

## Use of bokashi in germination and initial development of cucumber and tomato seedlings

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### ABSTRACT

This study aimed to evaluate the effect of the diluted application of Bokashi on seed germination and seedling development of tomato (*Solanum lycopersicum*) and cucumber (*Cucumis sativus*). The experiment was conducted in two stages: the first, in the laboratory, analyzed germination with two treatments: T1 (control), irrigated with water, and T2 (Bokashi), with a diluted solution (10 mL L<sup>-1</sup> of water). Germination was monitored in a B.O.D. (Biochemical Oxygen Demand) incubator at 25 °C under a 12-hour photoperiod for 9 days. The results showed that Bokashi reduced tomato germination by 17%, but did not affect cucumber germination, which remained 100%. In the second stage, in a greenhouse, the seedlings were transplanted into pots with Ultisol (argissolo) and monitored for 33 days, receiving 150 mL of water daily. Parameters such as height, number of leaves, leaf area, and stem diameter were evaluated weekly, with no significant differences at the end. There were also no changes in physiological parameters (chlorophyll content, stomatal conductance, chlorophyll a transient fluorescence, and pigment content), indicating that Bokashi did not affect these variables. Thus, the results indicate that Bokashi use did not improve germinative performance or seedling development.

**Keywords:** Organic fertilizer, Biofertilizer, Chlorophyll fluorescence.

## USO DO BOKASHI NA GERMINAÇÃO E NO DESENVOLVIMENTO INICIAL DE MUDAS DE PEPINO E TOMATE

### RESUMO

Este estudo teve como objetivo avaliar o efeito da aplicação diluída de Bokashi na germinação de sementes e no desenvolvimento de mudas de tomateiro (*Solanum lycopersicum*) e pepino (*Cucumis sativus*). O experimento foi conduzido em duas etapas: a primeira, em laboratório, analisou a germinação com dois tratamentos: T1 (controle), irrigado com água, e T2 (Bokashi), com solução diluída (10 mL L<sup>-1</sup> de água). A germinação foi monitorada em incubadora B.O.D. (Biochemical Oxygen Demand) a 25 °C, com fotoperíodo de 12 horas, durante 9 dias. Os resultados mostraram que o Bokashi reduziu em 17% a germinação do tomateiro, mas não afetou o pepino, que manteve 100% de germinação. Na segunda etapa, em casa de vegetação, as mudas foram transplantadas para vasos com argissolo e acompanhadas por 33 dias, recebendo 150 mL de água diariamente. Parâmetros como altura, número de folhas, área foliar e diâmetro do colo foram avaliados semanalmente, sem diferenças significativas ao final. Também não houve alterações nos parâmetros fisiológicos (teor de clorofila, condutância estomática, fluorescência da clorofila a e teor de pigmentos), indicando que o Bokashi não afetou essas variáveis. Assim, os resultados indicam que o uso do Bokashi não promoveu melhorias no desempenho germinativo nem no



desenvolvimento das mudas.

**Palavras-chave:** Adubo orgânico, Biofertilizante, Fluorescência da clorofila.

## 1. Introduction

The seed is the primary means of sexual multiplication in most cultivated plant species, and its study and technology are fundamental for advancing agricultural management, conserving genetic resources, and plant breeding (Carvalho and Nakagawa, 2012; Marcos-Filho, 2015). High-vigor seeds are crucial because they have a greater capacity to mobilize and utilize their reserves, resulting in rapid, uniform seedling establishment in the field (Finch-Savage and Bassel, 2016; Tunes et al., 2021). This characteristic is decisive, since the formation of a high-quality initial stand directly influences performance and productivity (Peske, Vilella, Meneghello, 2019).

The growing demand for seeds of high physiological and sanitary quality for more productive and sustainable agriculture has intensified monitoring across all stages of the seed industry's production process (Imolesi, 2001). However, despite recent advances, there are still gaps in understanding the relationships between fertilization and the physiological and sanitary quality of seeds, especially regarding the mechanisms involved and the variability across species and production systems (Imolesi, 2001; Oliveira et al., 2003). In this sense, interest has grown in alternative inputs and more sustainable practices that can influence not only plant development but also seed quality (Abreu et al., 2014).

