Implementation of Bacteria Isolate as a Fertilizer and Bioinsecticide Agent for the Mealybug

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Received: 23 November 2022, Revised: 28 September 2023, Available Online: 28 November 2023

Abstract – This research is about the implementation of bacteria in papaya plantation soil in Kebumen that has been isolated and characterized and purified. This study aims to determine the potential of local isolate bacteria from papaya agricultural soil: 1). as PGPR bacteria or fertilizing bacteria for chili, eggplant and tomato plants and 2) as a natural bioinsecticide. mealybugs on papaya plants. The samples used were bacteria from the preliminary isolation that had been carried out, namely from the genera *Bacillus*, Pseudomonas, Erwinia, Exyguobacterium, Serratia, Stenotropomonas, and Acinetobacter. The study was conducted using 50 stems of each plant. Bioinsecticides are applied to papaya trees that are attacked by mealybugs. Measurement of plant growth was measured at 7 days and 30 days after planting. As for bioinsecticides, spraying was carried out 3 times. The results of the study can be concluded in the form of the implementation of bacteria as PGPR which can increase the growth of cayenne pepper (*Capsicum frutescens*), purple eggplant (*Solanum melongena* L) and tomato (*Solanum lycopersicum* Mill) and can be used as a bioinsecticide to reduce mealybugs (*Paracoccus marginatus*) on papaya plants (*Carica papaya* L.).

Keywords: *PGPR, biofertilizer, bioinsecticide*

INTRODUCTION

Inorganic chemical fertilizers are fertilizers that have been used by farmers for a long time, which ultimately causes a decrease in soil quality. The use of synthetic chemical fertilizers is a factor that causes soil to harden, resulting in reduced soil porosity, low oxygen availability, and the decreased of presence of soil bacteria (Krasilnikov et al., 2022). Chemical residues left in the soil can be transported by plant production and consumed by humans which can harm health (Sharma & Singhvi, 2017). Biofertilizer fertilization can increase soil fertility because microbes can increase fertilization efficiency, fertility, and soil quality. Biofertilizer is a fertilizer that contains various microbes and can increase growth by providing the nutritional needs of plants. Biofertilizers have many advantages, including providing macro elements, not damaging the soil structure or not becoming dense, and not damaging the environment (Zhou et al., 2022). Microbial diversity can influence soil fertility, in other words, the population of microorganisms which is an indicator of soil fertility levels (Maron et al., 2018).

The use of chemical fertilizers in the cultivation of crops can accelerate growth and optimize plant productivity. However, the long-term use of chemical fertilizers also has an impact on soil fertility and the population of soil microorganisms. The implementation of chemical insecticides to control mealybug pests (*Paraccocus marginatus*) using chemical pesticides has proven to be effective and easy to use. On the other hand, dependence on chemical pesticides can cause resistance, and environmental pollution and impact human health (Sabarwal et al., 2018). Therefore, it is necessary to implement an agricultural system that returns to the natural system, namely an organic farming system. The implementation of local rhizobacteria that have the ability to solubilize phosphate, such as the *Bacillus* and *Pseudomonas* groups, plays an important role in maintaining the fertility and diversity of soil microbes. (Aarab et al, 2015). The variety of plant growth-promoting rhizobacteria which have many benefits means that it is necessary to develop promoting plant growth rhizobacter (PGPR).

METHOD

This research was carried out using a quantitative experimental method using a 4 x 3 factorial experiment arranged in a completely randomized design (CRD) with 3 treatment factors and 3 types of test plants with 50 repetitions. Fertilizer and bioinsecticide concentrations with M1 (microbial dose 10^6), M2 (microbial dose 10^8), M3 (microbial dose 10^{10}) and M4 (without microbial application). The formulation for administering the inoculant was carried by two-level dilution of the stock inoculant in NB media (number of microbial cells 10^{10} CFU/ml). Dilution is carried out by adding sterile distilled water. T1 is a chili plant, T2 is an eggplant plant, and T3 is a tomato plant. Fertilization is carried out every week for 3 weeks by spraying 5 ml/plant on the roots. For bioinsecticide treatment, several plants attacked by mealybugs are sprayed once a week, for 3 weeks. The tools used in the research were hoes, measuring tapes, stationery, scales, autoclaves, laboratory glass equipment, inoculation needles, plant sprayers with a capacity of 2 L. The materials used were median bacteria, compost, *Bacillus, Pseudomonas, Erwinia, Exyguobacterium, Serratia, Stenotropomonas, Acinetobacter*, chili seeds, eggplant seeds, tomato seeds, papaya plants, labels, raffia rope, polybags measuring 25 x 40 cm and homogenized soil. The data observed were plant length (cm), number of branches of each plant and leaf width of each plant with a period of 7 days after planting (DAP) and 30 days after planting (cm). Also observe the number of fleas on papaya plants attacked by mealybug pests by spraying them once every 10 days. Data analysis was carried out using one-way ANOVA and a further LSD test was carried out with a significant level of 5%.

