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BIOMETRY AND ESSENTIAL OIL OF OREGANO GROWN UNDER DIFFERENT WATER DEPTHS AND ORGANIC FERTILIZER DOSES IN A PROTECTED ENVIRONMENT

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KEYWORDS

agronomic performance, irrigation, *Origanum vulgare* L.

ABSTRACT

Information related to the phenological growth of oregano grown in a protected environment associated with water and nutritional availability is scarce. In this context, this study aimed to investigate the performance of oregano plants grown with bokashi organic fertilizer under water deficit. The experiment was carried out in randomized blocks in a 6×4 factorial scheme, consisting of six levels of water replacement (60, 70, 80, 90, 100, and 110% of crop evapotranspiration – ETc) and four bokashi doses (0, 100, 200, and 300 g m^{-2}), with five replications. The number of branches, leaf area, mass accumulation, and oil concentration and yield were evaluated to characterize the plant performance. The data were analyzed using analysis of variance, multivariate analysis for equation determination, construction of response surface plots and dendrogram charts, and linear correlation. The interaction of the factors water replacement and bokashi organic fertilizer application was significant for the analyzed variables. Morphological components presented better responses under conditions of high water replacement and bokashi organic fertilizer application. The deficit irrigation adopted during cultivation associated with bokashi organic fertilization potentiated the concentration and yield of oregano essential oil.

INTRODUCTION

Oregano (*Origanum vulgare* L.) is a spice plant used as food and in pharmacology. The plant is extremely responsive to irrigation during cultivation, which may enhance its biomass production (Fasolo et al., 2019). Proper management of water replacement generates water savings in the production system, as it allows better use of irrigation periods throughout the crop cycle and provides a quantitative and qualitative increase in plants (Santos et al., 2018). Agronomic management through irrigation aims to meet the water demand of plants, which requires planning and study to understand the response of plants to water deficit (Boeira et al., 2020).

The management and natural dynamics of soil microorganisms, in constant adaptation, especially when subjected to different water availability, make them interesting indicators of soil fertility and production potential. Bokashi, a material fermented by beneficial microorganisms of interest, stands out among the affordable agroecological-based biofertilizer options (Maluf et al., 2015).

In many ways, soils with a rich biota present a better condition for plants. It favors the first layers by altering the pH, making the nutrients already present in the soil accessible to plants, enabling better phenological development and, therefore, increasing productivity (Machado, 2017). Knowing the changes and their interference is essential to identify appropriate management strategies (Salles et al., 2017).

This study aimed to investigate the influence of water replacement and organic fertilization on plant growth characteristics, essential oil yield, and water use efficiency of *Origanum vulgare* L. grown in a protected environment.

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MATERIAL AND METHODS

The experiment was carried out at the Technical Irrigation Center (CTI) belonging to the State University of Maringá (UEM), whose geographic coordinates are 23°25′57″ S and 51°57′08″ W, with an altitude of 542 m. The local climate is characterized as Cfa, with average temperatures of 22 °C, solar radiation of 14.5 to 15 MJ m^{-2} day^{-1}, and annual evapotranspiration ranging from 1000 to 1100 mm (Nitsche et al., 2019). The temperature and relative humidity data inside the protected environment were obtained with an automatic weather station and are shown in Figure 1.

