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Use of Biostimulants to Reduce Chemical Fertilizers of Shallot Plants

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Abstract The use of biostimulants from endophytic bacteria enriched with seaweed is still rarely used in shallot plants. This study was conducted to determine the effect of reducing NPK chemical fertilizers and adding biostimulants to shallot plants in the highlands. The research was conducted in Lembang, Indonesian Vegetables Research Institute, Indonesia (IVEGRI) from January to July 2021. A two-factor Randomized Complete Block Design used two types of biostimulant formulation (biostimulant A, and biostimulant B), and the chemical fertilizer dose factors (7 levels with 3 replications. The observed parameters included plant height, number of leaves, clump fresh weight and dry weight of bulbs per sample and per hectare, as well as the Relative Agronomic Effectiveness (RAE). The results showed that the application of 75% NPK plus biostimulant at a dose of 3ml/L applied as much as 5X gave the same RAE value as NPK 100%.

Keywords Biostimulant, Chemical, Endophytic-Bacteria, Fertilizer, Shallot

1. Introduction

Shallots (*Allium ascalonicum* L.) are one of Indonesia's main vegetable crops besides chili, potato and garlic. Shallots have high economic value [1], and have many benefits as spice and as traditional medicine because it contains the amino acid *Alliin* which functions as an antibiotic. [2], as natural pesticides and others.

The potential and benefits of shallots cause the demand for shallots was increased along with the increasing population of Indonesia. The increasing demand was not matched by the production; Hence, the excess demand was met by imports. The high volume of shallot imports is due to the fact that shallots in Indonesia are still seasonal yield. Therefore, the demand for shallots in certain seasons is met by imports. Shallots import activity makes Indonesia a considerable importer of shallots [3]. The production of shallots shows a positive development towards the demand for shallots over time. Indonesian shallot production in 2020 is 1,815,445 tons/ha, increasing in 2021 to 2,004,590 tons/ha [4]. Therefore, a more significant increase in shallot production is needed.

One of the efforts to increase the growth of shallot plants is the utilization of biostimulants. Biostimulants are natural or synthetic organic compounds, and are not fertilizers, which can promote growth [5]–[7] and improve plant response to environmental stress [8]–[12]. The utilization is able to stimulate and modify plant physiological processes such as respiration, photosynthesis, nucleic acid synthesis, and absorption of ions [13]. According to Calvo et al. [14] there are several sources of biostimulants that have been developed in agriculture, including microbial inoculants such as bacteria [12], [15]–[22] or fungus [23]–[25]), humic acid [26]–[32], fulvic acid [32], [33], amino acid [34]–[37], seaweed extract [31], [38]–[43], microalgae [44] and plant extract [45]–[48]. These biostimulants can be used separately or in consort with more than one type of biostimulant [31], [32], [49], [50].

The use of seaweed to stimulate plant growth has been widely carried out, such as in the tagetes [43], dates [51], okra [52], sugarcane [10], beans [41], [53]–[55], tomato [42], [56], mustard [38], chilli [49], [57], [58], shallot [59], [60], etc.

Biostimulant from seaweed extract contains nutrients, amino acids, cytokinins, auxins, laminaran, fucoidan, alginate, and betaine which stimulate plant metabolism, therefore it can increase plant growth and yield. [60] and can also prevent disease in plants [39], [61]. *Sargassum* sp. seaweed has bioactive compounds that can function as antibacterial compounds, such as flavonoids, saponins, tannins, and phenols. [62]. Several studies have shown that the use of seaweed biostimulants can increase plant growth and productivity. Kumar and Sahoo reported that the use of liquid seaweed extract with a concentration of 20% on wheat plants has increased plant growth [63]. According to Vijayanandh et al. [54] the use of liquid extract of *Sargassum wightii* type seaweed with a concentration of 1.5% has increased the growth of beans According to Azri [60] seaweed extract was able to increase the weight of tubers or clumps and the production of shallots by 3.86 grams and 2.99 tons per ha, respectively. Furthermore, it is known that the response of plants to seaweed is influenced by the type of seaweed and the plants or varieties used [49]. *Ulva lactuca* seaweed has a better effect than *Sargassum* on chili plants [49]. Therefore, in this current study only *Ulva lactuca* was used.

