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Potential of Rhizosphere Bacteria Microcapsules Isolated from Sinabung Volcano in Stimulating Arabica Coffee Growth (*Coffea arabica* L.)

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ABSTRACT

Coffee is one of the results commodity plantations in Indonesia that have mark quite economical high among plant plantation others and play a role important as source foreign exchange. This research used CRD (Complete Randomized Design) factorial method consisting of 2 factors, 16 treatments, 2 repetition. Data obtained analyzed with use analysis of variance. From the results study treatment best in giving microcapsules and immersion to suspension bacteria rhizosphere attached, to the microcapsules addition, results highest obtained in treatment I2 with amount microcapsules 15 g (13.64 cm) and for the lowest data in treatment I1 with amount `10 gr (11.84 cm). Best treatment results also affect the diameter of the highest diameter plant found in treatment B3 (1.64) mm and treatment lowest at B2 (1.35) mm. Research results showed that rhizosphere bacteria microcapsules from the soil of the Sinabung volcano eruption were effective in stimulating the growth of arabica coffee.

Keywords: Microcapsules, Rhizosphere, Sinabung Volcano

INTRODUCTION

Coffee is one of the plantation commodities in Indonesia that has a fairly high economic value among other plantation crops and plays an important role as a source of foreign exchange for the country. Several areas that are used as centers for coffee production and cultivation in Indonesia, one of which is Tanah Toraja. coffee not only plays an important role as a source of foreign exchange but is also a source of income for no less than one and a half million coffee farmers in Indonesia (Rahardjo, 2012). In general, coffee is used as a processed product in the form of a drink that comes from the processing and extraction of coffee beans.

Coffee plants (*Coffea* sp.) are one of the plantation crops in Indonesia whose commodities are taken into account in strengthening the country's foreign exchange. Based on data from the Direktorat Jenderal Perkebunan, arabica coffee production reaches >1,000 tons which is dominated in North Sumatra, Aceh, South Sulawesi, and several other regions (Irmeilyana et al., 2019). The area of coffee plantations is dominated by smallholder plantations of 95.37% with robusta coffee covering 81.96% and Arabica coffee covering 18.04% (Kementerian Pertanian Republik Indoneisa, 2017).

Environmental conditions are one of the requirements for plant cultivation, including Arabica coffee. If environmental conditions are in accordance with the growing requirements of a plant, it is easy to cultivate the plant (Warsito, et. al., 2023). Indonesia with its tropical climate is an ideal and potential area for cultivating

coffee plants, one of which is in Lembang District, West Bandung Regency. The BPS of West Bandung Regency in 2018 stated that Lembang District is at an altitude of 1,312-2,084 m above sea level with its highest point at the peak of Mount Tangkuban Perahu, with an average temperature of 17-27ºC which is very suitable for the growing requirements of Arabica coffee plants. Arabica coffee grows well in tropical highland areas, the quality of Arabica coffee is also influenced by the distribution of rain and air temperature (Syakir & Surmaini, 2017).

Coffee is known as a drink that has a high caffeine content (Muhibatul, 2014). Caffeine is a methylxanthine alkaloid compound (purine base) that is in the form of white crystals and is psychoactive. Caffeine in coffee is known to have benefits when consumed by humans and also has adverse effects on the body if consumed at certain body conditions and in high caffeine levels. Caffeine consumption is useful for increasing alertness, eliminating drowsiness and improving mood. Caffeine also helps physical performance by increasing endurance and increasing muscle contractions (Ennis, 2014). Excessive caffeine consumption can cause tooth discoloration, bad breath, increased stress and blood pressure if consumed in the morning, insomnia, heart attacks, strokes, male infertility, digestive disorders, addiction and even premature aging (Farida et al., 2013).

MATERIALS & METHODS

Sample or Participant

This study was done with use CRD (Complete Randomized Design) factorial method consisting of from 2 factors, 16 treatments, 2 replications. Treatment time immersion seed with addition suspension bacteria consists of from: B0: without immersion, B1: 6.5 Hours; B1: 7.5 Hours; B3: 8.5 Hours. Addition microcapsule bacteria consists of from I 0 : 0 gr; I1: 10 grams; I2: 15 grams; I3: 20 grams. Data obtained analyzed with use analysis of variance. Results of analysis variance to be continued with distance test Duncan's double.

Isolation Bacteria Rhizosphere

Land taken from area rhizosphere plant around the slopes of Mount Sinabung. Land taken on depth 5-10 cm Then entered to in bottle Which has been sterilized for brought to laboratory. As many as 1 gram sample land entered into the 9 ml solution NaCl 0.85%. Sample vortex during 10 minutes furthermore dilution is carried out glow until dilution 10-6. Each as much as 100 µl the dilution suspension was grown using *the Pour Plate Method* using NA media. Bacterial cultivation was carried out in duplicate. The pour plate results were then incubated for 24 hours.

