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CROP PRODUCTION

Physiological responses of oregano under different water management and application of fermented bokashi compost

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ABSTRACT. Growing conditions such as water supply and soil fertility influence oregano morphological development and physiological responses. Our study aimed to analyse the physiological responses of oregano plants grown under different water conditions and bokashi application rates. The experiment was carried out in a greenhouse under a randomized block design and a 3 x 4 factorial scheme. Treatments encompassed three water replacement levels (60, 80, and 100% crop evapotranspiration - ETc) and four bokashi rates (0, 100, 200, and 300 g m^{-2}), with five replications each. Oregano seedlings were transplanted and grown in a spacing of 0.3 m between plants and 1 m between bed rows. After 60 days, treatments were evaluated for photosynthetic rate (A), stomatal conductance (Gs), internal CO_2 rate (Ci), transpiration (E), and water-use efficiency (WUE). Data underwent variance analysis by F-teste, multivariate analysis, and Pearson's linear correlation. Oregano physiological responses were significantly influenced by water replacement level and the application rate of fermented bokashi compost. The multivariate analysis allowed us to analyse the interaction effect between water replacement level and bokashi rate on photosynthesis, stomatal conductance, internal CO_2, and transpiration.

Keywords: organic fertilization; *Origanum vulgare* L.; water management.

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Introduction

Oregano (*Origanum vulgare* L.) belongs to the *Lamiaceae* family. It is used as a spice and aromatic plant and presents important bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial actions (Pezzani, Vitalini, & Iriti, 2017; Oniga et al., 2018). Morphological development and physiological responses are influenced by biotic and abiotic conditions during cultivation (Alvarez, Quilaleo, Mazzoni, & Ridiero, 2019; Oliveira, Santos, Souza, & Santos, 2017; Skoufogianni, Solomou, & Danalatos, 2019).

Water management is closely related to oregano plant development and physiological activity, having a direct influence on mass accumulation, nutrient extraction, and composition of phenolic compounds, oil yield, and final product quality (Hancioglu, Kurunc, Tontul, & Topuz, 2020; Maass, Cespedes, & Cardenas, 2020; Virga et al., 2020).

The use of fermented compounds, such as bokashi, can improve soil chemical, physical, and biological traits, favouring the development of plants of interest (Quiroz & Céspedes, 2019; Cortés-Tello & Jaramillo-López, 2020). Moreover, nutritional management is directly related to plant metabolism, acting in the production of bioactive compounds in aromatic and medicinal plants (Singh, Ali, & Irfan Qureshi, 2017).

The use of bokashi, together with other management practices, reduce the temperature and evaporation of water in the soil (Lasmini, Nasir, Hayati, & Edy, 2018; Shin, Diepen, Blok, & Bruggen, 2017), which can partially mitigate the consequences of water stress and contribute to food production and quality (Olle, 2020). The application of fermented bokashi compost into the soil can thus improve growing conditions, favouring physiological activity with a potential impact on crop yield.

Therefore, given the lack of studies on the use of bokashi in oregano cultivation, this study aimed to analyse the effect of water conditions and application of fermented bokashi compost on the physiological responses of oregano plants.

Material and methods

Study area and experimental design

The experiment was carried out in a greenhouse at the State University of Maringá, Maringá, Paraná State, Brazil (23°25'57" S, 51°57'08" W, and 542-m altitude).

The study was performed in a randomized complete block design and 3 x 4 factorial scheme, with three water replacement levels (60, 80, and 100% of crop evapotranspiration - ETc) and four fermented bokashi compost rates (0, 100, 200, and 300 g m^{-2}), with five replications.

Cultivation

Seedlings were acquired from a commercial nursery and transplanted 30 days after sowing, with about 7 cm in height. The plants were transplanted into bed rows (0.5 m wide and 1.5 m long) spaced in 0.3 m between plants and 1.0 m between rows. Each plot consisted of five plants, with the three central plants being considered useful.

