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# Effects of *Bacillus Sp.* on The Growth of Immature Plants In Year 1 Robusta Coffee Clones

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A B S T R A C T

Coffee (*Coffea sp.*) is a plantation crop that is widely cultivated and an export commodity that has an important role for the economy in Indo-nesia. The productivity of coffee cultivation in Indonesia is still not optimal due to several factors, one of which is the lack of innovative tech-nology that can increase the production of coffee plants. An important phase in the cultivation of coffee plants is in the phase of young plants or immature plants in year 1. In this phase, proper maintenance treatment is needed for the purpose of preparing the plant for optimal growth. The application of technology to increase the production of coffee plants can be done through the application of Bacillus sp. which can func-tion as bacteria that can stimulate plant growth and by using superior clones. The purpose of this study was to determine the effect of Bacillus sp. on the growth of immature plants in year 1 on several robusta coffee clones. The method of this research was using split plot design which was arranged in a randomized complete block design (RCBD) with 2 factors and 3 replications. Factor I as the main plot consisted of several clones with 4 levels, namely the Cabutan clone, BP 409, BP 936, and BP 939. Factor II as the subplot was the concentration of Bacillus sp. consisted of 3 levels, namely 0 gr/l, 30 gr/l and 60 gr/l. The results of this study indicate that there is no interaction between the application of Microbial Bacillus sp. with some of robusta coffee. While the application of Bacillus sp. significantly increase stem diameter and there were differences in the growth characteristics of several coffee clones on all observed variables.

## INTRODUCTION

Coffee (Coffea sp.) is a plantation crop that is widely cultivated and an export commodity that has an important role for the economy in Indonesia. Indonesia is the 4th coffee producer in the world after Brazil, Vietnam and Columbia. In Indonesia, there are three types of coffee that are commonly cultivated, namely Arabica coffee, Robusta coffee and Liberica coffee. Arabica coffee has the characteristics of small leaves, smooth shiny and the length of the leaves is 12-15 cm x 6 cm with a fruit length of 1.5 cm. Robusta coffee has large leaves with a length of about 20 cm x 10 cm wavy and fruit

length of about 1.2 cm. Liberica coffee has the characteristics of dense, large, shiny leaves, large fruit up to 2/3 cm, but the seeds are small (AAK, 1988).

Robusta coffee is a type of coffee that is widely cultivated by farmers in Indonesia. Robusta coffee is more resistant to disease, especially Hemileia vastatrix disease compared to Arabica coffee. Robusta coffee is grown at an altitude of 0 to 1,000 masl, but the ideal altitude is in the range of 400-800 masl and can grow well at soil acidity levels or pH 5-6.5 with an average temperature of 21oC to 24oC. Rainfall that is suitable for robusta coffee cultivation is between 2000-3000 mm/year and has a higher production than Arabica coffee (Asmak, 2018).

Land use for coffee cultivation in Indonesia continues to change from year to year. In 2017 it is known that the coffee cultivation area owned by the people was 1.192 million hectares and in 2018 it was 1.194 million hectares. Production of coffee plants from this land area in 2017 was 685.80 thousand tons (575.3 kg/ha) and in 2018 with the addition of cultivated land area from the previous year coffee production decreased to 685.79 thousand tons (574, 4 kg/ha) (BPS, 2018). The lack of use of innovative technology that can increase the production of coffee plants is one of the factors causing the suboptimal production of coffee plants cultivated by farmers, especially when the plants are young or immature plants.

One of the important phases in the cultivation of coffee plants is when they enter the phase of young plants or immature plants in year 1. In this phase of immature plants, proper maintenance treatment is needed for the purpose of preparing the plant for optimal growth so that when the plant enters a productive period or called also yielding plants (TM) are able to provide optimal results (Junaedi et al., 1999). Another important factor to obtain optimal productivity is the use of superior clones that are recommended as planting material for robusta coffee cultivation, including BP 409, BP 963 and BP 939.

These clones have advantages and different morphological and physiological characteristics. With these differences in each clone, of course there will also be differences in responses to various cultivation factors used such as fertilization and the provision of growth regulators (Rusli et al., 2015). The application of technology to increase the production of coffee plants can also be done through the application of *Bacillus sp*. which can function as bacteria that can stimulate plant growth.

