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Boosting strawberry yield and fruit sweetness with humic substances and biofertilizer in soilless cocopeat-based culture

Peningkatan hasil dan tingkat kemanisan stroberi dengan bahan humat dan pupuk hayati dalam budidaya tanpa tanah berbasis cocopeat

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ABSTRACT

Farmers usually apply more chemical fertilizers to enhance the yield of strawberries in soilless culture. Humic substances and biofertilizers are now considered essential nutrient sources in sustainable agriculture. The objective of the greenhouse experiment was to observe the effect of humic and fulvic acid (humic substances), and biofertilizer on root growth, nitrogen (N) and phosphor (P) of the growing media, populations of N-fixing and P-solubilizing bacteria in the root zone, and yield of strawberry grown in the cocopeat-based substrate. The experiment was set up in a randomized block design consisting of seven treatments and four replications. The treatments included humic acid, fulvic acid, and biofertilizer in single or combined applications. All data were subjected to analysis of variance and followed by Duncan's Multiple Range Test with $p<0.05$. All treatments received 50% of the recommended dose of NPK fertilizer. The results showed that all treatments did not affect root length and dry weight. Mixed application of humic substances and biofertilizer together with NPK fertilizer increased total N and P as well as the population of N-fixing bacteria in the growth substrate. Still, P-solubilizing bacteria did not grow in the cocopeat-based substrate. Moreover, mixing humic substances and biofertilizer increased strawberries' fruit yield and sweetness levels.

ABSTRAK

Petani biasanya menggunakan pupuk kimia untuk meningkatkan hasil stroberi dalam kultur tanpa tanah. Saat ini sumber nutrisi lain yaitu bahan humat dan pupuk hayati dianjurkan untuk pertanian ramah lingkungan. Percobaan rumah kaca ini bertujuan untuk mengamati pengaruh asam humat dan fulvat, yang merupakan bahan humat, serta pupuk hayati terhadap pertumbuhan akar, nitrogen (N) dan fosfor (P) media tanam, populasi bakteri pengikat N dan pelarut P di zona perakaran, serta hasil stroberi yang ditanam pada substrat berbasis cocopeat. Percobaan disusun dalam rancangan acak kelompok yang terdiri atas tujuh perlakuan dan empat ulangan. Perlakuan terdiri atas pemberian asam humat, asam fulvat, dan pupuk hayati dalam aplikasi tunggal atau kombinasi. Data penelitian dianalisis dengan analisis ragam dan dilanjutkan dengan Uji Jarak Berganda Duncan dengan $p <0.05$. Seluruh perlakuan termasuk kontrol diberi 50% dosis rekomendasi pupuk NPK. Hasil penelitian menunjukkan bahwa semua perlakuan tidak mempengaruhi panjang dan bobot kering akar. Aplikasi campuran bahan humat dan pupuk hayati bersama dengan pupuk NPK meningkatkan total N dan P serta populasi bakteri penambat N pada substrat pertumbuhan; tetapi bakteri pelarut P tidak tumbuh pada substrat berbahan dasar cocopeat. Selain itu, campuran bahan humat dan pupuk hayati meningkatkan hasil buah dan tingkat kemanisan stroberi.

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INTRODUCTION

The demand for strawberries (*Fragaria* x *ananassa* var Duchesne) is now increasing in Indonesia due to the awareness of human health related to the vitamin C and antioxidants in the fruit (Giampieri et al., 2014; Sumarlan et al., 2018). The strawberry is not native to Indonesia; these popular fruits have been cultivated for decades in various mountainous regions where the temperature was more suitable for strawberry cultivation.

In order to increase the yield, farmers generally rely on chemical fertilizers for available nutrients, mainly nitrogen (N) and phosphorus (P). The role of N and P are essential; N plays a significant role in photosynthesis and plant growth (Shen et al., 2022); meanwhile, P is vital for adenosine triphosphate (ATP) production to supply chemical energy in plant metabolisms (Cruz et al., 2005). The N deficiency resulted in plant necrosis and stunted growth (Veazie et al., 2020), while the lack of P delayed generative growth and fruit ripening (Abobatta and Abd Alla, 2023).

