Effectiveness of Rhizospheric Bacteria Microcapsules Addition on the Growth of Cocoa Leaves (Theobroma cacao L.)

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Abstract

Cocoa (Theobroma cacao L.) is one of the main plantation commodities which plays an important role in the Indonesian economy, and 3rd ranked in the world for cocoa producers. One effort to increase cocoa productivity was the use of biological fertilizer. The aim of this research was to determine the effect of using rhizosphere bacterial microcapsules as biofertilizer on the vegetative growth of cocoa leaves. The research design used was a Completely Randomized Design, with two factors, the first was immersion the cocoa beans using a rhizosphere bacterial suspension consisting of A0=0 hours; A1=8 hours; A2=16 hours and A3=32 hours and the second factor is the addition of microcapsules consisting of C0=0 gram(g); C1=10gr, C2=20gr, C3=30gr. Combination The treatment was repeated twice so that 32 plants were obtained. For the parameter of observing the number of leaves, the best results were shown in the 8 hour immersion treatment with an average number of leaves of 12.63, followed by the treatment of giving 20 grams of capsules with an average number of leaves of 11.75. The lowest number of leaves was shown in the 16-hour and 32-hour immersion treatments at 10.75. Meanwhile, for the observation parameters of leaf area, the best results were shown in the 32 hour immersion treatment with an average leaf area of 73.49 cm² and the lowest results were shown in the non-immersion treatment with an average leaf area of 59.88 cm². The results of the analysis of variance showed that all treatments had no significant effect.

Keywords: Cocoa, Bacteria rhizosphere, Vegetative leaf
INTRODUCTION

Cocoa is commodity flagship that has mark important, that is as mainstay ex or (Economics, 2017). Seed kakao is material standard main used in chocolate production, a popular product that is in demand consumers around the world (Manalu, 2018). Cocoa also plays a role in push regional development and development agroindustry. The Central Statistics Agency (BPS) recorded production of cocoa in Indonesia was 667 thousand tons with wide land 1.46 million hectares in 2021. Cocoa Production Results in 2022 would enhancement to 688 thousand/ton, with a land area of 1.46 million hectares. Terms growing need condition good drainage soil. Problem cultivation cocoa usually minimal innovation and technology in cultivation cocoa so that lots very tree cultivated cocoa not enough treated with Good (Ariningsih et al., 2021). Beside that ‘s low productivity cocoa because not yet use seeds quality cocoa and lack of innovation in technological development for cocoa farmers (Managanta, 2020) Accompanied by use fertilizer inorganic in a way constantly and not wise, no balanced with use fertilizer organic or fertilizer biological so that cause land become hard work and productivity decrease (Rezta & Dawam, 2018) fertilizer biological consists from various like, mycorrhiza, fungi and bacteria, both symbiotic with plant nor that life free in the environment (Kartikawati et al., 2017).

One of solution For overcome matter the is substitute or complementary usage fertilizer inorganic with fertilizer biological or fertilizer organic as well as application exercise land conservation (Herdiyanto et al., 2015). Fertilizer biological (biofertilizer) is product biology active consists on microorganisms in the rhizosphere soil (Nuraini et al., 2020) The rhizosphere is a type of bacteria that lives around plant roots (Sopialena et al., 2023). Meanwhile, Plant Growth Promoting Rhizobacteria (PGPR) was taken from the roots of the plants which play a role increase growth plants, also protect plant from attack pathogen. (Shofiah & Tyasmoro, 2018). Biofertilizer based on Local Microorganisms as fertilizer biological potential in increase productivity and sustainability land. This matter reported by (Damayanti et al., 2022). Bacteria was known capable endure live in conditions extreme like salt water lakes, hot springs, calderas mountains, sea in, and others (Sabdaningsih et al., 2013). Bamboo is known own ability high adaptiveness because can adapt with fast to change nor stress environment (Yulistiana et al., 2020). Bamboo roots contains a lot of colonized bacteria (Pseudomonas fluorescens), bacteria This can role increase solubility of P in land, certains train from Pseudomonas sp., which is able to stimulate plant growth in several ways (Husnibuda et al., 2017).

