The role of endophytic bacteria and mycorrhizae fungus as plant growth inducer of white turmeric

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Abstract. The use of endophytic bacteria and mycorrhizae fungus as plant growth inducer has been known. Endophytic bacteria could promote the growth of their host plant and Mycorrhizae could promote the growth of almost all species of plants. This study was aimed to investigate the effect of endophytic bacteria (Bacillus paramycoides KPU2, Klebsiella quasipneumoniae KPU4, Klebsiella pneumoniae KPR4.2, Burkholderia lata KPR8, Burkholderia metallica KPR9, and Kosakonia sachari KPD5), mycorrhizae fungus, and consortium both of the potential microbes on the growth of white turmeric. The experiment was set up at lath house for 10 months. The results of this study showed that single inoculation was better than that multi strains inoculation. Single endophytic bacteria inoculation and mycorrhizae inoculation produced the higher tuber dry weight of white turmeric (1059.33 gr and 918 gr, respectively). Endophytic bacteria were the most efficient inoculant compared to the other inoculant or treatment and significantly increased the number of leaf and sapling (14.33 and 2.83, respectively). The maximum plant height was observed in mycorrhizae inoculation (109.75 cm). Based on the microscopic observation of root infection, endophytic bacteria and mycorrhizae fungus could infected the root of white turmeric with the percentage of root infection is 25.55%. The results suggested that single endophytic bacteria and single mycorrhizae inoculation can be employed for plant growth inducer of white turmeric.

1. Introduction
White turmeric is a crop which is intended as herbal medicine. This crop acts as a functional food because it contains several active compounds that have beneficial values for health. Research on the benefits of white turmeric extract as an anticancer, analgesic, anti-inflammatory, anti-oxidant, anti-infective and antipyretic has been widely researched, but there is still a chance to find another active compound that have health benefits to mankind. There have been increase in the demand for white turmeric over the last decade because of its medical values, however this is not accompanied by plant productivity [1].

The low productivity of white turmeric is caused by the poor quality of seeds. The low productivity is also caused by farmers who have been using a lot of chemicals such as fertilizers, pesticides and herbicides. The utilization of chemicals for a long period of time and at high doses will reduce the soil quality [2].

To maintain productivity for a viable crop production, maintenance of soil fertility with addition of fertilizers is needed. In this research, to increase the productivity and quality of white turmeric plants...
can be done by treating BIOVAM biofertilizer and endophytic microbes in order to obtain quality seeds.

BIOVAM is a biofertilizer containing mycorrhizae fungi which is able to absorb nutrients especially phosphate. Mycorrhizae fungi forms a mutualism symbiosis relation with plant roots and interact with specific microbes that can increase plant growth under stressful conditions [3]. This symbiosis will increase the absorption of water, mineral and plant resistance to biotic and abiotic stresses. In addition, mycorrhizae fungi can play a role in restoring land degradation stabilizing dry land [4]. Inoculation of mycorrhizae fungi in plant seeds will make the plants stronger and more resistant when transferred to the field [5]. The use of mycorrhizae to increase the growth of white turmeric has been reported by [6]. They concluded that mycorrhizae arbuscular fungi inoculant isolated from *Hornstedtia scyphifera* Steud. rhizosphere showed compatibility with white turmeric but did not gave significant effect on the growth of turmeric plants after 12 weeks observation.

Endophytic bacteria are bacterial that colonize inside of healthy plant tissue but not lead any pathogenic reaction. Endophytic bacteria have mutualism symbiosis with their host plant and may play an important biological role. They can promote the plant growth (providing nitrogen, producing indolic compounds, phosphate solubilization, enzymatic activity) and also induce plant defence reactions and tolerance to stress environmental [7, 8]. The diversity of endophytic bacteria from medical plant-Zingiberaceae is very high. There were 20 endophytic bacteria isolated from turmeric plant (*Curcuma longa*) belonging to 12 different genera [9]. There are 107 endophytic bacteria from ginger plant (*Zingiber officinale*) grouped into 16 genera and 207 endophytes were isolated from white turmeric (*Curcuma zedoaria*) and belonging to 23 genera, but their use as agricultural inoculants in plants remain limited [10, 11].

The utilization of mycorrhizae fungi and endophytic microbes is expected to produce quality seeds of white turmeric that have high economic value, and produce a standardized herbal medicine. In addition, their use in plant is a solution in increasing the productivity of plants, environmentally friendly, and reducing the use of chemical fertilizers. Endophytic bacteria interact collaboratively with host plant, and are easy to cultivate in vivo [10]. Thus, the investigation of the role of mycorrhizae fungi and endophytic bacteria as white turmeric plant growth promoter is important to be developed.