In this context, Bokashi has been used as an organic input to improve soil quality, thereby increasing microbial activity and nutrient availability (Lasmini et al., 2018). However, its potential effects on the early stages of plant development, such as germination and early seedling growth, remain poorly explored. Considering that Bokashi contains beneficial microorganisms and fermentation-derived organic compounds, the hypothesis is raised that its application, even at diluted concentrations, can act as a biostimulant during seed imbibition, favoring initial metabolic processes and seedling establishment (Bocoli et al., 2020).

Within this sustainable perspective, crops such as cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* L.) stand out for the challenges they face in their production systems. Cucumber is an annual vegetable with a wide global distribution, recognized for its health benefits and socioeconomic role in Brazil, where it generates employment and drives the economy (Afrendi et al., 2024). On the other hand, tomato, widely used in the processing industry (pulp, sauces, soups), is one of the main horticultural products in the country, with production accounting for more than 50% of the

national cultivated area (Conab, 2019; Pedroso et al., 2023). To meet all the necessary demand, according to Conab (2021), fertilizer expenses account for the main production costs for these crops. This experiment hypothesized that applying diluted Bokashi during seed imbibition would improve seedling growth and development.

Thus, the objective was to evaluate the effect of diluted Bokashi solution application during seed imbibition on germination and initial growth parameters of tomato (*Solanum lycopersicum*) and cucumber (*Cucumis sativus*) seedlings.

## 2. Material and Methods

The experiment was carried out at the Federal Fluminense University (UFF) between July and September 2024. Part of the tests occurred in the laboratory, in a B.O.D (Biochemical Oxygen Demand) incubator, and the second stage of the experiment, when the seedlings were transplanted into pots, was conducted in a greenhouse located in the municipality of Niterói, RJ (22° 54' S, 43° 08' W and altitude of 6.0 meters). The structure is a galvanized arch, covered with low-density polyethylene (LDPE) film and equipped with a lateral closure with a black screen (70% shading mesh). The greenhouse has dimensions of 7.50 m wide by 15.00 m long, with a height under the gutter (post height) of approximately 4.00 m. The climate of the region is semi-humid tropical, classified as Aw according to Köppen, characterized by dry winters and rainy summers. The average annual temperature is 23 °C, and the average annual precipitation is 1,200 millimeters (mm). During the experiment, the maximum temperature and relative air humidity inside the greenhouse were monitored using an E5000 model meteorological station (Irriplus), with the data presented in the results.

Between July 22 and August 2, 2024, the first phase of the experiment was conducted in a laboratory environment focused on germination evaluation. The seeds were placed on ISOFAR® qualitative filter paper in glass Petri dishes and kept in a B.O.D incubator at 25 °C, with a 12-hour photoperiod. To prepare the solution, 10 mL (2 sachets) of Bokashi Isla® were diluted in one liter of water, and the filter paper was moistened with 5 mL of this solution in each Petri dish. The Bokashi used consists of an organic compound formed from a balanced mixture of plant- and mineral-origin materials, such as poultry litter, dolomitic limestone, castor cake, wheat bran, bone meal, and ashes or charcoal. In

addition, its composition includes a liquid fraction containing water, milk, effective microorganisms (EM), and crystal sugar, which stimulate microbial activity, thereby gradually increasing nutrient availability and improving media conditions (EMBRAPA, 2016).

For germination analysis, commercial seeds of *Cucumis sativus* and *Solanum lycopersicum* (Isla®) were used, distributed in two treatments. In the first treatment (T1, control), the seeds were irrigated exclusively with water. In the second treatment (T2), they received irrigation with a 1% Bokashi solution (10

mL L<sup>-1</sup>). The seeds were imbibed on filter paper moistened with the respective Bokashi solution, without prior drying, and incubated in a B.O.D. type incubator under continuous monitoring until germination stabilized.

