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RESEARCH PAPER

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PGPR Indigenous Peat Soil as Seed Biopriming and Bioprotectant in Early Growth of Corn Plant with *Fusarium sp* Inoculation

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Abstract

The mechanisms of PGPR in increasing plant growth include biofertilizers, biostimulants, phytohormones, producing terophores, dissolving phosphates, and bioprotectant agents. Seed biopriming allows bacteria to enter/attach to the seed and is effective in promoting seed emergence and suppressing disease. The purpose of this study was to analyze PGPR indigenous from peat soil as seed biopriming for the initial growth of corn plants inoculated with *Fusarium sp. in vivo*. The study was conducted in the laboratory, using agar media for seeding sweet corn seeds to be organized into a single factor completely randomized design, consisting of 12 treatments and 2 controls, to be repeated 3 times. The results showed that the treatment significantly affected the root dry weight variable and did not differ significantly from the root roasting variable, plumula length, plumula wet weight, plumula dry weight, and root wet weight and *Fusarium* disease attack. The biopriming treatment using rhizobacteria *Bacillus cereus* gave effect to the highest root dry weight, significantly different from the treatment of *Burkholderia cepacia* + *Fusarium sp.* and *Brevibacillus laterosporus* + *Fusarium sp.* Seed biopriming treatment on *Fusarium sp.* disease resistance inoculated on corn seeds showed no significant effect. The use of biopriming contributes to the reduction of inputs of environmentally unfriendly synthetic products.

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Introduction

Plant Growth Promoting Rhizobacteria (PGPR) is a group of bacteria in the root area of plants that can increase plant growth and crop yields. Some of the mechanisms played by PGPR in enhancing plant growth, as a biofertilizer, producing phytohormones, producing siderophores, dissolving phosphates, and as a protectionist agent (Arif *et al.*, 2017; Etesami and Adl, 2020; Li *et al.*, 2020; Hidayati *et al.*, 2022). The application of PGPR on various crops such as barley, chickpeas, canola, cotton, corn, beans, rice, vegetables, and wheat has been shown to have a positive effect on growth and sustainable agriculture (Arif *et al.*, 2017; Chauhan *et al.*, 2015; Ernita, 2015.; Etesami and Adl, 2020; Li *et al.*, 2020; Hidayati *et al.*, 2022). Favorable PGPR can be applied directly to the ground, or applied in plant tissue or through seed inoculation. At the beginning of plant growth, the seed inoculation method is more appropriate. Biopriming of seeds with an inoculum of live bacteria is referred to as involving the application of rhizobacteria to plant growth promoters, increasing the speed and uniformity of germination.

Seed Biopriming is the application of beneficial microbes on the seed by inserting/attaching to the seed. Triggers rapid germination and early growth (Mahmood *et al.*, 2016; O'Callaghan, 2016). Seed Biopriming is effective in promoting seed emergence and suppressing disease. This involves encapsulating microbial inoculants (e.g. biocontrol agents) onto seeds with coating agents (O'Callaghan, 2016; Chin *et al.*, 2022b).

Preliminary research results by Hidayati *et al.* (2022) that PGPR exploration in acidic peat soil found PGPR isolates (*Burkholderia cepacia*, *Pseudomonas luteola*, *Bacillus cereus*, *Bacillus subtilis*, and *Brevibacillus laterosporus*) as potential bioprotectants against *Fusarium sp.*, so it is necessary to research their use as biopriming in germination and early growth of sweet corn seeds because they contain biofertilizers and biostimulants as IAA, GA₃ and HCN. The purpose of this study was to analyze indigenous PGPR as seed biopriming and bioprotectant for the early

growth of corn plants inoculated with *Fusarium sp.*

Materials and methods

The study was carried out at the Phytopathology Laboratory, Universitas Lambung Mangkurat, from December 2021 to January 2022.

Activities include the preparation of tools and planting material, making agar media, seed treatment, seed growers and observation. Tools used include 3 cm diameter test tubes, test tube racks, measuring cups, tweezers, stationery, rulers, analytical scales, and documentation tools. Materials used: agar, aluminum foil, *Fusarium* fungus, bacteria collection from exploration collection of Central Kalimantan peatlands (Hidayati *et al.*, 2022).

