponding to each treatment, with four boxes per treatment.

The organic coriander seeds were obtained from "Environmental Seed Producers". The number of plants per unit area was experimentally determined using previous reports as a reference. The amount of seeds in a gram was counted for each vegetable; the obtained value was extrapolated to determine theoretically the grams required to obtain the desired plant density. The seeds were weighed and sown in the gutters, with a density of 31.25 g seed/m^{-2}. During cultivation time, the plants were watered daily for about 15 min by a drip system with a slope to allow excess water to drain from the gutters. The irrigation time was adjusted to keep substrate moisture close to 80%. Fertilization was done by spraying, distributing 10 Lt per gutter of the organic substrate "Axe-Root" (8.18% urea; 8.18% P_2O_5, 8.18% K_2O_5, 2.5 ppm L-cisteine, 2.3 ppm auxines, 5% aminoacid, 3% carbohidrats) from the Axeb Biotech company. The concentration of fertilizer used for foliar spraying was 50% and 100% of the recommended by the manufacturer (1.5 mL/Lt and 3 mL/Lt, respectively). The time of harvest was determined in a preliminary stage in which we estimated the time required for coriander to reach its maximum development before the flowering stage.

The number of samples (analyzed plants) from each cultivation box was determined by the following formula for finite populations [24]:

$$n = \frac{Z^2 pqN}{Nd^2 + Z^2 pq} \tag{1}$$

where: n = sample size; Z = 1.96; 95% confidence; p = expected proportion (50%); q = 1-p; N = population size; d = 5% Accuracy

3.2. Harvest

Harvesting was done when the plants reached a specific point, which was different for each vegetable. It was carried out at 6:00 h to prevent the heat from affecting the quality product. The size, color and fresh weight of the plants were evaluated on the day of harvest. The plants were divided to determine dry weight, antioxidant capacity and volatile compounds. For dry weight measurements, plants were dried at 60 °C for 72 h. The samples used for determining antioxidant capacity and volatile compounds were frozen with liquid nitrogen and stored at −80 °C until biochemical analysis.

3.3. Evaluation of Fresh Weight (FW) and Dry Weight (DW)

On harvest day, 16 plants per treatment were weighed individually, and the value in mg was expressed as FW. The dry weight of each plant was determined by placing the previously weighed plants in a drying oven without ventilation at a temperature of 60 °C for three days; after this period, the plants were weighed again and the value in mg was expressed as DW.

3.4. Evaluation of Antioxidant Capacity

The antioxidant capacity can be evaluated based on the chemical reactions involved in electron transfer. The method we used was the one proposed by [25]; we evaluated the antioxidant capacity of several substances against the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH●) in a methanolic solution of a deep violet color, which it gradually lost with the addition of samples with antioxidants. The samples were macerated individually in the presence of liquid nitrogen; the resulting powder was resuspended in anhydride methanol (99.8%) and stirred for 1 h; the mixture was then separated by centrifugation, the supernatant was stored at 4 °C and the pellet was resuspended in acetone (70%) with stirring for 1 h. After centrifugation, the precipitate was discarded and the supernatant was added to the previous extraction [26]. We took 60 μL from the extracted supernatant and added them to 3 mL of a 0.1 mM solution of 2,2 diphenyl-1-picrylhydrazyl (DPPH). After 45 min, we evaluated absorbance at a wavelength of 517 nm [27].

3.5. Solvent Extraction by Soxhlet

Fresh leaves and stems (50 g) were placed in a soxhlet extraction system, adding 200 mL of ether anhydride to the distillation flask as organic solvent. The extraction was carried out for 120 min at a temperature of 50 °C. The recovered solvent containing the sample was cooled to −20 °C and sealed until the concentration of volatile compounds reached a certain volume for subsequent analysis. The extractions were performed in duplicate.

3.6. Sample Concentration

The organic phases were concentrated at 30 °C using a Vigreux column at atmospheric pressure; the concentrated compounds were kept frozen (−20 °C) and tightly sealed until further analysis.

