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Escalation of Coffee Plant (*Coffea arabica* L) By Addition of Microcapsules From IAA (Indole Acetic Acid) Producing-Endophytic Bacteria

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Abstract

The growth of coffee (Coffea arabica L) *plants is highly dependent on the quality of the seeds and fertilization. One of the newest innovations in the field of organic farming is the use of endophytic bacteria as potential candidates to be developed become biofertilizers. Endophytic bacteria that produce IAA (Indole acetic acid) are able to produce phytohormones that can accelerate plant growth. The purpose of this researeh was to determine the effectiveness of endophytic bacterial microcapsules for the growth of Arabica coffee plants. This research was conducted using the factorial CRD (Completely Randomized Design) method consisting of 2 factors, 16 treatments, 2 replications. The first factor was immersion of endophytic bacterial suspension consisting of B0: without immersion; B1:8 hours; B2 :9 hours; B3: 10 hours. Microcapsules addition of endophytic bacteria consisting of I0 : 0 gr; I1 : 5 gr; I2 : 10 gr; I3 : 15 gr. The results from isolation was obtained four isolates of endophytic bacteria. The IAA test showed that the four isolates were able to produce IAA. Observation of plant height showed that the best treatment was treatment B1 of (16.31 cm). Observation on total of leaves, best treatment were B1 and B2 (10.57 strands). For the leaf area parameter, the best treatment was B3 of (27.36 cm^2). Test results showed that application of suspension and microcapsules of endophytic bacteria significantly increased growth of coffee.*

Keywords: *Arabica Coffee, Endophytic Bacteria, IAA*

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INTRODUCTION

Coffee plant (*Coffea Arabica* L) has an important meaning for national coffee plantation not only as a source of foreign exchange but also as a source of income for one and a half million people in Indonesia. Based on data from Central Statistics Agency (BPS, 2022), Indonesia's coffee production will reach 774.6 thousand tons in 2021. This number has increased by 2.75% from previous year which amounted to 753.9 thousand tons. Opportunity for coffee market was very wide because most of coffee production was exported to foreign countries and rest is produced domestically.

Growth of coffee plants was highly dependent on quality of seeds, fertilization, care and planting media used at start of planting (Dewantara, 2017). According to research (Sulaeman et al., 2017), fertilization was an important factor to obtain plants that can grow and develop properly. There are still many farmers in Indonesia used inorganic (chemical) fertilizers in long term to have a negative impact on environment such as reducing production and organic matter, soil microbial population and increasing soil. According to research (Parmila et al., 2019) replacement alternative of inorganic fertilizers was used biofertilizer. Biofertilizer can improve plant growth and development.

One of newest innovations in organic farming used of endophytic bacteria as potential candidates to be developed into biofertilizers (Gusmaini et al., 2019). Endophytic bacteria was bacteria that lived in plant tissues in symbiosis with their host plants (Kurniasih, 2021). Endophytic bacteria that produced IAA (*Indole Acetic Acid*) was able to produce phytohormones that could accelerate plant growth. Results of research (Arista, 2017) availability of IAA hormone could stimulate root growth. (Asyiah et al., 2020) reported that endophytic bacteria containing IAA hormones from *Bacillus* sp. group was able spurred growth in coffee plants. Used of endophytic bacteria as biofertilizer was able to maintain soil fertility and became a solution to increase the growth of coffee plants in sustainable and friendly manner environment. The aim of this research was to determine diversity and characteristics of IAA hormone-producing endophytic bacteria from coffee plants and determine effectiveness endophytic to stimulate growth of Arabica coffee plants.

