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Rhizobacteria Induce Growth Promotion and Fusarium Wilt Disease Suppression in Watermelon

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Abstract: Watermelon (*Citrullus lanatus*) cultivation is important for Malaysia's agricultural sector, accounting for approximately 8.8% of tropical fresh fruit production in 2021, primarily concentrated in Kelantan, Pahang, Johor, and Terengganu. Production dropped from 2016 to 2021, but showed resilience recently, bouncing back to 136 kilotonnes in 2022. Watermelon serves as a key export commodity, reaching markets in Singapore, China, and the Middle East. However, Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *niveum* (Fon), poses significant challenges, resulting in yield losses ranging from 30% to 80%. The disease thrives in warm, moist climates and can persist in soil for years, posing a long-term threat to cultivation. Management strategies like using resistant crop varieties, rotating crops, and cultural practices are used, but chemical control is limited by environmental concerns and other restrictions. Continuous watermelon cultivation depletes soil nutrients and increases pathogen buildup due to the crop exhausting essential nutrients and promoting disease-causing organisms, thereby reducing soil health and crop yield. Plant growth-promoting rhizobacteria (PGPR) show promise in enhancing plant growth, suppressing pathogens, and improving soil sustainability. This study aims to evaluate the effectiveness of selected rhizobacteria species in promoting watermelon growth and suppressing Fusarium wilt *in vitro*. The tested rhizobacteria were identified as *Pseudomonas atagonensis*, *P. resinovorans*, *P. germanica*, *P. alcaligenes*, and *P. glycinae*. The production of fluorescence pigments was assessed using King's B medium and were detected in *P. atagonensis* and *P. glycinae*. The bacteria were further evaluated for their plant growth-promoting abilities, revealing that *P. atagonensis*, *P. resinovorans*, *P. germanica*, and *P. glycinae* exhibit nitrogen fixation, phosphate solubilization, and potassium solubilization activities. Fusarium wilt disease suppression was assessed via dual culture techniques by measuring the inhibition zone. Results indicated that *P. atagonensis* exhibited the highest inhibition zone (10 mm), followed by *P. glycinae* (5.3 mm) and *P. alcaligenes* (4 mm) compared to the control. Overall, this study suggests that *Pseudomonas* species can induce growth promotion and suppress Fusarium wilt disease in watermelon.

Keywords: Rhizobacteria, Pseudomonads, plant growth promotion, disease suppression, Fusarium wilt, watermelon

1. Introduction

Watermelon (*Citrullus lanatus*) cultivation is a key part of Malaysia's agricultural sector, significantly contributing to the national economy. In 2021, watermelons accounted for about 8.8% of the total tropical fresh fruit production, mainly in Kelantan, Pahang, Johor, and Terengganu. The leading production area is Bachok in Kelantan with 19,914 metric tons, followed by Rompin, Pahang (13,386 mt), and Kota Tinggi, Johor (7,684 mt). Despite a decrease in production from 192,000 metric tons in 2016 to 127,000 metric tons in 2021, the sector has shown resilience with recent production increasing to 136 kilotons in 2022, a near 8% rise from the previous year (Kasron et.al., 2023). Additionally, watermelon and other melons are among the high-value fruits targeted in national policies aiming to increase production and ensure a sufficient domestic supply while also boosting export capabilities. Malaysian watermelons are also exported to several countries, including Singapore, China, and the Middle East, indicating a strong presence in the international market.

Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *niveum* (Fon), presents significant agronomic challenges for watermelon cultivation, impacting both yield and fruit quality significantly. This pathogen is one of the most severe soil-

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borne pathogens globally, capable of causing yield losses in watermelon ranging from 30% to 80% or even more (Rahman et al., 2021). The early symptoms start with the loss of turgor in leaves and advances to more severe symptoms including yellowing, wilting, and necrosis, eventually leading to plant death (Pamela et al., 2023). Fusarium wilt thrives in warm, moist soil conditions, which are common in tropical and subtropical climates like Malaysia, and can persist in the soil for many years through chlamydospores, posing a long-term threat to watermelon cultivation. The economic impact of this disease is profound, not only due to direct production losses but also due to the costs associated with managing the disease.

