J Bangladesh Agril Univ 19(4): 413–420, 2021 https://doi.org/10.5455/JBAU.130787

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau

Original Article

Eco-friendly Management of Blast Disease in Aromatic Rice by Formulated Antagonistic Fluorescent Pseudomonads under Natural Epiphytotic Condition

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ARTICLE INFO

Article history
Received: 12 Oct 2021
Accepted: 14 Nov 2021
Published: 31 Dec 2021

Keywords
Blast,
Aromatic rice,
Management,
Pseudomonas fluorescens (BdPf),
Bio-pesticide

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Abstract

The experiment was conducted in the field of Central Farming System (CFS) of Bangladesh Agricultural University under natural epiphytotic condition to evaluate the performances of formulated biopesticides comprising rhizopheric fluorescent Pseudomonads in aromatic rice plants *cv*. Kalizira. Antagonistic *Pseudomonas fluorescens* (BdPf) formulated in different carrier materials *viz*. talc, bentonite, palm oil, coconut oil and glycerin were applied for disease management. Root dipping of seedlings followed by foliar application with bacterial suspension at three stages of rice plants was done in order to reduce incidence and severity of blast disease. Disease incidence and severity were recorded at 30, 60 and 90 days after transplanting (DAT). Among 15 treatment combinations (T_1 = 10% BdPf-6 talc, T_2 = 10% BdPf-6 bentonite, T_3 = 1% BdPf-6 palm oil, T_4 = 1% BdPf-6 coconut oil, T_5 = 1% BdPf-6 glycerin, T_6 = 10% BdPf-7 talc, T_7 = 10% BdPf-7 bentonite, T_8 = 1% BdPf-7 palm oil, T_9 = 1% BdPf-7 coconut oil, T_{10} = 1% BdPf-7 glycerin,T_{11} = 10% BdPf-8 talc, T_{12} = 10% BdPf-8 bentonite, T_{13} = 1% BdPf-8 palm oil, T_{14} = 1% BdPf-8 coconut oil, T_{15} = 1% BdPf-8 glycerin), application of *Pseudomonas fluorescens*-8 (BdPf-8) formulated in talc (T_{11}) and bentonite (T_{12}) on the foliage significantly increased the vegetative, yield parameters, Benefit Cost Ratio (BCR) and reduced blast incidence and severity over control at every growth stage. Treatments T_{11} (10% BdPf-8 talc) and T_{12} (10% BdPf-8 bentonite) also gave the highest yield 2.56 t/ha and 2.50 t/ha with highest BCR 1.88 and 1.84 respectively. So, these findings reveal that biopesticide formulated with fluorescent Pseudomonads (BdPf-8) in talc and bentonite can be applied in the field for effective management of blast disease in aromatic rice.

Introduction

Rice blast [*Pyricularia oryzae* (teleomorph *Magnaporthe oryzae*)] is one of the most destructive diseases of rice (*Oryza sativa* L.) worldwide (Ou, 1985). It is the most common disease in irrigated rice of both temperate and subtropical areas of East Asia (Bonman et al., 1991). Even in less blast conducive environments, such as rain-fed lowland rice areas in the subtropics, serious epidemics occur where aromatic cultivars are grown (Ou, 1985). The pathogen is most common on leaves, causing leaf blast during the vegetative stage, or on nodes, neck and panicle branches during the reproductive stage, causing node and neck blast, respectively (Bonman, 1992). Leaf blast lesions decrease the photosynthetic rate of individual leaves (Bastiaans, 1991). Neck blast is considered the most destructive phase of the disease and can occur without being preceded by severe leaf blast (Zhu et al., 2005). It is difficult to control this disease due to having numerous pathogenic races in all rice growing regions of the world, including Bangladesh (Khan et al., 2014). Panicle infection causes complete yield loss (Ou, 1985). Yield reduction by neck blast infection is twice severe compare to leaf blast (Hwang et al., 1987). Different types of blast caused 40% yield loss in Dinajpur region (Rashid et al., 2011).

In Bangladesh, farmers cultivate hybrid, high yielding and local varieties of rice in different parts of the country. In general, rice is classified by its length, thickness and aroma. Aromatic rice is known for its characteristics fragrance when cooked and it fetches higher price in rice market than non-aromatic rice (Ashrafuzzaman et al., 2009). Locally adopted cultivars Kalizira, Sakkorkhora, and BRRI dhan34 have small grain and persistent aroma. Aromatic rice varieties have

Cite This Article

Chakraborty, S., Islam, M.M., Silvi, S.S., Khokon, M.A.R., 2021. Eco-friendly Management of Blast Disease in Aromatic Rice by Formulated Antagonistic Fluorescent Pseudomonads under Natural Epiphytotic Condition. *Journal of Bangladesh Agricultural University*, 19(4): 413–420. https://doi.org/10.5455/JBAU.130787

occupied about 12.5% of total rain-fed lowland rice cultivation (Dutta et al., 2002). The production cost of fine rice per hectare is very low compared to that of coarse rice. The income potential is higher in aromatic fine rice cultivation, since its cultivation does not normally require additional expenditure on fertilizer, pesticides and irrigation. The average yield of high-yielding rain-fed lowland rice is 3.4 t/ha, while the average yield of aromatic rice is 2-2.3 t/ha (Shakeel et al., 2005).