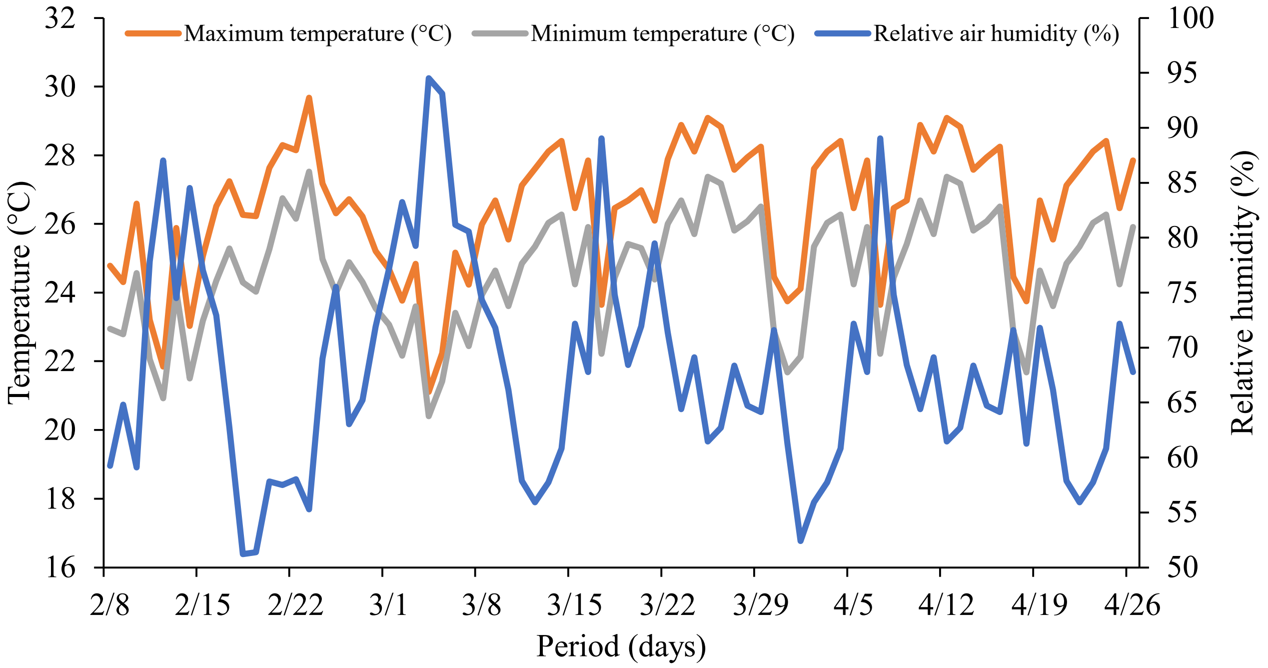


FIGURE 1. Temperature (°C) and relative humidity (%) data during the cultivation period.

The experimental design consisted of randomized blocks in a 6×4 factorial scheme, with six levels of water replacement (60, 70, 80, 90, 100, and 110% of crop evapotranspiration – ETc) and four bokashi organic fertilizer doses (0, 100, 200, and 300 g m^{-2}), with five replications.

Constant-level water table lysimeters were installed inside the protected environment to determine the daily water requirement, with daily replacement. A localized irrigation system, with self-compensating drippers spaced at 0.3 m, flow of 4 L h^{-1}, and line pressure of 20 mWC, was used for water replacement. The irrigation system had Christiansen's uniformity coefficient (CUC) of 94% at the beginning of the experiment.

The soil had the following chemical parameters: pH ($CaCl_2$) = 6.6; phosphorus = 6.13 mg dm^{-3}; potassium = 0.51 $cmol_c$ dm^{-3}; calcium = 6.43 $cmol_c$ dm^{-3}; magnesium = 1.87 $cmol_c$ dm^{-3}; aluminum = 0.13 $cmol_c$ dm^{-3}; hydrogen = 4.3 $cmol_c$ dm^{-3}; cation exchange capacity = 9.45 $cmol_c$ dm^{-3}; base saturation = 53.11%; and organic matter = 1.02%. Fertilization was carried out with cured bovine manure (3 kg m^{-2}), added to the soil fifteen days before oregano planting, considering the nutrient contents in the soil and the recommendations by Pauletti & Motta (2017). The bovine manure presented the following chemical parameters: 12.87 g kg^{-1} of nitrogen, 0.31 g kg^{-1} of phosphorus, 17.22 g kg^{-1} of potassium, 7.41 g kg^{-1} of calcium, 5.43 g kg^{-1} of magnesium, 1.87 g kg^{-1} of copper, 0.13 g kg^{-1} of iron, 4.43 g kg^{-1} of manganese, and 5.03 g kg^{-1} of zinc.

Bokashi organic fertilizer was applied associated with the manure, according to the treatment. The bokashi organic fertilizer presented the following chemical parameters: 41 g kg^{-1} of nitrogen, 1.4 g kg^{-1} of phosphorus, 21.5 g kg^{-1} of potassium, 25.7 g kg^{-1} of calcium, 10.8 g kg^{-1} of magnesium, and 1.8 g kg^{-1} of sulfur.