Another effort to improve the growth of shallot plants is the addition of endophytic microbes from the type of endophytic bacteria [64], [65]. Endophytic microbes are microbes that live in plant tissues, and have a symbiotic mutualism with their host plants. Endophytic microbes obtain nutrients from plant metabolism and protect plants against herbivores, insects, or pathogenic tissues [66]. Endophytic microbes have the potential to stimulate plant growth by producing phytohormones such as IAA, GA3, and cytokinins. Gusmaini et al stated that endophytic bacteria had a positive effect in increasing growth, production of fresh and dried herbs, and andrographolide production in bitter plants compared to controls [67]. The use of beneficial bacteria as biostimulants in various plants has also been carried out, including rice or corn [68]–[72], wheat [30], blackpepper [73], white turmeric [74], soybean[75], moringa [46], tea plant [76], carnation [77] lettuce [78], strawberry [79], eggplant [80], chilli [49], [65], [81]–[86], tomatoes [87]–[89], cucumber [12] garlic [90], shallot [19]–[21], [91], [92].

Several ways to use various types of biostimulants in shallot plants are using growth regulators [25], [34]–[36], [93]; using bacteria [19]–[21], [91], [92], fungus [25], seaweed [59], [60]. A combination of biostimulants based on seaweed and bacteria has been carried out on tomato plants [50] and chilli [49], however, information regarding the use of this combination in shallots is still very limited. Through this research, it is hoped that information about the effect of using seaweed biostimulants enriched with endophytic bacteria on shallots cultivated by reducing chemical fertilizers can be obtained.

2. Materials and Methods

This research was conducted at the Experimental Site of Indonesian Vegetable Research Institute (IVEGRI) in Lembang, West Bandung, West Java, Indonesia from January to July 2021. This research used a Randomized Complete Block Design by using two types of biostimulant formulation namely (biostimulant A, and biostimulant B), and fertilizers as treatment, with treatment details as followed:

1. Control
2. NPK 75% + biostimulant 3ml/L (3X)
3. NPK 75% + biostimulant 3ml/L (5X)
4. NPK 75% + biostimulant 5 ml/L (3X)
5. NPK 75% + biostimulant 5 ml/L (5X)
6. NPK 75% + biostimulant 10 ml/L (3X)
7. NPK 100%

The application of biostimulants was carried out at the time according to the treatment. The used material was shallot *Trisula* variety. Shallot bulbs were planted in silver black mulched beds with a spacing of 20 x 20 cm. The observed parameters included:

1. Plant height. Plants were measured from the ground surface to the growing point (Saputra, 2016) on 3, 5, and 7 WAP.
2. Number of leaves. Counted on 3, 5, and 7 WAP.
3. Clump fresh weight. Plants aged 6 WAP were removed and cleaned from the soil and then weighed.
4. Dry weight. The dry weights of bulbs and plant stoves were weighed after drying in an oven at 80^{o}C until the dry weight was constant.
5. Relative Agronomic Effectiveness (RAE), was counted with formulas :

$$\mathrm{RAE} = \frac{\text{tested fertilizer yield} - \text{control}}{\text{Standard fertilizer yield} - \text{control}} \times 100$$

RAE score ≥ 100% declared that the tested fertilizer was effective compared to standard treatment.

For the data analysis, the collected data were tabulated and analyzed for variance using the SPSS program and further tested using Duncan test if there were significant differences in the treatments tested.

3. Results and Discussions

The results of plant vegetative growth (plant height and number of leaves) at 3 and 5 WAP (weeks after planting) are shown in Table 1 and Table 2. Shallot was planted on March 29th, 2021 and the application of biostimulant formula (spraying) was 3 or 5 times. In general, biostimulants A and B gave different responses when combined with fertilizer treatments for plant height, number of leaves, and dry weight. This was because the type or dose of biostimulant gave a different response even when combined with the same fertilizer. Tuhuteru's research [21] stated that the amount of growth hormone (IAA) produced by shallots depends on the type of bacteria given and the shallot variety used.