In Bangladesh, almost all of the aromatic rice varieties are highly susceptible to neck blast due to a favorable environment during flowering (Khan et al., 2014). The use of pesticides is costly as well as environmentally undesirable. Thus, there is a need to develop strategies considering sustainable eco-friendly components that are useful over a broad geographic area. Among such new strategies, bio-control agents appear promising in blast management in some countries. *Pseudomonas fluorescens* and other related species have been the objective of particular interest because of their safety, widespread distribution in very diverse habitats, remarkable ability to survive adverse conditions due to the development of endospores, and production of compounds that are beneficial for agronomical purposes. The enzyme activity from these bacteria has multiple functions to keep unwanted pathogens population under check as well as to promote vegetative growth (Schirmbock et al., 1994; Lorito et al., 1996).

Application of bio-agents for plant disease control is a new concept in Bangladesh. On the other hand, many effective bio-agents are formulated and commercially available in many developed countries. Application of bio-agents for plant disease control is safe and sustainable. Moreover, introduction of commercial production of effective bio-agents may widen the scope of entrepreneurship. Effective bio-agents as well as their formulation are the prime concern for production at commercial scale. Therefore, this present study was undertaken to investigate the efficacy of formulated antagonistic *Pseudomonas fluorescens* for environmentally safe management of blast disease in aromatic rice.

Materials and Methods

The experiment was conducted in the field of Central Farming System (CFS) of Bangladesh Agricultural University during Aman season started from July and ended in December, 2020. A very popular local aromatic rice cultivar (Kalizira) was used in this experiment. Experimental plots consisting of 10 m^2 in area (2.5 meter wide by 4 meter long) with an additional of 1 meter width planted next to the adjacent plot as buffer zone were lined out in the field and arranged in randomized complete block design (RCBD). Nitrogen (N), phosphorous (P) and potassium (K) fertilizers were applied based on the standard recommended rate in all the treatments. Line to line 25 cm and plant to plant 15 cm spacing was maintained throughout the experiment.

Treatment combination

T_0 = Control, T_1 = 10% BdPf-6 talc, T_2 = 10% BdPf-6 bentonite, T_3 = 1% BdPf-6 palm oil, T_4 = 1% BdPf-6 coconut oil, T_5 = 1% BdPf-6 glycerin, T_6 = 10% BdPf-7 talc, T_7 = 10% BdPf-7 bentonite, T_8 = 1% BdPf-7 palm oil, T_9 = 1% BdPf-7 coconut oil, T_{10} = 1% BdPf-7 glycerin, T_{11} = 10% BdPf-8 talc, T_{12} = 10% BdPf-8 bentonite, T_{13} = 1% BdPf-8 palm oil, T_{14} = 1% BdPf-8 coconut oil, T_{15} = 1% BdPf-8 glycerin.

Bacterial isolates selection and formulation development

Previously isolated *Pseudomonas fluorescens* from rice rhizosphere soil were screened for their antagonistic potential against *M. oryzae* following dual culture method described in an earlier paper (Chakraborty et al., 2020). Isolates, BdPf-6 (GenBank: MN256392.1) (100% homology with GenBank: KC345028.1- *Pseudomonas fluorescens* strain psf6), BdPf-7 (GenBank: MN256393.1) (100% homology with GenBank: KC952984.1- *Pseudomonas putida* strain LB22) and BdPf-8 (GenBank: MN256394.1) (100% homology with GenBank: MG011565.1 - *Pseudomonas putida* strain 39) which inhibited the growth of *M. oryzae* completely, were utilized in the present study for developing formulation and field evaluation for blast disease management in aromatic rice. Formulation was made using different kinds of carrier materials like talc powder, bentonite, palm oil and glycerin. The formulations were developed with slight modifications of the methods developed by Vidhyasekaran and Muthamilan (1995) and Suryadi et al. (2013) described in. One kilogram of talc, 10g of Carboxy methyl cellulose (CMC), 15g of $CaCO_3$ and 40mL of 50% glycerol were mixed well and autoclaved and then 400mL suspension (10^9 CFU/mL) of *Pseudomonas* spp. isolates were added under aseptic conditions. Mixed products were air dried on sterile aluminium foil for 24 h with occasional stirring under laminar airflow cabinet. Formulated solid products (around 35% moisture content) of *Pseudomonas fluorescens* was passed through a 250µm mesh sieve to attain the desired particle size and packed in sterilized zipper poly bags, sealed and stored at refrigerator prior to use.

Field evaluation of formulated products

In this experiment, the formulated products were applied during transplantation of seedlings and at different growth stages of aromatic rice plants. The roots of 20 days old Kalizira rice seedlings were dipped into *Pseudomonas* spp. suspension of powder formulation (@ 10%) for overnight and 3 hours in case oil-based formulation (@ 1%). The treated seedlings were then transplanted into the field (Khan et al., 2017). Formulated products were sprayed on the foliage making suspension with distilled water for 4 times *viz*. 20, 35, 50 and 65 DAT (Suryadi et al., 2013; Khan et al., 2017). Data on vegetative, yield parameters, disease incidence and severity of the disease was recorded at different growth stages. Percent disease incidence and severity were estimated following James (1974) and IRRI (1996) respectively. The grain yield was estimated by examining yield performance and breaking the yield into its components following Shakeel et al. (2005). Benefit cost ratio (BCR) was calculated following method by Mehmood and Sabir (2011).

