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The Effect of Biofertilizer under Drought: Vitamin C, Starch Content, Biomass and Root Anatomy of Shallot (*Allium cepa* L.)

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ABSTRACT

Shallots (*Allium cepa* L.) is a plant that requires sufficient water and is susceptible to drought stress. The use of biofertilizer is employed because it contains microorganisms to enhance nutrient availability and assist plant growth under abiotic stress. The biofertilizer used in this study is the Siswanti formula (EnWie Biofertilizer) which contains *Bacillus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Streptomyces* sp., *Pseudomonas*, *Azospirillum*, *Azotobacter, Rhizobium* and *Trichoderma* sp. This study aims to analyze the physiological and anatomical responses of shallot bulbs following the application of biofertilizer under drought stress. The doses of biofertilizer used were 10 L/ha, 15 L/ha, and 20 L/ha with field capacity levels of 25%, 50%, 75%, and 100%. Parameters tested included vitamin C content, starch content, cortex thickness anatomy and dry weight of shallot roots. The method used to determine the vitamin C content was UV-VIS spectrophotometry, while the starch content research used the Nelson-Smogy method. The method used in making shallot root specimens was the embedding method. The results of this study showed that the application of biofertilizer affects the physiology and anatomy of *Allium cepa* L. under drought stress. The application of 15 L/ha biofertilizer increases vitamin C content, starch content, and cortex thickness. Meanwhile, 10 L/ha biofertilizer increases root dry weight.

Keywords: Biofertilizer; Drought stress; Shallots; Spectrophotometry

INTRODUCTION

Shallot (*Allium cepa* L.) is a perennial herbaceous plant from the Amaryllidaceae family that grows in various parts of the world. Medicinal plants have long been used as therapeutic drugs, giving them commercial value. Various plant varieties have been studied, analyzed, and characterized for their medicinal value based on their biological compounds (Dogara *et al*., 2022). Shallots can be considered a horticultural commodity for traditional medicine because they are widely used as antimicrobial agents and show anticancer, antidiabetic, antioxidant, antiplatelet, antihypertensive, antidepressant, anti-inflammatory, and antiparasitic effects (Renanda & Astuti, 2024). Shallots contain derivative antimicrobial compounds that can inhibit microorganisms such as bacteria, viruses, fungi, and parasites (Maldovan *et al*., 2022) Shallots can prevent cancer development because they contain therapeutic agents *(Jumi et al*., 2023). Additionally, shallots are beneficial for asthma as they contain quercetin and prostaglandins (Hanwate et al., 2024)

Shallot plants have a shallow root system and are vulnerable to drought, so adequate irrigation is needed to maintain plant growth (Sansan et al., 2024). Drought stress in shallots will disrupt the plant's physiological and biochemical processes (Seleiman *et al*., 2021). Drought is one of the most prominent abiotic environmental factors that affects plant growth and secondary metabolite content (Gana et al., 2022). Shallots contain various secondary metabolites such as flavonoids, tannins, saponins, essential oils, kaempferol, flavonglycosides, phloroglucinol, dihydroalliin, cycloalliin, methylalliin, quercetin, polyphenols, and sulfur in the bulb (Setiawan et al., 2021). One of the secondary metabolites in shallots is Vitamin C (Akram et al., 2017).

Vitamin C, or ascorbic acid (AsA), is the most abundant antioxidant in plant tissues and can directly protect cells from oxidative stress caused by ROS through the detoxification of oxidation products generated by abiotic stress (Akram *et al*., 2017). Drought conditions will affect the biosynthesis of vitamin C. According to research by Mugwanya *et al.* (2025), the vitamin C content in shallot bulbs decreased by 70% and 40% under water deficit conditions of 40% to 52%. Additionally, a study by Seminario *et al.* (2017) found a decrease in vitamin C content in soybean and wheat plants due to drought conditions. Drought is a form of abiotic stress that can affect plant physiology (Siswanti & Riesty Okky, 2021).

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Shallot plants have a shallow root system and are vulnerable to drought, so adequate irrigation is needed to maintain plant growth (Sansan *et al.*, 2024).

The application of biofertilizer can be a solution to cope with drought stress in plants because it can enhance growth under abiotic stress and improve the physical, chemical, and biological properties of soil affected by the use of chemical fertilizers (Pangestuti & Siswanti, 2021) and increase chlorophyll content and the growth rate and of spinachunder abiotic stress (Siswanti & Riesty, 2021). Biofertilizer is an organic fertilizer that contains microorganisms to provide nitrogen and essential nutrients for plants (Siswanti & Khairunnisa, 2021). The biofertilizer used in this study is the Siswanti formula (EnWie Biofertilizer) which contains *Bacillus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Streptomyces* sp., *Pseudomonas*, *Azospirillum*, *Azotobacter, Rhizobium* (Siswanti & Umah, 2021) and *Trichoderma* sp. (Siswanti, 2015). These nine microorganisms are nitrogen-fixing, phosphate-solubilizing, organic matter-decomposing, and growth-regulating substance-producing microbes (Khairunnisa & Siswanti, 2022). The application of 10 L/ha of biofertilizer showed the best conditions in terms of the number of leaves, plant height, and fresh weight of shallot plants (Pakpahan *et al.*, 2022) and increase the metaxylem diameter of *Amaranthus tricolor* L. stem. (Riesty & Siswanti, 2021). Meanwhile, research by Firmansyah *et al.* (2015) found that 50 Kg/ha biofertilizer provided the highest dry bulb weight yield in shallot plants.