METHOD

Tools and Materials

Tools in this research were petri dishes, test tubes, test tube racks, measuring cups, beaker glass, erlenmeyer, autoclave, oven, spatula, needle loops, incubator, hot plate, stir bar, analytical balance, sprayer, laminar water flow, glass bottles, aluminum foil, cotton, knife, polybag, bunsen, microscope and shaker. And Materials in this research were roots and stems of coffee, media Nutrient Agar (NA), distilled water, alcohol 70%, chlorine solution, $CaCl_2$, sodium alginate, inulin, poultry manure, top soil, rice husk charcoal, NaCl 0,9%, crystal violet, safranin, acetone, alcohol, iodine, L-tryptopan, peptone and salkowsky reagent.

Research Methods

This research was conducted using factorial CRD (Completely Randomized Design) method consisting of 2 factors, 16 treatments, 2 replications. Treatment of seed immersion time with addition of endophytic bacterial suspension consisted of: B0: without immersion, B1: 8 Hours; B1: 9 Hours; B3: 10 Hours. Microcapsule addition of endophytic bacteria consisted of I0: 0 gr; I1: 5 gr; I2: 10 gr; I3: 15 gr. Data obtained were analyzed using analysis of variance. Results of analysis of variance were continued with Duncan's multiple range test.

Isolation of Endophite Bacteria

Endophite bacteria was isolated from coffee roots and stem. Isolation of endophite bacteria using the method (Singh et al., 2022) modified. Before isolation, surface of roots and stems of coffee was sterilized.

Measurement of IAA (*Indole Acetic Acid*) from Endophite Bacteria

Potential test of IAA producing-endophytic bacteria used streak plate method. Isolates were inoculated on flat Nutrient Agar media supplemented with tryptophan at a concentration of 100 ppm and incubated at room temperature for 48 hours. Salkowski reagent was dripped onto endophite bacterial colonies. Colonies that has been dripped with Salkowski reagent was stored in a dark room for 30 minutes. Positive result was indicated by a change in color of colonies became red (Herlina et al., 2017).

Preparation and Sterilization of Planting Media

Planting medium used topsoil, roasted husks and chicken manure with a ratio of 50%: 25%: 25%. All media was mixed and put in polybag. Polybag was taped before being put into autoclave, then sterilized for 10 hours. Sterilized planting medium was immediately brought to greenhouse which has been sterilized by spraying 0.4% formalin.

Immersion Seeds with Suspension of Endophite Bacteria

Collection of endophytic bacteria solution was carried out by adding 10 ml of 0.9% NaCl solution in 1 petri, stirred using triangular stir bar. Coffee beans were soaked at ratio of 8 hours, 9 hours and 10 hours in a container covered with aluminum foil to keep it steril.

Producing of Microcapsules From Endophite Bacteria As Biofertilizers

A total of 14.7 g $CaCl_2$ dissolved in a volumetric flask with 1000 ml of destilled water and stiirred homogeneous. Sterilize solution used autoclave at 121°C for 15 minutes. Put steril alginate solution containing endophytic bacteria suspension into needle and drop into the 0.1M $CaCl_2$ solution. Formed microcapsules were allowed to stand for 1 hour. To remove $CaCl_2$ residues, microcapsules were filtered and rinsed with distilled water (Panichikkal et al., 2021)

Observation Parameters

Parameters observed in this research were chaeacterization of endophytic bacteria, measurement of endophytic auxin, plant height (cm), total of leaves (strands), leaf area (cm^2). Observational data was taken once a month.

RESULTS AND DISCUSSION

Isolation and characteristics of Endophytic Bacteria from Coffee Plant Roots and Stems

From Isolation of endophytic bacteria from roots and stems of coffee (*Coffea arabica* L), four isolates of endophytic bacteria were obtained, 2 isolates from stems and 2

isolates from roots. 4 isolates had characteristics that varied both in terms of morphology and coloring. Results of isolation and characteristics of endophytic bacteria were showed in Table 1.

