

ARTICLE

Nutrient allocation and growth responses of *Oncidium baueri* to different Bokashi application methods

Alocação de nutrientes e respostas de crescimento de *Oncidium baueri* a diferentes métodos de aplicação de Bokashi

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Abstract

Orchids require tailored fertilization protocols, yet the high-potential native Brazilian species *Oncidium baueri* Lindley lacks a validated cultivation protocol. This study evaluated the effects of Bokashi organic fertilizer on its growth, nutrient allocation, and photosynthetic activity to establish the first standardized protocol for this species. The experiment was conducted in a completely randomized design, comparing a conventional chemical fertilizer with five Bokashi application strategies (substrate-incorporated, surface-applied, and foliar sprays) at two doses: 10 g L⁻¹ and 20 g L⁻¹. Over a 12-month period, we monitored phytometric parameters (growth and biomass), chlorophyll *a* fluorescence, and the macronutrient and micronutrient content in different plant tissues (leaves, pseudobulbs, and roots). All Bokashi treatments outperformed the chemical control in promoting vegetative growth, with the substrate-incorporated 20 g L⁻¹ dose yielding the greatest increases in shoot length (359%), pseudobulb number (138%), and total biomass. The application method directly influenced nutrient partitioning; foliar sprays enhanced potassium (K) absorption in aerial tissues, while substrate-based methods were more effective for sulfur (S) and zinc (Zn). Photosynthetic efficiency (Fv/Fm) remained optimal across all treatments, indicating no physiological stress. Principal Component Analysis (PCA) confirmed these distinct tissue-specific nutrient accumulation patterns. We conclude that substrate-incorporated Bokashi is a highly effective and sustainable alternative to chemical fertilizers. This study provides a scientifically validated protocol for optimizing *O. baueri* cultivation, contributing to both the advancement of sustainable horticultural practices and the commercial valuation of this native ornamental species.

Keywords: bokashi, chlorophyll fluorescence, nutrient allocation, orchid fertilization, sustainable horticulture.

Resumo

Orquídeas exigem protocolos de fertilização específicos, mas a espécie brasileira de alto potencial ornamental, *Oncidium baueri* Lindley, carece de um protocolo de cultivo validado. Este estudo avaliou os efeitos do fertilizante orgânico Bokashi no crescimento, alocação de nutrientes e atividade fotossintética, visando estabelecer o primeiro protocolo padronizado para a espécie. O experimento foi conduzido em delineamento inteiramente casualizado, comparando um fertilizante químico convencional com cinco estratégias de aplicação de Bokashi (incorporado ao substrato, em superfície e via foliar) em duas doses: 10 g L⁻¹ e 20 g L⁻¹. Durante 12 meses, foram monitorados parâmetros fitométricos (crescimento e biomassa), a fluorescência da clorofila *a* e o teor de macro e micronutrientes nos tecidos (folhas, pseudobulbos e raízes). Todos os tratamentos com Bokashi superaram o controle químico no estímulo ao crescimento vegetativo, sendo que a dose de 20 g L⁻¹ incorporada ao substrato gerou os maiores incrementos no comprimento da parte aérea (359%), número de pseudobulbos (138%) e biomassa total. O método de aplicação influenciou diretamente a partição de nutrientes; a pulverização foliar otimizou a absorção de potássio (K) nos tecidos aéreos, enquanto os métodos via substrato foram mais eficazes para enxofre (S) e zinco (Zn). A eficiência fotossintética (Fv/Fm) permaneceu ótima em todos os tratamentos, indicando ausência de estresse fisiológico. A Análise de Componentes Principais (PCA) confirmou os distintos padrões de acúmulo de nutrientes específicos para cada tecido. Concluímos que o Bokashi incorporado ao substrato é uma alternativa sustentável e altamente eficaz. Este estudo fornece um protocolo cientificamente validado para otimizar o cultivo de *O. baueri*, contribuindo para o avanço de práticas horticolas sustentáveis e para a valorização comercial desta espécie nativa.

Palavras-chave: alocação de nutrientes, bokashi, fertilização de orquídeas, fluorescência da clorofila, horticultura sustentável.

Introduction

The fertilization of orchids is a determining factor for their successful cultivation, as these plants are renowned for their exotic and diverse flowers. Unlike other species, orchids possess delicate root systems and limited absorption capacity, demanding balanced and less concentrated nutrition. An appropriate nutritional management promotes vigorous growth, abundant flowering, and greater resistance to diseases, requiring protocols tailored to the specific needs of each species (Hoshino et al., 2023).

