Soil bacteria abundance in application of biopesticides (Bacillus aryabhattai) in swampland, South Kalimantan

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Abstract. In rice cultivation, farmers tend to use chemical fertilizers and pesticides. The prolonged intensive use of agrochemicals can decrease soil quality. Chemical pesticides can be replaced with biopesticides to maintain and improve soil fertility, also to prevent the attack of plant-disturbing organisms on crops. The aim of this study was to determine the total population abundance of soil bacteria and soil quality after the application of biopesticides in the swampland in Jejangkit District. This research was accomplished in Jejangkit District, Barito Kuala Regency, South Kalimantan, which started from June to December 2019. The study used a Randomized Completely Block Design (RCBD) with three treatments and six replications. The treatments are Super Intensive and Actual Swamp (RAISA), Indonesian Agricultural Environment Research Institute’s (IAERI) Biopesticide, Galam Biopesticide. The variety of rice used is Inpar a 2. The plot size is 900 m² by applying the Jarwo Planting system. The results revealed that the application of biopesticides could increase the total population of soil bacteria from \(10^4\) to \(10^6\) cfu mL\(^{-1}\). Moreover, N and P nutrient content increase from low to moderate (0.19 to 0.4) % and (13.25 to 38.87) mg P\(_2\)O\(_5\) 100g\(^{-1}\), respectively.

1. Introduction
Swamplands are only planted with rice once a year. After the rice was harvested, the land is left uncultivated. Usually, farmers use local rice that has harvest time more than six months after sowing, so that it is difficult for farmers to plant rice twice or more a year. Local rice is preferred by farmers because the seeds are available, do not need a lot of chemical fertilizers, low seed requirements (5-10 kg ha\(^{-1}\)), resistant to pests, diseases, and swampy conditions, and also have a good market in South Kalimantan [1].

One of the strategies to achieve the productivity of rice farming is the application of technological innovations that are in sync with agricultural resources in a location (specific location). Each of these innovations must support each other and have a better effect on plant growth and productivity [2]. Farming in tidal swamps is already supported by technological innovations that are specific to the characteristic of the lands, including innovations in water micro-management, land-planning management, amelioration, fertilization, selecting superior varieties that are adaptive and have high productivity, and agricultural tools and machinery [1]. Rice cultivation in swamps depends on typological conditions and the type of overflow. The arrangement of swampland is usually carried out in a surjan planting system [3].

Adding soil optimizers and fertilizers is an important factor for improving soil conditions and increasing productivity. Soil amendments can be in the form of lime or dolomite, husk ash, and other agricultural organic matter. Enhancing fertilizer's efficiency in swamps is achieved by the application...
of fertilizers according to the availability of nutrients in the soil and plant types. Low soil pH conditions in tidal swamplands can inhibit the availability of macronutrients, like phosphorus, calcium, magnesium, and others needed for the growth of rice plants. The high intensity of pests and diseases in swampland is caused by the location of swamp lands, which are usually close to the forest especially the newly-cleared land and the narrowness of the area used for planting high-yielding varieties so that pests and disease attacks are focused [3]. Biopesticides are available for the protection of crops against insect pests, fungal, and bacterial diseases and weeds [4]. Biopesticides is the naturally occurring formulations made from substances that control pest by non-toxic mechanism [5]. Biopesticide can be derived from animals, plants and microorganisms which can be used for the management of pests injurious [6]. In the development of biopesticides, it is necessary to develop strains with the right host compatibility, competitiveness, and tolerance to abiotic stress. Various strains activity or an efficient consortium of strains with various activities such as N fixation, phosphate dissolving, and/or bio control properties would be the appropriate choice. Appropriate application technology will increase adoption by end-users [7].