Each treatment had ten replications of 20 seeds (200 seeds per treatment, Figure 1). Irrigation was performed daily, with Bokashi solution applied only during germination.



**Figure 1.** Seeds of *Cucumis sativus* and *Solanum lycopersicum* during the germination process on filter paper inside glass Petri dishes and stored in a B.O.D. incubator.

The germination rate was monitored daily, and stabilization of the treatments was observed on the ninth day for tomato and on the fourth day for cucumber after sowing. The germination percentage and the germination speed index (GSI) were determined. The calculation of the percentage followed the guidelines of the Rules for Seed Analysis (Brasil, 2009), while the GSI was obtained by the equation (EQ (1)), according to Maguire (1962), with adaptation by Moraes et al. (2012):

$$GSI = \left(\frac{G1}{N1}\right) + \left(\frac{G2}{N2}\right) + \dots + \left(\frac{Gn}{Nn}\right)$$

Where: G1, Gn = number of germinated seeds in the first and subsequent counts; N1, Nn = days after sowing in the first and subsequent counts.

At the end of the laboratory count, the second stage of the experiment began, focused on seedling cultivation, conducted between August 2 and September 4, 2024, for a total of 33 days. In this stage, 2 L containers were filled with medium-textured Ultisol (argissolo), collected from the surface horizon (0-20 cm), totaling 12 experimental units per crop, with six assigned to each treatment (T1 and T2). It was decided to use the soil in its natural fertility state, without corrections or prior chemical fertilization, so that the soil's intrinsic properties would not mask or interfere

with the physiological conditioning with Bokashi carried out in the initial phase.

Each container received three seedlings, with the pot considered the experimental unit. For growth monitoring, one seedling per container was randomly selected as a representative subsample of the experimental unit.

Unlike the previous stage, in this phase all seedlings were kept under the same water conditions, with standardized daily irrigation of 150 mL of water per container. The experimental units were kept in a greenhouse during this period.

Climatological data for the location were collected once a week between August 7 and September 4, 2024, from the greenhouse's interior. The information obtained was processed in Excel, and the maximum values for temperature (°C), relative air humidity (%), total daily radiation (W/m<sup>2</sup>), and evapotranspiration (mm) were analyzed.

Throughout the experiment, one plant per replication was randomly selected from the three present in the container for weekly follow-up. Each treatment had six replications, totaling 12 units per species. Growth parameters (height, number of leaves, leaf area, and stem diameter) were counted from 7 DAT (days after transplanting). Height was measured with the aid of a ruler, from the stem base to the apical meristem of the stem, and expressed in cm. The number of leaves was

obtained through direct counting of a sample unit from each container and expressed in units. The stem diameter was measured with a caliper at the stem base and reported in mm.

Leaf area (LA) was estimated based on the dimensions of the leaf located in the median region of the plant, using the average length (L) and width (W). For tomato, the formula  $LA = [(L \times W)/2] \times CF$  was applied, with a correction factor (CF) equal to 0.59, as proposed by Reis et al. (2013). For the cucumber, the equation  $LA = [(L \times W) \times 0.739] - 0.00104$  was used, as reported by Yang et al. (1990).

At the end of the experiment, 33 days after transplanting (DAT), growth analyses and physiological parameters were performed:

**Chlorophyll content of leaves:** Chlorophyll measurement was performed by reading the most expanded leaves, randomly selected, using the Minolta SPAD-502 chlorophyll meter.

**Stomatal conductance:** Stomatal conductance was determined in six replications per treatment using the SC-1 Leaf Porometer (Decagon Devices) and expressed in  $\text{mmol}/(\text{m}^2\text{s})$ .

