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Growth and Yield of Shallot (*Allium cepa* L. Aggregatum Group) with Application of Amino Acid Biostimulant Dosages

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ABSTRACT

Intensive shallot cultivation needs high application rates of inorganic fertilizers that can cause environmental problems. Therefore, it is essential to lessen the rate of inorganic fertilizers by environmentally friendlier approaches, such as the application of biostimulants like amino acids. The present study determined the most effective dosage and application method of amino acid biostimulant to increase shallot yield and growth while using only half the amount of inorganic fertilizers. The research was arranged in a randomized complete block design with two factors and four blocks as replications. The first factor was the dose of amino acids biostimulant (0, 0.5, 1, and 2 L/ha), and the second factor was the application method (through leaves and soil). Data were observed on nitrogen (N), phosphorus (P), potassium (K), manganese (Mn), boron (B), indole acetic acid (IAA), gibberellin, zeatin, kinetin, nitrate reductase activity (NRA), chlorophyll as well as the growth and yield of shallot. The results showed that the application of amino acids biostimulants increased IAA, gibberellin, and kinetin content in both application methods. Amino acids biostimulants increased N, P, K, B, Mn, and chlorophyll. Amino acids biostimulant 1 L/ha was the best dosage to increase leaf diameter, leaf dry weight, total dry weight, number of bulbs (5.63 per plant; 44%), and productivity (16.46 tons/ha; 33.77%). The application through the leaves improved NRA, leaf area, and crop growth rate. It was indicated that amino acid biostimulant through leaves provides a useful instrument for plant growth, allowing the reduction of inorganic fertilizer without compromising crop yields.

Keywords: Amino acids, macro and micronutrients, phytohormones, supplement

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INTRODUCTION

Shallot has high economic and strategic values with consequently intensive cultivation, which generally needs a high inorganic fertilizer application. The required inorganic shallot fertilizers are nitrogen: phosphorus: potassium (NPK) 15:9:20 300 kg/ha, zwavelzure ammonium (ZA) 200 kg/ha, and potassium chloride (KCl) 75 kg/ha, as recommended based on research by Pangestuti et al. (2022) in tropical lowland and regosol soil. Intensive farming with high levels of inorganic fertilizers negatively affects the environment, including air, water, and soil pollution, which is followed by the phenomenon's production of greenhouse gases, disease resistance, nutritional deficiencies, and toxicity to organisms above and below the soil (Ning et al., 2017; Tyagi et al., 2022). Therefore, reducing the amount of inorganic fertilizers and replacing them with friendlier organic materials seems necessary (Souri et al., 2019; Souri & Sooraki, 2019). Amino acids represent the most common and effective compounds integrated in fertilizer manufacturing as well as one of the most known biostimulants in cropping systems (Mohammadipour & Souri, 2019; Souri, 2016; Souri et al., 2018; Souri & Hatamian, 2019).

Biostimulants are substances that contain one or more compounds or microorganisms formulated to stimulate plant growth, as well as mechanisms for regulating plant resistance to stress conditions from both biotic and abiotic sources, improving microbiological rhizosphere activity and soil enzymes, and optimizing the photosynthesis process to improve crop production with low application doses (du Jardin, 2015; Sharma et al., 2014; Yakhin et al., 2017). The use of biostimulants has been reported can increase the activity of vital enzymes involved in the metabolism of carbon and nitrogen, hormone activity as well as physiological and biochemical changes in plant tissues (Alfosea-Simón et al., 2020; Sheng et al., 2020). In horticultural crops, the use of biostimulants enables the reduction of fertilizer without a significant reduction in yield or quality (Noroozlo et al., 2019; Souri & Bakhtiarizade, 2019), as well as increasing essential micronutrients (Fe, Cu, and Zn) for human health (Mannino et al., 2020).

Amino acids are the fundamental components of proteins, play important roles in metabolic processes and transport, also serve as precursors for active substances, and influence physiological activity in plant growth (Moormann et al., 2022; Popko et al., 2018). Amino acid-based biostimulants can regulate the absorption and assimilation of nitrogen in plants as mediated by enzyme activity (Noroozlo et al., 2020). Applying amino acids increased nitrate, amino acid, and total nitrogen contents in leaves and yield of soybeans by up to 21% (Teixeira et al., 2018). Several studies have suggested that amino acid-based biostimulants applied through leaves increased antioxidant activity, pigment content, and production in pepper; chlorophyll, ion transport, photosystem II activity, assimilation of vital nutrients like N, P, Ca, Mg, S, and stress response at different levels of nitrogen application in spinach and lettuce (Carillo et al., 2019; Mola et al., 2020; Parađiković et al., 2011; Tsouvaltzis et al., 2020); they also increased the number of pods, seeds, phenol, flavonoids, and yields of soybean (Kocira, 2019). Meanwhile, amino acids from plants applied through the roots can increase reductase activity, chlorophyll and iron (Fe) content, leaf area, number of fruits, and yield of tomato (Cerdán et al., 2013; Souri et al., 2017).