Within this context, *Oncidium baueri* Lindley, a native Brazilian species with vibrant flowers and considerable hardiness, emerges as a promising candidate for commercial cultivation. However, its ongoing domestication process requires in-depth knowledge of its ecological and nutritional requirements to unlock its full ornamental potential (Cunha et al., 2024). Establishing specific cultivation protocols, particularly regarding fertilization, is therefore essential.

Given the sensitivity of orchids, organic fertilizers that promote slow nutrient release represent a promising approach. Among them, Bokashi - an organic compost derived from the fermentation of materials with beneficial microorganisms - is known to enhance soil quality, nutrient availability, and overall plant health (Tong et al., 2021; Vilcherrez-Atoche et al., 2022). Its benefits, such as improved soil structure and increased microbial activity, align well with the delicate needs of epiphytic plants.

Despite these potential advantages, a significant knowledge gap exists, as there is no standardized protocol for the application of Bokashi in orchid cultivation. Key questions regarding the optimal dosage, frequency, and application method remain unanswered, highlighting the need for scientific investigation to maximize its benefits without causing harm (Hoshino et al., 2021; Netto et al., 2021; Hoshino et al., 2023).

Nonetheless, this study aimed to identify the best dosage and application method for Bokashi by analyzing its effects on the vegetative growth, nutritional status, and Photosystem II activity in *Oncidium baueri* Lindley.

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Material and Methods

The experiment was conducted with *Oncidium baueri* Lindley plants, which had undergone 12 months of acclimatization and originated from asymbiotic germination in a tissue culture laboratory. For the experimental setup, seedlings with a total length of 12 ± 0.98 cm and three pseudobulbs were used. These seedlings were planted in 14 cm diameter pots (measured at the upper edge). The substrate consisted of a 1:1 (v v⁻¹) mixture of pine bark and charcoal.

The treatments consisted of: (CTRL) a chemical control with a 20:20:20 fertilizer applied at a dose of 2 g L⁻¹; (BOK-CL) a burlap bag containing 10 g Bokashi placed on the substrate surface on the day of the experiment setup; (BOK-10S) 10 g Bokashi per liter of substrate, incorporated on the setup day; (BOK-20S) 20 g Bokashi per liter of substrate, also incorporated on the setup day; (BOK-10F) 10 g Bokashi diluted in one liter of water; and (BOK-20F) 20 g Bokashi diluted in one liter of water. For treatments involving dilution (BOK-10F, BOK-20F), The solutions were prepared and kept in the dark for 6 hours; after this period, the solution was filtered through paper filters before application. For treatments (CTRL), (BOK-10F), and (BOK-20F), 30 mL of the solution was applied via foliar spray every 15 days between 6:00 PM and 7:00 PM from February 2023 to February 2024, ensuring that the spray covered the entire plant. The Bokashi used contained castor bean cake, rice bran, wheat bran, bone meal, basalt rock powder, and strains of *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilus*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Saccharomyces cerevisiae*.

The plants were maintained in a greenhouse with 70% shade netting, an average temperature of 25 ± 0.4 °C, and relative humidity ranging from 60% to 70%. Irrigation was automated by sprinklers, adjusted to the plants' needs during different seasons, with daily irrigation during warmer months and every other day during colder months.

After one year of cultivation, phytometric data were collected, including: number of leaves (NL), shoot length (SL), number of pseudobulbs (NP), number of shoots (NS), length of the longest root (LLR), fresh shoot mass (FSM), fresh root mass (FRM), dry shoot mass (DSM), dry root mass (DRM), and the sum of fresh and dry masses, represented by total fresh mass (TFM) and total dry mass (TDM). To obtain dry mass, samples were placed in kraft paper bags and dried in a forced-air oven at 65 °C until a constant mass was reached, over the course of one week.

On the day the experiment was dismantled, the youngest fully expanded leaves were selected for physiological analyses. Chlorophyll *a* fluorescence variables were measured using a portable OS1p fluorometer (OptiSciences, Hudson, NY, USA). The leaves were dark-adapted for 30 minutes using FL-DC clips, and basal fluorescence (F_0) was measured with diffuse modulated light for 0.1 s (10% intensity). Subsequently, the leaves were exposed to a saturating light pulse ($8250 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 0.8 s to measure the maximum quantum yield of Photosystem II (F_v/F_m). The effective quantum yield of Photosystem II ($\Phi\text{PSII} = \Delta F/F_m'$) was measured

at 10:00 AM on light-adapted leaves exposed to photosynthetically active radiation (PAR) of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$, quantified at the time of analysis. Basal fluorescence (F') and maximum fluorescence (F_m') of light-adapted leaves were determined before and after exposure to the saturating light pulse ($8250 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 0.8 s, respectively, and ΔF was calculated as the difference between F_m' and F' .