Farmers grow strawberries in the soilless substrate in which N and P content is insufficient for plant growth and production. Chemical fertilizers are widely used in soilless substrates because they provide high amounts of readily available nitrogen (N) and phosphorus (P). On the other hand, its continuous and excessive use does not support sustainable agriculture since inorganic fertilizer has the potency to reduce the yield and farmer's revenue (Bilong et al., 2022). A way to save the dose of chemical fertilizers in horticultural crops is the application of biostimulants and biofertilizers (Suwandi et al., 2017; Parađiković et al., 2019).

Over the last few decades, natural humic substances, including humic acid (HA) and fulvic acid (FA), have been utilized in agriculture as biostimulants (Calvo et al., 2014; Shah et al., 2018). Both compounds are necessary for the metabolism, signaling, and hormonal regulation of the growth and development of plants (Parađiković et al., 2019). Humic substances are reported to stimulate plant proton pumps across the cell membranes to promote root growth (Zandonadi et al., 2016). Humic acid is suggested to improve fruit yields, such as tomatoes (Haghighi and da Silva, 2013) and leafy vegetables (Ugur et al., 2013) in hydroponic systems. In potted strawberries, 8-week applications of mixed HA and FA enhanced photosynthetic pigments and photosynthetic efficiency (Neri et al., 2002). Foliar application of HA and salicylic acid increased strawberry fruit yield, sweetness, and mineral content (Aghaeifard et al., 2016).

Biofertilizers are widely used in sustainable agriculture; their active ingredients are soil microbes, mainly plant growth-promoting rhizobacteria (PGPR). The N-fixer bacteria (NFB) and P-solubilizer microbes (PSB) are among the essential microbes formulated in biofertilizers. The PGPR usually produces important phytohormones for plants, including Indole-3-acetic acid (IAA) as well as gibberellins and cytokinins (Mali and Bodhankar, 2009; Fukami et al., 2017; Patel and Saraf, 2017). Faculty of Agriculture Universitas Padjajaran has developed a consortia biofertilizer containing N-fixer bacteria and P-solubilizer microbes. Biofertilizer has been proven to increase the growth of various crops (Hindersah et al., 2017; Kalay et al., 2019; Hindersah et al., 2021a).

In the soilless system, the biofertilizer reduces the NPK fertilizer dose in potted strawberry seedlings (Hindersah et al., 2022). However, the mixed-use of biofertilizer and humic substances has not yet been done. The objective of this experiment was to determine the effect of humic substances, including HA and FA, combined with biofertilizer on the total N and P of the growing media, populations of N-fixing bacteria, and P-solubilizing bacteria in the rhizosphere and yield of potted strawberry.

MATERIALS AND METHODS

The experiment was conducted in the strawberry field belonging to CV Bumi Agro Technology Farm in Cisarua (6°48'01.9"S 107°34'02.1"E); a mountainous area in Bandung Barat District, West Java. The farm was located 1,250 m above sea level. The daily temperature during the experiment was 17-31 °C and 24 °C on average, while the humidity was 46.2-97.1% and 72.61% on average.

The growth substrate for the 'Festival' variety of strawberry, supplied by CV Bumi Agro Technology Farm, comprises a mixture of cocopeat, chicken manure, and sheep manure in a volume ratio of 8:2:1 (Hindersah et al., 2021b). The substrate had an acidity (pH) of 6.9; and contained organic-C of 43.1%, total N of 1.1%, C/N of 39.1, P_2O_5 of 0.007%, and K_2O of 0.21%. The exchangeable Ca and Mg of substrate were 30 cmol/100 and 1.42 cmol/100g, respectively. Before mixing with the cocopeat, the pH of chicken manure was 5.86 and contained 2.74 x 10^{11} CFU/g of total bacteria, 1.1 x 10^5 CFU/g of total fungi, and 5.6 x 10^6 NFB, while PSB was not detected.