Table 1. Characteristics and isolation of endophytic bacteria from coffee stems and roots

Isolate	Characterization						Gram
	Colony Morphology				Cell Morphology		
	Color	Form	Edge	Elevation	Form	Setup	
SP1BK	White	Lobate	Irreguler	Raised	Basil	Diplo	+
SP2BK	White	Irreguler	Irreguler	Plateau	Basil	Diplo	+
SP1AK	White	Irreguler	Irreguler	Plateau	Basil	Diplo	+
SP2AK	White	Lobate	Irreguler	Flat	Basil	Diplo	+

The Ability of Root and Stem Bendophite Bacteria In Coffee Plants To Produce IAA Hormones

Endophytic bacteria from coffee roots and stems that produce IAA were marked by formation of a reddish color. Four isolates showed positive results. The results of IAA hormone levels were showed in Table 2.

Table 2. IAA hormone levels produced by endophytic bacteria from roots and stems of the coffee plant (*Coffea arabica* L)

Isolate kode	IAA
SP1 AK	+
SP2 AK	+
SP1 BK	+
SP2 BK	+

Plant Height (cm)

Observations of plant height were observed in 1, 2, 3, 4, and 5 months after planting (MAP). Based on results of observations and analysis of variance, it was known that immersion treatment affected on growth of coffee plants (*Coffea arabica* L). Results showed significantly different effect on plant height (cm) in observation 1st MAP, and 5th MAP and had a very significant different effect on 2nd MAP, 3rd MAP, 4th MAP. However, it had no significant effect in treatment of microcapsules at 1st MAP, 2nd MAP, 3rd MAP, and 5th MAP. Microcapsules addition was very significant at 4th MAP observations.

Interaction effect of immersion variations and provision of microcapsules had no significant effect on observation of plant height measurements (cm) on growth of coffee (*Coffea arabica* L). Duncan's distance test results were showed in Table 3.

Table 3. Average height of coffee plants *(Coffea arabica* L) by immersion of endophytic bacteria and microcapsule addition.

Treatment	Average Plant Height (cm)				
	1st MAP	2nd MAP	3rd MAP	4th MAP	5th MAP
Immersion Treatment (B)					
B0 = 0 Hours	4.13^{bAB}	6.25^{cB}	7.31^{cC}	9.68^{cC}	12.59^{cD}
B1 = 8 Hours	5.25^{aA}	7.11^{bAB}	9.76^{aA}	12.9^{aA}	16.31^{aA}
B2 = 9 Hours	4.4^{abAB}	7.89^{aA}	8.36^{bB}	11.29^{bB}	15.81 ^{aAB}
B3 = 10 Hours	3.98^{bB}	6.30^{cB}	8.59^{bB}	15.35^{bB}	14.50^{bBc}
Microcapsule adition (I)					
I0 = 0 gr	4.23^{aA}	6.9^{aA}	8.83^{aA}	11.7 ^{aA}	14.85^{aA}
I1 = 5 gr	4.99^{aA}	6.95^{aA}	8.20^{aA}	10.58 ^{bB}	14.21^{aA}
I2 = 10 gr	4.29^{aA}	6.73^{aA}	8.38^{aA}	111.09^{abAB}	15,14^{aA}
I3 = 15 gr	4. 26^{aA}	6.96^{aA}	8.63^{aA}	11.7625^{aA}	15.01^{aA}

Total of Leaves (strands)

Total of leaves was observed at 3, 4, and 5 months after planting (MAP). Interaction of immersion variations and microcapsules addition had no significant effect on observation of total leaves (strands) on coffee growth (*Coffea arabica* L). Duncan's distance test results were showed in Table 4.

Table 4. Average number of leaves of coffee plant (*Coffea arabica* L) by immersion of endophytic bacteria and microcapsule addition.

