Journal of King Saud University - Science 36 (2024) 103228

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Journal of King Saud University - Science

journal homepage: www.sciencedirect.com

Full Length Article

Field performance assessment of formulated *Pseudomonas fluorescens* for enhancing plant growth and inducing resistance against rice blast disease

Shila Chakraborty, Md. Morshedul Islam, Md. Atiqur Rahman Khokon *

Department of Plant Pathology, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

ARTICLE INFO

Keywords:
Disease incidence and severity
Formulation
H_2O_2
Phenol
Pseudomonas fluorescens

ABSTRACT

To control blast disease in the rice field under natural conditions, three antagonistic isolates of rhizospheric *P. fluorescens* were formulated in talc, kaolinite, PVP, and vegetable oil using an RCBD with three replications. At various phases of the rice plants' growth, all of the products markedly accelerated plant growth and yield-contributing characteristics. Pf-8, compounded in talc (5 %) and vegetable oil (2 %) among the three isolates of *P. fluorescens*, significantly elevated vegetative and yield parameters with higher (2.11 and 2.08) benefit-cost ratios, respectively. At 90 days after transplanting (DAT), T_{11} (5 % Pf-8 Talc) and T_{13} (2 % Pf-8 Vegetable oil) showed a significant reduction in blast incidence (76.81 %, 75.45 %) and severity (71.57 %, 69.82 %), with the largest populations of *P. fluorescens* (9.60×10^{10} and 9.51×10^{10}) in the rhizosphere. Moreover, a significantly increased level of phenol and hydrogen peroxide (H_2O_2) was found in *Pseudomonas*-treated leaves (Pf-8) at 30, 60, and 90 DAT indicating a strong relationship with rice blast disease reduction. Further, blast incidence and severity showed a negative correlation with vegetative parameters, yield parameters, phenol, and H_2O_2. Thus, it may be claimed that using formulated *P. fluorescens* (Pf-8) to manage rice blast in the field could be an alternate strategy to using chemicals.

1. Introduction

In Bangladesh, various biotic & abiotic factors impede rice yield. One of the most deadly and common diseases affecting irrigated rice in temperate and sub-tropical regions of East Asia is the blast of rice (*Magnaporthe oryzae*). Under extreme infection circumstances, the projected production loss in the farmer's field due to blast disease is 56.9 % and 65.4 % in rainfed and irrigated ecological systems, respectively (Hossain et al., 2017). Surprisingly, BRRIDhan 28, the most popular rice variety which covers about 40 % of the Boro season in Bangladesh has been reported to suffer notable production losses as a result of the rice blast (Mahmud and Hossain, 2018).

The most efficient way to control blast disease is still to apply chemical fungicides such as tebuconazole, azoxystrobin + difenoconazole, trifloxystrobin + tebuconazole, and tricyclazole (Mohiddin et al., 2021). However, reckless application of these fungicides results in the fungicide's resilient development, which may eventually drive up the agricultural production cost and destroy the biodiversity of aquatic and soil systems. Moreover, the adverse consequences of fungicides on non-target species (carcinogenicity, high residual toxicity, and severe toxic qualities) and the possible risk of polluting the environment restrict the recurrent and sole use of fungicides (Xin et al., 2020; Xu et al., 2021).

In comparison to chemical fungicides, the use of PGPR as a natural component ensures several benefits, including increased and precise disease suppressive efficiency, a decreased likelihood of pathogen resistance, and the ability to manage disease both directly through their antagonistic behavior against pathogens and indirectly through inducing systemic resistance (ISR) that poses the minimal risks to non-target organisms and environment (Ons et al., 2020). *P. fluorescens* have shown enormous potential to develop resistance against various plant-associated pathogens (Choudhary et al., 2007; De Vleesschauwer et al., 2008). Upregulation of different enzyme activities related to disease resistance like SOD, CAT, PO, dehydroxyascobate reductase, S-transferase, & glutathione has been documented by Prabhukarthikeyan et al. (2018). *P. fluorescens* also has been documented as the most effective PGPR against a range of infectious pathogens like *Fusarium*

Abbreviations: PGPR, Plant growth-promoting rhizobacteria; BAU, Bangladesh Agricultural University; RCBD, Randomized Complete Block Design; BRRI, Bangladesh Rice Research Institute; CFU, Colony Forming Unit; BCR, Benefit-cost ratio; IAA, Indole Acetic Acid; PVP, Polyvinylpyrrolidone; DMRT, Duncan's Multiple Range Tests; ANOVA, Analysis of Variance; CMC, Carboxy Methyl Cellulose; ROS, Reactive Oxygen Species.

