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Research Article

Formulation and Evaluation of *Purpureocillium lilacinum* as a Plant Growth-Promoting Fungal Biofertiliser for *Brassica chinensis*

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ABSTRACT

This study explored the development of a biofertilizer formulation using *Purpureocillium lilacinum*, a plant growth-promoting fungus (PGPF), and evaluated its efficacy on *Brassica chinensis* var. *parachinensis*. Fungal isolates were obtained from soil samples through serial dilution plating and screened for plant growth-promoting traits, particularly auxin (IAA) and gibberellic acid (GA_3) production. Two potential *P. lilacinum* (Srk_P_06 and Srk_P_12) exhibited good IAA and GA_3 production respectively at 36.0 µg mL^{-1} and 58.1 µgmL^{-1} for IAA while 15.7 µg mL^{-1} and 11.2 µg mL^{-1} for GA_3 showing their potential of being used as an active component in biofertiliser formulation. *P. lilacinum* strain Srk_P_12 bound to zeolite and later formulated under different percentage in combination with NPK fertiliser (15:15:15) to be tested under controlled conditions. Synergistic effects were observed, with co-application of 40:60 (zeolite:NPK) resulting in a 17 % increase in plant weight compared to NPK alone. These findings demonstrate *P. lilacinum*'s function as growth enhancer offering a sustainable alternative to conventional fertilization practices. Further research is warranted before the microbe can be commercialised such as field validation trials and also molecular characterization of plant-microbe interactions to optimise application protocols for diverse cropping systems. This work is hoped to contribute to the advancing microbial-based strategies for sustainable agriculture practices by reducing the used of chemical inputs in farmers practice and thus help to maintain the biodiversity of the ecosystem in the area.

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INTRODUCTION

The extensive application of chemical pesticides and fertilisers has prompted significant concerns among researchers regarding the long-term sustainability of these practices in agricultural ecosystems. These concerns encompass detrimental effects on soil structure, environmental health, and most critically, the potential risks to human health. In response to this, many researchers have advocated for the adoption of green technologies as sustainable alternatives to conventional agricultural practices which has the potential in maintaining ecological balance and also for a long term agricultural viability (Diab et al. 2024; Gamage et al. 2024).

Purpureocillium lilacinum is a soil-borne fungus known for its dual role as a plant growth promoting microorganism and an effective biological control agent (Khan & Tanaka 2023). *Purpureocillium lilacinum* is commonly isolated from the rhizosphere of various plant species and exhibits notable pH tolerance, thriving in temperatures ranging from 8 to 38 °C (Khan & Tanaka 2023). *P. lilacinum* demonstrates significant biocontrol potential against insect pests, nematodes and phytopathogenic fungi such as *Rhizoctonia solani* and *Cytospora chrysosperma* (Khan & Tanaka 2023; Rigobelo et al. 2024). *Purpureocillium lilacinum* also enhances plant growth through various mechanisms (Yang et al. 2015; Khan & Tanaka 2023; Rigobelo et al. 2024). Given these attributes, *P. lilacinum* can be considered as a crucial element of Integrated Pest Management (IPM) systems

The use of *Purpureocillium lilacinum* as a plant growth-promoting fungus (PGPF) has not been extensively documented in scientific literature. Farias et al. (2018) developed a fungal consortium comprising *P. lilacinum*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Pochonia chlamydosporia*, and *Trichoderma asperellum* to assess their plant growth-promoting effects on soybean, corn and maize. While the results demonstrated a significant positive impact on the plant growth, the specific contribution of *P. lilacinum* could not be differentiated due to the consortium-based approach. In a more targeted study, Rigobelo et al. (2024) reported that *P. lilacinum* has been used as a biological control agent of nematode and PGPF. However, in this study, we utilised *P. lilacinum* to control *Ralstonia solanacearum.*

With the growing global demand for sustainable agricultural practices such as utilisation of biodiversity for agriculture usage, the development of *P. lilacinum*-based commercial biopesticides and PGPF will definitely become a key focus of agricultural research and innovation. This study aims to evaluate the potential of *P. lilacinum* as a plant growth-promoting agent and also assessing its feasibility for enhancing crop productivity.