Data analysis

Three important traits *viz*. growth parameters, yield parameters and disease parameters were considered at different growth stages of rice plant. Considering the growth parameters, plant height, no. of tillers/hill and no. of leaves /hill were recorded. For yield parameters, no. of panicles/hill, total grains/panicle, panicle length and yield were recorded. For disease parameters, no. of diseased panicles/hill, no. of diseased leaves/hill, no. of healthy grains/panicle, no. of diseased grains/panicle were recorded. The data were statistically analyzed following the analysis of variance (ANOVA) and mean differences were adjudged by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) using a computer operated program MSTAT-C.

Results and Discussion

Effect of formulated pseudomonads on the incidence and severity of blast disease of aromatic rice cv. Kalizira under natural epiphytotic condition

All treatments of formulated pseudomonads showed significant differences in incidence and severity of blast disease at different growth stages (Figure 1 and 2). Incidence of blast ranged from 13.33 to 80.00% while disease severity ranged from 20.00 to 90.00%. Significant and highest reduction in leaf and panicle blast incidence was recorded in 10% talc and bentonite formulation with BdPf-8 compared to control. Similar kind of response was also found in case of leaf and panicle blast severity where highest suppression of blast disease severity was recorded in T_{11} (10% BdPf-8 talc) and T_{12} (10% BdPf-8 bentonite). We hypothesized that root inoculation and foliar application of the formulated pseudomonads may show antagonism against other soil-borne microorganisms and might also produce antibiotics, siderophores, hydrolytic enzymes, phytohormones and/or other volatile extra-cellular metabolites which are responsible for promotion of vegetative growth and suppression of blast incidence and severity. Chakraborty et al. (2020) reported that talc and palm oil formulation of BdPf-8 showed potential suppression of rice blast incidence (71.35%) and severity (67.5%) under artificially inoculated condition. Suryadi et al. (2013) and Vidhyasekaran and Muthamilan (1995) also reported about the application of bacterial consortium A6 (*B. firmus*E65, *B. cereus* II.14, and *P. aeruginosa*C32b) in rice fields for blast disease suppression. The use of *Pseudomonas fluorescence* strains 4-15 and 7-14 also showed growth inhibitory activity against *P. oryzae* with a percentage of 59% and 47% (Gnanamanickam and Mew, 1992).

Effect of formulated pseudomonads on the vegetative growth of aromatic rice cv. Kalizira under natural epiphytotic condition

Formulated Pseudomonads were applied on the foliage of rice to examine their effect on growth promotion of aromatic rice cv. Kalizira at 30, 60 and 90 DAT (Table 2 and 3). There was no effect of treatments on plant height, number of tillers/hill, number of leaves/hill recorded up to 30 DAT. But, at 60 and 90 DAT all treatment combinations showed significant positive effect on vegetative growth parameters (plant height, number of tillers/hill, number of leaves/hill, number of panicles/hill) and negative effect on disease parameters (number of diseased leaves/hill, number of diseased panicles/hill) of rice at all growth stages compared to untreated control. Bacterial isolates and carrier materials both can influence the performances. However, among the treatments bacterial isolate BdPf-8 formulated in talc and bentonite showed better performance on vegetative growth compared to untreated control. The highest increment of plant height, no. of tillers/hill, no. of panicles/hill was observed 31.8, 55.6 and 100.0% over control respectively at 60 DAT. The similar trend was also followed 21.8, 55.6 and 40.0% over untreated control treatment (Table 1 and Table 2). Bakhshandeh et al. (2015) reported that application of bioagents (*Pseudomonas fluorescens* and *Pseudomonas putida*) at seedling and other growth stages significantly increased vegetative parameters compared to untreated control treatment which comply with the findings of the present research work. We hypothesized that secondary metabolites and antifungal compounds are released by *Pseudomonas* spp. which thus activate different activate growth promoting enzymes and activate pathogenesis related proteins in rice plants (Figure 3 and Figure 4) which eventually reduce disease incidence and severity. The *Pseudomonas* strains are reported to induce plant growth by producing plant growth regulators like gibberellins, cytokinins and indole acetic acid (Lifshitz et al., 1987; Dubeikovsky et al., 1993) which can either directly or indirectly modulate the plant growth and development. Growth promotion was also observed by the application of

PGPR (Pierson and Weller, 1994; Duffy and Weller, 1995).

