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Effectiveness of potassium humate and endophytic bacteria from *Serratia marcescens* strain NPKC3_2_21 in increasing root weight of paddy cultivation in acidic soil

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Abstract. *Sutio G, Iskandar, Indriyati LT, Djajakirana G. 2023. Effectiveness of potassium humate and endophytic bacteria from* Serratia marcescens *strain NPKC3_2_21 in increasing root weight of paddy cultivation in acidic soil. Biodiversitas 24: 6316-6322.* Phosphorus (P) deficiency is a significant factor limiting rice agricultural production in acidic soil. The availability of P in acidic soil is generally insufficient because a substantial portion of P is absorbed by Al^{3+} and Fe^{2+}, rendering it inaccessible to plants. This P deficiency in acidic soil hampers proper root growth. However, this limitation can be overcome by the application of potassium humate and endophytic bacteria. The objective of this study was to investigate the effects of phosphate fertilizer with and without potassium humate and/or endophytic bacteria on root growth in acidic fields. The research involved two treatment factors. The first factor comprised four levels of ex-madura phosphate rock fertilizer: 100% (1), 75% (2), 50% (3), 25% (4), and no application of phosphate rock fertilizer (5) and the second factor included potassium humate (A), endophytic bacteria from *Serratia marcescens* strain NPKC3_2_21 (B), a combination of potassium humate and *S. marcescens* endophytic bacteria (C), and the absence of both potassium humate and *S. marcescens* endophytic bacteria (D). The results revealed a positive interaction between phosphate fertilizer, the combination of potassium humate, and *S. marcescens* endophytic bacteria. Therefore, utilizing endophytic bacteria from *S. marcescens* and potassium humate could be an effective strategy for increasing root biomass in rice plants grown in acidic soil with a deficiency in available P.

Keywords: Acidic soil, endophytic bacteria, humic acid, P-solubilization, root biomass

INTRODUCTION

Acidic soil poses a significant challenge to agriculture and crop production in various regions worldwide, including Indonesia. Acidic soil is defined as soil with a pH less than a neutral pH (less than 7) (Cihacek et al. 2021). One of the primary challenges posed by acidic soil is aluminum ion (Al^{3+}) toxicity and phosphorus (P) deficiency (Cihacek et al. 2021). P availability in acidic soil is generally limited because a significant portion of P is absorbed by aluminum (Al^{3+}) and iron (Fe^{2+}) in the form of aluminum phosphate ($AlPO_4$) and iron (II) phosphate ($Fe_3(PO_4)_2$) (Sutio et al. 2023). P nutrients have a substantial impact on the productivity of food crops, such as lowland rice cultivation on acidic soil, where P constitutes 0.2% of the plant's dry weight (Prasad and Chakraborty 2019).

These factors may directly restrict growth and development of below-ground plant parts such as root. The challenges presented by acidic soil, including P deficiency and Al^{3+} toxicity, hinder proper root growth and decomposition due to reduced microbial activity and root decomposability (Wang et al. 2020). High levels of Al^{3+} affect the root development and growth (Cihacek et al. 2021). The initial and most obvious symptom which caused by Al toxicity is inhibition of root growth and injured roots are characteristically stubby with reduced growth of the main axis an inhibited lateral root formation (Thakuria and Hazarika 2016).

This limitation can be overcome by using potassium humate and the endophytic bacteria of *S. marcescens*, which play an important role in protecting plant roots against P deficiency in acidic soil. Potassium humate is commonly utilized as a source of K (Okba et al. 2021) enhances root growth, increases cell division as well as improves productivity (Abdellatif 2017; Aytaç 2022; Dinçsoy and Sönmez 2019). Higher doses of humate increase P availability and reduce the influence of Al^{3+} cations (Thu et al. 2022). In addition to using potassium humate to release P fixed by Al^{3+} cations, employing P-dissolving endophytes can be another solution. One of the endophyte bacteria proven to have beneficial effects on plants is *S. marcescens*. *Serratia marcescens* is one such endophytic bacterium that has an important role in rice cultivation (Sutio et al. 2023).