MATERIALS AND METHODS

Soil sample processing and fungal isolation

Approximately 10 g of peat soil collected from MARDI Sessang Research Station was suspended in 100 mL of sterile distilled water in a 500 mL conical flask. The mixture was homogenised by agitation on an orbital shaker at 250 rpm for 1 hr. Subsequently, 150 µL of the soil suspension was aseptically transferred and spread onto Potato Dextrose Agar (PDA) plates (Jeffrey et al. 2024). A serial dilution series (10^{-2} to 10^{-7}) was prepared using sterile distilled water to optimise isolation of fungus. Petri dish plates were incubated at room temperature for 5 days. After 5 days, pure culture of fungal colonies was subcultured onto new PDA petri dishes for the growth of pure cultures for further analyses.

Screening of Plant Growth Promoting characteristic

The procedure was adapted from Iqbal and Hasnain (2013) with minor modifications. The fungal spore suspension was incubated at 28 ± 2 °C for

48 hours, after which the culture broth was standardised to a density of 10^7 CFU mL^{-1} using a hemocytometer. Two milliliters of the broth were transferred into a microcentrifuge tube and centrifuged at 10,000 rpm for 30 minutes. The supernatant was then combined with Salkowski's reagent in a 2:1 (v/v) ratio and incubated for 30 mins in dark to allow color formation. The development of a pink chromogen was taken as evidence of indole-3-acetic acid (IAA) production. The IAA concentration was quantified spectrophotometrically at 530 nm using NanoDrop spectrophotometer. A standard calibration curve, was generated using commercial available IAA (Sigma-Aldrich), for determination of the fungal IAA secretion levels. The experimental procedure for GA_3 screening was conducted in accordance with the method established by Pandya and Desai (2014), without modifications. *Purpureocillium lilacinum* was grown in a conical flask containing 100 mL of nutrient broth at 35 °C for 48 hrs. After that, the fungal cells samples were centrifuged at 10,000 rpm for 20 minutes. The pH of the resulting supernatant was adjusted to 2.5 using HCl, followed by extraction through liquid–liquid partition method (ethyl acetate/$NaHCO_3$). The ethyl acetate phase was then collected and quantified using NanoDrop spectrophotometer at 254 nm to obtained the amount of GA_3 produced. A standard calibration curve was generated using commercially available gibberellic acid (GA_3; Sigma-Aldrich) and quantified spectrophotometrically at 254 nm using NanoDrop spectrophotometer.

Screening of antagonistic activity

Fungal isolates were subcultured on fresh PDA and incubated at at 28 ± 2 °C for 7 days. The bacterial pathogen *Ralstonia solanacearum* (MMCC10070), obtained from the MARDI Microbial Culture Collection (MMCC, Malaysia), was cultured on Tetrazolium Chloride Agar (TZC) at 28 ± 2 °C for 24-48 hours. Antimicrobial activity was assessed using the Kirby-Bauer diffusion method (Bauer et al. 1966), with modifications. *Ralstonia solanacearum* was lawns on TZC plate and the fungal mycelial plugs (5 mm diameter) were inoculated to the agar plate and incubated for 24 hrs at 28 ± 2 °C. All assays were performed in triplicate manner under controlled conditions. Formation of clear zone indicate anti-bacterial activity. Antimicrobial activity was quantified by calculating the percentage inhibition using the formula:

$$I=[(a-b)/a]\times 100\ \%$$

I: Percent inhibition (%),
a: Diameter growth of inhibition zone (mm)
b: Fungi plug size (mm)

Identification of soil fungi

Genomic DNA (how the prepare genomic DNA, write down in method). was extracted from the fungal isolates using the QIAamp® DNA Mini Kit (QIAGEN, Germany), in accordance with the manufacturer's protocol. Amplification of the internal transcribed spacer (ITS) region was performed using polymerase chain reaction (PCR) with universal fungal primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-TCCGTAGGTGAACCTGCGG-3'). Each PCR reaction was conducted in a final volume of 25 µL, comprising 0.1 µg of genomic DNA, 10 pM of each primer, 1× Taq polymerase buffer, 1.5 mM $MgCl_2$, 0.2 mM of deoxynucleotide triphosphate (dNTP), and 1 U of Taq DNA polymerase. The thermal cycling protocol are as follows; initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 40 seconds, and extension at 72 °C for 35 seconds, with a final extension step at 72 °C for 7 minutes according to Iyanyi et al. (2021) with little modifications. PCR amplicons were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany), and the purified products were subsequently subjected

