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Application of Microgranules from Endophyte Bacteria in Stimulating Vegetative Growth of Arabica Coffee (*Coffea arabica* L.)

Kabul Warsito[1], Nur Asmaq[2], Indra Irawan[3], Muhammad Taupik[4],

[1,2,3,4]Universitas Pembangunan Panca Budi, Indonesia

Corresponding Author: Kabul Warsito

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ABSTRACT

Increase the vegetative growth of arabica coffee, needs to be innovation in the fertilizer used. The latest innovation in fertilizer manufacturing technology is to form endophytic bacterial cell cultures into microgranules using encapsulation techniques. The application of microgranules to Arabica coffee plants is carried out using the factorial CRD (Completely Randomized Design) method consisting of 2 factors, 16 treatments, 2 replications. The treatment of seed immersion time with the addition of endophytic bacterial suspension consists of: B0: without immersion, B1: 6.5 Hours; B1: 7.5 Hours; B3: 8.5 Hours. The addition of endophytic bacterial microgranule consisted of I0: 0 gr; I1: 5 grams; I2: 10 grams; I3: 15 grams. The data obtained were analyzed using variance analysis. The results of the variance analysis were continued with multiple distance tests. Isolation of coffee roots and stems obtained 8 isolates of endophytic bacteria. From the results of the research, the growth of coffee in suspension immersion and microgranule administration was very influential, the best plant height was in treatment B2 (14.20 cm) and the lowest treatment was in B0 (9.96 cm). While the diameter of the best treatment was in B3 (1.66 mm) and the lowest treatment was in B0 (1.39 mm).

Keywords: Microgranules, Endophytes, Arabica Coffee

INTRODUCTION

Coffee plant commodities that are often cultivated in Indonesia and are widely favored by the public are arabica coffee (*Coffea arabica*) and Robusta (*Coffea canephora*). Arabica coffee is better known than Robusta coffee because its coffee is of high quality, has a characteristic pungent aroma after the beans are roasted, a delicious taste and a strong and complex aroma, is very famous and has high economic value from 80 species of coffee in the world (Ika Priantari., et. al., 2022).

Public interest in coffee during the end of this decade continues to grow. Coffee is not only a drink that is identical to being drunk by the elderly, but coffee is also enjoyed by young people today. In addition, not only across generations, coffee is also enjoyed by various genders, which are usually enjoyed by men, now many women also like to drink coffee (Hafni, 2020, Tamilmani & Pandey, 2015).

North Sumatra is a potential place for coffee development. In 2023, coffee production in North Sumatra reached 71,588 tons with a planting area of 79,388.64 (BPS, 2021). Various types of coffee plants are spread across several districts. One type of coffee that is developed is Arabica coffee (*Coffee arabica L.).* In North Tapanuli district, the community is developing more Arabica

coffee production and in 2021, Arabica coffee production (*Coffee arabica L.*) in North Tapanuli reached 16,036 tons (North Tapanuli Potential Report Book; 2021).

Endophytic bacteria are bacteria that live in the host plant tissue without causing symptoms of disease (Bhore, 2010). Endophytic bacteria enter plant tissue generally through the roots, but parts of the plant that are directly exposed to air such as flowers, stems and cotyledons, can also be the entry route for endophytic bacteria. These microorganisms can live in vascular vessels or in intercellular spaces, (Zinniel, 2002) roots, stems, leaves and fruits (Simarmata et al., 2007).

MATERIALS & METHODS

Research Method

This study was done with use CRD method (Design Random Complete) factorial consisting of from 2 factors, 16 treatments, 2 replications. Treatment time immersion seed with addition suspense bacteria endophyte consists of from: B0: without immersion, B1: 6.5 Hours; B1: 7.5 Hours; B3: 8.5 Hours. Addition microgranule bacteria endophyte consists of from I 0 : 0 gr; I1: 5 grams; I2: 10 grams; I3 : 15 grams. Data obtained analyzed with use analysis variance. Analysis results variance to be continued with distance test Duncan's.

Preparation and Sterilization of Planting Media

The planting media used in this study were topsoil that had been cleaned from weeds, broiler chicken manure and rice husk charcoal. With a ratio of (1:½:½), topsoil: 50%, broiler chicken manure: 25% and rice husk charcoal: 25%. Sterilization was carried out at a temperature of 150^0 C for 10 hours.

Immersion Seed with Suspension of Bacteria Endophyte

The collection of endophytic bacteria solution was done by adding 10 ml of 0.9% NaCl solution into 1 petri dish, and stirring using a triangular stirring rod. The rubber seeds were soaked for 12 hours, 24 hours and 36 hours in a container covered with aluminium foil to keep it sterile.

Microgranules Producing from Endophytic Bacteria as Biofertilizer

Sterile alginate and inulin containing 100 ml of endophytic bacterial suspension are added to 300 grams of tapioca flour. The addition of starch is useful as an adhesive in making fertilizer but should not exceed 30%. The less adhesive added, the less likely the granular fertilizer will be damaged and quickly damaged when packaged (Lisa et al., 2015). The extraction method was chosen because it is easy and inexpensive to operate, provides high usability in its processing, and does not experience various possibilities such as when using the spray-drying technique (Suryani et al., 2019) when adding endophytic bacterial suspension solution.