RESULT AND DISCUSSION

Collected data were analyzed using the LSD test showed that the three groups of variables received significantly different influences. The *Capsicum frutescens* L. plant group at 7 DAP and 30 DAP treated with microbial fertilizer obtained the highest average leaf width (Table 1).

Table 1 The growth of chili plant after the microbial treatment

Type of fertilizer	7 DAP		30 DAP	
	Leaf width (cm^2)	Stem number	Leaf width (cm^2)	Stem number
PGPR	2.5^a	3^a	3.1^a	3.8^a
Manure	2.1^b	2.5^b	2.6^b	2.9^b
Control	1.5^c	1.5^c	1.8^c	1.8^c

^{a,b,c} Different letters indicate that the test results are significantly different

Fertilizer was applied 3 times with an application period every 7 days. Bacterial inoculation was done by spraying 5 ml/plant of the bacterial liquid culture (10^6, 10^8, 10^{10} CFU/mL) on the planting medium close to the roots. The isolate used in this study was identified as having the ability to produce the phytohormone Indole-Acetic Acid (IAA), phosphate solvent, and nitrogen fixer (data not shown). Microbial fertilizers and manure as biofertilizers contain bacterial inoculants and other microbes that are able to fertilize nutrients in the soil. Microorganisms can increase the activity of breaking down organic materials containing lignin and cellulose in the soil. This activity can produce compounds whose results can increase biomass, reduce disease and weeds, and increase soil fertility and health. These microbes, among others, have a role as agents for phosphate compounds. Phosphate-solubilizing and nitrogen-fixing bacteria can also produce growth regulators such as IAA (*indole-acetic acid*) and gibberellin hormones which can help leaf growth, and tissue development and increase plant biomass (Zhao et al., 2019). Phosphate solubilizing bacteria can also produce organic acid compounds such as citric acid, succinic acid, and glyoxalate which can bind the elements Al, Mg, Ca and Fe so that phosphorus can be dissolved and absorbed by plant roots (Permatasari & Nurhidayati, 2014). According to (Suherman et al., 2018), biological fertilizer has a greater influence on the canopy width and leaf area of chili plants than other treatments.

The large number of branches on a chili plant indicates that the plant has the ability to produce more fruit compared to plants that have few branches (Yahya et al., 2022). Stimulation of the IAA hormone which can increase the number of plant branches is carried out by phosphate solubilizing microorganisms which can produce IAA. The IAA hormone, namely the endogenous auxin hormone, will stimulate plant growth, cell division and branch formation in plants (Walida et al., 2019). In addition, nitrogen-fixing bacteria will bind nitrogen to plants so that plant growth will increase. Nitrogen in the soil plays a role in the formation of chlorophyll and protein synthesis, thus increasing the photosynthesis process causes better plant growth (Suherman et al., 2018).

Table 2. The growth of purple eggplant after the microbial treatment

Type of fertilizer	7 DAP		30 DAP	
	Leaf width (cm^2)	Stem number	Lebar daun (cm)	Leaf width (cm^2)
PGPR	4^a	3^a	3.6^a	5.6^a
Manure	3.8^b	2.5^b	2.6^b	3.9^b
Control	3.2^c	1.5^c	1.5^c	1.6^c

[a,b,c] Different letters indicate that the test results are significantly different

The results of the LSD test analysis on eggplant leaf width variables at 7 DAP and 30 DAT showed that the application of different types of fertilizer showed significantly different effects on eggplant plants (Table 2). The treatment of providing microbial fertilizer resulted in the widest leaf width compared to the application of the other two types of fertilizer.

The wide leaf width of a plant indicates the plant's high photosynthetic ability. The wider the leaf surface, the easier it is for sunlight to enter and be received by the leaf cells. The width of the leaves is influenced by the nutrients absorbed by the plant. (Manuhuttu et al., 2018) explained that plants need nitrogen in the process of vegetative plant growth and produce plants with large leaf areas. Microbial fertilizer and manure contain IAA-producing microbes including *Bacillus* sp., *Pseudomonas* sp. and *Erwinia* sp. (Herlina et al., 2016).