Management strategies are essential and involve using disease-resistant varieties, rotating crops, and adopting better farming practices to lower soil moisture and stop fungus spread. However, chemical treatments often have limited effectiveness, especially in fields with heavy pathogen infestations. Controlling Fusarium wilt in watermelons typically requires a mix of approaches, including chemical fungicides, crop rotation, and growing resistant varieties. Chemical fungicides, such as prothioconazole, are registered for use against Fusarium wilt, but their effectiveness is often limited, particularly in fields with high levels of pathogen infestation (Petkar et al., 2019). Environmental concerns associated with these methods include the potential for chemical residues in the environment and the risk of developing fungicide resistance in pathogen populations. Additionally, the reliance on resistant cultivars can lead to a narrowing genetic base in cultivated watermelon varieties, potentially reducing genetic diversity and resilience. Integrated management strategies, which combine multiple control methods, are increasingly recommended to address these limitations and enhance the sustainability of watermelon production (Keinath, 2019). Continuous cultivation of watermelons on the same land can lead to a range of soil-related challenges that detrimentally impact crop health and yield. One major concern is soil depletion; repeated cropping can exhaust essential nutrients, necessitating an increased reliance on fertilizers. This not only disrupts soil fertility balance but also escalates farming costs (Gu et al.,2022). Moreover, the buildup of soil pathogens, especially Fusarium wilt, is a significant issue. The spores of this pathogen can survive in the soil for many years, and with each crop cycle, their concentration increases, escalating disease pressure on subsequent plantings (Tian, 2023). This persistent problem is compounded by the limited effectiveness of crop rotation, as some pathogens can survive without their host.

Rhizobacteria, specifically plant growth-promoting rhizobacteria (PGPR), play a significant role in enhancing the growth and health of watermelon plants through various mechanisms. Studies have shown that PGPR can improve plant growth by increasing nutrient availability, enhancing phytohormone production, and promoting root and shoot development. These bacteria also offer protection against various phytopathogens and help plants cope with abiotic stresses like salinity and drought (Kong and Liu, 2022) (de Andrade, 2023). One of the key benefits of PGPR is their ability to modulate the plant's photosynthesis, which is closely linked to the plant's defense mechanisms against pathogens. By enhancing photosynthesis, PGPR can improve the overall energy status of the plant, which supports growth and boosts the plant's innate immunity (Su et al, 2024). Furthermore, PGPR can induce systemic resistance in plants, which serves as a defense mechanism against a range of pathogens. This is achieved through the production of antimicrobial compounds that directly inhibit pathogens or by triggering the plant's immune responses, thus providing a more holistic form of protection against diseases (Kong and Liu, 2022). These attributes make PGPR an invaluable component of sustainable agricultural practices, offering a way to reduce reliance on chemical fertilizers and pesticides, thereby enhancing soil quality and sustainability (de Andrade, 2023). Previous studies have demonstrated the general benefits of rhizobacteria in agriculture, yet research specifically targeting rhizobacteria that promote growth and suppress Fusarium wilt in watermelon remains limited. This study aims to address this research gap by evaluating the effectiveness of selected rhizobacteria species in promoting plant growth and suppressing *Fusarium oxysporum* f. sp. *niveum*, the causative agent of Fusarium wilt.

2. Material and methods

2.1 Preparation of Microbial Cultures

The microbial cultures tested in this study were isolated from agricultural soil in Cameron Highlands and identified using a molecular approach by PCR amplification of the 16S rRNA gene (Haron et al., 2023). The study assessed the efficacy of five pseudomonads against *Fusarium oxysporum* f. sp. *niveum* (Fon). These bacteria were cultured on Luria-Bertani Agar medium and incubated at room temperature for 24 hours to promote robust growth before they were used in further experiments. A pure culture of Fon was maintained through regular subculturing on Potato Dextrose Agar (PDA) medium and kept at room temperature for 10 days, during which the growth of the colony expanded to the plate edges.