to sequencing by Apical Scientific Sdn. Bhd. (Selangor, Malaysia). The obtained most potential fungus sequence was subjected to BLASTN analysis with the libraries of sequences at the National Center for Biotechnology Information (NCBI) nucleotide database to determine the fungal taxonomic identity (www.ncbi.nlm.nih.gov). Phylogenetic reconstruction was performed using the Maximum Likelihood method with the Kimura 2-parameter model (Kimura 1980). A bootstrap consensus tree based on 1000 replicates was generated to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). All evolutionary analyses were conducted using MEGA X (Kumar et al. 2018).

Formulation of *Purpureocillium lilacinum*

Selected *Purpureocillium lilacinum* was grown in 500mL Potato Dextrose Broth (PDB) in a 2L conical flask with continuous agitation for 5 days. Spores count of 10^8 was determined using hemacytometer. Zeolite will be used as the carrier to house *Purpureocillium lilacinum*. Zeolite powder was added at the amount of 500 g L^{-1} and later 2 % (v/v) of glycerol was also added into the suspension. The suspension was further agitated at 8000 rpm for another 24 hrs to facilitate the binding of fungal spores onto the zeolite surface. After 24 hrs, the suspension was filtered and air dried for 1-2 days under sterile condition before it was kept in a ziplock bag for future usage.

Greenhouse testing on *Brassica chinensis*

Coco peat sourced from a local market in Selangor, Malaysia was used as the planting medium for *Brassica chinensis*. Five gram (5 g) of the mixture of Zeolite and NPK green fertilizer was used for each *B. chinensis* in the pot. The experimental treatments, outlined in Table 1, were applied twice during the study: once on the 1st day and again on the 15th day after planting. No artificial lighting was used to influence plant growth. Plants were manually irrigated each morning and evening. Ambient temperature was monitored and recorded twice daily at approximately 09:00 and 17:00. Plants were harvested 30 days after planting, and fresh weight, dry weight, and root length were quantified for each treatment. The experiment followed a randomised complete block design with five replicates per treatment. All results obtained were analysed using Tukey's HSD Test. Fresh weight was measured immediately after harvesting. Dry weight was determined after oven-drying the plants at 55 °C overnight. To prevent moisture absorption, dried samples were promptly transferred to pre-weighed Ziplock bags upon removal from the oven. Dry weight (g) was calculated as:

$$\text{Dry weight} = (c + d) - (e)$$

c: Weight of dried plant
d: Ziplock bag
e: Weight of empty Ziplock bag

RESULTS AND DISCUSSION

Fungal enumeration, plant growth promoting and antagonistic activity

A total of 12 fungi isolates were isolated from the soil samples collected. All the 12 isolates were evaluated for their ability to produce Indole-3-Acetic Acid (IAA) and Gibberellic Acid (GA_3), and the results are presented in Table 3. It was observed that 5 of the isolated fungi showed the ability to produce IAA with isolate Srk_P_12 giving the highest reading of 58.9 μgmL^{-1}. Four of the fungi isolated were observed to produce GA_3 with the highest observed to be 15.0 μgmL^{-1}. Two best fungi (Srk_P_06 and Srk_P_12) were selected and identified to be *Purpureocillium lilacinum* (Table 2). Phylogenetic tree constructed showed the close relateness of these 2 strain to *P. lilacinum* (Figure 1).

Table 1. Experimental design of treatment used.

Treatment	Zeolite inoculated with *Purpureocillium lilacinum* (%)	NPK green fertilizer (%)
T0: Zeolite	100 (without *Purpureocillium lilacinum*)	0
T1: Zeolite + *Purpureocillium lilacinum* : NPK (4:1)	80	20
T2: Zeolite + *Purpureocillium lilacinum* : NPK (3:2)	60	40
T3: Zeolite + *Purpureocillium lilacinum* : NPK (2:3)	40	60
T4: Zeolite + *Purpureocillium lilacinum* : NPK (1:4)	20	80
T5: NPK	0	100