The number of branches on an eggplant plant indicates the plant's ability to produce fruit. Fruit will appear from the resulting lateral branches, while the main stem that grows upwards will continue to produce new leaf shoots. The number of branches on a plant is influenced by the production of the hormones auxin and cytokinin that the plant produces (Muller & Leyser, 2011). Aside from that, plant growth and development are significantly influenced by soil nutrients. Phosphate in the soil in dissolved form (phosphorus) plays a role in the growth of shoots and branches, as well as the maturation of plants (Kim & Li, 2016). The availability of phosphate in plants is influenced by the capabilities of the plant, microbes, and the surrounding environmental conditions. PGPR provides phosphate-solubilizing bacteria that are mineralized and can be absorbed by plants (Kumar & Verma, 2019).

Table 3 The growth of tomato plant after the microbial treatment

Type of fertilizer	7 DAP		30 DAP	
	Leaf width (cm^2)	Stem number	Lebar daun (cm)	Leaf width (cm^2)
PGPR	5.3[a]	27.9[a]	12.8[a]	30.6[a]
Manure	4.2[b]	20.7[b]	10.9[b]	27.1[b]
Control	3.5[c]	15.5[c]	6.9[c]	23.7[c]

[a, b, c] Different letters indicate that the test results are significantly different

The data in Table 3 shows that the main effect of microbial fertilizer application, namely PGPR bacteria (*Bacillus* sp., *Pseudomonas* sp., *Erwinia* sp., *Exyguobacterium* sp., *Serratia* sp., *Stenotropomonas* sp., *Acinetobacter* sp.) is significantly different from the manure and control application treatments.

This growth shows that the application of PGPR bacteria to the soil can provide benefits for the growth of tomato plants. The ability of bacteria to produce phytohormones can increase plant cell development, increase nutrient absorption, especially P and N elements, and increase enzyme activity in plants (Baihaqi et al., 2018). The N element absorbed by the roots is used for overall growth, especially stems, branches, and leaves. Based on this description, plants given PGPR treatment can help plants activate their cells and maintain the rate of photosynthesis so they can produce more leaves and branches compared to manure and control treatments (Backer et al., 2018).

Plant height in the application of PGPR bacteria was greater than in the manure and control treatments. Bacillus bacteria in PGPR can dissolve phosphate so that element P becomes available. *Pseudomonas flourescens* is a PGPR bacterium which also acts as a phosphate solvent which helps mobilize nutrient absorption so that plants are able to obtain P elements easily (Suryatmana et al., 2022). The P elements obtained are then used by plants to support their growth and increase the metabolism and photosynthesis of tomato plants.

Based on the research data obtained, the intensity of mealybug attacks decreased with each observation carried out every three days. The research was carried out by observing 20 papaya trees that were attacked by mealybug pests. The first observation before the application of bioinsecticides, mealybug pest attacks reached 100% in each treatment, while in the last observation of spraying bioinsecticides, mealybug pest attacks decreased.

Bioinsecticides derived from PGPR are biopesticides made from bacteria (*Bacillus* sp., *Pseudomonas* sp., *Erwinia* sp., *Exyguobacterium* sp., *Serratia* sp., *Stenotropomonas* sp., *Acinetobacter* sp.) These bacteria can produce anti-pathogenic compounds or metabolites such as siderophores, B-1,3-glucanase, chitinase, antibiotics and cyanide (Suganthi et al., 2021). These compounds function as biologically effective pest/disease controllers, so they are environmentally friendly. The antipathogenic compounds in bioinsecticides are able to infect mealybugs through natural holes. Chitinase which enters the large intestine of the nematode has the ability to kill the host because it can produce toxins and exoenzymes. This toxin can disrupt the digestive system of mealybugs and can even cause death to the pests.

CONCLUSION

Implementation of bacteria as PGPR which can increase the growth of chili paper (*Capsicum frutescens*), purple eggplant (*Solanum melongena* L) and tomato *(Solanum lycopersicum* Mill) plants and can be used as a bioinsecticide to reduce mealybugs (*Paracoccus marginatus*) on papaya plants (*Carica papaya* L.).

The use of PGPR in the field of plant cultivation needs to be followed up as a potential and commercial organic material. PGPR can be used as a biofertilizer, bioinsecticide and other products. Further research is needed for the use of PGPR as another biotechnology product.

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