Rhizosphere soil contains diverse types of bacterial genera called rhizobacteria, which show beneficial effects on plant growth (Triani et al., 2022). Soil bacteria, which inhabit the rhizosphere, greatly influence the plant growth process. (Nuraini et al., 2020). Such rhizosphere bacteria are referred to as PGPR (Plant Growth Promoting Rhizobacteria) or Rhizobacter triggers the growth of rice sprouts (Hamdayanty et al., 2022). Based on these conditions, it is necessary to carry out research on the effect of giving microcapsules of rhizosphere bacteria to cocoa plants in the hope that it will help plants in the availability of nutrients. (Sopialena et al., 2023). The aim of this research is
to determine the effectiveness of administering rhizosphere bacterial microcapsules on the
growth of cocoa plants. Based on previous research which explains that soil bacteria,
which inhabit the rhizosphere, have a great influence on the growth process of production
plants and the quality of peanuts (Marom et al., 2017).

METHOD
Tools and materials
The tools used in this research were glass measuring, beaker glass, erlenmeyer,
tube reaction, rack tube reaction, petri dish, autoclave, oven, spatula, needle ose,
inoculator, hot plate, rod stirrer, dropper, Bunsen, lighter api, micro pipette, balance
analytical, sprayer, laminar air flow, bottle glass, aluminum foil, cotton, cutter knife,
polybag, microscope binoculars. Materials used in this research was soil rooting plant
bamboo, Nutrient Agar (NA) Media, distilled water, 70% alcohol, CaCl2, NaCl, Natrium Alginate from inulin, fertilizer dirt poultry, top soil, charcoal rice husk, NaCl 0.9%,
Crystal Violet, Safranin, Acetone alcohol.

Research methods
Study use Factorial Completely Randomized Design (CRD). by two factors. First
factor was immersion rhizosphere bacteria (A) consists of on 4 levels: A0 (Without
treatment), A1 (2 hours), A2 (4 hours) and A3 (6 hours). Second factor was microcapsules
(P) consist level: P0 (without Capsules), P1 (10g/polybag), P2 (20g/polybag), P3
(30g/polybag). Observed changes consists from amount leaves, quantity wide leaf.

Isolation Bacteria Rhizosphere
Rhizosphere bacteria was taken from the soil from area rooting plant bamboo,
Soil samples rhizosphere diluted with fusion method glow from 10-7 to 10-5, then taken
0.1 ml each dilution For distributed on Nutrien Agar (NA) media (Hardiansyah et al.,
2020). Different colonies was observed in characteristics macroscopic, size colony, form
colony, elevation colony, edge colony, color colonies, and surfaces colony, then separated
and purified. (Lengkong et al., 2022) Isolate bacteria rhizosphere that has purified
observed based on characteristics its biological, based color colony, form colony, edge
colony, elevation colony. Characterization morphology observed cells covers form cell,
structuring cell as well as gram staining (Bagus et al., 2014)

Producing of Microcapsules bacteria rhizosphere as a biofertilizer
Prepared solution alginate sterile that has been contain suspension bacteria
rhizosphere entered to in syringe Then dripped to in 0.1M CaCl2 solution. shut up during
one hour up formed dense microcapsules, then microcapsules are formed moved to in
distilled water sterile and stirred in a way slowly use a shaker during one hour for remove
CaCl2 residue, then strain (Yudiastuti et al., 2022)

Microcapsules Application on Cocoa Plant
Application microcapsules consortium bacteria rhizosphere given at age plant 3
weeks after plant it, then give it away dose in accordance each dose treatment.
RESULTS AND DISCUSSION

Characterization morphology colonies and cells bacteria rhizosphere

Explain that research data results characterization and gram staining of bacteria rhizosphere bamboo as following in table 1,

