

Application Techniques of Photosynthesis Bacteria and Its Effect on The Growth and Yield of Local Bantul Shallot Variety (*Allium cepa* **var. aggregatum cv. Crok Kuning)**

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ABSTRACT

Shallots represent a pivotal commodity catering to household needs in Indonesia; however, their production has failed to meet the escalating demand. Consequently, technological interventions to enhance production are imperative, with one promising opportunity being the application of photosynthetic bacteria (PSB). One may apply PSB through direct soil infusion or foliar spraying. This study seeks to elucidate the differential impacts of varied PSB application techniques on the growth and yield of the local Bantul shallot variety. Conducted from September to December 2022. the research employed a Complete Randomized Block Design (RCBD), incorporating a fertilization factor with four tiers: absence of fertilizer, NPK fertilizer 16:16:16 + PSB via pouring, NPK fertilizer 16:16:16 + PSB via spraying, and NPK fertilizer 16:16:16. Each treatment underwent ten replications. Subsequent to data acquisition, variance analysis was employed, followed by an honestly significant difference test (HSD Tukey) at a 5% error rate. Outcomes revealed that PSB provision led to heightened root length, chlorophyll content, nitrate reductase activity, fresh and dry weights of roots and shoots, bulb count per clump, fresh and dry weights of bulbs per clump, and overall productivity. The optimal PSB application technique, identified as pouring into the growing media, resulted in a significant 31.28% improvement in shallot productivity.

Keywords*: Photosynthetic bacteria, EPS, ALA, Rhodobacter, Siderophore*

1. INTRODUCTION

Shallots constitute a vital commodity and a staple for the Indonesian populace. However, within the context of Indonesia, shallots face the challenge of increasing demand outpacing production capacity. The demand and consumption of shallots in Indonesia exhibit a 1.18% annual growth rate. Notably, the Indonesian populace's per capita consumption of shallots reached 27.72 kg/capita/year in 2019 (Indonesian Central Agency of Statistics, 2019).

In 2021. the shallots production in Bantul Regency achieved a volume of 169.008 quintals. Nevertheless, as of now, this augmented production has exclusively addressed the local market demands within Bantul, falling short of meeting the broader needs of the entire province (Rahmawati, 2022). The shallots production in the Yogyakarta region remains seasonal, akin to agricultural yields in general. This condition leads to an inability to meet the demand for shallots among consumers outside of the harvest season, necessitating importation to fulfill requirements (Fauzan, 2016). Farmers in Bantul face issues accessing modern farming tech, relying on less efficient traditional methods. Inconsistent irrigation worsens instability, especially outside rainy seasons. Climate change adds to the struggle with rising temperatures and unpredictable rainfall, disrupting crop growth and lowering yields (Triyono et al., 2021). One viable intensification approach to augment shallot production involves the utilization of local varieties with high production potential, such as Crok Kuning, coupled with the application of beneficial microorganisms. Photosynthetic bacteria are a promising alternative to enhance plant photosynthesis, leading to increased production.

Photosynthetic bacteria (PSB) are phototrophic bacteria capable of performing photosynthesis under both oxygenic and non-oxygenic conditions (Lee et al., 2021). Photosynthetic bacteria are widely distributed in aquatic ecosystems such as lakes, seas, rivers, sediments, moist soils, and environments characterized by high salinity (Takeuchi & Numata, 2019). Species of photosynthetic bacteria that have been extensively cultivated include *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris*. The capabilities of these bacteria stem from the presence of various pigments, encompassing chlorophyll, carotenoids, and their derivatives. Photosynthetic bacteria exhibit versatility, thriving in aerobic and anaerobic environments, and demonstrating the capacity to utilize organic and inorganic substances for nitrogen (N_2) and carbon dioxide (CO_2) fixation. (George et al., 2020).

