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Biofertilizers improve growth rate, nitrate reductase activity, and productivity of shallot (*Allium cepa* L.) under drought stress

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ABSTRACT. Shallot (*Allium cepa* L.) is one of Indonesia's horticultural crops and represents as superior commodity. However, shallots are highly susceptible to drought and require sufficient water for growth. Biofertilizers contain various microbes that provide nutrients and increase the resistance of shallots to drought stress. This study aimed to determine the effect of biofertilizers on the growth rate, nitrate reductase activity, and productivity of shallot plants under drought stress. The research was conducted using a Factorial Completely Randomized Design with two factors. The first factor was the doses of biofertilizer with 0, 10, 15, and 20 L ha^{-1}. The second factor was the drought stress with treatments of 25, 50, 75, and 100% field capacity. Parameters measured included plant height rate, leaf number rate, number of tillers, tuber wet weight, tuber dry weight, and nitrate reductase activity. Data were analyzed by ANOVA using SPSS followed Duncan's Multiple Range Test (DMRT) at 5% significance level. The results showed that biofertilizer application did no significantly different on the growth rate, but significantly affected the number of tillers, tuber weight, and nitrate reductase activity of shallots under drought stress. The optimum dose of 10 L ha^{-1} biofertilizer increased the number of tillers (9.33). The optimum dose of 20 L ha^{-1} biofertilizer increased the growth rate, tuber wet weight (4.46 g), tuber dry weight (0.63 g), and nitrate reductase activity (1.11 μmol NO_2^- g^{-1} leaf wet weight h^{-1} of incubation). It is concluded that biofertilizer application, particularly at 10–20 L ha^{-1}, can improve shallot performance under drought stress and is recommended as a drought mitigation strategy in shallot cultivation.

Keywords: biofertilizer; drought stress; growth rate; nitrate reductase; shallots

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INTRODUCTION

Shallot (*Allium cepa* L.) is one of the horticultural plants in the Liliaceae family that is widely used as a seasoning (Wiyatiningsih *et al*., 2021; Alami *et al*., 2023). This plant has a relatively high nutritional content, containing sugar, carbohydrates, water, protein, vitamins, fiber, potassium, vitamin C, vitamin B6, and minerals (Deepthi *et al*., 2021; Chakraborty *et al*., 2022). This high nutrient content increases shallot production from year to year. However, this plant is highly susceptible to drought stress because it has shallow roots, so water absorption through the roots is limited (Polakitan *et al*., 2022; Rahmawati & Yasvi, 2024). Drought is an abiotic stress that causes the decline in shallot production so that adequate water availability is needed for its growth.

Drought can affect plant morphology, inhibit photosynthesis, and reduce shallot plant yield (Gedam *et al*., 2021; Sansan *et al*., 2024). Many studies prove drought stress affects plants' molecular physiology, morphology, and biochemistry (Bijalwan *et al*., 2022; Sansan *et al*., 2024). Research by Forotaghe *et al*. (2021) showed that water deficit significantly reduced shallot growth, yield, and affected shallot plants physiological and biochemical properties. Research by Ghodke *et al*. (2018) demonstrated that water deficit stress significantly reduces shallot yield. Drought also affected nitrate reductase activity, a key enzyme that catalyzes the reduction of nitrate to nitrite in nitrogen assimilation (Pagalla & Jannah, 2023). Nitrate reductase activity shows positive correlation with plant productivity, water insufficiency leads to decreased productivity and consequently reduced enzyme activity (Putra *et al*., 2020). Furthermore, limited water availability inhibits nitrogen transport, which further exacerbates the decline in nitrate reductase activity (Siswanti & Agustin, 2014). Similar findings by Gloser *et al*. (2020) revealed suppressed nitrate reductase activity in water-deficient leaves and roots of *Pisum sativum*, *Vicia faba*, and *Nicotiana tabacum* plants.

Biofertilizer is one way to increase shallot production and plant resistance to drought stress (Siswanti & Rachmawati, 2011; Anli *et al*., 2020; Ammar *et al*., 2023). Research by Siswanti & Rachmawati (2011) showed that adding biofertilizer to IR-64 rice plants with drought stress treatment can reduce plant stress. This biofertilizer is made from cow urine and contains various microorganisms such as *Streptomyces sp*., *Azotobacter* sp., *Bacillus* sp., *Saccharomyces* sp., *Lactobacillus sp*., *Azospirillum* sp., *Pseudomonas* sp., *Rhizobium* sp., and IAA hormone-producing bacteria that can support plant growth (Siswanti, 2015). These microorganisms colonize the rhizosphere and the inside of the plant, increasing nutrient availability and improving plant fertility (Jain, 2019; Tamiru, 2023). Demir *et al*. (2023) reported that adding biofertilizer positively affected growth parameters, yield, quality, and concentration in broccoli and lettuce plants. Research by Palupi & Siswanti (2023) also showed that biofertilizers can increase the growth of *Brassica juncea* L. Siswanti *et al*. (2022) also reported that the use of biofertilizers can affect the growth and productivity of Lurik peanut (*Arachis hypogaea* L. var. Lurikensis).

