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MINI REVIEW

The potential of *Pseudomonas fluorescens* as biological control agent against sheath blight disease in rice: a systematic review

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Abstract

Rice is a staple food source for more than 3.5 billion people. However, rice production can be hampered by several factors and disease is one of them. Sheath blight disease caused by *Rhizoctonia solani* is a serious disease that can cause huge losses. Several control methods have been practised by rice growers to manage the disease; however, chemical fungicides remain a main preference. Unfortunately, heavy reliance on chemical fungicides could lead to many other problems including resistant development, environmental pollution, and harmful to non-target organisms and humans. Managing rice disease through biological control is considered an alternative approach. Exploration of *Pseudomonas fluorescens* as a biocontrol agent against rice disease pathogens is an explicit example of biological approach. The potential of this bacterium has been highlighted by many researchers. This review provides insight into the potential of *P. fluorescens* against *R. solani* in rice. Three databases were used to collect reliable articles with four keywords. The Preferred Reporting Items for the Systematic Reviews approach were used to systematically screen the research articles and evaluate eligibility and exclusion. A total of 5930 articles were found but only 22 articles were selected after eliminating the duplicate articles and further eligibility was screened based on title and abstract. The highest number of published articles on *P. fluorescens* as a bioagent to control sheath blight was from India. The agent was used either alone or in combination with seed, root, soil, and foliar treatments to control sheath blight. Most of the research findings showed the agent successfully reduced the disease severity, promoted plant growth and increased rice yield. However, there are obstacles to commercial *P. fluorescens*-based products due to the instability of the agent at different soil types. More intensive research works are needed to enhance the efficacy of the agent in the real environmental setting.

1. Introduction

Rice (*Oryza sativa* L) is one of the most significant food crops, providing nearly half of the world's daily nutritional consumption. Global rice production is expected to reach a record 509.9 million tonnes (milled basis) in 2021 and 2022. Meanwhile, global rice consumption and residual usage are expected to reach a record 510.3 million tonnes in 2021 and 2022 (USDA 2022).

Rice plants can be infected by various diseases that cause a yield reduction. Rice diseases can be caused by numerous pathogen groups including bacteria, fungi, nematodes, and viruses (Singh *et al*., 2016). The most common diseases in rice are sheath blight, bacterial blight, blast disease, and sheath rot. Rice sheath blight, caused by *Rhizoctonia solani*, is one of the most widespread rice diseases, causing damage to the quality and significant losses in rice production around the world (Yu *et al*., 2017). Sheath blight disease disrupts grain filling and reduces rice production by 39%, but this loss can rise up to 50% in terms of kg/ha of milled whole grain rice due to weakened grains that break during milling. Once sheath blight infections reach 90% of the plant height, milled rice yields are expected to drop by 46% (Suman *et al*., 2017).

Rhizoctonia solani is a soil-borne fungal pathogen that attacks rice and causes significant losses in rice production. The sclerotia of the fungus live in the soil and are spread by irrigation water. The sclerotia infect the leaf sheaths at the base of the culms, causing water-

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soaked circular to oblong grey-green spots. Cobweb-like mycelia spread externally in moist conditions, and sclerotia are formed on damaged plant parts. By causing lesions on complete tillers from the water line to the flag leaf, the fungus spreads to the top section of the foliage. The fungus spreads both inside and between hills when healthy foliage comes into contact with infected foliage (Rabindran and Vidhyasekaran, 1996). Currently, the suitable management of sheath blight disease is by using biological control (Suman *et al*., 2017).

Pseudomonas fluorescens is one of the common biocontrol agents used for managing foliar infection by a soil-borne disease. *Pseudomonas fluorescens* is among the most potential rhizosphere bacteria, as they not only suppress disease but also promote plant development (Radja Commare *et al*., 2002). Numerous studies have been explored and reported by researchers. This review paper provided insight into the potential of *P. fluorescens* against *R. solani* in rice.

2. Materials and methods

The Preferred Reporting Items for Systematic Reviews (PRISMA) approach were used in this study from three databases of Web of Science, SCOPUS, and Science Direct. Then all the findings were further screened for eligibility and exclusion. The review process entails several stages, including identification, screening, eligibility, and data abstraction and analysis.

2.1 Database sources

Three databases were used to identify sources for 250 research articles. Further revisions to the criteria resulted in the removal of duplicate articles, resulting in a total of 230 articles. A total of 230 articles were screened during the screening stage, which involved reviewing all journal articles and selecting those that were relevant to the issue. The articles were further reduced to 199 following the title screening and to 31 articles following the abstract screening. This quantity features articles discussing the potential of *P. fluorescens* in controlling rice sheath blight disease. The flow diagram shown in Figure 1 depicts the article screening process. Non-indexed journals are used as sources of information for literature published in journals that are not indexed by major bibliographic databases such as Web of Science, SCOPUS, or Science Direct. Non-indexed journals were not used as the primary source of data in this analysis, but rather to supplement data from indexed articles.

2.2 Systematic review literature process

The methodology for this study is a systematic review literature (SLR). A SLR of the literature is to identify, assess, and synthesize any empirical data that meets the specified qualifying criteria for a particular research question.