This study was organized into a single-factor complete randomized design, consisting of 12 treatments and 10 controls, each of which was repeated 3 times so that there were 42 experimental units. Treatments are as follows:

Ko = positive control

KF = control negatif (*Fusarium*)

P1 = *Pantoea stewartii*

P2 = *Burkholderia cepacia* (TB 4.7)

P3 = *Pseudomonas luteola* (KB 4.3)

P4 = *Bacillus cereus* (TSA 2.1)

P5 = *Bacillus subtilis* (TSA 2.2)

P6 = *Brevibacillus laterosporus* (TSA 4.2)

P7 = *Pantoea stewartii* (TB 4.3) + *Fusarium*

P8 = *Burkholderia cepacia* TB 4.7 + *Fusarium*

P9 = *Pseudomonas luteola* (KB 4.3) + *Fusarium*

P10 = *Bacillus cereus* (TSA 2.1) + *Fusarium*

P11 = *Bacillus subtilis* (TSA 2.2) + *Fusarium*

P12 = *Brevibacillus laterosporus* (TSA 4.2) + *Fusarium*

Execution

Preparation of sterile agar media with a ratio of 6g l⁻¹. The medium is autoclaved for 20 minutes at a pressure of 212 atm. Poured in a test tube with a tube diameter of 3 cm as high as 10 cm and then covered with aluminum foil, and put incubated.

Inoculation

The use of antagonist bacteria with a density of 10^9 CFU ml⁻¹ results in suspension analysis with UV Vis spectrophotometry with a wavelength of 625 nm. *Fusarium* fungus as a pathogen with a density of 10^6 CFU ml⁻¹, calculation of the density with a haemocytometer. Corn seeds are washed 3 times with sterile water, then soaked in the suspension solution for 60 minutes and planted in agar media.

After 24 hours of seeding, inoculation was given *Fusarium sp* according to the treatment. The trick is to drip a suspension of the pathogen on the seed as much as 50µm with a spore density of 10^6 CFU ml⁻¹ Observation variables of this study are as follows:

Explore the roots. Calculated the length of all growing roots, unit cm.

Plumula length. Measured from the base of the plumula to the tip, units of cm.

Wet weight and dry weight of the top/plumula.

Plumula is separated from the root part, then weighed

wet weight. Dry weight is done at 78°C for 24 hours.

Wet weight and dry weight of roots: roots that have been separated, weighed wet weight (grams). Dry weight (gram) is weighed after 78°C temperature ovenization for 24 hours.

Fusarium disease attacks. Observed from the symptoms in the sown seeds.

Statistical analysis

Statistical analysis using software SPSS statistic 17.0. Tukey's honest significance test was used to carry out post-hoc comparisons of differences among means, applying a significance threshold of $p < 0.05$.

Results

The results showed that the treatment of rhizobacteria significantly affected the root dry weight variable and did not significantly affect the root roaming variable, plumula length, plumula wet weight, plumula dry weight, and root wet weight on germination and early growth of sweet corn plants (Table 1).

Table 1. The results of the analysis of variation at the level of 5% and 1%.

Variable	5 DAS	6 DAS	7 DAS
Explore the roots	0.7989 ^{ns}	1.3328 ^{ns}	1.3298 ^{ns}
Plumule length	0.9030 ^{ns}	0.8113 ^{ns}	0.8591 ^{ns}
Wet weight of plumule			0.9692 ^{ns}
Plumule wet weight			1.1339 ^{ns}
Root wet weight			0.7842 ^{ns}
Root dry weight			2.4978 [*]
Plant wet weight			0.9840 ^{ns}
Plant dry weight			1.5097 ^{ns}
Disease Attack			1.0292 ^{ns}

Note: DAS = days after seedling

* = significant, p -value > 0.05 ; ns = non-significant; p -value < 0.05 .

Table 2's average dry weight of roots test results showed that biopriming treatment using *Bacillus cereus* (P4) gave the highest root dry weight compared to other treatments, although only significantly different from the treatment of *Burkholderia cepacia* + *Fusarium* (p8) and *Brevibacillus laterosporus* + *Fusarium* (p12). According to Savini (2016), *Bacillus cereus* showed

diverse biological activities, such as suppression of microbial diseases in plants, also potentially along with potentiation of the insecticidal effectiveness of *Bacillus thuringiensis*. *Bacillus cereus* *Bacillus cereus* is used for Bio self-healing (Hwang *et al.*, 2022). The treatment P3 gives the longest plumula length which is 12.03 cm at 7 days after seedling compared to other treatments (Fig.1).

Table 2. The average value of the dry weight of the roots at the beginning of growth.

Treatment	Dry weight root (g)	Treatment	Dry weight root (g)
K	0.00703 ab	P6	0.01677 ab
F	0.01150 ab	P7	0.01007 ab
P1	0.01340 ab	P8	0.00137 a
P2	0.01390 ab	P9	0.00693 ab
P3	0.01022 ab	P10	0.00367 ab
P4	0.02017 b	P11	0.00787 ab
P5	0.00970 ab	P12	0.00200 a
BNJ 5%	0,01749		

Note: DAS = days after seedling

* = significant, p -value > 0.05; ns = non-significant; p -value < 0.05.

The treatment P4 showed the root variable had longest range of 44,33cm (Fig.2).