Macronutrient content in different plant parts (leaves, pseudobulbs, and roots) was determined from dried tissues. These tissues were ground using an analytical mill (IKA VR A11 model), and macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (Fe, Mn, Zn) were quantified. The nitro-perchloric acid digestion method was used to quantify P and S by colorimetry; Ca, Mg, Fe, Mn, and Zn were quantified by atomic absorption spectrophotometry, while K was measured by flame photometry. N content was obtained through sulfuric digestion and quantified using the Kjeldahl method, as described by Silva (2009). Macronutrient results were expressed in g kg⁻¹, while micronutrient results were expressed in mg kg⁻¹.

The pH and electrical conductivity were determined using a 1:5 (v v⁻¹) extraction ratio of substrate and deionized water, with measurements taken using portable pH and conductivity meters after 60 minutes of agitation on a shaker table.

The experiment was arranged in a completely randomized design with 10 replicates per treatment, each replicate represented by one pot containing a single plant. Phytometric data were collected from all 10 replicates. Photosystem II activity data were collected from seven plants per treatment as described previously. Nutritional data were collected from five plants per treatment, with each plant divided into leaves, pseudobulbs, and roots before the drying process, allowing separate analysis of these three regions.

All collected data were subjected to normality (Shapiro-Wilk) and homogeneity of variance (Levene) tests, followed by analysis of variance (ANOVA) to identify significant differences among treatments. Tukey's test at a 5% significance level was used to determine significant differences among treatments and for nutrient analyses in different plant parts. Principal Component Analysis (PCA) was performed after data preprocessing (mean centering) to identify similarities and correlations within the dataset, including treatments, plant structures (root, leaf, and pseudobulb), and nutrients.

Results and Discussion

Bokashi treatments had a significant impact on phytometric variables compared to chemical fertilizer (T1), demonstrating the efficacy of organic fertilizer in promoting the growth of *Oncidium baueri*. The mean values for the number of leaves (NL), shoot length (SL), and number of pseudobulbs (NP) were consistently higher in treatments T3 and T4, both involving Bokashi incorporated into the substrate. BOK-20S showed the best results, with an average of 31.14 leaves and 7.14 pseudobulbs, representing an increase of 138% in NP and 359% in SL compared to the initial values.

Table 1. Number of leaves (NL), shoot length (SL), number of pseudobulbs (NP), number of shoots (NS), electrical conductivity (EC), hydrogen potential (pH), and longest root length (LRL) of *Oncidium baueri* plants

Treatments:	NL	SL	NP	NS	EC	pH	LRL
CTRL	18.14bc	53.6ab	4.28bc	0.57a	0.03c	7.97b	16.47a
BOK-CL	26.14ab	51.27ab	5.71ab	1.71a	0.02ab	8.74a	17.60a
BOK-10S	29.57a	54.8a	6.14a	0.71a	0.01a	7.75bc	16.91a
BOK-20S	31.14a	54.71a	7.14a	0.57a	0.02ab	7.58c	23.66a
BOK-10F	15.85c	52.7ab	4.28bc	0.42a	0.02ab	7.54c	22.39a
BOK-20F	19.85bc	44.98b	3.85c	1.71a	0.02ab	7.54c	22.39a
CV (%)	23.45	10.9	16.32	32.7	27.06	2.57	26.59

Different letters denote statistically significant differences according to Tukey's test ($p < 0.05$).

This performance can be attributed to the gradual nutrient release promoted by Bokashi and its contribution to increasing organic matter in the substrate, improving water retention and the availability of essential nutrients (Hata et al., 2020). These factors are crucial for epiphytic orchids, whose root systems are adapted to efficiently capture nutrients in limited environments. According to Hoshino et al. (2023), organic fertilizers can enhance the activity of beneficial microorganisms, such as *Bacillus subtilis* and *Lactobacillus plantarum*, which assist in the production of plant hormones like auxins and in making nutrients available. This microbial activity is fundamental: the microorganisms break down complex organic matter, mineralizing nutrients like nitrogen and phosphorus and chelating micronutrients, which makes them readily available for root absorption over an extended period (Subedi, 2025). This avoids

the salinity stress that can be caused by high concentrations of soluble salts from chemical fertilizers, a critical factor for sensitive orchid roots. Furthermore, microorganisms like *Bacillus subtilis* are known to produce siderophores, which enhance iron uptake, and solubilize phosphates, directly contributing to the vigorous root and shoot development observed in the substrate-incorporated treatments (Jalal et al., 2023).

BOK-20S had the longest root length (LRL), with an average of 23.66 cm, indicating that Bokashi incorporated into the substrate can promote better root development (Fig. 1). Although no statistical difference was observed for the number of shoots (NS), the numerical increase in BOK-10S and BOK-20S may suggest a tendency towards greater development under these treatments, indicating an efficient nutritional response, though not statistically significant in this parameter.