From the results of experiments conducted by Puspita (2018), it is known that the bacteria *Bacillus sp*. can also play a role in accelerating plant growth by producing growth-regulating hormone IAA which affects plant growth. *Bacillus sp*. also able to produce other growth-promoting compounds, including auxins, cytokinins, and IAA (Tinendung et al., 2014).

## METHOD

### Location and Time

Research on the effect of *Bacillus sp*. on the growth of immature plants in year 1 on several robusta coffee clones (Coffea canephora Pierre) this was carried out on March 3, 2021 until May 26, 2021 and took place in a coffee plantation owned by the people in Curahpo Village, Curahdami District, Bondowoso Regency.

### Tools and Materials

Analytical balance, measuring cup, sprayer, large and medium bottles, meter, SPAD chlorophyll meter, stationery, caliper, digital camera, Robusta coffee plant clones BP 409, BP 936, BP 939 and extracted clones, Bacillus microbes sp. in the form of products (BIONANO), millimeter block paper, label paper, insulation and aquades/water.

**Data Analysis**

The experiment was carried out using a Divided Plot Design with the basic RAK pattern consisting of 2 factors with 3 replications. Factor I as the main plot consisted of several clones consisting of the Cautan clone (K0), BP 409 clone (K1), BP 936 clone (K2) and BP 939 clone (K3). Factor II as a sub-plot is the concentration of *Bacillus sp*. consisting of 0 gr/l, 30 gr/l and 60 gr/l *Bacillus sp*. The data obtained were analyzed using analysis of variance and if there was a significant difference between treatments, a further test was carried out using Duncan's multiple distance test at a level of 5%.

**Research Procedures**

1) The plant samples used were selected based on their almost uniform growth and were not attacked by disease, then the amount taken was in accordance with the research needs, 2) Land preparation before treatment application included cleaning the research area and making plates on each plant sample as well as giving basic fertilizer using Urea 20gr, SP36 25gr and KCL 15gr for planting. 3) Application of *Bacillus sp*. available in the BIONANO product with several tried concentrations dissolved in 1 liter of water and then applied to plants by spraying as much as 50 ml of plants using a sprayer. The application is given to the lower surface of the plant leaves which is carried out in the morning and applied once. 4) Maintenance is carried out every 2 weeks during the study in the form of weeding the research area and plant plates. 5) Observations were made every 2 weeks for 3 months, the variables observed were plant height, stem diameter, number of leaves, leaf chlorophyll content and leaf area.

**RESULT AND DISCUSSION**

The results of the analysis of variance in Table 4.1 show that the interaction of *Bacillus sp*. and Multiple Clones had no significant effect on all observed variables. The main influence of microbial factors *Bacillus sp*. no significant effect on all observation variables except the stem diameter observation variable. While the main influence of the Multiple Clones factor significantly affected all observation variables.

Table 1. Summary of Results of Variation (F-Count) on All Observation Variables

| No | Observation Variable | F-Count Value | | |
|---|---|---|---|---|
| | | Microbes *Bacillus sp*. (B) | Clone (K) | Combination (B x K) |
| 1 | Plant Height (cm) | 1.16 ns | 67.83 ** | 1.49 ns |
| 2 | Rod Diameter (mm) | 6.99 ** | 19.71 ** | 0.59 ns |
| 3 | Number of Leaves (strands) | 0.60 ns | 17.61 ** | 1.19 ns |
| 4 | Leaf Chlorophyll Value (SPAD units) | 0.18 ns | 13.32 ** | 0.86 ns |
| 5 | Leaf Area (cm2) | 0.45 ns | 5.21 * | 1.00 ns |

Description: ** Very Real Difference, * Real Difference, ns Not Real Difference

**Effect of Interaction Between Microbial Applications of *Bacillus sp*. and Several Robusta Coffee Clones Against Immature Crop Growth Year 1**

The interaction between the application of *Bacillus sp*. and some clones had no significant effect on all observed variables. This is because several robusta coffee clones tested, including plucked clones, BP 409, BP 936 and BP 939, had the same response to *Bacillus sp*. These clones are thought to have genetic similarity so that the response to the treatment is the same.