Several studies reported the benefits of *S. marcescens*. *Serratia marcescens* belongs to the group of Plant Growth Promoting Rhizobacteria (PGPR) and serves as a biological control agent and biofertilizer (Abreo and Altier 2019; Kalayu 2019) due to its ability to solubilize P (Gong et al. 2022). One specific strain of *S. marcescens,* NPKC3_2_21, has been reported to promote plant growth by converting P from an unavailable form into an available form into an accessible form through the production of organic acid compounds that can chelate and stabilize insoluble Al^{3+} and Fe^{2+} cations (Mohamed et al. 2018; Sutio et al. 2023). Additionally, another strain, *S. marcescens* S217, has been associated with the promotion of rice plant growth (Kotoky et al. 2019). Similarly, *Serratia* spp bacteria can also significantly enhance plant growth by facilitating the uptake of nutrients such as P (Kshetri et al. 2019).

Some researchers have reported that humate compounds positively interact with endophytic bacteria regarding plant root growth (Canellas and Olivares 2017; Galambos 2020; Silva 2021). Humic acid can increase the activation of signal transduction, hormone metabolism, transcription, protein metabolism, transportation, defense, and growth-related processes as a response to endophytic bacteria (Galambos 2020). However, the interaction between humate and the specific strain of *S. marcescens* strain NPKC3_2_21, which could solubilize P in acidic soil condition that usually suffers from deficiency P and toxicity Al^{3+}, is still unknown. The comprehensive research regarding the interaction between humate and *S. marcescens* strain NPKC3_2_21 in acidic soil condition is still needed and the result must be evaluated to its own condition.

Therefore, the aim of this study was to determine the effect of potassium humate and endophytic bacteria *S. marcescens* strain NPKC3_2_21 on root biomass of rice plant in acidic soil. Thus, these treatments could serve as cost-effective and environmentally friendly technologies to enhance rice plant productivity in acidic soil.

MATERIALS AND METHODS

Study area

The research was conducted on a 2000 m² plot of land in Kolam Kanan Village, Barambai District, Barito Kuala Regency, South Kalimantan Province, Indonesia, at coordinates 3°01'13.2" S latitude and 114°41'28.2" E longitude. This location was characterized by an acidic rice field with a pH level of 4.0. The experiment was carried out from April to August 2023.

Methods

The experiment was conducted in a split-block design with two treatment factors: the first factor involved the application of ex Madura phosphate rock fertilizer at varying levels, and the second factor involved the use of potassium humate and/or *S. marcescens* strain NPKC3_2_21 bacteria. There were a total of 20 treatments, each with three replicates, resulting in a total of 60 treatment combinations. The first factor consisted of four levels of ex Madura phosphate rock fertilizer: 100% (1), 75% (2), 50% (3), 25% (4), and no phosphate rock fertilizer application (5), corresponding to 400 kg/ha, 300 kg/ha, 200 kg/ha, 100 kg/ha, and 0 kg/ha. The second factor included the application of potassium humate (A), *S. marcescens* strain NPKC3_2_21 endophytic bacteria (B), a combination of potassium humate and *S. marcescens* strain NPKC3_2_21 endophytic bacteria (C), and the absence of both potassium humate and *S. marcescens* strain NPKC3_2_21 endophytic bacteria (D).

The experimental unit was a map made of boards coated with black mulch plastic with a size of 4 x 4 m. The area for one experimental plot was 16 m^2 with a plot spacing of 4 m. The planting distance used was 20 x 20 cm, with the number of seedlings planted three seedlings/planting hole. An experimental plot contained 324 plants. The number of sample plants per experimental plot was 17 plants. There was a trench with a size of 1 m and a depth 30 cm as barrier between the plots. Each plot had water outlets and inlets.