The biofertilizer is developed by the Soil Biology Laboratory of the Faculty of Agriculture Universitas Padjajaran; it is composed of NFB Azotobacter, Azospirillum, and Acinetobacter; and PSB Pseudomonas and Penicillium. The bacterial and fungal densities were at least 10^7 and 10^5 CFU/mL, respectively. Based on a previous study, all bacteria produced phytohormones (Hindersah et al., 2021a).

Experimental design

The design of the pot experiment was a randomized block design with seven treatments and four replications. The treatments were as follows: A = control; B = humic acid 0.9 kg/ha; C = fulvic acid 1 L/ha; D = consortium biofertilizer 3L/ha; E = consortium biofertilizer + humic acid; F = consortium biofertilizer + fulvic acid; G = consortium biofertilizer + humic and fulvic acid. Before application, the biofertilizer, HA, and FA were diluted at 5%, 12%, and 0.1% in water, respectively. Before dilution, the amount of humic acid, fulvic acid, and biofertilizer per plant was 0.018 g, 0.02 mL, and 0.06/plant, respectively. All the doses and concentrations are based on the recommendation. Each replication consisted of 2 pots; one for soil and plant analysis at vegetative stadia and another for yield parameters.

Experimental setup

The strawberry cv. 'Festival' was grown in 2 kg of substrates in 30 cm × 30 cm black polyethylene pots. Six-week-old strawberry seedlings were obtained from the runner of the mother plant. Single strawberry seedlings were planted at a depth of 5 cm in the middle of the substrate. Biofertilizers, HA and FA, were applied twice at four weeks and six weeks after transplanting by soil dressing. The granule NPK fertilizer (16:16:16) was applied 2 g a week after planting (Palupi et al., 2017); a similar NPK dose was given every month for four months. The NPK fertilizer was placed on the 2 cm deep circular band 3 cm away from the stem (Hindersah et al., 2021b). All plants were placed outdoors under the transparent plastic shade; the distance between pots was 20 cm.

Parameters and statistical analysis

Root length and root dry weight, total N and P of the substrate, and population of NFB and PSB in the substrate around the roots were analyzed when strawberry plants entered the late vegetative phase 8 weeks after planting (WAP). In order to obtain the root dry weight, roots were wrapped in a paper bag and stored at 70 °C for two days until constant weight. The total N and P of substrates were analyzed by the Kjeldahl method according to the Association of Official Analytical Chemists (AOAC) methods for proximate analysis (AOAC, 2012). The NFB and PSB were counted by using the Total Plate Count method (Ben-David and Davidson, 2014) in James Nitrogen Free malat-Bromthymol agar (Baldani et al., 2014; Setiawati et al., 2023) and Pikovskaya agar (Sankaralingam et al., 2014; Fitriatin et al., 2020) respectively. Fruits were harvested six times until 16 weeks after planting. The fruit weight, number of fruit, and average weight of single fruit and sugar solution were measured from all harvested fruits. The sugar solution of the fruits is determined by the Brix refractometer (Vee Gee Scientific BTX-1, China). All data were subjected to the analysis of variance at $p \leq 0.05$; if the treatment significantly affected the parameter, then the Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ was performed. Statistical analysis was done by IBM SPSS Statistics version 24.

RESULTS AND DISCUSSIONS

The combination of BF and Humic substances had no significant effect on root dry weight and length (Table 1). The biofertilizers did not influence the root parameters due to environmental factors such as the pH of the substrate. Hindersah et al. (2022) verified that the consortia biofertilizer increased plant growth and nitrogen and phosphorus uptake of strawberry seedlings grown in pots with the soilless substrate. In the current research, the pH of substrates was 6.9; the strawberry prefers the substrate with slight acid. The substrate acidity at week 7 varied between 6.6-6.81 (Table 1) but there was no significant difference between treatments. This acidity is higher than the optimal pH for strawberries.