Amino acid-based biostimulants (Amiboost®) are made from plant-based organic materials (sugar cane and corn) and fermented using microbes to produce L-amino acids that plants can absorb. The nutritional content of the amino acids biostimulant consists of 6.5% nitrogen, 2.0% phosphorus, 1.5% potassium, 0.1% boron, 0.1% manganese, and 10% total amino acids. Considering the problems associated with inorganic fertilizers, using amino acids-based biostimulants can be a promising method for reducing the use of inorganic fertilizers, which can support sustainable agriculture. Shallot has never been exposed to amino acids-based biostimulants. Therefore, studies regarding the impact of the application of amino acids-based biostimulants (Amiboost®) must be conducted to determine the ideal dosage and efficient application method on the growth and yield of shallot.

MATERIALS AND METHODS

Material Preparation

The research was conducted from March to June 2022 at the Tri Dharma Fields Laboratory, Faculty of Agriculture, Universitas Gadjah Mada, Bantul, Special Region of Yogyakarta Province, Indonesia (07°48'17"S and 110°24'45"E) at 107 m a.s.l. Throughout the experiment, the average temperature was 30.5°C, the humidity was 34%, the soil temperature was 34.57°C, and the amount of sunlight intensity was 38,259 lux. The shallot variety was Gamaba, and the biostimulant product for amino acids was Amiboost® (Korea). The amino acids biostimulants used contain macronutrients, micronutrients, and total amino acids, and the elemental content was 6.5% nitrogen, 2.0% phosphorus, 1.5% potassium, 0.1% boron, 0.1% manganese, and 10% total amino acids.

Experimental Design

The experiment was arranged in a randomized complete block design with two factors and four blocks as replications. The first factor was the dose of amino acid biostimulants (0, 0.5, 1, and 2 L/ha), and the second factor was the application method (through leaves and soil). There are eight treatment combinations. In a 95 cm × 80 cm field plot, 15 bulbs were planted with 20 cm × 15 cm spacing. The planting medium was regosol soil.

In the present study, amino acids biostimulants were applied twice, 14 and 28 days after planting (DAP), with a volume of 1,000 L/ha. The amino acids biostimulant application methods involved applying through the leaves by directly spraying it on the leaves and through the soil by watering it directly into the soil around the plants. Fertilization was applied three times, the time before planting, at 28 and 42 DAP, by giving half the recommended rate of inorganic fertilizer based on research by Pangestuti et al. (2022). The fertilizer rate before planting was manure 20 ton/ha (Dharma Jaya, Indonesia), SP36 130 kg/ha (Petro, Indonesia), NPK 16:16:16 100 kg/ha (Mutiara®, Indonesia), and ZA 85 kg/ha (Petro, Indonesia); at 28 DAP, it was NPK 15:9:20 75 kg/ha (Mutiara®, Indonesia), ZA 100 kg/ha ha (Petro, Indonesia); and at 42 DAP, it was NPK 15:9:20 75 kg/ha ha (Petro, Indonesia), KCl 75 kg/ha ha (Petro, Indonesia).

The Analysis of the Plant Tissue, Phytohormones, Nitrate Reductase, and Chlorophyll

The plant tissue, phytohormones, nitrate reductase, and chlorophyll were analyzed 35 DAP. Plant tissue analysis included N, P, K, B, and Mn. Tissue N content was examined utilizing wet destruction and semi-automatic distillation equipment Kjeldahl UDK 139 (Velp Scientifica, Italy). The P contents were examined utilizing wet destruction and a GENESYS™ 10 UV-Visible spectrophotometer (Thermo Fisher Scientific, USA). The K contents were examined utilizing wet destruction and a PFP7C flame photometer (Rose Scientific Ltd., Canada). Tissue B content was analyzed using the dry destruction and a GENESYS™ 10 UV-Vis spectrophotometer. The Mn content was analyzed using dry destruction and atomic absorption spectrophotometry (AAS) (Thermo Fisher Scientific, USA). Phytohormone analysis measurements, including IAA, gibberellin, zeatin, and kinetin, were analyzed using the Linskens and Jackson (1987) method and high-performance liquid chromatography (HPLC, Thermo Fisher Scientific, USA). Nitrate reductase was analyzed spectrophotometrically at a wavelength of 540 nm using the method described by Jaworski (1971). Nitrate reductase activity (NRA; in µmol/NO_2/hr) is measured using the following equation:

$$\text{NRA} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \frac{1{,}000}{\text{Leaf fresh weight (mg)}} \times \frac{1}{\text{Incubation time (hour)}} \times \frac{50}{1{,}000}$$

Chlorophyll contents were analyzed spectrophotometrically at 645 nm and 663 nm wavelengths using the Coombs et al. (1985) method. The formula was used to determine chlorophyll *a* (mg/g), chlorophyll *b* (mg/g), and total chlorophyll (mg/g):

$$\text{Chlorophyll } a = (12.7 \times A_{663}) - (2.69 \times A_{645})$$
$$\text{Chlorophyll } b = (22.9 \times A_{645}) - (4.68 \times A_{663})$$
$$\text{Total chlorophyll} = (20.2 \times A_{645}) + (8.02 \times A_{663})$$

Growth and Yield

Plant growth and yield variables, including plant height (cm), number of leaves, leaf diameter (mm), leaf area (cm^2), net assimilation rate (mg/cm^2/week), crop growth rate (mg/cm^2/week), root dry weight (g), leaf dry weight (g), total dry weight (g), harvest index, number of bulbs per plant, and productivity (ton/ha) were analyzed. The leaf surface area was measured using leaf area meters software WInDIAS 3 (United Kingdom). The dry weight of roots, leaves, and bulbs was measured during a 48-hr period in an oven set to 80°C.