2.2 Detection of Fluorescent Pigment Production in *Pseudomonas* spp.

The fluorescence production of all tested Pseudomonads was assessed using King's B medium. The bacteria were streaked onto agar plates containing this medium and then incubated at room temperature for 72 hours. After incubation, the plates were examined under UV light at 360 nm to detect the presence of any fluorescent pigments. If the *Pseudomonas* spp. produced pyoverdine, the characteristic fluorescent siderophore, a bright yellow-green fluorescence was emitted under the UV light. This fluorescence served as a significant indicator of the presence of *Pseudomonas* species.

2.3 Plant Growth Promoting Ability Assay

The ability of each isolate to fix nitrogen was determined using Jensen's nitrogen-free medium. The cultures were incubated at room temperature with four replications for a week, with daily observations of bacterial growth. The ability of each isolate to solubilize phosphate was tested on Pikovskaya Agar; each isolate was streaked onto the agar and observed in four replicates. A clear zone around the colony indicated successful phosphate solubilization. A similar method was employed using Aleksandrow Agar to assess potassium solubilization capabilities. The isolates were streaked and maintained at room temperature, with a clear zone appearing around the colony after 72 hours indicating positive activity.

2.4 Biocontrol Assay Against *Fusarium oxysporum* f.sp. *niveum*

All tested *Pseudomonas* spp. were evaluated for the ability to suppress Fon using the dual culture technique. Potato dextrose agar (PDA) medium was prepared and poured into petri dishes with a diameter of 9 cm. A fungal plug (5 mm) of Fon was placed at the centre of the plate with a bacterial line of *Pseudomonas* spp. streaked with 2 cm distance from each other. Control plates utilized water as a negative control and chlorothalonil as a positive control, replacing the bacteria. The inhibition zone between the fungus and bacteria was observed after 4 days of incubation at room temperature by measuring the distance in millimetres (mm). Treatments were arranged in a completely randomized design (CRD) with three replications. Data were analysed using analysis of variance (ANOVA). Differences between means were identified using the Least Significant Difference (LSD) test at a significance level of $P<0.05$. Statistical analysis was conducted using SAS software, version 9.4.

3. Results and Discussion

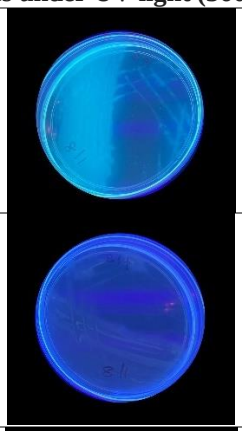
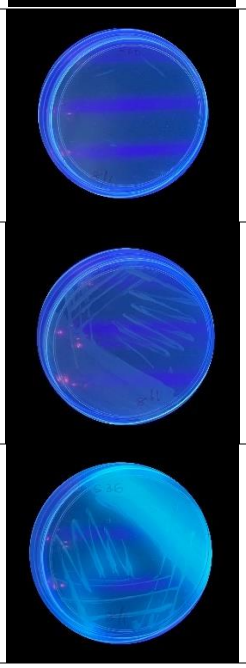
The rhizobacteria isolates were identified using a molecular method based on the 16S rDNA sequence (Table 1), showing a similarity value of 98 – 100% with references in GenBank. Five *Pseudomonas* species were identified as *P. atagonensis, P. resinovorans, P. germanica, P. alcaligenes* and *P. glycinae.* Previous studies have highlighted *Pseudomonas* species as eco-friendly biocontrol agents that suppress a wide range of plant pathogens through multiple mechanisms (Siddiqui & Akhtar, 2021). These bacteria compete for nutrients and space, produce antibiotics and lytic enzymes, induce systemic resistance in plants, and alter microbial community structures in the rhizosphere (Wu et al., 2020). Additionally, *Pseudomonas* spp. serve dual roles as plant growth-promoting rhizobacteria (PGPR) and biocontrol agents against soilborne pathogens, crucial for sustainable agriculture (Bhat et al., 2021). PGPR enhance plant growth and development by fixing nitrogen, solubilizing phosphorus, producing phytohormones, and inducing systemic resistance against pathogens (Nazari et al., 2022). Specifically, *Pseudomonas* spp. improve nutrient uptake, modulate phytohormone levels, facilitate root development, and suppress pathogens through antagonism and induced systemic resistance (Ahemad & Khan, 2021).