Vitamin C is often used as a general standard in the analysis of secondary metabolites because of its easily measurable chemical properties. The analysis of secondary metabolites, such as vitamin C, serves as an indicator to ensure accuracy and validity. Vitamin C has relatively stable chemical stability, is easy to handle, possesses good antioxidant properties, and is frequently used in studies involving oxidation and reduction. Moreover, there are many well-established and standardized methods for measuring vitamin C, such as titration, spectrophotometry, and chromatography. Based on this background, research on the effect of biofertilizer under drought stress conditions on shallot (*Allium cepa* L.) has not been extensively conducted. Therefore, this study aims to investigate the effect of biofertilizer application on vitamin C, starch content, biomass and root anatomy of shallot (*Allium cepa* L.) under drought stress.

MATERIALS AND METHODS

Materials

The materials used in the research are as follows: for the vitamin C content test, materials required include shallot bulbs, ascorbic acid, and distilled water. For the starch content test, materials required include shallot bulbs, distilled water, anhydrous glucose, Nelson reagents A and B (25:1), and arsenomolybdate reagent. For the preparation of cross-sectional slides of shallot roots, materials required include shallot roots, FAA solution (5% formalin, 5% glacial acetic acid, and 70% alcohol), 80% alcohol, 95% alcohol, 100% alcohol, alcohol/xylene (3:1), alcohol/xylene (1:1), alcohol/xylene (1:3), xylene, xylene/paraffin (1:9), pure paraffin, glycerin, albumin, 1% safranin, and Canada balsam.

Methods

The equipment used for the following in the testing of vitamin C content requires instruments such as the Pa224 Ohaus analytical balance and the Genesys 150 UV-Vis Spectrophotometer Thermo Scientific. For starch content testing, the required instrument is the Genesys 150 UV-Vis Spectrophotometer Thermo Scientific. In the preparation of cross-sectional preparations of shallot roots, equipment such as the Olympus CX23LEDRFS binocular microscope, optilab advance V2 optical camera, and Histo Core Leica rotary microtome are necessary. Subsequently, the equipment used for measuring the dry weight of shallots includes the Pa224 Ohaus analytical balance and an oven.

Research Design

The following research design is used:

Table 1. Research Design

Explanation:

The variations in biofertilizer concentration used are as follows:

A1: Biofertilizer at a dose of 0 L/ha (control); A2: Biofertilizer at a dose of 10 L/ha; A3: Biofertilizer at a dose of 15 L/ha; A4: Biofertilizer at a dose of 20 L/ha

The variations in drought stress levels used are as follows:

B1: Without drought stress (0%) (with 100% field capacity); B2: 75% field capacity; B3: 50% field capacity; B4: 25% field capacity

Table I. Research Design

Field Capacity	**Biofertilizer Dosage**			
	A1 (0 L/ha)	**A2 (10 L/ha)**	**A3 (15 L/ha)**	**A4 (20 L/ha)**
B1 (100%)	A1B1	A2B1	A3B1	A4B1
B2 (75%)	A1B2	A2B2	A3B2	A4B2
B3 (50%)	A1B3	A2B3	A3B3	A4B3
B4 (25%)	A1B4	A2B4	A3B4	A4B4

Vitamin C Content Test

Preparation of Vitamin C Stock Solution 100 ppm

Ascorbic acid is weighed as much as 50 mg and placed in a 500 mL volumetric flask, then dissolved to the mark.

Preparation of Calibration Curve Solution

A number of 100 ppm vitamin C solution is pipetted and placed in a 100 mL volumetric flask with concentrations of 1, 3, 5, 7, 9, and 11 ppm respectively, then topped up with distilled water to the mark.

Determination of Maximum Wavelength and Measurement of Calibration Curve Solution

Vitamin C solution concentrations of 1, 3, 5, 7, 9, and 11 ppm are measured for maximum wavelength within the absorbance range of 200-400 nm with distilled water as the blank. Subsequently, the absorbance results for each concentration at the maximum wavelength are used to create a calibration curve and calculate the linear regression equation.