* Corresponding author.
E-mail address: atiq.ppath@bau.edu.bd (Md.A.R. Khokon).

https://doi.org/10.1016/j.jksus.2024.103228
Received 14 February 2024; Received in revised form 22 April 2024; Accepted 25 April 2024
Available online 26 April 2024
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spp., *Rhizoctonia* spp., *Magnaporthe oryzae, Sarocladium oryzae,* etc. (Reddy et al., 2009; David et al., 2018). For creating a favorable microclimate and supporting long-term microbial survivability, PGPR cells are designed in forms that can be used in future applications. Numerous liquids and solid formulations using PGPR were developed to enhance crop productivity (Prabhukarthikeyan et al., 2018; Lobo et al., 2019). These formulations contain carrier materials and additives/adjuvants that are inexpensive, safe, and simple to produce inoculants (Kumaresan and Reetha, 2011; Suryadi et al., 2013; Lee et al., 2016). Kaolinite and talc are good carrier materials because of their fineness, lightweight, and good capacity to absorb bacterially-laden liquids. Water-, oil-, or polymer-based ingredients are employed in liquid formulations to improve adhesiveness, durability, emulsifier & dispersion properties. PVP is an affordable, safe polymer and has been reported to be employed in the production of inoculants due to their thermal stability, and adhesive qualities that might improve the adherence of bacterial cells to plant parts, and viscous nature, which could slow down the bioinoculants' drying process.

In the present study, an investigation on the efficacy *of P. fluorescens* formulated in talc, kaolinite, vegetable oil, and PVP was carried out to manage rice blast disease in the field under a natural epiphytotic environment.

2. Methodology

2.1. Study location and treatment combinations

In 2021, during the boro season, the study was conducted in the experimental field of BAU Farming System using an RCBD with three replications. In the present research, a blast-susceptible rice cultivar (BRRIDhan 28) was employed. The study used 10 plants per replication, with each experimental plot of 10 m^2 (4 m × 2.5 m) and maintaining line-to-line & plant-to-plant spacing of 25 cm. All of the treatments received the necessary amounts of potassium, phosphorus, and nitrogen fertilizers from the BRRI. The supplementary table 1 contains a list of treatment combinations employed in this trial.

2.2. Selection of bacterial isolates and developing formulation

Previously isolated and characterized rice rhizospheric *P. fluorescens* were assessed for their capacity to suppress the mycelial growth of *M. oryzae* by the dual culture method as described by Chakraborty et al. (2021). Isolates, Pf-6 (MN256392.1), Pf-7 (MN256393.1), and Pf-8 (MN256394.1) which previously exhibited complete growth inhibition of *M. oryzae*, were utilized for developing new formulations (supplementary table 2) and field trial for blast disease control. Formulations were prepared following the method of Vidhyasekaran and Muthamilan (1995) & Suryadi et al. (2013) with slight modification and stored in an ambient room condition (23 ± 2 ℃).

2.3. Preliminary evaluation of formulated products by germination assay

A series of concentrations of the formulation were selected for primary evaluation. To assess the percentage of germination, the formulated *P. fluorescens* (1 %, 3 %, and 5 %-solid and 0.5 %, 1 %, and 2 %-liquid formulations) and distilled water-treated rice seeds were evenly distributed over moistened sterile blotter discs in Petri dishes (ISTA, 2005). The treatments were kept at 25 ± 2 °C for fourteen days in an incubator, and the germination percentage of the seeds was estimated using the following formula:

$$\%Seedgermination = \frac{Totalno.ofexaminedseeds}{Totalno.ofgerminatedseeds} \times 100$$

2.4. Evaluation of bacterial formulations under natural epiphytotic conditions

In this study, the formulations were applied during the transplantation of seedlings as well as at different growth phases of rice plants. The roots of 20 days seedlings were immersed overnight into mineral clay-based formulation (5 %) & 3 h in oil, polymer formulation, and bacterial suspension (2 %) (selected from the preliminary evaluation of different concentrations of formulations), and subsequently, the seedlings were planted in the field. Each treatment was applied to the foliage 4 times *viz.* 20, 35, 50, and 65 days after transplanting before the anticipated time of infection for panicle attack (the panicle formation stage) (Prathuangwong et al., 2013).