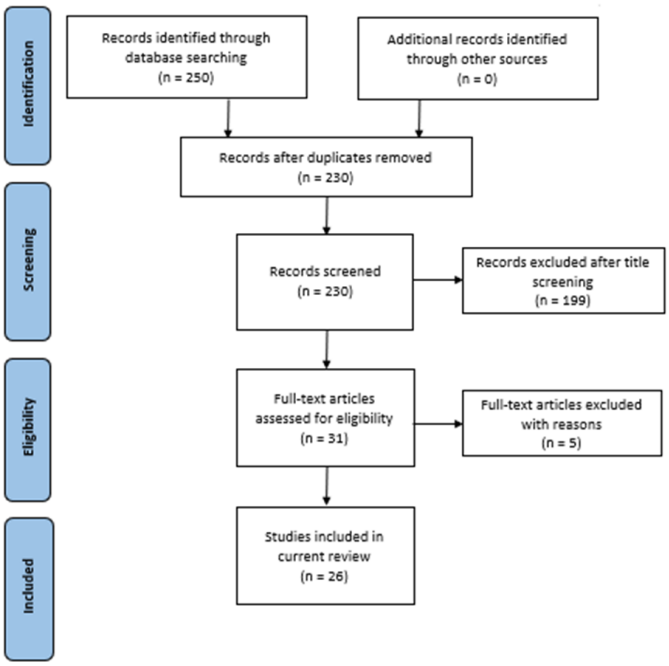

Figure 1. The figure shows the SLR screening process.

2.2.1 Identification

Identification of information sources. The investigation began with a literature search for the terms "*Pseudomonas fluorescens*", "biological control" or "biocontrol", "sheath blight", "disease", and "rice" or "paddy". The title of each document is used to determine the initial relevance of the manuscript. The title indicates that the content of the article includes issues related to *P. fluorescens* controlling sheath blight in rice, we collected the complete reference of the article, including author, year, title, and abstract, for further review. This is achieved using three databases which are Web of Science, SCOPUS, and Science Direct, the databases that are frequently accessed by researchers from various disciplines.

2.2.2 Screening

This stage is critical in identifying all relevant studies on the issue of the potential of *P. fluorescens* in controlling sheath blight disease in rice. In addition, this stage also contains several stages. The first level of screening requires a search through any article journal using the Web of Science, SCOPUS, and Science Direct search engines, and the inclusion of keywords such as "*Pseudomonas fluorescens*" "biological control or biocontrol" "sheath blight" "disease" and "rice or paddy", a total of 250 articles were collected. Then, a second stage in the screening stage took place which involved reviewing all the journal articles and selecting those related to the issue, a total of 230 articles were

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screened. However, 199 sources were excluded after the title content screening due to being irrelevant to the topic. The remaining thirty-one articles were then subjected to a third-level screening, in which journal eligibility was determined.

2.2.3 Eligibility

The third stage is an eligibility evaluation for 31 chosen articles. The titles, abstracts, and main contents of all articles were extensively reviewed to ensure that the articles met up the inclusion criteria and were suitable for use in the current study to achieve the research objectives. As a result, a total of 5 articles were removed due to duplication and lack of empirical data. Finally, a total of 26 articles were selected for further analysis.

2.2.4 Data extraction and analysis

The articles will be evaluated, reviewed, and analyzed following the eligibility process. This paper will discuss the results in detail. The reviews will be based on individual articles that are relevant to and address the research issue. The studies were collected in order to find relevant themes and sub-themes for the current study by reading the titles, abstracts, and entire body content of the articles, the data were extracted and placed under the relevant themes and sub-themes. An integrative review was conducted in the form of a synthesis of several research designs such as qualitative, quantitative, and mixed methods in a review. To accomplish the study's objectives, data extraction is accomplished by reading the complete articles. Mendeley was used to cite the information. For data analysis, the categories of data that were extracted qualitatively and quantitatively were synthesized. The data were derived from secondary sources by collecting and extracting data from another survey published in a peer-reviewed paper. Finally, journals written by various authors were compared in order to obtain the desired results.

3. Results

3.1 Biological control agent

According to Vidhyasekaran and Muthamilan (1999), an interesting alternative approach to control disease on rice is to use biological control methods. It has been reported that the lesion length of sheath blight disease on rice is reduced when rice seeds are treated with certain antagonistic bacteria. The use of plant biological control methods is becoming increasingly popular in most crops. However, its use is still in its early stages in rice ecosystems for various reasons. Since rice is a crop that is grown on wetlands, the survival, growth, and establishment of biological control agents are dubious. An efficient ShB disease management strategy is only possible if the biocontrol agents used in rice-based cropping systems survive, proliferate, establish, and control the ShB pathogen while having a synergistic growth-promoting effect on the crop. Additionally, the biological control agent should be capable of inducing systemic resistance, aiding in disease control (Vijay Krishna Kumar *et al*., 2009).