Determination of Vitamin C Content in Red Onion Bulbs

The samples that is 0.5 grams of red onion bulbs are cut and ground. The sample is added to 10 mL and filtered with filter paper. One mL of the filtered solution is pipetted and placed in a 10 mL volumetric flask and topped up with distilled water to the mark. The sample solution is then measured for absorbance using the linear regression equation. The data obtained were further analyzed using the formula below,

$$\text{Vitamin C content} = \frac{x\left(\frac{mg}{L}\right)\times volume\ (L)\times fp}{sample\ weight\ (mg)} \times 100$$

(Rahmawati *et al.*, 2022)

Starch Content of Shallots

Ten mg of anhydrous glucose is dissolved in 100 mL of water. Dilutions are made to obtain concentrations of 2, 4, 6, 8, and 20 ppm. Each diluted solution is filled with 1 mL of standard glucose, and one tube is filled with 1 mL of water as a blank. Then, Nelson-Smogyi reagent is added. The reaction tubes are boiled and then cooled to room temperature (25°C). After cooling, 1 mL of arsenomolybdate solution is added, mixed until homogeneous, then 7 mL of distilled water is added and mixed until homogeneous. The absorbance is measured using a UV-visible spectrophotometer with a wavelength of 540 nm.

A number of 1.25 grams of red onion bulbs are ground and added to 20 mL of water, then filtered with filter paper. Subsequently, 1 mL of the filtered solution is added to 50 mL and topped up with water to the mark. Then, 1 mL of the sample solution is taken and 1 mL of Nelson-Smogyi reagent is added. The same procedure as for the preparation of the glucose standard curve is followed thereafter. The data obtained were further analyzed using the formula below.

$$\text{Reducing sugar} = \frac{\frac{mg}{L}\,curve \times fp}{sample\ weight(gr)\times 1000} \times 100\%$$

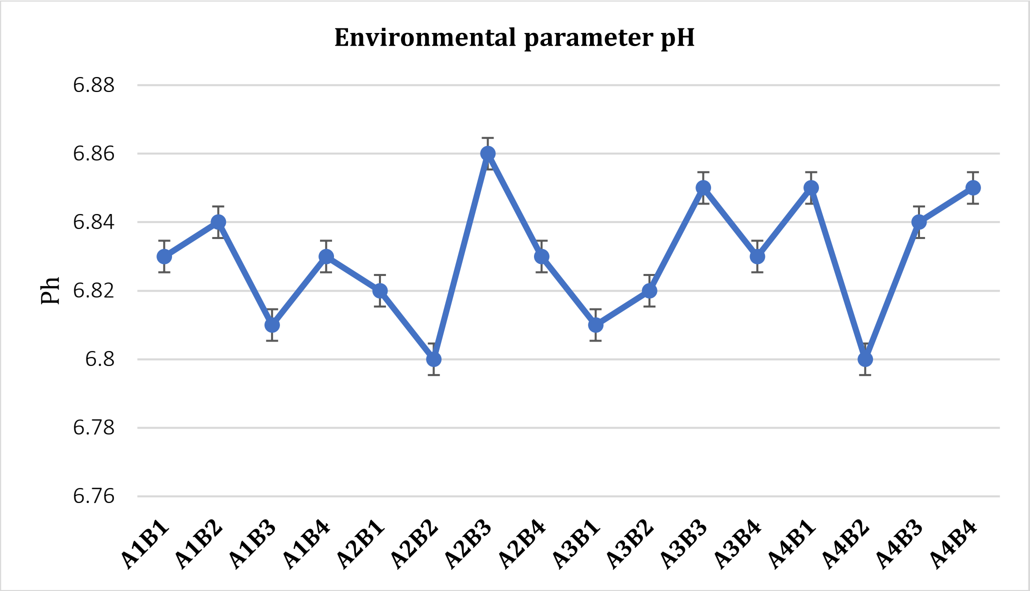
Starch Content: % Reducing Sugar × 0.9 (Ardiansyah *et al.*, 2018)