Note: Only 5 g of treatment used per pot of *Brassica chinensis*

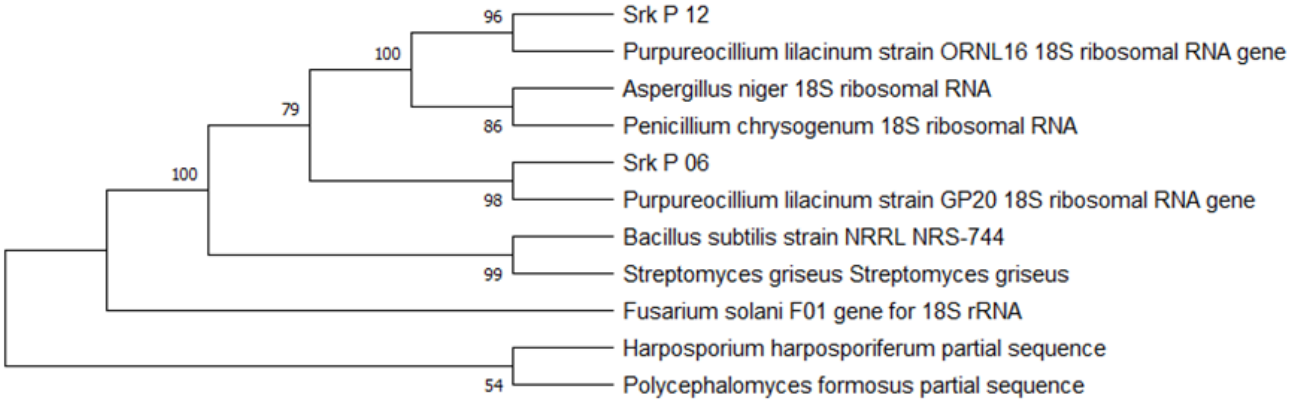

Figure 1. Phylogenetic tree constructed for Srk_P_06 and Srk_P_12

Baron et al. (2020), noted that *Purpureocillium lilacinum* was a good candidate for plant growth promoting microbes. In a study conducted by Moreno -Salazar et al. (2020), the researchers showed that *P. lilacinum* produces IAA which is essential for the growth of plant. García-Latorre et al. (2025), showed that *P. lilacinum* isolated from healthy leaves of *Trifolium subterraneum* produces 4.43 µg $mL^{-1}$of IAA and 451.85 µg $mL^{-1}$of GA_3. This is in contrast with the results obtained in this study, where the highest IAA and GA_3 obtained was 58.1 µg $mL^{-1}$and 15.7 µg mL^{-1} respectively showing that *P. lilacinum* isolated form Malaysia soil produce more IAA but less GA_3. With the isolation these plant growth hormone producers, it showed that *P. lilacinum* not only having the potential to control plant disease such as *Ralstonia solanacearum* but also a good producer of plant hormones.

The potential of *P. lilacinum* to be used as a biological control for *Ralstonia solanacearum* has not been well studied. Due to that in this study, we studied the potential used of *P. lilacinum* against *R. solanacearum*. Currently attention on controlling of plant pathogens, such as *R. solanacearum*, *Sclerotinia sclerotiorum* and *Phytophthora capsici* has been focus on *Purpureocillium* sp. (Yang et al. 2015; Hu et al. 2020; Gomes et al. 2023). Shanmugam et al. (2024), in their study also stated the benefit of using *P. lilacinum* together with other microbes to control disease (*R. solanacearum*) of tomato in Tamil Nadu, India. From our observation, 9 isolates in our study showed the ability to produce inhibition zones against *R. solanacearum*. The highest percentage of inhibition was observed from isolate Srk_P_11 which is 74.1 % (Table 3). Interaction of *Purpureocillium lilacinum* with plants provide lots of benefits. These fungi has the ability to decompose organic matter and also release important nutrients (nitrogen, phosphorus, and kalium) that can be easily uptake by the plants. *P. lilacinum* also produces phytohormones that stimulate plant

Table 2. Sequences for Srk_P_06 and Srk_P_12 with the blastn result.