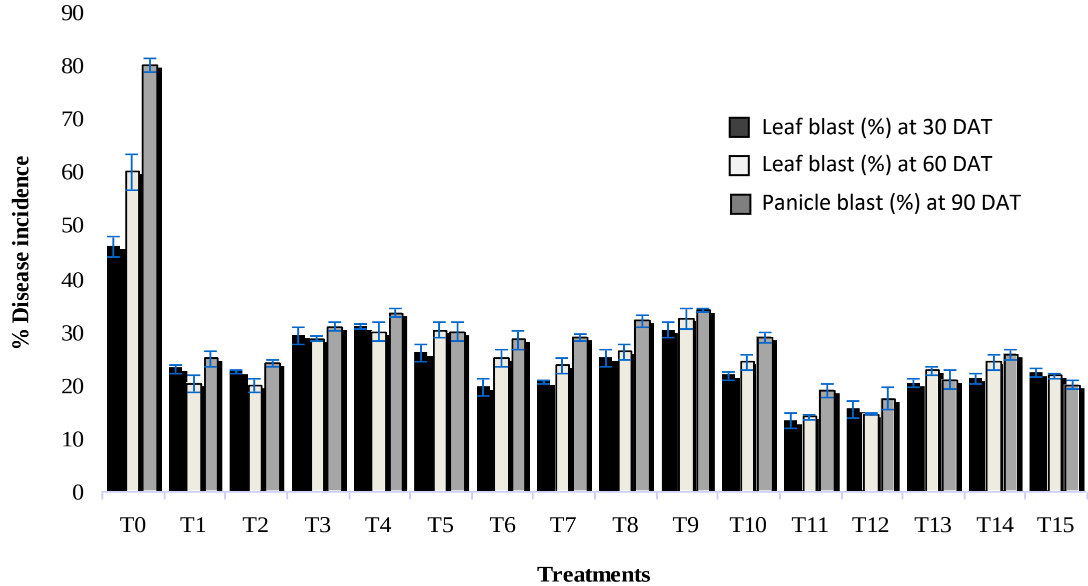

Figure 1. Effect of different treatments of formulated pseudomonads on the incidence of blast of aromatic rice cv. Kalizira at 30, 60 and 90 DAT under natural epiphytotic condition

NS = Non significant, ** = 1 % level of significance, CV = Co-efficient of variation, Here, T_0 = Control, T_1 = 10% BdPf-6 talc, T_2 = 10% BdPf-6 bentonite, T_3 = 1% BdPf-6 palm oil, T_4 = 1% BdPf-6 coconut oil, T_5 = 1% BdPf-6 glycerin, T_6 = 10% BdPf-7 talc, T_7 = 10% BdPf-7 bentonite, T_8 = 1% BdPf-7 palm oil, T_9 = 1% BdPf-7 coconut oil, T_{10} = 1% BdPf-7 glycerin,T_{11} = 10% BdPf-8 talc, T_{12} = 10% BdPf-8 bentonite, T_{13} = 1% BdPf-8 palm oil, T_{14} = 1% BdPf-8 coconut oil, T_{15} = 1% BdPf-8 glycerin.

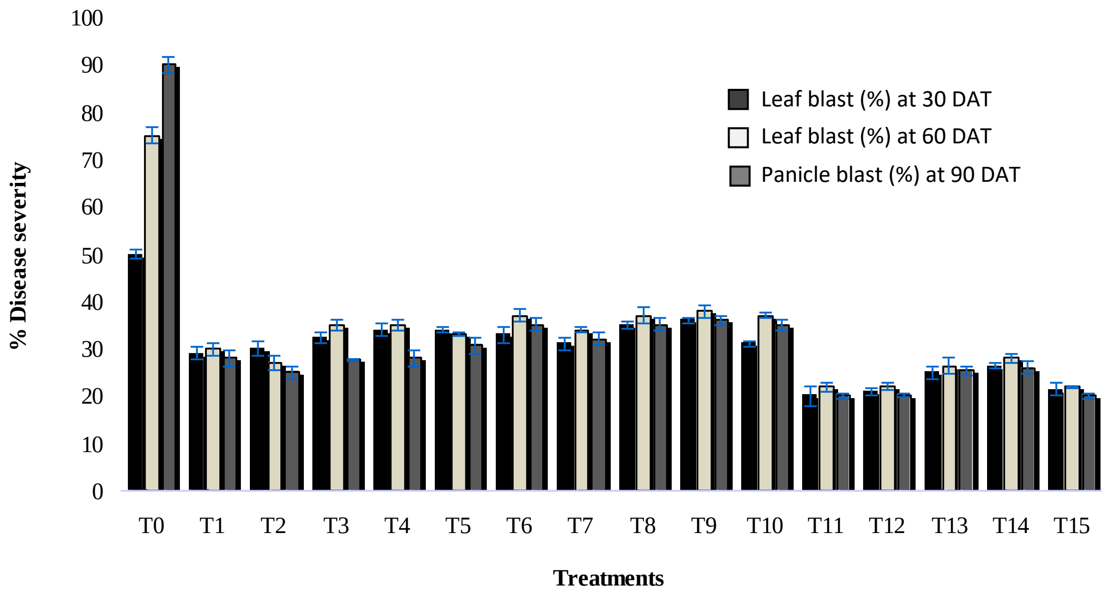

Figure 2. Effect of different treatments of formulated pseudomonads on the severity of blast of aromatic rice cv. Kalizira at 30, 60 and 90 DAT under natural epiphytotic condition under natural condition

NS = Non significant, ** = 1 % level of significance, CV = Co-efficient of variation, Here, T_0 = Control, T_1 = 10% BdPf-6 talc, T_2 = 10% BdPf-6 bentonite, T_3 = 1% BdPf-6 palm oil, T_4 = 1% BdPf-6 coconut oil, T_5 = 1% BdPf-6 glycerin, T_6 = 10% BdPf-7 talc, T_7 = 10% BdPf-7 bentonite, T_8 = 1% BdPf-7 palm oil, T_9 = 1% BdPf-7 coconut oil, T_{10} = 1% BdPf-7 glycerin,T_{11} = 10% BdPf-8 talc, T_{12} = 10% BdPf-8 bentonite, T_{13} = 1% BdPf-8 palm oil, T_{14} = 1% BdPf-8 coconut oil, T_{15} = 1% BdPf-8 glycerin.