Several studies indicate that applying Photosynthetic Bacteria (PSB) can enhance plant resilience to abiotic stressors, such as salinity in maize crops (Feng et al., 2019), salinity in green beans (Talaat, 2019) and flooding in soybeans (Kang et al., 2021). PSB is also reported to mitigate plant stress levels under biotic constraints. A study conducted by Su et al. (2019) demonstrated that the inoculation of tobacco plants with the bacterium Rhodopseudomonas palustris GJ-22 enhanced resistance to tobacco mosaic virus. In a parallel study conducted by Nookongbut et al. (2020), it was observed that the bacterium R. palustris KTSSR54 exhibited the capability to suppress pathogenic agent growth responsible for brown spot (*Bipolaris oryzae*), black leaf spot (*Curvularia lunata*), and blast (*Magnaporthe oryzae* Anamorph *Pyricularia oryzae*) in rice plants. Yu et al. (2022) noted that the bacterium Rhodobacter sphaeroides produces isoprene, inhibiting Gram-positive bacteria's growth. Several PSB varieties, functioning as Plant Growth-Promoting Rhizobacteria (PGPR), also demonstrate the capacity to produce other secondary metabolites, such as lipopeptides, siderophores, and Extracellular Polymeric Substances (EPS), which effectively suppress fungal pathogens (Andreolli et al., 2019; Faria et al., 2020).

In its development, Photosynthetic Bacteria (PSB) has become widely utilized as a biofertilizer in agriculture, as it functions as plant growth that may promote rhizobacteria (PGPR). The direct application of PSB into the soil or foliar onto plant canopies is implemented to enhance soil fertility and increase overall crop production (Naik et al., 2020; Surachat et al., 2022). According to Tuhuteru et al. (2017), PGPR application in sandy coastal land shows promise for improving shallot growth and yield, with specific combinations of cultivars and bacteria strains proving the most effective. A similar finding by Agung and DIara (2019) study, direct soil application of biostimulants significantly increased shallot growth and yield in sandy coastal land. PGPR consists of Rhodopseudomonas, which has also been reported to mitigate the sensitivity of shallot plants to drought stress. This bacteria predominantly suppresses drought stress's impact during the vegetative growth phase (Pratiwi et al., 2024).

The direct application of *Rhodobacter sphaeroides* to other plants effectively increases yield. Application of *Rhodobacter* sp. as biofertilizer in pineapple plants has been reported to enhance soil phosphorus (P) availability by 25.3-33.9%, elevate plant height by 3.56-4.10%, and increase yields by up to

12.1%, all while requiring a reduced amount of phosphorus fertilizer (Huu et al., 2022). Another study observed that applying photosynthetic bacteria *R. palustris* combined with straw to the soil increased sesame seed yield. This enhancement was attributed to the mechanism of providing high quantities of nitrogen (N) and phosphorus (P) in the soil (Khuong et al., 2023). The capability of PSB bacteria, when applied to the soil, lies in their capacity to produce siderophores, rendering ions NH_4^+ dan $PO₄³⁻$ readily available (Nookongbut et al., 2019). PSB also exhibits the capability to synthesize indole-3-acetic acid (IAA) hormone and 5-aminolevulinic acid (ALA) to stimulate plant growth (Khuong et al., 2020).

The study by Xu et al. (2016) revealed that when subjected to foliar application of R. palustris bacteria, stevia plants exhibited an increase in photosynthetic rate and biomass. In Chinese kale plants, the foliar application of PSB has been associated with an augmentation in leaf quantity and surface area (Panunggul, 2023). Rice plants subjected to foliar application of PSB also demonstrated increased productive tiller count, chlorophyll content, root length, grain yield, 1000-grain weight, and harvest index (Yen et al., 2022). No investigations have yet been undertaken to compare the varied application techniques of PSB and their consequential influence on the growth and yield of crops. Furthermore, research on PSB's effects on shallot plants remains absent in the existing literature. Consequently, the primary aim of this study is to elucidate the differential impacts of varied PSB application techniques on the growth and yield of the local Bantul shallot variety, specifically the Crok Kuning cultivar.

2. MATERIALS AND METHODS

2.1. Research Site and Materials

The research was conducted from September to December 2022 at the Biotech Botanical Garden Research Farm owned by PT. Biotek Cipta Kreasi (- 7° 41' 50.27", 110° 23' 10.39"). The materials employed in this study included bulbs of the Crok Kuning shallot variety (a local Bantul cultivar), NPK 16:16:16 fertilizer, Photosynthetic Bacteria (*Rhodobacter* spp. and *Rhodopseudomonas* spp.), and labels. Various tools were utilized, including a hoe, bucket, dibble, ruler, scale, beaker glass, cutter, oven, knapsack sprayer, and Spektronik 21-D Milton Roy.

