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The effect of beneficial microorganism as biofertilizer application in hydroponic-grown tomato

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ABSTRACT

The use of N_2-fixing bacteria and growth hormone-producing rhizobacteria delivers nitrogen, enhances nutrients absorption by plants, and reduces the usage of inorganic fertilizers. Implementing biofertilizer in the hydroponic system as a means to reduce application of synthetic nutrient is recently in interest due to economic, food safety, and sustainability factors. This study determines the effects of biofertilizer dose on tomato yields in the hydroponics system. A randomized block design was utilized that consisted of seven treatments, namely 100% inorganic fertilizer and 0% biofertilizer (control), and various doses of inorganic nutrient combined with 25%, 50%, 75%, and 100% biofertilizer. The result illustrated that the application of biofertilizer augmented the population of endophytic bacteria, *Azotobacter* sp., *Azospirillum* sp., phosphate solubilizing bacteria, and nitrogen content. The distinct combination of biofertilizer did not alter the phosphorus and potassium content compared to control samples however resulted in superior tomato grade. The 50% inorganic fertilizer and 100% biofertilizer combination amplified the weight of the fruit by 36% compared to the control. This finding indicates that the application of biofertilizers in the hydroponic system for tomato plants is not only beneficial in minimizing the dosage of inorganic fertilizers but also enhancing the fruit quality.

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1. INTRODUCTION

Soil is a natural planting medium that supports plant productivity, serves as a habitat for organisms that actively participates in providing nutrients, promoting growth, and protecting plants. Nevertheless, not all types are appropriate for optimal crop production, namely marginal and contaminated soils. The inadequacy of fertile soils have promoted to soil-free practice being recurrently implemented in the urban farming. Advantageously, agricultural wastes, such as paddy husk and cocopeat, are also being reutilized as planting media in hydroponic systems for their biomass abundance.

Tomato (*Lycopersicon esculentum* Mill.) is an all-year round commodity in the tropics that constitutes the Indonesian diet and possesses high economic value. According to the Ministry of Agriculture, the crop's production volume in 2018 was 976,809 tons, and the demand increased to 1,023,270 tons in 2020 (Kementan, 2021). The current production rate has not been able to meet this high demand in Indonesia. Production can be intensified by cultivation techniques such as hydroponic system that comprises utilization of mineral nutrient solutions in water destitute of soil (Resh, 2022). This system offers excellent advantages in areas with low water availability and narrow spaces. It is also suitable for high-density agriculture to achieve maximum yield and can deliver a soilborne disease-free environment as well as proper nutrition-irrigation in accordance with plant requisites (Mitsanis et al., 2021).

Nutrients play an indispensable role in the hydroponic system. They are prerequisites for support growth directly associated with crop yield. Determination of the accurate dose, especially for tomato plants, is crucial due to their diversified nutritional requirement for each cultivar. The availability of minerals for the crop is obtained from the application of inorganic fertilizers in the form of nutrient solutions containing macronutrients such as N, P, K, Ca, and

Mg as A solution as well as micronutrients including Fe, B, Mn, Cu, Na, Mo, and Zn as B solution (Bailey & Ferrarezi, 2017).

Expediently, application of microorganisms can deplete the destructive effects caused by high doses of inorganic fertilizers. Augmentation of beneficial microorganisms in the form of biofertilizer or bacteria consortia can be employed for growth optimization by proliferating nutrient availability and absorption rate when applied to the soil, seeds, and plants. Addition of beneficial microbes assists in amplifying the soil fertility through fixation of atmospheric nitrogen, dissolving insoluble phosphates, and producing plant growth promoting substances around their habitat (Mahanty et al., 2017) by colonization of the rhizosphere or plant tissues (Malusá & Vassilev, 2014).

Employment of beneficial microorganism in the controlled growth system can improve efficiency and extend the microbial activity due to absence of extensive competition in the rhizosphere (Woitke & Schitzler, 2005). In the hydroponic system of tomato, plants inoculated with rhizobacteria delivered comparatively superior yield to the control treatment in the first four weeks of harvesting period. Selection of rhizobacteria is based on the attributes to promote plant growth and biological control performance (Gul et al., 2012). Inoculation of biological agents (PGPR and AMF) in a hydroponic system of substrate culture augments the growth and yield of cherry tomato plants. Tomatoes fruit per plant with treatment of biological agents weigh higher than the one without their application (Aini et al., 2019). The yield of a tomato hydroponic system can be optimized through the application of beneficial microorganisms in various form such as biofertilizer that is not only capable of providing nitrogen and soluble phosphate but also aids in triggering growth hormones for overall development of the plant. Biofertilizer also includes N-fixing endophytic bacteria that is capable of supplying nitrogen through plant tissues should there be any hindrances in nutrient absorption through plant roots.

N_2-fixing bacteria (NFB) and phosphate solubilizing bacteria (PSB) are some of the common active inoculants in the Indonesian commercial biofertilizer market. The function of biofertilizer containing microbial consortium is to fixate N_2, dissolve phosphorus, produce plant growth hormones, reduce usage of inorganic nutrients, and improve the comprehensive quality of the environment. NFB, such as *Azotobacter* sp., *Azospirillum* sp., and N_2-fixing endophytic bacteria can colonize plants in distinct areas, including the rhizosphere, rhizoplane, and tissues. Kandel et al. (2017) reported that endophytic bacteria have been identified as the diverse array of microbial communities that reside intercellularly in the plant tissues for majority of their life cycle symbiotically. In the hydroponic system, phosphate solubilizing, and other beneficial microbe can promote root development through the production of growth hormone, which enhances nutrient absorption. Some PSB can also facilitate plant development by expanding root surface area due to their ability to synthesize indole acetic acid (IAA). This compound is the predominantly abundant auxin produced, and it has been recognized as a vital factor contributing to the stimulation of root development in plants (Cataldi et al., 2020).