Treatment	Average number of leave (strands)		
	3th MAP	4th MAP	5th MAP
Immersion Treatment (B)			
B0 = 0 Hours	4.50^{aA}	6.00^{bB}	9.25^{aA}
B1 = 8 Hours	5.25^{aA}	7.75^{aA}	10.75^{aA}
B2 = 9 Hours	4.50^{aA}	6.25^{bB}	10.75^{aA}
B3 =10 Hours	4.50^{aA}	6.25^{bB}	10.25^{Aa}
Microcapsule adition (I)			
I0 = 0 gr	4.40^{aA}	6.05^{aA}	10.00^{aA}
I1 = 5 gr	4.75^{aA}	6. 50^{aA}	10.25^{aA}
I2 = 10 gr	4.75^{aA}	6.25^{aA}	10.25^{aA}
I3 = 15 gr	4.75^{aA}	6.50^{aA}	10.15^{aA}

Leaf Area (cm^2)

Leaf area was taken in 5th month after planting. From analysis of variance, results obtained were not significantly different. Duncan's distance test results were showed in Table 5.

Table 5. Average number of leaves of coffee plant (*Coffea arabica* L)) by immerstion endophytic bacteria and microcapsules addition.

Treatment	Average Leaf Area (cm^2)
Immersion Treatment (B)	
B0= 0 Hours	20.381^{Aa}
B1 = 8 Hours	26.975^{aA}
B2 = 9 Hours	26.31^{aA}
B3 = 10 Hours	27.36^{aA}
Microcapsule adition (I)	
I0 = 0 gr	20.3815^{aA}
I1 = 5 gr	24.5652^{aA}
I2 = 10 gr	24.7515^{aA}
I3 = 15 gr	22.9687^{aA}

Discussion

Characteristics of Endophytic Bacteria from Coffee Plant Roots and Stems

IAA hormone-producing bacteria were characterized based on colony morphology, namely colony shape, elevation, edges and color, as well as cell morphology through bacterial staining, namely cell shape and grammatical characteristics of bacteria. Based on results of bacterial isolation and endophytic characteristics on coffee plants, four different isolates were obtained, then all isolates showed for gram (+) types of bacteria from IAA-producing endophytic bacterial isolates. From previous research, six different endophytic bacterial isolates were obtained, namely *Bacillu*s sp., *Pseudomona*s sp., *Klebsiella* sp., *Xanthomonas* sp. (Aizar & Parlina, 2017). These results were corroborated by (Yenny, 2016) which obtained 7 isolates of endophytic bacteria from coffee plants. From results, different characteristics were obtained which were observed in terms of colony shape, bacterial morphology and bacterial physiology. Results was accordance with previous research (Silitonga et al., 2017) which stated that growth of microorganisms on solid media was characterized by different colony shapes such as round, irregular and so on.

The Ability of Root And Stem Endophite Bacteria In Coffee Plants To Produce IAA Hormones

From all isolates of endophytic bacteria originating from roots and stems of coffee, all of them had potential produced IAA hormones. From results of characteristics of endophytic bacteria, it was found that genus of *Bacillus* bacteria containing IAA hormone levels showed (+) results. These results were in accordance with previous research (Astriani & Murtiyaningsih, 2018) which revealed that *Baccilus* sp. bacteria were able produced 48 ppm of IAA hormone for 48 hours of incubation period with using 5 mg/ml tryptophan. (Pertiwi et al., 2020) reported that IAA-producing endophytic bacteria isolated from roots of Robusta and Arabica coffee plants as many as 12 endophytic bacterial isolates, each endophytic bacterial isolate had a different potential in producing IAA hormone. Bacteria that are capable of producing IAA will turn red when

dropped by Salkowski because of interaction between IAA and Fe formed complex compounds. Color produced by endophytic bacterial isolates after adding Salkowski reagent was unstable, color will quickly form and then fade. Stability of high color density after addition of reagent will determine high concentration of IAA and unstable color changes could be detected again by adopting time standard between addition of reagent and absorbance reading.