2.2. PSB based *Rhodobacter* **spp. dan** *Rhodopseudomonas* **spp. Production**

The propagation of *Rhodobacter* spp. and *Rhodopseudomonas* spp. Isolates were carried out in the laboratory using an NC medium. An amount of 0.8 g NB medium was supplemented with 2 g NaCl. The medium was homogenized and sterilized using an autoclave at a 121 ºC temperature and a 1 atm pressure for 15 minutes. Subsequently, the medium was retrieved and cooled. The two bacterial isolates were then inoculated into NC + NaCl 2 g medium and incubated with incandescent light for approximately 4 days. Once the starter was ready, 10 mL was extracted for further cultivation. Nitrogen and carbon sources (malic acid and sodium glutamate) were introduced into a container containing 100 mL of RO water. After thorough mixing, 10 mL of *Rhodobacter* spp. and *Rhodopseudomonas* spp. Bacterial starters, respectively, were added to the container and stirred until homogeneous (non-aggregated). Following uniform mixing, the container was sealed and placed in a light-exposed area for 5 days.

From the 100 mL starter, RO water was added again until reaching 1 L and exposed to light for an additional 5 days. Subsequently, RO water was added to the 1 L starter until reaching 20 L, and it was again exposed to light for 5 days until the PSB color turned reddish.

2.3. Research Design

The initial stages of the research encompassed the creation of treatment beds, seed selection, seed incision, and seed planting. Treatment beds were constructed with 1 m in width and 5 m in length dimensions. Concurrently, seed selection for the Crok Kuning shallot variety involved choosing large-sized seeds devoid of pathogenic elements. The selected seeds underwent incision at their growing points and were allowed to undergo overnight incubation. Planting involved burying the bulbs to threequarters of their depth. Routine care procedures included watering and weed management.

The research adopted a Complete Randomized Block Design (RCBD) with a single factor comprising the type of fertilization. The fertilization factor consisted of four levels: no fertilization (P0), NPK fertilizer 16:16:16 at a dosage of 650 kg/ha + PSB 10 mL/L applied through pouring (P1), NPK fertilizer 16:16:16 + PSB 10 mL/L applied through foliar spraying (P2), and only NPK fertilizer 16:16:16 at a dosage of 650 kg/ha. Each experimental unit was replicated ten times, resulting in 40 experimental units. NPK fertilizer application was performed as basal fertilization (-7 days after planting) at 14. 21. and 35 days after planting. PSB application was carried out weekly at 10 mL/L. The pouring treatment (P1) involved evenly irrigating PSB onto the shallot roots, providing approximately 10 mL of PSB per plant. The foliar spraying treatment (P2) was executed by uniformly spraying PSB onto the canopy using a hand sprayer. Observational parameters included root length, fresh root weight, dry root weight, leaf count, plant height, chlorophyll A and B content, nitrate reductase enzyme activity, fresh shoot weight, dry shoot weight, bulb count per clump, fresh bulb weight per clump, dry bulb weight per clump, and overall productivity.

Figure 1. Research Flow Diagram

2.4. Chlorophyll Content Analysis

The chlorophyll content of the leaves was measured using the method developed by Coombs et al. (1985). The procedure commenced with carefully selecting the central part of red shallot leaves, weighing precisely 1 g, and then grinding the leaves within a mortar. Subsequently, the crushed material was combined with 20 ml of an 80% acetone solution. The resulting mixture was gently ground once more, filtrated using Whatman filter paper, and then transferred to a reaction tube. The absorbance of the solution was measured using a Milton Roy Spektronik 21D spectrophotometer at targeted wavelengths of 645 um and 663 um, corresponding to the absorption peaks of chlorophyll a and b. The concentrations of chlorophyll a and b were determined using the following formula:

Chlorophyll A (mg g^{-1}) = (0.0127 x A663– $0.00269 \times A645 \times 20 \text{ mL}$ (1)