Data Analysis

All observational data were examined utilizing analysis of variance followed by Tukey's test with a significance level of 95%. The analysis used version 3.2.2 of the R statistical computing platform (The Austrian R foundation).

RESULTS AND DISCUSSION

Plant Tissue Analysis

Tissue analysis was carried out on the macro and micronutrients in shallot leaves. The highest accumulation of N content was 1.02% in the leaf tissue at a dose of 1 L/ha through the leaves (Figure 1). Plants need N for metabolism in preparing proteins, making up leaf chlorophyll, cell division, and stimulating vegetative plant growth (Zhang et al., 2013). In shallot, N can increase the number of leaves, plant height, stem diameter, bulbs, and yield (Biru, 2015; Kemal, 2013). Furthermore, N deficiency in plants can occur due to the application of inorganic fertilizer (NPK) at half the recommended rate. Navarro-León et al. (2022) stated that the total N content accumulated in lettuce plant tissue increased by up to 30% when L-amino acid-based biostimulants were applied with N fertilizer only 30% and 60%. Amino acids play roles as different physiological signaling factors in plant processes. In *Arabidopsis thaliana*, the glutamate receptor can bind to other amino acids. Amino acids activate the receptor and trigger physiological processes to regulate nitrogen uptake (Miller et al., 2007). Moreover, based on the application method, the N content accumulated in higher concentrations in the application through the leaves. Biostimulants containing amino acids could become nutrients for microbes when applied through the soil; hence, it is possible for biostimulants that are used through the soil to be easily captured and decomposed by bacteria in the soil. Jones et al. (2009) reported that soil microorganisms consume 30–40% of amino acids for respiration and the remaining amino acids-C are used to produce and maintain cell biomass.

P content accumulated was the highest at 0.3% at a dosage of 0.5 L/ha through the leaves (Figure 1). P is a necessary nutrient that plays an aspect in the preparation of adenosine diphosphate (ADP), adenosine triphosphate (ATP), deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). Furthermore, P can stimulate root growth, strengthen stems, and increase onion bulbs' weight and size (Anbes et al., 2018). According to Khan et al. (2019), spraying individual amino acids improved the uptake of N (67.44%), P (66.57%), and K (50.55%) and the yield of lettuce.

The highest accumulation of K content was 3.5% at an amino acid biostimulant dose of 1 L/ha through leaves (Figure 1). K is involved in several biochemical and physiological processes. Tränkner et al. (2018) stated that K regulates stomata for photosynthetic carbon dioxide fixation

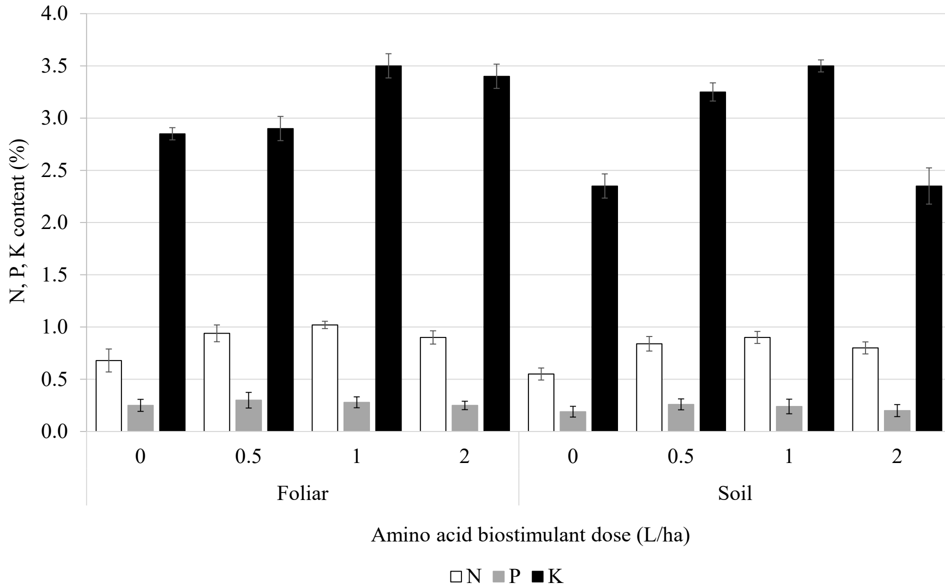

Figure 1. Tissue analysis of N, P, and K content in shallot leaves 35 days after planting

as transport as well as photoassimilates consumption. K also activates the enzyme adenosine triphosphate synthase (ATP), which affects the plasma membrane (Juhaszova et al., 2019). Jiku et al. (2020) stated that K can increase garlic's bulb weight, size, and yield. Al-Karaki and Othman (2023) reported that biostimulants containing amino acids enhance N, P, K, and Mg content in lettuce leaves by 20.5%, 25.1%, 19.8%, and 58.5%.