This study observed the effect of beneficial microorganism augmentation in the hydroponic system contributing to the microorganism population and tomato yield. While beneficial microbe-plant association has been widely explored, only a few studies have been conducted with consortium microbes on tomato plant. To date, no investigations have determined the effects of *Azotobacter* sp., *Azospirillum* sp., N_2-fixing endophytic bacteria, and PSB on tomato when incorporated as the biofertilizer. In this study, three species of nitrogen fixer and one species of phosphate solubilizer were evaluated for their colonization abilities, impact on nutrient absorption, tomato yield, and inorganic fertilizers usage reduction.

2. MATERIAL AND METHODS

The experiment was conducted from April to August 2019 at Agriculture Faculty Greenhouse Hydroponic Facility, Universitas Padjadjaran located in Ciparanje village, Jatinangor District (06°55'02,2" S, 107°46'20,2" E). The location has an altitude of 715 meter above the sea level with annual temperature of 23°C–34°C and 70%–80% relative humidity. The rainfall during research in the study location was 10.0–90.5 mm/month. The research was systematized as a randomized block design with seven treatments and four replicates that encompassed distinct combinations of beneficial microorganism consortium (BMC) and inorganic nutrient solution doses (Table 1). Treatment A (control) was treated solely with inorganic nutrient solution. Meanwhile B and C treatments were both given 100% density of BMC mixture with an addition of 50% and 70% recommended dose of inorganic nutrient solution. D, E, F, and G treatments were given 25%, 50%, 75%, and 100% density of BMC mixture and 100% recommended dose of inorganic nutrient solution.

Observational parameters included N_2-fixer population (*Azotobacter* sp., *Azospirillum* sp.), PSB, nitrogen, phosphate, and potassium content (absorption), weight, and yield of tomato plants. Leaf samples for N, P, and K content analysis were collected from twelve petioles leaves opposite or below top flower cluster at mid bloom 3rd cluster (Jones et al., 1991).

Table 1. Treatments of nutrient compound and beneficial microorganism density dose

Treatments	Density Population of Beneficial Microbes Consortia (%)	Recommended Dose of Nutrient Solution (%)
A	0	100
B	100	50
C	100	75
D	25	100
E	50	100
F	75	100
G	100	100

Note: density population of Beneficial microbes 100% was 4.5 × 10^8 CFU mL^{-1}

Figure 1. Hydroponic tomato plant with drip irrigation system in greenhouse at 5 WAP

The yield parameters included the number of tomatoes per individual plant, the total weight of fruit per plant, and the grade of tomatoes weight harvested from first until third bunches. The data obtained were analyzed for the F-test by MS Excel 2019 software and analyzed for variance by SPSS version 16.0. ANOVA test was followed by Duncan Multiple Range test at $p \leq 0.05$.

2.1. Isolation and preparation of BMC

The microorganism consortium comprised of *Azotobacter* sp., *Azospirillum* sp., PSB, and N_2-fixing endophytic bacteria that were isolated from the rhizosphere and root tissue of tomato plant respectively. Isolation of bacterial groups was carried out using selective media for each microbial group, Ashby's, Okon's, and Pikovskaya's media for *Azotobacter*, *Azospirillum*, and PSB isolation respectively. Morphological and physiological characteristics of isolated bacteria were observed using Bergey's Manual of Systematic of Archaea and Bacteria (Whitman, 2015). Isolation of N_2-fixing endophytic bacteria was carried out in accordance with the method described by Baldani et al. (2014) and Setiawati et al. (2023) and grown in JNFb media. The four bacteria inoculants were tested against each other by conducting a compatibility test (Prasad & Babu, 2017). Bacterial cultures were streaked on nutrient agar plates for every single bacterial culture in the plate, the same isolates were streaked vertically on the first streaked isolates. The plates were incubated at 37°C for 48 h and the inhibition zone was observed and recorded. The inhibition zones were not visible after 48 h in all tested colonies. Prior to the study, pathogenic test was carried out on tobacco leaves. Hypersensitivity reactions test were conducted by injecting pure bacterial suspension into tobacco leaf tissue (methods based on Munif et al. (2021)). No symptomatic development of chlorosis and disease was observed up to 4 days in tobacco leaves.

The preparation of one liter of BMC mixture was as follows: 5% of pure inoculant was added into one liter sterile distilled water with 3% molasses and 0.1% yeast extract (this formulation referred as 100% BMC density). Incubation was carried out at the room temperature (±25°C) for 3 days on a 120 rpm rotary shaker. After incubation, each inoculant was enumerated to ensure the average colony formation unit was above 2×10^8 CFU mL^{-1}. Inoculants were mixed with the ratio of 1:1:1:1 prior to its incorporation in the experimental treatment.

2.2. Preparation of inorganic nutrients solution

The nutrient compositions for tomatoes (*Lycopersicon esculentum* Mill. Var. Valoasis RZ) were categorized into two solutions: A solution and B solution. The A solution consisted of 25 g of $Ca(NO_3)_2$, 12.5 g of KNO_3, and 1 g of $Fe(SO_4)$. On the other hand, the B solution consisted of 20 g of $MgSO_4$, 5 g of KH_2PO_4, 0.025 g of $CuSO_4$, 0.05 g of $MnSO_4$, 0.05 g of H_3BO_3, 0.05 g of $ZnSO_4$, and 0.01 g of $(NH_4)_2MoO_4$. Each nutrient compounds mixture was dissolved in 1 L of distilled water and used as a mother solution for the vegetative phase.

