Plant growth promoting effects of indigenous rhizosphere and endophytic bacteria on soil fertility and black pepper (*Piper nigrum*) yield at Bangka Belitung

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Abstract Black pepper cultivation in Bangka Belitung is commonly followed a shifting cultivation method. This is due to the low soil fertility. The study aimed to obtain the best combination and concentration of indigenous rhizosphere and endophytic bacteria to improve soil fertility and enhanced growth and yield of black pepper. The study was set up in a complete randomized block design, 9 treatments and 3 replications. The treatments were the combinations of the types and concentrations of the bacterial isolates, i.e. (1) Control, (2) Endophytic bacteria A, (3) Combination of Endophytic bacteria B + Bb. chosinensis+ Rhizobacteria A, (4) Combination of Rhizobacteria B + Endophytic bacteria A+ Endophytic bacteria C, (5) Combination of Endophytic bacteria B + Endophytic bacteria C + Endophytic bacteria D at 10^3 and 10^5 cpu ml^-1 respectively. Endophytic bacteria were capable to boost the growth and yield of black pepper, also improved the soil chemical properties. The best treatment combinations were the mixture of of bacteria, i.e. (1) Psedomonas sp.+ Brevibacillus sp+Mesorhizobium sp., (2) Bb. sp1 +Bb. sp2+Bacillus sp., and (3) Brevibacillus sp +Bb sp1+Bacillus sp. The study implies that rhizosphere and endophytic bacteria are potential to be developed for reducing fertilizer use in black pepper cultivation.

Keywords: Biofertilizer, production, soil chemical properties.

1. Introduction

Bangka Belitung is the largest white pepper producer at Indonesia. Its production is around 80-97% of the total white pepper production in Indonesia [1]. Commonly, the pepper cultivation in Bangka Belitung uses a shifting cultivation system, where the crop survives for 5-6 years or maximum 3-4 harvesting times and then when the crop productivity decreases, farmers will cultivate new land. Some problems in the pepper cultivation in this region are stem rot disease caused by *Phytophthora capsici* and low soil fertility.

The low soil fertility was indicated by soil properties (chemical and physical characteristic). The total of exchanged-base content was 0.68%, cation exchange capacity was 11.07%, C-organic was 1.61%, and base saturation was 11.07%. The soil texture also was dominated by sand 63.99%, silt 10.34%, and clay 25.67% [2]. Thus, the nutrient and water holding capacity of the soil were very low. Therefore, the nutrients and water will be easily lost carried by groundwater flow.
Increasing land productivity and crop yield can be done by improving soil fertility through application of microorganisms, including plant growth promoting bacteria (PGP). The origin of the bacteria can be obtained from rhizosphere (rhizobacteria) and internal plant tissues (endophytic bacteria). The role of beneficial bacteria may affect the chemical properties of the soil through a direct mechanism, such as (1) fixing nutrients, especially N fixation [3;4] and P solubilization [5;6;7] and (2) synthesizing phytohormones or compounds that regulate plant growth [8;9].

Furthermore, bacteria can produce organic materials through the mineralization processes affecting soil characteristics (the physical and chemical properties). This is positively associated with the C/N ratio of bacteria [10]. Clay possesses higher levels of organic matter decomposition and lower C/N ratio, than sandy soils. The role of soil microbes in the decomposition processes of organic matters is important. This is related to the level of N mineralization per unit of microbial biomass [10].

The role of soil bacteria in improving soil fertility and promoting plant growth are expected to enhance black pepper productivity. This research aimed to obtain the best combination and concentration of indigenous rhizosphere and endophytic bacteria to improve soil fertility and increase black pepper yield at Bangka Belitung.

2. Materials and methods

This research was executed at the Microbiology Laboratory of Indonesian Spices and Medicinal Crops Research Institute, Bogor, West Java; and farmer plantations of Bangka Belitung Province, Indonesia, from January to December 2017. The black pepper plants used in the study were 5-6 years old and have been harvested 3-4 times. Bacterial isolates used were from rhizospheres and endophytes.