The viability of bacteria in the formulations was assessed at various time points after storage by serial dilution of 1 g or 1 mL of product with double distilled water and 0.1 mL suspension was dispersed over the King's B medium and incubated for 2 days at 28℃. The following formula was utilized for computing CFU:

$$CFU/mL = (No.\ of\ colonies \times Dilution\ factor)/Volume\ of\ the\ culture\ plate$$

Biochemical analysis of treated plants was assayed using leaves. The measurement of phenol and H_2O_2 was conducted following the methods Ding et al. (2019), Alexieva et al. (2001), & Heath and Packer (1968), respectively. Data on disease incidence (%), severity (%), vegetative, & yield attributes were recorded at different growth stages. Percent disease incidence and severity (leaf and panicle blast), grain yield, and BCR were estimated following James (1974), Rais et al. (2018), and Chakraborty et al. (2021), respectively.

2.5. Data analyses

Minitab 18 software was used to assess the statistical analysis of the data and computing correlation coefficient. DMRT was utilized to evaluate the treatment differences that were deemed significant after a one-way ANOVA revealed such differences.

3. Results and discussions

3.1. Effect of formulated P. fluorescens on germination percentages in laboratory condition

The highest germination percentage was observed in seeds bioprimed with 5 % solid and 2 % liquid formulations of Pf-6, Pf-7, and Pf-8 ranging from 90 to 100 %, while the control (water) recorded the least with 34 % germination (supplementary table 3). In comparison to the control, treating seeds with bacterial strains enhanced seed germination and seedling height because the bacterial strains stimulated the production of phytohormones such as cytokinins auxins, & gibberellins. PGPR like *P. aeruginosa* actively colonizes around plant roots, produces IAA, salicylic acid, and siderophore, and thus increases plant growth and yield (Hariprasad et al., 2014).

3.2. Effect of formulated P. fluorescens on vegetative and yield contributing parameters of rice under natural epiphytotic conditions in the field

The efficacy of formulated *P. fluorescens* on vegetative & yield attributes of rice cv. BRRIdhan 28 in the field under natural epiphytotic conditions were evaluated. All the formulations showed statistically significant variation in different vegetative growth parameters like no. of leaves, no. of tillers/hill, no. of panicles/hill, plant height, and yield contributing characters such as no. of healthy grains/panicle, total grains/panicle, panicle length compared to untreated control plots (Table 1, 2, 3 and 4). Among them, Pf-8 formulated in talc and vegetable oil performed better than the others. This finding confirms that the

Table 1
Effect of different treatments on vegetative parameters of BRRIDhan 28 in the field under natural conditions at 30 DAT (tillering stage).

Treatments	Plant height (cm)	No. of tillers/hill	No. of leaves/hill
T_0	31.67 d	2.66 f	10.67 f
T_1	40.67 a	4.00 b-e	12.00 d-f
T_2	40.00 ab	5.00 ab	15.00 a-c
T_3	34.67 b-d	3.00 ef	13.33 c-e
T_4	32.33 d	3.33 d-f	11.33 ef
T_5	32.00 d	3.33 d-f	11.67 d-f
T_6	40.57 a	5.33 a	14.67 a-c
T_7	40.00 ab	4.66 a-c	14.00 b-d
T_8	34.00 cd	3.33 d-f	11.33 ef
T_9	32.00 d	4.33 a-d	13.00 c-f
T_{10}	31.67 d	3.66 c-f	12.00 d-f
T_{11}	42.53 a	5.33 a	16.70 a
T_{12}	41.33 a	4.33 a-d	15.33 a-c
T_{13}	42.00 a	5.00 a	16.33 ab
T_{14}	40.67 ab	5.00 ab	15.00 a-c
T_{15}	32.00 d	3.33 d-f	11.00 ef
Level of significance	**	**	**
CV (%)	**9.64**	**16.79**	**9.55**