Table 1. Effect of different treatments of formulated pseudomonads on the vegetative parameters of aromatic rice cv. Kalizira under natural epiphytotic condition at 30 DAT (tillering stage) and 60 DAT (booting stage)

Treatments	30 DAT			60 DAT				
	Plant height(cm)	No. of tillers/hill	No. of leaves/hill	Plant height(cm)	No. of tillers/hill	No. of panicles/hill	No. of leaves/hill	No. of infected leaves/hill
T_0	50.6	5.0	15.0	66.0d	9.0c	7.0e	55.0	14.0a
T_1	48.0	6.0	18.0	82.0ab (24.2)	13.0ab (44.4)	12.0abc (71.4)	60.0	6.0e (- 57.1)
T_2	51.3	6.0	18.0	82.0ab (24.2)	12.0abc (33.3)	12.0abc (71.4)	52.0	6.0e (- 57.1)
T_3	42.6	5.0	15.0	78.0bc (18.2)	10.0bc (11.1)	10.0cd (42.8)	50.0	11.0ab (- 21.4)
T_4	46.3	5.0	15.0	74.0c (12.1)	10.0bc (11.1)	10.0cd (42.8)	56.0	8.0cde (- 42.9)
T_5	46.0	6.0	18.0	78.6bc (19.1)	11.0abc (22.2)	9.0de (28.6)	56.7	8.0cde (- 42.9)
T_6	48.0	6.0	17.0	75.0c (13.6)	11.0abc (22.2)	11.0bcd (57.2)	55.0	10.0bcd (- 28.6)
T_7	47.0	6.0	15.0	78.0bc (18.2)	11.0abc (22.2)	11.0bcd (57.2)	55.0	10.0bcd (- 28.6)
T_8	48.0	6.0	18.0	77.7bc (17.7)	10.0bc (11.1)	9.0de (28.6)	50.0	10.0bcd (- 28.6)
T_9	48.0	6.0	15.0	75.0c (13.6)	10.0bc (11.1)	7.0e (0.0)	50.0	9.0bcde (- 35.7)
T_{10}	50.0	6.0	18.0	77.6bc (17.5)	11.0abc (22.2)	7.0e (0.0)	53.0	11.0bc (- 21.4)
T_{11}	52.0	8.0	22.0	85.0a (28.8)	14.0a (55.6)	13.0ab (85.7)	65.0	6.0e (- 57.1)
T_{12}	50.0	7.0	18.0	87.0a (31.8)	12.0abc (33.3)	14.0a (100.0)	60.0	7.0de (- 50.0)
T_{13}	48.0	5.0	15.0	82.0ab (24.2)	11.0abc (22.2)	12.0abc (71.4)	55.0	6.0e (- 57.1)
T_{14}	48.0	5.0	18.0	75.0c (13.6)	10.0bc (11.1)	11.0bcd (57.2)	60.0	8.0cde (- 42.2)
T_{15}	50.0	6.0	15.0	82.0ab (24.2)	12.0abc (33.3)	10.0cd (42.8)	50.0	6.0e (- 57.1)
Level of significance	NS	NS	NS	**	**	**	NS	**
CV (%)	7.8	17.0	14.4	13.0	16.7	20.0	11.2	20.0

NS = Non significant, ** = 1 % level of significance, CV = Co-efficient of variation, Here, T_0 = Control, T_1 = 10% BdPf-6 talc, T_2 = 10% BdPf-6 bentonite, T_3 = 1% BdPf-6 palm oil, T_4 = 1% BdPf-6 coconut oil, T_5 = 1% BdPf-6 glycerin, T_6 = 10% BdPf-7 talc, T_7 = 10% BdPf-7 bentonite, T_8 = 1% BdPf-7 palm oil, T_9 = 1% BdPf-7 coconut oil, T_{10} = 1% BdPf-7 glycerin, T_{11} = 10% BdPf-8 talc, T_{12} = 10% BdPf-8 bentonite, T_{13} = 1% BdPf-8 palm oil, T_{14} = 1% BdPf-8 coconut oil, T_{15} = 1% BdPf-8 glycerin. Figures in the parentheses indicate % increase or decrease over control treatment.

Table 2. Effect of different treatments of formulated pseudomonads on the vegetative parameters of aromatic rice cv. Kalizira under natural epiphytotic condition at 90 DAT (maturity stage)