**Chlorophyll a transient fluorescence:** For chlorophyll fluorescence analysis, leaves were dark-adapted for 30 minutes using a closed clip. The evaluation was performed with a portable fluorometer Handy PEA (Hansatech Instruments, King's Lynn, Norfolk, UK), and the parameters were calculated according to the methodology proposed by Strasser and Strasser (1995) and Tsimilli-Michael and Strasser (2008).

**Pigment content:** Photosynthetic pigment content was determined according to the methodology described

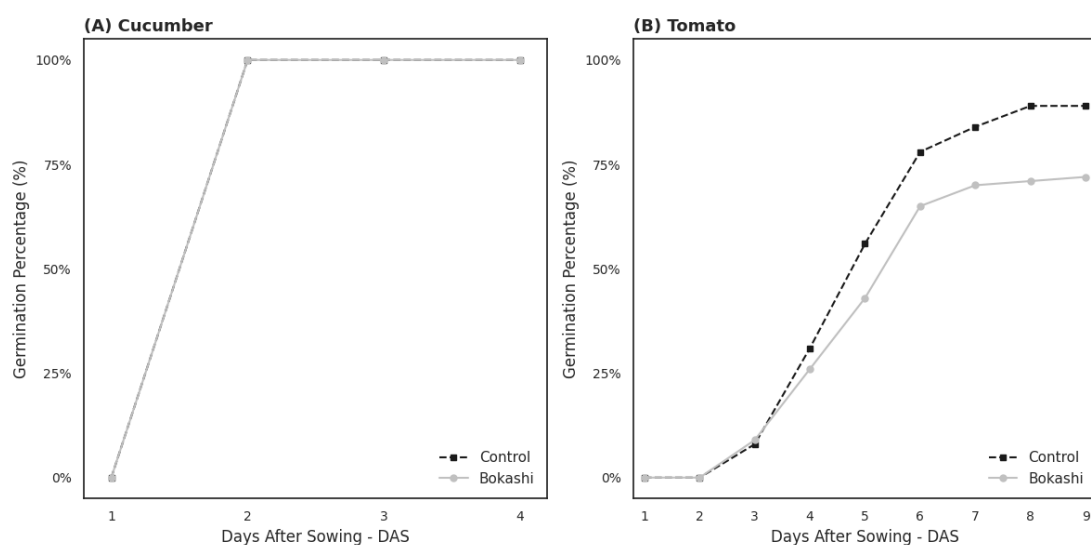
by Arnon (1949). For this, 0.030 g of the sample was macerated in 20 mL of 80% acetone. The values obtained were calculated using the equations of Hendry and Grime (1993) and expressed in  $\mu\text{mol g}^{-1} \text{FM}$ .

**Statistical analysis:** The data were submitted to analysis of variance (ANOVA) with the aid of the SISVAR® program, adopting a significance level of 5%. Multiple-comparison tests were not necessary because there were only two treatments.

### 3. Results and Discussion

Seed germination of tomato and cucumber showed differences between treatments. In the tomato, germination started on the third day in both treatments. Still, the control showed a faster, more efficient rate, reaching 89.0% in 9 days, while the Bokashi solution reached 72.0%, indicating a lower germination percentage than the control (Figure 2A). For cucumber, the application of the Bokashi solution did not interfere with the process, as both treatments achieved 100% germination (Figure 2B).

The trend in germination percentage was not reflected significantly in the Germination Speed Index (GSI). For both crops, the control values were numerically higher than those for the Bokashi treatment, but the difference was not statistically significant. This result indicates that, despite the reduction in final germination observed in the tomato, the germination speed of viable seeds was not affected by the treatment, suggesting that Bokashi solution may have affected some seeds, without altering germinative dynamics of viable seeds (Table 1).



**Figure 2.** Germination percentage of cucumber (A) and tomato (B), from the first to the ninth day after sowing (DAS), with and without the application of a solution containing 1% of Bokashi diluted in water.

**Table 1.** Germination speed index (GSI) of cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*), from the first to the ninth DAS, with and without application of a solution containing 1% of Bokashi diluted in water.