Dry Weight of Shallot Roots

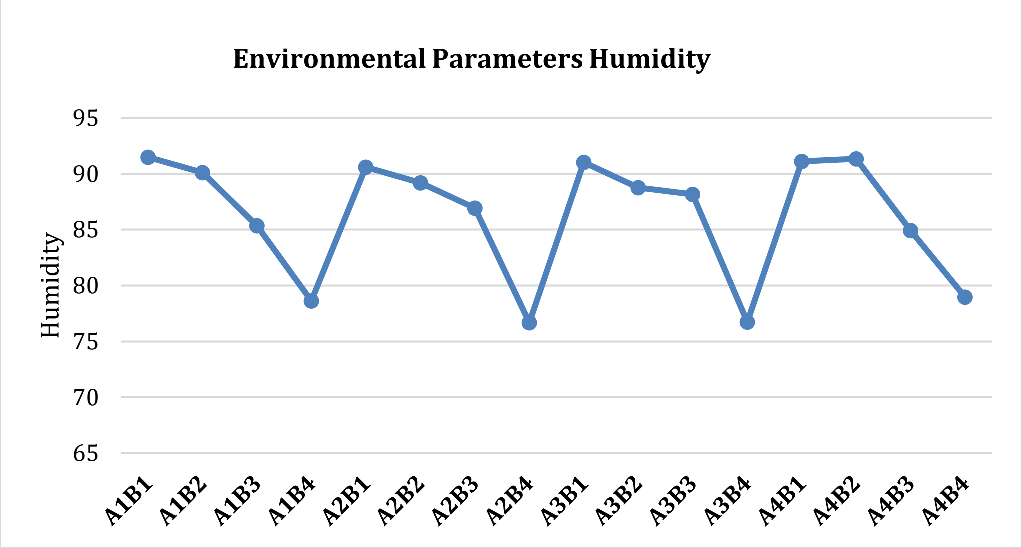
Shallot plants were cut at the root neck, then the roots were weighed using an analytical balance. The measurement of shallot root dry weight was as follows: shallot bulbs were dried in an oven at 80°C until the weight of the shallot roots remained constant. The procedure was repeated three times for each treatment.

Anatomy of the root : cortex thickness

The root anatomy parameters of red onions are analyzed using the embedding method. Plant root samples are processed through fixation, dehydration and dealcoholization, infiltration, wrapping, slicing, coloring, and receiving (Sutikno, 2006). Firstly, the roots are cut transversely. Secondly, the red onion roots are fixed by immersion in FAA solution. Thirdly, the FAA solution is drained, and replaced with 70% alcohol, left to stand for 30 minutes. Fourthly, after 30 minutes, the 70% alcohol is drained and replaced with 1% safranin, and left to stand for 24



(a)



(b)

Figure 1. (a) Enviromental parameters of pH and (b) Enviromental Humadity Parameter of *Allium cepa* L.

hours. Fifthly, washing and dehydration are carried out (Sutikno, 2006).

Sixth, de-alcoholization is performed. Seventh, the xylene solution is replaced with a xylene-paraffin mixture at a ratio of 1:9, then placed in an oven at 57°C for 24 hours. Eighth, infiltration is carried out, wherein the xylene-paraffin mixture is replaced with pure paraffin. Ninth, embedding is carried out. Tenth, sectioning is done using a rotary microtome. Eleventh, mounting is performed. Twelfth, staining is carried out with 1% safranin in 70% alcohol. Successive slides are immersed in xylene, xylene, alcohol/xylene 1:3, alcohol/xylene 1:1, alcohol/xylene 3:1, 100% alcohol, 100% alcohol, 95% alcohol, 80% alcohol, and 70% alcohol, with each immersion lasting for 3 minutes. Afterward, the slides are immersed in 1% safranin solution in 70% alcohol for 1 hour. Thirteenth, sealing is performed, where the slices are covered with Canada Balsam. Then, the preparations are observed using an optical microscope. The steps are as follows: the microscope is prepared by attaching the Optilab Advance V2 camera to the eyepiece tube, then the specimen is photographed using the Optilab Advance V2 camera (Sutikno, 2006; Palupi & Siswanti, 2023).

RESULTS

Environmental Conditions

Based on the results in Figure 1, it is known that the pH values in each treatment are not significantly different, meaning that the treatment of different doses of biofertilizer and field capacity does not affect the pH value. However, in terms of humidity parameters, there is a significant difference. In Figure 1, it can be seen that the humidity value will decrease as drought stress increases. The highest moisture measurement was found in treatment A1B1 (biofertilizer with a dose of 0 L/ha) with a moisture level of 91,49 RH.

Vitamin C Content of Shallot Bulbs

Based on table II, it is known that on average, the application of biofertilizer shows significant results that are not significantly different. Meanwhile, the average field capacity results show significantly different results, with the highest value at 50% field capacity. The treatment with the highest field capacity at 25% has results that are not significantly different from the control treatment (100% field capacity). This indicates that biofertilizer helps shallot plants to produce higher or equal amounts of vitamin C compared to the control treatment under water deficit conditions. The interaction results of biofertilizer application with drought stress that produced the highest vitamin C were in treatment A3B3 (biofertilizer dose of 15 L/ha and 25% field capacity).

Starch Content of Shallot Bulbs un

Based on table III, it is known that, on average, the application of biofertilizer shows significantly different results. The application of a biofertilizer dose of 15 L/ha was able to produce the highest starch content compared to other treatments. Meanwhile, the average field capacity results show significantly different results, with the highest starch content at 50% field capacity. The treatment with the highest field capacity at 25% has results that are not significantly different from the control treatment (100% field capacity). This indicates that biofertilizer helps shallot plants to produce higher or equal starch content compared to the control treatment under water deficit conditions. The interaction results of biofertilizer application with drought stress that produced the highest vitamin C were in treatment A3B1 (biofertilizer dose of 15 L/ha and 0% field capacity).