The variables wet weight shoots, dry weight shoots, wet weight roots, and dry weight roots in observations

on 7 days after seedling (DAS) (Fig. 3). Fusarium disease causes abnormal and non-growing seeds, as shown in Fig.6. Primary root elongation, lateral root growth, and adventitious root production variabel are shown in Figs.4 and 5.

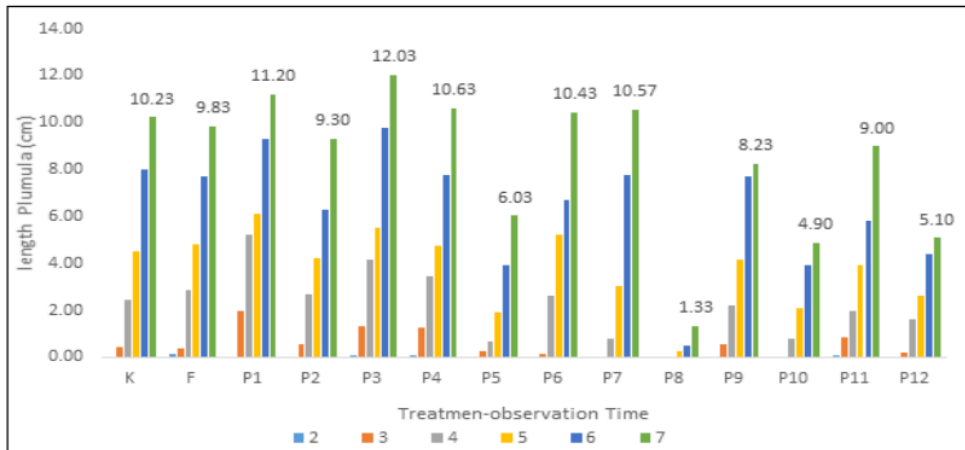


Fig. 1. Growth of plumula length (cm) for 7 days after seedling.

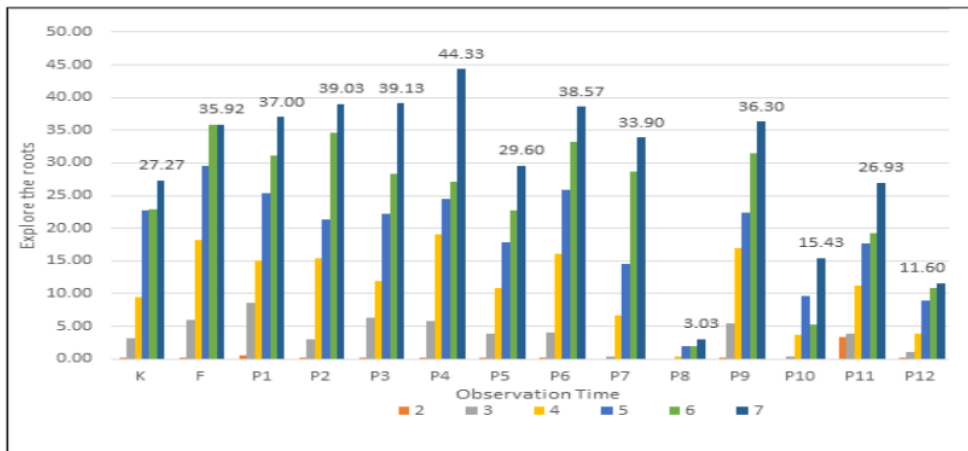


Fig. 2. Root roaming (cm) for 7 days after seedling.

Discussion

Bacterial isolates were obtained from rhizosphere *Zea mays saccharata* in peatland with pH range of 3.0-4.2. This bacterium has been tested in vitro as a potential bioprotectant against corn stem rot disease caused *Fusarium sp* (Prasetyo and Wahyu, 2019; Elfiati *et al.*, 2021; Hidayati *et al.*, 2022; Purwanto *et al.*, 2022). Additionally, this isolate also has the potential as a biofertilizer. These isolates can be employed as biopriming and PGPR for the

development and yield of maize plants in peatlands since they originate from acidic land (extreme land).

For sustainable agricultural production, rhizobacteria-mediated seed bio-priming promotes plant development and resilience. By releasing carboxylates in insoluble P-enriched media (phosphate rock), *Bacillus cereus* has a significant potential for P dissolution and mobilization (Arif *et al.*, 2017).