The stages of acidic rice field cultivation began with the application of systemic herbicide 55 days before planting, followed by two rounds of plowing the land at 50 and 25 days before planting. Experimental plots were established 20 days before planting, and ex Madura phosphate rock fertilizer was applied four days prior to planting. Seed preparation involved soaking the seeds for 20-22 days before planting, fertilizing the seedbed 7-10 days after sowing, and transplanting the seeds at 21-25 days after planting (DAP). The first fertilization performed at one DAP, with seed replanting for non-germinating or snail-eaten seeds taking place seven DAP. The second fertilization was conducted at 20-21 DAP, while the third performed at 42-43 DAP. Pest control involved the application of synthetic insecticide (active ingredients: chlorantraniliprole) at 21, 28, and 35 DAP, and biological fungicides (*Metarhizium anisopliae*, *Beauveria bassiana, Streptomyces thermovulgaris, Geobacillus thermocatenulatus*) application at 25, 30, 40, 55, 70, 73, 76, 79, and 82 DAP.

Observational parameters

Root weight samples were collected at 125 DAP from one plant located in the same row and column per plot. These roots were carefully dug to a depth of 30 cm, washed clean, and their fresh weight was measured. To determine dry root weight, the roots were dried in a drying house at a temperature of 52°C for 72 hours and weighed to achieve the same water content.

Data analysis

Data analysis was performed using the SMARTSTATXL application, including variance analysis (ANOVA) and Pearson correlation. Mean values were compared using the honest significant difference calculations or the Tukey test at a significance level of $p<0.05$. An F-test was employed at a 95% confidence interval for further analysis.

RESULTS AND DISCUSSION

The results of the field test, assessing the effectiveness of potassium humate and *S. marcescens* strain NPKC3_2_21 endophytic bacteria through the administration of various levels of phosphate rock doses on fresh root weight in acidic soil are presented in Table 1.

Based on the results of the variance analysis, it was observed that an interaction existed between phosphate rock dosage and application of potassium humate and *S. marcescens* strain NPKC3_2_21 on the fresh root weight of rice plants cultivated in acidic soil. This interaction is evident through the calculated F-value, which exceeds the critical F-table value of 0.05, signifying statistical significance at the 5% significance level.

Figure 1 shows that the most effective treatment combination for achieving maximum root fresh weight was using both potassium humate and *S. marcescens* strain NPKC3_2_21, without the addition of phosphate rock fertilizer (5C).

Furthermore, the results of variance analysis showed a significant interaction between phosphate rock dosage, humate application and the presence of *S. marcescens* strain NPKC3_2_21 on root dry weight in acidic soil rice cultivation (Table 2). The calculated F-value surpassed the critical F-table value of 0.05, signifying statistical significance at a 5% significance level.

This result showed that the most effective treatment combination to increase the dry weight of rice plant roots involved the application of potassium humate and endophytic bacteria, without the addition of phosphate rock fertilizer (5C) (Figure 2). The interactive effects of humate and *S. marcescens* strain NPKC3_2_21 on root weight in acidic soil are presented in Figures 1 and 2. It was observed that concurrent use of humate and *S. marcescens* strain NPKC3_2_21, without the addition of phosphate rock significantly improved both the dry and fresh weights of rice plant roots under acidic soil conditions (5C). This treatment outperformed control and single treatment options. Notably, providing phosphate rock fertilizer at varying levels had no significant impact on the fresh or dry weights of the roots. The treatment 5C (10 kg potassium humate + 1 kg *S. marcescens* endophytic bacteria + without phosphate rock) emerged as the most effective approach for increasing rice plant root weight. The interaction between application of humate and *S. marcescens* endophytic bacteria did not yield a significant difference in root weight parameters among treatments.