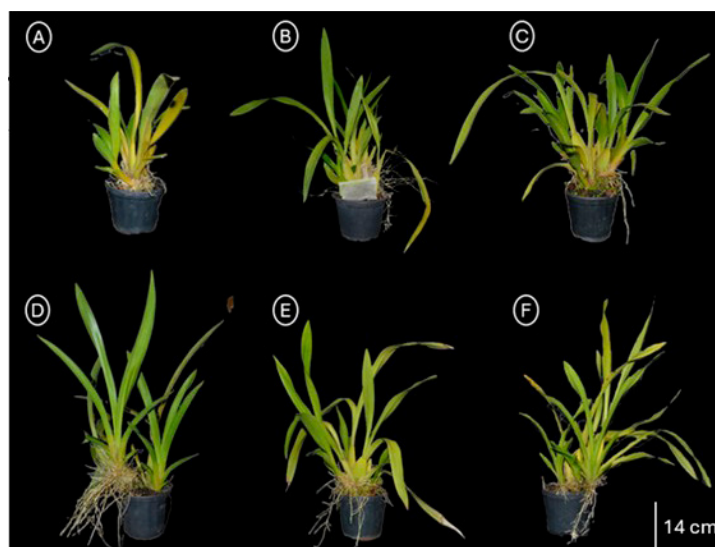


Fig. 1. *Oncidium baueri* orchid plants at the end of the experimental period showing treatment effects. Plants were photographed at the conclusion of the experiment to document treatment responses. (A) Control treatment. (B) Controlled-release fertilizer sachets. (C) Bokashi organic fertilizer at 10 g per liter of substrate. (D) Bokashi organic fertilizer at 20 g per liter of substrate. (E) Foliar application of Bokashi at 10 g per liter of water. (F) Foliar application of bokashi at 20 g per liter of water.

Regarding biomass data, BOK-20S outperformed the other treatments in all parameters, including shoot fresh mass (SFM), root fresh mass (RFM), and total fresh mass (TFM, the sum of shoot and root fresh mass), with averages of 215.45 g, 121.19 g, and 336.64 g, respectively.

This suggests that Bokashi incorporated into the substrate provides a continuous nutrient source, promoting greater biomass accumulation, as shown in Table 2.

Table 2 – Fresh weight and dry weight biomass measurements of *Oncidium baueri* plants following one-year bokashi organic fertilizer treatment.

Treatment:	Fresh weight (g)		
	Shoot	Root	Total
CTRL	128.86ab	81.36b	210.23ab
BOK-CL	198.68ab	106.39ab	305.07ab
BOK-10S	178.31ab	64.97b	243.29ab
BOK-20S	215.45a	121.19a	336.64a
BOK-10F	141.36ab	88.17b	229.53ab
BOK-20F	108.06b	70.24b	178.33b
CV (%)	30.34	26.21	24.3
Treatment:	Dry weight (g)		
	Shoot	Root	Total
CTRL	15.59b	14.43a	30.02ab
BOK-CL	22.84ab	18.37a	41.21ab
BOK-10S	20.78ab	11.19a	31.98ab
BOK-20S	26.32a	20.31a	46.64a
BOK-10F	15.5b	14.67a	30.17ab
BOK-20F	13.74b	10.39a	24.13b
CV (%)	26.28	27.14	27.7

Different letters denote statistically significant differences according to Tukey's test ($p < 0.05$).

For dry mass data, differences were observed in shoot dry mass (SDM) and total dry mass (TDM), with BOK-20S (20 g Bokashi incorporated into the substrate) being superior to all other treatments, with averages of 26.32 g and 46.64 g, respectively. The lowest values were observed in T6 for both SDM and TDM, with 13.74 g and 24.13 g, respectively. Netto et al. (2021) emphasizes the importance of combining foliar applications with organic matter-rich substrates to maximize orchid growth.

In terms of photosynthetic data, the initial fluorescence (F_0) was highest in BOK-10F, with an average of 213, surpassing all other treatments, as was the maximum fluorescence (F_m) (Fig. 2). No differences were observed for F_v/F_m and F_v/F_0 , indicating that the

light absorption efficiency by reaction centers was consistent, with all values corresponding to plants with properly functioning photosystems. As reported by Taiz et al. (2021), photosynthetic efficiency is often preserved in well-nourished plants, provided that critical nutrients for Photosystem II function, such as Mg and Fe, are adequately supplied. The stability of F_v/F_m across all treatments, including the high-dose Bokashi application (BOK-20S), is a significant finding. It suggests that organic fertilization promoted vigorous growth without inducing photochemical stress, which can occur with nutrient over-supply or imbalance (Maxwell and Johnson, 2000). The slow-release nature of Bokashi likely maintained a balanced nutrient environment, preserving the integrity and repair capacity of the photosynthetic apparatus.