The composition of A solution for the generative phase was 30 g of $Ca(NO_3)_2$, 5 g of KNO_3, and 1 g of $Fe(SO_4)$ dissolved in 1 L water and the solution B consisted of 20 g of $MgSO_4$, 10 g of KH_2PO_4, 2.5 g of K_2SO_4, 0.025 g of $CuSO_4$, 0.05 g of $MnSO_4$, 0.05 g of H_3BO_3, 0.05 g of $ZnSO_4$, and 0.01 g of $(NH_4)_2MoO_4$ dissolved in 1 L of distilled water. Solutions were drained into clean barrels and labeled A, B, C, D, E, F, and G in accordance with the experimental treatments. Each barrel contained 50 L homogenized mixture of A and B solutions. The 100% inorganic treatment contained 2 L of A solution, 2 liters of B solution, and 46 L of water (for A, D, E, F, and G treatments). The 75% inorganic treatment contained 1.5 L of A solution, 1.5 L of B solution, and 47 L of distilled water. The 50% inorganic treatment contained 1 L of A solution, 1 L of B solution, and 48 L of distilled water (B).

The inorganic nutrient solution used was developed from the Controlled Culture Laboratory, Faculty of Agriculture, Universitas Padjadjaran, specifically for tomato plants. Development of hydroponic nutrition for tomato plants was based on the macro and micronutrient requirements in the vegetative and generative phases of tomato plants.

2.3. Preparation of growing media and tomato seedling

The growth media utilized in this study were rice husk and cocopeat charcoal media in a volume ratio of 3:1. The weight of media that comprised of husk and cocopeat were 1.65 kg and 0.55 kg, respectively. The total media weight for each polybag was 2.2 kg. The media were mixed evenly and inserted into 40 cm × 50 cm polybags. The media were mixed with 2 L of clean water until it became saturated. Incubation was carried out up to 48 hours or until the water retention reached field capacity. Polybags were arranged randomly.

Tomato seeds were planted in pot tray using rice husk charcoal. The seeds were put into the planting hole at depth of 1 cm, then covered again with charcoal husk until the seeds were not visible. Pot trays were filled with one tomato seed per hole. Seeds were watered daily, supplied 250 mL of nutrients until 14 days, and maintained until those were ready to be transplanted into the hydroponic system.

2.4. Hydroponic nutrient delivery system

The nutrient solution was applied through drip irrigation. Ten milliliters of biofertilizer was injected around the plant roots post transplantation to polybags. The effect of both macro and micronutrients contained in biofertilizers can be

ignored because the control treatment used the identical solution without microbes. Biofertilizer were injected in the growth medium in accordance with the density of each treatment under humid conditions. Plant nutrients in the barrel were streamlined through a hose to a polybag known as drip irrigation system (Fig. 1). Nutrient solutions were reapplied three times a day as follow: 50 mL in the morning, afternoon, and evening respectively. The dose was increased by 50 mL per polybag every week. The nutrients solutions were applied for 120 seconds and timed by a stopwatch.

2.5. Microbial analysis

The plant growth media samples were collected at 7 weeks after planting (WAP) at the depth of 15–30 cm at four distinct spots near root zone in each polybag and the sample was thoroughly blended to obtain a composite sample. The number of populations of N_2-fixer bacteria (NFB) and PSB were determined by serial dilution plate count technique. The media utilized to count population of N-fixing endophytic bacteria, *Azotobacter* sp., *Azospirillum* sp., and PSB were JNFb, Ashby, Okon, and Pikovskaya agar media respectively. One milliliter aliquot of sample was pipetted into sterile test tube, then serially diluted in six set of test tubes that resulted in serial dilution ratio 10^{-1}–10^{-6}. Furthermore, 0.1 ml of solution from fifth (10^{-5}) and sixth (10^{-6}) dilution was added into another sterile petri dish, after which 15 ml of 45°C sterile molten agar media was poured into the suspension under aseptic condition; the petri dish was swirled gently to distribute and homogenize the microbial cells and was then incubated at 30°C–35°C for 48 h. The growth of the bacterial colony in the plate were counted and recorded appropriately at the end of the incubation time. The endophytic diazotrophic microorganisms from leaves tissue of tomato were isolated using nitrogen free semi-solid media (Ji et al., 2014). Isolating microbe endophytic from leaves tissue was carried out through the following steps: Sample of the plant tissue surface was sterilized by 70% ethanol for 1 min, then washed by 1.2% (w/v) NaClO solution, and shaken for 15 min. The sterilized samples were then washed thrice (15 min each) with sterile distilled water. Surface sterilized samples were mashed by sterilized mortar and pestle, and then were inoculated on nitrogen free semi-solid agar media in sterile petri dish. The samples prepared were incubated at 30°C for 2 days.

3. RESULTS

3.1. Microbial activity of PGPR biofertilizer

Table 2 showed differences in acetylene reduction of three nitrogen fixers. *Azospirillum* produces higher nitrogenase, IAA hormone, and kinetin activities in comparison to *Azotobacter* and endophytic bacteria. Whereas *Azotobacter*, although known to act as a nitrogen fixer, could produce high levels of zeatin and gibberellin hormones compared to the other two N-fixing bacteria.