3.7. Analysis by Gas Chromatography

Once concentrated, the isolated extracts were analyzed by gas chromatography using a Thermo Scientific equipment Ultra Trace GC model 101408 manufactured by Thermo Fisher Scientific Company, (Waltham Massachusetts, USA), with a flame ionization detector (FID). We used an apolar capillary column HP-5 (850 m × 0.20 mm, 0.25 µm; agilent). The carrier gas used was helium with a flow of 1.5 mL/min. The split ratio was 60:1. The analysis was performed using the following temperature program: the oven temperature started at 40 °C and increased to 300 °C at a rate of 15 °C/min, then kept isothermally at 350 °C for 5 min. The temperatures of the detector and injector were set at 300 and 250 °C, respectively. In addition, 1 µL of concentrated sample was injected in duplicate (experimentally determined).

3.8. Identification of Volatile Compounds

Two commercial standards of limonene and linalool were injected into the gas chromatograph under the same conditions as the samples in order to identify them in the test samples. A mixture of compounds previously identified by mass spectroscopy under the same conditions was also used to identify the peaks of compounds found in the test samples [20].

3.9. Gas Chromatography-Mass Analysis (GC-MS)

The samples were analyzed using an Agilent chromatograph 6890N model, version N.05.04, coupled to a mass detector 5973 N (GC/MSD) also manufactured by Agilent Technologies. Santa Barbara California USA.; the column used was a nonpolar (HP-5 100 µm ID × 10 m × 0.34 µm); the carrier gas used was helium at a flow of 0.4 mL/min in constant flow mode with vacuum compensation. The injection port was kept at a temperature of 250 °C GC/MSD, and the interface at 300 °C. We used a split injection with 1 µL of sample at 100:1; the temperature program used was as follows: initial temperature of 70 °C and increases of 5 °C/min to a final temperature of 225 °C; the detector was used with a scan range of 25–800 amu, and scan rate of 1.38 scans/sec (experimentally determined).

3.10. Statistical Analysis of the Results

The statistical analysis, which included analyzes of variance (ANOVA) to compare means and the means comparison test (LSD), was performed using the software Statgraphics centurion (Version XVI) from StatPoint technologies Inc. (Warrenton, VA, USA). Single variance analysis was used to evaluate the data of the characteristics of the substrates; and a multifactorial ANOVA was used to analyze the factors affecting the quality of products. To determine whether there was interaction between the factors, we analyzed the experiment by two-way ANOVA.

4. Conclusions

We do not recommend using the substrates evaluated here by themselves for growing vegetables; they should be mixed, making sure that the final mixture has proper porosity, conductance and pH characteristics, as the mixtures proposed in this work (Mixture 1: proportion 1:1:1, Dzidzilché leaves:coconut fiber:tezontle gravel; Mixture 2: proportion 4:3:3, Dzidzilché leaves:coconut fiber:tezontle gravel).

Fertilization is directly related to crop productivity [10]; however, there is no general effect or a universal concentration for growing a specific species, as the absorption of nutrients by plants also depends on the chemical composition of the soil, pH, soil and air moisture. It is therefore necessary to evaluate the effect of the substrate and fertilization separately, but also any interaction between both factors that could create production synergies. In the present study, we evaluated the effect of two substrate mixtures, two fertilizer concentrations and the interaction between these two factors on the quality of coriander grown in organic systems. To evaluate the quality of coriander, we considered different parameters such as size, biomass, antioxidant capacity and aroma, the latter of which was evaluated by chromatography.

The visual evaluation (length, number of leaves and stem diameter) of coriander plants showed no significant differences due to the concentration of fertilizer used; therefore, we recommend using 50% of the amount of fertilizer, which could significantly reduce production costs. Regarding the substrate mixture, we suggest using substrate mixture M1, since the stem diameter of the plants grown under this treatment was thicker, which gives the appearance of vigor.

In terms of value added (antioxidant capacity and aroma), all plants grown under the different treatments showed similar antioxidant activity; however, regarding aroma, the plants grown under the M150 treatment showed the greatest concentration of dodecanal and decanal, and the second highest concentration of 2-octenal; we can thus conclude that the best overall treatment is M150. In general, this study showed that organic farming conditions do not reduce the quality parameters of the crops when using adequate fertilization and irrigation management practices.

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