Improper land clearing will cause pyrite oxidation. Pyrite accumulates in flooded soils that contain lots of dissolved organic and sulfates. Pyrite will then be oxidized into sulfuric acid when the drainage carries oxygen through the stagnant soil. Then acidic sulfate soil will be formed if the acid production exceeds the neutralization ability of the parent material, decreasing the soil pH (to less than 4). This is the beginning of the destruction of the swampland due to soil acidity and the forming of toxic elements. The content of Fe$^{2+}$, Al$^{3+}$, H$^+$, and SO$_4^{2-}$ in the area with drainage will be higher than in the area without it. A drainage system that is poorly developed can reduce the quality of soil and water [8]. Another problem with swamps is the low availability of nutrients in swamps, so it requires chemical and biological fertilizer additions to get optimal results [9].

Various soil factors can increase the availability of nutrients and plant productivity, one of which is soil microbial organisms [10]. The increasing use of synthetic fertilizers causes a decrease in its efficiency. It can be increased by using microbes such as N-fixing microbes, P and K solvents, and plant growth promoters. The use of soil fertilizing microbes can provide nutrients for plants, protect roots from pests and diseases, provide growth-regulating metabolites, and stimulate roots [11]. Apart from having the ability to increase the availability of N, P, and K nutrients, microbes also can provide micronutrients to support plant production [12].

To increase soil fertility and crop production can use synthetic fertilizers combined with organic fertilizers inoculated with Bacillus aryabhattai bacteria. Bacillus aryabhattai is a nitrogen-fixing bacterium, and it also has a role in decomposing organic matter into simple compounds so that it is available for plants [13]. The results showed that Bacillus aryabhattai isolate has the potential to mobilize soil P and increase growth, yield, and P assimilation in soybean and wheat crops [14].

Nitrogen (N) is one of the most important elements for plant growth and yield. Nitrogen is widely involved in the stress response (stress) in plants, and also has a role in complex host-pathogen interactions [15]. Moreover, N nutrients include inorganic compounds (nitrates and ammonium) and organic compounds (all amino acids). Elemental N is an important nutrient for plant growth, development, and response to stress and plant production [16]. Phosphorus (P) is one of the main growth-limiting nutrients of plants even though it is abundant in both inorganic and organic forms [17].

The availability of P in soil is strongly influenced by soil pH. In acidic soils, P will combine with Al and Fe to form Al-P and Fe-P, lowering the effectiveness of P fertilization because some of the P will turn into a form that is not available to plants. Plants that suffering from P deficiency result in stunted growth, imperfect roots, inhibited flowering, and fruit ripening and impaired protein formation [18]. Potassium is also a macro element needed by plants. Potassium deficiency can inhibit photosynthesis and photosyntheate absorption [19]. In rice plants, K fertilizer tends to produce higher grain [20]. The objective of this research was to determine the abundance of the total population of soil bacteria and the quality of the soil in the swamplands.
2. Materials and methods
The research was carried out in the field from January to December 2019 and superimposed on SERASI activities in Jejangkit Subdistrict, Barito Kuala Regency, South Kalimantan Province.

2.1. Material
Field materials used Inpara 2 rice seeds, the treatments are Super Intensive and Actual Swamp (RAISA) (P1), Indonesian Agricultural Environment Research Institute’s biopesticide (P2), Galam biopesticide (P3). RAISA plots are using superior varieties, legowo 2:1 planting system, water management, controlling plant pests. In this activity, IAERI’s biopesticide plays a role in controlling the pest and biofertilizers. The ingredients of IAERI’s biopesticides (P2) are neem leaves, mahogany leaves, turmeric, liquid smoke, cow urine, Bacillus aryabhattai, and water. Meanwhile, the materials used to make biopesticide made from local resources (P3) consist of Galam leaves, cow urine, clean water, liquid smoke, and Bacillus aryabhattai. The tools used during the research were hoes, water pumps, roller meters, scales, swing nets, gauges, taper buckets, plastics, sample bottles, scoops, and measuring cups.