Chlorophyll B (mg g^{-1}) = (0.0229 x A645– $0.00468 \times A663$) \times 20 mL (2) **2.5. Nitrate Reductase Activity Analysis**

189 Nitrate Reductase Activity (NRA) was observed once, precisely at 35 Days After Planting (DAP). The central portion of red shallot leaves was sampled, weighing 0.2 g, and cut into square pieces with a 1 mm width. These cut samples were placed in light-proof plastic tubes and supplemented with 5 mL of 0.1 M phosphate buffer solution. The plastic tubes were then stored in a lightprotected chamber for 24 hours. After this incubation period, 0.1 mL of 0.05 M $NaNO₃$ solution was added to the tubes, followed by another 2-hour incubation in a light-protected environment at room temperature. Meanwhile, reaction tubes were prepared, adding 0.2 mL of 1% sulfanilamide solution and 0.2 mL of 0.02% N-ethyl-1-naphthylamine solution. After the incubation, 0.1 mL of the solution was extracted into the prepared reaction tube and allowed to stand for 10–15 minutes. The solution's color would be pink, indicating nitrate reductase activity. Following this color change, 2.5 mL of distilled water was added to the reaction tube, bringing the total volume to 3 mL, and then mixed thoroughly. The resulting solution's absorbance was read at a wavelength of 540 nm using a spectrophotometer. NRA levels were expressed in micromoles of NO_2^{-1} g/hour, calculated using the formula: $NRA = ASxA0xFWxT \mu mol NO₂/g/hour$ (3)

- $AS =$ absorbance at 540 nm
- A0 = standard absorbance
- $FW = lead$ fresh weight
- $T =$ incubation time.

2.5. Data Analysis

The collected data were subsequently analyzed using analysis of variance (ANOVA). In the event of a significant effect revealed by the ANOVA, further analysis was conducted employing Tukey's Honestly Significant Difference (HSD) test at a significance level of 5%. Data analysis was performed utilizing R-4.3.1 software, explicitly using the Agricolae package.

3. RESULTS AND DISCUSSION

The growth resulted from the intricate interplay among genotype

factors, environmental elements, and human management practices. One pivotal environmental factor influencing growth is the presence of microorganisms in the plant rhizosphere. *Rhodobacter* spp. bacteria represent a subset of Purple Non-Sulfur Bacteria (PNSB) known for their potential role as Photosynthetic Bacteria (PSB). This bacterial type can stimulate plant growth through biofertilization and biostimulation mechanisms. (Lee et al., 2021). Table 1 illustrates that applying *Rhodobacter* spp. can stimulate root growth when administered through pouring and foliar spraying compared to treatments involving only NPK fertilizer or no fertilization. PSB bacteria applied to the soil can produce external Indole-3-Acetic Acid (IAA) hormones, which plants subsequently utilize. By accumulating external IAA, the roots of shallot plants can exhibit increased length. These research findings align with Sakpirom et al. (2017) assertion that PSB bacteria can produce ALA, IAA, and NH_4^+ , thereby playing a role in stimulating root formation. Kantha et al. (2015) also reported that rice plants subjected to salinity stress exhibited root elongation when PSB was applied foliar due to the high production of ALA.

Table 1. Growth and Physiological Parameters of Shallot Under Varied Fertilization **Treatments**

Note: The presented numbers represent the means \pm standard error. Similar letters adjacent to the mean values indicate statistically nonsignificant differences based on the Tukey Honestly Significant Difference (HSD) test (α = 5%).

When applied foliar, PSB significantly influences root length but not as effectively as when applied in the soil. These results diverge from Xu et al. (2016), who stated that stevia plants subjected to foliar PSB application still exhibited similar increases in root length as those applied to the soil. Robust root growth can stimulate the aboveground growth of shallot plants. However, in this study, no significant impact was observed from PSB application, whether through pouring or spraying, on the plant height and leaf count of red shallot. Nevertheless, PSB application affected the plant's greening level and physiological processes.