Dry Weight of Shallot Roots

Based on Table IV, it is known that the average application of biofertilizer shows significantly different results. The biofertilizer dose that produced the highest root dry weight was 10 L/ha. Meanwhile, the average field capacity results also show significantly different results, with the highest value found at 75% field capacity. The highest field capacity treatment at 25% had results that were not significantly different from the control treatment (100% field capacity). This indicates that biofertilizer helps shallot plants to produce higher or equal root dry weight compared to the control treatment under water deficit conditions. The interaction result of biofertilizer application with drought stress, which produced the highest starch content, was in treatment A1B2.

Cortex Thickness in Shallot Roots

Shallot root anatomy

Figure 2. Transversal section of *Allium cepa* L. root: co - cortex; en – endodermis; p – pericycle; mx – metaxylem

Figure 3. *Allium cepa* L. root anatomy (A) A1B1 (B) A1B2 (C) A1B3 (D) A1B4 (E) A2B1 (F) A2B2 (G) A2B3 (H) A2B4 (I) A3B1 (J) A3B2 (K) A3B3 (L) A3B4 (M) A4B1 (N) A4B2 (O) A4B3 (P) A4B4

Cortex Thickness in Shallot Roots

Based on table V, it is known that the average application of biofertilizer shows significantly different results. The biofertilizer application that produced the highest cortex thickness was at a dose of 15 L/ha. Meanwhile, the average field capacity results also show significantly different results, with the highest value found at 25% field capacity. The interaction result of biofertilizer application with drought stress, which produced the highest starch content, was in treatment A3B4 (biofertilizer dose of 15 L/ha and field capacity of 25%).

DISCUSSION

Environmental Conditions

The cultivation of shallots is carried out in a greenhouse to minimize biases towards unwanted treatments. Shallot plants require well-drained, crumbly-textured soil and good water drainage (Sansan et al., 2024). Soil pH affects the activity of microorganisms and nutrient availability. Acidic soil tends to have more microelements compared to neutral to alkaline soil (Gentili *et al.*, 2018)

The soil pH for shallot plants under drought stress conditions ranges from 6.7 to 6.85. The highest pH values tend to occur under drought stress conditions. This indicates that under drought stress, the pH becomes more alkaline, which means drought inhibits bacterial function (Toriq & Puspitawati, 2023). Microbes under drought stress conditions do not thrive optimally

Table II. Vitamin C content of shallot bulbs under different field capacity treatments and biofertilizer application

Field Capacity	Biofertilizer Dosage (L/Ha)				Average
	A1 (0 L/ha)	A2 (10 L/ha)	A3 (15 L/ha)	A4 (20 L/ha)	
B1 (100%)	0.27 ± 0.01^{bcde}	0.26 ± 0.0^{abc}	0.25 ± 0.03^{ab}	0.26 ± 0.0^{abc}	0.26 ± 0.01^{x}
B2 (75%)	0.29 ± 0.0^{def}	0.26 ± 0.01^{abc}	0.29 ± 0.01^{cde}	0.27 ± 0.0^{abcd}	0.27 ± 0.01^{yz}
B3 (50%)	0.26 ± 0.01^{abc}	0.29 ± 0.01^{ef}	**0.3 ± 0.02^{f}**	0.26 ± 0.01^{abc}	**0.28 ± 0.02^{z}**
B4 (25%)	0.27 ± 0.01^{abcd}	0.27 ± 0.01^{bcd}	0.25 ± 0.02^{a}	0.28 ± 0.0^{cde}	0.27 ± 0.01^{xy}
Average	0.27 ± 0.01^{o}	0.27 ± 0.01^{o}	**0.28 ± 0.02^{p}**	0.27 ± 0.01^{o}	

Numbers followed by the same letter in a column and row are not significantly different in the DMRT test at a significance level of 95% (α = 0.05).

Table III. Starch content of shallot bulbs in treatments of field capacity and biofertilizer application

Field Capacity	Biofertilizer dosage (L/Ha)				Average
	A1 (0 L/ha)	A2 (10 L/ha)	A3 (15 L/ha)	A4 (20 L/ha)	
B1 (100%)	22.08 ± 0.05^{cd}	21.2 ± 1.19^{ab}	**23.21 ± 0.5^{f}**	21.58 ± 0.11^{bc}	21.99 ± 0.96^{x}
B2 (75%)	23.02 ± 0.16^{ef}	24.5 ± 0.46^{g}	23.11 ± 0.24^{ef}	23.13 ± 0.11^{ef}	23.44 ± 0.68^{y}
B3 (50%)	24.83 ± 0.08^{g}	21.26 ± 0.11^{ab}	25.64 ± 0.11^{h}	23.17 ± 0.26^{ef}	**23.73 ± 1.75^{y}**
B4 (25%)	21.65 ± 0.24^{bc}	20.75 ± 0.43^{a}	22.48 ± 0.27^{def}	22.45 ± 0.08^{de}	$21.\ 83 \pm 0.78^{x}$
Average	22.90 ± 1.28^{p}	21.93 ± 1.66^{o}	**23.61 ± 1.29^{q}**	22.58 ± 0.69^{p}	

Numbers followed by the same letter in a column and row are not significantly different in the DMRT test at a significance level of 95% (α = 0.05).