Despite the absence of statistical significance, humate application in the 5C treatment resulted in better fresh and dry root weights compared to other treatments. The simultaneous application of humate and endophytic bacteria in 5C treatment increased the average fresh weight of roots by 888 grams, compared to only 480 g in the control (Figure 1). Similarly, the average dry weight of roots in the 5C treatment was 545 grams, whereas in the control it was only 253 grams (Figure 2). This suggests that application of potassium humate alongside *S. marcescens* endophytic bacteria, without the addition of phosphate, significantly increased root biomass. An increase in root weight values indicated the development of well-developed roots, exemplified by an increase in root weight.

At 90 days after sowing, the roots of rice plants in all treatments were cut carefully and cleaned from soil between the roots. Figure 3 shows that the root system was significantly affected by acidity and P deficiency and showed negative structural changes in control (5D), while acid-stressed plant without applying potassium humate and *S. marcescens* strain NPKC3_2_21 (D treatments) showed more dwarf and shorter roots than other treatments. Phosphate rock fertilizer dose showed a nonsignificant effect on all root morphology parameters. However, potassium humate and *S. marcescens* strain NPKC3_2_21 showed a positive result without adding P fertilizers by significantly increased root volume (Figure 3) and root biomass (Figures 1 and 2).

Table 1. Variance analysis of fresh root weight

Variance analysis	DF	SS	MS	F-Count	P-Value	F-0.05	F-0.01
Repetition (U)	2	72736.2871	36368.1436	0.903 tn	0.443	4.459	8.649
Phosphate rock dose (D)	4	256771.3330	64192.8333	1.593 tn	0.266	3.838	7.006
Error a	8	322305.7371	40288.2171				
Treatment (P)	3	109851.6718	36617.2239	1.992 tn	0.136	2.922	4.510
D x P	12	513937.0183	42828.0849	2.330 *	0.030	2.092	2.843
Error b	30	551416.2838	18380.5428				
Total	59	1827018.3311					

Note: * = real difference at level of 5%; tn = no real difference

Table 2. Variance analysis of root dry weight

Variance analysis	DF	SS	MS	F-Count	P-Value	F-0.05	F-0.01
Repetition (U)	2	7042.3074	3521.1537	0.236 tn	0.795	4.459	8.649
Phosphate rock dose (D)	4	144226.8020	36056.7005	2.414 tn	0.134	3.838	7.006
Error a	8	119510.7670	14938.8459				
Treatment (P)	3	49704.3488	16568.1163	1.822 tn	0.164	2.922	4.510
D x P	12	286853.9116	23904.4926	2.629 *	0.016	2.092	2.843
Error b	30	272775.0446	9092.5015				
Total	59	880113.1814					