Table 2. Nitrogenase activity and release of hormones including organic acids by biofertilizer microbes

Microbes in biofertilizer	Acetylene Reduction Assay (nmol C_2H_4/g dry wt/hour)	Auxin (ppm)	Cytokinin (ppm)		
		IAA	Zeatin	Kinetin	Gibberellin
Azotobacter sp.	183.250	13.624	16.320	12.442	29.015
Azospirillum sp.	555.642	15.966	10.619	14.475	21.397
Endophytic bacteria	257.036	13.023	9.011	10.324	16.001
		Organic acids (ppm)			
Phosphate Solubilizing Bacteria	Citric	Ferulic	Coumaric	Malic	Pyruvic
	128.064	101.013	94.619	5.147	1.556

Table 3. Effect of biofertilizers and inorganic fertilizer on the population of beneficial bacteria of tomatoes in the hydroponic growing media

Treatments	Endophytic (CFU g^{-1})	*Azotobacter* sp. (CFU g^{-1})	*Azospirillum* sp. (CFU g^{-1})	PSB (CFU g^{-1})
A = 100% inorganic fertilizer without biofertilizer (control)	$0.38 \times 10^{6\ a}$	$6.0 \times 10^{5\ a}$	$4.4 \times 10^{6\ a}$	$0.1 \times 10^{8\ a}$
B = 50% inorganic fertilizer + 100% biofertilizer	$0.80 \times 10^{6\ b}$	$23.7 \times 10^{5\ b}$	$6.3 \times 10^{6\ ab}$	$1.23 \times 10^{8\ b}$
C = 75% inorganic fertilizer + 100% biofertilizer	$1.55 \times 10^{6\ c}$	$5.3 \times 10^{5\ a}$	$7.0 \times 10^{6\ ab}$	$1.53 \times 10^{8\ bc}$
D = 100% inorganic fertilizer + 25% biofertilizer	$1.45 \times 10^{6\ c}$	$2.5 \times 10^{5\ a}$	$11.9 \times 10^{6\ c}$	$1.53 \times 10^{8\ bc}$
E = 100% inorganic fertilizer + 50% biofertilizer	$1.30 \times 10^{6\ c}$	$6.4 \times 10^{5\ a}$	$9.7 \times 10^{6\ bc}$	$2.23 \times 10^{8\ c}$
F = 100% inorganic fertilizer + 75% biofertilizer	$1.34 \times 10^{6\ c}$	$2.3 \times 10^{5\ a}$	$5.5 \times 10^{6\ ab}$	$1.23 \times 10^{8\ b}$
G = 100% inorganic fertilizer + 100% biofertilizer	$1.45 \times 10^{6\ c}$	$6.0 \times 10^{5\ a}$	$4.0 \times 10^{6\ a}$	$1.66 \times 10^{8\ bc}$

Note: means with the same letter in a column were not significantly different ($p \geq 0.05$)

Table 4. The effect of biofertilizer and inorganic fertilizer on the N, P, and K content of RJ *Valoasis* tomato plants in the hydroponic system

Treatments	N (%)	P (%)	K (%)
A = 100% inorganic fertilizer without biofertilizer (control)	3.85[a]	1.13[a]	3.55[a]
B = 50% inorganic fertilizer + 100% biofertilizer	4.26[ab]	1.20[a]	3.88[a]
C = 75% inorganic fertilizer + 100% biofertilizer	4.18[ab]	1.20[a]	3.25[a]
D = 100% inorganic fertilizer + 25% biofertilizer	4.08[ab]	1.14[a]	3.77[a]
E = 100% inorganic fertilizer + 50% biofertilizer	5.40[b]	1.24[a]	3.74[a]
F = 100% inorganic fertilizer + 75% biofertilizer	4.28[ab]	1.18[a]	4.12[a]
G = 100% inorganic fertilizer + 100% biofertilizer	4.27[ab]	1.17[a]	4.19[a]

Note: means with the same letter in a column were not significantly different ($p \geq 0.05$)

Table 5. Effect of biofertilizer and inorganic fertilizer on the weight and number of fruits per plant in the hydroponic system

Treatments	Fruit weight per plant (Kg)	Number of fruits per plant
A = 100% inorganic fertilizer without biofertilizer (control)	3.47[a]	26[a]
B = 50% inorganic fertilizer + 100% biofertilizer	4.72[a]	30[a]
C = 75% inorganic fertilizer + 100% biofertilizer	4.25[a]	32[a]
D = 100% inorganic fertilizer + 25% biofertilizer	4.27[a]	29[a]
E = 100% inorganic fertilizer + 50% biofertilizer	3.85[a]	28[a]
F = 100% inorganic fertilizer + 75% biofertilizer	4.47[a]	30[a]
G = 100% inorganic fertilizer + 100% biofertilizer	3.07[a]	23[a]

Note: means with the same letter in a column were not significantly different ($p \geq 0.05$)

3.2. Beneficial microbial population

Duncan multiple range test demonstrated that the fertilizer treatment influenced the population of microbes (Table 3). The lowest populations of beneficial bacteria (N_2-fixing endophytic bacteria and PSB) were detected in control treatments of all samples. Inorganic fertilizer and BMC mixture significantly increased the population of endophytic and PSB is all treated samples. The rhizosphere of tomato plants inoculated with biofertilizer has a higher endophytic bacteria population than the control. High dose of inorganic fertilizer (75%–100%) elevated the endophytic bacteria population. Although lower doses of treatment (50% inorganic fertilizer and 100% biofertilizer) caused higher endophytic bacterial colonization than the control, it was lower than that caused by other doses of the same treatment.

The treatment of biofertilizer and inorganic nutrients exhibited significantly contrasting results on the population of *Azospirillum* sp. Increased amount of biofertilizers decreased the *Azospirillum* sp. population. The highest number of populations of *Azospirillum* sp. was in D treatment (25% Biofertilizer + 100% Inorganic fertilizer) but was not statistically different from 50% biofertilizer + 100% inorganic fertilizer treatment. Meanwhile, the *Azotobacter* and *Azospirillium* population showed varied response toward the treatment. *Azotobacter* population was higher after the application of 50% inorganic fertilizer + 100% biofertilizer (B treatment). *Azospirillum* was the highest in treatment D and E, with 100% dose of inorganic growth nutrient and 25%–50% of BMC mixture.