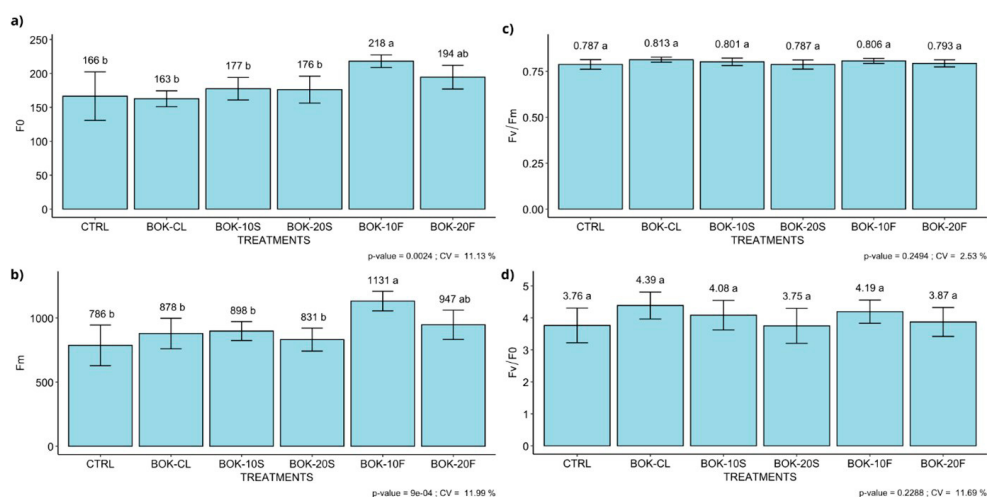


Fig. 2. Graphs showing the means and standard deviations of analysis parameters for photosystem II activity in *Oncidium baueri* subjected to different bokashi doses and application methods

In the leaf nutrient analysis, significant differences were observed for K, Ca, S, and Zn. For potassium (K), foliar application (BOK-10F and BOK-20F) outperformed other forms of Bokashi application and chemical fertilization (CTRL). The highest value was recorded in BOK-20F, with 26 g kg⁻¹. This result is consistent with the high mobility of

potassium within the plant and its efficient absorption through the leaf surface. Foliar sprays provide immediate availability of K⁺ ions to the photosynthetic tissues, where it plays a crucial role as an enzymatic cofactor and in regulating stomatal function (Fageria et al., 2009). For calcium (Ca), the best averages were obtained with Bokashi incorporated

into the substrate at the lower dose (BOK-10S) and with foliar application at the higher concentration (BOK-20F), with the highest value recorded in T3 (40.096 g kg⁻¹). In contrast to potassium, calcium is primarily absorbed by young roots and transported via the xylem with very low mobility in the phloem. This explains why substrate-based application (BOK-10S) was more effective for Ca accumulation than foliar sprays, as the nutrient was directly available to the primary absorption sites (White and Broadley, 2003). For sulfur (S) and zinc (Zn), Bokashi incorporated into

the substrate stood out compared to foliar application, with T4 showing the highest S value (0.788 g kg⁻¹) and BOK-10S the highest Zn value (14.09 mg kg⁻¹) (Table 3).

It is also noteworthy that certain nutrients, such as N, P, and Mg in leaves, did not show statistically significant differences across treatments, indicating that their supply was adequate regardless of the Bokashi application method or the chemical control, or that their concentrations were maintained within optimal ranges for *Oncidium baueri*

Table 3 Nutritional analysis of *Oncidium baueri* under different fertilization treatments, showing macronutrient and micronutrient concentrations in plant tissues (leaves, pseudobulb, leaves + pseudobulb, and root). Values represent mean concentrations per treatment and tissue for nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), and zinc (Zn).