Biological control is the method of reducing the population of a pest or pathogenic organism through the use of biological control agents such as parasites, predators, and microbes. It is frequently regarded as a safe and dependable method of plant disease management. By minimizing *Rhizoctonia solani* infection, microorganisms such as plant growth-promoting rhizobacteria (PGPR) could protect the production. PGPR are free-living bacteria that have been proven to play an active role in phytohormone biosynthesis (gibberellic acid, indole acetic acid, and abscisic acid), improve nitrogen absorption, promote phosphate solubilization, and inhibit pathogen toxin production. *Pseudomonas fluorescens* and several *Bacillus* spp. are excellent PGPR strains for reducing ShB infection in rice (Singh *et al*., 2019).

One growing field of research for the management of various phytopathogenic agents is the application of PGPR. The mechanisms of PGPR indicate that these strains provided resistance in plants against a wide variety of diseases, including the induction of systemic resistance (ISR). These bacteria are capable of inhibiting or preventing phytopathogen damage (Krishnamurthy and Gnanamanickam, 1997; Suman *et al*., 2017).

3.2 Pseudomonas fluorescens

Pseudomonas is Gram-negative, aerobic Gamma proteobacteria that belong to the Pseudomonadaceae family (Peix *et al*., 2009). They have a strictly respiratory metabolism and are catalase-positive and chemoorganotrophic Pseudomonads are abundant in a variety of soil ecosystems due to their genetic diversity and metabolic flexibility. They are common inhabitants in the plant rhizosphere, where they promote plant growth and work as a biological control agent against soil-borne phytopathogens (Das *et al*., 2020). *Pseudomonas fluorescens* has gotten a lot of attention as potential biological control agents for plant pathogens that are mostly found in the soil (Choi *et al*., 2006). The sheath blight disease is caused by *R. solani*, a soilborne fungus that causes damage to the foliar. Bacterial colonization of plant surfaces, nutrient competition, siderophore generation, and antimicrobial metabolite production are several of the bacterial traits that help

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prevent soilborne plant disease. The suitable biocontrol agents for this disease should survive in both the rhizosphere and phyllosphere. As has been found in previous studies, *P. fluorescens* is able to control plant pathogenic fungi. The production of iron-chelating antibiotics and siderophores as well as inducing systemic resistance by *P. fluorescens* can cause antagonism to the fungus (Haque and Khan, 2021).

Under scanning electron microscopy, *P. fluorescens* induced considerable physiological and morphological changes in *Rhizoctonia* hyphae, including flattening, swelling, knotting, bursting, crumpling, shriveling, and cytoplasm leakage. The capacity of *P. fluorescens* strains to suppress plant diseases and boost plant growth and development allows for their long-term potential for use as biocontrol agents in many varieties of crops. The advantageous effects of *P. fluorescens* on plants have been related mostly to their ability to compete with *R. solani* for space and substrate through a combination of antibiosis, mycoparasitism, and competition for space. *P. fluorescens* is extremely diversified, not just in terms of adaptation, but also in terms of the metabolites it produces (Singh *et al*., 2016). Plant Growth Promoting Rhizobacteria (PGPR) strains are among the biocontrol agents that can survive in the rhizosphere. *Pseudomonas fluorescens* is one of the biocontrol agents that can survive in both the phyllosphere and rhizosphere, as it has a beneficial effect on the promotion of disease growth (Rabindran and Vidhyasekaran, 1996; Radja Commare *et al*., 2002; Akter *et al*., 2014). *P. fluorescens* has been applied to seeds and the rhizosphere because of its biological control traits. Several research and reports have shown that fluorescens pseudomonads can induce disease resistance in leaf diseases. Figure 2 shows the countries that study *P. fluorescens* controlling sheath blight disease in rice.

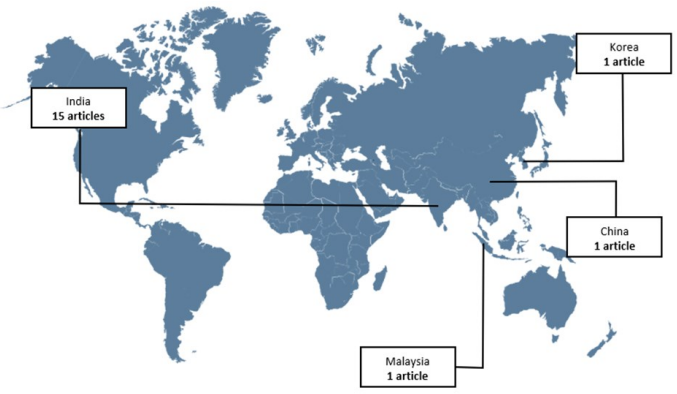

Figure 2. The figure shows the countries that study about *Pseudomonas fluorescens* controlling sheath blight disease in rice.

3.3 Mechanism

A plant's ability to withstand pathogen attack is dependent on both induced active defence mechanisms and preformed barriers. Numerous crops have been discovered to have induced defence mechanisms against many diseases using abiotic and biotic inducers. Pathogens, chemicals, plant growth-promoting rhizobacteria (PGPR), and plant products are all examples of classical inducers. They lead to the activation of defence genes encoding chitinase, peroxidase, β-1,3-glucanase, and enzymes that are involved in phytoalexin production. According to Nagarajkumar *et al*. (2004), the main mechanism involved is parasitism involved in *P. fluorescens*, the biological control of plant diseases. The involvement of enzymes such as β-1,3-glucanase and chitinase, in this process, can degrade cells. It has been shown that early and enhanced expression of these genes confers on plants -induced systemic resistance (ISR) against a variety of diseases. *Pseudomonas fluorescens* strains appear to be promising for inducing ISR.