Figure 2 shows that the highest accumulated Mn content was 17.51 ppm at a dosage of 0.5 L/ha through the leaves. Meanwhile, the Mn content decreased in plants without amino acid biostimulants. Mn is an element that plants require for photosynthesis, increasing the nitrate reductase enzyme's activity and the production of photosynthetic pigments, carotenoid content, and membrane stability index (Shahi & Srivastava, 2018).

The highest accumulation of B content was 134.63 ppm at an amino acid biostimulant dose of 1 L/ha through the leaves (Figure 2). In the absence of amino acids biostimulant dose, the boron content was merely 2.02 and 8.02 ppm. B plays a role in developing and growing new cells in merismatic tissue and could influence root cell elongation and division, phenol metabolism, carbohydrate metabolism, cell wall structure, and auxin synthesis (González-Fontes et al., 2016; Li et al., 2016). In addition, amino acids influence other micronutrients' availability and soil absorption by acting as chelating agents in the soil, translocating micronutrients in the phloem, and affecting root morphology (Halpern et al., 2015). In tomatoes, the application of biostimulants that contain amino acids and microelements (boron) could increase plant growth by up to 48.5% and the number of fruits by up to 105.3% (Francesca et al., 2020).

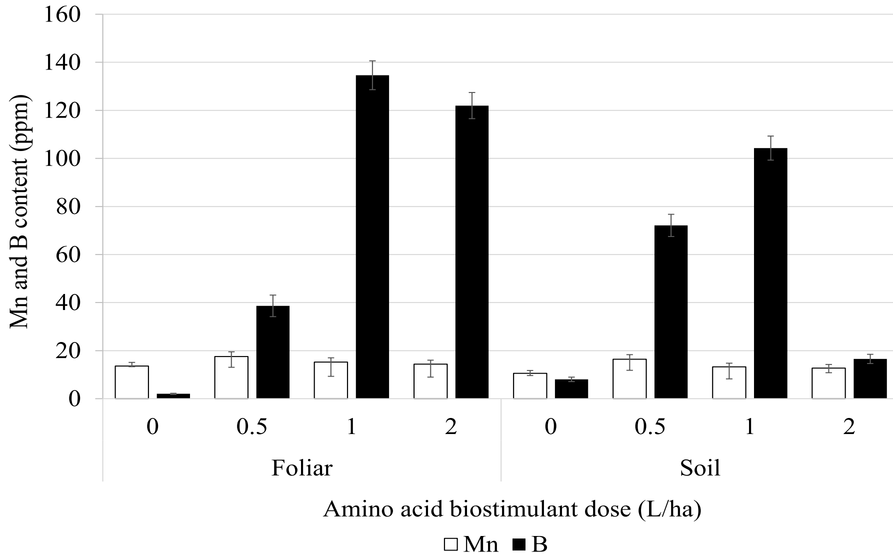

Figure 2. Tissue analysis of manganese (Mn) and boron (B) content in shallot leaves 35 days after planting

Phytohormone Analysis

The phytohormone content in shallot was observed in the leaf organs 35 DAP. Table 1 shows an interaction between the dosage of the amino acids biostimulant and the application method for IAA, gibberellin, and kinetin, whereas zeatin showed no interaction.

Amino acids biostimulant dose of 0.5–2 L/ha through leaves and soil had significantly higher IAA and gibberellin content than without amino acids biostimulants through leaves or soil. The highest IAA content was found at amino acids biostimulant dose of 2 L/ha through the soil (5.81 mg/g) but was not significantly different with amino acids biostimulant dose of 0.5–2 L/ha through leaves. Meanwhile, the highest gibberellin content was found at an amino acids biostimulant dose of 1 L/ha through the leaves (5.25 mg/g). Auxin content has increased in plants due to amino acids in biostimulants. Amino acids are considered precursors for auxin (IAA) synthesis, essential in regulating cell division and elongation processes (Yue et al., 2021). L-tryptophan promotes auxin production in plants, enhancing plant growth and productivity when foliar and seed are applied (Mustafa et al., 2018). Auxin promotes the formation of root and lateral fibers, thereby optimizing the absorption of water and minerals in plants. Gibberellins can increase cell division and growth, which will cause stem elongation and increase the number of internodes (Liu et al., 2019).