This is supported by research conducted by Sobari et al (2018), regarding the use of manure with *Bacillus sp*. as a phosphate solvent in several robusta coffee clones, namely BP 308, SA 237, BP 42, BP 358 and BGN 371, showed that there was no significant interaction. The absence of a significant interaction indicated that all the clones tested had the same response to the given treatment. The tested Robusta coffee clones are thought to have genetic similarity so that they show the same response to the treatment given. Research conducted by Baon (2011), states that a superior clone of robusta coffee with the initial name BP (Besoekisch Proefstation) is the result of breeding where the parent material is the same, namely using the results of its introduction in the Congo area.

**Effect of Microbial Application *Bacillus sp*. Against Immature Plant Growth Year 1 In Several Clones Of Robusta Coffee (Coffea Canephora Pierre).**

The results of the analysis of variance in Table 4.1 show that the application of *Bacillus sp*. gave a significantly different effect on stem diameter, while the other variables showed no significant effect. From the results of Duncan's multiple distance test, the main influence of microbial factors is *Bacillus sp*. to the stem diameter in Figure 4.1 shows that the application of *Bacillus sp*. with treatment B1 (*Bacillus sp*. concentration 30 g/l) and B2 (*Bacillus sp*. 60 gr/l) treatment gave a better increase in plant stem diameter growth than treatment B0 (*Bacillus sp*. 0 gr/l concentration).

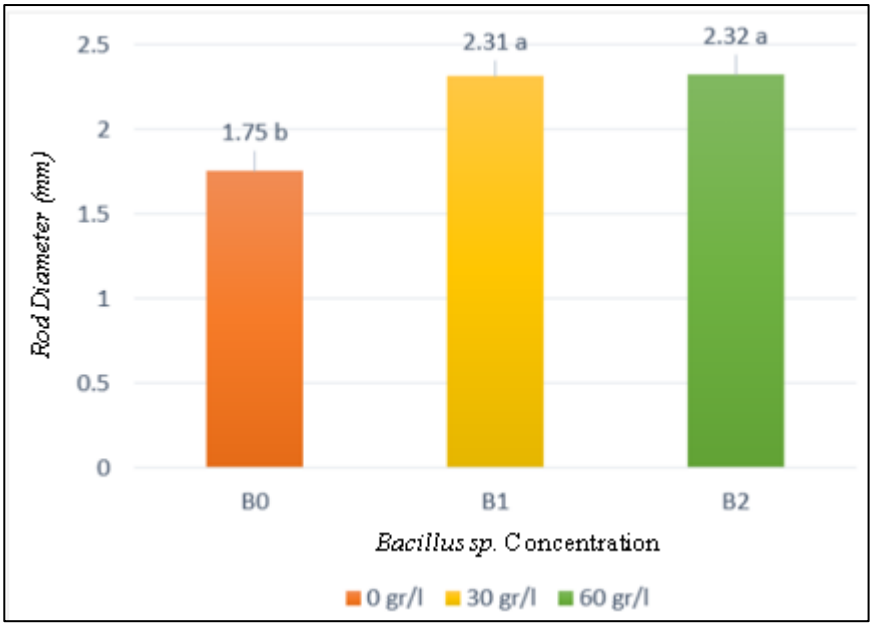


Figure 1. Effect of *Bacillus sp*. to rod diameter

The presence of *Bacillus sp*. Plants are known to be able to produce phytohormones to stimulate plant growth. *Bacillus sp*. enter into plant tissues through plant roots and other parts of plants such as flowers, lenticels on stems or natural wounds and leaves through stomata. Then *Bacillus sp*. that have entered the plant tissue will colonize where it enters or spread to all parts of the plant through the transport network (Zulkifli et al, 2016). *Bacillus sp*. can have a function as a growth promoter for plants because these bacteria are able to produce phytohormones or growth-promoting hormones in plants in the form of Indole 3-Acetic Acid or IAA which can increase plant growth (Husna et al., 2019). IAA is an endogenous auxin that has a role in cell enlargement, inhibits the growth of side shoots, stimulates abscission, is able to play a role in the formation of xylem and phloem transport networks and plays a role in root development and elongation, so that it can increase plant vegetative growth, one of which is stem diameter (Puspita). et al., 2018).