According to Table 1. the chlorophyll A content in red shallot plants administered using PSB through pouring was the highest (0.261 μ g g⁻¹ leaf), followed by treatments that did not differ significantly, i.e., PSB pouring (0.084 µg) g^{-1} leaf), NPK (0.071 µg g^{-1} leaf), and without fertilizer (0.054 μ g g⁻¹ leaf). Meanwhile, for the chlorophyll B content variable, pouring PSB had the highest effect (0.069 μ g g⁻¹ leaf), equivalent to the NPK treatment (0.045 μ g g⁻¹ leaf) and higher than the spraying PSB application $(0.025 \text{ µg g}^{-1}$ leaf) and without fertilizer $(0.023 \text{ µg g}^{-1}$ leaf). These research outcomes align with Yen et al. (2022) in rice. Plants sprayed with PSB experienced an increase in chlorophyll content due to ALA synthesized by bacteria. 5-aminolevulinic acid is a significant precursor to chlorophyll (Wu et al., 2019). When PSB is poured into the

soil, the elevated chlorophyll content is likely attributable to higher ALA production when PSB bacteria are more active.

Moreover, Ge & Zhang's (2019) research shows that *Rhodopseudomonas palustris* strain G5 enhances cucumber seedling growth and sugar content under salt stress by boosting chlorophyll levels. In another set of results, treatment with PSB-06 demonstrated effectiveness in alleviating chlorotic symptoms in tomato leaves compared to the control, exhibiting increased chlorophyll levels at day 15 and day 45 (Nunkaew et al., 2014). Chlorophyll is the primary machinery used by red shallot plants to conduct photosynthesis. The essential component in the chlorophyll structure is nitrogen, thus necessitating an adequate external nitrogen supply for optimal photosynthesis. This result is supported by research conducted by De Oliveira Siqueira Lino et al. (2023), where *R. palustris* affects gas exchange and nitrate reductase activity in mango cv. 'Keitt' grown in the Brazilian semiarid. Nitrate reductase is an enzyme that plays a crucial role in the reduction chain of nitrate to ammonia. In this process, nitrate reductase activity transforms absorbed nitrate by the roots into nitrite, converted into ammonium. After this, ammonium is converted into the amino acids glutamine and asparagine amide groups. This process yields δaminolevulinic acid as a chlorophyll precursor, concurrently with proline and arginine (Kishorekumar et al., 2020).

Table 2. Shoot and Root Growth Parameters of Shallot under Various Fertilization Treatments.

Note: The presented numbers represent the means \pm standard error. Similar letters adjacent to the mean values indicate statistically nonsignificant differences based on the Tukey Honestly Significant Difference (HSD) test $(\alpha = 5\%)$.

From Table 1. it is evident that the nitrate reductase enzyme activity in the poured and sprayed PSB treatments has the same value (0.071 and 0.061 µmol NO_2 g^{-1} h⁻¹), higher than the NPK fertilizer-only and no fertilizer treatments (0.032 and 0.018 μ mol NO₂ g⁻¹ h⁻¹). Applying PSB as an additional fertilizer can also enhance the effects of chemical fertilizers (in this experiment, NPK) in chlorophyll formation. Upon further observation, PSB can be applied through pouring to increase chlorophyll formation effectiveness. When PSB is poured into the soil, shallot plants exhibit higher efficiency in chlorophyll formation compared to plants with PSB sprayed onto the canopy.

Plant growth is an outcome of photosynthesis, where the assimilates produced are accumulated in plant organs. Table 2 presents various growth parameters of shallot plants influenced by applying the biological fertilizer PSB. Based on Table 2. red shallot plants treated with sprayed PSB exhibit the highest fresh and dry shoot weights (33.30 g; 3.58 g), followed by NPK fertilizer (28.44 g; 2.19 g), poured PSB (24.32 g; 2.60 g), and no fertilizer (21.58 g; 2.53 g) Wong et al. (2014) discovered that PSB *Rhodopseudomonas palustris* can enhance the fresh and dry weight of mustard plants. Tobacco plants treated with *R. palustris* bacteria and mycorrhiza increased the number of leaves and the fresh and dry weight of both shoots and roots (Hua et al., 2009). Concerning the leaf number parameter, no significant differences were observed between the effects of PSB and chemical fertilizer NPK (Table 1). However, foliar application of PSB resulted in increased fresh and dry weights of the shallot plants' shoots (Table 2).