Biofertilizer is an effective strategy to support crop fertility, which is cost-effective and environmentally friendly compared to chemical fertilizers (Daniel *et al*., 2022; Ammar *et al*., 2023). The application of biofertilizers is likely to contribute to a sustainable agricultural economy and improve global food security. However, there had been no study exploring the formulation of cow urine-based biofertilizer specifically for shallots, especially under drought stress conditions. Previous studies on cow urine-based biofertilizers were still limited to major food crops such as rice (Siswanti & Rachmawati, 2011; Siswanti & Rachmawati, 2013) and broad-leaf horticultural crops such as spinach and chili (Siswanti & Lestari, 2019; Khairunnisa & Siswanti, 2021; Siswanti & Umah, 2021), but did not cover the physiological response of shallots. In fact, shallots were sensitive to water deficits, while the interaction between cow urine biofertilizers and their drought tolerance mechanisms had not been revealed. Therefore, this study aimed to investigate the effect of biofertilizer application on the growth rate, productivity, and nitrate reductase activity of shallot plants under drought stress. Application of biofertilizers has the potential to serve as an effective agronomic strategy for enhancing shallot tolerance to drought stress through the optimization of growth, yield, and plant physiological mechanisms.

MATERIALS AND METHODS

Plant material and experimental design. The research was conducted in Sawitsari greenhouse, Faculty of Biology for shallot planting and Biochemistry Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, from October 2023 to January 2024. Shallot seeds (Tajuk variety) were planted in polybags measuring 20 x 20 cm, with two seeds per polybag. The planting medium used was a mixture of paddy soil and manure in a ratio of 1:1. The research was conducted using a Factorial Completely Randomized Design with two factors, the first factor was the doses of biofertilizer (0, 10, 15, and 20 L ha^{-1}) and the second factor was the drought stress (25%, 50%, 75%, and 100% of field capacity), so that 16 treatment combinations were obtained. Each treatment was conducted in three replications. Parameters measured in this study were plant height rate, leaf number rate, nitrate reductase activity, number of tillers, and tuber biomass.

Biofertilizer and drought treatment. The biofertilizer used was a formula found by Siswanti (2015) with a mixture of cow or goat urine and microbial starter with a ratio of 49:1. The microbial starter consists of bacteria, such as *Bacillus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Streptomyces* sp., *Azospirillum* sp., *Pseudomonas* sp., *Azotobacter* sp., *Rhizobium* sp., and IAA hormone-producing bacteria. Biofertilizer was applied every ten days, starting seven days after planting (DAP) by watering it into the growing medium at the following doses: control, 10, 15, and 20 L ha^{-1}. All applications were applied before drought stress induction to avoid compromising the experimental stress conditions. Drought stress treatments were imposed by regulating irrigation to 25%, 50%, 75%, and 100% of field capacity. Drought stress is given after 7 DAP until harvest. The drought stress level was determined by measuring the field water capacity. The soil was watered until saturated, allowing excess water to drain out of the polybag. The drained water was collected and observed until no more droplets fell (indicating that field capacity was reached). The absorbed water volume was calculated using the formula V1 – V2, where V1 was the initial watering volume and V2 was the volume of drained water. The result of this calculation showed the water volume required to achieve 100% field capacity. This result was then used as a reference to determine watering volumes for 25%, 50%, and 75% field capacity (Devy & Nawfetrias, 2012).

Growth rate and productivity measurements. The growth rate was measured every ten days after two weeks of treatment, with five observations taken until harvest. Plant height was measured using a ruler, while the number of leaves was counted manually. The plant productivity is calculated as the number of tillers, wet weight, and dry weight of tubers. The number of tillers was determined by counting the total number of tillers growing around the shallot plants. The number of tillers was counted every ten days after 7 DAP. The wet weight and dry weight of tubers were measured after harvest by weighing using an analytical scale.