2. Materials and Methods

2.1. Materials

The bacterial strains that used in this research are endophyte bacteria and isolated from white turmeric (*Curcuma zedoaria* Rosc.). The bacteria are *B. paramycoides* KPU2, *K. quasipneumoniae* KPU4, *K. pneunomaiae* KPR4.2, *B. lata* KPR8, *B. KPR9*, and *K. sachari* KPD5. The bacteria are sub cultured on Nutrient Agar (NA) medium. Vesicular Arbuscular Mycorrhizae (VAM) is a collection of the Plant Symbiotic Microbial (PSM) Laboratory, Research Center of Biotechnology, Indonesian Institute of Science. The inorganic fertilizer used are NPK. White turmeric rhizome is collection of Balai Penelitian Tanaman Rempah dan Obat (Balittro-Bogor).

2.2. Methods

Bacterial culture for inoculation preparation

Selected bacteria were grown in Nutrient Broth (NB) and incubated for 24 hours under shaking (100 r.min⁻¹). Vesicular Arbuscular Mycorrhizae (VAM) that used in this research consist of *Acalauspora* and *Scutellospora* in the form of infected biomass.

2.3 White turmeric cultivation

White turmeric plants were planted during November, 2019 and harvest in September, 2020. The studies were carried out at screen house, Research Center of Biotechnology, Indonesian Institute of Science. One seed-rhizome of 30 g was planted at the depth of 8 cm in each polybag. The polybags
were filled with 5 kg of soil, compost, rice husk (ratio 3-1-0.5) and the polybags were placed randomly in the screen house.

Treatments were Randomized Complete Design (RCD) with 6 fertilizer treatment combination, there are the following; control-no add fertilizer (KO), chemical fertilizer 100% (KN), endophytic bacteria inoculum (ENDO), vesicular arbuscular mycorrhizae inoculum (VAM), endophytic bacteria and vesicular arbuscular mycorrhizae inoculum (ENDO+VAM) dan commercial biofertilizer (POH SM). Each treatment combination consists of 6 replications. Chemical fertilization was applied on 15, 75, 120, and 160 days after planting. The doses of chemical fertilizer were 1, 25 g NPK per polybag. Water was applied as required every day for proper seedling emergence and plant growth.

2.4 Data collection

The growth parameter (plant height, number of leaves, the width of leaf, and the number of sapling were observed after 7 months after planting when the turmeric have developed full canopy formation and the main shoot terminated leaf formation. Rhizomes were harvested and collected at 10 months after planting. Rhizome samples were kept in paper bag and were rinse with clean water and dried in oven at 65-70°C until its completely dry.

2.5 Statistical analysis

The data were analyzed by analysis of variance (ANOVA) and if there were significant different the analyses were continued by Duncan’s multiple-range test (DMRT) at 95% confidence interval (α = 0.05).

2.6 Root infection

At the end of the observation, the percentage of VAM infection was observed using the root staining method [12]. Infection rate of VAM on the host plant root and dependence of plant seeds on mycorrhizae (Mycorrhizal depency) were observed. Mycorrhizal Dependency (MD) or dependence of turmeric plant seeds on VAM observed by a formula based on [13]: where DW is dry weight of plant

3. Results and Discussion

In the previous study, the endophytic bacteria isolates were isolated from white turmeric plant. The endophytic bacterial isolates were characterized by their ability to promote the turmeric plant growth and their antimicrobial and antioxidant activity (the paper in published process). They were identified based on morphology and molecularly. The selected bacteria isolates were Bacillus paramycoides KPU2, Klebsiella quasipneumoniae KPU4, Klebsiella pneumoniae KPR4.2, Burkholderia lata KPR8, Burkholderia metallica KPR9, and Kosakonia sachari KPD5. Based on the research, the ENDO treatment significantly increased number of leaves and number of sapling compared with the other treatment. The NPK treatment significantly increased the width of the leaf. The plant height showed non-significant in all treatment.

In this study, ENDO, VAM, ENDO+VAM increase the number of leaves and sapling compared with the control (KO) and KN. This was due to high concentration of nutrients in ENDO and VAM, which, in turn, would increase organic matter decomposition and subsequent nutrient release (Figure 1).