When PSB is sprayed onto the shallot foliage, *Rhodobacter* spp. bacteria produce exopolysaccharides (EPS), stimulating leaf growth and metabolic activities, particularly photosynthesis. (Batool et al., 2017; Nookongbut et al., 2019; Sakpirom et al., 2017). Thus, the production of primary metabolites in the shoot region occurs more rapidly. Conversely, onion plants administered PSB by pouring exhibit higher fresh and dry root weights than other treatments (Table 2).

This phenomenon represents a continued effect of exogenous IAA hormone PSB bacteria produced in the rhizosphere zone. The dry weight of shallot roots treated with PSB is equivalent to shallots fertilized with NPK alone. Examining Table 2 reveals that PSB exhibits different mechanisms when applied to the foliage by spraying and to the roots by pouring.

Treatment	Bulb Count per Clump	Fresh Weight of Bulb per Clump (g)	Dry Weight of Bulb per Clump (g)	Productivity (ton/ha)
No Fertilizer	5.60 ± 0.12 c	9.34 ± 0.85 b	0.58 ± 0.05 b	13.01 ± 1.32 c
Poured	7.50 ± 0.18 a	12.96 ± 1.10 a	1.31 ± 0.09 a	24.30 ± 2.45 a
Sprayed	6.70 ± 0.15 b	12.38 ± 1.05 a	1.18 ± 0.08 a	20.74 ± 2.10 b
NPK	6.60 ± 0.16 b	11.22 ± 0.98 ab	0.83 ± 0.07 a	18.51 ± 1.88 b
CV (%)	2.14	11.54	8.45	10.14

Table 3. Shallot Yield Parameters under Various Fertilization Treatments

Note: The presented numbers represent the means \pm standard error. Similar letters adjacent to the mean values indicate statistically nonsignificant differences based on the Tukey Honestly Significant Difference (HSD) test (α = 5%).

Production parameters are the primary goals in shallot cultivation. Based on Table 3. it is evident that the application of PSB fertilizer through pouring can increase the number of bulbs per clump by 7.5. When PSB is applied by spraying, it yields a statistically similar number of bulbs, approximately 6.7. compared to shallots treated with NPK alone, which produces 6.6 bulbs per clump. Without any fertilization, shallots respond by producing only 5.6 bulbs per clump. The yield of shallot in each treatment is shown in Fig 2. Applying PSB fertilizer also influences the fresh weight of bulbs per clump. Shallot plants

treated with PSB through pouring and spraying differ significantly from those not fertilized. The increase in the percentage of fresh bulb weight in the PSB pouring treatment compared to NPK is 15.51%. Meanwhile, when PSB is applied by spraying, there is a 10.53% increase in fresh bulb weight compared to NPK fertilizer. The improvement in yield in this study aligns with research on several plant types, such as stevia. (Xu et al., 2016); mustard green (Wong et al., 2014); soybean (Kang et al., 2021); sesame (Khuong et al., 2020); and rice (Kantachote et al., 2016; Nookongbut et al., 2020; Yen et al., 2022).

Figure 2. Yield of shallots in each treatment applied

The fresh weight of bulbs per clump significantly influences the

productivity of shallot plants. With PSB application through pouring, shallot plants achieve the highest productivity, namely 24.3 tons/ha. PSB treatment by spraying and NPK do not differ significantly, with 20.74 tons/ha and 18.51 tons/ha productivities, respectively. The increase in productivity in the PSB pouring treatment is 31.28%, while in the PSB spraying treatment, it is 12.04% compared to NPK fertilizer. For the variable of dry bulb weight per clump, the PSB pouring, PSB spraying, and NPK treatments do not differ significantly statistically but are higher than those without fertilizer.

4. CONCLUSION

Applying PSB as a biofertilizer can stimulate growth and increase the yield of Crok Kuning shallot varieties due to the mechanism of increased nitrate reductase activity and chlorophyll content. The application of PSB fertilizer through pouring can enhance productivity by 31.28%, making it highly recommended for widespread use by shallot farmers.

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