Nitrate reductase activity measurements. Nitrate reductase activity (NRA) was measured by picking the third leaf of shallot plants between 09.00-10.00 as an observation sample, with three repetitions for each treatment. The leaves were finely sliced, taken as much as 200 mg, then put into a buffer solution of $Na_2HPO_4.2H_2O$ and $NaH_2PO_4.2H_2O$ at pH 7.5 with a volume of 5 mL each in a dark tube and closed, then soaked for 24 hours. After that, the buffer solution was discarded and replaced with a new buffer solution of 5 mL, then 0.1 mL of 5 M NaNO3 was added to each tube and incubated for two hours. The filtrate of 0.1 mL was taken and put into a test tube filled with 0.2 mL of 1% sulfanilamide reagent dissolved in 3 N HCl and 0.2 mL of 0.02% napthylethylendiamide solution. Aquadest was then added to as much as 2.5 mL. The solution was then measured for absorbance using a spectrophotometer at 540 nm (Ende *et al*., 2022). The formula for calculating nitrate reductase activity is as follows (Affifah & Siswanti, 2022):

$$\text{Nitrate Reductase Activity (NRA)} = \text{NO}_2\text{– } (\mu\text{mol}) \times \frac{5\text{ mL}}{0.1\text{ mL}} \times \frac{1000\text{ mL}}{200\text{ mg}} \times \frac{60\text{ minutes}}{\text{incubation time (minutes)}}$$

Data analysis. Data were analyzed using SPSS ver. 26 with ANOVA. If there was a significant effect, it was continued with the Duncan Multiple Range Test (DMRT) at the 95% confidence level (α = 0.05%).

RESULTS AND DISCUSSION

Plant Growth Rate. The growth rate in this study was described by the increase in plant height and number of leaves measured every ten days. The rate of plant height and number of leaves with various doses of biofertilizer and drought stress are presented in Fig. 1 and Fig. 2. Based on Fig. 1 and Fig. 2, the growth pattern of plant height and number of leaves of shallot plants at the age of 7 to 47 days after planting (DAP) follows a bell-shaped curve. This is characterized by the plant growth rate, which initially slows down, then increases and over time decreases until senescence. In this study, the growth rate of plant height and leaf number was expressed as an increase in the height and number of leaves per unit time. A decline in growth rate only indicated that the increase in the height or number of leaves per unit time became smaller, not an actual reduction in height or the number of leaves already formed. At the beginning of vegetative growth, biofertilizer application had not been able to increase the plant growth rate significantly (Table 1). According to Siswanti & Rachmawati (2011), at the beginning of growth, biofertilizer does not directly provide nutrients for plant growth because microbes in biofertilizer need sufficient time to break down organic matter in the soil into nutrients available for shallots. The biofertilizer application produced a rapid plant growth rate at 17 DAP when plant growth reached its maximum point. Biofertilizer contains various types of microorganisms that provide N, P, and K elements as well as phytohormones to increase the growth rate of shallot plants (Allouzi *et al*., 2022; Chaudhary *et al*., 2022; Kumar *et al*., 2022). Nutrients, especially nitrogen (N), play a role in spurring plant growth, especially in the vegetative phase (Leghari *et al*., 2016; Sabur *et al*., 2021).