Plant height at 100% NPK treatment was not significantly different from plant height on NPK 75% with biostimulant treatment, both biostimulant A and biostimulant B formulas at 3 WAP. The treatment of 75% NPK with biostimulant application showed relatively better plant height than 100% NPK and complete control (100% NPK). After observation at 5 WAP, it was shown that the application of biostimulant formula, precisely in the 75% NPK treatment at a dose of 5 ml/l and applied 5 times, resulted in the highest plant height at 38.1 cm. Meanwhile, the treatment of biostimulant B formula that was applied at a dose of 3 ml/L for 5 times resulted in higher plant height than the standard and control NPK. However, in statistical analysis, plant height at 5 WAP in the treatment that gave the highest plant height showed no significant difference from the control (Figure 1).

These results were in line with the results of Hadiawati's research [25] that Trisula variety of shallot with Trichoderma treatment showed a significant difference in plant height with the control at 40 days after planting (DAP) and 60 DAP, while for 20 DAP it was not significantly different from the control. Furthermore, it was said that the plant height of the control was 26.94 cm and 31.57 cm at 20 DAP and 40 DAP respectively. This figure was not much different from the results of this study which produced a plant height of Trisula variety 26.8 cm and 38.0 cm at 3 and 5 weeks after planting (WAP) respectively. The increase in plant height of shallot with biostimulant treatment compared to the control in this study was not as high as the increase in plant height in the research conducted by Hadiawati which could increase 50% higher than the control. This might be due to differences in the soil of the planting location where in Hadiawati's research the planting location was at an altitude of 52 m above sea level (asl), alluvial soil with a sandy loamy texture with a neutral pH (6.52) and temperatures ranging from 20-33 with an average temperature of 35. Whereas in this study, the planting location was in the highlands of 1250 m asl, andisol soil with a dusty loamy texture. Shallots are bulbous plants that grow well in sandy textured soil, required full light and grow well in lowlands. This caused the height of shallot plants in the highlands to be less responsive than when planted in the lowlands.

Table 1. Shallot plant height on biostimulant and fertilizers treatment

No	**Treatment**	**Plant height of shallot plant (cm)**			
		Biostimulant A		**Biostimulant B**	
		3 WAP	5 WAP	3 WAP	5 WAP
1.	Control	26.8 a	38.0 a	30.1 a	36.9 a
2.	NPK 75% 3ml/L (3X)	28.1 a	36.4 b	30.0 a	37.2 ab
3.	NPK 75% 3ml/L (5X)	26.8 a	35.9 b	30.2 a	**38.3 a**
4.	NPK 75% 5 ml/L (3X)	27.2 a	37.5 a	31.4 a	36.5 b
5.	NPK 75% 5 ml/L (5X)	27.1 a	**38.1 a**	30.5 a	37.7 ab
6.	NPK 75% 10 ml/L (3X)	25.2 a	35.5 b	30.7 a	38.5 a
7.	NPK 100%	25.6 a	34.0 c	29.6 a	37.2 ab

Note: numbers followed by the same letter were not significantly different at the 5% level of Duncan test.