Preparation and Sterilization of Planting Media

The planting media uses topsoil, burnt rice husks and chicken manure with a ratio of 50%:25%:25%. All media are mixed and put into polybags. Polybags are taped first before being put into the autoclave, then sterilized for 10 hours. The sterilized planting media is immediately taken to a greenhouse that has been sterilized by spraying 0.4% formalin.

Seed Immersion with Rhizosphere Bacterial Suspension

Endophytic Bacteria Collection of endophytic bacteria solution was done by adding 10 ml of 0.9% NaCl solution in 1 petri dish, stirred using a triangular stirring rod. Coffee beans were soaked for 8 hours, 9 hours and 10 hours in a container lined with aluminum foil to keep them sterile.

Making Microcapsules from Rhizosphere Bacteria As Biofertilizer

A total of 14.7 g of CaCl2 was dissolved in a measuring flask with 1000 ml of distilled water and stirred homogeneously. Sterilize the solution using an autoclave at 121°C for 15 minutes. Insert a sterile alginate solution containing an endophytic bacterial

suspension into the needle and insert it into a 0.1M CaCl2 solution. The formed microcapsules were left for 1 hour. To remove residual CaCl2, the microcapsules were filtered and rinsed with distilled water (Panichikkal et al., 2021).

Observation Parameters

The parameters observed in this study were the characterization of endophytic bacteria, measurement of endophytic auxins, plant height (cm), number of leaves (blades), leaf area (cm2). Observation data were taken once a month.

RESULT

Isolation and Characteristics of Rhizosphere Bacteria in Mount Sinabung Soil

From the results of rhizosphere bacterial isolates that have been obtained from various places in the Mount Sinabung area, various bacterial isolates were obtained, namely: Simpang Empat (SE) from SE, 3 isolates were obtained, ranging from shape, color and type of soil pH as in the table and here are some photos of the results of the microcapsules that have been made.

Table 1. The Results of 3 Isolates were Obtained

Species	Elevation	Margin	Whole	Color	Soil pH
SE 1 sp 1	Convex	Smooth	Round	White	7
SE 1 sp 2	Convex	Smooth	Round	Cream	7
SE 1 sp 3	Umbonate	Smooth	Round	White	7
SE 2 sp 1	Convex	Smooth	Round	White	7
SE 2 sp 2	Convex	Rhizoid	Rhizoid	White	7
SE 2 sp 3	Convex	Smooth	Round	Cream	7
SE 3 sp 1	Flat	Irregular	Irregular	White	7
SE 3 sp 2	Convex	Smooth	Round	Yellow	7
SE 4 sp 1	Flat	smooth	Round	White	7
SE 4 sp 2	convex	irregular	Irregular	White	7
SE 4 sp 3	Convex	Rhizoid	Rhizoid	White	7
SE 5 sp 1	convex	Rhizoid	Rhizoid	White	7
SE 5 sp 2	convex	smooth	Smooth	White	7
SE 6 sp 1	Flat	Irregular	Irregular	Yellow	7
SE 6 sp 2	Flat	Filamentous	Filamentous	White	7
GK1 sp 1	Flat	Rhizoid	Rhizoid	White	6.4
GK1 sp 2	Convex	Smooth	Smooth	Cream	6.4
GK1 sp 3	Convex	Irregular	Irregular	Cream	6.4
GK2 sp 1	Flat	Irregular	Irregular	White	6.7
GK2 sp 2	Convex	Smooth	Round	White	7
GK2 sp 3	Convex	Irregular	Irregular	Orange	7
GK2 sp 4	Umbonate	Smooth	Round	White	7
GK 3 sp 1	Umbonate	Smooth	Round	Cream	7
GK 3 sp 2	Raised	Irregular	Irregular	Cream	7
GK 3 sp 3	Convex	Smooth	Smooth	Yellow	7
GK 4 sp 1	Convex	Rhizoid	Rhizoid	White	7
GK 4 sp 2	Flat	Filamentous	Filamentous	White	7
GK 4 sp 3	Umbonate	Smooth	Round	White	7
GK 4 sp 4	Flat	Smooth	Round	White	7
GK 5 sp 1	Convex	Smooth	Round	White	7
GK 5 sp 2	Umbonate	Smooth	Round	Yellow	7
GK 5 sp 3	Convex	Smooth	Round	Red r	7
NT 1 sp 1	Flat	Rhizoid	Round	White	6.5
NT 1 sp 2	Flat	smooth	Round	White	6.5
NT 2 sp 1	Flat	Smooth	Round	White	6.1
NT 2 sp 2	Flat	Irregular	Irregular	Yellow	6.1
NT 3 sp 1	Flat	filamentous	Filamentous	White	6.3
NT 3 sp 2	Flat	Rhizoid	Rhizoid	White	6.3

