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Evaluation of Strawberry Seedling Growth in Various Planting Media Amended with Biofertilizer

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Abstract.
The quality of strawberry seedlings is impacted by the composition of the growing medium. Due to its good drainage and high water-holding capacity, coco-peat is now widely used as a major component of strawberry substrate; however, coco-peat has a low nutrient content. By combining coco-peat with manure and biofertilizer, more nutrients can be provided. This study investigated the effect of the growing medium composition with biofertilizer inoculation on the growth and quality of strawberry seedlings in a mountainous area using a pot experiment. With six treatments and five replications, the experiment was set up in randomized block design. The treatments involved substrate composition, which included cocopeat and chicken manure in ratios of 2:1, 2:3 and 3:2, and biofertilizer inoculation (with and without). Nitrogen-fixing bacteria and phosphate-solubilizing microbes were included in the biofertilizer. The results showed that substrate composition affected plant height, shoot and root dry weight, chlorophyll content, and nitrogen and phosphorus uptake by seedling shoots, but not the surface area of leaves on six-week-old seedlings. The addition of biofertilizer to substrates made up of cocopeat and chicken manure in ratios of 2:3 and 3:2 increased plant growth and nitrogen and phosphorus uptake.

Keywords: Chicken namnure, Cocopeat, N_2-fixer bacteria, P-solublizer Microbes.

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1. Introduction

Strawberry (Fragaria x ananassa Duch.) was introduced to mountainous area of West Java, and become a popular and high-priced fruit due to their flavor and taste. Strawberry is being cultivated in tropical area for reducing their imports from subtropical countries. Cultivation of strawberry in tropic can performed almost throughout the year in soil or soilless cultivation. Nowadays, coco peat is widely used as a major component for strawberry's substrate in due to good in drainage and high in water holding capacity.

The compositions of growth media is one of the factor that determines strawberry seedling quality and hence the yield. The used of cocopeat as a substrate component is common in strawberry nursery and production because of its porosity and relatively good water holding capacity [1]. In the nursery, cocopeat growth substrate enriched with chiken manure support the growth of strawberry seedling with NPK fertilizer [2]. Mixing vericompost with cocopeat and perlite is reported to produce tasty strawberry and enhance the yield [3].

The main disadvantage of cocopeat is low in plant nutrient content. The nutrient composition of cocopeat in [1] include 0.39% of total Nitrogen (N), 0.41% of phosphorous (P) and 2.39% of Potassium (K). The composition of another cocopeat was 0.14% N, 0.04 % P, 0.65% K+, 9.75% Ca2+ dan 0. 25% Mg2+ [4]. Therefore, it is a common practice to mix cocopeat with manure and fertilizer. In order to maintain strawberry plant quality, inorganic fertilizer mainly N, P and K is commonly introduced. In the cocopeat-based nursery, nutrient solution composed of N, P, K and Mg supplied to mother plant increased the number of runner and rooting plantlets of individual plant during three consecutive years [5].

Vegetative phase of plant needs not only macro and micro-nutrient but also secondary metabolite phytohormones that function as biostimulants. Phytohormones such as gibberellins (GAs) auxins mainly indole acetic acid (IAA) and cytokinins (CKs) are growth hormones that regulates plant growth and development [6]. In strawberry plant, growth hormones such as GA3 and CKs were very effective in increasing the vegetative growth, flowering and yield of the fruits either in temperate or tropical area [7][8]. Application of exogenous cytokinins is effective to alter the cytokinin and auxin ratio in plant tissue and hence to regulate vegetative growth of strawberry as well as yield and fruit quality [9].

The rhizobacteria is well known soil beneficial microbes that synthesis and secrete growth hormones. Moreover, some rhizobacterial species contribute to plant growth by providing major nutrient through N2 fixation and P solubilization of unavailable soil phosphorous. In soilless growth media, the exogenous phytohormones content might be limited; and the N and P level are low. Introduction rhizobacteria formulated in mixed biofertilizer is suggested to fulfill the needs of growth hormones and major nutrient mainly nitrogen for seedling growth. The advantage of biofertilizer in strawberry cultivation have been documented. Mixed application of N-fixer Azotobacter and phosphate solubilizing bacteria at the rate of 6 Kg/ha clearly increased the height, number of leaves and crowns diameter of strawberry [9]. Inoculation of N-fixer Azotobacter and Azospirillum following a recommended dose of N fertilizer is suggested to produce strawberry in green house [10].