Table 1. Characterization and Gram Staining of Bacteria Rhizosphere Bamboo

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Characterization</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Color</td>
<td>Morphology Colony</td>
</tr>
<tr>
<td>HS3</td>
<td>Yellow</td>
<td>Circular</td>
</tr>
<tr>
<td>HS4</td>
<td>Milk white</td>
<td>Spindle</td>
</tr>
<tr>
<td>HS5</td>
<td>white</td>
<td>Irregular</td>
</tr>
<tr>
<td>RS1</td>
<td>Yellow</td>
<td>circular</td>
</tr>
<tr>
<td>RS2</td>
<td>putih susu</td>
<td>circular</td>
</tr>
<tr>
<td>RS3</td>
<td>Yellow</td>
<td>circular</td>
</tr>
<tr>
<td>MS1</td>
<td>White</td>
<td>Irregular</td>
</tr>
<tr>
<td>MS2</td>
<td>Yellowish</td>
<td>Irregular</td>
</tr>
<tr>
<td>MS3</td>
<td>Milk white</td>
<td>Circular</td>
</tr>
</tbody>
</table>

Total of Leaves (strands)

The results of observations and analysis of variations in the total of cocoa leaves (Table 1) showed that immersion and microcapsules addition of rhizosphere bacteria to cocoa plants (Theobroma coca L) gave no significant effect on the growth of the number of leaves. The immersion treatment and administration of rhizosphere bacterial microcapsules had no interaction or had no significant effect on the measurement of total leaf on cocoa plants (Theobroma coca L). After carrying out the Duncan test, it could be seen in table 2.

Table 2. The average total of cocoa plant leaves in response to immersion and microcapsules addition.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average number of Leaves (Sheets)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 WAP</td>
</tr>
<tr>
<td>Immersion (A)</td>
<td></td>
</tr>
<tr>
<td>A0 = 0 Hours</td>
<td>4.13 aA</td>
</tr>
<tr>
<td>A1 = 8 hours</td>
<td>3.17 aA</td>
</tr>
<tr>
<td>A2 = 16 hours</td>
<td>4.50 aA</td>
</tr>
<tr>
<td>A3 = 32 hours</td>
<td>4.25 aA</td>
</tr>
<tr>
<td>Microcapsule addition (C)</td>
<td></td>
</tr>
<tr>
<td>C0 = 0 gr</td>
<td>4.38 aA</td>
</tr>
<tr>
<td>C1 = 10 gr</td>
<td>4.25 aA</td>
</tr>
<tr>
<td>C2 = 20 gr</td>
<td>4.00 aa _</td>
</tr>
<tr>
<td>C3 = 30 gr</td>
<td>4.00 aa _</td>
</tr>
</tbody>
</table>
Area of leaves (cm²)

Leaf area was observed 8 weeks after planting (WAP). Based on the results of the analysis of variance calculations, Table 2 shows that the immersion treatment on the leaf area of cocoa plants (*Theobroma cacao* L) gave a non-significant different effect. Table 2 shows that the treatment immersion has a different effect not significant in the leaf area of cocoa plants at 8 weeks after planting (WAP). However, there was no interaction between the immersion treatment and the administration of microcapsules of bamboo root soil rhizosphere bacteria had a real effect, but there was no interaction between treatments immersion and giving microcapsules have no effect real.

Table 3. Area average leaf plant cocoa (*Theobroma cacao* L) against immersion and adding microcapsules.

<table>
<thead>
<tr>
<th>Treatment Immersion (S)</th>
<th>Area average leaves (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0 = 0 Hours</td>
<td>59.88 aA</td>
</tr>
<tr>
<td>A1 = 8 hours</td>
<td>60.06 aA</td>
</tr>
<tr>
<td>A2 = 16 jam</td>
<td>66.59 aA</td>
</tr>
<tr>
<td>A3 = 32 jam</td>
<td>73.49 aA</td>
</tr>
<tr>
<td>Microcapsule addition (I)</td>
<td></td>
</tr>
<tr>
<td>P 0 = 0 gr/ polybag</td>
<td>61.28 aA</td>
</tr>
<tr>
<td>P1 = 10 gr/ polybag</td>
<td>67.51 aA</td>
</tr>
<tr>
<td>P2 = 20 gr/ Polibag</td>
<td>68.87 aA</td>
</tr>
<tr>
<td>P3 = 30 gr/ Polibag</td>
<td>62.32 aA</td>
</tr>
</tbody>
</table>

