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PHYTOBENEFICIAL TRAITS AND GROWTH PROMOTION OF SSR IN RICE SHEATH BLIGHT CONTROL

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ABSTRACT

The destructive impact of Rhizoctonia solani, the disease's causal agent, on rice harvests necessitates strategies for long-term management of the illness. In this work, SSR bacteria were taken from the rice rhizosphere in Besut, Malaysia, and their biocontrol and growth-promoting activities were examined. In vitro experiments were used to screen for phytobeneficial characteristics in the first round of 31 SSR isolates. Some of these characteristics were phosphate solubilisation, volatile chemicals, hydrogen cyanide, indole-3-acetic acid, and the capacity to create diffusible antibiotics and extracellular metabolites. Thereafter, eight potent isolates were subjected to a greenhouse experiment with MR219 rice at UMT. After being infected with R. solani and treated with SSR, they were evaluated for disease severity and traits related to plant growth. Disease incidence and susceptibility were shown to be significantly reduced by SSR treatments, as proven by statistical analysis employing ANOVA and Duncan's test ($p < 0.05$) with the lowest incidence seen in isolate SSR24 (2016). Improved plant development was aided by SSR treatment-induced increases in phosphate availability and IAA production. Their detection brought attention to the potential of the selected isolates, Serratia marcescens and Pseudomonas aeruginosa, as biofertilizers that are harmless to the environment. These findings should pave the way for more environmentally friendly ways of cultivating rice in further research into optimising SSR formulations and evaluating their efficacy in the field.

Keywords: *Rice sheath blight, Rhizoctonia solani etc.*

INTRODUCTION

As a main source of nutrition, rice (Oryza sativa L.) is essential for about half of the world's population. Nevertheless, biotic and abiotic stressors might occasionally restrict its production. Rhizoctonia solani causes rice sheath blight, which is one of the most economically significant fungal diseases impacting rice production globally. Because it hinders tillering, reduces grain filling, and weakens plants generally, the disease causes production losses of 20% to 50% under ideal circumstances. Sustainable and environmentally friendly disease control strategies are sought after due to growing worries about pathogen resistance, soil health degradation, and environmental safety. For a long time, the standard method of controlling R. solani infections has been the use of chemical fungicides. One potential strategy involves making use of beneficial microorganisms, specifically silicon-solubilizing rhizobacteria (SSR), to reduce pathogenic infections and boost plant growth. Many people are interested in sustainable agriculture because of the many ways helpful bacteria may help. These include solubilising nutrients, producing phytohormones, causing systemic resistance (ISR), and directly attacking plant diseases. Plants become more resistant to fungal diseases like sheath blight and other structural defences when soil silicon availability is increased by microorganisms that dissolve silicon in soil.

Rhizoctonia solani diseases/rice sheath blight

During tillering and flowering, the soil-borne necrotrophic fungus Rhizoctonia solani infects rice plants. Therapy becomes more complicated due to the fact that disease-carrying sclerotia dwell soil and plant waste. A wet lesion on the underside of leaves is the most common symptom of sheath blight. Significant lodging, insufficient grain filling, and lesion growth/merging-related output losses occur. R. solani cannot be effectively controlled with conventional medicines alone because of its wide host range and incredible adaptability. Chemical fungicides, cultural methods, and resistant rice types are some of the modern methods used to manage sheath blight. There are valid concerns about the misuse of fungicides for human and environmental health, and the efficiency of resistance breeding has been greatly hindered by resistance's polygenic nature. Biological management approaches, such as plant growth-promoting rhizobacteria (PGPR), have gained popularity as a means to manage R. solani infections and enhance crop yield.