Oregano seedlings of the cultivar 494 were produced in 128-cell plastic trays, previously filled with the MecPlant® commercial substrate, with sowing carried out on January 5, 2021, and kept in a greenhouse. The seedlings were planted 30 days after sowing (DAS) in experimental units composed of beds (3 × 0.5 m), whose clayey-textured soil (72% clay, 16% silt, 7% fine sand, and 5% coarse sand) is classified as a dystroferric Red Nitosol (Santos et al., 2018). The three central plants of the five plants grown in each bed were evaluated.

Plant height (PH) was determined at 80 days after planting (DAP), before the plants were harvested and sent to the laboratory of post-harvest technology at UEM. The components were separated, identified, and quantified as follows: the number of branches (NB) by counting the branches and the total shoot fresh biomass (TSFM), leaf fresh biomass (LFM), stem fresh biomass (SFM), and root fresh biomass (RFM) by weighing on an analytical scale (±0.001 g). The leaf area index (LAI) was determined in fresh leaves using the LI-COR® LI-3100 equipment.

The plant components (leaves, stems, and roots) were dried in a forced-air circulation oven at 65 °C until constant weight to obtain the leaf dry mass (LDM), stem dry mass (SDM), and root dry mass (RDM).

A supercritical fluid extraction unit, consisting of a 42-mL volume 316S stainless steel extractor with 260-mesh screens at the top and bottom to avoid clogging the line or the passage of any material, coupled to a thermostatic bath

(Haake, model K15, Brazil) for monitoring and controlling the extraction temperature, was used to extract the essential oil. A high-pressure pump (Palm Tecnologia em Alta Pressão, model G100, Brazil), specific for CO_2 pumping, responsible for feeding the solvent, had the pressure monitored during the experiment using a manometer. The operating conditions of the supercritical extraction of the DLM ground sample (1.0±0.005 g) were defined based on Rodrigues et al. (2004), Rodrigues et al. (2008), and Vargas et al. (2013), being carried out under pressure conditions of 80 bar, a flow rate of 1000 g h^{-1}, a temperature of 30 °C, and time of 100 min. The samples and collection tubes were weighed on an analytical digital scale (e = ±0.0001 g).

Water consumption was considered as the sum of the water volume applied daily during the oregano cycle in irrigation management at the time of %ETc of each treatment. Water use efficiency was determined by the ratio between the total dry mass (g) and water consumption (L). The total dry mass corresponds to the leaf, stem, and root dry mass.

The data were subjected to analysis of variance (ANOVA) using the F-test, with a 5% significance. A multivariate analysis was performed when a significant interaction was observed, with adjustment equations obtained for the estimated value and response surface plots being constructed.

Linear correlation analysis was performed between the variables water replacement, bokashi, root fresh mass, root dry mass, number of branches, leaf-to-stem dry mass ratio, plant height, shoot fresh mass, and shoot dry mass. Dendrogram charts were constructed for the variables oil content, shoot dry mass accumulation, and oil yield to compare the cultivation conditions. The data analysis was performed using the software SISVAR version 5.6 (Ferreira, 2019), Microsoft Excel®, Past4.06®, and Statistica 8®.

RESULTS AND DISCUSSION

Table 1 shows that the interaction between the water replacement level (L) and bokashi application (D) was significant ($p<0.05$).

TABLE 1. Summary of analysis of variance relative to water replacement level (L) and bokashi application (D) in oregano cultivation in a protected environment.

Variable	P-value			Mean	CV (%)
	Water depth (L)	Dose (D)	L*D		
Shoot fresh mass	*	*	*	50.22	14.73
Shoot dry mass	*	*	**	10.19	21.20
Root fresh mass	*	*	**	6.08	26.71
Root dry mass	*	*	*	3.26	27.53
Leaf-to-stem dry mass ratio	*	*	*	2.61	24.95
Number of branches per plant	*	*	**	24.64	13.67
Height	*	*	**	29.14	11.47
Leaf area index (LAI)	*	*	**	126.82	5.66
Oil content	*	*	*	0.70	12.05
Oil yield	*	*	**	7.08	22.78

** Significant at $p<0.05$; * significant at $p<0.01$; ns not significant ($p>0.05$).

The management of oregano cultivation had a significant effect on the response variables and the interaction between the two factors (replacement x bokashi) (Table 1). A multivariate analysis was performed considering the significant factorial interaction (replacement x bokashi) and quantitative variables to characterize the components under the study conditions. Table 2 shows the adjusted equations.

TABLE 2. Multivariate equations for the response variables of oregano grown under different conditions of water replacement and bokashi application.