3.3. N, P, and K content of tomato leaves

Table 4 displayed that plant N content in leaves treated with 50% biofertilizer and 100% nutrient inorganic fertilizer was higher compared to the control (treatment A). While the application of various composition of biofertilizers and inorganic nutrients caused negligible differences in P and K content of tomato plants.

3.4. Tomato yield

The weight and number of tomatoes fruit harvested from plants treated with various biofertilizer doses were not significantly different compared to plants without biofertilizers (Table 5). In terms of fertilizer efficiency, treatment B (50% inorganic fertilizer + 100% biofertilizer) elevated the fruit weight by 36.02% compared to the control treatment. In terms of N, P, and K uptake of individual plants, the N uptake of treatment E, which was higher than the control, did not exhibit results that were in line between the weight and number of fruits produced (Table 6).

Table 7 displays the correlation between plant nutrition properties, beneficial bacteria, and yield of tomato plant. The weight of yield tomatoes was strongly correlated with the number of tomatoes (r = 0.743). The number of tomatoes has moderate correlation with the number of grade C tomatoes (r = 0.404), while the increase in the number of grade C fruits decreased the number of grade B tomatoes (r = −0.450). Among the types of bacteria applied, *Azotobacter* has strong correlation with the number of grade A tomatoes (r = 0.489), while endophytic bacteria correlated with grade C tomatoes (r = 0.377). PSB has strong correlation with the P concentration of plants (r = 0.625) and the population of N-fixing endophytic bacteria (r = 0.522).

4. DISCUSSION

Besides the quantity of grade A fruit, beneficial microorganism demonstrated no significant effect toward tomato's agronomical parameters such as weight and number of fruits per plant. The combination of 50% inorganic nutrient and 100% beneficial microorganism mixture yielded in higher grade A tomatoes, suggests that the reduction of inorganic nutrient and addition of beneficial organism mixture can enrich tomato fruit quality. The appropriate

combination of biofertilizer and inorganic fertilizer can assist plants absorb more nutrients by optimizing distribution of available nitrogen to plants. The treatment comprised of 50% biofertilizer and 100% inorganic fertilizer was the optimal concentration (100% dosage of inorganic fertilizer without biofertilizer) for effective distribution of nitrogen to plants in comparison to the control. Nonetheless, the amount of inorganic fertilizer was lowered by 50%, the N_2-fixing bacteria in the biofertilizer provided adequate amount of readily accessible N in the hydroponic system for tomato plants.

The application of 100% inorganic fertilizer with biofertilizers does not warrant an increase in yield in comparison to the application of lower doses of inorganic fertilizer (Table 6); attributable to N loss from volatilization induced by high temperature in the tropic. The liquid biofertilizer that was applied 4 times also plays a profound role in nutrient availability with some fertigation systems being capable of reducing nutrient loss (Barzee et al., 2019). Meanwhile the quantity of tomato with the application of 100% biofertilizer with 50% inorganic fertilizer increased fruit weight by 36% with an average fruit weight of 157.50 g per fruit. The results of this research imply that the biofertilizer application with low inorganic fertilizer dosage could aid in preventing over usage of high price inorganic fertilizers to a significant level without compromising the yield of tomatoes. Biofertilizer application with lower inorganic fertilizer practice not only results in improved fruit quality but also enhances the plant medium health as well as microbial diversity.

The application 100% biofertilizer with 50% inorganic fertilizer resulted in the production of tomatoes in A category with an average weight of 157.50 g, meanwhile the application of other treatments, mostly, resulted in the production of B category (Table 6). However, although the results are, statistically, not significantly different from the control, the application of B treatment (100% Biofertilizer + 50% Inorganic fertilizer) can increase fruit weight by 35.97% and produce more tomatoes in 'A' grade compared to the control. The weight of tomato fruit is influenced by the number of tomatoes produced (Table 7). In this study, more grade C fruits were produced than grade A and B. The population of *Azotobacter* was strongly correlated with grade A tomatoes. *Azotobacter* through its N-fixing activity can distribute ammonium in the vicinity of plant roots making it readily accessible for plant absorption. Increasing the supply of N to plants will proliferate the biomass and size of tomatoes as well. The role of endophytic bacteria and PSB on fruit weight appears to have a positive correlation. Based on correlation analysis, the endophytic population correlated with the increase in C grade tomatoes. PSB was strongly correlated with the increase in the endophytic population. PSB activity can fulfill the phosphorus demand for the growth of the endophytic population. Phosphorus solubilizing bacteria may aid the growth of plants by stimulating the biological nitrogen fixation bacteria (Alori et al., 2017). This study illustrates that soil benefit microorganisms in biofertilizer influences the weight and grade of tomatoes yield although not all correlated strongly with tomato fruit size and weight. A study conducted by Zhao et al. (2022) demonstrated that the application of biofertilizers can improve the physical and chemical properties of the soil compared to the control treatment. Some soil chemical properties were significantly different ($p < 0.05$) due to the biofertilizer gradient. The benefits of the biofertilizer consortium have been proven to improve the physicochemical properties of the soil, which is also supported by previous research.