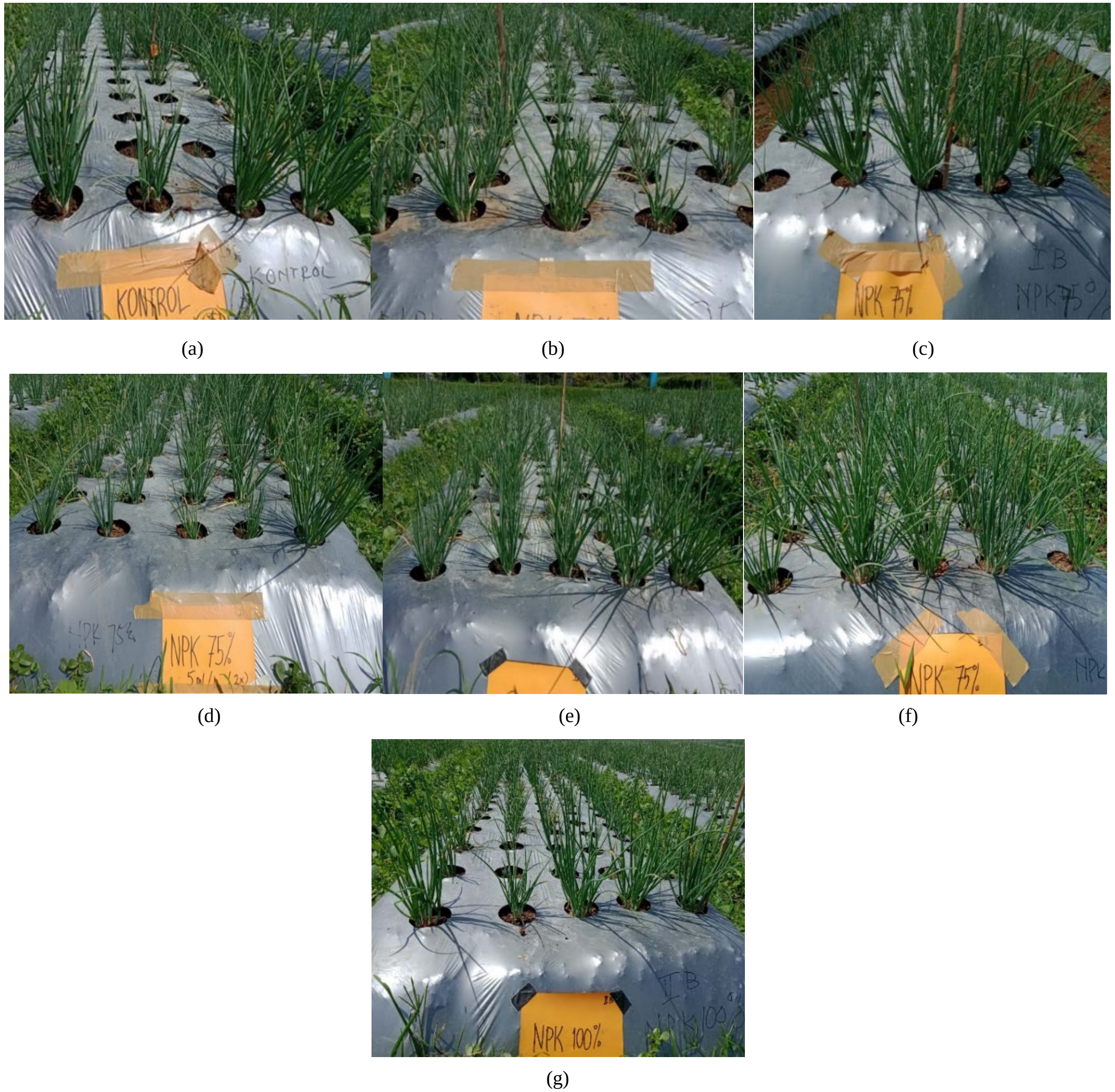

Figure 1. Appearance of shallot plants with treatment : (a) Control; (b) NPK 75% 3ml/L (3X); (c) NPK 75% 3ml/L (5X); (d) NPK 75% 5 ml/L (3X); (e) NPK 75% 5 ml/L (5X); (f) NPK 75% 10 ml/L (3X); (g) NPK 100%

Based on Table 2, it showed that the number of leaves per plant at the observation of 5 WAP on 75% NPK treatment combined with biostimulant A at the dose of 3 ml/L and applied 3 times, and treatment of biostimulant B at the dose of 3 ml/L and applied 5 times combined with 75% NPK had the highest number of leaves compared to the control and standard 100% NPK.

This result was different from the results of Hadiawati's research [25] which stated that the number of shallot leaves Trisula variety at 40 DAP was not significantly different between all treatments tested. The number of leaves looked significant at 60 DAP. Trichoderma and chemical fertilizer treatments had more leaves than other treatments. The number of leaves in this study ranged from 27.3 - 36.0 at 5 WAP, which was higher than the number of leaves in Hadiawati's study at 40 DAP which ranged from 22.47 - 27.67. This might be due to the soil in the highlands is more fertile than in the lowlands so that the growth of leaves was more.

In Table 3, it showed that the combination treatment of inorganic fertilizer with biostimulant A and the combination of inorganic fertilizer with biostimulant B gave a significant effect on the total dry weight of 10 shallot plant clumps. All treatments showed that the dry weight values of 10 shallot plant clumps were higher than the control. The dry weight of 10 shallot plant clumps from standard NPK treatment was not significantly different from the combination treatment of inorganic fertilizers with both biostimulant formula A and formula B. This was in line with the results of Aini's research [19] which found that the dry weight of shallot plants increased with the addition of Rhizobacteria.