Note: * = real difference at level of 5%; tn = no real difference

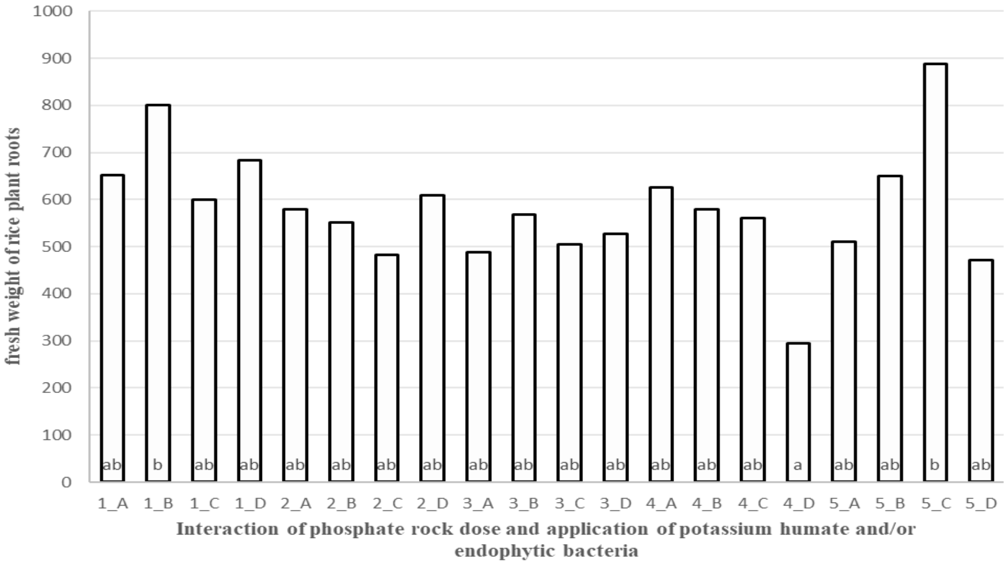

Figure 1. Interaction of phosphate rock dose and application of potassium humate/*S. marcescens* strain NPKC3_2_21 on the fresh weight of rice plant roots

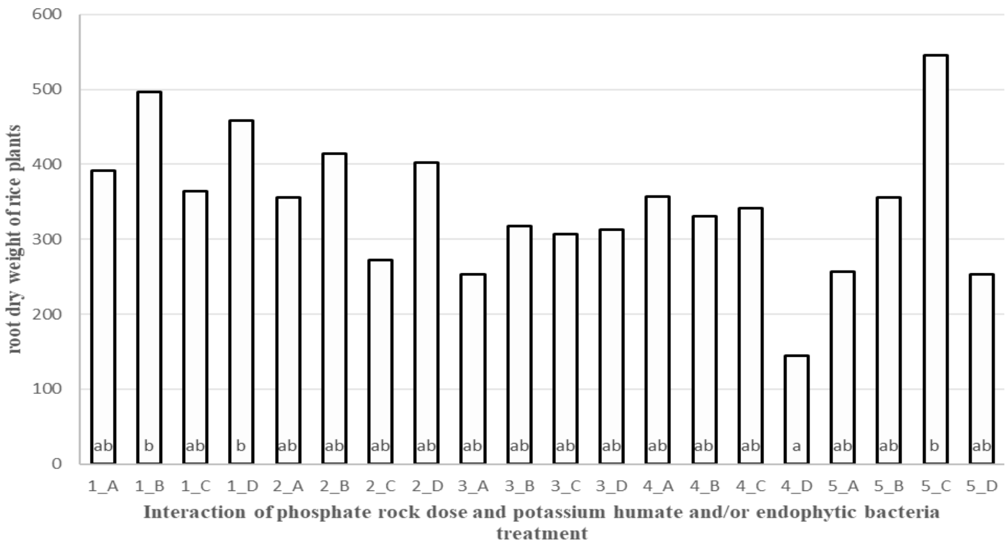

Figure 2. Interaction of phosphate rock dose and potassium humate/*S. marcescens* strain NPKC3_2_21 treatment on the dry root weight of rice plants

Discussion

In the last decades, root biomass was adopted for assessing plant growth and was considered a sensitive growth parameter and indicator in plant stress physiology. The challenges of acidic soils, including P deficiency and Al^{3+} toxicity, impair plant root growth. The limitation can be overcome by using potassium humate and endophytic bacteria of *S. marcescens* strain NPKC3_2_21, which are essential in protecting plant roots against P deficiency in acidic soil and also affects the root biomass of rice plants. Humate and endophytic bacteria offer distinct advantages and benefits for plant roots in acidic soil. Results of the present study revealed that the most effective treatment combination was simultaneously applying humate and *S. marcescens* endophytic bacteria, even without adding phosphate rock fertilizer. In this regard, 5C treatment revealed root biomass at 888 grams of fresh-weight roots and 545 grams dry weight of dry-weight roots, respectively (p <0.005), compared to the control. These findings indicate that potassium humate and the endophytic bacteria of *S. marcescens* strain NPKC3_2_21 promote investment in root biomass under acid-stress conditions. At the same time, P fertilizer had no significant effect on increasing root biomass to all treatments.