Treatments	GSI	
	Tomato	Cucumber
Control	0.371849 a	0.534439 a*
Bokashi	0.307257 a	0.530187

\*Same letters in the column do not differ statistically from each other by Tukey's test at 5%. Values representing the mean of n=10.

When analyzing the climatological data collected during the greenhouse experiment (August to September 2024), a slight variation in the environment was observed. The 12th DAT presented the greatest difference, especially regarding temperature, which reached a maximum of 33.64 °C, while the average of the other days was approximately 28 °C. According to Embrapa (2013), the ideal temperature range for cucumber cultivation is 20-30 °C, indicating that the conditions were favorable for the species' development. As for the relative air humidity, the 12th DAT also stood out, registering 65%, while the general average was 80%. Regarding radiation, the highest availability occurred on the 26th DAT, while the lowest was

recorded on the 5th DAT. Evapotranspiration averaged 0.8 mm per day.

The growth of tomato and cucumber, evaluated by stem height (cm), stem diameter (mm), number of leaves (units), and leaf area (cm<sup>2</sup>), remained similar across treatments throughout the experiment. No statistically significant differences were observed between the control and diluted Bokashi application. This result may be related to the fact that the Bokashi application was restricted to the germination phase, possibly limiting the manifestation of effects on vegetative growth, as this input is more commonly associated with improving soil conditions and microbial activity.

**Table 3.** Chlorophyll content of leaves of *Cucumis sativus* (cucumber) and *Solanum lycopersicum* (tomato) at 44th DAS, with and without application of a solution containing 1% Bokashi diluted in water during germination.

Treatment	Relative Chlorophyll Content	
	Tomato	Cucumber
Control	25.18 a	14.12 a*
Bokashi	24.82 a	14.22 a

\*Same letters in the column do not differ statistically from each other by Tukey's test at 5%. Values representing the mean of n=6.

**Table 4.** Stomatal conductance in leaves of *Cucumis sativus* (cucumber) and *Solanum lycopersicum* (tomato) at 44th DAS, with and without application of a solution containing 1% Bokashi diluted in water during germination.

Treatment	Stomatal Conductance (mmol m <sup>-2</sup> s <sup>-1</sup> )	
	Tomato	Cucumber
Control	245.32 a	210.37 a*
Bokashi	282.87 a	192.95 a

\*Same letters in the column do not differ statistically from each other by Tukey's test at 5%. Values representing the mean of n=6.

The chlorophyll content in the leaves at the end of the experiment indicated that both tomato and cucumber showed similar concentrations between the control treatment and the Bokashi solution application, without statistically significant differences (Table 3). The chlorophyll content of leaves, which is correlated with leaf nitrogen content (Leonardo et al., 2013), can reflect the nutritional condition of plants. These results suggest that diluted Bokashi application did not affect this specific aspect of plant nutrition, particularly nitrogen.

The analysis of stomatal conductance also revealed no significant difference between treatments, indicating that stomatal opening for gas exchange occurred similarly in both conditions assessed (Table 4). Given that greater soil nutrient availability is positively associated with stomatal conductance (Santos et al.,

2010), the results suggest that diluted Bokashi application did not significantly affect this physiological parameter during the study period.

Regarding the content of photosynthetic pigments extracted with acetone, the results show that there were no statistically significant differences between treatments (Control and Bokashi) for any of the evaluated variables (chlorophyll a, chlorophyll b, and carotenoids) in either tomato or cucumber seedlings (Table 5). These pigments are fundamental for light absorption and for protection against oxidative damage, and variations in their levels can reflect differences in photosynthetic efficiency and the physiological state of plants (Force et al., 2003).