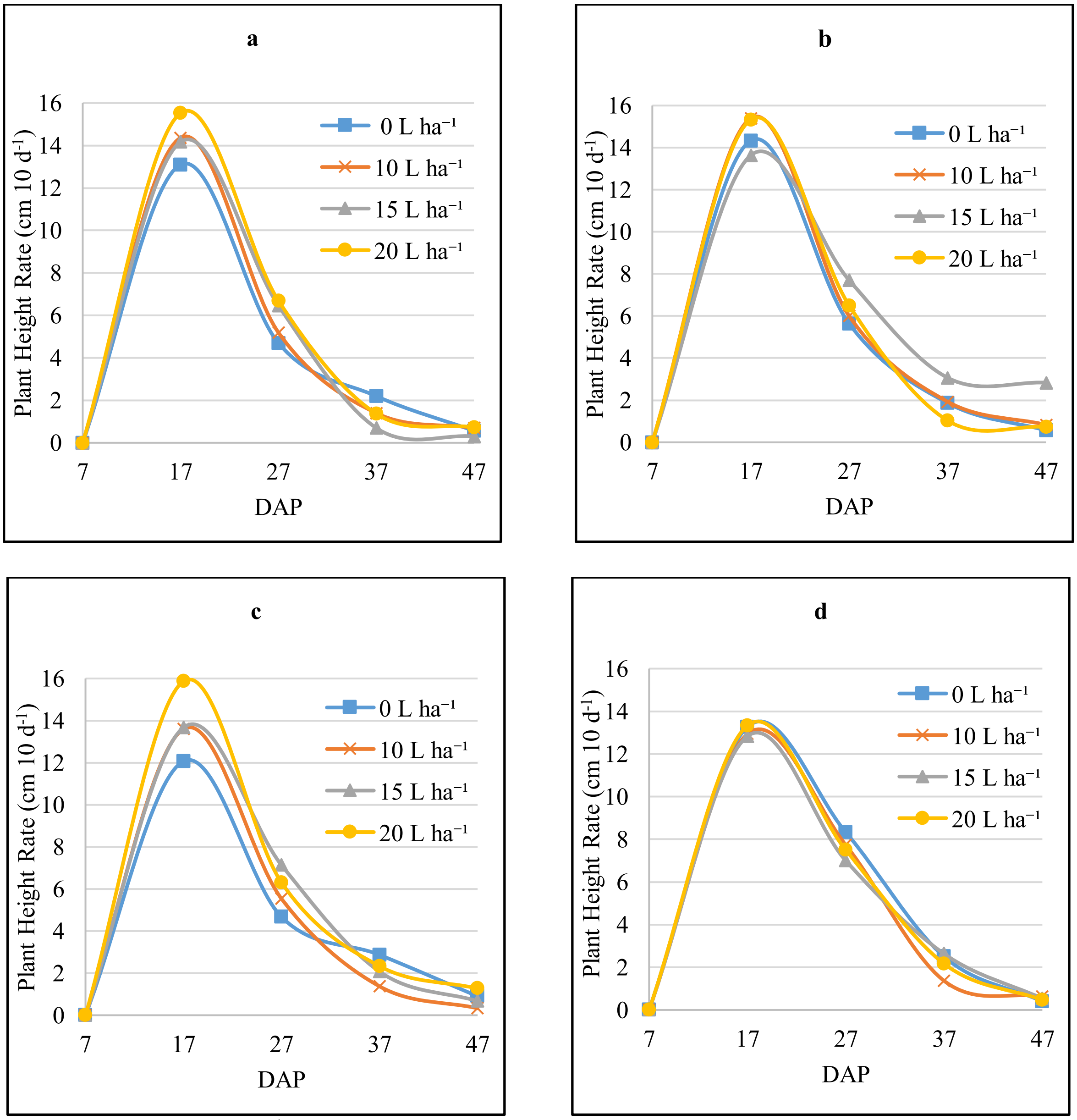

Fig. 1. Plant height rate (cm 10 d^{-1}) of shallot plants in biofertilizer treatments under drought stress: a. Control (no stress); b. 75% field capacity; c. 50% field capacity; d. 25% field capacity. DAP: Days After Planting

Meanwhile, during the generative phase, there was no significant increase in the variation of biofertilizer dosage (Table 1). Siswanti *et al*. (2019) stated that plants that have entered the generative phase optimize nutrients from biofertilizers for forming reproductive organs, so these nutrients are not fully utilized for plant vegetative growth. In general, the biofertilizer treatment dose of 20 L ha^{-1} tends to increase the plant growth rate optimally, both the height and number of leaves of shallot plants under drought stress in the vegetative phase. This result is based on research by Siswanti & Umah (2021), where the biofertilizer dose of 20 L ha^{-1} increased the plant height and the number of leaves of *Amaranthus tricolor* L. The same results were shown in the research of Palupi & Siswanti (2023), which examined the plant height and number of leaves of *Brassica juncea* L., showed the highest results after biofertilizer application in hydroponic systems under salinity stress conditions.

Meanwhile, the drought stress treatment reduced the plant growth rate, which was indicated by the growth rate at 25% field capacity (Fig. 1d & 2d) lower than the 100% (Fig. 1a & 2a), 75% (Fig. 1b & 2b), and 50% (Fig. 1c & 2c) field capacity treatments. Low water content will reduce turgor pressure, which causes the process of cell elongation and enlargement to be disrupted, ultimately resulting in a decreased growth rate (Manurung *et al*., 2019; Wach & Skowron, 2022).