These pseudomonads were assessed for their fluorescence production (Table 2), a key trait for identification and taxonomic classification within the *Pseudomonas* genus. This characteristic is not only crucial for distinguishing between species but also implicates the bacteria's ecological roles due to its association with the production of secondary metabolites like pyoverdine and pyocyanin. These compounds have significant biological activities, influencing their applications in agriculture by promoting plant health and disease resistance. Furthermore, the fluorescent property of these bacteria provides insights into their metabolic status and environmental adaptability, which is essential for developing effective biotechnological applications (Yu et al., 2022).

Table 1: Identification of rhizobacteria based on 16S rDNA gene sequencing

Isolate Code	Species Identity	GenBank Accession No.	Sequence Similarity (%)
S9.4.1	*Pseudomonas atagonensis*	NR_181838.1	99.5
S14.25.2	*Pseudomonas resinovorans*	NR_112062.1	98.0
S30.17.3	*Pseudomonas germanica*	NR_181838.1	100
S33.19.4	*Pseudomonas alcaligenes*	NR_113646.1	99.5
S36.21.5	*Pseudomonas glycinae*	NR_179889.1	100

Table 2: Detection of fluorescent pigment production in Pseudomonas species

Isolate Code	Pseudomonas Species	Fluorescence	Plates under UV light (360 nm)
S9.4.1	*P. atagonensis*	Fluorescent	
S14.25.2	*P. resinovorans*	Non-fluorescent	
S30.17.3	*P. germanica*	Non-fluorescent	
S33.19.4	*P. alcaligenes*	Non-fluorescent	
S36.21.5	*P. glycinae*	Fluorescent	

Several reviews have underscored the role of fluorescent *Pseudomonas* spp., particularly *P. fluorescens*, as potent biocontrol agents in agriculture. They combat soil-borne pathogens by mechanisms such as siderophore production and the synthesis of antifungal compounds, notably hydrogen cyanide and exoproteases, as demonstrated by *P. fluorescens* CFBP2392 (Riera et al., 2023). Such strains have been effectively utilized in bio-friendly formulations to significantly reduce diseases caused by pathogens like *Fusarium oxysporum* and *Rhizoctonia solani* in greenhouse settings (Abo-Zaid

et al., 2023). Additionally, their compatibility with commercial fungicides enhances their utility in integrated disease management strategies, maintaining efficacy against diseases such as those caused by *R. solani* (Amit et al., 2023). These capabilities highlight the integral role of fluorescent *Pseudomonas* spp. in advancing sustainable agricultural practices by reducing reliance on chemical pesticides and enhancing crop resilience.

Table 3 indicates that *P. atagonensis*, *P. resinovorans*, *P. germanica*, *P. alcaligenes*, and *P. glycinae* exhibit key plant growth-promoting traits, including the ability to fix nitrogen, and solubilize phosphate and potassium. *P. atagonesis* and *P. resinovorans* demonstrated strong nitrogen-fixing and phosphate-solubilizing activities, while *P. glycinae* exhibited strong phosphate and potassium-solubilizing activities, outperforming other isolates.