Strain Number	Sequence	Blastn result
Srk_P_06	cctctggcaccgttcgagaaatcaaagcttttgggctctgggggggagtatggtcg-caaggctggaacttaaaggaattgacggaagggcaccaccaggggtggagcctgcggcttaatttgactcaacacgggacaacgtactaggtccagacatacgaaggattgacagaatacagctctttcataatcatatgggtagtggtgcatggcccttcttagttggtggagcgatttgtctggttaattccgataacgacagagaccctgacctgcacactagtgcccgcttggttcaagcgcggtgcctctgagagggacaatgggtggcaaacccatggaagtgctgggccaaaacaggtctgtgatgcccttagatgttctgggccgcacgcgcgctacactgatggcggcagcgagccacgctggactgacaaggctggctgatcttgtgaaacgccatcgtgctagggatagagcattgcaatttgctcttgaacgaggaatccctagtaagcgcaagtcatcagcttgcgttgattacgtccctgccctttgtacacaccgcccgtcgctactaccgattgaatggcttagtgagatctccggatcggtgccctggtgtccgccctggtgctgagaagctgatcaaacttggtcatttagaggaagtaaaagtcgtaacaaggtttccgtaggtga	*Purpureocillium lilacinum*
Srk_P_12	taagcaattatacggcgaaactgcgaatggctcattatataagttatcgtttatttga-tagcaccttactacttggataaccgtggtaattctagagctaatacatgctaaaaatcccgacttcggaaggatgtatttattagattaaaaaccaatgccctctgggctctctggtgattcatgataacttctcgaatcgcatggccttgcgccggcgatggttcattcaaatttcttccctatcaactttcgatgtttgggtagtggccaaacatggttgcaacgggtaacggagggttagggctcgatcccggagaaggagcctgagaacatccaaggaaggcagcaggcgcgcaaattacccaatcccgacacggggaggtagtgacaataaatactgatacagggctcttttgggtcttgtaattggaatgagtacaatttaaatcccttaacgaggaacaattggagggcaagtctgttgccagcagccgcggtaattccagctccaatagcgtatattaaagttgttgtggttaaaaactcgtagttgaaccttgggcctggctggccggtccgcctcaccgcgtgcactggtccggccgggccttccctctgtggaaccccatgcccttcactgggtgtggcggggaaacaggacttttactttgaaaaattagagtgctccaggcaggcctatgctcgaatacattagcatggaataatgaaataggacgtgcggttctatttgttggtttctaggaccgccgtaatga	*Purpureocillium lilacinum*

Table 3. Production of Indole-3-Acetic Acid (IAA) and Gibberellic Acid (GA_3) by isolated fungi.

Isolate no.	Phytohormone producer (mean ± SD µg mL^{-1})		Percentage of antibacteria activity against Ralstonia solanacearum (mean ± SD %)
	IAA	GA_3	
Srk_P_01	0.0	0.0	44.0 ± 6.3^{k}
Srk_P_02	0.0	5.6 ± 0.36^{h}	51.5 ± 2.6^{k}
Srk_P_03	32.6 ± 0.47^{c}	8.4 ± 0.40^{g}	55.6 ± 4.8^{j}
Srk_P_04	0.0	0.0	56.9 ± 4.0^{j}
Srk_P_05	0.0	0.0	34.5 ± 5.1^{k}
Srk_P_06	36.0 ± 0.74^{b}	15.7 ± 0.75^{e}	0.0
Srk_P_07	36.8 ± 1.15^{b}	0.0	68.7 ± 2.0^{i}
Srk_P_08	0.0	0.0	65.7 ± 3.7^{i}
Srk_P_09	0.0	0.0	0.0
Srk_P_10	0.0	0.0	0.0
Srk_P_11	27.3 ± 0.72^{d}	0.0	74.1 ± 1.6^{i}
Srk_P_12	58.1 ± 0.91^{a}	11.2 ± 0.40^{f}	67.4 ± 1.2^{i}

Value (mean ± standard deviation) with different alphabets within the same column are significantly different by Tukey's test at $p \leq 0.05$.

growth. (Tariq et al. 2020; Rigobelo et al. 2024)

Glasshouse trial analysis

The used of *Purpureocillium lilacinum* as a plant growth promoting fungi was not well studied. *Purpureocillium lilacinum* strain Srk_P_12 (Figure 2) was chosen for further investigation in the pot trail due to its good attributes in IAA and GA_3 properties. It was observed that T3 which is the combination of 40 % Zeolite (inoculated with *P. lilacinum*) and 60 % NPK provide a better growth for *Brassica chinensis* not just the weight but also the growth of the root. By comparing T5 (positive control) with T3, we observed an increased in *Brassica chinensis* treated under T3 showed an increased in mean weight of 17 % but there is no differences in the mean root elongation for both treatments (Table 4).