Variable	Equation	R^2
Shoot fresh mass (g)	$\hat{Y} = -30.2158 + (0.9861xW) + (0.2336xB) - (0.0037xW^2) + (0.0007xWxB) - (0.0006xB^2)$	0.75
Shoot dry mass (g)	$\hat{Y} = -1.4499 + (0.1109xW) + (0.0448xB) – (0.0003xW^2) + (8.3032E^{-5}xWxB) – (0.0001xB^2)$	0.80
Root fresh pasta (g)	$\hat{Y} = 13.4237 – (0.1801xW) + (0.02449xB) + (0.0011xW^2) – (0.0005xWxB) + (8.2161E^{-5}xB^2)$	0.70
Root dry mass (g)	$\hat{Y} = 2.5204 + (0.0196xW) + (0.0156xB) – (7.038E^{-5}xW^2) – (0.0004xWxB) + (7.9709E^{-5}xB^2)$	0.73
Leaf-to-stem dry mass ratio	$\hat{Y} = 4.9482 + (0.0019xW) – (0.0169xB) – (0.0002xW^2) + (0.0001xWxB) – (2.3787E^{-6}xB^2)$	0.88
Number of branches per plant	$\hat{Y} = 13.4237 – (0.1801xW) + (0.0249xB) + (0.0011xW^2) – (0.0005xWxB) + (8.2161E^{-5}xB^2)$	0.66
Height (cm)	$\hat{Y} = 16.3412 + (0.139xW) – (0.0009xB) – (0.0001xW^2) + (0.0003xWxB) – (6.2681E^{-5}xWxB)$	0.85
Leaf area index (cm^2)	$\hat{Y} = -132.5186 + (3.6178xW) + (0.7478xB) – (0.0131xW^2) – (0.0005xWxB) – (0.0016xB^2)$	0.74
Oil content (%)	$\hat{Y} = 1.1751 – (0.0081xW) + (0.0024xB) + (1.56E^{-5}xW^2) – (1.4457E^{-5}xWxB) – (2.3333E^{-6}xB^2)$	0.89
Oil yield (g)	$\hat{Y} = -1.0786 + (0.1078xW) + (0.0594xB) – (0.0006xW^2) – (0.0002xWxB) – (8.0699E^{-5}xB^2)$	0.71

*Equations with a significance coefficient equal to and/or lower than 10% ($p<0.1$).
**W = water replacement (% ETc); B = bokashi dose (g m^{-2}).

Figure 2 shows three-dimensional response plots constructed from the fitted equations.