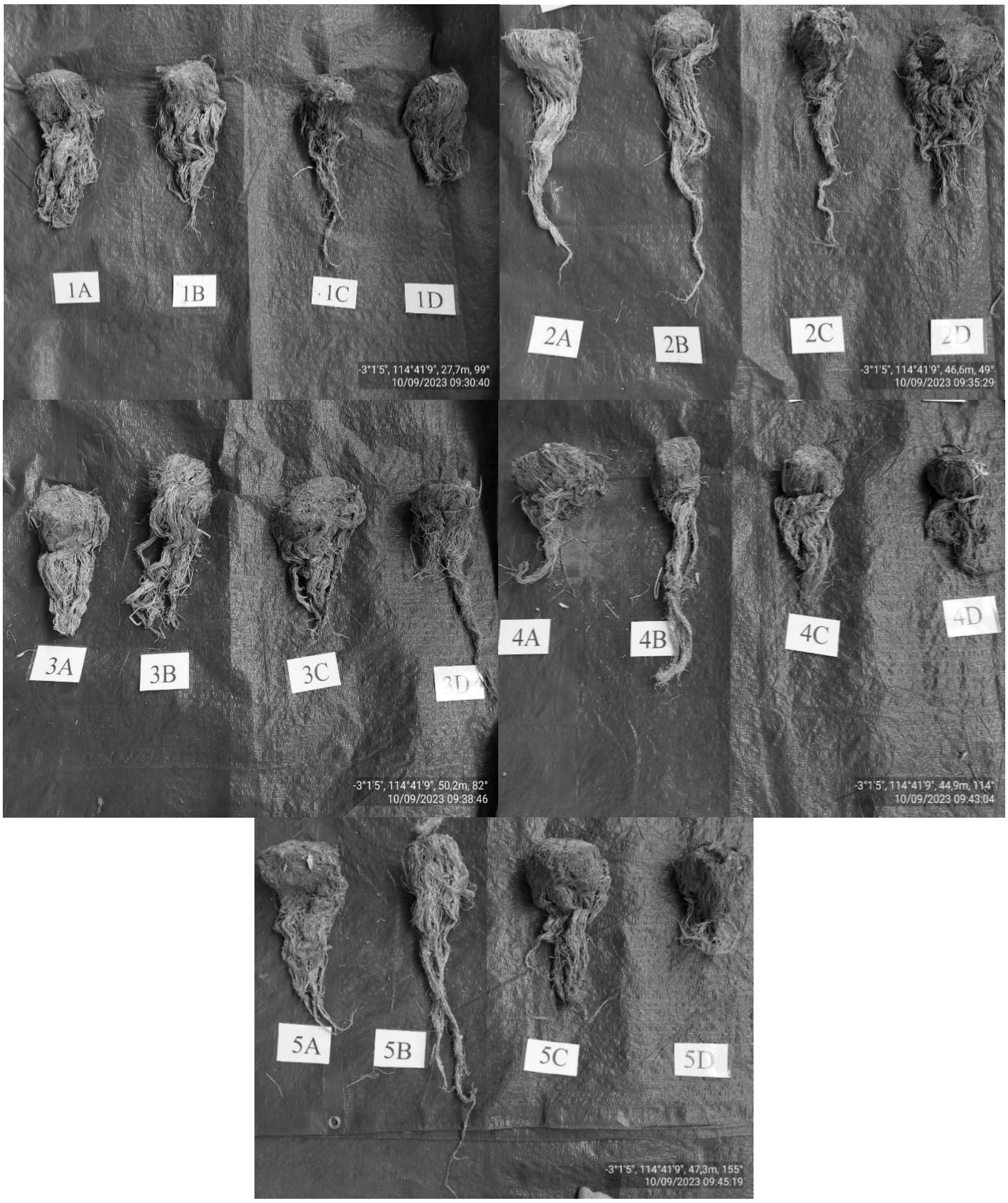

Figure 3. Condition of rice plant roots in various treatment combinations

Results revealed that humate compounds positively interact with *S. marcescens* strain NPKC3_2_21 endophytic bacteria. Simbiosis between humate and *S. marcescens* endophytic bacteria confers acid tolerance in rice plant effect in roots. This interaction might occur directly or indirectly via several mechanisms causing the promotion of plant root biomass. A connection exists between the influence of humate on the development and morphology of rice plant roots and the identification of bacterial taxa selected under these conditions. The synergy between humate and microbiota benefits plants, in this case, *S. marcescens* strain NPKC3_2_21, since both can modulate the root physiological processes. This aligns with the opinion of Silva et al. (2021), who stated that the advantages of humate may stem from its direct impact on plant metabolism or through the stimulation of plant microbiota.