Although there was no effect on root traits, the application of biofertilizer combined with HA and FA has the potency to produce better root growth compared with humic- or fulvic acid application with and without biofertilizer inoculation. Root dry weight and plant length with biofertilizer+HA+FA were 12-64% and 13-25% higher, respectively, compared to other treatments, although they did not significantly differ from the control.

Table 1. Effect of humic substance and consortia biofertilizer on roots biomass of 7-week-old strawberry on soilless substrates

Treatments		Root dry weight (g)	Root length (cm)	Substrate acidity
A:	Control	1.18 a	25.06 a	6.81 a
B:	Humic acid (HA)	1.47 a	24.02 a	6.68 a
C:	Fulvic acid (FA)	1.82 a	24.74 a	6.77 a
D:	Biofertilizer (BF)	1.99 a	27.22 a	6.79 a
E:	BF + HA	2.16 a	27.00 a	6.73 a
F:	BF + FA	1.64 a	23.96 a	6.83 a
G:	BF+HA+FA	2.42 a	30.86 a	6.81 a

Numbers followed by the same letters were not significantly different based on DMRT at $p < 0.05$

Cocopeat-based substrates contained various amounts of total N and P, but the difference was relatively small (Kamaluddin et al., 2022). Based on DMRT at $p < 0.05$, the total N and P of substrates were determined by treatments (Table 2). In the current study, the control plants had lower total N and P, while plants treated with biofertilizer combined with humic substances (G treatment) significantly had a higher content of both major nutrients. All treatments received half of the recommended dose of NPK; enriching substrate with biofertilizer and both humic substances increased total-N and total-P by 74.6% and 54%, respectively. The substrate treated with biofertilizer and fulvic acid showed less increase in both parameters (54% and 27.9%). Mixed biofertilizer in this study contained P-solubilizing microbes as well as N-fixing bacteria that enable to provide of available N; moreover, all bacteria in this liquid biofertilizer produced phytohormones mainly auxin (Hindersah et al., 2021) that play a role in root development. Furthermore, better root growth will facilitate the uptake of N and P. Unfortunately, at the end of the experiment, the P-solubilizing bacteria could not be isolated from the substrate.

Table 2. Effect of humic substance and consortia biofertilizer on N and P content of substrates of 8-week-old strawberry

Treatments		Total-N (%)	Total-P (%)
A:	Control	2.01 a	0.68 a
B:	Humic acid (HA)	2.26 b	0.76 b
C:	Fulvic acid (FA)	2.15 ab	0.66 a
D:	Biofertilizer (BF)	2.69 bc	0.77 b
E:	BF + HA	3.10 c	0.87 c
F:	BF + FA	2.27 bc	0.71 ab
G:	BF+HA+FA	3.49 d	1.05 d

Numbers followed by the same letters were not significantly different based on DMRT at $p < 0.05$

The mixed application of biofertilizer and humic substance enhances the NFB population in the root-affected substrate for growing strawberries (Figure 1). Application of either HA or FA resulted in a similar NFB count to the control. Mixed application of biofertilizer, HA, and FA led to the highest NFB population, approximately 153% higher than the control and humic substances treatments (B and C). Biofertilizer inoculation alone caused a 22% higher NFB population than the control. It was increased in the NFB population in G treatment caused by the root growth enhancement (Yigit and Dikilitas, 2008; Zhang et al., 2021), which in turn increases the root exudates that serve as a nutritional source for the heterotrophic bacteria (Sun et al., 2021).