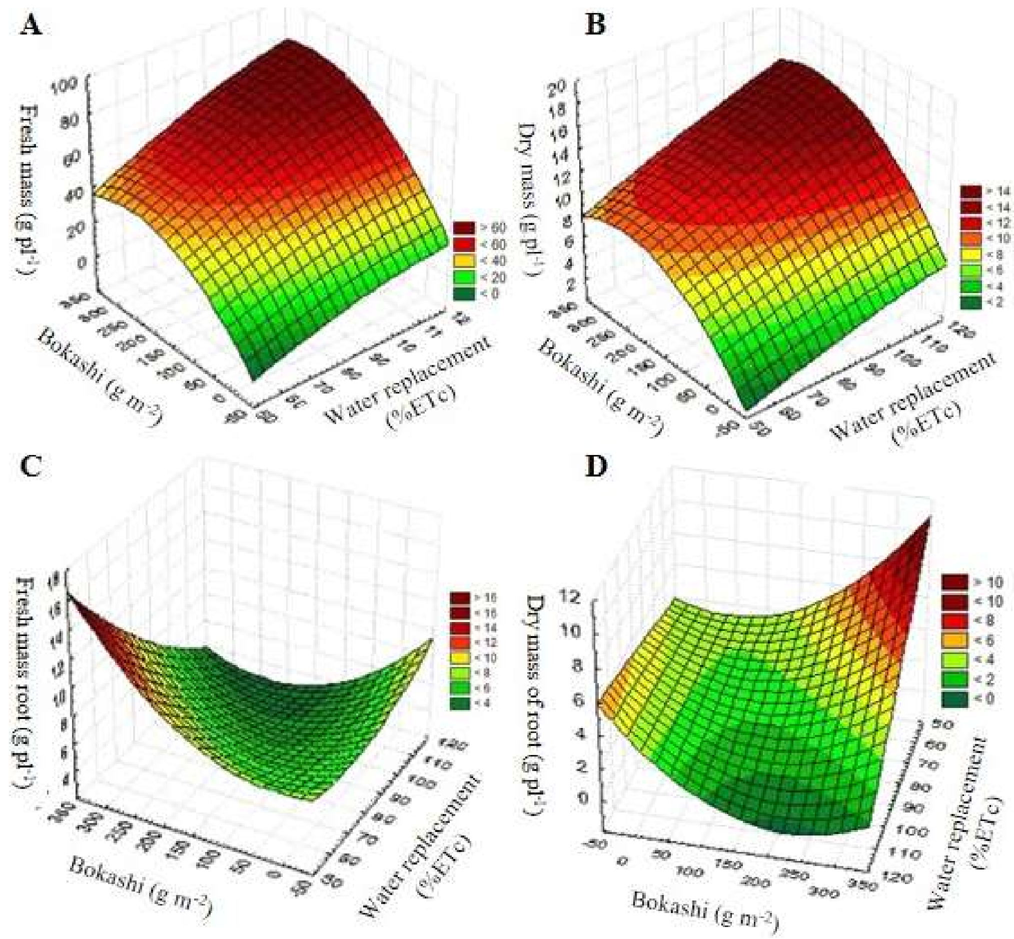


FIGURE 2. Plant components of oregano grown under different water replacement conditions and bokashi doses. A) Shoot fresh mass; B) shoot dry mass; C) root fresh mass; D) root dry mass.

Shoot biomass accumulation (Figure 2A and B) showed an increasing trend with an increase in the water replacement level, especially when associated with the bokashi application. Vegetative development is affected under water deficit conditions, causing a reduction in crop yields (Taiz et al., 2017). Cell elongation in plants under stress conditions is inhibited due to reduced turgidity pressure (Novello et al., 2020), and the accumulation of photoassimilates and metabolites necessary for cell division decreases (Baghalian et al., 2011).

Novello et al. (2020) analyzed the effect of soil water availability on basil development under different water replacements and found that plant height, shoot fresh mass, and leaf water content were influenced by different stress levels, significantly reducing the values of the analyzed variables. Borges et al. (2016) attributed the drastic reduction in dry mass accumulation in *Petroselinum crispum* (family Apiaceae) plants to lower irrigation water depths.

Bokashi organic fertilization in the crop cycle favored nutrient availability, soil water retention, and plant development (Machado, 2017). Factors such as water availability and soil fertility stand out in oregano (*Origanum vulgare* L.) cultivation in terms of plant physiological responses, mass accumulation, and chemical composition (Oliveira et al., 2017; Vierga, 2020; Hancioglu et al., 2020).

The root mass accumulation (Figure 2C and D) trend was inversely proportional to the shoot, with an increase as a function of the water stress condition. Higher root development is observed under water stress conditions as a strategy to increase the explored area in the soil, consequently increasing water absorption (Kapoor et al., 2020). Bokashi application under water stress conditions contributes to root mass accumulation, with an inverse effect under total and/or excess replacement conditions (Figure 2C and D). This fact may be associated with bokashi changes in the soil at high doses and the interaction of organic fertilization with other crop components (Oliveira et al., 2017; Olle, 2021).

The leaf-to-stem mass ratio showed a reduction mainly due to the bokashi application (Figure 3A). The number of branches (Figure 3B), plant height (Figure 3C), and leaf area index (Figure 3D) were higher under the replacement condition close to 100% of ETc with the bokashi organic compost application.