They are referred to as PGPR because they enhance plant growth and development in addition to increasing plant defence genes. PGPR are root colonizers in general and are classified into many genera and species. The majority of strains are from *Pseudomonas* spp., with fluorescent pseudomonads particularly notable. Numerous studies on the biological control mechanisms of fluorescent pseudomonads have demonstrated that different strains give systemic resistance to plants susceptible to a variety of plant diseases in a variety of crops (Nandakumar, Babu, Viswanathan, Raguchander *et al*., 2001). Lipopolysaccharides extracted from the outer membrane of *P. fluorescens* produced a higher accumulation of phytoalexins, increased amounts of phenolics, pathogenesis-related (PR) proteins, and salicylic acid, indicating that pseudomonads are involved in ISR. Fluorescent pseudomonads have the ability to survive endophytically in a variety of host plants (Krishnamurthy and Gnanamanickam, 1997).

3.3.1 Chitinase and peroxidase activity

When bacteria, viruses, fungi, or other pathogens infect plants, host-encoded proteins called pathogenesis-related proteins (PR-proteins) are activated and accumulate to protect the plant from pathogen attack. The PR proteins are critical in the defence of plants against infections. Chitinase is a significant PR protein because it catalyzes the breakdown of chitin (a significant component of the fungal cell wall) (Ramamoorthy *et al*., 2001). It was established that moderately resistant rice cultivars had increased chitinase activity and were less vulnerable to disease than susceptible cultivar IR58. Chitinase has long been considered a desirable donor gene in genetic engineering. It was first reported that transgenic tobacco seedlings containing a bean chitinase gene were more resistant to

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R. solani infection and showed a delayed start of disease symptoms (Zeng *et al*., 2011).

The involvement of peroxidases and chitinases in plants against diverse diseases has been proposed that increased activity of these defensive enzymes plays an indirect or direct role in the establishment of systemic resistance in plants against infections. According to Thara and Gnanamanickam (1994), *P. fluorescens* strains exhibited low or absent chitinase activity in the study. Nevertheless, two chitinase isoforms (35 and 28 kDa) and five peroxidase isoforms (PO1-PO5) were detected in the study, three peroxidase isoforms (PO3-PO5) and 35 kDa chitinase were revealed to be the particular determinants of ISR. Antagonism may be attributed to the production of antibiotics or siderophores or chitinases by these bacteria (Nandakumar, Babu, Viswanathan, Raguchander *et al*., 2001).

Pseudomonas treatment of rice resulted in the induction of systemic resistance against *R. solani* through an increase in chitinase and peroxidase activities. Increased induction of pathogenesis-related chitinase isoforms in rice treated with *Pseudomonas* in response to *R. solani* infection suggests that increased chitinase plays an important role in disease suppression (Radjacommare *et al*., 2004). Furthermore, in the culture media, *Pseudomonas* strains produced chitinase. It is thought that induced chitinase, peroxidase, and bacterial chitinase all contribute to the reduction of ShB disease growth in rice, either indirectly or directly. When the pathogen was inoculated, the plants produced more chitinase and peroxidase activity, regardless of *Pseudomonas* treatment. Although the increase in peroxidase and chitinase activity began 24 hours after challenge inoculation, it continued to rise and remained significantly higher from 96 to 164 hours in plants treated with *Pseudomonas*. In untreated or *Pseudomonas* -treated plants that had not been infected with the pathogen, there were no significant changes in chitinase and peroxidase activity. Some *Pseudomonas* strains produced similar amounts of chitinase in rice plants, however, there was strain variation in peroxidase activity.

The increased chitinase activity on the chitin medium indicates that *Pseudomonas* strains are capable of degrading the complex chitin polymer that serves as the primary component of fungal cell walls. Thus, because the fungal cell wall contains chitin, bacteria in the plant rhizosphere may have produced more chitinase. The increased chitinase activity could be one of the reasons for increased disease reduction despite the induction of less peroxidase in plants. It has been demonstrated that amending soil with chitin-containing amendments reduces plant diseases caused by fungi and nematodes (Nandakumar, Babu, Viswanathan, Raguchander *et al*., 2001). Rice may have developed systemic resistance as a result of the activation of peroxidase and chitinase defensive mechanisms. Additionally, *P. fluorescens* produce salicylic acid, which acts as a local and systemic signalling molecule in plants, inducing resistance.

3.3.2 Oxalic acid detoxifying enzymes

In a previous study, the *P. fluorescens* strain was capable of detoxifying oxalic acid (OA), a toxin produced by *R. solani*, and a plasmid from the *P. fluorescens* strain included gene(s) implicated in the OA detoxification process (Nagarajkumar *et al*., 2004). The study indicated that multiple mechanisms may be involved in *P. fluorescens* inhibition of *R. solani*. Furthermore, OA is a pathogenicity factor produced by a variety of sclerotium-producing fungi, including *Sclerotinia sclerotiorum*. Some fungi produce OA before mycelial development during pathogenesis. It has been indicated that the OA produced by the fungus precipitates calcium from the middle lamellae to form calcium oxalate crystals, exposing pectic materials to enzymatic degradation.