** = 1 % level of significance, CV = Co-efficient of variation

application of formulated *P. fluorescens* has an impact on the physiological processes of rice at various vegetative stages. The application of bio-agents like *P. fluorescens* increases vegetative growth and yield through the production of amino acids, vitamins, and phytohormones, including IAA, cytokinins, and gibberellins, and nutrient solubilization/uptake. Moreover, bacterial solubilization of insoluble phosphate in the soil may be the cause of the yield increase in treated plots compared to control. These bacteria demonstrated a useful function in plant growth promotion and P absorption through the breakdown of inorganic insoluble phosphate. Yield increases in a variety of crops have been documented since *P. fluorescens* isolates are recognized as plant growth promoters (Yasmin et al., 2016; Mishra et al., 2023). Moreover, statistically significant and the highest yield of rice was also recorded in T_{11} (5.68 t/ha) and T_{13} (5.63 t/ha) with the highest benefit-cost ratio (2.11 and 2.08) respectively. The direct growth promotion with increased hormones and mineral uptake by PGPR could improve the efficacy of disease control and then achieve a significant increase in yield. The higher selling prices and lower manufacturing costs in the case of plants treated with formulated products are thought to be the reason for the higher BCR. An experiment by Adhikari (2009) also revealed a higher BCR in the organic carrot production system (1.52) than in the inorganic

Table 2
Effect of different treatments on vegetative parameters of BRRIDhan 28 in the field under natural conditions at 60 DAT (booting stage).

Treatments	**Plant height (cm)**	**No. of tillers/hill**	**No. of panicles /hill**	**No. of infected panicles /hill**	**No. of leaves/hill**	**No. of infected leaves/hill**
T_0	53.17 c	10.67 c	10.00 f	12.00 a	60.00 c	36.00 a
T_1	76.67 b	18.00 ab	12.67 e	7.66cde	71.00 c	26.00 de
T_2	74.00 b	19.33 ab	14.00 c-e	7.00 de	73.00 bc	25.00 de
T_3	58.67 c	14.67 a-c	12.33 e	10.67 ab	73.33bc	27.00 cd
T_4	54.00 c	14.00bc	13.67 de	8.00c-e	70.00 c	27.00 cd
T_5	55.00 c	14.33a-c	13.33 e	8.66 cd	71.67bc	27.00 cd
T_6	76.67 b	19.33 ab	16.67 a-c	6.00 e	90.00 ab	31.00 b
T_7	70.67 b	18.33 ab	16.00 b-d	7.33 de	90.00 ab	30.00 bc
T_8	56.00 c	14.33a-c	13.00 e	12.00 a	71.00 c	29.67b-d
T_9	54.33 c	14.67a-c	14.33c-e	12.67 a	73.30bc	30.33bc
T_{10}	55.33 c	14.67a-c	13.67 de	11.33 ab	73.33bc	31.00 b
T_{11}	88.67 a	20.00 a	17.33 ab	6.00 e	100.0 a	22.00 f
T_{12}	76.00b	19.33 ab	18.67 a	7.33 de	96.67 a	25.00 de
T_{13}	87.00 a	20.00 a	17.67 ab	6.00 e	97.00 a	20.67f
T_{14}	77.03 b	19.00 ab	18.67 a	7.00 de	90.00 ab	26.00 de
T_{15}	56.00 c	14.67a-c	14.00 de	9.66bc	73.33 bc	34.00 a
Level of significance	**	**	**	**	**	**
CV (%)	**8.68**	**17.45**	**8.99**	**12.85**	**12.48**	**8.42**

** = 1 % level of significance, CV = Co-efficient of variation

Table 3
Effect of different treatments on vegetative parameters of BRRIDhan 28 in the field under natural conditions at 90 DAT (ripening stage).