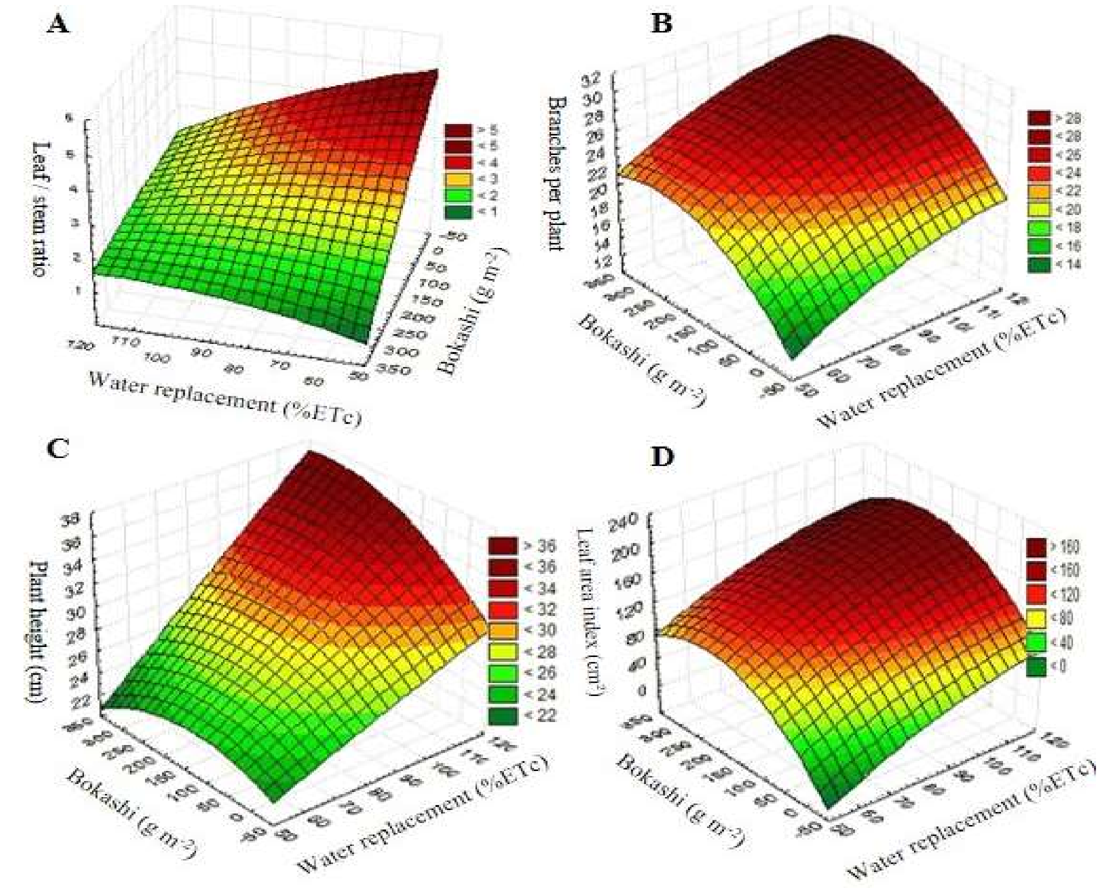


FIGURE 3. Morphological components of oregano grown under different water replacement conditions and bokashi doses. A) Leaf-to-stem dry mass ratio; B) branches per plant; C) plant height; D) leaf area index.

Oregano shows changes in morphological development and distinct physiological activity according to the analyzed cultivar when subjected to water deficit conditions, and changes may involve plant architecture characteristics, reduction in leaf area, and photosynthetic rate (Mohammadi et al., 2021; Pereyra et al., 2021). Plants under water deficit conditions decrease leaf water potential, regulate stomatal opening, reducing photosynthesis and transpiration, and invest in chlorophyll *b* production (França et al., 2017).

Water deficit impairs plant metabolism and development characteristics, but it can be advantageous when it comes to the extraction and composition of the oil obtained from cultivation under stress (Kulak et al., 2019). In the study, the water deficit condition caused an increase in oil content (Figure 4A) although reductions in shoot dry mass yields were observed (Figure 2B), corroborating the results obtained by Minei et al. (2019) and Hancioglu et al. (2020), who obtained not only a higher percentage of essential oil but an increased quality of the extracted oil in terms of phenol and flavonoid contents and antioxidant activity.