A high amount of oxalate before the development of a fungus may make plant tissue more vulnerable. The potential of the culture filtrate to induce electrolyte leakage and reduce seedling vigour was significantly reduced when *P. fluorescens* PfMDU2 was cultured in a KMB medium containing OA. This could be attributed to *P. fluorescens* PfMDU2 detoxification or catabolization of OA. When *P. fluorescens* PfMDU2 was grown in the presence of OA, SDS-PAGE analysis revealed that multiple proteins were secreted into the culture medium, suggesting that these proteins may have played a role in OA detoxification.

Several strains of *Pseudomonas fluorescens*, *Pseudomonas cepacia*, *Pseudomonas putida*, and *Pseudomonas aeruginosa* have been effectively utilized for the biological management of plant diseases. Toxic metabolites produced by fungal infections, on the other hand, are known to inhibit *P. fluorescens* of antimicrobial efficacy against several soil-borne phytopathogenic fungi (Nagarajkumar *et al*., 2005). The suppressive effect of fungal toxins on the production of antibiotics by *P. fluorescens*, which is critical in biocontrol activity, may impair the biocontrol efficacy of toxin-sensitive *P. fluorescens* strains against soil-borne diseases. *P. fluorescens*, which are insensitive to toxic metabolites produced by plant pathogens due to their ability to detoxify toxins, can be used to provide effective biological control of soil-borne diseases.

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Numerous rhizobacteria have been developed as biocontrol agents against fungal diseases of crop plants due to their ability to detoxify fungal pathogens' toxins. Numerous strains of *P. fluorescens* have been effectively utilized to control rice ShB biologically. Since the fungus *R. solani's* ability to persist in the soil as sclerotia and create OA, it would be perfect to develop an antagonistic strain of *P. fluorescens* capable of detoxifying the OA.

3.4 Potential of Pseudomonas fluorescens

According to Devi *et al*. (1989), *P. fluorescens* strains were able to suppress sheath blight disease development in IR 20 and TKM 9 rice varieties. Their findings indicated that *P. fluorescens* strain, Pfr 11 reduced disease symptoms by 31% in IR 20 variety and 74% in TKM 9 variety. Meanwhile, field experiments found that bacterial seeds grown on IR 50 and TKM 9 varieties showed reduced severity compared to those grown from non-bacterial seeds. Treatment using NF2 strains reduced disease severity by 65% in IR 50 variety and by 72% in TKM 9 variety. Based on this study, greenhouse experiments showed that selected antagonistic bacterial strains could reduce the pathogenic activity of *R. solani*. Meanwhile, their on-field experiments revealed that the biological control of ShB disease is possible with appropriate seed sterilization. Furthermore, the bacterization raises grain production.

Pseudomonads have also been found in previous studies to have the ability to reduce rice disease. In IR 50, *P. fluorescens* strain 7-14 the infection of ShB disease is 15%, compared to infected control, which is found 100% infection (Krishnamurthy and Gnanamanickam, 1997). The phenazine-like antifungal antibiotic appears to be the key contributor to disease suppression in a comprehensive genetic analysis of Pf7–14 (Gnanamanickam, 2009). Among the 14 biological control agents, treatment with the *P. fluorescens* 4aYN11 strain in the greenhouse achieved an average control efficiency of 56.50%, which is equal to the 91% control efficiency achieved by the traditional fungicide validamycin used in China. These results indicate that strain 4aYN11 potentially stable bio-control that provides a broad inhibitory capacity against the 11 pathogens tested. Simultaneously, promising bio-control strains have shown the capability of screening biological control agents against pathogens with a high genetic variety. Interestingly, despite its low antagonistic activity, 4aYN11 got one of the highest evaluations of the 14 strains, the greatest mean control effectiveness, and the broadest spectrum effect on disease control (Yu *et al*., 2017).

The potential of *P. fluorescens* in controlling ShB disease has been proven through previous studies that have been conducted by researchers (Table 1). According to Rabindran and Vidhyasekaran (1996), strain *P. fluorescens* PfALR2 is the most potential strain among the four strains isolated. The disease intensity was 45, 36, 42, and 43% in PfALR1, PfALR2, PfK1, and PfK2 respectively under greenhouse conditions. Strain *P. fluorescens* Pf1 in study Vidhyasekaran and Muthamilan (1999), found the lowest disease index is 6.8% using a combination of four treatments (seed + root + soil + foliage) with powder-based formulation under greenhouse conditions. The single and mixture of *P. fluorescens* also play a role in controlling sheath blight disease. The application of talc-based formulations with a combination of four treatments for *Pseudomonas* strains either individually or as a mixture has reduced sheath blight under greenhouse conditions. In a study from Nandakumar, Babu, Viswanathan, Sheela *et al*. (2001), under the glasshouse study, the percent of disease index strain *P. fluorescens* PF1 is 40.0% and FP7 is 41.3% compared to control which is 75.0%. The study found that the *P. fluorescens* strain mixture PF1+FP7 has the minimum disease index which is 33.3%, provided sheath blight protection comparable to or better than fungicide treatment, and that vigorous growth promotion by the bacteria increased grain yield. In other studies, *P. fluorescens* strain Pf1 was found to have a disease index of 37.83%, FP7 of 40.27% and a combined Pf1+PF7 of 34.72%, compared to infected control, which is found 75.22% (Radja Commare *et al*., 2002).