Despite the importance of biofertilizer in strawberry growth, biofertilizer consumption in Indonesia include in small fruits and vegetable production in Indonesia is low. Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran have developed a mixed biofertilizer of N fixing bacteria and P solubilizing microbes. All the microbes exclude the P solubilizing fungi produce the phytohormone. This liquid biofertilizer have recorded to increase the growth of caysim [11] and nutmeg seedling [12] in potted-soil culture. Nonetheless the effectivity of biofertilizer on soilless media has not been studied. The objectives of this greenhouse experiment were to 1) observe the effect of different compositions of growth media and biofertilizer inoculation towards nitrogen and phosphorus uptake, and the growth of strawberry seedlings and 2) finding the best composition of growth media for strawberry seedling growth in soilless media.

2. Methodology

The pot experiment was carried out on February to April 2019 in the greenhouse belong to Bumi Agrotechnology Farm located in Cisarua, in Bandung Barat Regency of West Java, Indonesia. Cisarua district is a tropical mountainous area with the altitude of 1,225 m above sea level while the average annual temperature of 17 0C -26 0C and humidity of 70-90%.

The experiment has been laid on the completely randomized block design with six treatments and five replications. The treatment was the composition of cocopeat and chicken manure for the growth substrate i.e 2:1, 2:3, 3:2 (v/v) with and without biofertilizer inoculation. Before experiment, the major nutrients content of each substrates were analyzed (Table 1) for Each replication consisted of three seedlings. The treatments and replications were chosen randomly within the parent plant with the single or two stolons.

Table 1: Nutrient charactersitics of each planting media composition before experiment.

Chemical properties	Unit	Ratio of cocopeat to chicken manure		
		2:1	2:3	3:2
C-organik	%	46,74	43,58	44,52
C/N	-	39,29	33,39	35,62
N	%	1,19	1,32	1,25
P_2O_5	%	0,003	0,025	0,025
K_2O	%	0,19	0,15	0,14
Kadar Air	%	73,29	71,76	75,23

2.1. Experimental establishment

The molasses-based biofertilizer prepared by Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The liquid inoculant consists of N-fixing bacteria (*A. chrooococcum*, A. *vinelandii*, *Azospirillum* sp., and endophytic *Acinetobacter* sp) and P-solubilizing microbes (*Bulkholderia cepacea*, and *Penicillium* sp.). The density of bacteria and fungi based on serial dilution plate method [13] was 10^7 CFU/mL and 10^5 CFU/mL respectively.

The 6 months-old strawberry parent plants were growing in the greenhouse in a 3-m polyvinyl chloride (PVC) rectangular container with the distance of 25 cm (fig 1a). The PVC containers for nursery are placed in the scaffold at the greenhouse (Fig 1a). The substrate for producing the parent plants was mixed of cocopeat and chicken manure at the volume ratio of 2:1. The parent plants were chosen purposively based on the single stolon they produce. Seedlings for this pot experiment were intact runner (stolon) that was still attached to the parent plants. The 2-weeks old stolon required for the treated-seedlings had already 2-cm roots, 0.5-cm crown stem diameter and 2 trifoliate leaves.

Planting media were prepared manually by mixing the cocopeat and chicken manure evenly in a bowl. The composition of both ingredients was according to the treatments described above. As much as 40 g of substrates were put in the 12 cm x 5.5 cm perforated black polyethylene bag (polybag). Biofertilizer were applied to the growth media by injecting 2 mL liquid biofertilizer in order to obtain the final bacterial and fungal cell density of 10^7 and 10^5 CFU/g substrate. The inoculated-substrate were then incubated for six days in the greenhouse prior to planting the runner. One rooted-runner with two open trifoliate leaves from single parent plants (Fig 1b) were grown in polybag contained growth media. The treated seedlings were put in the PVC in adjacent to their parent plants (Fig 1c).

Figure 1: Parent plants growing in the greenhouse (a) with stolon (b) treated by mixed biofertilizer while still attaching to their parents (c).

Compound N-P-K fertilizer (16-16-16) was applied to parent plant with the dose of 5 mg per plant which was applied every two weeks until 6 weeks after planting. The fertilizers were put in 3 cm-depth holes at 10 cm from parent plants stem. Seedlings were watering everyday with 100 ml of ground water until the water percolate through the holes in the bottom of polybag. During the experiment, the pest was controlled manually; parent leaves with powdery mildew diseases were cut by using clean scissor. necrotic leaves due to tip burn and flowers – if any – in parent plant were cut.

2.2. Parameters and statistical analysis

The growth and nutrient status parameter of seedlings included plant height, leaves number, leaf area, shoot to root ratio (S/R), total chlorophyll content, and N and P content and uptake of shoot. Leaf area was determined by using an automatic digital image analysis [14]. The chlorophyll a and b were extracted from the leaves by utilizing methanol prior to chlorophyll (Chl) measurement by spectroscopic method at absorbance of at 663.6 nm for Chl a, 646.6 nm for Chl b [15].