NUTRICIONAL ANALYSIS									
Treat:	Leaves								
	g kg ⁻¹						mg kg ⁻¹		
	N	P	K	Ca	Mg	S	Fe	Mn	Zn
CTRL	1.442a	1.22a	19.772b	34.370bc	3.673a	0.628ab	253.1a	96.520a	4.940ab
BOK-CL	2.142a	1.29a	18.357b	38.672ab	3.728a	0.742ab	316.4a	86.520a	3.140b
BOK-10S	2.044a	1.17a	18.250b	40.096a	3.254a	0.712ab	293.89a	79.680a	14.090a
BOK-20S	1.512a	1.433a	17.678b	32.811c	3.269a	0.788 a	338.5a	107.210a	5.428ab
BOK-10F	2.66a	1.33a	25.321a	33.812c	3.304a	0.522b	286.5a	94.600a	6.160ab
BOK-20F	0.882a	1.41a	26.000a	39.391a	3.802a	0.630ab	364.1a	111.490a	10.050ab
Treat:	Pseudobulb								
	N	P	K	Ca	Mg	S	Fe	Mn	Zn
	CTRL	0.224a	1.68ab	10.178bc	33.126a	2.823bc	0.375c	305.43ab	27.170a
BOK-CL	0.490a	1.15b	7.964c	34.278a	2.893bc	0.825 a	162.42b	24.680a	0.92b
BOK-10S	0.644a	1.349ab	8.678bc	27.255a	2.727c	0.572bc	275.72ab	25.000a	3.32ab
BOK-20S	1.134a	1.827ab	10.037bc	27.545a	3.766ab	0.667ab	188.42b	54.910a	4.778ab
BOK-10F	0.644a	2.01a	11.642ab	28.760a	4.150a	0.381c	265.46ab	55.900a	8.6ab
BOK-20F	1.092a	1.67ab	14.821a	34.608a	3.108bc	0.518bc	392.87a	41.840a	15.44a
Treat:	Shoot (Leaves + pseudobulb)								
	N	P	K	Ca	Mg	S	Fe	Mn	Zn
	CTRL	1.666a	2.913ab	29.921b	67.496ab	6.496ab	1.004c	558.55b	123.690a
BOK-CL	2.632a	2.45b	26.321b	72.951a	6.622ab	1.567a	478.86b	111.200a	4.06b
BOK-10S	3.066a	2.528b	26.928b	67.351ab	5.980b	1.288abc	569.61ab	104.680a	17.41ab
BOK-20S	2.646a	3.26ab	27.714b	60.357b	7.034ab	1.451ab	527.3b	162.120a	10.207ab
BOK-10F	3.304a	3.351a	36.964a	62.573b	7.454a	0.904c	552.05b	150.500a	14.76ab
BOK-20F	1.974a	3.056ab	40.821a	73.999a	6.909ab	1.154bc	757.14a	153.330a	25.49a
Treat:	Root								
	N	P	K	Ca	Mg	S	Fe	Mn	Zn
	CTRL	1.330a	1.470a	3.071bc	19.411b	1.991a	0.795a	869.14ab	86.330sb
BOK-CL	4.774a	1.596a	2.607c	22.751ab	2.130a	0.825a	1039.27a	77.230b	9.72a
BOK-10S	0.938a	1.162a	4.428bc	22.924ab	2.350a	0.788a	770.73b	55.400b	11.54a
BOK-20S	0.224a	1.709a	4.500bc	19.784b	2.141a	0.665a	1039.9a	64.010b	8.73a
BOK-10F	1.596a	1.190a	5.535ab	22.316ab	2.275a	0.745a	853.45ab	109.860ab	5.81a
BOK-20F	4.550a	1.485a	7.607a	27.235a	2.17a	0.788a	1060.9a	147.520a	12.3a
Treat:	Total								
	N	P	K	Ca	Mg	S	Fe	Mn	Zn
	CTRL	2.996a	4.383ab	32.992b	86.907bc	8.487a	1.8b	1427.69b	210.02ab
BOK-CL	7.406a	4.046ab	28.928b	95.703ab	8.752a	2.394a	1518.14b	188.43ab	13.78ab
BOK-10S	4.004a	3.690b	31.357b	90.275abc	8.330a	2.071ab	1340.35b	160.08b	28.95ab
BOK-20S	2.870a	4.969a	32.214b	80.141c	9.176a	2.115ab	1567.2ab	226.13ab	18.942ab
BOK-10F	4.900a	4.541ab	42.500a	84.889bc	9.729a	1.652b	1405.5b	260.36ab	20.57ab
BOK-20F	6.524a	4.571ab	48.428a	101.234a	9.080a	1.942ab	1818.05a	300.85a	37.79a

Different letters denote statistically significant differences according to Tukey's test ($p < 0.05$).

In pseudobulbs, known for their storage capacity, nutrients such as P, K, Mg, S, Fe, and Zn showed significant differences. As critical storage organs for water and nutrients in sympodial orchids like *Oncidium*, pseudobulbs reflect the plant's long-term nutritional status, making them excellent indicators of nutrient allocation efficiency (Zhang et al., 2021). Biweekly foliar application was superior for most nutrients, except for sulfur, where BOK-CL had the highest value (0.825 g kg⁻¹). The highest phosphorus (P) and magnesium (Mg) averages were recorded in BOK-10F, with 2.01 g kg⁻¹ and 4.15 mg kg⁻¹, respectively. For potassium (K), iron (Fe), and zinc (Zn), T6 showed the highest values, indicating that dosage and application method directly influence nutrient absorption.