Table IV. Dry weight of roots in drought stress treatment and biofertilizer application

Field Capacity	Biofertilizer Dosage (L/Ha)				Average (mg)
	A1 (0 L/ha)	A2 (10 L/ha)	A3 (15 L/ha)	A4 (20 L/ha)	
B1 (100%)	18 ± 15.13^{a}	31.67 ± 7.51^{a}	18 ± 14^{a}	29.67 ± 26.08^{a}	24.34 ± 7.36^{xy}
B2 (75%)	**34.33 ± 3.22^{a}**	26 ± 9.64^{a}	25.33 ± 19.3^{a}	24.33 ± 7.23^{a}	**27.5 ± 4.61^{y}**
B3 (50%)	17.67 ± 7.02^{a}	27.67 ± 5.13^{a}	23.33 ± 9.02^{a}	11.67 ± 10.5^{a}	20.09 ± 6.95^{xy}
B4 (25%)	14.33 ± 8.51^{a}	17.67 ± 6.43^{a}	18.67 ± 8.62^{a}	20.33 ± 12.74^{a}	17.75 ± 2.53^{x}
Average (mg)	21.08 ± 8.99^{o}	**25.75 ± 5.89^{o}**	21.33 ± 3.57^{o}	21.5 ± 7.59^{o}	

Numbers followed by the same letter in a column and row are not significantly different in the DMRT test at a significance level of 95% (α = 0.05).

because they cannot perform metabolism efficiently. Generally, the pH range of the soil in the research medium is still tolerable by shallot plants (Siebielec *et al.*, 2020)

Based on the results obtained, humidity levels decrease as drought stress increases (Zhang *et al.*, 2022). This is consistent with the analysis of environmental parameters measuring pH. According to the results obtained, humidity levels decrease as drought stress increases. This is in line with the analysis of environmental parameters measuring pH, where drought stress conditions create unfavorable environmental conditions (Toriq & Puspitawati, 2023).

Vitamin C Content of Shallot Bulbs

The parameter determination of vitamin C levels in shallot bulbs is carried out to understand the biochemical response of shallot bulbs in facing drought stress. The application of biofertilizers showed no significant difference in vitamin C levels. A comparison at 50% field capacity yielded vitamin C levels. This may be due to the plant's response to drought stress, which is also determined by species, drought stress period, and severity (El-mageed *et al.*, 2016)

The application of biofertilizers at 15 L/ha had the highest average vitamin C levels compared to treatments of 10 L/ha and 20 L/ha.

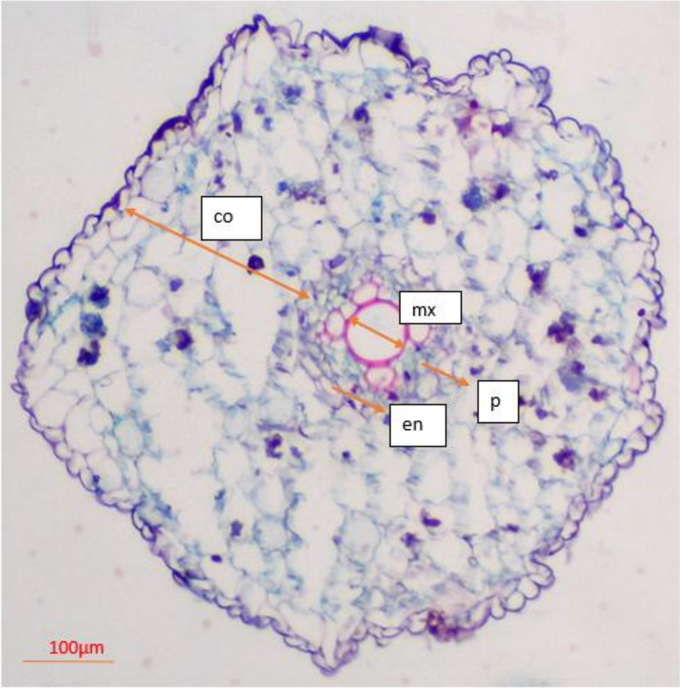


Figure 2. Transversal section of *Allium cepa* L. root: ko - cortex; en – endodermis; p – pericycle; mx – metaxylem