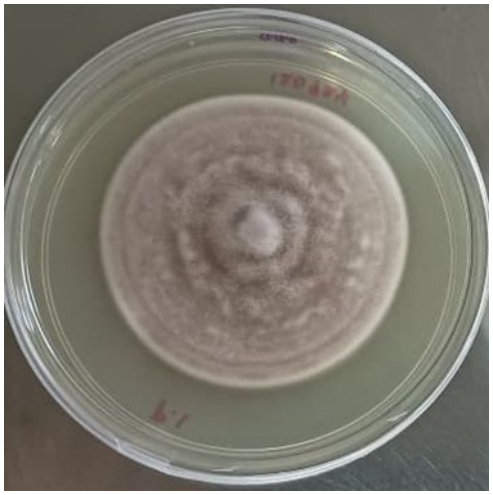

Figure 2. *Purpureocillium lilacinum* subcultured on Potato Dextrose Agar.

Table 4. Weight and root length for each treatment.

Treatment	Weight (mean ± SD g)	Dry weight (mean ± SD g)	Root length (mean ± SD cm)
T0	13.43 ± 1.068^{a}	0.672 ± 0.053^{e}	15.0 ± 3.000^{j}
T1	18.35 ± 2.430^{b}	1.285 ± 0.170^{f}	12.1 ± 3.568^{k}
T2	19.25 ± 1.130^{b}	1.925 ± 0.113^{h}	9.2 ± 0.289^{l}
T3	28.95 ± 0.477^{d}	2.895 ± 0.048^{i}	15.3 ± 0.577^{j}
T4	20.00 ± 0.979^{b}	1.600 ± 0.078^{g}	12.3 ± 1.258^{k}
T5	23.88 ± 0.501^{c}	2.886 ± 0.060^{i}	15.6 ± 0.777^{j}

Value (mean ± standard deviation) with different alphabets within the same column are significantly different by Tukey's test at $p \leq 0.05$.

It is well known that auxin help plant growth by promoting root and shoot development, stimulating fruit growth, and aiding in regulating flowering while gibberellic acid helps to promote plant growth and mediate plants biochemical and physiological responses (Farhad et al. 2022). According to Baron et al. (2020), the higher IAA production by *P. lilacinum* the plant dry weight will increase. *Purpureocillium lilacinum* strain Srk_P_12 which has the highest IAA production was chosen and tested for its impact on plant growth. Treatment T3, showed the highest dry weight of 2.895 g among all treatment. Apart from this, it also has a root length of 15.3 cm which is comparable to the root length of treatment with NPK fertilizer (T5).

CONCLUSIONS

The current study highlighted the promising bioactive potential of *Purpureocillium lilacinum* strain Srk_P_12, isolated from peat soil, in promoting plant growth and suppressing phytopathogens. Quantitative analysis revealed that the strain synthesises significant levels of indole-3-acetic acid (IAA) at 58.1 µg mL^{-1} and gibberellic acid (GA_3) at 11.2 µg mL^{-1}, both of which are key phytohormones involved in root development, cell elongation, and overall plant vigor. Additionally, Srk_P_12 exhibited notable antagonistic activity against Ralstonia solanacearum, achieving 67.4 % inhibition, suggesting its potential role in biocontrol strategies. Pot trials demonstrated that a formulation comprising Srk_P_12 combined with 40 % zeolite and 60 % NPK fertiliser resulted in a 17 % increase in the biomass of Brassica chinensis, underscoring its efficacy as a plant growth-promoting fungus (PGPF). To validate these findings under agronomic conditions, further investigations including large-scale field trials, formulation stability assessments, and long-term efficacy studies are essential. These steps will ensure the reproducibility, scalability, and commercial viability of Srk_P_12-based bioformulations for sustainable agriculture.

AUTHOR CONTRIBUTION

J.L.S.H. designed the research, analysed, and wrote the manuscript, N.A.N. and H.H. collected and analysed data.

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CONFLICT OF INTEREST

There is no conflict of interest.

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