Treatments	Plantheight (cm)	No. of tillers/ hill	No. of panicles/hill	No. of infected panicles/hill	No. of leaves/ hill	No. of infected leaves/hill
T_0	87.17 d	15.67 c	13.00 c	13.67 a	83.34 d	45.67 a
T_1	119.3 b	24.50 a	20.33 b	13.00 a	89.03 c	43.33 a
T_2	118.7 b	24.00 a	19.00 b	13.05 a	88.00 c	42.34 a
T_3	98.67 c	19.67 b	18.67 b	12.00 ab	98.33 b	30.00 bc
T_4	94.00 c	19.00 b	18.00 b	11.33 ab	95.00 b	30.00 bc
T_5	95.00 c	19.67 b	18.67 b	12.67 ab	98.33 b	29.00 bc
T_6	118.7 b	24.33 a	17.33 b	8.00 cd	87.07 c	34.30 b
T_7	119.7 b	25.65 a	24.67 a	8.00 cd	88.03 c	35.35 b
T_8	96.00 c	19.33 b	18.33 b	13.00 ab	96.67 b	35.00 b
T_9	94.33 c	19.67 b	18.67 b	12.00 ab	98.33 b	32.00 bc
T_{10}	93.67 c	19.67 b	18.67 b	12.67 ab	98.33 b	34.00 b
T_{11}	125.0 a	25.00 a	24.00 a	7.00 d	121.7 a	22.00 d
T_{12}	121.7 ab	24.67 a	18.67 b	8.67 cd	97.07 b	29.30 bc
T_{13}	125.0 a	25.00 a	25.00 a	7.00 d	125.0 a	21.00 d
T_{14}	117.3 b	24.00 a	18.00 b	12.00 ab	98.03 b	27.33 c
T_{15}	95.67 c	19.67 b	18.57 b	10.33 bc	98.33 b	28.00 c
Level of significance	**	**	**	**	**	**
CV (%)	**8.27**	**11.48**	**12.21**	**14.84**	**10.03**	**9.56**

** = 1 % level of significance, CV = Co-efficient of variation

Table 4
Effect of different treatments on yield parameters of BRRIDhan 28 in the field under natural conditions at 120 DAT (harvesting stage).

Treatments	No. of panicles/ hill	Total grains/ panicle	No. of healthy grains/ panicle	No. of diseased grains/ panicle	Panicle length (cm)	Yield (t/ ha)	BenefitCost Ratio
T_0	15.00 b	185.33 e	117.00 d	76.33 a	18.00 d	0.67 g	0.57 h
T_1	24.33 a	198.35 bc	165.33 a	39.00 e	23.00 b	4.64 ce	2.02 b
T_2	23.33 a	200.00 b	164.67 a	39.05 e	22.57 b	4.74 be	1.96 bc
T_3	18.67 b	194.00 d	185.00 bc	40.00 e	22.67 b	4.63 ce	1.73 e
T_4	18.00 b	187.00 e	139.33 c	47.67 bc	18.00 d	4.73 be	1.68 e
T_5	18.67 b	187.34 e	136.67 c	50.67 b	18.00 d	3.50 f	1.53 f
T_6	23.33 a	185.68 e	153.00 b	52.67 b	27.00 a	5.40 ac	1.90 c
T_7	24.67 a	185.00 e	152.00 b	33.00 f	27.00 a	5.23 ae	1.96 bc
T_8	18.33 b	192.35 d	151.33 b	41.00 de	22.33 bc	4.50 e	1.12 g
T_9	18.67 b	186.00 e	140.00 c	46.00 b-d	19.67 b-d	4.53 de	1.14 g
T_{10}	18.67 b	191.00 d	143.33 bc	47.67 bc	18. 67 cd	4.51 de	1.10 g
T_{11}	24.00 a	205.00 a	168.33 a	32.00 f	27.67 a	5.68 a	2.11 a
T_{12}	23.67 a	200.00 b	166.65 a	33.33 f	27.33 a	5.33 ac	2.00 b
T_{13}	25.00 a	205.00 a	167.68 a	32.33 f	28.33 a	5.63 a	2.08 a
T_{14}	24.67 a	199.03 bc	165.70 a	33.33 f	27.00 a	5.30 ad	1.82 d
T_{15}	18.67 b	190.00 d	145.00 bc	45.00 c-e	22.00 bc	3.53 f	1.68 e
Level of significance	**	**	**	**	**	**	**
CV (%)	**12.21**	**11.24**	**11.5**	**9.94**	**8.40**	**6.24**	**1.47**