Observation Parameters

The parameters observed in this study were the characteristics of endophytic bacteria, plant height (cm) and stem diameter.

RESULT

Isolation and Characteristics of Endophytic Bacteria from Roots and Stems

Coffee Plants From the isolation of endophytic bacteria from the roots and stems of coffee (*Coffea arabica L*), four isolates of endophytic bacteria were obtained, 2 isolates from the stems and 2 isolates from the roots. The 4 isolates have varying characteristics both in terms of morphology and color. The results of the isolation and characteristics of endophytic bacteria are presented in Table 1.

Table 1. The Results of The Isolation and Characteristics of Endophytic Bacteria

Species	Colony Color	Elevation	Edge (Margin)	Form (Whole)
Sp. 1 Ar	White	Flat	Filamentous	Filamentous
Sp. 2 Ar	White	Flat	Rhizoid	Rhizoid

Sp. 3 Ar	White	Flat	Irregular	Irregular
Sp. 4 Ar	White	Flat	Lobate	Irregular
Sp. 5 Ar	White	Flat	Filamentous	Rhizoid
SP. 1 Br	White	Flat	Irregular	Filamentous
SP. 2 Br	White	Flat	Irregular	Rhizoid

Plant Height (cm)

Plant height observations were conducted in months 1, 2, 3, 4, and 5 Month after planting (MAP). Based on the results of observations and analysis of variance, it is known that the immersion treatment affects the growth of coffee plants (*Coffea arabica L*). The results showed a significantly different effect on plant height (cm) in the 1st MAP and 5th MAP observations, and had a very significant effect on the 2nd MAP, 3rd MAP, and 4th MAP. However, it did not have a significant effect on the microgranule treatment at 1 MAP, 2 MAP, 3 MAP, and 5 MAP. The addition of microgranule was very significant in the 4th MAP observation (Kabul Warsito et al., 2023).

The effect of interaction of immersion variation and microgranule administration did not significantly affect the observation of plant height measurement (cm) on coffee growth (*Coffea arabica L*). The results of the Duncan distance test are shown in Table.

Table 2. The Results of the Duncan Distance Test

Treatment	**Average Plant Height (mm)**				
Immersion Treatment (B)	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP
B0 = 0 Hours	6.09 ^{aA}	6.55 ^{aA}	5.53 ^{dD}	9.66 ^{aA}	9.96 ^{dD}
B1 = 6.5 Hours	6.31 ^{aA}	6.33 ^{aA}	12.14 ^{aA}	11.03 ^{aA}	14.43 ^{aA}
B2 = 7.5 Hours	5.99 ^{aA}	5.45 ^{aA}	11.60 ^{abB}	11.71 ^{aA}	14.20 ^{bcBC}
B3 = 8.5 Hours	6.36 ^{aA}	7.36 ^{aA}	12.21 ^{abcA}	12.26 ^{aA}	15.89 ^{aA}
Microgranule Addition (A)					
A0 = 0 grams	6.15 ^{aA}	6.58 ^{aA}	10.66 ^{bA}	10.59 ^{aA}	13.01 ^{bA}
A1 = 10 grams	6.41 ^{aA}	6.63 ^{aA}	11.11 ^{abA}	11.50 ^{aA}	14.06 ^{abA}
A2 = 15 grams	5.91 ^{aA}	6.39 ^{aA}	9.85 ^{aA}	10.45 ^{aA}	14.94 ^{abA}
A3 = 20 grams	6.28 ^{aA}	6.10 ^{aA}	9.85 ^{abA}	12.13 ^{aA}	12.46 ^{aA}

Stem Diameter (mm)

Based on the results of observations and variance analysis, it is known that the effect of immersion and adding microgranules on the growth of coffee (*Coffea arabica L*) no significant effect on stem diameter (Setiawan et al., 2023). The interaction of the influence of immersion and the addition of microgranule did not have a significant effect on stem diameter. Diameter measurement data (mm) on coffee growth (*Coffea arabica L*) after being tested using the Duncan Distance Test is shown in Table.

Table 3. Data (mm) on coffee growth (*Coffea arabica L*) after being tested using the Duncan Distance T

Treatment	**Average Plant Diameter (mm)**				
Immersion Treatment (B)	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP
B0 = 0 Hours	0.48 ^{aA}	1.34 ^{dD}	1.41 ^{dD}	1.36 ^{cC}	1.39 ^{bcBC}
B1 = 6.5 Hours	0.49 ^{aA}	1.70 ^{aA}	1.71 ^{aA}	1.48 ^{aA}	1.48 ^{aA}
B2 = 7.5 Hours	0.65 ^{aA}	1.70 ^{abAB}	1.69 ^{abAB}	1.64 ^{bcABC}	1.64 ^{abcABC}
B3 = 8.5 Hours	0.42 ^{aA}	1.76 ^{abAB}	1.78 ^{abcABC}	1.66 ^{abAB}	1.66 ^{aA}
Microgranule Addition (A)					
A0 = 0 grams	0.51 ^{aA}	1.60 ^{aA}	1.56 ^{bA}	1.53 ^{aA}	1.53 ^{aA}
A1 = 10 grams	0.59 ^{aA}	1.60 ^{aA}	1.68 ^{abA}	1.53 ^{aA}	1.53 ^{aA}