Why Silicon Is Necessary for Plant Protection

Among silicon's many beneficial uses is fortifying plant defence mechanisms. Although silicon isn't strictly necessary for plant growth, studies have demonstrated that plants that store silicon in their tissues are better able to withstand stress, have higher mechanical strength, and are less susceptible to fungal infections. The rice plant builds a physical defence against dangerous illnesses by accumulating monosilicic acid [$Si(OH)_4$] in its epidermal layers, which it then utilises to cover its leaves, stems, and roots. Some research suggests that silicon may inhibit fungal colonisation and disease progression by activating metabolic defences. Plant defences include phytoalexins, phenolic chemicals, and PR proteins that are involved in disease. Although soil silicon has several applications, its insoluble forms make it not always easily accessible. By converting insoluble silicate minerals into forms that plants can utilise, silicon-solubilizing rhizobacteria (SSR) substantially enhance plants' nutrient absorption and disease resistance. Because SSR can boost silicon bioavailability, it benefits plant health, R. solani resistance, and sustainable agriculture.

Silicon-Solubilizing Microbes in Bromelain

One kind of PGPR is SSR, which may solubilise silicate minerals and improve the availability of silicon. There are a number of ways in which these microbes boost plant health. Organic acids such gluconic acid, citric acid, and 2-keto-gluconic acid are released by SSR during silicate solubilisation, an important process. Plants can make use of monosilicic acid, a byproduct of these acids' breakdown of insoluble silicates. The increased absorption of silicon fortifies plant cell walls and enhances resistance to fungal infections. In addition to antibiotics, SSR produces a variety of volatile and non-volatile antifungal compounds, including as siderophores, hydrogen cyanide (HCN), and others. By lowering mycelial development, inhibiting pathogen colonisation, and interfering with fungal metabolism, these chemicals make R. solani grow slower. By activating defense-related gene expression pathways dependent on salicylic acid (SA) and jasmonic acid (JA), these bacteria play a substantial role in inducing systemic resistance (ISR) in rice plants. This allows plants to react swiftly when faced with disease threats. Plant resistance to sheath blight is enhanced by better root architecture and increased water and nutrient absorption. SSR enhances plant nutrition by solubilising phosphate and generating indole-3-acetic acid (IAA), a vital phytohormone involved in root elongation and nutrient uptake. At last, SSR invades the rhizosphere and root surfaces to outcompete R. solani for vital nutrients and ecological niches, which aids in lowering pathogen establishment and illnesses in plants in general.

How Effectively SSR Controls R. solani and Promotes Plant Growth

Several investigations have shown that SSR can improve rice sheath blight and increase plant development. It is probable that the generation of volatile and diffusible metabolites is responsible for the significant antifungal activity of some SSR isolates against R. solani in dual culture plate investigations. When comparing biomass accumulation and disease incidence, greenhouse studies have also demonstrated that SSR-treated rice plants outperform their untreated counterparts. The effectiveness of Serratia marcescens and Pseudomonas aeruginosa isolates as SSRs in inhibiting R. solani was recently proven in a research. These isolates did more than just stop fungal growth; they also made plants healthier by increasing their vitality via phosphate solubilisation, silicon absorption, and IAA synthesis. Due to its capacity to both boost growth and suppress infections, SSR might be a viable alternative to chemical fungicides in sustainable rice agriculture.

REVIEW OF LITERATURE

Fatemeh Abdi et.al. (2020) Rice fields in northern Iran struggle due to a lack of easily available, highly soluble phosphorus. The effects of silicon fertiliser and plant-growing microbes in 2017 and 2018 in Amol split plots may be studied using a randomised complete block design (RCBD) with three replications. Thank you for your assistance. Soil spraying with nano-silicon, potassium, and calcium silicates, as well as a control group, made up the bulk of the experiment. A 100 kg control plot, a 50 kg plot with Herbaspirillum seropodicae bacteria, and a 50 kg plot containing mycorrhiza fungus (Gholusmosseae) were the three subplots that made up the experiment. The control group exhibited longer internodes lodging index (L4LI), greater PY, and lower grain filling time compared to the silicon resource group. The combination of phosphorus and bacteria resulted in an optimal I3LI of 49.54% and an I4LI of 52.36%. The largest Y-value achieved was 4542 kg ha-1 with a chemical phosphorus and nano-Si spray of 4670 kg ha-1. Spraying with nano-silicon and using microorganisms that contain phosphorus enhances nutrient absorption. To increase rice yields and decrease blast disease, spray nano-silicon and add microbes to chemical fertiliser.