Plant Height (cm)

Growth of coffee plants (*Coffea arabica* L) by suspension immersion treatment of endophytic bacterial obtained highest data at 5 MAP in treatment B1 within 8 hours (16.31 cm). Lowest plant height in treatment B0 without immersion (12.59 cm). Treatment of microcapsules, highest results were obtained in treatment I2 with 10 g microcapsules (15.14 cm) and for lowest data in treatment I1 with 5 gr (14.21 cm). Results of this research were better compared using organic fertilizer from tofu waste (16.16 cm). (Marziah et al., 2020) from these results, endophytic bacteria were able to provide effectiveness for coffee nurseries. Accordance research (Putri et al., 2016) reported that endophytes containing IAA hormones were known to be able stimulated growth of pepper plants. Growth increase in coffee plants was influenced by phytohormones produced by endophytic bacteria, hormones referred to are auxin, ethylene, and cytokines (Herlina et al., 2017).

Total of Leaves (strands)

Observing total of leaves on coffee plants (*Coffea arabica* L) obtained highest data at 5 MAP in treatments B1 and B2 by immersing of 8 and 10 hours (10.75 strands). For lowest data in treatment B0 without immersion (control) (9.25 strands). Treatment of microcapsules addition, highest data were in treatments I1 and I2 with doses of 5 and 10 gr/polybag (10.25 strands) and lowest result was at no treatment (10.00 strands). These results were better than using NPK with a dose 5 g/polybag chemical fertilizers (8.78 strands) (Sari et al., 2019). Results accordance with previous research (Manguntungi et al., 2018) reported that application of endophytic bacterial biofertilizers to oil palm seedlings did not had a significant effect on total of leaves. It caused treatment using endophytic microbes contained in biofertilizer had not been able to significantly affect plant growth, therefore dose of microcapsules was further increased.

Leaf Area (cm^2)

Observing leaf area were observed in last month of observation. Based on results of observations and analysis of variance, showed that had no significant effect on leaf area. Highest data for immersion treatment was in treatment B3 with 10 hours of immersion (27.38 cm^2). For lowest data at control treatment (20.74 cm^2). Whereas for treatment of microcapsules with widest leaves in B3 treatment with a dose of 15 g/polybag (22.96 cm^2). Smallest leaf area in treatment I0 without treatment (control) with a leaf width (20.74 cm^2). Results accordance with (Arista, 2017) reported that phytohormones IAA (*Indole Aceti Acid*) played a role in cell enlargement and elongation, growth and development in plants. IAA hormone was an endogenous auxin which played a role in cell enlargement, inhibits growth of side shoots, stimulates abscission, played a role in

formation of xylem and phloem tissue and also influences root development and elongation (Herlina et al., 2016).

Effectiveness of Microcapsules From Endophytic Bacteria on Growth of Coffee (*Coffea arabica* L)

Endophytic bacterial microcapsules consist of alginate and inulin which have their respective functions in manufacture of endophytic bacterial microcapsules, function of inulin is as a food reserve for bacteria after it becomes a soft capsule while alginate functions as a gel former or soft capsule (Hartono et al., 2013). Microcapsule probiotics use a matrix form of alginate. Alginate has been tested to increase probiotic survival by 80-95% (Suryani et al., 2019). Alginate also acted as protective layer for microbial cells against abiotic stresses. Immobilization in alginate polymers increases 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 controlled release of beneficial plant microbes onto soil. Natural polysaccharides are directly utilized by plants which act as plant development and defense molecules (Liao et al., 2019). Endophytic bacteria containing IAA hormone were converted into microcapsules directly applied to plants by pouring capsule granules directly into plant root area and then plants directly absorb biological fertilizer through plant roots.

CONCLUSION

From this research showed that,

1. The isolation of endophytic bacteria from roots and stems of coffee (*Coffea arabica* L) obtained 4 isolates, 2 isolates from stems and 2 isolates from roots. IAA hormone test for 4 isolates showed positive contain IAA hormone.
2. Highest plant height data was treatment B1 (16.31 cm). Highest data in total of leaves was treatment of B1 and (10.75 strands) and highest data for leaf area was treatment B3 (27.36 cm^2).
3. The application of suspension and microcapsules of endophytic bacteria significantly increased growth of coffee.

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