The increase in the height plant and the width of leaf as a result of the application of NPK fertilizer was due to the fact that the soil of the growing media lacked essential nutrients responsible for growth and yield of turmeric [14]. Turmeric is a crop that can exhaust soil fertility quickly. Therefore, due to its long gestation period (about 8 months) and high productivity, it requires high fertilizer input [15]. NPK fertilizer has an effect on turmeric. It was due to nitrogen in NPK fertilizer promoting leaf growth and required to form proteins and chlorophyll, while P contributes to root development, energy transfer reactions, and cell division and multiplication and K contributes to stem development, cell
division, formation and translocation of carbohydrates, and mainly tuber/rhizome development in roots [16].

![Figure 1](image_url)

**Figure 1.** Effect of fertilizer treatment on plant height, number of leaves, the width of the leaf, and number of sapling of turmeric plant 7 months after planting. (KO = un-inoculated microbial, KN = chemical fertilizer, ENDO = endophytic inoculation, VAM = mycorrhizae inoculation, ENDO+VAM = endophytic and mycorrhizae inoculation, POH SM = Commercial fertilizer.

The results show the significant or higher yields of turmeric with ENDO treatment compared with another treatment, followed by VAM, ENDO+VAM and commercial POH-SM treatment (Figure 2). This is due to ENDO contains potential microbes that are capable to increase the efficiency of nutrient uptake by plant. The selective bacteria are also capable of producing growth hormones-Indole Acetic Acid (IAA), substitute nutrients, especially nitrogen, and providing phosphate which can support the turmeric plant growth. Meanwhile, VAM can dissolve insoluble phosphate becomes available to plants. Phosphate availability depends on solubility, the amount of solvent, the distance from the phosphate ion to the root plant. VAM and ENDO can support the plant to absorb them.

The highest yield was obtained from the turmeric grown with ENDO followed by VAM, because the plants with these treatments has been known, produce a higher number of leaves and saplings. The number of leaves and saplings influence the number of rhizomes. The tuber weight of turmeric is increasing with the increasing of the number of leaves and saplings.
Figure 2. Effect of fertilizer treatment on tuber weight of turmeric plant-10 month after planting. (KO = uninoculated microbial, KN = chemical fertilizer, ENDO = endophytic inoculation, VAM = mycorrhizae inoculation, ENDO+VAM = endophytic and mycorrhizae inoculation, POH SM = Commercial fertilizer.

Figure 3. Effect of fertilizer treatment on tuber weight of turmeric plant after harvested. (KO = uninoculated microbial, KN = chemical fertilizer, ENDO = endophytic inoculation, VAM = mycorrhizae inoculation, ENDO+VAM = endophytic and mycorrhizae inoculation, POH SM = Commercial fertilizer.

Figure 3. showed the microbial infection in turmeric plant tissue in treatment of ENDO, VAM, and ENDO+VAM. Based on microscopic observations, on the root tissue of the turmeric plant, it was seen that there was microbial colonization in plant roots that were inoculated by microbes, both ENDO and VAM. Meanwhile, in the control and KN treatment were not seen the presence of root infection. Inside the roots that were inoculated with VAM, it was seen that VAM was dependent on turmeric which affected the tuber weight value.

The percentage of root infection of turmeric seedlings inoculated with VAM and ENDO+VAM showed moderate-high criteria (Table 1). The criteria for high infection occurred in the ENDO+VAM
inoculation treatment. This showed that VAM is able to collaborates with microbes to infect turmeric plants.

The association between Mycorrhizae fungi and plants can be identified by the presence of infection. Mycorrhizae fungi infection can be seen from the presence of structures produced by VAM, including: hyphae, arbuscular, mycelia, vesicles and spores [17]. Based on the observations, several mycorrhizal structures were found in the roots, including hyphae and vesicles (Figure 3). Mycorrhizae fungi that infect the roots of the host plant will produce hyphae strands so that mycorrhizal plants will be able to increase their capacity to absorb nutrients and water. The increasing absorption of nutrients and water in mycorrhizal plants can improve plant growth under drought stress conditions [18].

Table 1. The Infection rate percentage of VAM on the white turmeric root.

| Treatment | Infection rate percentage (%) | Criteria |
|-----------|-------------------------------|----------|
| KO        | 13.4                          | Poor     |
| KN        | 17.6                          | Poor     |
| VAM       | 51.3                          | Good     |
| ENDO+VAM  | 66.3                          | Good     |

According to the Institute of Mychorrizal Research and Development, mycorrhizal colonization is categorized as follows, very poor (0-5%), poor (6-25%), moderate (26-50%), good (51-75%) and very good (> 75%). The KO and KN treatments showed low root infection. This is caused the soil used as a planting medium contains indigenous mycorrhizae. Mycorrhizae colonization of turmeric roots on VAM and ENDO+VAM treatment showed high root infection. These results indicate the compatibility of VAM with host plants in the mechanism of nutrient exchange, mycorrhizal viability and host sensitivity. This is also influenced by the type of mycorrhizae fungi that are inoculated and their host plants [19].