Based on transient chlorophyll a fluorescence data, the Bokashi treatment elicited distinct responses among

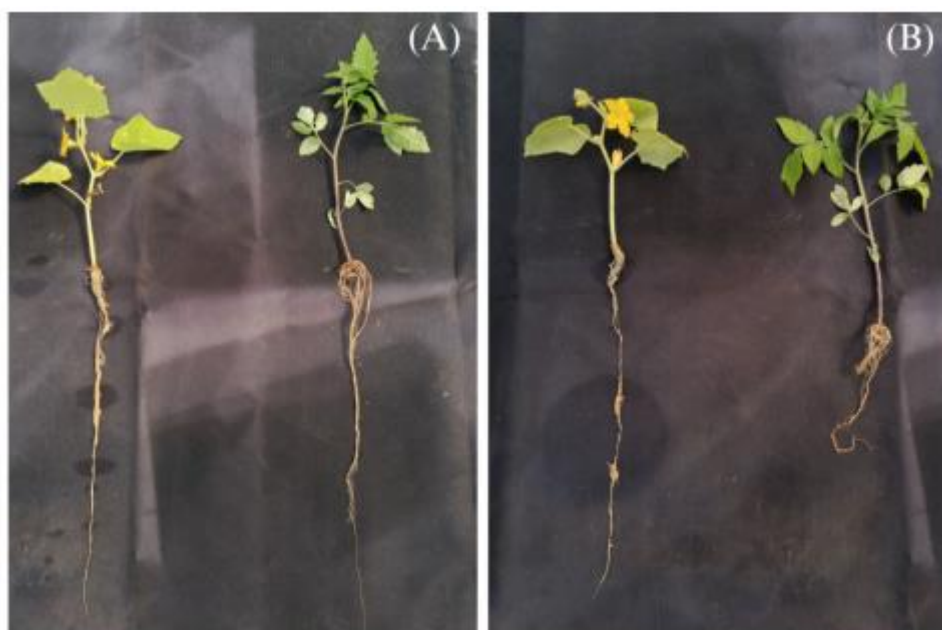
the evaluated species. In tomato, trends of alteration were observed in parameters related to photochemical performance, including reductions in efficiency indices ( $F_v/F_m$  and  $F_v/F_o$ ) and performance indices ( $PI_{abs}$  and  $PI_{total}$ ), as well as an increase in energy dissipation ( $DI_o/RC$ ). These results may indicate an initial disturbance in Photosystem II function. However, such changes were not statistically significant and should be interpreted with caution. In addition, despite these physiological trends, no differences in plant growth were observed, suggesting that eventual changes in the

photosynthetic apparatus were not severe enough to compromise development (Figure 3).

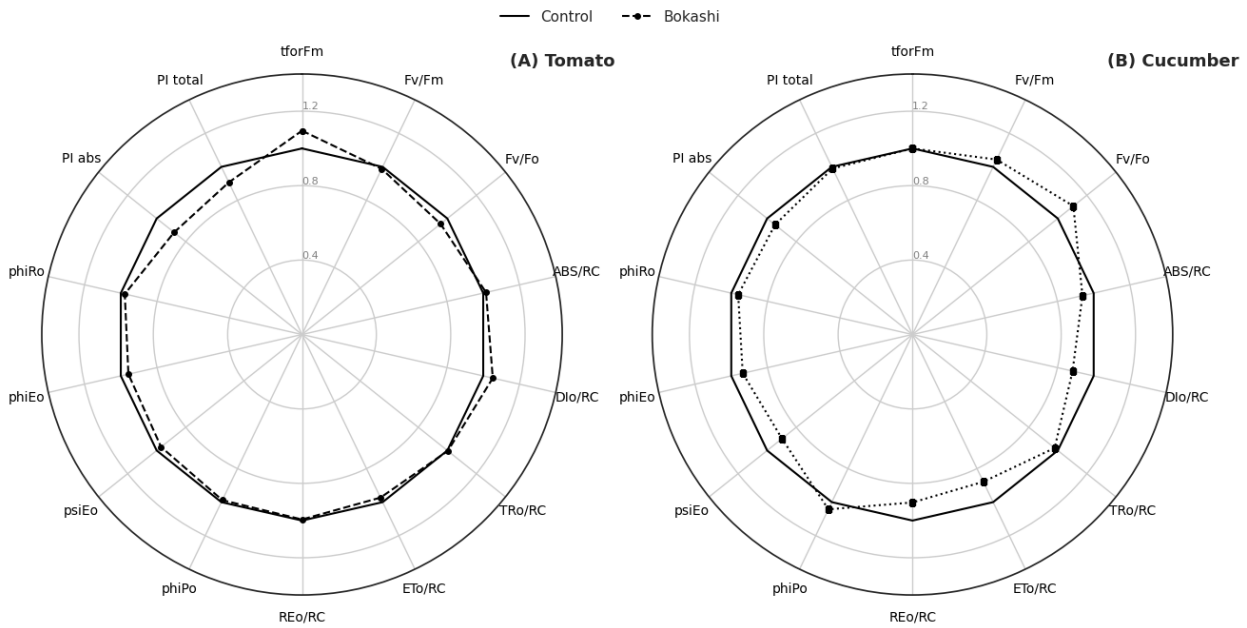
For the cucumber, values remained within the range considered adequate, while performance indices ( $PI_{abs}$  and  $PI_{total}$ ) showed only minor variations. In addition, no expressive increases in energy dissipation ( $DI_o/RC$ ) were observed, suggesting maintenance of the energetic balance of the photosynthetic apparatus. These results confirm the absence of significant responses in growth variables (Figure 4).