The soil in the area is characterized as a *Latossolo Vermelho distroférrico* according to the Brazilian Soil Classification and shows a correlation with a Ultisol in the Soil Taxonomy (Santos et al., 2018). The soil physical composition is 72% clay, 16% sand, and 12% silt, with a density of 1.10 t m^{-3}. The soil chemical parameters are 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%, organic matter = 1.02%, and pH ($CaCl_2$) = 6.6. In the soil preparation, we applied 2 kg m^{-2} of compost (bovine manure), which allowed an input of nutrients estimated at 560 g N, 30 g P_2O_5, 22 g K_2O, 39 g CaO, 20 g MgO, and 6 g S.

Fermented bokashi compost was produced on a family property in the municipality of Ubiratã, Paraná State, Brazil (24°33′18″ S, 52°58′40″ W, and 507-m altitude) following the method of Siqueira and Siqueira (2013). Efficient microorganisms (EM) were collected in a native forest and inoculated in a mixture of wheat bran (55%), soybean bran (40%), bone meal (3%), and dolomitic limestone (2%). This compost had an average density of 350 kg m^{-3}, a water content of 10%, and a chemical composition of 46 kg t^{-1} N, 16 kg t^{-1} P, 10 kg t^{-1} K, 18 kg t^{-1} Ca, 10 kg t^{-1} Mg, and 2 kg t^{-1} S.

Water was replaced using a drip irrigation system. Drippers were spaced 0.3 m apart and had a flow rate of 4 L h^{-1} and a uniformity coefficient of 94%. Irrigation was performed daily (9:00 a.m.). The amount of water applied was determined as a function of Etc, which was monitored through constant groundwater lysimeters installed inside the protected environment.

Gas exchange

After 60 days of transplantation, gas exchange was analysed using a portable photosynthesis system (LI-6400XT, Li-COR) equipped with the following settings: flow rate = 500 µmol s^{-1}, Photosynthetic Photon Flux Density (PPFD) = 1,200 µmol m^2 s^{-1}, and Mixer reference CO_2 = 400 µmol mol^{-1}, with measurements taken at room temperature. The evaluated parameters comprised: photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapour (Gs, mol m^{-2} s^{-1}), internal CO_2 rate (Ci, mmol m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}), water-use efficiency (WUE, mmol CO_2 mol^{-1} H_2O), which is the A/E ratio. The readings were made from 8:30 to 10:30 a.m., during monitoring.

Data analysis

Data were subjected to analysis of variance (ANOVA) by F-test, using the SISVAR software (Ferreira, 2019). Having obtained a significant effect for interaction between factors (water level and bokashi rate), the data were subjected to multivariate analysis, and mathematical models were estimated for each response variable (A, Gs, Ci, and E), with their respective coefficients of determination. From the fitted equations, three-dimensional plots were constructed. The Surfer™ software was used for multivariate analysis and 3D plots. The data were also analysed by Pearson's linear correlation using the Microsoft Excel™ software.

Results

The interaction between water replacement levels and bokashi application rates into the soil had a significant effect on the physiological variables analysed (Table 1).

Table 1. Photosynthetic rate (A), stomatal conductance (Gs), internal CO_2 (Ci), transpiration (E), and water-use efficiency (WUE) in oregano (*Origanum vulgare* L.) grown in a protected environment under different water replacement and bokashi application conditions.

Bokashi rate ($g\ m^{-2}$)	WR[(1)] (% ETc)	A ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Gs ($mol\ m^{-2}\ s^{-1}$)	Ci ($mmol\ m^{-2}\ s^{-1}$)	E ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	WUE ($mmol\ CO_2\ mol^{-1}\ H_2O$)
0	60	12.80	0.09	92.14	4.25	4.25
	80	15.21	0.11	166.12	4.41	5.07
	100	20.85	0.35	135.83	5.09	5.39
100	60	13.11	0.10	143.29	4.38	4.60
	80	17.77	0.15	193.91	5.07	5.87
	100	22.74	0.36	251.00	5.39	7.07
200	60	14.91	0.11	177.37	4.60	4.94
	80	18.57	0.15	228.68	5.87	6.13
	100	23.83	0.56	270.76	6.28	6.28
300	60	19.43	0.34	201.16	4.94	4.38
	80	22.52	0.51	266.49	6.13	5.41
	100	25.10	0.75	282.57	7.07	5.09
WR		**	*	*	*	*
Bokashi		***	***	**	***	***
WR* Bokashi		***	**	**	***	***
CV (%)		13.16	26.69	24.79	21.36	13.47