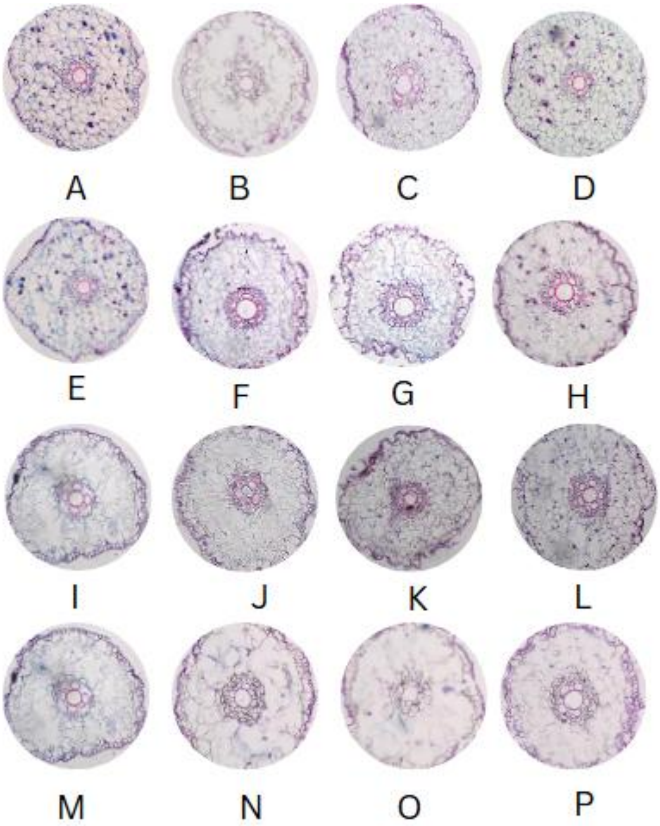


Figure 3. *Allium cepa* L. root anatomy
(A) A1B1 (B) A1B2 (C) A1B3 (D) A1B4 (E) A2B1 (F) A2B2 (G) A2B3 (H) A2B4 (I) A3B1 (J) A3B2 A3B3 (L) A3B4 (M) A4B1 (N) A4B2 (O) A4B3 (P) A4B4

Table V. Cortex thickness (µm) of roots in drought stress treatment and biofertilizer application

Field Capacity	Biofertilizer Dosage (L/Ha)				Average
	A1 (0 L/ha)	**A2 (10 L/ha)**	**A3 (15 L/ha)**	**A4 (20 L/ha)**	
B1 (100%)	160.97 ± 8.15^{bc}	212.71 ± 22.81^{f}	141.61 ± 3.41^{a}	175.94 ± 5.04^{cd}	172.81 ± 30.09^{x}
B2 (75%)	191.57 ± 5.26^{de}	173.87 ± 4.2^{cd}	172.99 ± 3.29^{cd}	214.01 ± 3.69^{f}	188.11 ± 19.27^{y}
B3 (50%)	175.51 ± 2.63^{cd}	146.96 ± 13.22^{ab}	231.99 ± 17.04^{g}	185.1 ± 14^{de}	184.89 ± 35.33^{y}
B4 (25%)	199.35 ± 10.9^{ef}	173.5 ± 5.0^{cd}	**241.05 ± 1.72^{g}**	188.95 ± 9.87^{de}	**200.71 ± 28.91^{z}**
Average	181.85 ± 17.1^{o}	176.76 ± 27.08^{o}	**196.91 ± 47.64^{p}**	191 ± 16.28^{p}	

Numbers followed by the same letter in a column and row are not significantly different in the DMRT test at a significance level of 95% ($\alpha = 0.05$).

The application of biofertilizers at 10 L/ha contained fewer bacteria than the biofertilizers at 15 L/ha. Meanwhile, the application of biofertilizers at 20 L/ha resulted in lower vitamin C levels. This might occur because, even though the microbes in the higher dose of biofertilizers are abundant, there is competition among microbes in decomposing organic matter (manure), thus hindering the availability of NPK elements during the decomposition process (Sriwahyuni & Parmila, 2019). This is evidenced by research from Nunilahwati et al. (2022), which states that applying 100 kg/ha of biofertilizer resulted in lower growth of mustard greens compared to the 80 kg/ha treatment. Although a higher amount of biofertilizer was provided, it led to competition among bacteria in breaking down organic matter.

Ascorbic acid, or vitamin C, contains bioactive compounds with potent antioxidant properties to neutralize cellular oxidative stress. At 50% field capacity, the vitamin C content is higher than at 25% field capacity. This is possibly because, at 50% drought stress, the plant reaches an optimal point to combat oxidative stress. Meanwhile, at higher drought stress levels (25% field capacity), the plant can no longer effectively maintain or enhance this adaptation due to excessive stress. However, at the highest drought stress level, there is no significant difference compared to the control treatment. This means that biofertilizers can enable the plant to produce secondary metabolites similar to the control treatment without drought stress.