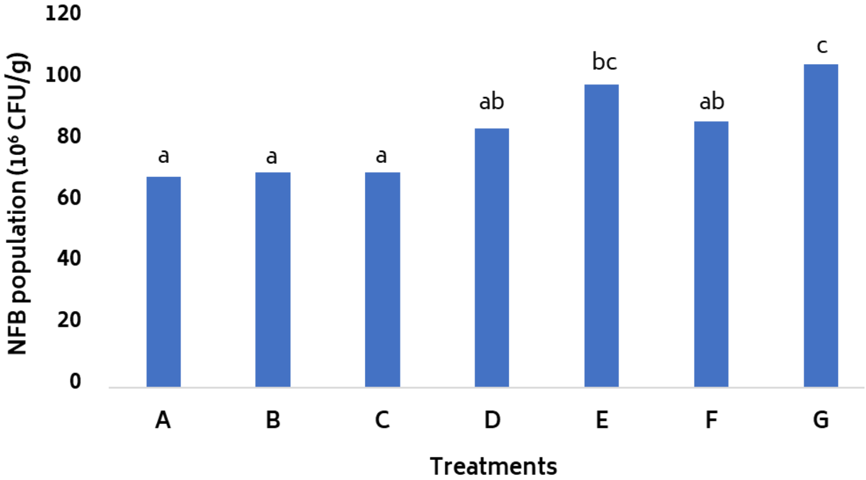

Figure 1. The population of N-fixing bacteria (NFB) in cocopeat-based substrates of strawberry after humic substances and biofertilizer application. A: control, B: humic acid, C: fulvic acid, D: biofertilizer, E: biofertilizers + humic acid; F: biofertilizers + fulvic acid; G: biofertilizer + humic and fulvic acids. Values with the same letters on the bar were not significantly different based on DMRT at $p < 0.05$

In the current experiment, the PSB was not grown in Pikovskaya media (Figure 2); the PSB contained in biofertilizer did not proliferate in cocopeat-based substrates. The absence of PSB in Pikovskaya media was verified with no halo zone around any colonies grown on the agar. Pour and Streak Methode on agar are usually used to test P-solubilization by soil bacteria (Pande et al., 2017; Wang et al., 2022). The absence of PSB in substrates was due to competition with other microbes, unsuitable substrates for microbial growth, and insufficient nutrient availability for PSB proliferation; that reasons are supported by the research results of PSB application on strawberry soil (Sharon et al., 2016). The Pikovskaya media is a specific media for detecting the presence of PSB since it contains non-soluble Calcium phosphate (Manoharan et al., 2018). Organic production by PSB will reduce the pH and induce better phosphate solubilization. The soilless substrate did not contain unavailable inorganic phosphate, but the substrate contained a significant amount of available P from the fertilizer; therefore, the PSB activity will cease.

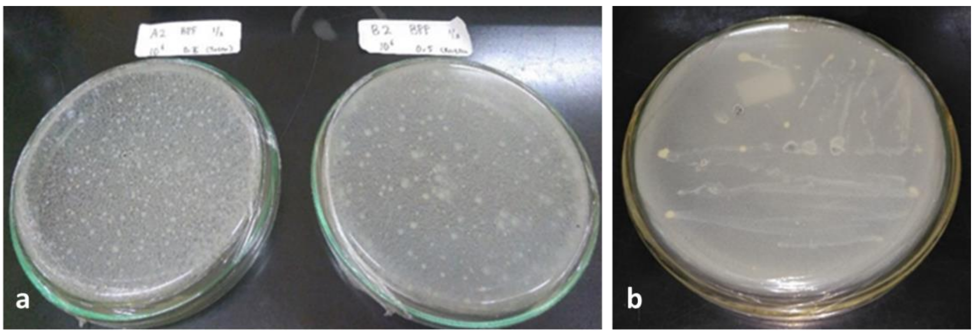

Figure 2. Colonies of bacteria isolated from strawberry substrates showed no halozone after 72 h incubation at 30 °C by using pour method (a) and streak method (b) on Pikovskaya plate agar

The fruit weight of a strawberry plant was clearly increased when the substrates were treated with biofertilizer mixed with HA (E) and HA + FA (G), even though the fruit number and weight per fruit were unchanged (Table 3). Nonetheless, fruit diameter increments were shown by plants treated with biofertilizer and mixed with BF and FA (Table 3); regardless of fruit diameter, the red color of fruit harvested from plants with BF and FA was more intense (Figure 3). Plants treated with mixed biofertilizer and HA and FA (G treatment) had 77% more fruit weight than the control.