[(1)]Water replacement (WR) was performed daily as a function of crop evapotranspiration (ETc), which was determined in a constant level water table. ***significant at $p < 0.01$ probability error; ** significant at $p < 0.05$ probability error; * significant at $p < 0.1$ probability error; ns non-significant.

Given the significance of the interaction between the analysed factors (Table 1), mathematical models with respective coefficients of determination were built for photosynthesis (Equation 1), stomatal conductance (Equation 2), internal CO_2 (Equation 3) and transpiration (Equation 4) using multivariate analysis. However, when analysing interaction value, the water-use efficiency (WUE) response did not show such interaction, as its mathematical model had R^2 below 0.5.

$$A = (0.2016 * ETc) + (0.01942 * Bokashi) - 0.14467 \quad R^2 = 0.95 \quad (1)$$

$$Gs = (0.0086 * ETc) + (0.001115 * Bokashi) - 0.556 \quad R^2 = 0.81 \quad (2)$$

$$Ci = (203875 * ETc) + (0.38567 * Bokashi) - 20.1734 \quad R^2 = 0.88 \quad (3)$$

$$E = (0.035375 * ETc) + (0.0050267 * Bokashi) + 1.706 \quad R^2 = 0.90 \quad (4)$$

where in:
A- photosynthetic rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$);
Gs- stomatal conductance ($mol\ m^{-2}\ s^{-1}$);
Ci- internal CO_2 ($mmol\ m^{-2}\ s^{-1}$);
E- transpiration ($mmol\ H_2O\ m^{-2}\ s^{-1}$);
ETc- water replacement (%).
Bokashi- fermented bokashi compost ($g\ m^{-2}$).

From the fitted equations, three-dimensional plots were constructed for photosynthetic rate, stomatal conductance, internal CO_2, and transpiration (Figure 1).

When analysing the response surface for variables (Figure 1), we observed that they tended to be similar, which is reflected in the linear correlation values obtained (Table 2).

Discussion

The morphological development and photosynthetic activity of plants are related to genetic and environmental characteristics (Taiz, Zeiger, Möller, & Murphy, 2017). In oregano (*Origanum vulgare* L.) cultivation, factors such as quality and intensity of incident light, temperature, water availability, and soil fertility are related to physiological responses, mass accumulation, and chemical composition (Oliveira et al., 2017; Virga, 2020; Hancioglu et al., 2021). For the water levels supplied, the severe water deficit when 60% of ETc was replaced decreased average values of physiological responses under all application rates of bokashi compost (Table 1).

Plants can adapt to adverse conditions, improving their photosynthetic and water-use efficiencies (Oliveira & Gualtieri, 2017). According to Bell, Schwartz, Mcinnes, Howell, and Morgan (2020), plant responses to water deficit depend on the amount of water lost by transpiration, application losses, and duration of stressful conditions in the environment.