Table 3 showed the same RAE value between RAE value on the treatment of 75% NPK with biostimulant A or B at the dose of 3 ml/L for 5 times application and RAE value on the standard NPK treatment (RAE value was 100%). These results indicated that the combination treatment was effective on the growth and yield of the shallot Trisula variety.

Table 2. Leaves number of shallot on biostimulant and fertilizers treatment

No	Treatment	Number of leaves at 5 WAP	
		Biostimulant A	Biostimulant B
1.	Control	27.3 d	33.3 b
2.	NPK 75% 3ml/L (3X)	**36.0 a**	36.0 ab
3.	NPK 75% 3ml/L (5X)	33.7 b	**37.7 a**
4.	NPK 75% 5 ml/L (3X)	33.7 b	36.0 ab
5.	NPK 75% 5 ml/L (5X)	30.7 c	34.7 ab
6.	NPK 75% 10 ml/L (3X)	34.0 b	35.0 ab
7.	NPK 100%	33.3 b	35.0 ab

Note: numbers followed by the same letter were not significantly different at the 5% level of Duncan test.

Table 3. Dry weight of shallot and RAE on biostimulant and fertilizers treatment

No	Treatment	Dry weight of shallot bulbs (kg)			
		Biostimulant A		Biostimulant B	
		Dry weight/ 10 clumps	RAE (%)	Dry weight/ 10 clumps	RAE (%)
1.	Control	0.66 c		0.56 d	
2.	NPK 75% 3ml/L (3X)	0.75 a	69.2	0.66 ab	83.3
3.	NPK 75% 3ml/L (5X)	**0.78 a**	**92.3**	**0.68 a**	**100.0**
4.	NPK 75% 5 ml/L (3X)	0.68 c	15.4	0.63 bc	58.3
5.	NPK 75% 5 ml/L (5X)	0.74 ab	61.5	0.63 abc	58.3
6.	NPK 75% 10 ml/L (3X)	0.69 bc	23.1	0.61 c	41.7
7.	NPK 100%	**0.79 a**	**100.0**	**0.68 a**	**100.0**

Note: numbers followed by the same letter were not significantly different at the 5% level of Duncan test

Table 4. Dry Weight of Shallot Bulbs on Biostimulant A and Biostimulant B Treatment

No	Treatment	Dry weight of shallot bulbs (ton/ha)	
		Biostimulant A	Biostimulant B
1.	Control	3.0 d	2.6 c
2.	NPK 75% 3ml/L (3X)	3.7 ab	3.6 ab
3.	NPK 75% 3ml/L (5X)	**3.8 a**	**3,9 a**
4.	NPK 75% 5 ml/L (3X)	3.2 bc	3.7 a
5.	NPK 75% 5 ml/L (5X)	3.1 c	3.1 bc
6.	NPK 75% 10 ml/L (3X)	3.1 c	3.8 ab
7.	NPK 100%	**4.0 a**	**3.9 a**

Note: numbers followed by the same letter were not significantly different at the 5% level of Duncan test

The shallot production (Table 4) showed that the application of NPK fertilizer combined with biostimulant A or biostimulant B was not significantly different with 100% NPK fertilizer only. The use of biostimulants with a dose of 75% NPK with biostimulant A or B at the dose of 3 ml/L for 5 times application could reduce the use of NPK by 25%. This was in line with the results of Rokhminarsi's research [94] which stated that using Azola mycorrhiza on shallots can reduce 40% of chemical fertilizers. So the use of biostimulants could be recommended to reduce the use of synthetic chemical fertilizers on shallot.

4. Conclusions

The use of NPK 75% 5 ml/L (5X) of biostimulant A resulted in the highest plant height, while the treatment of biostimulant B applied at NPK 75% 3ml/L (5X) resulted in higher plant height than the control NPK treatment. The biostimulant A at application with NPK 75% 3ml/L (3X) and the biostimulant B with NPK 75% 3ml/L (5X), resulted in the highest number of leaves. The heaviest dry weight of 10 shallot plant clumps of both biostimulant formula A and formula B were not significantly different from the combination treatment of control NPK treatment. The treatment of biostimulant B with NPK 75% 3ml/L (5X) gave the same RAE value with NPK 100%. It could reduce 25% of the use of NPK fertilizer. However, there were no significant differences between both biostimulants at NPK 75% 3ml/L (5X) to the NPK control treatment for the shallot's dry weight.

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