It is also noteworthy that certain nutrients, such as N, P, and Mg in leaves, did not show statistically significant differences across treatments, indicating that their supply was adequate regardless of the Bokashi application method or the chemical control, or that their concentrations were maintained within optimal ranges for *O. baueri*.

In root analysis, BOK-20F stood out for K, Ca, and Mn, demonstrating the superiority of foliar application with a 20 g L⁻¹ Bokashi solution in promoting the fixation and absorption of these elements. For iron (Fe), treatments BOK-CL, BOK-20S, and BOK-20F were the most effective, indicating that this element can be well absorbed through both foliar application and substrate incorporation of Bokashi at 20 g L⁻¹ or through slow-release Bokashi (BOK-CL).

When analyzing the total nutrient concentration in the shoot (leaves + pseudobulbs), biweekly foliar application (BOK-10F and BOK-20F) was more efficient than other application methods, outperforming conventional mineral fertilization. Sulfur was the only nutrient whose absorption was not optimized by foliar application, with the highest values observed in BOK-CL.

The results of this study confirm the efficacy of Bokashi as a sustainable alternative to chemical fertilizers in orchid cultivation. Bokashi treatments

not only promoted greater vegetative growth but also ensured adequate nutrition levels while preserving the plants' photosynthetic efficiency.

These findings corroborate previous studies, such as those by Hoshino et al. (2021), which highlight the potential of Bokashi to optimize the development of ornamental plants through slow nutrient release and the promotion of microbial activity in the substrate. Furthermore, the diverse responses among foliar and substrate treatments suggest that combining both strategies may be the most efficient approach to maximizing Bokashi's benefits. This points towards an integrated nutrient management (INM) strategy, where substrate application of Bokashi builds soil health and provides a baseline of slow-release nutrition, while targeted foliar applications can supplement key mobile nutrients during critical phenological stages, such as flowering or active vegetative growth (Aulakh and Malhi, 2005).

From a commercial perspective, the use of Bokashi in *Oncidium baueri* not only contributes to environmental sustainability but also enhances the value of this native species as a high-potential ornamental plant in the market. Standardizing application protocols, based on studies like this, could drive its large-scale adoption.

Principal Component Analysis (PCA) revealed the influence of treatments on the nutritional composition of plant structures. In the score plot, samples were differentiated by tissue and treatment, while the loading plot showed the nutrients' influence on the principal components. Roots clustered in the lower-left quadrant of the score plot, indicating lower nutritional variability, while leaves and pseudobulbs showed greater variation. PC1 (33.5%) was negatively influenced by Mn, N, Zn, and S, indicating higher concentrations of these nutrients in roots, while PC2 (18.7%) was positively impacted by K, Ca, and Mg, associating leaves and pseudobulbs with these macronutrients. This distribution reflects the functional specialization of tissues in nutrient absorption and allocation (Fig. 3).