Treatments	Plant height (cm)	No. of tillers $hill^{-1}$	No. of panicles $hill^{-1}$	No. of diseased panicles $hill^{-1}$	No. of leaves $hill^{-1}$	No. of diseased leaves $hill^{-1}$
T_0	87.0d	9.0c	10.0bcde	8.0a	77.0cde	28.0a
T_1	100.0abc (14.9)	13.0ab (44.4)	12.0abc (20.0)	3.0de (- 62.5)	91.0ab (18.2)	13.0fg (- 53.6)
T_2	102.0ab (17.24)	12.0abc (33.3)	12.0abc (20.0)	3.0de (- 62.5)	84.0bcd (9.1)	13.0fg (- 53.6)
T_3	98.0abc (12.6)	13.0ab (44.4)	10.0bcde (0.0)	6.0bc (- 25.0)	91.0ab (18.2)	21.0b (- 25.0)
T_4	94.0bcd (8.1)	11.0abc (22.2)	10.0bcde (0.0)	8.0a (0.0)	78.0cde (1.3)	16.0de (- 42.8)
T_5	94.0bcd (8.1)	12.0abc (33.3)	9.0cde (10.0)	6.0bc (- 25.0)	84.0bcd (9.1)	17.0de (- 39.3)
T_6	105.0a (20.7)	11.0abc (22.2)	11.0abcd (10.0)	6.0bc (- 25.0)	77.0cde (0.0)	20.0bc (- 28.6)
T_7	100.0abc (14.9)	11.0abc (22.2)	11.0abcd (10.0)	7.3b (- 8.7)	77.0cde (0.0)	20.0bc (- 28.6)
T_8	98.0abc (12.6)	10.0bc (11.1)	9.0cde (- 10.0)	5.0cd (- 37.5)	70.0e (- 9.1)	21.0b (- 25.0)
T_9	95.0bcd (9.2)	9.0c (0.0)	7.0e (- 30.0)	5.0cd (- 37.5)	71.0e (- 7.8)	18.0cd (- 35.7)
T_{10}	98.0abc (12.6)	11.0abc (22.2)	7.0e (- 30.0)	6.0bc (- 25.0)	76.0de (- 1.3)	20.0bc (- 28.6)
T_{11}	105.0a (20.7)	14.0a (55.6)	13.0ab (30.0)	2.0e (- 75.0)	98.0a (27.3)	12.0g (- 57.1)
T_{12}	104.0a (19.5)	14.0a (55.6)	14.0a (40.0)	3.0de (- 62.5)	90.0ab (16.9)	11.0g (- 60.7)
T_{13}	106.0a (21.8)	14.0a (55.6)	12.0abc (20.0)	3.0de (- 62.5)	84.0bcd (9.09)	12.0g (- 57.1)
T_{14}	93.0cd (6.9)	12.0abc (33.3)	11.0abcd (10.0)	3.0de (- 62.5)	85.0bc (10.4)	16.0de (- 42.8)
T_{15}	95.5bc (9.8)	14.0a (55.6)	10.0bcde (0.0)	3.0de (- 62.5)	91.0ab (18.2)	15.0ef (- 46.4)
Level of significance	**	**	**	**	**	**
CV (%)	6.2	20.0	19.4	20.0	5.9	20.0

NS = Non significant, ** = 1 % level of significance, CV = Co-efficient of variation, Here, T_0 = Control, T_1 = 10% BdPf-6 talc, T_2 = 10% BdPf-6 bentonite, T_3 = 1% BdPf-6 palm oil, T_4 = 1% BdPf-6 coconut oil, T_5 = 1% BdPf-6 glycerin, T_6 = 10% BdPf-7 talc, T_7 = 10% BdPf-7 bentonite, T_8 = 1% BdPf-7 palm oil, T_9 = 1% BdPf-7 coconut oil, T_{10} = 1% BdPf-7 glycerin, T_{11} = 10% BdPf-8 talc, T_{12} = 10% BdPf-8 bentonite, T_{13} = 1% BdPf-8 palm oil, T_{14} = 1% BdPf-8 coconut oil, T_{15} = 1% BdPf-8 glycerin. Figures in the parentheses indicate % increase or decrease over control treatment.

Table 3. Effect of different treatments of formulated pseudomonads on the yield parameters of aromatic rice cv. Kalizira under natural epiphytotic condition at 120 DAT (harvesting stage)

Treatments	No. of panicles hill^{-1}	Total grains panicle^{-1}	No. of healthy grains panicle^{-1}	No. of diseased grains panicle^{-1}	Panicle length (cm)	Yield (t/ha)	BCR
T_0	10.0bcde	87.0e	36.0a	51.0a	23.0abc	0.75g	0.77 h
T_1	12.0abc (20.0)	100.0b (14.9)	89.0fg (147.2)	21.0fg (- 58.8)	24.0ab (4.3)	2.0c (166.7)	1.80 b (133.7)
T_2	12.0abc (20.0)	98.0bc (12.6)	78.0gh (116.7)	20.0gh (- 60.7)	21.0abcd (- 8.6)	2.01c (168.0)	1.78 bc (131.2)
T_3	10.0bcde (0.0)	90.0de (3.4)	67.0ef (86.1)	23.0ef (- 54.9)	20.0bcd (- 13.0)	1.67 d (122.7)	1.55 e (101.2)
T_4	10.0bcde (0.0)	91.0de (4.6)	63.0d (75.0)	28.0d (- 45.1)	22.0abcd (- 4.3)	1.56 de (108.0)	1.50 e (94.8)
T_5	9.0cde (- 10.0)	92.0d (5.7)	62.0d (72.2)	30.0d (- 41.2)	23.3abc (1.3)	1.45 ef (93.3)	1.35 f (75.3)
T_6	11.0abcd (10.0)	93.0d (6.9)	71.0efg (97.2)	22.0efg (- 56.8)	20.0bcd (- 13.0)	1.98 c (164.0)	1.72 c (123.4)
T_7	11.0abcd (10.0)	90.0de (3.4)	66.0e (83.3)	24.0e (- 52.9)	19.3cd (- 16.1)	1.96 c (161.3)	1.78 bc (131.1)
T_8	9.0cde (- 10.0)	92.0d (5.7)	56.0b (55.5)	36.0b (- 29.4)	18.0d (- 21.7)	1.60 de (113.3)	1.00 g (29.8)
T_9	7.0e (- 30.0)	93.0d (6.9)	60.0c (66.7)	33.0c (- 35.2)	18.3d (- 20.4)	1.57 de (109.3)	1.02 g (32.46)
T_{10}	7.0e (- 30.0)	90.0de (3.4)	60.0d (66.7)	30.0d (- 41.2)	20.0bcd (- 13.0)	1.32 f (76.0)	1.01 g (31.1)
T_{11}	13.0ab (30.0)	105.0a (20.6)	87.0h (141.7)	18.0h (- 64.7)	25.0a (8.6)	2.56 a (241.3)	1.90 a (146.7)
T_{12}	14.0a (40.0)	107.0a (22.9)	92.0i (155.5)	15.0i (- 70.6)	24.0ab (4.3)	2.50 a (233.3)	1.88 a (144.2)
T_{13}	12.0abc (20.0)	100.0b (14.9)	78.0efg (116.7)	22.0efg (- 56.8)	19.3cd (- 16.1)	2.45 a (226.6)	1.84 ab (139.0)
T_{14}	11.0abcd (10.0)	94.0cd (8.0)	69.0e (91.6)	25.0e (- 50.9)	20.0bcd (13.0)	2.23 b (197.3)	1.64 d (113.0)
T_{15}	10.0bcde (0.0)	94.0cd (8.0)	72.0efg (100)	22.0efg (- 56.8)	25.0a (8.6)	2.11 bc (181.3)	1.50 e (94.8)
Level of significance	**	**	**	**	**	**	**
CV (%)	19.4	3.2	5.8	5.8	12.8	3.0	1.2