The research was set up in a randomized block design, single factor, 3 replications, and 9 treatments. The treatments were a combination of the kind and concentration of bacteria. The treatments consisted of (1) Control (K0), (2) Endophytic bacteria A, at 10^3 cfu/ml (K1), (3) Combination of Endophytic bacteria B + Bb. chosinensis+ Rhizobacteria A at 10^5 cfu/ml (K2), (4) Combination of Rhizobacteria B + Endophytic bacteria A+ Endophytic bacteria C at 10^5 cfu/ml (K3), (5) Combination of Endophytic bacteria C + Endophytic bacteria D at 10^5 cfu / ml (K4), (6) Endophytic bacteria A at 10^6 cfu/ml (K5), (7) Combination of Endophytic bacteria B + Bb. chosinensis+ Rhizobacteria A at 10^6 cfu/ml (K6), (8) Combination of Rhizobacteria B + Endophytic bacteria A+ Endophytic bacteria C at 10^6 cfu/ml (K7), and (9) Combination of Endophytic bacteria B + Endophytic bacteria C + Endophytic bacteria D at 10^6 cfu/ml (K8).

The basic fertilizations applied were 10 kg manure/plant applied at the beginning of the trial, whereas NPKMg fertilizer (300 g/plant) was applied one month after the first application of bacteria. The bacteria (1000 ml/plant at 10^3 and 10^6 concentration) were applied 6 times every month. The parameters observed were the leaf number, internode number, the length of fruit branch, canopy circumference, fruit branch number, flowers number, panicles number, and the fresh weight of pepper fruit. Soil analysis was conducted at the beginning and the end of the experiment to measure the organic C, pH, the content of N, P, and K. The data obtained were analysed using an analysis of variance and Tukey Test at 5% level for further analysis.

3. Results and Discussions

3.1. Pre-trial Soil Characteristic

The soil analysis result before the experiment indicated that the land had low soil fertility, indicated by low N, P, and K contents and high C/N ratio. Moreover, the soil texture was dominated by sand (Table 1). These soil conditions need to be improved by increasing organic matter and nutrients through bacteria application.
Table 1. The chemical and physical characteristics of the soil before trial

| Tested Parameter         | Value | Status  |
|--------------------------|-------|---------|
| N-Total (%)              | 0.14  | Low     |
| P$_2$O$_5$ available (ppm)| 19.99 | Low     |
| K (cmol(+)/kg)           | 0.24  | Low     |
| Na (cmol(+)/kg)          | 0.24  | Low     |
| Ca (cmol(+)/kg)          | 1.24  | Very low|
| Mg (cmol(+)/kg)          | 0.49  | Low     |
| C-organic (%)            | 2.47  | Low     |
| C/N-ratio                | 17.64 | High    |
| pH (H$_2$O)              | 5.16  | Acid    |
| pH KCl 1 M               | 4.50  | Acid    |
| Al dd (cmol(+)/kg)       | 0.89  | Very low|
| H (cmol(+)/kg)           | 6.74  | -       |
| Base saturation (%)      | 32.79 | -       |
| Texture                  |       |         |
| Sand (%)                 | 53.28 | -       |
| Silt (%)                 | 29.83 | -       |
| Clay (%)                 | 16.89 | -       |

3.2. Pepper Plant Growth After Bacteria Application

The growth of pepper plants treated with bacteria were varied and the K4, K5, and K6 treatments significantly increased all the growth parameters compared to control (K0) (Table 2). The effects among K4, K5, and K6 treatments were not significant. However, K6 treatment produced better plant growth.