The experiment showed that biofertilizer, in combination with HA and FA, increased the Total-N and total-P of substrates. Phosphorus, as well as N and K, are prominent macronutrients that have significant effects on strawberry yield as well as fruit quality in the soilless substrate (Wu et al., 2020). HA and FA have a role in stimulating plant growth (Parađiković et al., 2019) even though the effect was insignificant in current research.

The sweetness was slightly affected by humic substances and biofertilizer treatments. Nonetheless, mixed application of biofertilizer and humic substance increased the sweetness to 9.05; in this experiment, that is the highest Brix degree (Table 3). The titratable acidity reduction and brix increment have been shown by strawberries treated with HA, FA, P-solubilizing bacteria, and N-fixing bacteria (Cruz et al., 2022).

Table 3. Effect of humic substance and consortia biofertilizer on yield parameters of strawberry in a soilless substrate

Treatments		Yield per plant		Weight per fruit (g)	Fruit diameter (cm)	Brix degree
		Fruit weight (g)	Fruit number			
A:	C	18.8 a	2.1 a	8.8 a	2.4 a	8.15 a
B:	HA	20.8 ab	2.5 a	8.2 a	2.5 a	8.24 ab
C:	FA	20.1 ab	2.6 a	7.7 a	2.5 a	8.16 ab
D:	BF	23.2 ab	3.2 a	7.3 a	2.8 b	8.13 ab
E:	BF+HA	27.5 bc	2.9 a	9.6 a	2.5 a	8.74 bc
F:	BF+FA	24.7 ab	2.8 a	8.8 a	2.8 b	8.23 ab
G:	BF+HA+FA	33.4 c	3.5 a	9.4 a	2.5 a	9.05 c

Numbers followed by the same letters were not significantly different based on DMRT at p < 0.05

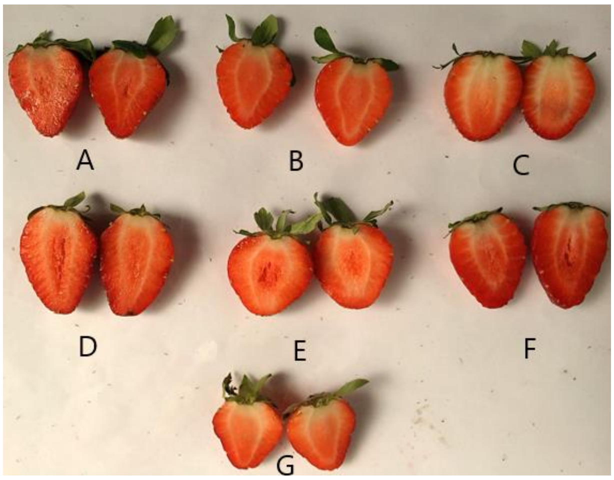

Figure 3. The difference in diameter of strawberry fruit grown in the soilless substrate with various humic substances and biofertilizer, A: control, B: humic acid, C: fulvic acid, D: biofertilizer, E: biofertilizers + humic acid; F: biofertilizers + fulvic acid; G: biofertilizer + humic and fulvic acids

The study demonstrated that HA, FA, and biofertilizers have a role in increasing total N and P content as well as NFB population in the substrate of strawberries. Moreover, the combined application of consortia biofertilizer, humic acid, and fulvic acid significantly resulted in fruit weight and sweetness increment of strawberries grown in cocopeat and chicken manure-based substrate. The experiment was performed in the soilless substrate; root growth did not hamper by physical constraints, so the effect of HA and FA as well as biofertilizer, did not show. However, the application of biofertilizer and humic substance might not change the root length and weight measured in this experiment. Humic substances and biofertilizers also play a role in other root structures, such as branching and root hair density which did not count in this study. An increase in fruit weight shows that humic and fulvic acids can play a role in the physiology of nutrient absorption (Canellas and Olivares, 2014).