NS = Non significant, ** = 1 % level of significance, CV = Co-efficient of variation, Here, T_0 = Control, T_1 = 10% BdPf-6 talc, T_2 = 10% BdPf-6 bentonite, T_3 = 1% BdPf-6 palm oil, T_4 = 1% BdPf-6 coconut oil, T_5 = 1% BdPf-6 glycerin, T_6 = 10% BdPf-7 talc, T_7 = 10% BdPf-7 bentonite, T_8 = 1% BdPf-7 palm oil, T_9 = 1% BdPf-7 coconut oil, T_{10} = 1% BdPf-7 glycerin, T_{11} = 10% BdPf-8 talc, T_{12} = 10% BdPf-8 bentonite, T_{13} = 1% BdPf-8 palm oil, T_{14} = 1% BdPf-8 coconut oil, T_{15} = 1% BdPf-8 glycerin. Figures in the parentheses indicate % increase or decrease over control treatment.

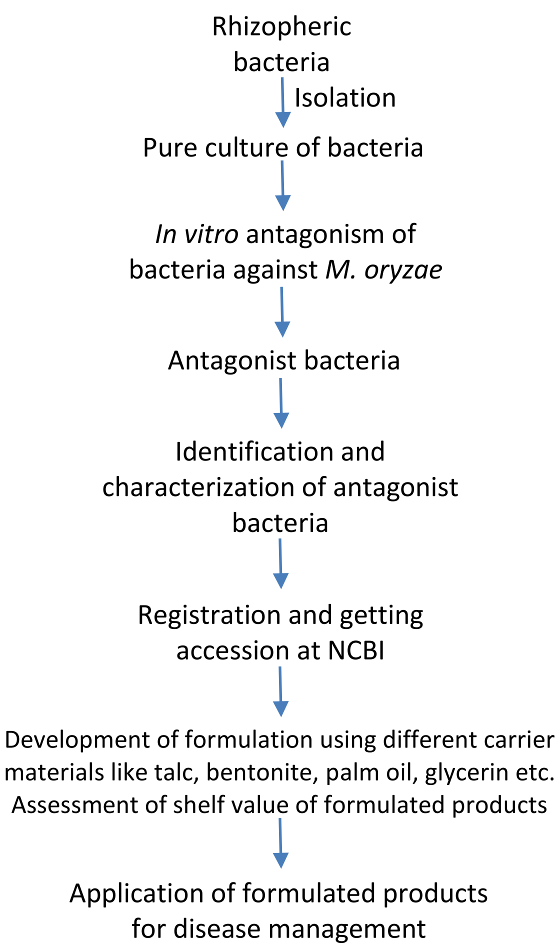

Figure 3. Flow chart of development of formulated bio-pesticides

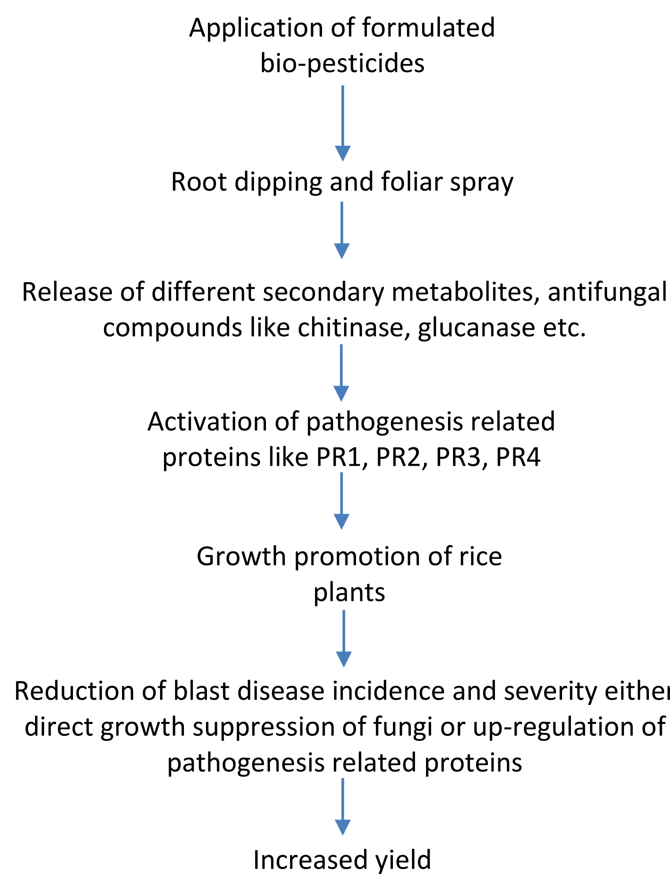

Figure 4. A hypothetical schematic diagram of mode of action of formulated bio-pesticide in blast disease management

The effect of different treatments of formulated pseudomonads on the number of panicles/hill, total grains/panicle, infected grains/panicle, healthy grains/panicle, length of the panicle, yield (t/ha) and BCR of aromatic rice cv. Kalizira was recorded at 120 DAT (Table 4). Likewise vegetative growth yield contributing characters were also significantly influenced by all treatments of formulated pseudomonads at different levels. At 120 DAT, no. of panicles/hill, total grain/panicle, no. of healthy grains/panicle, panicle length and yield were increased 40.0, 22.9, 155.5, 13.6 and 241.0% respectively over untreated control (Table 3). At 120 DAT, the highest decrement in diseased grain/panicle (70.6%) was also recorded in BdPf-8 formulated in talc and bentonite. Formulated products showed similar kinds of positive response on other yield contributing parameters like total grains/panicle, number of healthy grains/panicles, panicle length. The lowest yield (0.75 t/ha) was recorded in untreated control whether the highest yield was in T_{11} (2.56 t/ha) and T_{12} (2.50 t/ha) with highest BCR (1.90 and 1.88) (Table 3) indicating that maximum economic return can be obtained by the application of formulated pseudomonads. Additionally, formulated products significantly reduced disease parameters *viz*. number of infected leaves/hill, number of infected panicles/hill at all stages. So, these yield parameters significantly affected by all treatments, similar kind of research was also conducted by Chakraborty et al. (2020) where they applied formulated Pseudomonads on foliage against blast pathogen and found significant increment in yield and yield contributing parameters. The findings of the present study are in similar line with Lavakush et al. (2014) where they reported that different PGPRs were highly effective for increasing plant growth and yield. Singh and Sinha (2005) also reported the effect of *P. fluorescens* strains 1 and 5 against sheath blight and found that *P. fluorescens* of higher rate, *i.e.*, 8 g/L was highly effective in reducing disease severity (60.0%) and incidence (35.6%) and increasing grain yield (33.8%) and 1000-grain weight (12.9%) which also reveals the positive effect of *P. fluorescens* in the management of rice disease.