The essential elements especially the major nutrients N, P, and K are considered the most important among nutrients and factors limiting growth and yield of tomato plants. Since deficiency of these major nutrients can result in restricted plant growth, requisite maintenance by optimization of N, P, and K fertilizers are crucial. The combined effect of plant characteristics, soil properties, and root interactions with microbes can control nutrient availability in the rhizosphere. The concentration of nutrients and their availability in the rhizosphere were higher than in the bulk soil. The quantity and quality of root exudates are altered by the microbial activity in the rhizosphere, which influences rooting patterns and the nutrient availability in plants (Fenta & Assefa, 2017).

Nutrient provisioning from inorganic and biofertilizer combination was considered as adequate because the leaf absorption of N, P, and K results were not statistically different compared to the full dosage of inorganic fertilizer (Table 4). According to Jones et al. (1991), the N, P, and K content of tomato leaves is deemed commensurable if the content ranges between 3.0%–4.0%, 0.4%–1.0%, and 5.0%–9.0%, respectively. According to the above range, all tomato plants treated had received adequate nutrition of N and P, however were deficient of K. The absorption of nitrate stimulates the uptake of cations (K^+) (Tischner & Kaiser, 2007).

Table 6. The effect of treatments on the grade of tomatoes fruits

Treatment	A Grade (Fruit per plant)	B Grade (Fruit per plant)	C Grade (Fruit per plant)
	Mean ± Standard Deviation		
A = 100% inorganic fertilizer without biofertilizer (control)	1.50 ± 1.02 [a]	2.25 ± 1.89	2.75 ± 0.96
B = 50% inorganic fertilizer + 100% biofertilizer	3.50 ± 1.29 [b]	2.75 ± 1.26	1.25 ± 0.96
C = 75% inorganic fertilizer + 100% biofertilizer	2.00 ± 0.82 [a]	2.50 ± 2.38	3.50 ± 1.91
D = 100% inorganic fertilizer + 25% biofertilizer	1.75 ± 0.54 [a]	2.00 ± 0.82	3.50 ± 1.29
E = 100% inorganic fertilizer + 50% biofertilizer	2.50 ± 0.46 [ab]	1.25 ± 0.96	3.25 ± 0.54
F = 100% inorganic fertilizer + 75% biofertilizer	1.50 ± 1.02 [a]	4.50 ± 2.38	1.50 ± 1.29
G = 100% inorganic fertilizer + 100% biofertilizer	1.75 ± 1.26 [a]	1.25 ± 0.50	2.75 ± 1.71

Note: means with the same letter in a column were not significantly different ($p \geq 0.05$)

Table 7. Correlation analysis between plant nutrition properties, beneficial bacteria, and yield of tomato plant by Pearson method

	N Concentration (%)	P Concentration (%)	K Concentration (%)	Fruit Number (fruit plant^{-1})	A Grade (fruit plant^{-1})	B Grade (fruit plant^{-1})	C Grade (fruit plant^{-1})	Endophytic Population (cfu g^{-1})	Azotobacter Population (cfu g^{-1})	Azospirillum Population (cfu g^{-1})	PSB Population (cfu g^{-1})	Fruit Weight (g plant^{-1})
N Concentration (%)	1											
P Concentration (%)	0.043	1										
K Concentration (%)	0.048	−0.517(**)	1									
Fruit Number (fruit plant^{-1})	−0.101	0.302	−0.274	1								
A Grade (fruit plant^{-1})	0.134	0.006	0.048	−0.008	1							
B Grade (fruit plant^{-1})	−0.193	−0.088	0.103	−0.093	0.107	1						
C Grade (fruit plant^{-1})	−0.022	0.346	−0.091	0.404(*)	−0.340	−0.450(*)	1					
Endophytic Population (cfu g^{-1})	0.122	0.117	0.029	0.222	0.093	−0.128	0.377(*)	1				
Azotobacter Population (cfu g^{-1})	−0.056	−0.122	−0.004	0.002	0.489(**)	−0.219	−0.257	−0.321	1			
Azospirillum Population (cfu g^{-1})	0.094	0.101	−0.179	−0.082	−0200	0.200	0.022	0.167	−0.166	1		
PSB Population (cfu g^{-1})	0.234	0.625(**)	−0.204	0.119	−0.007	−0.129	0.266	0.522(**)	−0.218	0.366	1	
Fruit Weight (g plant^{-1})	0.047	0.163	−0.001	0.743(**)	0.251	0.018	0.046	0.213	0.096	−0.012	0.052	1

Note: ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

A high carbohydrate status increases the intake of ammonium, and ammonium inhibits the uptake of cations, which can induce Ca shortage and lower K levels in plants. It is likely that most of the N absorbed by tomato plants is in the form of ammonium (NH_4^+) cation, which affects K^+ uptake. The uptake of nitrogen in NH_4^+ or NO_3^- form is influenced by soil pH, temperature, and presence of other ions in the soil solution (Borgognone et al., 2013).

The treatment of biofertilizer and inorganic nutrients demonstrated significantly different results on the population of *Azospirillum* sp. An increased amount of biofertilizers receded the *Azospirillum* sp. population. It was suspected that the addition of inorganic fertilizer by more than 50% may have inhibited the development of *Azospirillum* sp. The population of N_2-fixing endophytic bacteria, *Azotobacter* sp., *Azospirillum* sp., and phosphorus solubilizing bacteria (PSB) increased compared to the population in the control. Inorganic fertilizers were known to cause fluctuation in putative abundance of plant growth promoting rhizobacteria (Reid et al., 2021) and altered the soil rhizobacterial community (Kavamura et al., 2020).