Table 1
IAA, gibberellin, zeatin, and kinetin content of shallot 35 days after planting

	Application method			
	Amino acids biostimulant dose (L/ha)	Foliar	Soil	Mean
Indole acetic acid (mg/g)	0	2.43 ± 0.10 c	2.38 ± 0.06 c	2.41 ± 0.05
	0.5	5.49 ± 0.26 a	4.46 ± 0.11 b	4.98 ± 0.24
	1	5.01 ± 0.12 ab	5.17 ± 0.16 ab	5.09 ± 0.10
	2	5.76 ± 0.18 a	5.81 ± 0.27 a	5.78 ± 0.15
	Mean	4.67 ± 0.35	4.45 ± 0.34	(+)
	CV (%)			7.67
	Amino acids biostimulant dose (L/ha)	Foliar	Soil	Mean
Gibberellin (mg/g)	0	2.31 ± 0.09 d	2.22 ± 0.02 d	2.26 ± 0.04
	0.5	3.68 ± 0.10 b	3.04 ± 0.15 c	3.36 ± 0.15
	1	5.25 ± 0.13 a	3.11 ± 0.04 c	4.18 ± 0.41
	2	3.27 ± 0.02 bc	3.67 ± 0.06 b	3.47 ± 0.08
	Mean	3.63 ± 0.28	3.01 ± 0.14	(+)
	CV (%)			5.56
	Amino acids biostimulant dose (L/ha)	Foliar	Soil	Mean
Zeatin (mg/g)	0	1.28 ± 0.07	1.27 ± 0.05	1.28 ± 0.04 q
	0.5	1.44 ± 0.05	1.63 ± 0.10	1.53 ± 0.06 p
	1	1.19 ± 0.02	1.39 ± 0.06	1.29 ± 0.05 q
	2	1.25 ± 0.02	1.39 ± 0.03	1.32 ± 0.03 q
	Mean	1.29 ± 0.03y	1.42 ± 0.04x	(-)
	CV (%)			8.29
	Amino acids biostimulant dose (L/ha)	Foliar	Soil	Mean
Kinetin (mg/g)	0	0.49 ± 0.01 c	0.48 ± 0.01 c	0.49 ± 0.00
	0.5	1.05 ± 0.03 b	1.35 ± 0.00 a	1.20 ± 0.06
	1	0.93 ± 0.02 b	1.29 ± 0.06 a	1.11 ± 0.08
	2	1.05 ± 0.05 b	1.07 ± 0.08 b	1.06 ± 0.04
	Mean	0.88 ± 0.06	1.05 ± 0.09	(+)
	CV (%)			9.10

Note. The Tukey's test at 5% revealed no significant differences between means that were followed by the same letters in a row or columns; (-) = No interaction between treatment; (+) = There was an interaction between treatment

Kinetin and zeatin are hormones belonging to the group of natural cytokinins that can process cell division. The dosage of amino acids biostimulants and the application method significantly affected the zeatin content in shallot plants. Applying an amino acids biostimulant dose of 0.5 L/ha had significantly higher zeatin content (1.53 mg/g) than other amino acids biostimulant doses. Meanwhile, based on the application method, the zeatin content was significantly higher in the application through the soil. For the kinetin hormone, applying amino acids biostimulant doses of 0.5–2 L/ha through the leaves and soil had a significantly higher kinetin content than without amino acids biostimulants. The highest kinetin contents were 1.35 and 1.29 mg/g at amino acids biostimulant doses of 0.5 and 1 L/ha, respectively, through the soil (Table 1). The high content of zeatin and kinetin in the application of amino acids biostimulants through the soil could be due to the synthesis of cytokinins in the root tips.

Cytokinin is synthesized in the roots and translocated to the shoots via the xylem vessels. The occurrence of cytokinin accumulation in leaves results from transportation through the xylem. Cytokinin has the function of encouraging cell division and differentiation in cooperation with auxins. Cytokinin can stimulate development by modulating plants' morphologies, physiology, and biochemistry. Furthermore, cytokinin causes the expression of phototropin involved in the opening of the stomata to increase stomatal conductance, regulates the synthesis of chlorophyll pigments, and regulates the production of crucial proteins necessary for the assembling and enzyme activation of the Rubisco (Gujjar et al., 2020). Hormones can also be synthesized through rhizobacteria in the soil, producing phytohormones. Rhizobacteria can use biostimulants containing amino acids through the soil to synthesize phytohormones.

The Analysis of Nitrate Reductase and Chlorophyll

Figure 3 shows that the highest NRA was 1.97 µmol/NO_2/hr at amino acids biostimulant dose of 1 L/ha. Meanwhile, based on the application method, NRA was highest in applications through the leaves (Figure 3). Nitrate reductase is the initial enzyme in the process of nitrate assimilation. Nitrate reductase analysis illustrates the high uptake of N in the plant body. There is a tendency to increase nitrate reductase at amino acids biostimulant doses. Ertani et al. (2009) stated that applying a hydrolysate protein-based fertilizer enhanced corn's glutamine synthetase and nitrate reductase activity, transforming nitrate to organic nitrogen. Kunicki et al. (2010) studied the impact of the biostimulant (Aminoplant), which contains an amino acid in spinach, which increased NRA—the improvement in nitrate reductase related to the chlorophyll content of plants. The highest chlorophyll *a*, *b*, and total content were found at amino acids biostimulant dose of 1 L/ha. Meanwhile, based on the application method, application through the leaves produced the highest total chlorophyll content (Figure 4). Similar studies showed that chlorophyll *a*, *b*, and total increased with the application of amino acid biostimulants in *Achillea millefolium* (Shafie et al., 2021), faba bean (Desoky et al., 2021), tomato (Alfosea-Simón et al., 2020), and lettuce (Tsouvaltzis et al., 2020). Furthermore, the application of protein hydrolysate from legumes at different N fertilization levels can also increase chlorophyll content in spinach and lettuce (Mola et al., 2020).