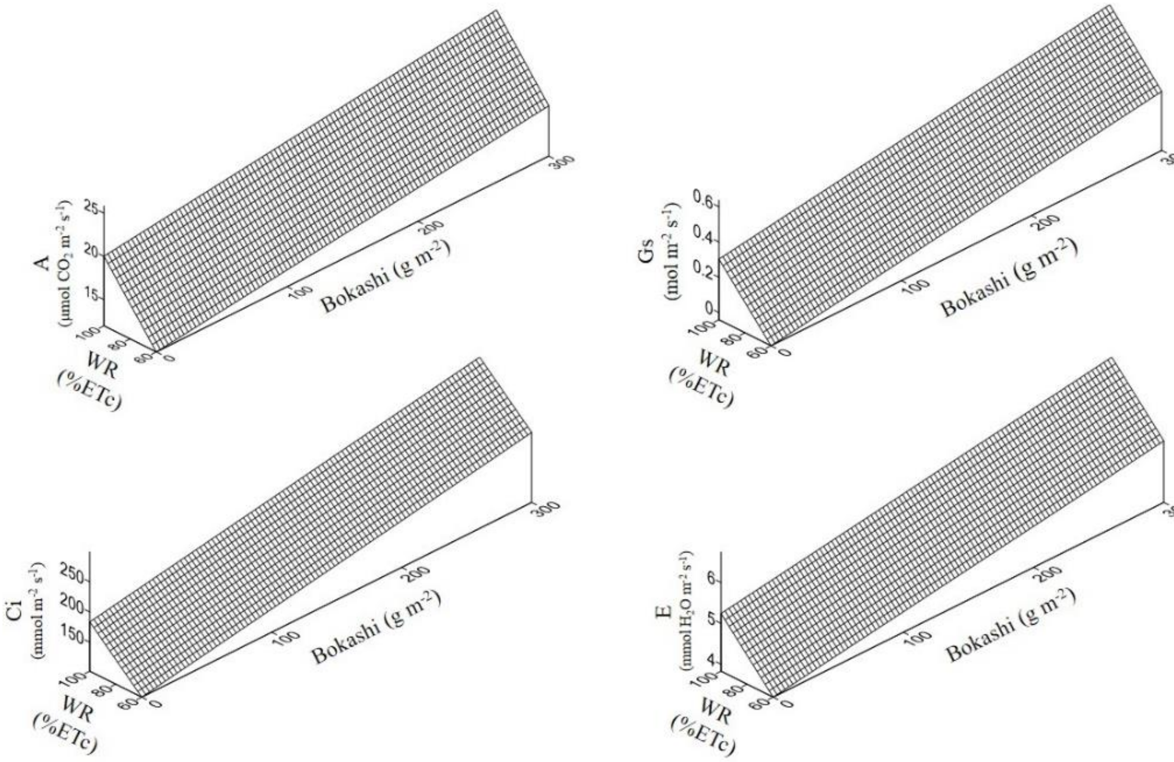

Figure 1. Three-dimensional response surface for the effect of interaction between water replacement (WR) and bokashi application rates on photosynthetic rate (A), stomatal conductance (Gs), internal CO_2 (Ci), and transpiration (E) in oregano leaves (*Origanum vulgare* L.) grown in a protected environment.

Table 2. Correlation between the factors and variables analysed.

	WR	Bokashi	A	Gs	Ci	E	WUE
WR	1.00	-	-	-	-	-	-
Bokashi	-	1.00	-	-	-	-	-
A	0.81	0.54	1.00	-	-	-	-
Gs	0.67	0.60	0.92	1.00	-	-	-
Ci	0.57	0.74	0.84	0.76	1.00	-	-
E	0.68	0.66	0.89	0.88	0.88	1.00	-
WUE	0.65	0.12	0.73	0.55	0.42	0.33	1.00

*WR - Water replacement; A-Photosynthetic rate; Gs - stomatic conductance; Ci - Internal CO_2; E - transpiration; WUE - water use efficiency.

Water deficit decreases photosynthetic efficiency due to stomatal conductance reduction (Peloso, Tatagiba, Reis, Pezzopane, & Amaral, 2017), as observed in plants under shading (Oliveira et al., 2017) and flooding (Oliveira & Gualtieri, 2017). Qualitatively, water shortage can affect the oregano plant growth and its essential oil yield without necessarily changing its composition (Virga, 2020).

According to Hancioglu et al. (2020), growing conditions in naturally dry environments decrease the sensitivity of oregano plants to water deficit but are resistant to quality parameters such as extract yield, contents of flavonoids and phenolic compounds, including improvements under low water consumption. We observed values of A, Gs, Ci, and E close to those reported by Oliveira et al. (2017), who evaluated oregano development under different shade nets and organic fertilization conditions.