Following six time bacterial applications on all treatments, the leaf number increased 5.71-50.32%, the internode number increased 1.69-26.45%, the newly emerged-flowers increased 21.55-119.77%, and the fruit branch length increased 10.16-18.82%. It can be assumed that microbes contained in K6 treatment produced more phytohormones to promote plant growth [11]. Plant growth hormones produced by microorganisms has been known to promote horticulture [12;13;14;15] and food crops [16]. The phytohormones are organic substances presented in a very small amount. However, they can affect morphology, physiology and biochemistry characteristic of the plants [16]. Phytohormones directly or indirectly synthesized by plant growth promoting microbes play important roles in regulating plant growth [18; 19]. Moreover, the microbes improve soil chemical properties, such as the content of N, P, K, and C-organic, as well as pH (Table 4). The study indicated that K4, K5, and K6 treatments promoted better plant growth than other treatments. Even though the bacteria concentration in the K4 (10$^5$ cfu/ml) was lower than those in the K5 and K6 (10$^6$ cfu/ml), the effects on the plant growth was similar to K5 and K6. Further study is required to understand the mechanism of microorganisms roles in the K4 treatment.
### Table 2. The effect of bacteria on the black pepper plant growth after 6 times applications

| Treatments | Leaves number/branch sample | Node number/branch sample | Fruits number/branch sample | Length of fruit branch (cm) |
|------------|-----------------------------|---------------------------|-----------------------------|-----------------------------|
| K0         | 30.61 d                     | 53.11 c                   | 7.17 e                      | 47.83 c                     |
| K1         | 36.87 d                     | 58.00 abc                 | 10.67 cd                    | 54.37 ab                    |
| K2         | 34.81 cd                    | 57.28 bc                  | 10.98 cd                    | 52.69 bc                    |
| K3         | 32.36 cd                    | 53.99 c                   | 8.71 de                     | 54.98 ab                    |
| K4         | 36.40 ab                    | 64.34 ab                  | 11.71 bc                    | 56.83 a                     |
| K5         | 42.52 ab                    | 66.89 a                   | 15.75 a                     | 54.44 ab                    |
| K6         | 46.32 a                     | 64.09 ab                  | 11.63 bc                    | 53.57 bc                    |
| K7         | 39.99 bc                    | 60.67 abc                 | 14.55 ab                    | 52.92 bc                    |
| K8         | 34.56 dc                    | 55.60 bc                  | 11.63 bc                    | 53.57 bc                    |

Note: Numbers followed by the same letter at the same column were not significantly different at Tukey Test 5%

K0 = control  
K1 = Endophytic bacteria A at $10^5$ cfu/ml  
K2 = combination of Endophytic bacteria B + Bb. chosinensis + Rhizobacteria A at $10^5$ cfu/ml  
K3 = combination of Rhizobacteria B + Endophytic bacteria A + Endophytic bacteria C at $10^5$ cfu/ml  
K4 = combination of Endophytic bacteria B + Endophytic bacteria C + Endophytic bacteria D at $10^5$ cfu/ml  
K5 = Endophytic bacteria A at $10^8$ cfu/ml  
K6 = combination of Endophytic bacteria B + Bb. chosinensis + Rhizobacteria A at $10^8$ cfu/ml  
K7 = combination of Rhizobacteria B + Endophytic bacteria A + Endophytic bacteria C at $10^8$ cfu/ml  
K8 = combination of Endophytic bacteria B + Endophytic bacteria C + Endophytic bacteria D at $10^8$ cfu/ml

#### 3.3. Yield of Black Pepper

The microbes application increased pepper production significantly compared to control, except for K1, K4 and K5 treatments. The K2, K3, K6, K7, and K8 treatments significantly enhanced pepper yield 14-42.9% (Figure 1).

![Figure 1. The effect of bacteria application on black pepper yield](image-url)
In the previous study, the tested microbes were able to produce phytohormone (auxin, cytokinin, and gibberellins), fix N from the air and dissolve P, as well as increase N, P, K, pH, and C-organic contents [20]. These characteristics supported the increase of plant growth and crop yield. The effects of microbe treatments on the treated black pepper plants improved the soil fertility (Figure 2). Phosphate, nitrogen, and potassium elements are essential and important nutrients for plant growth and yield. Acid soils have a high nutrient-binding capacity resulting in the very low nutrient availability in the soil. Moreover, the nutrients are bound by metal which also had high binding capacity characteristics. Thus, this causes nutrients are unavailable for plants. Despite the plants are applied with high dose fertilizer, they are unable to absorb the nutrients.