DISCUSSION
Characterization morphology colonies and cells bacteria rhizosphere

Isolation results in Table 1 explain that bacteria from land rhizosphere land root bamboo obtained nine isolates, among others color, shape, edge, elevation colony. No order and color colony diverse. Morphology cells contained in this research, shape cell isolate bacteria part big was round (cocus), and recorded only three isolate bacteria that have form bacilli (stem) cells. Research result This different with study (Fallo et al., 2023) Isolation results bacteria from land rhizosphere plant peanut pigeon pea (*Cajanus cajan* L) was obtained seven isolate. Seventh isolate own character colony that is size colony generally small and medium, color colony white, shape colony round, stringy, no regular, elevation colony flat, umbuniate, pulvinate, and convex. Edge of the colony own flat, wavy and curved characteristics. Based on results isolation and characterization bacteria rhizosphere pure that has been identified in a way morphology based on color colony form colony, edge colonies, and elevation colony (Syarif, 2021) Isolate bacteria HS4, RS1, MS1, MS2, MS3, are group bacteria producer of IAA (indole Acetic Acid). Based on characteristics biochemistry, known that IAA-producing bacteria are dominated by groups gram negative bacteria, bacteria rhizosphere capable produce bacteria like *Stenotrophomas maltophilia* and Arthrobacter (Patel & Saraf, 2017). (Naue, et al., 2022) state that gram positive bacteria on colored Gram stain purple, meanwhile
gram negative bacteria colored red. Two types were found form *Bacillus* bacteria, shaped diplobacilli stem coupled with two and Streptobacilli that is bacteria shaped joined stems elongated form chain (Nunki et al., 2020) Study (Susanti et al., 2015) prove that land rhizosphere bamboo nature deep suppressive soil push pathogen *P. palmivora* reason rotten base stem (damping off) and boost growth seeds pawpaw.

**Total of Leaves (Strands)**

The results of calculating the number of leaves based on analysis of variance showed that the results of calculating the highest leaf growth at 8 WAP in immersion treatment A1/8 hours= (12.63) and the lowest leaf growth in the treatment A0/control= (11.38) Application of microcapsules showed highest data was at C2/20gr= (11.75) level and the lowest at C0/0gr= (10.88). Research (Zit et al., 2023) used immersion treatment and addition of endophytic bacteria microcapsules from tea roots and stems in plants. tea, on treatment immersion had the highest data at 90 days after plant (DAP) in treatment S1 (6.75 strands ) while treatment addition The highest microcapsule data was at 90 HST in treatment I3 (6.88 strands ). (Dewi et al., 2022) revealed that the results of variance calculations on the average number of leaves at 60 DAP on the growth of cocoa seedlings were highest in chicken manure planting media K2= (8.20) and control planting media K0 and K4 goat manure planting media total leaf as much as (8.15 ) next is the planting medium dirt cattle (K3) total leaf as much (7.80 ) furthermore amount the fewest leaves namely the planting medium bokasi (K1) as much (7.40 ). This research result linear with the research above which states that on treatment immersion bacteria endophyte on coffee plants based on results duncant test calculations , total The leaves that obtained the highest data were soaked for 8 hours (10.75 pieces ) and the lowest data were without immersion B0= control . (9.25 strands ). Applicationmicrocapsules , highest data found in treatments I1 and I2 with doses of 5 and 10 gr/ polybag (10.25 pieces ) and results lowest on without treatment (10.00 strands ). (Purba et al., 2023)