The role of humic substances to improve nutrient uptake and stimulate plant growth has been described. Humic acid is attributed to the Krebs cycle in plant tissue due to its low molecular size (Nardi et al., 2007). They have a prominent role in specific physiological effects (Parađiković et al., 2019), including photosynthetic pigment synthesis (Neri et al., 2002). In the current study, the nutrient uptake in strawberry shoot did not analyze. Still, the slight increase in fruit weight (10% and 7%) after applying HA and FA explained that both humic substances have a role in nutrient metabolisms. Acidic properties are related to the deprotonated OH/OOH of the carboxylic, and phenolic might reduce the pH (De Melo et al., 2016). Before the experiment, the pH of the substrate was 6.9; the reduced pH possibly happened in the substrate with humic acid. Reduced acidity of substrate gave the advantage for strawberries since their optimum pH for better growth is 5.5-6.8 and 5.2-5.8 in soilless substrate (Sangiorgio et al. 2023).

Humic substances contain C, H, O, and N (Nardi et al., 2021) that probably contribute to the N increment of the substrate, which is shown by total N enhancement after humic substances application. The increase of the NFB population in the substrate might cause total N and P increments. Death and living cells of NFB possibly are being extracted during N and P measurement in the laboratory. Even though all treatments have no effect on root dry weight, the application of HA and FA had the potency to increase root dry weight.

A single application of biofertilizer only brought about a slight increase of N and P in the substrate. The NFB and PSB live in the proximity of root that provide root exudates as their carbon and other nutrition sources. Available nitrogen, formed through nitrogen fixation, can be released into the substrate. Surprisingly, biofertilizer inoculation also somewhat increased the total P of the substrate, even though in this current study, the phosphate solubilizing bacteria was not detected in all treatments. The increase of total P of the substrate with biofertilizer is possibly caused by the rise of secondary root growth, which is not analyzed in this pot experiment. The PSB Pseudomonas, as well as NFB Azotobacter and Azospirillum in general, produce phytohormone indole-acetic-acid (IAA) and cytokinin, while the PSB Pseudomonas that regulate root and shoot growth (Egamberdieva et al., 2017; Mali and Bodhankar, 2009; Fukami et al., 2017). The NFB Azospirillum is also reported to produce IAA (Fendrihan et al., 2014). Therefore, bacteria in biofertilizers in this study have a role as a biostimulant. The nature of some bacteria in the biofertilizer to produce organic acid plays a role in lowering the pH of the substrate (Ramalakshmi et al., 2008; Bouizgarne, 2022) that promotes strawberry growth.

The mixed application of humic substances and biofertilizer strengthens the impact, possibly due to nutrient metabolism improvement. In this experiment, those effects resulted in a significant total N and P increment as well as yield enhancement. The experiment's results agreed with the impact of HA in increasing sugar content in tomatoes (Haghighi and Da Silva, 2013); they yielded as well as the brix index of strawberries (Aghaeifard et al., 2016). Mixed NFB, PSB, and reduced potassium dose is the best way to increase strawberry yield (Bhagat & Panigrahi, 2020). Therefore, the current study demonstrated that mixing the humic substance of consortia biofertilizer (NFB and PSB) and application of reduced NPK fertilizer increased strawberry yield over conventional treatment.

CONCLUSION

The single and mixed application of HA, FA, and consortia biofertilizer did not affect the roots length and dry weight of strawberries grown in cocopeat-based substrate with low NPK fertilizer. Nonetheless, the combined use of humic substances and biofertilizer increased total N and P in the substrate and the population of N-fixing bacteria that colonized the near-root substrate. The experiment verified that P-solubilizing bacteria were not found in the cocopeat-based substrate, possibly because they did not solubilize the P due to the lack of unavailable P in the substrate. Mixed HA, FA, and biofertilizer resulted in strawberry yield and fruit sweetness level increment over conventional treatment. However, they did not affect fruit number and weight per fruit. For further research, we suggest counting all species of microbes formulated in this consortia biofertilizer. Moreover, the root volume, number of root branches, and density of roots hair need to be studied to verify whether the HA, FA, and biofertilizer influenced the detailed root parameter.

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