In a study conducted by Nagarajkumar et al., (2005), *P. fluorescens* strain PfMDU2, seed treatment followed by soil application of rice reduced the severity of sheath blight disease by 75% compared to the control, however, PfMDU2P$^-$ failed to control sheath blight disease. According to Mathivanan *et al*. (2005), when *P. fluorescens* and *T. viride* were used alone or in combination, as well as in Carbendazim-treated plots, the severity of sheath blight in rice was dramatically reduced. The combination application of *P. fluorescens* and *T. viride* resulted in minimum disease severity of 9.9%, compared to 18.8% in the control condition. When comparing biocontrol agents and fungicide-treated plots to control plots, disease reduction ranged from 42 to 47.3%. At the maximum tillering stage, when comparing all treatments with inoculation with *R. solani*, the maximum reduction in disease severity was found with T5 treatment (Seed treatment with DMP1 + *Rhizoctonia solani* inoculation) 41.12% followed by 30.47% in T6 (Root dipping with DMP1 + *Rhizoctonia solani* inoculation) when compared with controls (T1: *Rhizoctonia solani* inoculation only) (Suman *et al*., 2017).

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Table 1. The potential of *Pseudomonas fluorescens* in controlling sheath blight disease in rice

Strains	Disease reduction (%)	Disease severity (%)	Disease intensity (%)	Treatments	Formulation	References
P. fluorescens Pfr11	31-74%	-		Seed	-	Devi *et al*. (1989)
P. fluorescens PfALR2	-	-	34%	Seed + root + soil + foliage	Peat-based	Rabindran and Vidhyasekaran (1996)
P. fluorescens 7-14	15%	-	-	Seed + root + foliar	-	Krishnamurthy and Gnanamanickam (1997)
P. fluorescens Pf1	-	6.8%	-	Seed + root + soil + foliage	Talc-based	Vidhyasekaran and Muthamilan (1999)
P. fluorescens Pf1	29.7-42.2%	-	-	Seed + root + soil + foliage	Talc-based	Nandakumar, Babu, Viswanathan, Sheela *et al*. (2001)
P. fluorescens Pf1	-	37.83%	-	Seed + root + soil + foliage	Talc-based	Radja Commare *et al*. (2002)
P. fluorescens	42%	-	-	Seed + soil + foliage	Talc-based	Mathivanan *et al*. (2005)
P. fluorescens PfMDU2	75%	-	-	Seed + soil	Talc-based powder	Nagarajkumar *et al*. (2005)
P. fluorescens 4aYN11	56.50%	-	-	Seed + foliage	-	Yu *et al*. (2017)
P. fluorescens PDI	41.12%	-	-	Seed	-	Suman *et al*. (2017)

A review by Ganeshan and Kumar (2005), discussed that rice seeds treated with a *P. fluorescens* formulation seed treatment, during sowing, and at 30 days, seedlings demonstrated resistance to the disease, with disease incidence decreasing from 6.8 to 1.2. The complementing effect of silica and a foliar spray of the biological control agent, *P. fluorescens*, was examined in a field study. *Pseudomonas fluorescens* foliar spray dramatically decreased rice disease incidence and raised crop production. Rice seeds germinated at a rate of 26.3-52.6% when treated with the *P. fluorescens* strain. Additionally, *P. fluorescens* inhibited the ShB fungus *R. solani* mycelial growth and increased seedling vigour and yield of rice plants grown in the greenhouse and field. Rice treated with *Pseudomonas* resulted in the creation of systemic resistance to *R. solani* through an increase in chitinase and peroxidase activities.

These antagonistic bacteria are suitable for use after the maximum stage of crop tillering because the pathogens are less effective during flood conditions. The increase in root length, as well as seedling shoots, is due to seed treatment by antagonistic bacteria. The incidence of this disease is reduced due to the spraying of the leaves by using this antagonist. Previous studies reported that *P. fluorescens* bacterial strains effectively inhibited the growth of pathogenic mycelial on rice plants. According to Vijay Krishna Kumar *et al*. (2009), field application of the strain S-18 at a concentration of 3 x 10^9 cfu/ml significantly suppressed pathogen mycelial growth and resulted in a decrease of ShB disease incidence.

3.5 Treatments

Carbendazim, a fungicide that is frequently used in the field to control *R. solani*, demonstrated comparable efficacy against *R. solani* as the *Pseudomonas* strains used in the study. Fluorescent pseudomonads have also been demonstrated to be useful in the management of foliar disease when applied through root treatment. Pseudomonads migrate from root to foliage, and the bacteria have been observed to develop epiphytically (Rabindran and Vidhyasekaran, 1996). When applied to seeds, roots, or soil, *P. fluorescens* efficiently prevented rice sheath blight. Bacteria were found moving from seed to roots, stems, and leaves epiphytically. Other studies have demonstrated that pseudomonads may be isolated from the aerial sections of plants developed from bacteria-treated seeds.