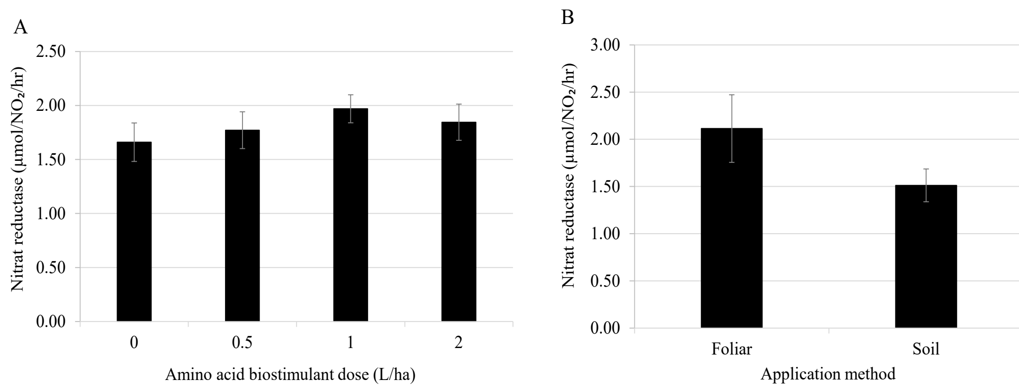

Figure 3. Nitrate reductase activity of shallot plants 35 days after planting based on (A) amino acids biostimulant dose and (B) application method

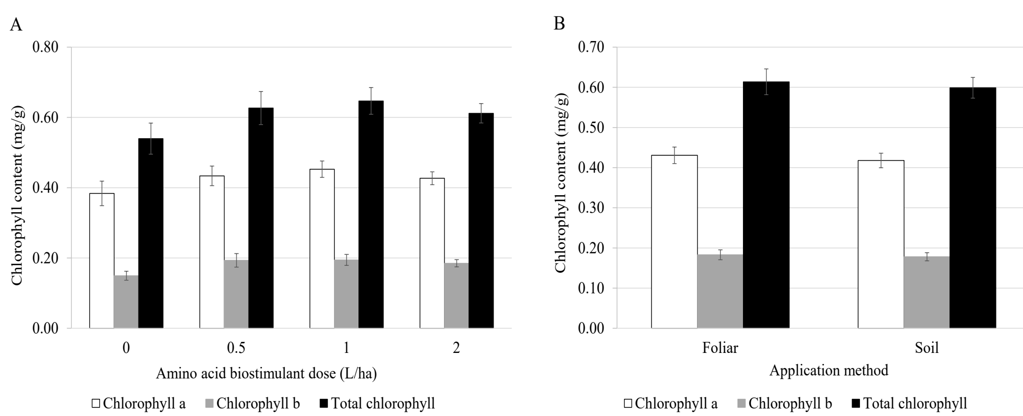

Figure 4. The chlorophyll content of shallot plants 35 days after planting based on (A) amino acids biostimulant dose and (B) application method

Growth and Yield

Growth indicators, including plant height, the number of leaves, and leaf diameter, were observed at 35 DAP. Table 2 shows no interaction between the dose of amino acids biostimulants and the application method. Individually, the dosage of amino acids biostimulants and the application method had the same effect on plant height and number of leaves. Meanwhile, in leaf diameter, amino acids biostimulant dosage of 1 L/ha was significantly higher than without amino acids biostimulant. The growth-stimulating effects of amino acids linked to an increase in the content of phytohormones (IAA and gibberellins) are responsible for the increase in plant leaf diameter. Auxin cooperates with gibberellins to promote cell division, growth, and elongation by activating cell wall structural proteins (Kou et al., 2021), positively affecting plant growth, including leaf diameter. Furthermore, providing amino acid biostimulants can increase the availability and absorption of nutrients, especially nutrient N, which plants require to stimulate their vegetative growth.

Table 2
Plant height, the number of leaves, and leaf diameter of shallot 35 days after planting

Amino acids biostimulant dose (L/ha)	Plant height (cm)	Number of leaves	The leaf diameter (mm)
0	38.04 ± 1.78 a	20.38 ± 1.60 a	3.93 ± 0.10 b
0.5	38.85 ± 1.46 a	22.42 ± 1.63 a	4.05 ± 0.14 ab
1	43.88 ± 1.19 a	25.17 ± 2.19 a	4.62 ± 0.22 a
2	39.88 ± 1.95 a	20.42 ± 2.23 a	4.37 ± 0.18 ab
Application method			
Foliar	41.25 ± 1.21 p	21.88 ± 1.37 p	4.38 ± 0.15 p
Soil	39.07 ± 1.22 p	22.31 ± 1.46 p	4.10 ± 0.11 p
Interaction	(-)	(-)	(-)
CV (%)	12.20	21.84	11.25

Note. The Tukey's test at 5% revealed no significant differences between means that were followed by the same letter in columns; (-) = No interaction between treatment

The highest leaf area was found on the application of a 1 L/ha amino acids biostimulant dose. Meanwhile, in the application method, the highest leaf area was found through the leaves (Figure 5). Leaves are plant organs that play an essential role as a place for photosynthetic activity and transpiration and as light receptors. Studies revealed that the nature of the biostimulants that induced plant growth was related to the expansion of the leaf surface (Calvo et al., 2014) and improved biochemical, thus raising metabolism in plant tissues (Sadak et al., 2014).