Table 3: Plant growth promoting abilities of Pseudomonas species

Isolate Code	Species Identity	Nitrogen Fixation	Phosphate solubilization	Potassium Solubilization
S9.4.1	*P. atagonensis*	+++	+++	++
S14.25.2	*P. resinovorans*	+++	+++	+
S30.17.3	*P. germanica*	+	++	+
S33.19.4	*P. alcaligenes*	++	++	-
S36.21.5	*P. glycinae*	+	+++	+++

+: Mild activity ++: moderate activity +++: strong activity - : no activity

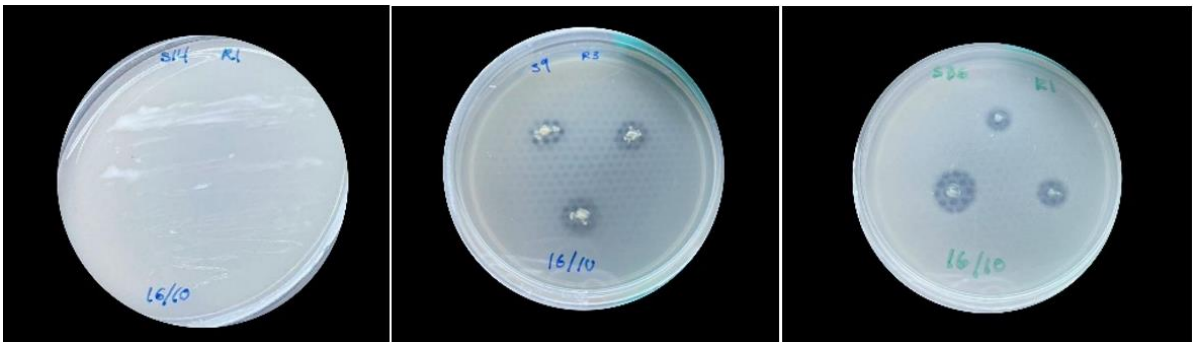


Fig. 1: Plant growth-promoting ability assay of *Pseudomonas* species after 72 hours of incubation at room temperature, indicating positive results; (A) *P. resinovorans* was grown abundantly on Jensen-free nitrogen agar, indicating a strong nitrogen-fixing activity. (B) A clear zone around the colony of *P. atagonensis* grown on Pikovskaya agar, indicating a strong for phosphate solubilization. (C) A clear zone around the colony of *P. glycinae* grown on Aleksandrow agar, indicating a strong activity for potassium solubilization