Plant Height (cm)

Plant height observations were conducted in months 1, 2, 3, 4, and 5 after planting (MAP). Based on the results of observations and analysis of variance, it is known that the soaking 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 microcapsule treatment at 1stMAP, 2ndMAP, 3rdMAP, and 5thMAP. The addition of microcapsules was very significant in the 4th MAP observation.

The effect of interaction of soaking variation and microcapsule 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 the table below.

Table 2. The Results of The Duncan Distance Test

Treatment	**Average Plant Height (cm)**				
Soaking Treatment (B)	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP
B0 = 0 Hours	5.99 ^{aA}	5.71 ^{aA}	6.68 ^{dD}	6.11 ^{cdCD}	9.71 ^{dBCD}
B1 = 6.5 Hours	6.36 ^{aA}	6.61 ^{aA}	11.14 ^{aA}	12.85 ^{aA}	14.91 ^{aA}
B2 = 7.5 Hours	5.96 ^{aA}	5.75 ^{aA}	11.89 ^{abAB}	11.78 ^{abcABC}	13.61 ^{bcABC}
B3 = 8.5 Hours	5.61 ^{aA}	5.76 ^{aA}	11.34 ^{abcABC}	13.65 ^{aA}	13.51 ^{abAB}
Microcapsule Addition (I)					
I0 = 0 grams	6.13 ^{aA}	6.46 ^{aA}	11.61 ^{bA}	10.13 ^{aA}	14.15 ^{aA}
I1 = 10 grams	6.24 ^{aA}	5.68 ^{aA}	10.85 ^{abA}	11.60 ^{aA}	11.80 ^{aA}
I2 = 15 grams	5.90 ^{aA}	5.94 ^{aA}	9.16 ^{aA}	10.91 ^{aA}	13.64 ^{aA}
I3 = 20 gr	5.66 ^{aA}	5.76 ^{aA}	9.41 ^{abA}	11.75 ^{aA}	12.16 ^{aA}

Plant Diameter (mm)

Based on the results of observations and analysis of variance, it is known that the effect of soaking and adding microcapsules on the growth of coffee (*Coffea arabica* L.) does not significantly affect the stem diameter. The interaction of the effect of soaking and adding microcapsules does not significantly affect the stem diameter. The measurement data of diameter (mm) on the growth of Coffee (*Coffea arabica* L) after being tested using the Duncan Distance Test are shown in the Table below.

Table 3. The Measurement Data of Diameter (Mm) on The Growth Of Coffee (*Coffea Arabica L*)

Treatment	**Average Plant Diameter (mm)**				
Immersion Treatment (B)	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP
B0 = 0 Hours	0.49 ^{aA}	1.48 ^{dB}	1.65 ^{aA}	1.64 ^{aA}	1.64 ^{cA}
B1 = 6.5 Hours	0.43 ^{aA}	1.68 ^{aA}	1.53 ^{aA}	1.53 ^{aA}	1.53 ^{aA}
B2 = 7.5 Hours	0.55 ^{aA}	1.79 ^{abAB}	1.33 ^{aA}	1.35 ^{aA}	1.35 ^{abcA}
B3 = 8.5 Hours	0.35 ^{aA}	1.65 ^{bcAB}	1.50 ^{aA}	1.54 ^{aA}	1.66 ^{aA}
Microcapsule Addition (I)					
I0 = 0 grams	0.50 ^{aA}	1.64 ^{aA}	1.61 ^{aA}	1.56 ^{aA}	1.56 ^{aA}
I1 = 10 grams	0.43 ^{aA}	1.63 ^{aA}	1.48 ^{aA}	1.49 ^{aA}	1.58 ^{aA}
I2 = 15 grams	0.41 ^{aA}	1.74 ^{aA}	1.45 ^{aA}	1.48 ^{aA}	1.50 ^{aA}
I3 = 20 gr	0.47 ^{aA}	1.59 ^{aA}	1.46 ^{aA}	1.53 ^{aA}	1.54 ^{aA}