The highest net assimilation and crop growth rates were found at amino acids biostimulant dosage of 1 L/ha. Meanwhile, in the application method, the highest net assimilation and crop growth rates were found through the leaves (Figure 6). According to Khan et al. (2019), applying amino acids to leaves increased photosynthesis efficiency and lettuce growth. The higher crop growth rate in plants can be due to the biostimulant composition, which contains various stimulants such as amino acids that can act as signaling molecules to improve fertilizer assimilation (Sheng et al., 2020). Amino acids are crucial for the growth and development of horticultural crops (du Jardin, 2015). Amino acids and proteins are the main components of living cells and play a crucial part in many cells' metabolic processes. Amino acid biostimulants greatly affect morphology, physiology, and biochemistry (Tadros et al., 2019).

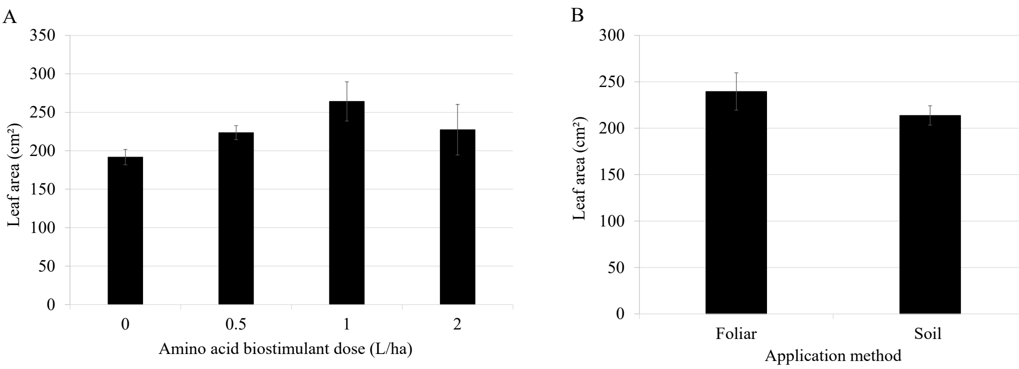

Figure 5. The leaf area of shallot 63 days after planting based on (A) amino acids biostimulant dose and (B) application method

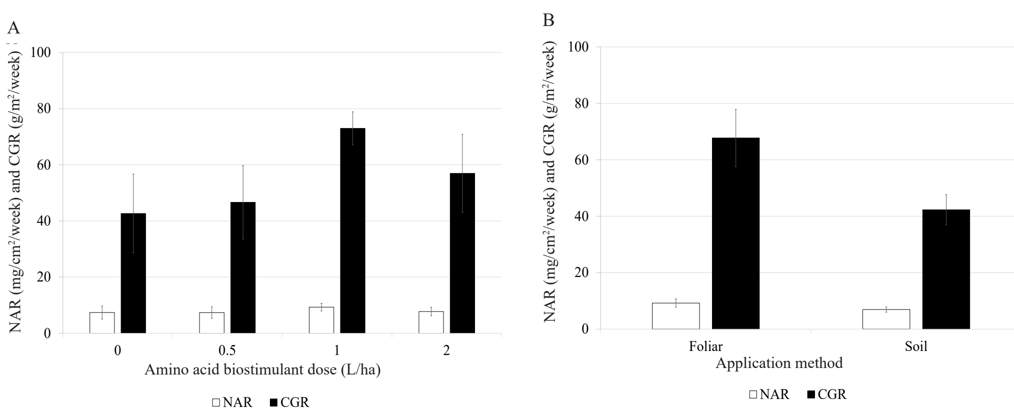

Figure 6. Net assimilation rate (NAR, mg/cm²/week) and crop growth rate (CGR, g/m²/week) of shallot at 5–9 weeks after planting based on (A) amino acids biostimulant dose and (B) application method

The growth parameters show no interaction between the dose of amino acid biostimulants and the application method on root dry weight, leaf dry weight, bulb dry weight, and total dry weight (Table 3). The dosage of amino acids biostimulants and the application method individually had the same effect on root and bulb dry weights. Meanwhile, the dose of amino acids biostimulants significantly affected leaf dry weight and total dry weight. Amino acids biostimulant dose of 1 L/ha resulted in significantly higher leaf dry weight and total dry weight compared to without the amino acids biostimulant. The increased dry weight of a plant occurs due to the formation of photosynthates into biomass stored in the plant body. This outcome is relevant to Navarro-León et al. (2022), where the application of 60 and 30% N fertilizer reduced the value of leaf biomass of lettuce, whereas adding amino acids biostimulants was able to improve both the fresh and dry weight of the leaves.