The analysis of phytohormone production from biofertilizer consortia implemented in this study revealed that each microorganism employed in this study produced auxin as well as cytokinin (Table 2). The application of biofertilizer can increase PGPR proliferation which functions to accelerate the process of plant growth to optimize the fruit production. Genus *Azospirillium* and *Azotobacter* are a well-known plant growth promoting rhizobacteria (PGPR). *Azospirillum brasilense* can produce auxin and gibberellin, two vital phytohormones (Zaheer et al., 2022). *Azotobacter*s fix nitrogen, however they primarily influence plant growth by generating growth precursors. *Azotobacter* and *Azospirillum* are the most important PGPR that contributes to the improvement of plant growth by the production of phytohormones in addition to the biological nitrogen fixation. The role of bacteria in biofertilizer in hydroponic tomato cultivation was not only for distribution of N nutrients, but also for the exudation of growth hormones that can stimulate root extension to absorb macro and micronutrients as well as accelerate the fruit formation. *Azotobacter* sp. inhabiting the rhizosphere plays a significant role in N distribution as well as in the secretion of plant hormones such as cytokinin, gibberellic acid, auxin, amino acids, and B-group vitamins (Rahimi et al., 2021). Although *Azotobacter* provides N in small amounts, it is beneficial for plants and other microbes encompassing the roots.

PSB can produce five carboxylic acid types (Table 2) to help unbind the orthophosphate from Al, Fe, Mg, or Ca fixation. The type of organic acid produced by PSB depends on the initial phosphorus availability. Gluconic acid was the predominant organic acid produced under insufficient phosphorus condition in growth media (Chen et al., 2016). Some of the most frequent organic acids include citric and pyruvic acids (Ribeiro et al., 2020). During the dissolution of soil mineral apatite, PSB released various organic acids such

as citric, ferulic, coumaric, syringic, and malic acids that were detected during the treatment (Kurnianta et al., 2019).

Plant growth promoting rhizobacteria (PGPR) consists of widely ranged bacterial strains that colonize plant roots and rhizosphere through composite mode of action toward plant growth and development. PGPR can increase agricultural crop productivity, induce plant protection against pathogens (Tsukanova et al., 2017), enhance resiliency against stress (Glick, 2012), increase plant mineral nutrition through fixation (Kuan et al., 2016), and phosphate mobilization (Mehta et al., 2015).

The population of endophytes in plants treated with the combination of biofertilizer and inorganic fertilizer were higher compared to the control (Table 3). This may be due to readily available nutrient for plant uptake, which directly affected the growth of tomato plant. Despite the adverse effect of inorganic fertilizer application toward endophytes diversity (Collavino et al., 2020), augmentation in the form of biofertilizer addition were known to increase the endophytic bacterial population in general (Yadav et al., 2021). Inhabiting inside the plant tissues, endophytic bacteria is safeguarded against direct contact to inorganic nutrient in tomatoes growth media. A mutualistic link between the bacteria and the plant is established once bacterial endophytes have colonized plant tissues. The bacterial endophytes act as producers of biologically active metabolites, while the plant provides the bacterial community with nutrients (Fouda et al., 2021).

Besides biofertilizer concentration, endophytic bacterial population fluctuation displayed in Table 3 was likely to be caused by physiological properties of plant tissue. Nutrition absorption triggers changes in plant osmotic pressure. Drastic changes such as increased osmotic pressure, decreased nitrogen and decomposition of plant tissue will affect endophytic bacterial population both in roots, canopies, and rice seeds (Mano et al., 2007). Endophytic bacterial population in the control treatment is the indigenous bacteria derived from rice husk and cocopeat charcoal growing media. The rhizosphere of many plants is occupied by bacteria because plant roots excrete root exudates containing nutrients that attract endophytic bacteria to inhabitat the plant root tissue. Microbes such as Streptomyces are also attracted to exudate components of plants root (Worsley et al., 2021). Plants are known to release up to 40% of their photosynthetically fixed carbon into the surrounding soil through their roots, resulting in proliferation of bacterial communities in the soil that are attracted to the root exudate substances as a specific nutrient and carbon resources which are metabolized by microbial population for their growth and colonization of the root plant (Haichar et al., 2016).

The inoculation of consortia biofertilizers to the growing media induces endophytic bacterial association with plants root, stem, and leaf tissue. Plant growth can be induced with the presence of *Azotobacter* and *Azospirillum* not only due to nutrient availability through free nitrogen fixation, but also because of the synthesis of growth-stimulating hormones, such as cytokinin, gibberellic acid, auxin, amino acids, and B-group vitamins (Rahimi et al., 2021).

High inorganic fertilizer in the planting medium will inhibit *Azotobacter* growth because it is sensitive to the availability of nitrate, nitrite, or ammonium in the growth media. Nitrates and nitrites can suppress *Azotobacter* activity. *Azotobacter* in the root rhizosphere of tomato plants will be in direct contact with nutrient solutions containing nitrate, nitrite, or ammonium in rice husk charcoal and cocopeat growing media, which may lead to the inhibition of nitrogenase activity. The short-term inhibitory effect on nitrogenase activity may be due to the presence of nitrate or nitrite. Nitrogenase in *Azotobacter chroococcum* can also be inhibited by various organic products formed because of ammonium assimilation. A study determined the inhibition in 42-hours old culture, at the stage when maximum biomass production was retrieved and the stationary stage began (Gutiérrez-Rojas et al., 2011).

Azospirillum sp. is a non-symbiotic bacteria with the ability to fix N_2 colonizing the root zone. Varying nitrogenase activity among microorganism can be considered as a common phenomenon (Tejera et al., 2005). The benefits of plants inoculated with *Azospirillum* was not only associated to its capacity to fix atmospheric N_2, but also to its ability to synthesize phytohormones, indole-3-acetic acid (Fukami et al., 2018). In the soil, the distinct impact on preponderance of *Azospirillum* in maize rhizosphere varied, contributing to distinct physio-chemical factors and history of land use patterns (Verma et al., 2011).