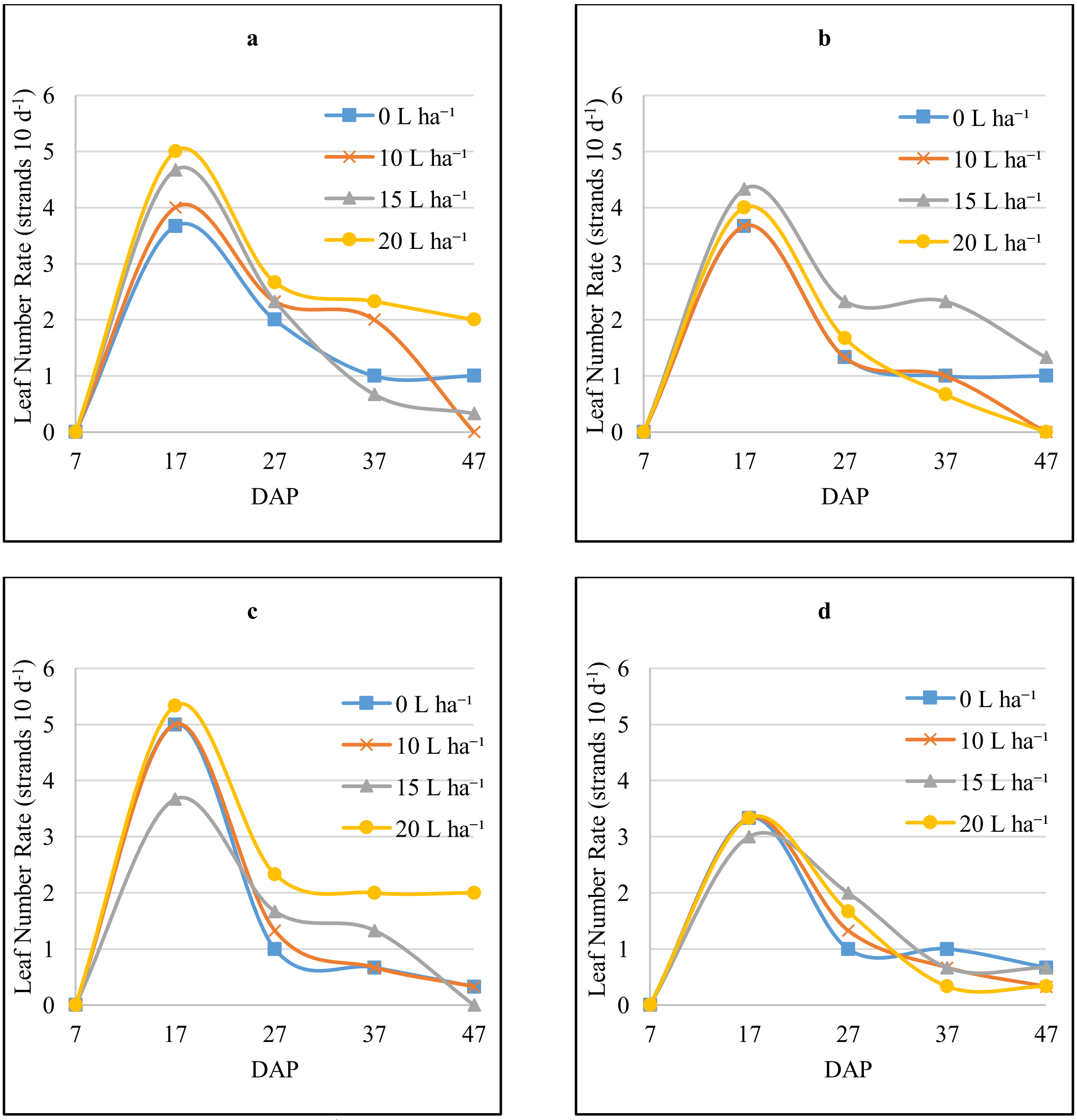

Fig. 2. Leaf number rate (strands 10 d^{-1}) of shallot plants in biofertilizer treatments under drought stress: a. Control (no stress); b. 75% field capacity; c. 50% field capacity; d. 25% field capacity. DAP: Days After Planting

Table 1. Mean plant height rate and leaf number rate of shallot (*A. cepa* L.) with various doses of biofertilizer under drought stress

Treatment	Plant Height Rate (cm 10 d^{-1})				Leaf Number Rate (strands 10 d^{-1})			
Biofertilizer (B)	DAP				DAP			
	17	27	37	47	17	27	37	47
0 L ha^{-1}	13.19^a	5.83^a	2.36^a	0.61^a	3.92^a	1.33^a	0.92^a	0.75^a
10 L ha^{-1}	14.08^a	6.12^a	1.52^a	0.63^a	4.00^a	1.58^a	1.08^a	0.17^a
15 L ha^{-1}	13.58^a	7.08^a	2.12^a	1.08^a	3.92^a	2.08^a	1.25^a	0.58^a
20 L ha^{-1}	15.02^a	6.75^a	1.73^a	0.80^a	4.42^a	2.08^a	1.33^a	1.08^a

Field Capacity (FC)								
100%	14.29^{a}	5.77^{a}	1.42^{a}	0.58^{b}	4.33^{a}	2.33^{a}	1.50^{a}	0.83^{a}
75%	14.68^{a}	6.46^{a}	1.98^{a}	1.24^{a}	3.92^{a}	1.67^{a}	1.25^{a}	0.58^{a}
50%	13.80^{a}	5.91^{a}	2.16^{a}	0.79^{ab}	4.75^{a}	1.58^{a}	1.17^{a}	0.67^{a}
25%	13.09^{a}	7.64^{a}	2.17^{a}	0.51^{b}	3.25^{a}	1.50^{a}	0.67^{a}	0.50^{a}
Interaction (B × FC)	ns	ns	ns	*	ns	ns	ns	ns

Notes: Mean values followed by different letters within a column are significantly different between treatments (DMRT test: $p<0.05$). (ns: not significant, (*): significant at $p<0.05$)

Number of tillers. The number of tillers of shallot plants with biofertilizer treatment and drought stress was presented in Table 2.