Doni et al. (2019) It was found that microbial inoculants are vital components of sustainable intensification because they aid to enhance plant health in addition to enhancing plant output. This was determined via research. There is a possibility that the use of these methods might meet the requirements for rice production, which would also contribute to the maintenance of environmental stability.

David et al. (2018) Evidence suggests that Pseudomonas fluorescens can act as a biological control agent. To do this, secondary metabolites and enzymes are produced, which effectively stunt the development of pests. The bacteria serve several purposes for the plant, including improving nutrient absorption, promoting overall health, and protecting it from pests.

Yong-qiang HAN et.al. (2018) Pests destroy rice, a global staple. Silicon (Si) makes rice more pest-resistant in controlled circumstances, but field research are scarce. In early- and late-season rice plots with 0, 75, 150, and 300 kg $SiO2$ ha^{-1}, the effects of silicon supplementation on insect pests, illnesses, and rice yield were investigated. Compared to control plots, plants treated with 300 kg SiO2 ha^{-1} demonstrated improved control of stem borer, leaf folder, and planthopper in three to five monitoring observations each season. Though it did not protect against late-season or rice sheath blight, experimental plots treated with 300 kg SiO2 ha^{-1} shown a reduction in the early-season rice blast disease index when contrasted with control plots. The control plots yielded 604 kg ha^{-1} of rice, whereas those treated with 300 kg SiO2 increased it by 16.4%. Our field study indicates that adding 300 kg SiO2 ha^{-1} can significantly minimise insect damage to rice. These findings suggest silicon amendment should be an important component of rice integrated pest control.

Dhir (2017)'s Biofertilizers such as Azospirillum and Trichoderma spp. can reduce the incidence of root-knot nematodes and leafhoppers, according to study on rice. These fertilisers improve plant health and promote naturally resistant crops. In addition to maintaining healthy soil, biofertilizers provide a balanced microbial habitat, which can help prevent the growth of pests and illnesses. By making crops more resistant to pesticides and reducing the need for chemical inputs, this helps with sustainable agriculture by strengthening pest resistance through integrated pest management.

OBJECTIVES OF THE STUDY

Following are the main Objective of this study:

1. To assess *Rhizoctonia solani's* impact on rice sheath blight and its management.
2. To evaluate the biocontrol potential of Silicon-Solubilizing Rhizobacteria (SSR) against plant pathogens.

HYPOTHESIS

Following are the main hypothesis of this study:

H_1: There is a significant impact of Rhizoctonia solani on the severity and management of rice sheath blight.

H_2: There is a significant biocontrol potential of Silicon-Solubilizing Rhizobacteria (SSR) against plant pathogens, including Rhizoctonia solani.

RESEARCH METHODOLOGY

Researchers in Besut, Malaysia, searched the rhizosphere soil for silicon-solubilizing rhizobacteria (SSR). After assessing the phytobeneficial characteristics of 31 isolates, 8 were advanced to the next stage of investigation. Researchers at UMT used a greenhouse experiment to look into how SSR affected MR219 rice. We evaluated the intensity of the illness, the rate of plant development, and the ability to identify isolates using the VITEK 2 system. The study employed an ANOVA analysis with SPSS ($p \leq 0.05$) and a randomised block design.