Table 3
Root dry weight, leaf dry weight, bulbs dry weight, and the total dry weight of shallot 63 days after planting

Amino acids biostimulant dose (L/ha)	Root dry weight (g)	Leaf dry weight (g)	Bulbs dry weight (g)	Total dry weight (g)
0	0.14 ± 0.01 a	1.41 ± 0.11 b	5.29 ± 1.46 a	7.01 ± 1.53 b
0.5	0.15 ± 0.02 a	1.83 ± 0.09 ab	6.72 ± 2.27 a	8.82 ± 2.40 ab
1	0.31 ± 0.13 a	2.13 ± 0.16 a	7.46 ± 0.85 a	10.23 ± 0.94 a
2	0.14 ± 0.03 a	1.86 ± 0.25 ab	6.76 ± 1.56 a	9.10 ± 1.55 ab
Application method				
Foliar	0.23 ± 0.07 p	1.88 ± 0.16 p	7.01 ± 1.13 p	9.35 ± 1.20 p
Soil	0.14 ± 0.01 p	1.73 ± 0.09 p	6.11 ± 1.10 p	8.22 ± 1.16 p
Interaction	(-)	(-)	(-)	(-)
CV (%)	10.82	15.70	14.81	18.06

Note. The Tukey's test at 5% revealed no significant differences between means that were followed by the same letter in columns; (-) = No interaction between treatment

The yield parameter indicates no interaction between the dosage of amino acids biostimulant and the application method on the number of bulbs per plant, harvest index, and the productivity of shallot. The dosage of amino acids biostimulants and the application method individually had the same effect on the harvest index. Applying an amino acids biostimulant dose of 1 L/ha was significantly higher in the number of bulbs and productivity than without an amino acids biostimulant. Meanwhile, it has the same effect based on the application method, both through leaves and soil (Table 4). Several studies reported that applying amino acids biostimulant had positive effects on the yield of vegetable crops such as lettuce (Bulgari et al., 2019), basil (Noroozlo et al., 2020), and mint (Tarasevičienė et al., 2021).

Inorganic fertilizer is ideal for plant cultivation because it provides nutrients for plants in the early stages of growth. The application of amino acid-based biostimulants (Amiboost®) could provide nutrition to the end stages of growth and positively affect yield with only 50% inorganic fertilizer. This result related to Klokić et al. (2020) that the effects of amino acid-based biostimulants increased

Table 4
The number of bulbs per plant, harvest index, and productivity of shallot

Amino acids biostimulant dose (L/ha)	The number of bulbs per plant (cm)	Harvest index	Productivity (ton/ha)
0	3.13 ± 0.35 b	0.65 ± 0.09 a	10.90 ± 1.12 b
0.5	4.88 ± 0.44 ab	0.67 ± 0.06 a	11.55 ± 0.86 b
1	5.63 ± 0.73 a	0.72 ± 0.03 a	16.46 ± 1.02 a
2	4.50 ± 0.82 ab	0.70 ± 0.06 a	11.87 ± 0.74 b
Application method			
Foliar	4.56 ± 0.56 p	0.67 ± 0.05 p	13.01 ± 0.83 p
Soil	4.50 ± 0.39 p	0.70 ± 0.03 p	12.38 ± 0.88 p
Interaction	(-)	(-)	(-)
CV (%)	27.14	27.46	22.01

Note. The Tukey's test at 5% revealed no significant differences between means that were followed by the same letter in columns; (-) = No interaction between treatment

plant growth and yield in conventional nutrition and prevented yield loss in low-input nutrition. Furthermore, Koleška et al. (2017) stated that the yield of tomatoes in two varieties improved by applying biostimulants containing amino acids up to 14 and 13% by reducing the amount of NPK nutrition. Kocira (2019) stated that biostimulants with free amino acids and *Ascophyllum nodosum* extracts increased soybean yields by up to 25%. Applying amino acids biostimulant (Perfectose) through the leaves enhanced fresh yield by up to 39%, dry yield by up to 55.3%, and the number of leaves by up to 22.8% of lettuce (Al-Karaki & Othman, 2023).

CONCLUSION

Application of amino acids-based biostimulants (Amiboost®) 0.5−2 L/ha to shallot plants with 50% reduced NPK fertilizer has positive effects on enhancing nutrient acquisition and assimilation (high N, P, K, B, and Mn accumulation), as well as an increased IAA, gibberellin, kinetin, zeatin, NRA, and chlorophyll. Amino acid-based biostimulant 1 L/ha was the best dosage to increase leaf diameter by 18%, plant growth rate by 42%, leaf dry weight by 56%, total dry weight by 30.88%, number of bulbs by 5.63 per plant (44%), and productivity by 16.46 ton/ha (33.77%). The application method through leaves increases NRA, leaf area, and crop growth rate. It was indicated that using amino acid-based biostimulant 1 L/ha through leaves makes it possible to reduce inorganic fertilizer with high growth and productivity of shallot.

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