Table 2. Number of tillers of shallot (*A. cepa* L.) with various doses of biofertilizer under drought stress for 67 DAP

Field Capacity (%)	Biofertilizer (L ha^{-1}) 0	10	15	20	Average
100	7.33 ± 1.53^{cde}	8.67 ± 1.53^{de}	9.00 ± 1.73^{e}	8.33 ± 1.53^{cde}	8.33 ± 1.50^{y}
75	6.33 ± 0.58^{bcd}	7.67 ± 0.58^{cde}	8.33 ± 1.15^{cde}	8.33 ± 0.58^{cde}	7.67 ± 1.07^{y}
50	4.33 ± 1.53^{ab}	**9.33 ± 1.53^{e}**	8.00 ± 1.00^{cde}	9.00 ± 1.00^{e}	7.67 ± 2.35^{y}
25	7.33 ± 1.53^{cde}	7.00 ± 1.73^{cde}	6.00 ± 0.00^{bc}	3.67 ± 1.15^{a}	6.00 ± 1.86^{x}
Average	6.33 ± 1.72^{o}	**8.17 ± 1.53^{p}**	7.83 ± 1.53^{p}	7.33 ± 2.43^{op}	

Notes: Mean values followed by different letters within a column or row are significantly different between treatments (DMRT test: $p<0.05$)

Based on the results of ANOVA analysis, biofertilizer dose treatment showed a significant effect ($p<0.05$) on the number of shallot tillers under drought stress (Table 2). The results showed that the biofertilizer treatment produced more tillers than the treatment without biofertilizer application (control). The highest number of tillers was showed by biofertilizer treatment with a dose of 10 L ha^{-1} at 50% drought stress. Biofertilizers contain various microbes that can decompose organic compounds into nutrients that plants can absorb. Nitrogen in biofertilizers can increase the vegetative formation of plants, such as the number of tillers. If the vegetative growth of plants is good, the number of tillers will also increase (Marlina *et al*., 2018). In general, the higher the dose of biofertilizer, the lower the number of tillers, with a dose of 10 L ha^{-1} producing the highest number of tillers. However, this dose resulted in a number of tillers that were not significantly different from the doses of 15 and 20 L ha^{-1}. This showed that even lower doses can increase the number of tillers similar to the doses of 15 and 20 L ha^{-1}. Doses that are too high contain more microbes, so there is competition between microbes in the rhizosphere area, which causes less nutrient uptake by plants (Siswanti *et al*., 2018; Riesty & Siswanti, 2021). Previous research by Siswanti & Rachmawati (2013) also showed that three varieties of rice plants produced the optimal tiller growth at a biofertilizer dose of 10 L ha^{-1}.

Based on Table 2, drought stress reduced the number of tillers, whereas the 25% field capacity treatment produced fewer tillers and significantly differed from the other treatments. The higher the drought stress, the lower the number of tillers. Tiller number was significantly influenced by the interaction between biofertilizer and drought stress. Biofertilizer effectiveness was highly dependent on soil water availability. Under severe drought stress (25% field capacity), tiller numbers decreased across all biofertilizer dose treatments compared to the control. In contrast, under moderate drought and non-stress conditions (50-100% field capacity), no significant differences in tiller numbers were observed between biofertilizer treatments. Notably, the 10 L ha^{-1} treatment at 50% field capacity did not show significant improvement over the control (without stress). These results demonstrate that biofertilizers can effectively increase tiller production even at 50% drought stress. For efficient irrigation under drought conditions, we recommend maintaining 50% field capacity water supply combined with biofertilizer application. Drought stress disrupts the microbial activity of biofertilizers in absorbing water so that the nutrients obtained by plants are less for the photosynthesis process. This impacts the amount of photosynthate that will be translocated to plant organs, which is also low, so the number of tillers formed is small (Istiqomah *et al*., 2016; Singh *et al*., 2018).

Tuber biomass. The tuber biomass measured in this study was the wet and dry weight of tubers. The results of the measurement of tuber biomass in the treatment of various doses of biofertilizer under drought stress were presented in Table 3.

Table 3. Tuber wet weight and dry weight of shallot (*A. cepa* L.) with various doses of biofertilizer under drought stress