RESULTS

Hypothesis testing:

Hypothesis	**Null Hypothesis (H_0)**	**Alternative Hypothesis (H_1)**	**Statistical Test**	**Expected Outcome**
H_1: There is a significant impact of *Rhizoctonia solani* on the severity and management of rice sheath blight.	H_{01}: *Rhizoctonia solani* has no significant impact on the severity and management of rice sheath blight.	H_{11}: *Rhizoctonia solani* significantly affects the severity and management of rice sheath blight.	ANOVA / t-test	Significant difference in disease severity across treatments.
H_2: There is a significant biocontrol potential of Silicon-Solubilizing Rhizobacteria (SSR) against plant pathogens, including *Rhizoctonia solani*.	H_{02}: SSR has no significant biocontrol effect against *Rhizoctonia solani* or other pathogens.	H_{12}: SSR exhibits significant biocontrol activity against *Rhizoctonia solani* and other pathogens.	ANOVA / t-test	Reduction in disease incidence and pathogen growth with SSR application.

The hypothesis testing table evaluates the biocontrol potential of Silicon-Solubilizing Rhizobacteria (SSR) and the influence of Rhizoctonia solani on the severity and management of rice sheath blight. Next, we will begin by testing the alternative hypothesis (H_{11}), which proposes that R. solani does, in fact, have a notable influence on sickness severity and management, against the null hypothesis (H_{01}) that it does not. Using t-tests or analysis of variance, we may determine if there is a statistically significant difference in the severity of the condition between treatments. The second hypothesis (H_2) assesses the biocontrol capabilities of SSR against various diseases, given that H_0 states that SSR has no discernible effect and H_1 states that SSR effectively suppresses R. solani and other plant infections. By lowering disease incidence and pathogen growth, statistical analysis should prove that SSR is efficient in biological disease control.

Bacterial Isolation and Soil Sampling

A mixture of diluted Rhizosphere soil from Besut, Terengganu, Malaysia, and soil grown on a magnesium trisilicate medium at 28±2°C for 24-36 hours was used to create the soil. The presence of halo zones allowed the identification of 31 isolates as silicon-solubilizing rhizobacteria (SSR).

Phytobeneficial Property Screening in a controlled environment

To assess the fungal inhibition, we used a contrast-culturist to incubate R. solani agar plugs on PDA plates containing SSR isolates for 5 days at a temperature of 28±2°C. We placed sealed SSR suspensions in NA and R. solani cultures in PDA and incubated them for 5 days to produce volatile chemicals. We used PIRG to measure growth inhibition.

$$\text{Inhibition in radial growth (\%)} = \frac{R1 - R2}{R1} \times 100\%$$

At 625 nm, the picrate test, which quantifies HCN production, undergoes a colour shift to a reddish-brown shade. To confirm that IAA was formed, Salkowski reagent was employed, which was detected at 530 nm. Through phosphate solubilisation studies, There were five very effective SSR isolates that were found by seeing a notable reduction in R. solani growth: SSR2, SSR13, SSR24, SSR25, and SSR26.

$$\text{Inhibition (\%)} = 1 - \left(\frac{\text{Treatment growth}}{\text{Control growth}}\right) \times 100\%$$

In NBRIP medium, the solubilisation of phosphate was assessed. Eight SSR were selected for their inhibitory and growth-promoting properties. Using tests for antibiotic diffusibility and extracellular metabolite production, we were able to identify five potent R. solani SSR inhibitors.

$$\text{Inhibition (\%)} = 1 - \left(\frac{\text{Treatment growth}}{\text{Control growth}}\right) \times 100\%$$

In a greenhouse experiment carried out at UMT, SSR inoculants were studied on MR219 rice. Prior to planting, the sterilised seedlings were immersed in SSR suspensions; distilled water was used as a control. Inoculation with R. solani was done twice: first at the pre-inoculation stage and again at the four-leaf stage. We checked the severity of the sickness and the plants' progress after a week.

$$\text{Disease incidence (\%)} = \frac{\text{Total No. of infected plants}}{\text{Total No. of plants}} \times 100$$