DISCUSSION

Characteristics of Rhizosphere Bacteria from Mount Sinabung Soil

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. 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, different endophytic bacterial isolates were obtained, namely *Bacillus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Xanthomonas* sp. (Aizar & Parlina, 2017). These results were confirmed by (Yenny, 2016) who obtained 39 rhizosphere 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 the treatment of rhizosphere bacterial suspension immersion obtained the highest data at 5 MAP in treatment B1 within 8.5 hours (14.91 cm). The lowest plant height was in treatment B0 without immersion (9.71 cm). Microcapsule treatment, the highest results were obtained in treatment I2 with a microcapsule amount of 10 g (14.14 cm) and for the lowest data in treatment I1 with an amount of 5 gr (13.64 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, rhizosphere bacteria are able to provide effectiveness in coffee seedlings. According to research (Putri et al., 2016) reported that rhizosphere containing the hormone IAA is known to be able to stimulate the growth of pepper plants. The increase in coffee plant growth is influenced by phytohormones produced by rhizosphere bacteria, the hormones in question are auxin, ethylene, and cytokines (Herlina et al., 2017).

Stem Diameter (mm)

Observations of the stem diameter of Arabica coffee plants show the growth in the diameter of Arabica coffee (*Coffea Arabica* L.) in the soaking treatment, significantly different results were obtained and treatment B3 (1.66 mm). The lowest data in treatment B1 (1.35 mm) and the addition of microcapsules did not have a significant effect on the measurement of stem diameter on the growth of Arabica coffee (*Coffea Arabica* L.). The highest data was in treatment I1 (1.58 mm), then the lowest data in treatment I2 (1.50 mm). This result is better than other studies using urea fertilizer with a coffee plant stem diameter of 0.49 mm (Pamungkas and Supijatno., 2017). This finding is in line with previous studies (Sudiarti, 2017) and (Setiawan et. al., 2023) 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 rhizosphere microbe application on oil palm seedlings (Sajar et. al., 2024).

The Effectiveness of Microcapsules from Rhizosphere Bacteria on the Growth of Coffee Plants (*Coffea arabica* L.)

Endophytic bacterial microcapsules consist of alginate and inulin which have their respective functions in making rhizosphere bacterial microcapsules, the function of inulin as a food reserve for bacteria after becoming a soft capsule while alginate functions as a gel former or soft capsule (Hartono et al., 2013). Microcapsule probiotics use an alginate matrix form. Alginate has been tested to increase probiotic survival by 80-95% (Suryani et al., 2019). Alginate also acts as a protective layer of microbial cells against abiotic stress. Immobilization of inalginate polymers increases the survival of microorganisms compared to conventional liquid bacterial cells which do not provide adequate protection for microorganisms (Stella et al., 2019). Encapsulation of

inoculated cells in polysaccharide polymers such as alginate is a technique that ensures the controlled release of beneficial plant microbes into the soil (Lubis et al., 2020). Natural polysaccharides are utilized directly by plants which act as plant development and defense molecules (Liao et al., 2019). Rhizosphere bacteria containing IAA hormones are converted into microcapsules which are directly applied to plants by pouring capsule granules directly into the plant root area then the plants directly absorb biofertilizers through the plant roots (Yanita et et al., 2024).

CONCLUSION

The results from plant growth measurements showed that the application of rhizosphere bacterial suspensions significantly enhanced the growth of coffee plants, particularly in terms of plant height. Treatments with bacterial suspensions led to an average plant height of 14.91 cm, which was higher compared to the control (without immersion). The use of microcapsules also had a positive effect on growth, with the highest plant height observed in treatments with 10 g of microcapsules (14.14 cm). These results support the notion that IAA-producing bacteria in the rhizosphere can stimulate plant growth.

In terms of stem diameter, while rhizosphere bacteria had a positive effect on the growth of coffee plants, the addition of microcapsules did not significantly impact this particular growth parameter. Nonetheless, the findings on stem diameter are still promising compared to other fertilization methods, such as urea-based fertilizers, which show lower growth results. Microencapsulation of rhizosphere bacteria using alginate and inulin proved to be an effective method for protecting bacterial cells from abiotic stress and ensuring their survival. This encapsulation technique offers a controlled release of beneficial microbes into the soil, improving their effectiveness as biofertilizers. By directly applying these microcapsules to the root zone, plants are able to absorb the beneficial bacteria, leading to enhanced growth and development.

Overall, this study highlights the significant role of rhizosphere bacteria, especially IAA-producing strains, in promoting the growth of coffee plants. The combination of bacterial treatment and microencapsulation shows potential for improving agricultural practices, offering an eco-friendly and sustainable alternative to chemical fertilizers. Further research with longer observation periods is needed to fully understand the long-term benefits of rhizosphere bacterial application, particularly in terms of plant development and soil health.

Declaration by Authors

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