In order to obtain the shoot dry weight, shoots were wrapped in paper bag and stored in the oven of 70 ^{0}C for two days until constant weight. The content of N and P in shoots was analyzed by Kjeldahl Method according to Association of Official Analytical Chemists (AOAC) methods for proximate analysis [16]. The N and P uptake of shoots was calculated by multiplying the N or P content in shoot by the shoot dry weight.

All data were subjected to the analysis of variance (Anova) at $p \leq 0.05$; if the treatment have influenced the parameter significantly then the Duncan's Multiple Distance Test at $p \leq 0.05$ was done [17]. Statistical analysis had been carried out by IBM SPSS statistics version 24.

3. Results and Discussion

The temperature in the greenhouse during the experiment were 170C in the early morning and 36 0C at noon; while the humidity was 65,4% at noon and 90% in the morning. In Indonesia [18], the strawberry tolerates to high temperature up to 26.1 0C but their best productivity is at 17-20 0C. From 90 treated seedlings, 5 were lightly attack by species of powdery mildew, possibly Sphaerotheca macularis [19].

3.1. Plant Growth

Based on Anova, planting media significantly affected shoot height, leaves chlorophyll and shoot to root ratio but did not affect the leaves area. At 6 weeks after planting (WAP) seedlings grown in substrate with cocopeat (C) and manure (P) of 3:2 was taller than control and another treatment (Table 2). Before experiment, the seedling shoot height was 6.36-6.86 cm with 2 trifoliate leaves. At 6 weeks after planting, all plants included the control one has 5 trifoliate leaves.

TABLE 2: Strawberry Seedlings Height in Various substrate Compositions of Cocopeat and Manure with and without Biofertilizer at 6 Weeks after planting.

Substrate composition[1]	Shoot height (cm) at 1-6 weeks						
	0	1	2	3	4	5	6
A : (2:1)	6.36	7.47a	8.43a	9.32a	9,98a	10.60a	11.27a
B : (2:3)	6.85	8.17b	9.05ab	9.93ab	10,65ab	11.36ab	11.99ab
C : (3:2)	6.67	8.16b	9.50bc	10.30bc	11,35b	12.03bc	12.63b
D : (2:1) + BF[2]	6.97	8.55bc	9.90cd	10.70c	11.37b	11.94b	13.27bc
E : (2:3) + BF	6.84	8.99cd	10.17d	11.42d	12.25c	12.87cd	13.63cd
F : (3:2) + BF	6.86	9.12d	11.19e	11.79d	12.45c	13.22d	13,97d

Numbers in a column followed by the same letters was not significantly different based on Duncan's Multiple Range Test at $p \leq 0.05$. [1]Volume ratio of cocopeat and manure, [2]Mixed biofertilizer

The leaf area (LA) of control seedlings was potentially lower than LA of seedling grown in planting media with C:P of 2:3 and 3:2 with and without inoculation (Fig 2), but statistically they are not different. Fig 2. showed that total chlorophyll content was increased in seedlings grown in various planting media composition compared to the control. Regardless of substrate composition, biofertilizer application on the substrate of seedling caused chlorophyll increment. The inoculation of biofertilizer in planting media with C:P of 2:3 and 3:2 clearly increased chlorophyll content compared to C:P of 2:1.

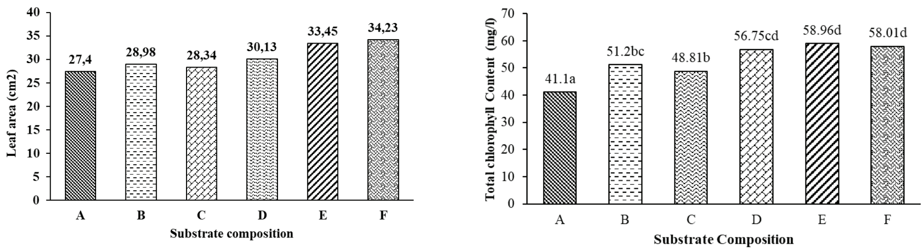

Figure 2: Leaf area and chlorophyll content of strawberry seedlings grown in various substrate composition with and without biofertilizer at 6 weeks after planting.

All shoot to root ratio (S/R) of strawberry at 6 WAP were more than 1 indicated that shoot growth was more rigorous than roots (Table 3). Surprisingly, the S/R of control were similar to that of seedlings grown in all substrate composition with bacterial inoculation. Regardless statistical analysis, the highest S/R was showed by seedling grown in planting media with C:P of 3:2 with biofertilizer.

TABLE 3: Effect of substrate compositions of cocopeat and manure with and without biofertilizer on shoot and root dry weight, and shoot to root ratio (S/R) at 6 weeks after planting.