Concerning bokashi, increases in A, Gs, Ci, and E values were observed as a function of the soil application rate, given the coefficients of the equations and positive linear correlation. When considering the soil organic matter content at the beginning of the experiment (1.02%), the bokashi application may have benefited soil fertility since it increased organic carbon content, and the amount of N-fixing and P-solubilizing bacteria (Lasmini et al., 2018).

Maass et al. (2020) applied different types of bokashi and observed increases in P contents in parsley leaves and the soil. Improvements in soil physical traits and sources of nutrients and microorganisms contribute to the development of plants and their responses to adverse conditions (Quiroz & Céspedes, 2019; Cortés-Tello & Jaramillo-López, 2020).

When analysing Pearson's linear correlation between factors (bokashi application rate and water replacement level) and variables (photosynthetic rate, stomatal conductance, internal CO_2, transpiration, and water-use efficiency), stronger relationships were observed between water replacement level and photosynthetic rate (0.81), and between bokashi application rate and stomatal conductance (0.74). Photosynthesis (Table 2) also showed a high correlation with water-use efficiency (0.73).

Although only physiological responses were analysed, water replacement and bokashi application conditions can also influence oregano morphological development and productivity. Some studies have shown increases in parsley (Maass et al., 2020), Cerrado quince (Santos, Vieira, Zárate, Carnevali, & Gonçalves, 2020), cabbage (Xavier, Santos, Costa, & Carmo, 2019), *C. adamantium* seedlings (Santos et al., 2019), and tomato (Anhar, Junialdi, Zein, Advinda, & Leilani, 2018) due to bokashi application. These studies also demonstrated that the best rate of bokashi varies with the variable analysed, species of interest, and growing conditions.

Reduction in soil water availability can affect plant development. Thus, according to Taiz et al. (2017), under low soil water content, plants close their stomata to ensure survival, while reducing gas exchange in photosynthetic activities. Concerning water replacement, oregano plants without water deficit had the production of reserves optimized through high CO2 concentrations, while those under 20 and 40% water restrictions tended to have their ecophysiological activities reduced. Oregano plants under a water restriction of 20% of ETc had their instantaneous and intrinsic water-use efficiencies maximized.

In the dynamics of physiological processes, plants automatically lose water to the atmosphere when stomata open to acquiring CO2 (Taiz et al., 2017). In this sense, Figure 1 shows that stomatal conductance (Gs) increased for all water replacement levels as a function of the bokashi rate applied to the soil. On the other hand, transpiration (i.e., loss of water in the form of vapour in oregano plants under severe water restriction - 40% of ETc) remained constant depending on the bokashi rate used.

For the same water replacement level, CO2 concentration, stomatal conductance (Gs), internal CO2 (Ci), and transpiration rate (E) increased as the rate of bokashi applied to the soil was raised.

Water stress can reduce leaf transpiration rate due to stomatal conductance reductions to limit water loss. According to Alvarenga et al. (2014), plants develop adaptations to meet water scarcity and tend to optimize water use in times of shortage in the soil (Gupta, Rico-Medina, & Caño-Delgado, 2020).

The addition of bokashi to the soil increased nutrient availability and improved microbiological activity efficiency. Our results (Figure 1) show that the supply of nutrients by bokashi application, besides stimulating the activity of specific microorganisms, such as growth-promoting bacteria naturally in low populations in the soil (Kaushal & Wani, 2016; Vimal, Singh, Arora, & Singh, 2017; Jardim et al., 2018), helped mitigate water stress (Jardim et al., 2018; Sinclair et al., 2017), thus improving plant performance in environments with moderate water limitation.

Under the experimental conditions studied, bokashi application to the soil brought benefits in terms of physiological responses for oregano plants grown under different water management conditions, thus showing the agronomic potential of such an organic compost.

Conclusion

Both water replacement level and bokashi fermented compost application rate significantly influence the physiological responses of oregano plants. The multivariate analysis allows evaluating the interaction effect of water level and bokashi application rate on the photosynthesis, stomatal conductance, internal CO_2, and transpiration in oregano leaves.

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