Table 1: Disease severity scale for rice sheath blight

Disease Scales	Disease Symptoms
0	Absolutely no lesion
1	Showing signs of soggy lesions
2	Surface necrosis developing on the leaf
3	Damage to the leaf area of less than 50%
4	A leaf area necrosis of more than 50%
5	Complete leaf section necrosis and leaf death

$$\text{Disease susceptibility index (DSI\%)} = \sum \frac{\text{No. of plant in the specific scale} \times \text{disease scale}}{\text{Total No. of plants}} \times 100$$

In order to evaluate the growth characteristics of the rice plants, we measured their height thirty days after planting. Using VITEK 2 technology, a number of SSR isolates were detected. Every experiment was conducted in a random order and assessed using SPSS using ANOVA and Duncan's test ($p \leq 0.05$). From soil samples collected from active rice rhizospheres, thirteenteen SSR isolates were identified; these isolates possess the capability to degrade silicon by means of organic acid generation. The volatile inhibitor of soil pathogens, hydrogen cyanide, was only generated by four isolates: SSR24, SSR25, SSR26, and SSR27. The pathogen inhibition rate was 84.55% in SSR12, which was associated with volatile chemical generation. Pathogen suppression varies among isolates due to antibiotic synthesis and competition, as shown by in vitro screening. With a concentration of 0.74 µg/mL, SSR31 was one of six isolates that achieved the highest level of IAA. Although it varied between samples, the rate of phosphate solubilisation was highest for SSR13 at 33.33 percent.

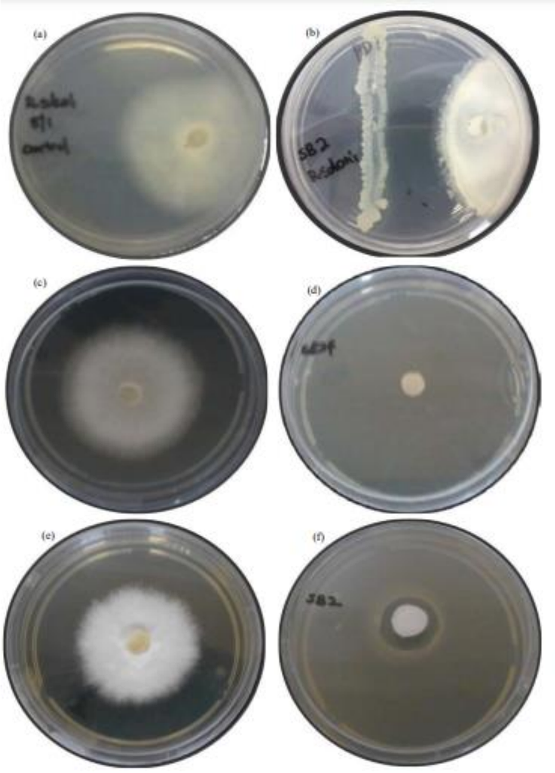

Figure: 1 SSR inhibited R. solani differently based on species, strain, and conditions.

The eight selected SSR isolates were evaluated for their capacity to inhibit R. solani growth and their capacity to stimulate plant development. Most antibiotics were produced by SSR24, SSR25, and SSR26 (PIRG 78.90%), while most extracellular metabolites were synthesised by SSR2 and SSR13 (PIRG 66.90%).

Table 2: In vitro screening evaluated the phytobeneficial and growth-promoting traits of SSR.