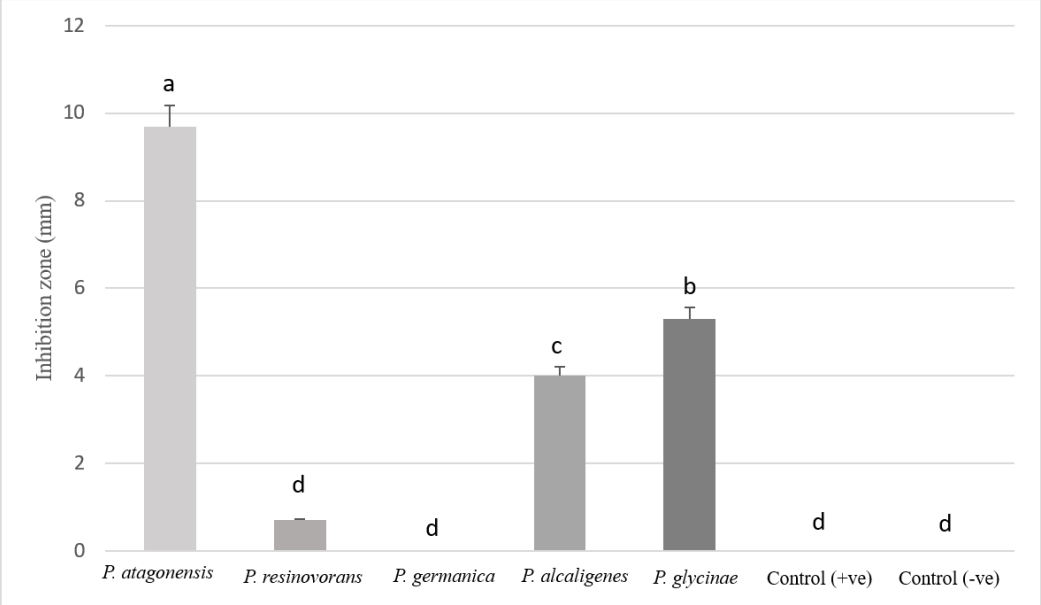


Fig. 2: Antifungal activity of *Pseudomonas* species on inhibition zone (mm) of *Fusarium oxysporum* f.sp. *niveum* after 4 days of incubation (Means within bars followed by the same letters are not significantly different, LSD test; P<0.05)

Fig. 3: Biocontrol assay of *Pseudomonas atagonensis* against *Fusarium oxysporum* f.sp. *niveum* on a dual culture plate showing an inhibition zone between the fungal colony and bacterial line, indicating antifungal activity after 4 days of incubation at room temperature

According to the biocontrol assay, all tested *Pseudomonas* species significantly (P<0.05) controlled the growth of *Fusarium oxysporum* f.sp. *niveum* (Fon) compared to chlorothalonil (positive control) and water (negative control), except for *P. Germanica* and *P.resinovorans*. *P. atagonensis* gave the biggest inhibition zone (9.7mm), indicating the highest antifungal activity, followed by *P. glycinae* (5.3mm) and *P. alcaligenes* (4.0mm). Research on Bacillus species against Fon indicates significant potential for biological control. Studies have shown that certain biocontrol agents, including *Exiguobacterium* sp., can inhibit the growth of Fon effectively. For instance, combinations of various biocontrol agents have demonstrated substantial inhibitory effects on Fon growth, with some combinations showing up to 60% inhibition. These agents work through mechanisms such as antagonism and the production of volatile organic compounds (VOCs) that suppress the pathogen's growth (Lan et al., 2022). Furthermore, various studies have explored the potential of different biocontrol agents against *Fusarium oxysporum* f. sp. *niveum* (Fon), the causal agent of Fusarium wilt in cucurbits. For instance, research by Smith et al. (2020) investigated the biocontrol activity of Trichoderma species and found significant inhibition of Fon growth in vitro. Similarly, Chen and colleagues (2019) evaluated the efficacy of bacterial strains from the genus Pseudomonas and observed promising results in suppressing Fon infection in greenhouse

experiments. Additionally, recent work by Garcia et al. (2021) highlighted the role of certain plant-associated bacteria, such as *Bacillus* spp., in mitigating Fusarium wilt symptoms and reducing disease incidence in field trials. These findings collectively underscore the potential of diverse biocontrol agents in managing Fusarium wilt in cucurbit crops.

4. Conclusion

In conclusion, this study identified five *Pseudomonas* species; *P. atagonensis, P. resinovorans, P. germanica, P. alcaligenes*, and *P. glycinae* as potential biocontrol agents and plant growth-promoting rhizobacteria (PGPR). Through various mechanisms such as plant growth promoting activities such as nitrogen fixation, phosphate and potassium solubilization, these *Pseudomonas* species contribute significantly to sustainable agriculture practices. Their fluorescence trait not only aids in taxonomic classification but also could signify their ecological roles and metabolic status. Furthermore, the tested *Pseudomonas* species especially *P. atagonensis* exhibited significant antifungal activity against *Fusarium oxysporum* f.sp. *niveum* (Fon), a notorious pathogen causing Fusarium wilt in watermelon. While further research on biocontrol agents has shown promising results, the highlighted potential of diverse biocontrol agents emphasizes their importance in managing plant diseases effectively and reducing reliance on chemical pesticides, thus advancing sustainable agricultural practices.

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