In the field, humate compounds offer numerous benefits to soil. Humate can enhance soil microbial activity and biological soil functions (Lumactud et al. 2022), improve soil structure (Li 2020), increase yield components and nutrient uptake (Thu et al. 2022), and boost the supply of essential nutrients while facilitating the assimilation of atmospheric CO2 (Tiwari et al. 2023). Several studies have reported positive effects of humic acid on root development, root length, root thickness, length, branching, and density, thus altering root architecture under abiotic stress (Abdelsalam 2017; Aytaç 2022; Canellas and

Olivares 2017; Li et al. 2019; Rahmi and Satriawan 2022; Shah et al. 2018; Tavares et al. 2020; Wang et al. 2020; Yang et al. 2023; Zhang et al. 2020).

Furthermore, aside from humate application, the role of endophytic bacteria was equally significant. Several microbes have the capacity to solubilize insoluble inorganic phosphorus compounds and make them available for plant uptake. *Serratia marcescens* NPKC3_2_21 has been reported to enhance plant growth by converting P from an unavailable form to an accessible form by producing organic acid compounds in making P nutrients available to the roots (Sutio et al. 2023). Several other researchers have also reported the positive effects of *Serratia marcescens* regarding its ability to promote plant growth and enhance root architecture (Abreo and Altier 2019; Jupatanakul et al. 2020; Kshetri et al. 2019; Li et al. 2022; Niu 2022; Sutio et al. 2023; Verma et al. 2021).

However, this research not indicates an interaction between the phosphate rock dosage and the treatment involving humate and *S. marcescens* endophytic bacteria mixture. Applying phosphate rock at any dosage did not significantly affect the optimization of root fresh weight and dry weight increase parameters. This interaction may be related to the possibility that the amount of total phosphorus (P-total) in acidic soil, already categorized as high, was predominantly absorbed by aluminum (Al^{3+}). Consequently, providing additional phosphate fertilizer at any dosage to acidic soil no longer impacted because the ground's P-total elements were already in the high category. P-total becomes functional only when it interacts with endophytic bacteria and potassium humate, making phosphorus available. According to Drewry et al. (2022), soil with optimal nutrient levels does not require additional fertilizer. Additionally, the type of fertilizer can influence phosphorus absorption in the ground, with phosphate rock fertilizer being less soluble in water than other types, such as SP36 or NPK.

In conclusion, the root growth promotion in rice plants cultivated in acidic soil was influenced by the application of endophytic bacteria of *S. marcescens* strain NPKC3_2_21 and humate. This interaction yielded a positive outcome, leading to a significant increase in root weight. The result showed better fresh and dry root weights compared to other treatments. The simultaneous application of humate and endophytic bacteria in the 5C treatment increases the average fresh weight of roots by 888 grams and the average dry weight of roots by 545 grams. In contrast, in control, the average fresh weight of roots and the average dry weight of roots were 480 and 253 grams, respectively. This presence of humate and endophytic bacteria of *S. marcescens* strain NPKC3_2_21 facilitated phosphorus availability in the acid soil and affected the root biomass of the rice plant. Farmers could use this strategy to understand the complementation effects of the interaction of endophytic bacteria *S. marcescens* strain NPKC3_2_21 and humate to further develop sustainable biofertilizers for rice production in acidic stress conditions and can mitigate the effects of acid stress on rice plant roots. More studies in the future are needed to develop a better understanding that gives an input in agricultural systems.

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