Variable	Field Capacity (%)	Biofertilizer (L ha^{-1}) 0	10	15	20	Average
Tuber wet weight (gram)	100	2.55 ± 0.34^{bcde}	4.34 ± 0.29^{f}	2.51 ± 0.42^{bcde}	2.97 ± 0.63^{cde}	3.09 ± 0.86^{y}
	75	2.57 ± 0.82^{bcde}	3.60 ± 1.15^{ef}	2.77 ± 0.19^{bcde}	2.90 ± 0.78^{cde}	2.96 ± 0.80^{y}
	50	1.63 ± 0.97^{ab}	3.27 ± 0.28^{de}	2.86 ± 0.51^{cde}	$\mathbf{4.46 \pm 0.74^{f}}$	3.05 ± 1.20^{y}
	25	2.05 ± 0.62^{abc}	1.39 ± 0.25^{a}	2.36 ± 0.17^{abcd}	2.87 ± 0.40^{cde}	2.17 ± 0.65^{x}
	Average	2.20 ± 0.74^{o}	3.15 ± 1.25^{p}	2.63 ± 0.37^{o}	$\mathbf{3.30 \pm 0.90^{p}}$	
Tuber dry weight (gram)	100	0.28 ± 0.03^{abcd}	0.59 ± 0.05^{f}	0.37 ± 0.14^{cde}	0.38 ± 0.09^{cde}	0.41 ± 0.14^{y}
	75	0.29 ± 0.07^{abcd}	0.41 ± 0.11^{de}	0.36 ± 0.02^{cde}	0.38 ± 0.14^{cde}	0.36 ± 0.09^{y}
	50	0.21 ± 0.11^{ab}	0.44 ± 0.03^{e}	0.31 ± 0.07^{bcde}	$\mathbf{0.63 \pm 0.07^{f}}$	0.40 ± 0.18^{y}
	25	0.25 ± 0.02^{abc}	0.17 ± 0.02^{a}	0.36 ± 0.04^{cde}	0.31 ± 0.04^{cde}	0.27 ± 0.08^{x}
	Average	0.26 ± 0.06^{o}	0.40 ± 0.17^{pq}	0.35 ± 0.07^{p}	$\mathbf{0.43 \pm 0.15^{q}}$	

Notes: Mean values followed by different letters within a column or row are significantly different between treatments (DMRT test: $p<0.05$)

Based on Table 3, it is known that the application of biofertilizer showed a significant effect on the wet weight and dry weight of tubers according to the DMRT test ($\alpha=0.05$). Biofertilizer treatment produced a higher wet and dry weight of tubers than those without biofertilizer (0 L ha^{-1}). The highest wet weight (4.46 g) and dry weight (0.63 g) of tubers were in the treatment of a biofertilizer dose of 20 L ha^{-1} at 50% drought stress. Biofertilizer contains nutrients needed for the photosynthesis process. The phosphorus element in biofertilizers plays an essential role in tuber formation (Fernandes *et al*., 2014; Gitari *et al*., 2018; Aarakit *et al*., 2021). In addition, phosphate-solubilizing bacteria such as *Pseudomonas* sp. and *Bacillus* sp. in biofertilizers also play a role in dissolving phosphate so that plants can absorb it and support the formation and enlargement of tubers (Siagian *et al*., 2019; Mayadunna *et al*., 2023). Previous research by Saharuddin *et al*. (2018) showed that tubers' wet and dry weight in three varieties of shallot plants increased after biofertilizer application compared to without biofertilizer. Research by Khairunnisa & Siswanti (2021) also reported that biofertilizers could increase the productivity of *Amaranthus tricolor* L. plants under salinity stress.

Wet and dry weight of tubers tended to increase with increasing biofertilizer doses, with a dose of 20 L ha^{-1} showing the highest results (Table 3). However, these results showed no significant difference with the 10 L ha^{-1} dose treatment. This means that the dose of 10 L ha^{-1} alone can increase the wet weight and dry weight of tubers. The 20 L ha^{-1} dose treatment produced the highest tuber weight because the microbes in the 10 and 15 L ha^{-1} doses contained fewer nutrients than the 20 L ha^{-1} dose, resulting in less photosynthate for tuber formation. Table 3 also shows that drought stress reduced tuber biomass. Tubers' wet and dry weights at 25% field capacity showed the lowest results and significantly differed from other treatments. Meanwhile, the field capacity of 50, 75, and 100% were not significantly different on the wet weight and dry weight of tubers. These results indicated that drought stress reduced tubers' wet and dry weight only at 75% drought stress (25% field capacity). The 10 L ha^{-1} treatment at 50% field capacity increased tuber wet weight by 1.64 g and dry weight by 0.23 g, indicating improved drought resistance. The availability of sufficient water supports microbial activity and metabolism to provide nutrients and support the photosynthesis process optimally. Drought stress inhibits photosynthesis, thus reducing tuber weight (Hafizh *et al*., 2021; Zaki & Radwan, 2022).

Nitrate reductase activity. The measurement results of nitrate reductase activity of shallot plants treated with various doses of biofertilizer under drought stress are presented in Table 4.