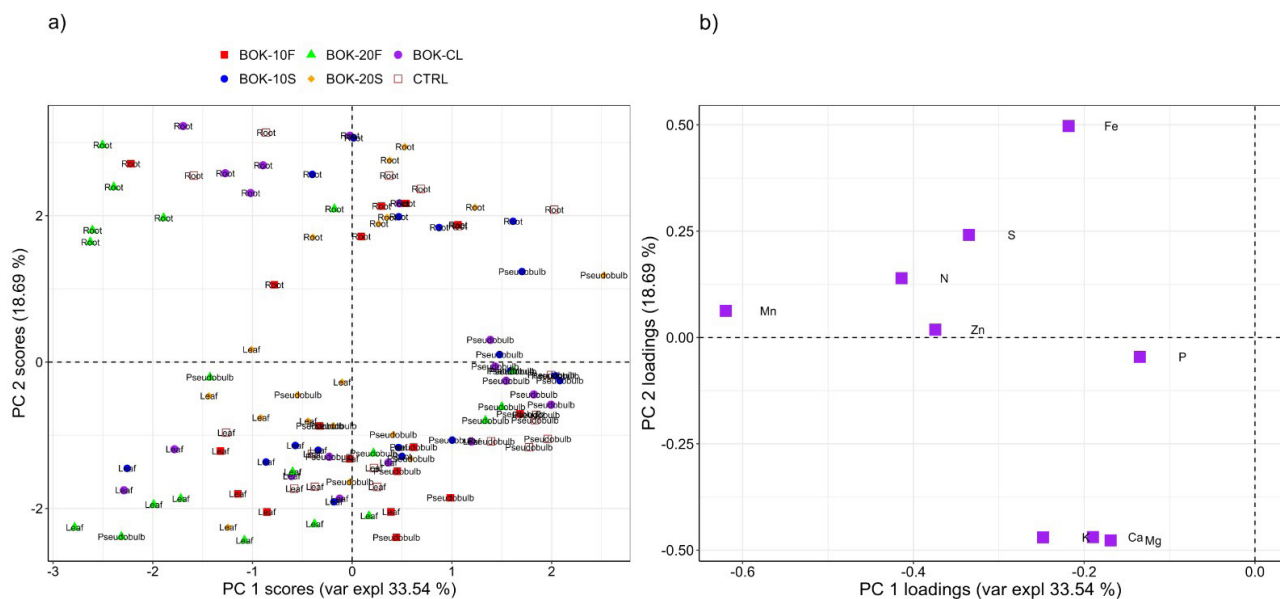


Fig. 3. Principal component analysis (PCA) of nutrient distribution in different plant tissues (leaf, pseudobulb, root) and treatments. PC1 explains 33.5% of the variance and PC2 explains 18.7%.

The accumulation of K, Ca, and Mg in the aerial parts is linked to osmotic regulation, photosynthesis, and cellular stability. K regulates stomatal opening and photoassimilate transport, controlling water and gas flux (Hasanuzzaman et al., 2018). Mg is essential for chlorophyll synthesis and enzymatic activation, driving carbon fixation and light energy conversion (Tränkner et al., 2018). Ca contributes to cell wall structuring and signaling, providing mechanical resistance to plant tissues (Xie et al., 2021).

Conversely, the higher concentration of Mn, Zn, Fe, N, and S in roots is due to the low mobility of these nutrients in the phloem and their importance in root metabolism. Fe and Mn are predominantly transported via the xylem and accumulate in roots and older leaves due to the formation of insoluble complexes, hindering their redistribution to aerial parts (Page and Feller, 2015). Zn, despite its intermediate mobility, tends to remain in roots due to its association with proteins

and metabolic enzymes essential for root function (Gupta et al., 2016). Additionally, N and S are crucial for amino acid and sulfur compound synthesis, being initially metabolized in roots before translocating to other tissues (Zayed et al., 2023). This distribution pattern highlights the role of roots as a reservoir of micronutrients, optimizing their absorption and primary metabolism, while leaves and pseudobulbs prioritize photosynthetic and structural functions, ensuring balanced plant growth (Chao and Chao, 2024).

Treatments directly influenced nutrient absorption and accumulation in plant tissues, reflecting distinct patterns according to the application method. The "10 g L⁻¹ foliar spray" treatment favored the absorption of K, Ca, and Mg predominantly in leaves and pseudobulbs, optimizing photosynthesis, osmotic regulation, and assimilate transport, resulting in greater biomass and vegetative growth (Hata et al., 2020). Conversely, the "10 g incorporated" and "burlap bag" treatments promoted higher

concentrations of Mn, Zn, N, and S in roots due to the low mobility of these nutrients in the phloem and the enhanced availability provided by soil microbiota, which increased root absorption (Keitan and Hadi, 2023). These results indicate that application methods directly modulate nutrient partitioning. Crucially, while foliar sprays efficiently delivered mobile nutrients to aerial parts, the superior overall growth and biomass in the substrate-incorporated treatment (T4) suggests that for *O. baueri*, establishing a robust nutritional foundation in the root zone - rich in S, Zn, and other microelements - is the primary driver for achieving maximum vegetative potential.

Conclusions

Fertilization with substrate-incorporated Bokashi at a dose of 20 g L⁻¹ proved to be the most efficacious treatment for promoting vegetative growth and biomass accumulation in *Oncidium baueri*, outperforming conventional chemical fertilization and other organic application methods. This method promoted a continuous and balanced supply of nutrients directly to the root system without inducing photosynthetic stress. Therefore, our findings establish the use of substrate-incorporated Bokashi as the first scientifically validated, sustainable, and high-yielding protocol for the vegetative development of *O. baueri*. Future research should build upon this foundation, investigating the long-term impacts of this protocol on the flowering cycle and commercial value of the plants, as well as exploring potential synergies with other organic inputs.

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Author Contribution

SPJ: Conceptualization, Data Curation, Methodology, Writing – Original Draft, Writing – Review & Editing. **VFAS:** Data Curation. **DPT:** Conceptualization. **JPCP:** Data Curation, Methodology. **HCO:** Funding Acquisition, Supervision. **RTF:** Funding Acquisition.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available upon request to the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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