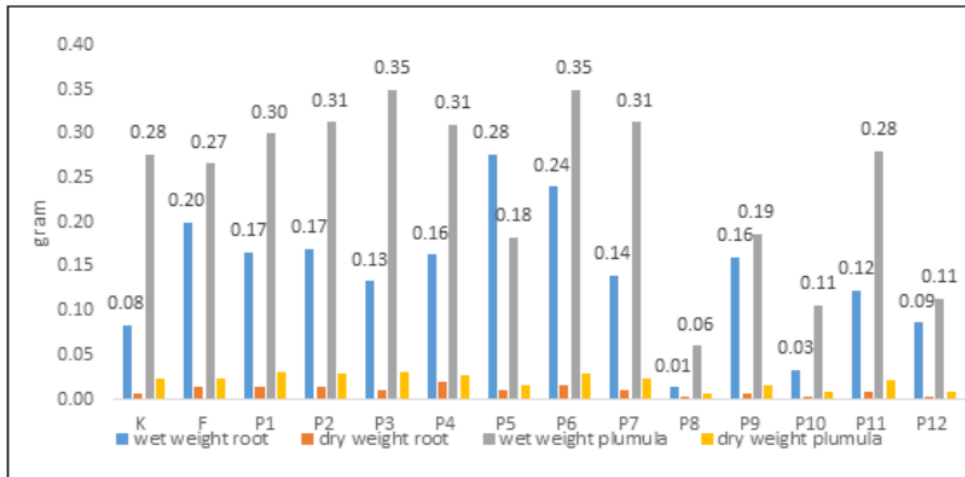


Fig. 3. Wet weight plumula, dry weight plumula, Wet weight roots, and dry weight roots.

The mechanism of increasing plant growth by endophytic bacteria can occur in several ways including folate compounds, nitrogen fixation, stimulating lateral root growth and producing growth hormones such as auxin, ethylene and cytokinin (Thakuria *et al.*, 2004).

Plants meet hormone needs through their ability to synthesize auxin hormones from microorganisms in their tissues.

Hormones IAA, GA3 and siderophore-producing bacteria. The result a counting IAA range of 12,99-29,60 ppm. The highest IAA produced is *Brevibacillus laterosporus* (TSA 42). The amount of IAA content that is relatively the same size causes many variables that are not different. GA3 produced *Pseudomonas luteola* (KB 43) of 0.220 ppm; producing siderophore range of 0.005 – 0.137 *Bacillus cereus* (TSA 21) produced the highest siderophore.



Fig. 4. Germination of corn age 7 days after seedling.

The result research by Larosa *et al.* (2013) showed that from peatland pH 3-5 obtained bacteria that produce the highest IAA 43.311 ppm with the addition

of 300ppm L - Tryptophan. Hormone IAA-producing by bacteria has the potential to combine with some plant physiology by incorporating the IAA they produce into the plant. Plants are more sensitive to changing the concentration of IAA they have. Root for example is one of the most sensitive organs to

fluctuations in IAA and is responsible for increasing the amount of exogenous IAA that is useful for the process of primary root elongation, lateral root formation and adventitious roots (Chauhan *et al.*, 2015). IAA produced by bacteria in plants increases the number of root hairs and lateral roots of plants).



Fig. 5. A. Growth of the plumula and radicle, B. Appearance of root growth.

Biopriming on lombok seeds as a bioprotectant (Chin *et al.*, 2022a; Mitra *et al.*, 2021). Use of *Bacillus subtilis*, *Pseudomonas fluorescense* as biopriming (Suriani *et al.*, 2019; Razad *et al.*, 2021; Prasetyo *et al.*, 2019; Zhao *et al.*, 2022).

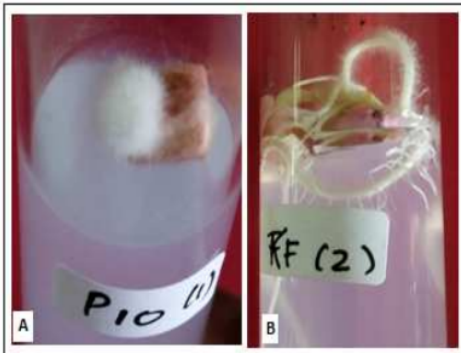


Fig. 6. A. Seeds affected by Fusarium; B. Not growing plumula.

Biopriming methods using PGPR inoculants are becoming more common in modern agriculture as an alternative to chemical treatments. They are more environmentally friendly and safer for future agriculture in addition to improving crop and soil health (Verma *et al.*, 2022).

Conclusion

The results showed that PGPR indigenous treatment as biopriming in the early growth of corn seed had a significant effect on the root dry weight variable and did not differ significantly from the root roaming variable, plumula length, plumula wet weight, plumula dry weight, and root wet weight and *Fusarium* disease attack. Biopriming treatment using rhizobacteria *Bacillus cereus* (P4) gives effect to the highest root dry weight, significantly different from the treatment of *Burkholderia cepacia* + *Fusarium* sp (P8) and *Brevibacillus laterosporus* + *Fusarium* sp (P12). Seed biopriming treatment against *Fusarium* sp. fungal disease resistance inoculated on corn seeds showed no significant effect. The use of biopriming contributes to the reduction of inputs of environmentally unfriendly synthetic products.

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