Table 4. Nitrate reductase activity (μmol NO_2^-/gram leaf wet weight/hour of incubation) of shallot (*A. cepa* L.) with various doses of biofertilizer under drought stress

Field Capacity (%)	Biofertilizer (L ha^{-1}) 0	10	15	20	Average
100	0.78 ± 0.03^{b}	0.80 ± 0.00^{b}	0.84 ± 0.05^{bc}	1.08 ± 0.02^{d}	0.88 ± 0.13^{y}
75	0.80 ± 0.02^{b}	0.86 ± 0.01^{bc}	0.95 ± 0.05^{c}	0.93 ± 0.09^{c}	0.88 ± 0.08^{y}
50	1.09 ± 0.14^{d}	0.93 ± 0.03^{c}	1.09 ± 0.10^{d}	$\mathbf{1.11 \pm 0.08^{d}}$	1.05 ± 1.11^{z}
25	0.85 ± 0.05^{bc}	0.61 ± 0.05^{a}	0.83 ± 0.07^{bc}	0.94 ± 0.01^{c}	0.81 ± 0.14^{x}
Average	0.88 ± 0.14^{p}	0.80 ± 0.13^{o}	0.93 ± 0.12^{q}	$\mathbf{1.02 \pm 0.10^{r}}$	

Notes: Mean values followed by different letters within a column or row are significantly different between treatments (DMRT test: $p<0.05$)

The results of the ANOVA analysis showed that the biofertilizer dose treatment significantly affected the nitrate reductase activity (NRA) of shallots under drought stress (Table 4). Biofertilizer treatment increased NRA compared to the treatment without biofertilizer, where all biofertilizer dose treatments showed significantly different results. The biofertilizer dose treatment of 20 L ha^{-1} with 50% field capacity produced the highest nitrate reductase activity (1.11 μmol NO_2^- g^{-1} leaf wet weight/hour of incubation). The higher the biofertilizer dose, the higher the nitrate reductase activity with a dose of 20 L ha^{-1} showing the highest results. Nitrate reductase is an enzyme that plays a role in catalyzing the reduction of nitrate to nitrite (Fu *et al*., 2018; Pagalla & Jannah, 2023). This enzyme reduces nitrate to nitrite and requires several components, including nitrate concentration (Kishorekumar *et al*., 2020). Biofertilizer contains nitrogen-fixing bacteria that can help provide nitrate (Sapalina *et al*., 2022). Nitrate is a substrate for the enzyme nitrate reductase to reduce nitrate to nitrite. Biofertilizer fertilization can increase the activity of nitrate reductase. Nitrogen levels increase as nitrate reductase activity increases (Chen & Huang, 2019). Biofertilizer dose of 20 L ha^{-1} produced the highest NRA because it contains more microbes that provide nutrients, especially nitrogen. Low biofertilizer doses contain fewer microbes, so microbial activity is lower in providing nutrients (Nunilahwati *et al*., 2022). Previous research by Affifah & Siswanti (2022) reported that a biofertilizer dose of 20 L ha^{-1} could increase NRA under salinity stress. Research by Soliman & Hamza (2016) also reported that biofertilizers could increase NRA in soybean plants.

However, the biofertilizer dose of 10 L ha^{-1} produced lower nitrate reductase activity than the treatment without biofertilizer (control). The result is possible because the control treatment, although not fertilized, still contains manure and paddy soil containing macro and microelements that increase nitrate reductase activity. Meanwhile, the 10 L ha^{-1} dose treatment also had the same paddy soil condition as the control. Thus, the 10 L ha^{-1} dose treatment contains more microbes from adding biofertilizer, so there is competition between existing microbes in the media and those newly given (Siswanti *et al*., 2018). This causes the nitrate reductase activity produced in the 10 L ha^{-1} dose treatment to be lower. Nitrate reductase activity positively correlates with plant productivity (Putra *et al*., 2020; Mapegau *et al*., 2023). This can be shown in the biofertilizer treatment, which has a higher tuber biomass than the treatment without biofertilizer (Table 3). High nitrate reductase activity will produce high tuber biomass as well.

Nitrate reductase activity is influenced by water availability (Sun *et al*., 2015; Putra *et al*., 2020; Ende *et al*., 2022). Drought stress reduces nitrate reductase activity (Table 4). The 25% field capacity treatment with biofertilizer fertilization produced the lowest NRA and significantly differed from the other treatments. The 75 and 100% field capacity treatments (control) produced an NRA that was not significantly different (Table 4). These results indicate that at 25% drought stress (75% field capacity), biofertilizer maintained nitrate reductase activity similar to the control (without drought stress). Drought stress can reduce nitrate reductase activity related to photosynthesis (Putra *et al*., 2020; Ende *et al*., 2022). Reducing nitrate to nitrite requires electrons from NADH or NADPH, which results from the bright reaction of photosynthesis (Putra *et al*., 2020; Prado, 2021). In addition, low water availability inhibits nitrogen transport, which has an impact on reducing nitrate reductase activity (Siswanti & Agustin, 2014).

CONCLUSION

The application of biofertilizers did not significantly affect the growth rate, but significantly influenced both productivity and nitrate reductase activity in drought stressed shallot plants. The application of biofertilizer at a dose of 10 L ha^{-1} is the most optimum dose to increase the number of tillers, while the dose of 20 L ha^{-1} is the most optimum to increase the growth rate, tuber wet weight, tuber dry weight, and nitrate reductase activity of shallot plants.

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