Based on duncant test results on observations Sunday to - 2WAP, 4WAP, 6WAP, 8WAP. With no results _ significant is different No real . (Purba et al., 2023) research (Depari et al., 2018) using the application of compost and giving NPK fertilizer (16:16:16) explained at the age of 12 WAP showed that the highest average number of leaves was in the application 338 g cocoa shell compost (K3) of 13, 83 strands. while the lowest was in the treatment of giving 113 g cocoa shell compost (K1) amounting to 13.10 pieces. In the NPK treatment (16:16:16) 8 g (N2) it was 13.69 strands, while the lowest was 0 g without giving NPK N0= 13.13 strands. Average amount leaves at 60 DAP shows that the treatment of giving chicken manure (K2) amount leaf seeds plant the most cocoa that is as much as 8.20, then planting media control (K0) and planting media dirt goat (K4) quantity leaf as much as 8.15, then planting media dirt cattle (K3) total leaf as much as 7.80, next amount the fewest leaves namely the planting medium bokasi (K1) as much as 7.40. (Dewi et al., 2022) . Biologically, these microbes and enzymes can work optimally and can convert nutrients that were previously difficult for plants to absorb into nutrients that are easily absorbed by plants so that fertilizer use becomes very efficient (Murniatyi et al., 2022) . PGPR from the rhizosphere of shallot plants shows that bacterial isolates are capable of producing the hormone IAA which has the potential to be a biofertilizer. (Handayani et al., 2013).
**Leaf Area (Cm²)**

Duncan test calculation results shows the results of calculating the highest leaf area at 8 WAP in the immersion treatment A3/32 hours=(84.03) Cm² and the lowest leaf growth in the treatment A0/control=(68.51)Cm². And in the treatment of giving microcapsules based on the Duncan test, the highest data was at the C2/20gr=(78.89)Cm² level and the lowest was at the C0/0gr=(70.11)Cm² level. Research (Prayoga et al., 2023) states the measurement results and variance in leaf area using immersion treatment and administration of endophytic bacterial microcapsules obtained from the roots and stems of cocoa plants which shows that the immersion treatment S3 = (65.77cm2) produces the largest leaf area, in addition The largest leaf microcapsules in treatment B1 = (66.64 cm2). This research is in line with the research above, the immersion treatment using endophytic bacteria from the roots and stems of rubber plants, had the highest yield at 5 WAP in the R2 treatment within 24 hours with an average leaf area of 53.19 cm². And the lowest leaf area was in treatment R0 (control) with an average plant leaf area of 48.61 cm². And for the microgranule addition treatment, the highest yield of tea plant leaf area at the age of 5 WAP was found in the J3 treatment of 20 g/ polybag (Arg, 2023). The results of variance analysis on plant height using various types of planting media on the growth of cocoa seedlings showed no significant effect. This is because the planting medium has adequate nutrients and water so that the plant height rate is almost the same.

The use of top soil in the cocoa plant nursery media produces almost the same seed height. This is because topsoil contains sufficient nutrients, good air and water management, has stable aggregates and good water holding capacity as a result of research (Manullang & Silalahi, 2019) using the M4 planting media composition (Top Soil + Husk Ash + Manure composition (1:1:2) gave the highest average cocoa leaf area results until the end of the observation with an average leaf area of 62.18 cm2, followed by M1 (Top Soil + Manure (1:1)) with an area of 59.03 cm2 and then M0 (Top Soil ) area of 55.3 cm2. Based on research (Cahyadi et al., 2017) Leaf growth plant caisin No influenced by fertilization, treatment with biological fertilizer and NPK fertilizer on the growth of the number of leaves did not have a significant effect plant who does not given fertilizer. Observation of the number of leaves in the treatment of several peanut varieties had a very significant effect, on the administration of rhizobacteria RB36 had the highest ability reaching 98.18 pieces and the lowest in the control, namely 68.8 pieces. The addition of pgpr also played a role in increasing the chloroplast content so that there was an increase in leaf expansion. This is supported. (Anjardita et al., 2018) which states that PGPR treatment can increase leaf chlorophyll from 23.81% to 28.22% 15 days after administering the PGPR formula.
CONCLUSION

1. Rhizosphere bacteria in the soil of bamboo roots produced gram negatives producing the hormone IAA.
2. Parameters for observing the number of leaves, the best results were shown in the 8 hour immersion treatment with an average total of leaves of 12.63, followed by application 20 grams of capsules with an average number of leaves of 11.75.
3. The results of measurements and calculations of analysis of variance showed that the immersion treatment and application of rhizosphere bacterial microcapsules did not have a significant effect.

REFERENCES


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