** = 1 % level of significance, CV = Co-efficient of variation

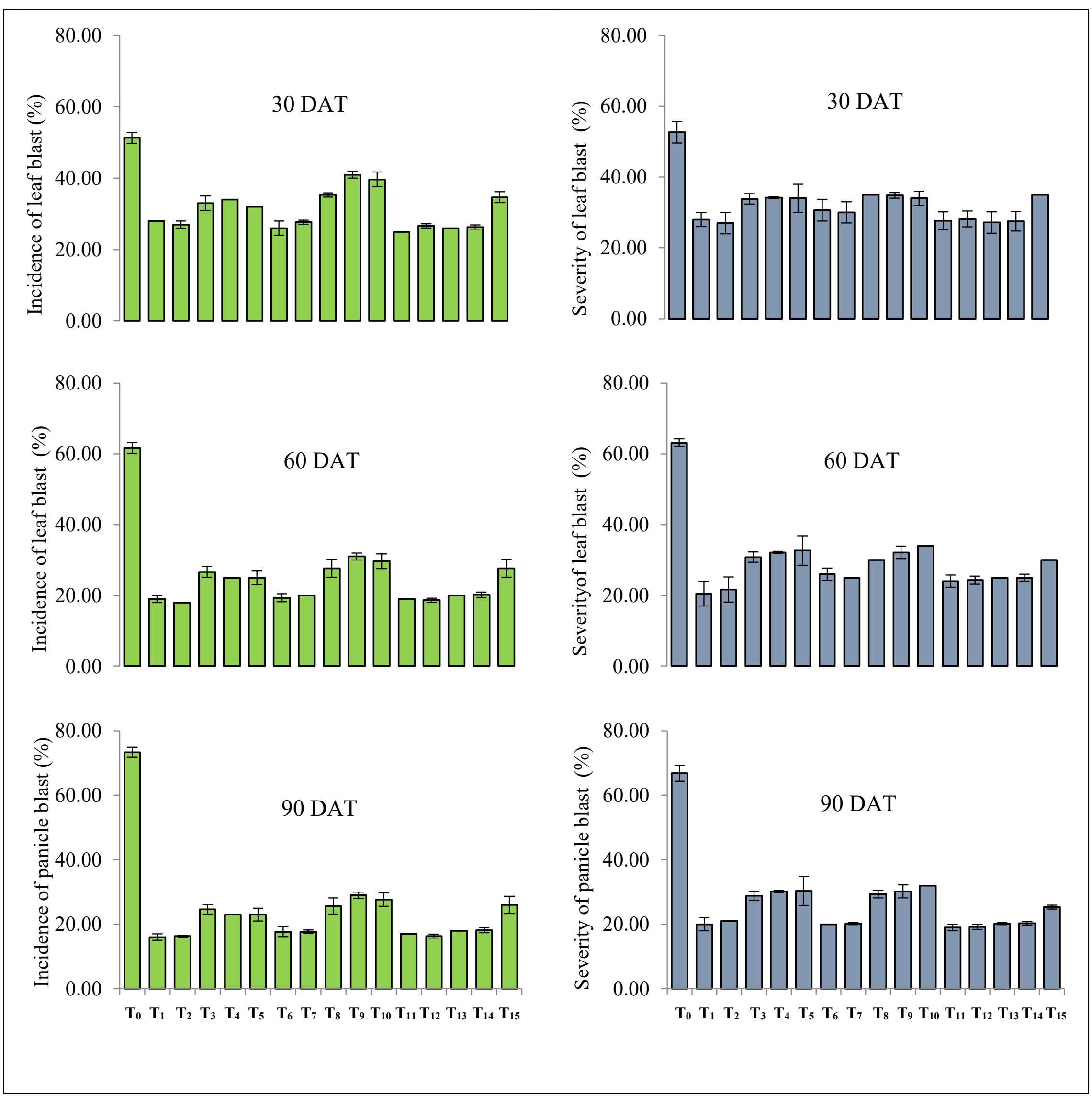

Fig. 1. Effect of different treatments on the incidence and severity of blast of rice at different growth stages.

one (1.44) due to the higher profit and lower production cost in the organic production system.