Substrate composition[1]	Dry weight (g)		S/R
	shoot	Root	
A: (2:1)	1.80	0.27	6.67 a
B: (2:3)	1.95	0.34	5.74 b
C: (3:2)	1.88	0.38	4.95 c
D: (2:1) + BF[2]	2.79	0.44	6.34 a
E: (2:3) + BF	3.85	0.55	7.00 a
F: (3:2) + BF	3.21	0.45	7.13 a

Numbers in a column followed by the same letters was not significantly different based of Duncan's Multiple Range Test at $p \leq 0.05$. [1]Volume ratio of cocopeat and manure, [2]Mixed biofertilizer

Nitrogen and Phosphorous Status in shoot

The effect of various composition of planting media did not change the N and P content of shoot but influenced N and P uptake by individual plant due to different shoot dry weight. At the late vegetative phase (6 WAP), the N and P content was about 1.92-2.07% and 0.05-0.09% respectively (Table 4). Jones et al. (1991) stated that sufficient N and P content in mature plant was 2.50-4% and 0.25-1% respectively. The results verified that both macronutrient content was low especially the P.

TABLE 4: Effect of various cocopeat to manure ratio with and without biofertilizer on nitrogen status in shoot of strawberry seedlings at 6 weeks after planting.

Substrate composition[1]	N content (%)	N uptake (mg/plant)	P content (%)	P uptake (mg/plant)
A: (2:1)	2.06	37.24 a	0.05	0.87 a
B: (2:3)	2.02	39.97 a	0.07	1.25 b
C: (3:2)	1.98	36.94 a	0.09	1.57 c
D: (2:1) + BF[2]	2,07	59.07 ab	0.07	1.84 d
E: (2:3) + BF	2.13	82.57 b	0.06	2.31 e
F: (3:2) + BF	2.07	67.35 b	0.06	1.80 d

Numbers in a column followed by the same letters was not significantly different based of Duncan's Multiple Range Test at $p \leq 0.05$. [1]Volume ratio of cocopeat and manure. [2]Mixed biofertilizer

The results showed that inoculation of mixed biofertilizer to seedling substrate increased leaves chlorophyll, shoot height and S/R significantly at 6 weeks but did not change the leaf area. The biofertilizer used in this experiment contained phytohormone-producing soil beneficial microbes [12] which is the prominent growth hormone to regulate plant growth. The IAA and CKs with the ratio of 1:2 promote the shoot growth

[20]. The GA in bacterial inoculant has a role to increase plant growth and this finding accord with the vegetative growth increment after introducing GA3 on strawberry [7]. Moreover, the ability of N-fixing bacteria (Azotobacter, Azospirillum and Acinetobacter) in biofertilizer can contribute to increase the availability of N for root uptake.

Table 4 showed that biofertilizer treated seedling have higher N uptake. The Nitrogen in major and essential macronutrient uptake in high amount during vegetative stage for their normal growth. Nitrogen is a part of carbon component if plant tissue; N is constituent of many plant cell substances such as chlorophyll, amino acids, proteins, nucleic acids, nucleotides, and coenzymes [20].

The P uptake of strawberry shoot at various substrate composition was increased following biofertilizer inoculation (Table 4). Phosphorous is major essential nutrient and integral part of chemical energy, Adenosine triphosphate. Sufficient amount of P is needed to provide the energy for plant metabolisms during whole stage of plant life including vegetative stage. Phosphorous has a prominent role in energy transfer, photosynthesis regulation, sugars and starches biosynthesis and carbon metabolism [20][21]. The sufficient Therefore, sufficient N and P uptake following biofertilization may improve the function of chlorophyll and plant growth, as well as inhibit necrosis due to nitrogen deficiency and stunting due to limited energy transfer.

Regardless of inoculation, the composition of substrate B and E (cocopeat: manure, 2:3) and substrate C and E (cocopeat: manure, 3:2) were more supportive on seedling growth compared to that of 2:1. Before experiment 2:3 and 3:2 substrates have water content of 72% and 75%, higher than the substrate A and D. The water content in soilless media dictated the plant growth since the water evaporation in soilless media was more intensive than soil. Moreover, the carbon to nitrogen ratio (C/N) of substrate 2:3 and 3:2 before experiment were lower than 1:2 (Table 1). In this experiment, the higher N and P content in substrates of 2:3 and 3:2 before experiment may involve in better plant growth.

4. Conclusion

Regardless substrate composition, inoculation of biofertilizer composed of N-fixing bacteria and P solubilizing microbes clearly increased plant height, shoot to root ratio, chlorophyll content, and N and P shoot content. Growing strawberry seedling with liquid biofertilizer in substrate with the volume composition of cocopeat and chicken manure of 2:3 and 3:2 resulted in highest root to shoot ratio, chlorophyll content as well as N and P uptake by shoot. This results suggested that in soilless media, biofertilizer application was effective to induce the growth of strawberry seedling at various composition of substrate.

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