	Dual culture	Volatile compound	Hydrogen cyanide	IAA production	Phosphate solubilising
Isolates	test (PIRG)	testing (%)	test OD (625 nm)	test (µg mLG1)	index (%)

SSR1	0.00e	4.88c,d	0c	0c	0e
SSR2	76.54a	52.03b	0c	0c	0e
SSR3	25.93b,c	20.33b,c,d	0c	0c	26.93a,b,c,d
SSR4	9.26c,d,e	26.02b,c,d	0c	0c	14.90e
SSR5	0.00e	6.50c,d	0c	0c	21.86d
SSR6	0.00e	19.51b,c,d	0c	0c	22.56c,d
SSR7	1.85d,e	23.58b,c,d	0c	0c	0e
SSR8	0.00e	13.82c,d	0c	0c	24.96b,c,d
SSR9	0.00e	30.08b,c,d	0c	0c	21.40d
SSR10	0.62e	12.20c,d	0c	0c	23.83b,c,d
SSR11	38.89b	52.03b,c	0c	0c	25.80b,c,d
SSR12	12.35c,d,e	84.55a	0c	0c	23.60b,c,d
SSR13	4.94c,d,e	26.02b,c,d	0c	0c	33.33a
SSR14	24.69b,c,d	17.07b,c,d	0c	0c	30.13a,b
SSR15	0.00e	22.76b,c,d	0c	0c	0e
SSR16	7.41c,d,e	16.26b,c,d	0c	0c	0e
SSR17	9.88c,d,e	29.27c,d	0c	0.04 b,c	21.76d
SSR18	4.32c,d,e	30.08b,c	0c	0.27a,b,c	0e
SSR19	1.23e	18.70b	0c	0.55a,b	0e
SSR20	9.88c,d,e	10.57c,d	0c	0c	24.33b,c,d
SSR21	10.49c,d,e	17.07b,c,d	0c	0 c	0e
SSR22	7.41c,d,e	23.58b,c,d	0c	0c	0e
SSR23	0.62e	16.26b,c,d	0c	0c	23.36b,c,d
SSR24	78.00a	38.21b,c	0.032b	0.13b,c	0e

SSR25	79.63a	37.40b,c,d	0.017b,c	0c	29.66a,b,c
SSR26	75.93a	13.01c,d	0.018b,c	0c	25.53b,c,d
SSR27	3.70c,d,e	13.82c,d	0.069a	0c	21.76d
SSR28	0.62e	13.01c,d	0c	0c	0e
SSR29	0.63e	18.70b,c,d	0c	0c	21.83d
SSR30	8.02c,d,e	36.59b,c,d	0c	0.27a,b,c	0e
SSR31	25.31b,c	0.00d	0c	0.74a	28.43a,b,c,d

Based on their capacity to boost plant growth and inhibit the development of R. solani, eight SSR isolates were chosen. Antibiotic production was dominated by SSR24, SSR25, and SSR26 (PIRG 78.90%), while extracellular metabolite synthesis was dominated by SSR2 and SSR13 (PIRG 66.90%).

Table 3: Selected SSR extracellular metabolite synthesis and diffusible antibiotics against R. solani.

Treatments	The Production of Disseminable Antibiotics (PIRG)	Metabolite Production Outside of Cells (PIRG)
SSR2	32.32^{b}	66.90^{a}
SSR12	23.83^{c}	3.07^{c}
SSR13	29.90^{bc}	66.90^{a}
SSR24	78.90^{a}	5.44^{c}
SSR25	78.90^{a}	45.63^{ab}
SSR26	78.90^{a}	52.72^{ab}
SSR27	18.59^{c}	42.55^{b}
SSR31	9.70^{c}	5.44^{c}

Table 4: Rice sheath blight severity based on disease incidence and susceptibility.

Treatments	Disease Incidence (%)	Disease Susceptibility Index (%)
Control	56.67^{a}	59.00^{a}

Treatments	Disease Incidence (%)	Disease Susceptibility Index (%)
SSR2	46.67^{ab}	26.67^{bc}
SSR13	33.33^{bc}	12.00^{bc}
SSR24	16.67^{d}	6.00^{c}
SSR25	36.67^{b}	16.00^{bc}
SSR26	20.00^{c}	12.00^{bc}

By suppressing R. solani, certain SSR isolates successfully reduced the incidence of rice sheath blight; the isolate with the lowest disease incidence, SSR24, had a rate of 16.67%. It is probable that the reduced disease susceptibility index in plants treated with SSR was caused by antibiotic synthesis and resistance mediated by silicon. While uninoculated plants did develop to a greater height, healthy plants, in particular, benefited from SSR treatment, which increased their growth rate through phosphate solubilisation.