3.3. Effect of formulated *P. fluorescens* on blast disease incidence and severity of rice under natural conditions in the field

Besides growth promotion and yield increment, the prevalence and severity of blast disease varied significantly at different growth stages after formulated *P. fluorescens* was applied to foliage (Fig. 1). The severity ranged from 19.00 to 67.00 %, and the blast incidence from 16.00 to 73.33 %. In comparison to the control (spraying with water), all *P. fluorescens* formulations showed a significant decrease in incidence and severity at all phases of growth. However, among all the treatments, T_{11}, $T_{12,}$ and T_{13} showed promising responses in reducing the percent incidence & severity of rice blast. The incidence and severity of the blast were more effectively controlled at 90 DAT (ripening stage). It is hypothesized that the reduction in the percent rice blast incidence & severity may be the result of utilizing the beneficial traits of *P. fluorescens* for controlling plant diseases that include competing for nutrients and space; antibiosis through the production of antibiotics, namely, pyrrolnitrin, pyocyanin and production of siderophores (pyoverdin), which restricts the iron availability necessary for the pathogenic growth. In addition, PGPR such as *Bacillus* spp. and *Pseudomonas* spp. can also diminish blast symptoms by interfering with the spore tip mucus and extrinsic matrix's adhesion from the leaf surface, which obstructs the effective adhering of *M. oryzae*. The functional ability of formulated products is described in Supplementary figure 4. The outcomes of the study are in line with the report of Amruta et al. (2018) who also noticed the reduction of blast and enhanced plant growth in rice for the spraying of *P. fluorescens and B. subtilis*.

3.4. Shelf life of *P. fluorescens* in different formulations

Three *P. fluorescens* isolates (Pf-6, Pf-7, and Pf-8) were tested for survival after being stored for up to ten months at intervals of thirty days (supplementary table 4). The CFU number was the highest in all formulations for the first three months. The highest CFU of Pf-8 was recorded in Talc formulation (8.51×10^8) after fresh preparation at 1st month after storage which was found 9.60×10^{10} CFU at 3 months after storage. The prepared products are capable of maintaining the highest level for up to four months, as seen by the slow drop in CFU numbers after that time. After 10 months of storage, the lowest CFUs were found. *P. fluorescens* can survive well throughout the study period. It can be concluded that solid and liquid formulations that had additives (CMC, $CaCO_3$, tritonX, etc.) supplemented with glycerol enhanced the shelf life and performance of the bacteria in the formulations as they provide nutrition, protect bacteria from drying out and also enhance the effectiveness of biocontrol efficacy. It is reported that the use of oil, additives like CMC, tryptone, glycerol, etc. with talc in the formulation maximizes the population of both *P. fluorescens, T. harzianum*, *T. viride*, *Bacillus* spp. (Rangeshwaran et al., 2010; Kala et al., 2013; Archana et al., 2015).

3.5. Effect of formulated *P. fluorescens* on total phenol and H_2O_2 contents in plants

To investigate the potential of *P. fluorescens* formulations to influence the defense system of rice plants, the total phenol content, and hydrogen peroxide (H_2O_2) levels were quantified in the treated plants (Table 5). Total phenol and H_2O_2 were measured at 30, 60, and 90 DAT to understand the longevity of the increased levels of these components. Total phenol content increased significantly with time in all treatments when compared to the constitutive level T_0 (control) at all periods. But at 90 DAT, the highest (403.5 μg g^{-1}) total phenol content was found in T_{11} (5 % Pf-8 Talc) followed by 401.6 μg g^{-1} in T_{13} (2 % Pf-8 Vegetable oil) compared to control (294.5 μg g^{-1}). A comparable pattern was recorded in case of H_2O_2 content. The highest H_2O_2 was estimated in T_{11} (85.17 nmol g^{-1} FW) and T_{13} (82.09 nmol g^{-1} FW) at 90 DAT. The findings revealed that both total phenol and ROS gradually increased and remained elevated up to the ripening stage which may increase tolerance against rice blast disease. Consequently, the application of formulated *P. fluorescens* reduces the occurrence and severity of blast disease by accumulating phenolics and ROS that enhance the plant's tolerance to the disease, ultimately leading to improved yield-contributing factors and overall rice yield. Several researches revealed that ROS & phenolic compounds produced by plants in reaction to pathogen infection are linked to the host's resilience (Xia et al., 2015; Zaynab et al., 2018).