2.2. Methods
The plot size in this research is 12 x 75 m with 80 cm spacing between plots. This research used a Randomized Completely Block Design (RCBD) with three (3) treatments and six (6) replications. The treatment includes:
1. RAISA (Chemical Pesticide) as Control
2. IAERI’s biopesticide
3. Galam biopesticide

Biopesticide application is carried out every 2 weeks, seeing the existing pest attack conditions. If the pest starts a lot the spraying interval is accelerated to once a week. The application of botanical pesticides was started at the age of 14 DAP to 84 DAP with a dose of 10 mL L⁻¹ of water. The RAISA plots were applied with chemical pesticides according to the treatment of the farmers.

The bacterial population analysis was carried out by the TPC method on PCA media. The TPC method was carried out by dissolving 1 g of sample with 9 mL of NaCl (0.9%) into the test tube. The dilution of this solution is 10⁻¹ and the dilution is carried out to 10⁻⁶ then stirred using a vortex. Furthermore, 0.1 mL of suspension of 10⁻² to 10⁻⁶ dilution was poured onto PCA medium using an Ependorph from a sterile strip. Furthermore, the solution was spread with a spreader dipped in an alcohol pad and heated. Then incubated at room temperature for 48 hours. Only 30–300 colonies were counted. In one treatment there were 6 replications, for each replication 5 diagonal soil sample points were taken. The five points of soil were composited. Then it will be composited from 6 replications. Analysis of soil characteristics was carried out in the IAERI laboratory.

3. Results and discussion

3.1 Total population of soil bacteria
The total population of bacteria in the initial soil of all treatment was not significantly different by 10⁴ cfu mL⁻¹. The bacterial population from high to low were RAISA > Galam biopesticide > IAERI’s Biopesticide consecutively. Soil test after harvest time showed that the highest total population of bacteria was IAERI’s biopesticide treatment (2.68 x 10⁶ cfu mL⁻¹), followed by Galam biopesticide (1.95 x 10⁶ cfu mL⁻¹) and RAISA control (3.5 x 10⁵ cfu mL⁻¹) as presented in table 1.
Table 1. The total population of bacteria in the soil at various treatments, Jejangkit 2019.

| Treatment         | Initial soil | Harvest soil |
|-------------------|--------------|--------------|
| RAISA             | 3.1 x 10^4 a | 3.5 x 10^5 c |
| IAERI’s Biopesticide | 2.9 x 10^4 a | 2.68 x 10^6 a |
| Galam Pesticide   | 3.0 x 10^4 a | 1.95 x 10^6 b |

The presence of *Bacillus aryabhattai* in IAERI’s biopesticide and Galam biopesticide increased the total bacteria in the soil. *Bacillus aryabhattai* has a role as a decomposer of soil organic matter, thereby increased soil fertility. *Bacillus aryabhattai* is also a phosphate solubilizing bacteria and non-symbiotic N bacteria [21]. At harvest, there was an increase in the number of soil bacterial populations for all treatments. Organic matter serves as a substrate for most soil microorganisms to grow and an increase in the bacterial population. Soil that contains a lot of organic matter is overgrown by various types and numbers of soil microorganisms, resulting in higher diversity and the population of microorganisms in the soil [22]. When the biopesticide is applied to plants, a small amount of the biopesticide solution will fall to the land. The existence of soil microorganisms, including the bacterium *Bacillus aryabhattai*, is used to transform organic matter into available compounds for rice plants [23]. Plant pest control using biopesticides is environmentally friendly. The application of biopesticides to rice plants in the tidal swamp can increase the total abundance of soil bacteria, as presented in figure 1.

![Figure 1](image-url)

**Figure 1.** The total population of bacterial at various treatment.

Biopesticides that fall in the soil when applied to rice plants increase the development of microorganisms in the soil. The role of microorganisms in organic agriculture is essential. The requirements for environmentally friendly agriculture need to be met: (1) preservation of biological diversity and ecological balance of natural biota; (2) physical, organic and chemical agricultural resources are maintained; (3) land and environment avoid contamination of chemical residues; (4) maintenance of land productivity sustainably without increasing input of production facilities [23].