**Figure 2.** The effect of bacteria application on soil chemical properties

| Treatments | N (%) | P (ppm) | K (cmol+ kg⁻¹) | C-organic (%) | pH |
|------------|-------|---------|----------------|---------------|-----|
| K0         | 0,17  | 0       | 0              | 5,6           | 6,2 |
| K1         | 0,175 | 50      | 0,05           | 5,8           | 6   |
| K2         | 0,18  | 100     | 0,1            | 6             | 6,2 |
| K3         | 0,185 | 150     | 0,15           | 6,2           | 6   |
| K4         | 0,19  | 200     | 0,2            | 6,4           | 5,8 |
| K5         | 0,195 | 250     | 0,25           | 6,6           | 5,6 |
| K6         | 0,2   | 300     | 0,3            | 6             | 6   |
| K7         | 0,205 | 0       | 0,35           | 6,4           | 5,8 |
| K8         | 0,2   | 0       | 0,4            | 6,6           | 5,6 |

K0 = control
K1 = *Endophytic bacteria A* at 10⁵ cfu/ml
K2 = combination of *Endophytic bacteria B* + *Bb. chosinensis* + *Rhizobacteria A* at 10⁵ cfu/ml
K3 = combination of *Rhizobacteria B* + *Endophytic bacteria A* + *Endophytic bacteria C* at 10⁵ cfu/ml
K4 = combination of *Endophytic bacteria B* + *Endophytic bacteria C* + *Endophytic bacteria D* at 10⁵ cfu/ml
K5 = *Endophytic bacteria A* at 10⁸ cfu/ml
K6 = combination of *Endophytic bacteria B* + *Bb. chosinensis* + *Rhizobacteria A* at 10⁸ cfu/ml
K7 = combination of *Rhizobacteria B* + *Endophytic bacteria A* + *Endophytic bacteria C* at 10⁸ cfu/ml
K8 = combination of *Endophytic bacteria B* + *Endophytic bacteria C* + *Endophytic bacteria D* at 10⁸ cfu/ml
The mechanism of P solubilization by P solubilizing bacteria had been studied intensively. The soil pH around the rhizosphere decreases and organic acids generated to promote mineralization process of soil organic matter complex [12]. Plant growth promoting bacteria application on raspberry plants increased plant growth and yield, as well as soil nutrient contents, such as N-total, P, K, Ca, Mg, Fe, Mn and Zn. [12].

3.4. Post-trial Soil Chemistry Characteristic
The effect of bacteria treatments on the soil nutrient contents, such as N, P, and K were varied. The study showed that the bacteria application increased nutrient availability in the soil and therefore improved plant growth and yield (Table 2 and Figure 1).

The enhancement of soil pH (Figure 2) indicated that nutrients are available and easily absorbed and utilized by the plants. The role of these nutrients by the bacteria applied to promote plant growth, flower and fruits formation of black pepper need to be investigated. Previous study showed that the presence of such soil microbes increased the nutrients availability through nutrients fixation and leaching losses prevention. This study implies that the application of endogenous rhizosphere and endophytic bacteria is promising in improving black pepper plant in Bangka Belitung Province. Further study is required to examine the stability of the bacteria combination on plant growth and yield of black pepper in the following year.

4. Conclusions
Combination applications of endogenous rhizosphere and endophytic bacteria increased the productivity of black pepper plants through improvements of soil fertility properties, such as N, P, K, C-organic, and pH. The best bacteria combination treatments were (1) Rhizobacteria B + Endophytic bacteria A + Endophytic bacteria C, (2) Endophytic bacteria B + Bb. chosinensis + Rhizobacteria A, and (3) Rhizobacteria B + Endophytic bacteria A + Endophytic bacteria C.

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