When treated as a root and seed treatment, the strain had a larger rhizosphere population than when applied as a seed treatment alone. In one of the two glasshouse studies, more soil application increased the rhizosphere population. Treatment with *P. fluorescens* efficiently suppressed rice ShB. Additionally, seed treatment was successful in decreasing disease severity. However, additional bacteria were applied to the root, which significantly lowered disease severity. Additional soil and foliar sprays effectively suppressed the disease (Vidhyasekaran and Muthamilan, 1999).

However, maximum disease control was observed when bacteria were applied using all four methods which are seed, root, soil, and foliage treatment Rabindran and

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Vidhyasekaran, 1996; Vidhyasekaran and Muthamilan, 1999; Nandakumar, Babu, Viswanathan, Raguchander *et al*., 2001). *Pseudomonas fluorescens* administered by all four methods was more successful at controlling the disease than the usual fungicide, carbendazim. According to Nandakumar, Babu, Viswanathan, Sheela *et al*. (2001), it was found that a specific *Pseudomonas* strain was used in a variety of applications, including root, seed, soil, and foliar, significant decreases in ShB development were observed when only one or two of the above applications were used. As an added feature, when compared to untreated plants, the width and length of sheath blight lesions on the *Pseudomonas*-infected plants were significantly decreased. The study found that using *Pseudomonas* as a seed treatment, soil application, root dip, and foliar significantly reduced the severity of the disease when compared to using either of the two treatments alone or in combination, and it was found to be as effective as fungicide treatment in reducing the severity of the disease. Although seed treatment alone can reduce disease incidence, the following application of *Pseudomonas* strains through soil application and root dipping increases the efficacy of seed treatment.

3.6 Formulation

Bacterial cell suspensions are unsuitable for large-scale field applications due to their storage, transportation, and handling difficulties. In previous studies, a formulation was produced in which the fluorescent pseudomonad persisted well for more than two months in the carrier medium. When introduced through seed or soil, fluorescent pseudomonads survive in the rhizosphere of rice. Ganeshan and Kumar (2005) discovered that peat, coir pith, and talc were the best substrates for cultivating *P. fluorescens.*

3.6.1 Peat-based formulation

After 60 days, peat is the ideal carrier material for growth. According to a previous study (Rabindran and Vidhyasekaran, 1996), the efficiency of peat-based formulations as a seed treatment for sheath blight control increased with increasing formulation dose. The effectiveness of controlling the disease was shown when the intensity of the disease was reduced to 34% using seed treatment at 20 g kg^{-1} of seeds. Dipping the roots into the water with peat-based bacterial formulation provides extra protection. The maximum level of control was achieved when peat-based formulations were applied at a concentration of 2.5 g^{-1} water which could control as much as 34% of the disease intensity. Meanwhile, root treatment using a concentration of 6 g^{-1} water could control 48% of the disease intensity.

The disease was suppressed by applying a peat-based formulation of *P. fluorescens* to the soil. Increased soil treatment with the peat-based formulation resulted in an increase in ShB control effectiveness. When a peat-based formulation of the bacterium was sprayed on rice leaf, the disease intensity was reduced. Improved control was achieved by increasing the formulation concentration. When sprayed on plants, the bacterium was found to persist on the foliage. Antibiotic-resistant *P. fluorescens* strains were found in the stems, roots, and leaves of plants produced from seeds treated with the bacterium's peat-based formulation.

In the field, the most effective method of disease management was to apply the peat-based formulation simultaneously to root, seed, soil, and leaf. *Pseudomonas fluorescens* populations growing in the phyllosphere and rhizosphere may have contributed to disease control. Control could involve both direct pathogen suppression and systemically generated resistance in rice plants. In both field studies, the peat-based formulation of the *P. fluorescens* strains not only inhibited rice ShB as effectively as carbendazim but also increased rice yield. The bacterium's highest population was discovered 15 days after sowing, and a significant population was seen up to 45 days afterwards. The bacterium could be found on roots, stems, and leaves when it was applied through soil or root. The antibiotic-resistant strain, on the other hand, moved less from the soil to the stems and leaves. When the bacteria's peat-based formulation was sprayed on foliage, the bacterium was found on stems and leaves.

Under greenhouse conditions, a single application strategy was efficient in decreasing the incidence of disease, but when the peat-based formulation was applied to seed, soil, root, and foliage in combination or seed, soil, and foliage in combination, the most effective control was obtained. The use of a combination of treatments was comparable to the use of carbendazim. In field studies, a combination of all four application treatments was the most successful for ShB control. In both trials, the bacterium in the peat-based formulation was as effective as a carbendazim spray. In both studies, the formulation enhanced the crop's grain yield. According to the studies, an adhesive appears to be necessary for increasing the efficacy of *P. fluorescens* as a rice seed treatment. Because bacterial cell suspension is difficult to apply on a huge scale, produced a peat formulation that allows bacteria to persist for at least two months.