Table 5
Effect of different treatments on phenol and H_2O_2 content of rice leaves.

Treatments	Phenol (μg g^{-1})			H_2O_2 (nmol/g FW)		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T_0	286.3 i	293.6 p	294.5 n	25.17 l	46.26 l	55.18 i
T_1	292.5 fg	330.6 e	390.6 e	54.27 c	73.13 c	77.17 c
T_2	293.4 ef	320.6 h	385.5 f	56.10 bc	75.30 b	81.00 b
T_3	290.6 gh	297.4 n	310.4 j	39.07 gh	55.09 i	61.00 fg
T_4	288.4 hi	311.4 j	315.4 i	36.03 i	59.13 g	65.08 e
T_5	291.0 gh	309.4 k	321.0 h	34.10 ij	53.19 j	57.10 hi
T_6	295.4 de	325.4 g	393.5 d	41.13 g	63.19 f	69.27 d
T_7	292.4 fg	326.5 f	391.0 e	44.20 f	60 21 g	66.17 e
T_8	297.6 d	301 m	307.5 k	38.07 h	52.09 j	58.21 gh
T_9	291.0 gh	295.5 o	300.4 m	33.21 j	49.23 k	59.57 gh
T_{10}	296.4 d	302.4 l	305.7 l	31.0 k	57.17 h	63.23 ef
T_{11}	305.5 a	358.6 b	403.5 a	59.08 a	78.17 a	85.17 a
T_{12}	301.0 c	355.5 c	396.4 c	51 06 d	70.07 d	76.31 c
T_{13}	304.6 ab	359.5 a	401.6 b	57.08 b	76.12 b	82.09 b
T_{14}	302.6 bc	351.0 d	390.6 e	41.11 g	65.13 e	71.10 d
T_{15}	295.5 de	312.6 i	364.6 g	46.20 e	60.18 g	66.17 e
Level of significance	**	**	**	**	**	**
CV (%)	**0.43**	**0.16**	**1.00**	**2.80**	**1.56**	**2.46**

** = 1 % level of significance, CV = Co-efficient of variation

3.6. Correlation matrices analyses

A correlation matrix was developed to analyze whether vegetative attributes, yield attributes, phenol, and H_2O_2 accumulation have any relation with disease incidence and severity (Supplementary figures 1, 2, and 3). The correlation analysis revealed a significant negative correlation among vegetative attributes, yield contributing attributes, phenol, and H_2O_2 content with the percent incidence & severity of blast disease during tillering, booting, and ripening stages. This result indicated the change in rice physiology affects vegetative growth and reduction in incidence and severity due to phenol and H_2O_2 accumulation by the application of formulated *P. fluorescens* treatments which ultimately enhance rice yield in the field. This outcome is in line with the findings of Chakraborty et al. (2021).

4. Conclusion

The locally isolated and formulated rhizobacteria from rice fields (Pf-8) can effectively suppress the incidence and severity percentage of rice blast disease and promote plant growth by modulating the total phenol and H_2O_2 levels. It is therefore concluded that bacterial isolate (Pf-8) has immense potential for commercialization. However, further research should be focused on overcoming extreme environmental conditions like salinity and drought. The application of nanotechnology in the formulation could enhance the efficacy.

Funding statement

The Peoples' Republic of Bangladesh's Ministry of Education (MoE) provided a research fund (Project no. 2018/504/MoE – LS 2017357), and the Ministry of Science and Technology (MoST) awarded the first author a National Science & Technology (NST) fellowship.

CRediT authorship contribution statement

Shila Chakraborty: Writing – original draft, Validation, Formal analysis, Data curation. **Md. Morshedul Islam:** Validation, Investigation, Data curation. **Md. Atiqur Rahman Khokon:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2024.103228.

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