3.2 Soil qualities
Swamplands have different characteristics and fertility levels depending on the type. In general, tidal swamps are acidic (pH < 5.5), have low fertility, and are deficient in micro-elements. The land used is acid sulfate land, which has been given lime that increases the pH soil. Table 2 shows some of the chemical characteristics of the initial and after treatments soil samples based on the results of...
laboratory analysis. Organic C content is at a low level because it is still below 2%, low organic C is one of the problems in tidal fields because it can affect other nutrients such as N elements. N levels are influenced by organic matter, if organic matter is low, N levels are also low [25].

**Table 2. Chemical properties of the soil at various treatment.**

| Chemical properties | Initial Condition | RAISA biopesticide | IAERI’s biopesticide | Galam biopesticide |
|---------------------|------------------|---------------------|----------------------|---------------------|
| pH H₂O              | 6.29             | 6.30                | 6.22                 | 6.59                |
| Organic C           | %                | 1.01                | 0.89                 | 0.84                | 0.89                |
| Total N             | %                | 0.19                | 0.31                 | 0.40                | 0.32                |
| Potential P         | (mg P₂O₅ 100 g⁻¹) | 13.25               | 38.87                | 31.92               | 35.08               |
| Potential K         | (mg K₂O 100 g⁻¹)  | 147.40              | 179.15               | 196.27              | 202.02              |
| Available P         | ppm              | 195.24              | 384.61               | 461.09              | 387.89              |
| Available K         | ppm              | 150.42              | 367.48               | 371.54              | 208.09              |
| CEC                 | cmol(+1)kg⁻¹     | 28.28               | 53.20                | 22.77               | 32.29               |
| Total P             | %                | 0.15                | 0.19                 | 0.21                | 0.42                |
| Total K             | %                | 0.15                | 0.18                 | 0.18                | 0.20                |

Increased levels of several nutrients in the soil, can be influenced by the presence of *Bacillus aryabhattai* bacteria. Bacillus species are known as plant growth-promoting agents along with *Bacillus aryabhattai*. Total N levels increased from low to moderate after treatment due to the ability of *Bacillus aryabhattai* for nitrogen fixation, which can also help the growth of the stem and root plant [26]. *Bacillus aryabhattai* is also a plant growth-promoting rhizobacteria that helps rhizobium bacteria in nitrogen fixation and increase nutrient uptake by the plant [27].

The potential P content of the initial soil increased from very low to moderate after treatment, as well as the available P and total P content increased after treatment. Besides nitrogen fixation function, *Bacillus aryabhattai* is used as a phosphate solvent due to its ability to convert insoluble P into soluble P form available for plant uptake through the process of releasing organic acids, ion exchange, and chelating [28]. *Bacillus aryabhattai* is a Phosphate Solubilization Bacteria (PSB) which can dissolve insoluble P and release soluble P that increases the available P in the soil [29].

Besides being a phosphate solubilizing bacteria (PSB), *Bacillus aryabhattai* is a potassium-dissolving strain [30]. In table 2, available K is increased with the IAERI’s biopesticide treatment compared to the initial condition and RAISA treatment. PSB can release other nutrients besides P such as N and K that are available in the rhizosphere soil, PSB can increase the amount of N, P, and K available in the soil [31].

4. Conclusions

Application of biopesticide that contain *Bacillus aryabhattai* increased the abundance of the total population of soil bacteria from 2.9 x 10⁴ to 2.68 x 10⁶ cfu mL⁻¹ with IAERI’s biopesticide treatment. In this study, the soil N and P nutrient also increased from low to moderate, (0.19 to 0.4%) and (13.25 to 38.87%) respectively. Application of Biopesticides that contain *Bacillus aryabhattai* increased the abundance of the total population of soil bacteria from 2.9 x 10⁴ to 2.68 x 10⁶. In this study, the soil N and P nutrient also increased from low to moderate, (0.19 to 0.4%) and (13.25 to 38.87%) respectively. The presence of *Bacillus aryabhattai* in biopesticides can improve the soil quality of tidal swamplands in Jeajngkit South Kalimantan and further provides better nutrition for plant growth.
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