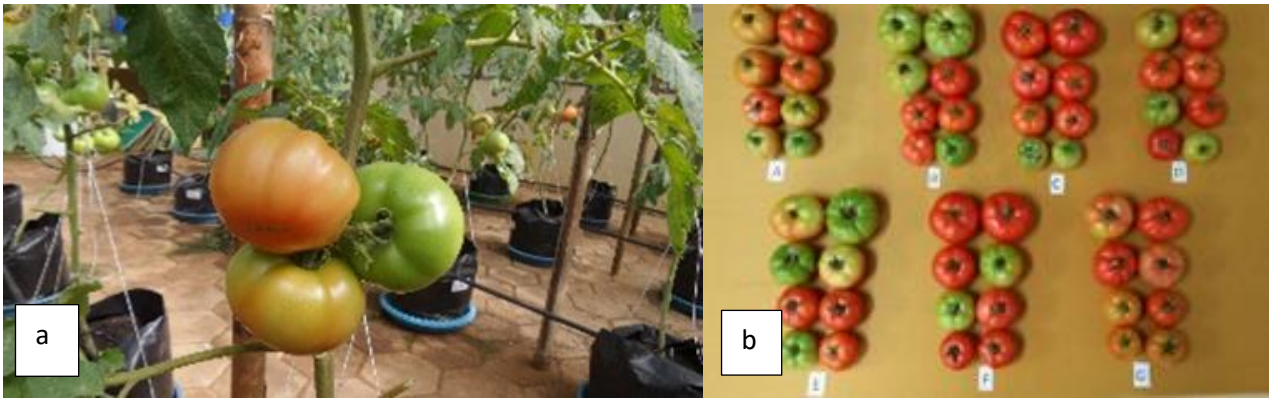

Figure 2. Tomatoes fruit harvested from the hydroponic system. (a) The tomatoes fruits in the first bunch of individual plant; (b) Tomatoes were harvested until the second bunch and were sorted based on their grade: Large (Grade A), Medium (Grade B), and Small (Grade C).

The application of biofertilizer proliferated the population of PSB compared to the treatment without biofertilizer. Plant treated with 50% of biofertilizer + 100% inorganic fertilizer demonstrated higher PSB population than other treatments although it was not different from the plant with 100% inorganic fertilizer + biofertilizer. In hydroponic systems where the supplied macro nutrients are readily accessible, the PSB cannot solubilize the unavailable P, however they utilize nutrients in growing media for development and potentially produce growth hormones. PSB is a promising biofertilizer and can supply plants with available phosphorous. The PSB also increases the efficiency of biological nitrogen fixation which is important for plant nitrogen supply (Kalayu, 2019). Moreover, PSB stimulates the plant growth by enhancing the availability of micronutrients through modification of the root morphology that aid in nutrient absorption from the soil (Fahsi et al., 2021).

Bacterial genera such as *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcous, Pseudomonas,* and *Serratia* belongs to extracellular plant growth promoting rhizobacteria (ePGPR) which colonize the outer part of the rhizosphere (Gupta et al., 2015). On the other hand, intracellular PGPRs including the endophytes are located generally inside the specialized structures of root cell. Moreover, the application of PGPR can promote plant growth and yield through direct and indirect mechanisms. Some PGPR species has the capability to solubilize phosphate, fix atmospheric nitrogen, and exude phytohormones such as auxin and cytokinin. PGPRs are also important in maintenance of nutrient absorption, root health, and tolerance to stress in deterring environment (Malhotra & Srivastava, 2009).

Fruit formation in tomato plants was strongly influenced by nutrients availability in soil (Liu et al., 2019). The availability of macro and micronutrients transforms fruit weight and number (Yang & Kim, 2020). This phenomenon occurs when the nutrient for meristem cell activation to facilitate photosynthesis and other physiological processes for plant growth are accomplished. Increased photosynthesis will elevate the organic matter, eventually multiplying the fruit's number and weight. However, severe nitrogen deficiency will reduce yield. Therefore, regulation of nitrogen supply in accordance with the plant requirement is essential for physiological maintenance (Truffault et al., 2019).

The average number of tomatoes produced until the third bunch was between 23–32 fruits per plant and the average weight of the fruit produced is between 3.07–4.72 Kg plant^{-1}. The tomatoes in bunch of individual plant were ripe enough to be picked at 7 WAP (Fig. 2). Each fruit has weight in the range 133.69–157.50 g. According to the Indonesian National Standard (SNI 01-3162-1992), the weight of fresh tomatoes is classified as: large > 150 g fruit^{-1}, medium 100–150 g fruit^{-1}, small < 100 g fruit^{-1}. The quality of tomatoes was determined by weight assessment of fresh tomatoes in accordance with the consumer demand.

5. CONCLUSION

The profound combination of biofertilizer and inorganic fertilizer in the hydroponic system can transform the population of endophytic bacteria, *Azotobacter* sp., *Azospirilum* sp., PSB, and plant N content. However, there was not a significant difference on P and K content of plants and the yield. The quality of tomato with the application of 100% biofertilizer with 50% inorganic fertilizer increased fruit weight by 36% with an average fruit weight of 157.50 g that is principally classified as grade A category. For future studies, the application of 50% inorganic fertilizer in the fertigation system with biofertilizer can be utilized to reduce nutrition loss from evaporation; consequently enriching nutrient availability and enhancing tomato yields.

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Declaration of Competing Interest

The authors declare that no competing financial or personal interests that may appear and influence the work reported in this paper.

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