A2 = 15 grams	0.50 ^{aA}	1.66 ^{aA}	1.68 ^{aA}	1.55 ^{aA}	1.55 ^{aA}
A3 = 20 grams	0.44 ^{aA}	1.64 ^{aA}	1.68 ^{abA}	1.54 ^{aA}	1.56 ^{aA}

DISCUSSION

Characteristics of Endophytic Bacteria from Coffee Plant Roots and Stems

AA hormone-producing bacteria are characterized based on colony morphology, namely colony shape, height, edge and color, and cell morphology through bacterial staining, namely cell shape and grammatical characteristics of bacteria (N. Lubis et al., 2020). Based on the results of bacterial isolation and endophytic properties in coffee plants, four different isolates were obtained, then all isolates showed gram (+) bacteria types from IAA-producing endophytic bacterial isolates. From previous studies, six different endophytic bacterial isolates were obtained, namely *Bacillus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Xanthomonas* sp. (Aizar & Parlina, 2017). These results were confirmed by (Yaninta Ginting et al., 2024) who obtained 8 endophytic bacterial isolates from coffee plants. From these results, different characteristics were obtained which were observed in terms of colony shape, bacterial morphology, and bacterial physiology. These results are in accordance with previous research (Silitonga et al., 2017) which stated that the growth of microorganisms on solid media is characterized by different colony shapes such as round, irregular and so on.

Plant Height (cm)

The growth of coffee plants (*Coffea arabica* L) with endophytic bacterial suspension immersion treatment obtained the highest data at 5 MAP in treatment B1 within 6.5 hours (14.43 cm) (Sajar et al., 2024). The lowest plant height was in treatment B0 without immersion (9.96 cm). Microgranule treatment, the highest results were obtained in treatment A2 with a microgranule count of 15 g (14.94 cm) and for the lowest data in treatment A3 with a count of 20 g (12.46 cm). The results of this study are better than the use of organic fertilizer from tofu dregs (16.16 cm). (Marziah et al., 2020) from these results, endophytic bacteria are able to provide effectiveness in coffee seedlings. According to research (Putri et al., 2016) reported that endophytes containing the hormone IAA are known to be able to stimulate the growth of pepper plants. The increase in coffee plant growth is influenced by phytohormones produced by endophytic bacteria, the hormones in question are auxin, ethylene, and cytokines (Herlina et al., 2017).

Stem Diameter (mm)

Observations of the diameter of tea plant stems show the growth of the diameter of coffee (*Coffea arabica* L) in the immersion treatment, significantly different results were obtained from treatment B2 (1.64 mm). The lowest data was in treatment B3 (1.66 mm) and the addition of microgranules did not significantly affect the measurement of stem diameter on coffee growth (*Coffea arabica* L) The highest data in treatment A3 (1.56 mm), then the lowest data in treatments A1 and A2 (1.53 mm). These results are better than other studies using urea fertilizer with a tea plant stem diameter of 0.49 mm (Pamungkas and Supijatno., 2017). This finding is in line with previous research (Sudiarti, 2017) which found that the use of microbes included in biological fertilizers did not have a significant effect on plant growth. Therefore, a longer observation period (more than two months) is needed to detect the statistically significant effect of endophytic microbe applications on oil palm seedlings (Yanita, et al., 2024).

Effectiveness of Microgranules on Coffee Plant (*Coffea arabica* L) Growth

The materials used to make microgranules use tapioca flour, alginate, inulin, and endophytic bacterial suspension solutions, where endophytic bacteria play a role in increasing the growth of host plants (Santoyo et al., 2016). Research (Nasrun & Nurmansyah, 2015) using *Bacillus* and Pseudomonas strains isolated from the rubber rhizosphere was able to inhibit

R.microporus by 72.69-90.94% using less *R.microporus* inoculum. According to (Sebola et al., 2019) several strains of basil have been used in agriculture as plant growth promoters and biological control agents. The results obtained from the observed parameters: plant height, number of leaves, and leaf area gave the best growth in the R2 treatment with a dose of 15 gr/polybag.

CONCLUSION

From this research, the results obtained are that;

a. Isolation of coffee roots and stems obtained 8 isolates of endophytic bacteria. Auxin test showed that six isolates were able to produce auxin.
b. Observation of plant height showed that the best treatment was in treatment B3 (15.89 cm). The highest figure was found in treatment B1 (1.66 mm) in stem diameter.
c. The results of the study showed that administration of endophytic bacterial suspensions and microgranule significantly increased the growth of Arabica coffee.

Declaration by Authors

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