Table 2. Mycorrhizal dependency (MD) of turmeric plant.

| Treatment   | Mycorrhizal dependency (%) | Criteria |
|-------------|----------------------------|----------|
| KO          | -                          | -        |
| KN          | -                          | -        |
| VAM         | 25.16                      | Moderate |
| ENDO+VAM    | 15.25                      | Moderate |

The dependency of white turmeric plant toward VAM inoculation is presented in Table 2. Mycorrhizal Dependency (MD) is defined by [13] as the degree of dependence on mycorrhizal conditions to produce maximum growth and yield at a certain soil fertility level. There are three classifications of MD, namely high (MD> 40%), moderate (10 <MD <40%) and low (MD <10%) dependence [20]. MD is influenced by several factors including the variety and ability of plants to utilize phosphate, root morphology and root physiology and the types of mycorrhizal fungi [21].

The value of the dependence of white turmeric plants on VAM inoculation ranged from 15.25 - 25.25% with moderate categories (Table 2). This means that white turmeric seeds without VAM inoculation can grow well, but if inoculated with VAM inoculants, they will show a better growth impact as reported in the results of this study. The low dependence value of turmeric plants with VAM maybe caused the white turmeric has a fibrous root system with a lot of root hairs, which causes the increasing nutrients absorption by the root surface area. Fibrous root systems that have many root hairs are generally less dependent on VAM infection than root systems that have few or no root hairs [22]. Plants that have less root hairs are dependent on VAM, because there is a symbiotic relationship that can replace the root hairs in absorbing nutrients [17].

The low dependence value of white turmeric on VAM caused the plant height and leaf width parameters were not significantly different. Not all plant species responded positively to mycorrhizae
inoculation. Plants that have a high dependence on the presence of mycorrhizae will show the real growth, on the other hand, they will not grow perfectly without any association with mycorrhizae [17].

![Figure 4](image_url)

**Figure 4.** Effect of fertilizer treatment on tuber weight of turmeric plant. (KO = un-inoculated microbial, KN = chemical fertilizer, ENDO = endophytic inoculation, VAM = mycorrhizae inoculation, ENDO+VAM = endophytic and mycorrhizae inoculation, POH SM = Commercial fertilizer.

VAM inoculation caused the increasing of the number of hyphae and root extension, and the available phosphate to plants so that plant growth was better. The high growth of root and shoot will result in the number of tubers (Figure 4). However, P fertilizer alone without N probably created nutrient imbalance, which in an antagonistic effect on vegetative growth. An imbalance or excessive of nutrients prevent ion formation, which causes trouble in nutrient absorption for plant [23].

The increasing of the growth and yield of white turmeric plants with mycorrhizal fungal treatment was due to the ability of VAM to form a mutualism symbiosis with white turmeric roots. The symbiosis of VAM with roots will form an interaction where mycorrhizae will stimulate root growth and help absorption of nutrients and water. Endophytic bacteria support plant growth and plant resistance through their ability to produce growth promoting substances. VAM and endophytic bacteria interact with each other during the root colonization process. Endophytic bacteria affect the formation and function of VAM, whereas VAM affects the bacterial population in the rhizosphere. The treatment of ENDO is better in increasing the yield of turmeric than commercial POH, so that it can be a candidate for biological fertilizer for turmeric and other crops.

4. Conclusion
The single microbial inoculation was better than that multi strains inoculation. Single endophytic bacteria inoculation and mycorrhizae inoculation produced the higher tuber dry weight of white turmeric. Endophytic bacteria were the most efficient inoculant compared to the other inoculant or treatment and significantly increased the number of leaf and sapling. The maximum plant height was observed in mycorrhizae inoculation. Based on the microscopic observation of root infection, endophytic bacteria and mycorrhizae fungus could infected the root of white turmeric with the percentage of root infection is 51,3% for mycorrhizae fungus treatment alone and 66,3% for combination between endophytic bacteria and mycorrhizae fungus treatment. The results suggested that the application of biofertilizer will give high yield in turmeric production, reduce cost and sustainable.

5. References
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