**Table 5.** Photosynthetic pigment content in leaves of cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*) at 44 DAS, with and without the application of a solution containing 1% of Bokashi diluted in water during germination.

Photosynthetic pigment content			
Crop and Treatment	Chlorophyll a ( $\mu\text{mol g}^{-1}$ )	Chlorophyll b ( $\mu\text{mol g}^{-1}$ )	Carotenoids ( $\mu\text{mol g}^{-1}$ )
Tomato			
Control	0.007553 a	0.014413 a	0.004553 a
Bokashi	0.006740 a	0.013730 a	0.004133 a
Cucumber			
Control	0.004627 a	0.013587 a	0.003340 a
Bokashi	0.004723 a	0.013590 a	0.003347 a



**Figure 3.** Cucumber and tomato seedlings, respectively. Control treatment (A) and treatment with a 1% diluted Bokashi solution (B), 44 days after sowing.



**Figure 4.** Chlorophyll *a* transient fluorescence parameters analyzed in leaves of *Solanum lycopersicum* (tomato) (A) and *Cucumis sativus* (cucumber) (B) at 44 DAS, with and without the application of a solution containing 1% of Bokashi diluted in water during germination.

#### 4. Conclusions

The diluted application of Bokashi did not significantly affect the development of tomato and cucumber seedlings under the evaluated conditions. Although tomato germination decreased, this difference did not translate into subsequent morphophysiological parameters. Thus, the application of Bokashi in germination, in the form and concentration used in this study, did not demonstrate significant benefits or harms to the development of the evaluated species, being limited to the specific application conditions adopted.

#### Authors' Contribution

Conceptualization and methodology: Fernanda Macedo de Araujo Azeredo, Nicole Pereira de Souza Rocha, Cristina Moll Hüther; data collection and curation: Fernanda Macedo de Araujo Azeredo, Natália Bastos da Silva, Sophia Carvalho de Souza, Gabrielle Botelho Guimarães dos Santos; formal analysis: Fernanda Macedo de Araujo Azeredo, Nicole Pereira de Souza Rocha; data interpretation: Fernanda Macedo de Araujo Azeredo, Nicole Pereira de Souza Rocha, Cristina Moll Hüther; project administration: Fernanda Macedo de Araujo Azeredo; supervision: Cristina Moll Hüther; original draft preparation: Fernanda Macedo de Araujo Azeredo; writing-review and editing: Nicole Pereira de Souza Rocha, Cristina Moll Hüther, Natália Bastos da Silva, Sophia Carvalho de Souza, Gabrielle Botelho Guimarães dos Santos. All authors read and approved the final version of the manuscript.

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