Another possible influencing factor is intraspecific interaction. This interaction occurs because two shallot plants are in the same polybag. Intraspecific interaction happens as the plants compete for space, light intensity, water, nutrients, and other ecological factors needed for each organism to live and grow, even in a supportive environment with the highest biofertilizer dose (Syafi'i *et al*., 2017).

Starch Content of Shallot Tubers

Starch is a key molecule in plants' responses to drought stress, high salinity, or extreme temperatures. The increase in starch content as a response to drought stress is often associated with increased tolerance. Plants generally remobilize starch to provide energy and carbon when photosynthesis is limited due to low water supply under drought conditions (Dien *et al*., 2019).

In the pharmaceutical industry, starch is one of the excipients that acts inertly as a filler, disintegrant, and binder in the formulation of solid dosage forms. Starch consists of two types of polysaccharides, namely amylose and amylopectin. Amylose is a linear chain polysaccharide that forms amorphous granules, while amylopectin is a branched polysaccharide that forms the crystalline part of sugar (Sulaiman *et al*., 2022). Starch has a hydrophilic carbohydrate chain, which is still difficult to penetrate because starch is made up of dense and complex particles (Mawarni *et al*., 2023).

The highest starch content from biofertilizer application was obtained at a dose of 15 L/ha. The application of biofertilizers indicates that the microbial content in the biofertilizer provides beneficial nutrients. Adequate nutrients in the planting medium increase glucose levels. This glucose increase enhances the conversion of glucose into acetyl-CoA, which **is** then transformed into fats through fatty acid synthesis (Siswanti *et al*., 2022)

At 50% field capacity, the highest starch content was produced compared to other treatments. Starch content tends to be higher under drought stress conditions than in control treatments. This is because, under abiotic stress conditions, starch emerges as a key molecule to mediate plant responses to conditions such as drought, high salinity, or extreme temperatures. Plants generally remobilize starch to provide energy and carbon when photosynthesis is limited.

Starch can act as a signaling molecule that engages in cross-talk with ABA-dependent signaling pathways to activate downstream components in the stress response cascade (Thalmann & Santelia, 2017).

Dry Weight of Shallot Roots

Roots are organs that play an important role in the absorption of nutrients, water, minerals, and other elements. Root growth is the most responsive variable to drought stress (Khalil *et al*., 2020). Plant dry weight is a parameter that indicates the accumulation of photosynthetic biomass in each part of the plant (Nova *et al*., 2020). The application of 10 L/ha of biofertilizer resulted in the highest root dry weight. Meanwhile, the treatment with field capacity resulted in the highest root dry weight at 75% field capacity. This may be because drought conditions affect root growth, which varies depending on species, growth stage, and the intensity and duration of the drought. Plants will increase the allocation of their resources to the roots in response to drought stress. This aims to enhance the plant's ability to search for and absorb water from soil affected by abiotic stress. This mechanism is intended to improve the plant's capability to seek and absorb water from dry soil. However, under severe or prolonged water deficit conditions, plants may not be able to maintain optimal root growth and eventually show reduced root dry weight (Handoyono et al., 2020; Ichsan, 2021). This is in line with research by Ginting et al. (2024), which shows that the higher the level of drought stress applied, the more the root dry weight decreases. Field capacity at 40% had the lowest results compared to field capacities at 80% and 60%.

Cortex Thickness Shallot Roots

The application of biofertilizer that resulted in the greatest cortex thickness was at a dose of 15 L/ha. The 15 L/ha dose did not significantly differ from the 20 L/ha dose. Meanwhile, the highest field capacity treatment was at 25% field capacity. When looking at the results of the cortex length of shallot roots based on the average comparison of field capacities, it can be seen that the cortex length increases with increasing drought stress. The application of biofertilizer resulted in an optimal increase in cortex thickness of shallot roots. This may occur due to the addition of external IAA obtained from the biofertilizer or the auxin hormone, which plays a role in cell expansion, xylem and phloem tissue formation, and root elongation (Salem *et al*., 2024).

This is in line with research conducted by Salem et al. (2024), which found that the administration of microbial strain *Bacillus* sp. could increase cortex and stele thickness by 49% compared to the control, thereby increasing lateral root diameter. The increase in phytohormone levels is accompanied by an increase in both the number and size of cells, resulting in an increase in diameter. Bacillus sp. strains can produce phytohormones such as auxin, gibberellin, ABA, cytokinin, ethylene, brassinosteroids, strigolactones, and jasmonates in the root zone, directly facilitating cell division, elongation, and differentiation in the meristem (Bhardwaj et al., 2014).

CONCLUSION

Based on the research conducted, it can be concluded that the application of biofertilizer affects the physiology and anatomy of *Allium cepa* L. under drought stress. The application of 15 L/ha biofertilizer increases vitamin C content, starch content, and cortex thickness. Meanwhile, 10 L/ha biofertilizer increases root dry weight.

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