The results of this study can also be seen that the application of *Bacillus sp*. showed no significant difference to the variables of plant height, number of leaves, leaf chlorophyll value and leaf area (Table 4.2). Although the results of the analysis of variance showed that the results were not significantly different, from these results it can be seen that the application of *Bacillus sp*. with concentrations of 30 g/l and 60 g/l showed a better growth increase compared to the application of *Bacillus sp*. concentration 0 gr/l.

Table 2. Effect of *Bacillus sp*. on plant height, number of leaves, leaf chlorophyll value and leaf area

| Concentration of *Bacillus sp*. | Plant height (cm) | Number of leaves (strands) | Leaf chlorophyll value (SPAD units) | Leaf area ($cm^2$) |
|---|---|---|---|---|
| 0 g/l (B0) | 13.67 | 31.33 | 57.90 | 202.29 |
| 30 g/l (B1) | 17.13 | 37.33 | 58.88 | 209.41 |
| 60 g/l (B2) | 18.61 | 32.17 | 58.10 | 215.59 |

Sumihar (2013), explained that the success of the use of endophytic bacteria that provide benefits in agriculture is not only determined by the quality of the cells in the inoculant, there are several other influences, including energy sources, application of inoculants and environmental factors (temperature, rainfall) as well as methods. product storage before use. Another opinion also states that an organism's life activity can be influenced by several things, including climate, soil and vegetation factors (Hakim et al., 1986).

The effect of *Bacillus sp*. on the growth of immature robusta coffee plants in year 1 had no significant effect on the observed variables of height, number of leaves, leaf chlorophyll content and leaf area, allegedly due to the influence of environmental factors or weather factors where at the research location, the weather can change at any time and research carried out during the rainy season.

Another assumption that causes the application of microbes *Bacillus sp*. on the growth of immature robusta coffee plants in 1 year, the effect was not significantly different on the above variables, it could be because the concentration used was still too low so that the effect or impact on plant growth was not optimal. From the results of research conducted by Simulingga et al (2015), it is stated that the use of biological fertilizers with doses or concentrations that are too low can cause the effect given to plant growth is not optimal, this is because the number of microorganisms that are too few are not able or not enough to have an effect on plant growth. significant effect on plant growth.

Another study conducted by Iswandi et al (1994), stated that the high population of microorganisms will affect plant growth. This statement is supported by the results of another study conducted by Puspita et al (2013), which stated that the higher the number of colonies of *Bacillus sp*. it will more quickly colonize the entered parts such as plant tissue, plant roots and other parts so as to assist in the absorption of nutrients and increase plant growth.

**Effect of Several Robusta Coffee Clones on Immature Crop Growth Year 1.**

The results of the analysis of variance in Table 4.1 show that there was a significant effect of several robusta coffee clones on immature plant growth in 1. Duncan's multiple spacing test results showed that the main effect of several clones had a significant effect on plant growth on all observed variables, namely plant height, diameter stem, number of leaves, leaf chlorophyll value and leaf area.

*1. Plant Height (cm)*

Duncan's multiple spacing test results showed that clone BP 409 had the highest plant height of 25.20 cm, which was significantly different from the other clones. While the plucked clones showed the lowest plant height, which was 3.96 cm. These results are in accordance with research conducted by Puslitkoka (2006), which states that the BP 409 clone has a large and sturdy crown. The results of Widodo's research (2015), states that the growth of plant height is related to the rate of photosynthesis, if the rate of photosynthesis in plants is high, it will be able to produce photosynthate in large quantities which plants use for growth. The photosynthetic capacity is influenced by the chlorophyll content, so that the chlorophyll content in the plant can be an indicator of the plant's photosynthetic ability. From the results of this study, it was also known that the BP 409 clone had a large leaf chlorophyll value, while the extracted clone had the lowest leaf chlorophyll value.