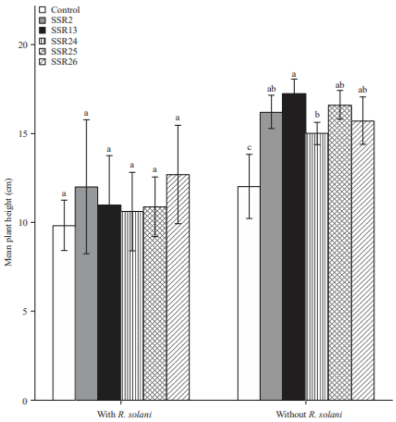

Figure: 2 Rice plant height at 30 days post-sowing with and without *R. solani* inoculation under SSR treatment.

Plant development was helped by phosphate solubilisation and IAA generation, although seedling growth may have been inhibited by HCN synthesis. When it came to managing rice sheath blight and boosting plant output, SSR13 and SSR24 showed the highest potential. It was discovered that Serratia marcescens and Pseudomonas aeruginosa were the specific isolates that improved rice's health and conferred resistance to R. solani.

DISCUSSION

The findings of this study have shown that silicon-solubilizing rhizobacteria (SSR) has the potential to be an efficient biocontrol agent against Rhizoctonia solani, the pathogen that is responsible for rice sheath blight. This potential has been proved by the findings of this study. The delivery of SSR inoculation resulted in a considerable reduction in both the occurrence of sickness and the intensity of sickness, according to experimental investigations that were carried out in greenhouses. SSR24 revealed the highest degree of suppression, which was assessed to be 16.67 percent disease incidence, when compared to the control group, which did not receive any therapy during the course of the study. The capacity of SSR isolates to create volatile chemicals, hydrogen cyanide (HCN), extracellular metabolites, and antibiotics was shown to be correlated with their antagonistic action. This link was found to be significant. It has been demonstrated that each of these compounds is capable of effectively suppressing the development of R. solani. In addition, the incorporation of SSR into the plant led to an increase in the plant's growth, which was shown to be beneficial. The production of considerable quantities of indole-3-acetic acid (IAA) and solubilising phosphate was attributed to isolates such as SSR13. Both of these compounds facilitated the plant's capacity to take in nutrients and contributed to the plant's overall vitality. Based on the findings of the statistical analysis, it was determined that the SSR therapy had a substantial influence on both the decrease of disease and the promotion of growth. This shows that it has the potential to act as an alternative to chemical fungicides that is both safe and favourable to the environment at the same time. However, because this research was carried out under controlled conditions, more field studies are required in order to evaluate the efficacy of SSR in agricultural settings that are representative of the real world. This is because the circumstances under which this research was carried out were controlled. In the years to come, the major focus of research should be on the creation of biofertilizers that are both safe for the environment and capable of being deployed on a wide scale in rice cultivation. This inquiry has to concentrate on improving the formulations of SSR and acquiring an understanding of the molecular interactions that they have with host plants. This is the primary focus of the investigation.

CONCLUSION

Finally, these results will be expanded upon in future studies to prove that SSR may greatly mitigate rice sheath blight and improve plant development generally. Deep molecular investigations will shed light on the processes driving their antibiotic synthesis, volatile chemical emission, and nutrient solubilisation; further research will optimise SSR formulations and prove their efficiency under field settings. More robust and productive rice farming techniques are on the horizon, thanks to the potential for isolates like SSR24 and SSR13 to be refined into environmentally benign biofertilizers that can replace chemical fungicides.

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