Analysis of correlation

Pearson correlation co-efficient was calculated at 30, 60 and 90 DAT to investigate the influence of disease incidence and severity on the vegetative and yield parameters under different treatment of formulated pseudomonads (Table 4). The correlation analysis revealed that disease incidence and severity was significantly and negatively correlated with (plant height, number of tillers/hill, number of leaves/hill and number of panicles/hill) at tillering, booting and ripening stage indicating the changes in plant physiology that promote vegetative growth and increase disease tolerance. Yield and yield contributing parameters of aromatic rice plants also had a significant and negative correlation with disease incidence and severity (Table 4) indicating that application of formulated pseudomonads reduces blast incidence and severity which eventually promote yield contributing parameters, yield and BCR in aromatic rice cv. Kalizira. We previously reported the significant and negative relation of vegetative and yield parameters with percent rice blast disease incidence and severity by applying formulated Pseudomonads in net house experiments (Chakraborty et al., 2020). Torres and Teng (1993) and Van Bruggen (1986) also reported similar relationship of vegetative parameters with disease incidence and severity in rice and dry bean (*Phaseolus vulgaris*) respectively.

Conclusion

The formulated product of *Pseudomonas fluorescens* showed significant effect to reduce the incidence and severity of blast disease of aromatic rice cv. Kalizira under natural epiphytotic condition at different growth stages. Both Talc and Bentonite formulation is effective for field application. The present research further emphasizes to explore the efficacy of these formulated products in aromatic rice in different agro-climatic zones of Bangladesh as well as to investigate the compatibility with commercial fungicides which might widen the scope of integration in the existing IPM schedules in rice and other cereal crops.

Acknowledgement

The study was jointly funded by the peoples' Republic of Bangladesh for awarding National Science & Technology (NST) fellowship and Ministry of Education (Project no. 2017/195/MoE), Bangladesh Agricultural University, Mymensingh, Bangladesh.

Author contribution

S.C.: Experiment set up, treatment application, manuscript draft. M.M.I.: Data collection, Analysis of data; S.S.S.: Data collection, data tabulation, developing graphs, M.A.R.K.: Design of experiment, finalizing manuscript, Supervision of experiments

Competing interests

There is no competing interest among the authors

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