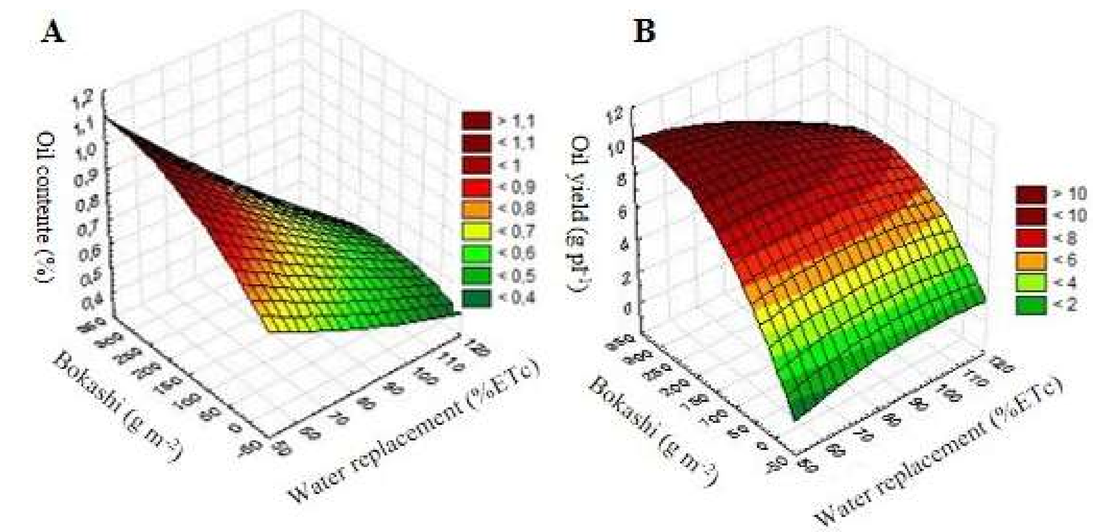


FIGURE 4. Leaf oil content (A) and oil yield (B) in oregano grown under different water replacement and bokashi doses.

Slight water deficit conditions may not cause significant differences in oil yield and changes are directly observed in the composition, mainly relative to the oleic acid, linoleic acid, linolenic acid, and palmitic acid contents in sunflower, safflower, and sesame (Ebrahimian et al., 2019).

According to Emrahi et al. (2021), the imposition of a moderate water deficit in oregano cultivation leads to an increase in essential oil yield, while the content of secondary compounds such as carvacrol is influenced at any level of deficit. However, the high carvacrol content is also obtained in cultivation with organic fertilization (Matłok et al., 2020).

The adoption of practices complementary to irrigation allows for mitigating the stress caused by water deficit imposition (Wenneck et al., 2021). Thus, the use of bokashi organic fertilizer can improve the soil, with a potential effect on the yield of several crops (Olle, 2021), with the plant leaves under conditions without bokashi fertilizer application presenting the lowest essential oil yields (Figure 4B).

Spice plants such as oregano, garlic, marjoram, and basil tend to have higher polyphenol and carotenoid contents when grown in an organic system (Hallmann & Sabała, 2020). In terms of mass accumulation, biofertilizers can be adopted as substitutes for chemical fertilizers without changes in yield (Nikou et al., 2019).

The linear correlation between the variables and the significance of this correlation were determined considering that the development of plant components is related to each other and reflects the cultivation conditions (Table 3).

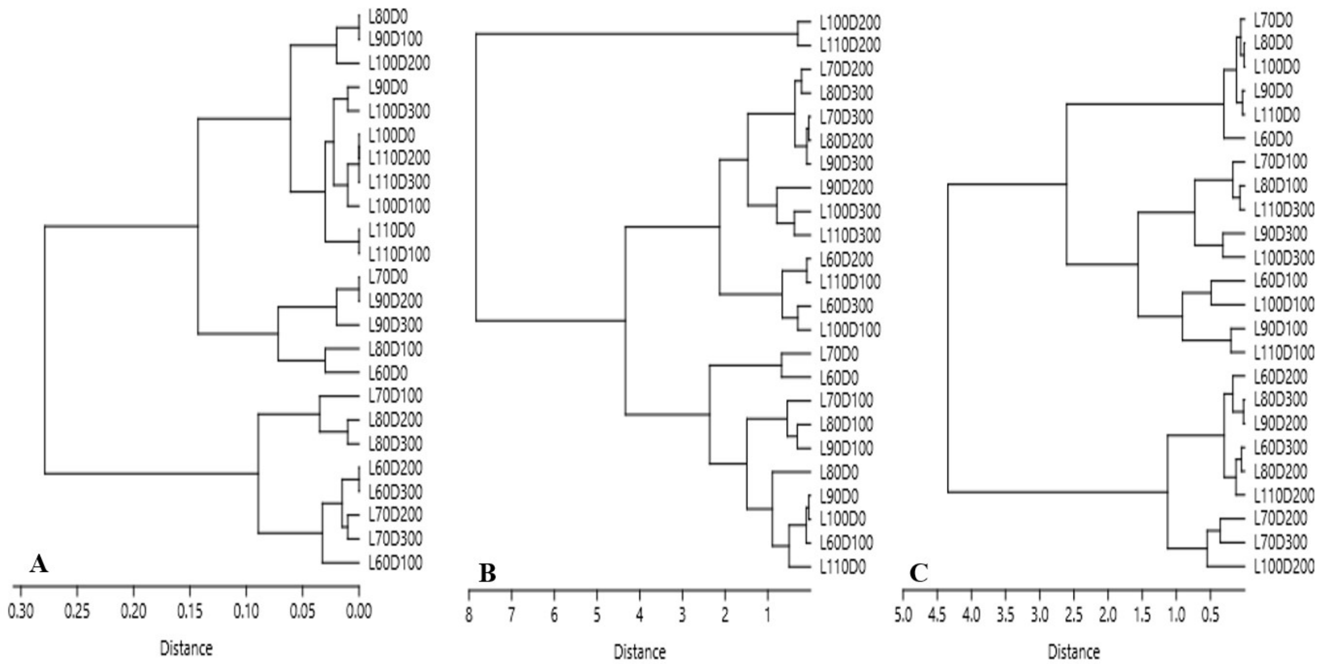
TABLE 3. Linear correlation between the variables water replacement (W), bokashi (B), root fresh mass (RFM), root dry mass (RDM), number of branches (NN), leaf-to-stem dry mass ratio (L/S), plant height (h), shoot fresh mass (SFM), and shoot dry mass (SDM) of oregano grown under different water replacements and bokashi doses.