*2. Root Diameter (mm)*

Duncan's multiple spacing test results showed that clone BP 939 had the highest stem diameter of 2.99 mm, which was not significantly different from clone BP 409 which had a stem diameter of 2.67 mm. While the plucked clones showed the lowest stem diameter of 0.61 mm. These results are consistent with research conducted by Puslitkoka (2006), which states that clone BP 939 has a wide, sturdy crown with regular branches and clone BP 409 has a large and sturdy crown with a crown diameter of approximately 2.7 meters. Khusna et al (2016), stated that the growth of stem diameter is related to the growth of plant height and number of leaves, if the growth of height and the number of leaves of the plant is high, it will also be followed by the growth of the diameter of the stem. The results of research by Jumin (2005), explained that the growth of stem diameter is determined by the rate of photosynthesis, if the rate of photosynthesis is high, it will produce a large amount of photosynthate to be used in plant vegetative growth. This is in accordance with the results of this study where it was known that clones BP 939 and BP 409 had high plant height, number of leaves and high leaf chlorophyll values. Meanwhile, uprooted clones showed low plant height, number of leaves and leaf chlorophyll values.

*3. Number of Leaves (strands)*

Duncan's multiple spacing test results showed that clone BP 939 had the highest number of leaves, namely 42.67 leaves, which was significantly different from the other clones. While the plucked clones showed the lowest number of leaves, namely 14.44 strands. Martade and Basri (2011), stated that the growth of the number of leaves is related to the rate of plant growth. If the plant growth rate is high, it will also be followed by the differentiation of cells that make up plant tissues or organs such as leaves. So that the vegetative growth of plants such as height and stem diameter will certainly be accompanied by an increase in the number of leaves. It is known from the results of this study that clone BP 939 has a high growth rate which can be seen in the stem diameter and high leaf chlorophyll value. While the uprooted clones had a low growth rate which could be seen from the plant height, stem diameter and low leaf chlorophyll value.

*4. Leaf Chlorophyll Value (SPAD unit)*

The results of Duncan's multiple spacing test showed that clone BP 409 had the highest leaf chlorophyll value of 61.07 units, which was not significantly different from clones BP 936 and BP 939, but significantly different from uprooted clones which had chlorophyll values. the lowest leaf is 52.22 units. The results of this study are in accordance with research conducted by Puslitkoka (2006), which states that clone BP 409 has a dark dark green leaf color, clone BP 936 has dark green leaf color but is not as dark as the leaf color of clone BP 409. While clone BP 939 has dark green leaves. green. Sakiroh

et al (2020), stated that the value of chlorophyll in plants is influenced by genetic and environmental factors (sunlight, carbohydrates, oxygen, nitrogen, magnesium, iron, water and temperature). If these environmental factors are in suitable conditions, then the value of leaf chlorophyll in plants will be optimal.

*5. Leaf Area ($cm^2$)*

Duncan's multiple spacing test results showed that clone BP 936 had the highest leaf area of 234.77 cm2, which was significantly different from the other clones. These results are in accordance with research conducted by Puslitkoka (2006), which states that the BP 936 clone has an elongated oval leaf shape with a slightly wide rounded leaf tip, blunt horizontally. Besides being influenced by genetic factors, Widodo (2015), stated that plant leaf area is also influenced by light intensity, humidity, nitrogen, temperature and water content in the soil. Humidity, temperature and light intensity affect transpiration and evaporation. If transpiration and evaporation are high, the availability of water for plants is insufficient, resulting in the cells in plant leaves shrinking and causing small leaf size. Water and nitrogen are needed by plants for the process of cell division and enlargement that occurs in plant meristem tissue during the exponential and linear growth phases.

## CONCLUSION

The effect of *Bacillus sp*. on the growth of immature robusta coffee plants in 1 year is significant effect on the stem diameter parameters with the highest growth increase of 36.6% at the concentration treatment of 60 gr/l *Bacillus sp*. There were differences in the growth characteristics of several Robusta coffee clones on the growth of immature plants in year 1 on all observation parameters and the clone that had the best growth was clone BP 939.

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