3.6.2 Talc/powder-based formulation

Pseudomonas fluorescens survived for more than 8 months in a moist, talc-based powder formulation (35% moisture content). The selection of an efficient strain, the presence of an effective bacterial population to control

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the disease, the development of carrier material for the bacteria, and the method of application all play a role in the development of fluorescent pseudomonad formulations for the diseases management of rice ShB (Vidhyasekaran and Muthamilan, 1999). In a previous study, the bacteria developed successfully in the rice rhizosphere when the formulation was applied as a seed treatment. The formulation was less effective at establishing the bacteria in the rhizosphere when applied by root treatment or soil application. Rice sheath blight was effectively controlled when the powder formulation was applied to seed, roots, soil, and foliage, and these treatment methods resulted in an improvement in crop grain yield in field trials. This study used the biological control agent *P. fluorescens* strain Pf1. In a greenhouse trial of treatment using the four treatments with talc-based powder formulation, the index of ShB disease was 6.8%, compared to infection control, which found 95% infection.

The bacteria grew successfully in the rice rhizosphere when the formulation was used as a seed treatment. The bacteria were less effective in the rhizosphere after root treatment or soil application of the formulation. When the powder formulation was applied to soil, seed, roots, and foliage, it effectively controlled rice sheath blight (Radja Commare *et al*., 2002). According to Nagarajkumar *et al*. (2005), the previous study showed that seed treatment followed by soil application of a talc-based powder formulation of *P. fluorescens* PfMDU2 significantly reduced ShB severity by 75% compared to untreated control plants, whereas the plasmid-deficient strain of *P. fluorescens*, PfMDU2P$^-$, failed to control ShB disease.

Rice sheath blight can be effectively managed, according to the study, a powder formulation of an effective strain of *P. fluorescens* is applied using appropriate procedures. When the powder formulation was applied to roots, seeds, soil, or foliage, the disease incidence was dramatically reduced. The most effective control of the ShB was obtained by using a combination of all four application methods. The development of a bacterium powder formulation with a shelf life of more than 8 months and an easy application method could be extremely valuable for large-scale field application and effective control of rice ShB. Farmers can be recommended to use talc formulations of biocontrol agents as one of their crop protection methods for managing rice ShB, either alone or in combination, and this approach could be expanded to other crops (Mathivanan *et al*., 2005).

4. Discussion

In recent years, beneficial microorganisms have become increasingly important as biocontrol agents, not only to control disease management and promote plant growth but also to reduce environmental pollution. Among bacterial antagonists, fluorescent pseudomonads, especially *P. fluorescens*, are considered to be among the most promising candidates for biological control. A possible biocontrol agent, *P. fluorescens*, has been demonstrated to be a fungal infection-resistant agent that prevents plant disease by protecting seeds and roots from fungal infection. They have been shown to increase the promotion of plant growth while simultaneously decreasing the severity of numerous fungal diseases.

According to the findings of this study, the bacterium *P. fluorescens* has the potential in controlling sheath blight in rice. Whether the bacterium was used alone or in combination (seed, root, soil, and foliar), sheath blight was successfully reduced, plant growth was promoted, and yield was increased. Although the combination of the four treatments with a powder-based formulation was more effective than using a peat-based formulation. This is due to the fact that *P. fluorescens* bacteria may survive for up to 8 months in a moist talc-based powder formulation with a humidity of 35%. Meanwhile, bacteria can survive for approximately 3 months in a peat-based formulation.

5. Conclusion

This systematic review obtains relevant research findings from the literature and discusses the potential of *P. fluorescens* in controlling soil-borne disease, sheath blight disease caused by *R. solani*. This disease causes a reduction in crop yields and rice quality. Currently, the use of resistant cultivars is not successful in controlling the ShB disease due to the low resistance level of the host. The use of fungicides such as carbendazim is effective in controlling disease, however, its use is limited due to its cost. Frequent use of pesticides also has adverse effects on human health, the environment, and soil biodiversity. It also causes long-term environmental difficulties due to pesticide accumulation in the environment. Because of the large use of chemicals and the environmental risks associated with long-term use, more environmentally friendly and safer ShB management methods were implemented. Biological control is widely accepted as a safe and effective method for solving these issues. The improvements have been made to improve and optimize biological control activities by combining several desirable traits of potential biocontrol organisms. Therefore, another alternative to control this disease is to use a bacterial antagonist that is *P. fluorescens*.

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Pseudomonas fluorescens is a biological control agent that has been demonstrated to be effective. This is due to the fact that these bacteria have great mechanisms for suppressing ShB. Numerous successful research conducted by scientists worldwide has demonstrated that various strains of *Pseudomonas* are capable of managing a variety of fungal, bacterial, and nematode diseases found in rice and other crops. Antimicrobial resistance is frequently more efficient than fungicides at controlling the disease. Applications using *P. fluorescens* as a biological control agent for soil-borne diseases are not commercialized due to disease control activities in different soils and poor bacterial survival in soil and inconsistent seeds (Choi et al., 2006). Therefore, more studies need to be conducted to discover the potential of the use of *P. fluorescens* in controlling ShB disease in rice. With much research evidence of the potential of the use of biological agents to control disease, this method can be commercialized more widely throughout the country.

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