	W	B	RFM	RDM	NB	L/S	h	SFM	SDM
B	-	-	-	-	-	-	-	-	-
RFM	-0.59*	0.24^{ns}	-	-	-	-	-	-	-
RDM	-0.53*	0.16^{ns}	0.93*	-	-	-	-	-	-
NB	0.46*	0.77*	-0.16^{ns}	-0.23^{ns}	-	-	-	-	-
L/S	-0.26^{ns}	-0.76*	-0.21^{ns}	-0.04^{ns}	-0.77*	-	-	-	-
h	0.88*	0.26^{ns}	-0.57^{ns}	-0.55*	0.65*	-0.42*	-	-	-
SFM	0.42*	0.75*	-0.22^{ns}	-0.28^{ns}	0.92*	-0.74*	0.71*	-	-
SDM	0.42*	0.72*	-0.20^{ns}	-0.28^{ns}	0.92*	-0.75*	0.70*	0.98*	-

* Significant ($p<0.05$); ^{ns} not significant ($p>0.05$).

The linear correlation analysis showed that water replacement has a negative correlation only with root mass, but root mass accumulation had a significant correlation only for plant height (Table 3). Bokashi showed a negative correlation only with the leaf-to-stem ratio, confirming the data shown in Figure 3A.

The cluster analysis by the dendrogram construction allows the characterization of similarity and divergence between management groups through the distances between components (Figure 5).



Legend: L = water replacement level (%ETc); D = bokashi dose (g m^{-2}).

FIGURE 5. Dendrogram of oil content (A), shoot dry mass accumulation (B), and oil yield (C) in oregano leaves submitted to different water replacement levels and bokashi fertilizer application to the soil.

The longest distances in the oil content dendrogram are related to the water replacement level, while bokashi application caused possible water stress mitigation for dry mass accumulation and oil yield, with less distances obtained for different water replacement conditions.

Multivariate analysis techniques allow the comparison of results based on the interaction of different factors and different applications (Kemsley & Marini, 2019). According to the development conditions of the study, the interaction of the analyzed factors (water replacement and bokashi fertilization) could not be adequately analyzed by conventional statistical methods.

The results show that oregano development presents better indices when grown under water replacement conditions close to crop evapotranspiration (100% ETc) and bokashi organic fertilizer application. However, water deficit conditions lead to the partial mitigation of stress by bokashi application. Further studies are needed to understand the physiological and metabolic aspects involving water replacement and bokashi application in oregano.

CONCLUSIONS

The interaction between water replacement factors and bokashi organic fertilizer application was significant for the analyzed variables.

Morphological components showed better responses under conditions of high water replacement and bokashi organic fertilizer application.

The deficit irrigation adopted during the cultivation associated with bokashi organic fertilization potentiated the concentration and yield of oregano essential oil.

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