Integrated Management Practices against an Emerging Bakanae Disease of Rice under the Hot-Humid Climate of Indo-Gangetic Plains of India

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Bakanae disease caused by *Fusarium fujikuroi* is emerging in India. In this work, fungicides and biocontrol agents were evaluated as seed, seedling treatment, and foliar spray(s) against bakanae disease in rice under field conditions. Carbendazim (50% WP) was found to be the most effective fungicide for seed and seedling root dip treatments. Soil drenching with 0.25% carbendazim (50% WP) 5 days before transplanting of seedlings was effective for large scale treatments. Foliar spray of 0.1% tebuconazole 50%+ trifloxystrobin 25% w/w (75 WG) significantly reduced the percentage of infected seeds (7.17%) compared with the control (32.50%). The minimum disease incidence (31%) was observed when seeds were treated with *Talaromyces flavus.* Individual treatments were integrated in the form of six management modules and evaluated under the field conditions of New Delhi (India) and Pusa Bihar (India) with the susceptible cultivars 'Pusa Basmati 1121' and 'Pusa 1176'. Bakanae disease incidence was lower in the modules tested compared with the inoculated control or the individual treatment. The most favorable results at two locations achieved the lowest disease incidence of 7% and 2.41%.

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INTRODUCTION

Rice is a major source of food for global populations. Rice farming is the largest single use of land for producing food, and the produce is consumed by nearly half of the world's population. Rice production is affected by different diseases of fungal, bacterial, viral, or nematode origin. India is the largest producer and exporter of basmati rice in the world. Pusa Basmati 1121 (PB 1121) is a landmark basmati rice variety in India. During the year 2017, the farmers cultivating PB 1121 earned an average US$ 1400/ha, making it a highly profitable enterprise. About 70% of the world basmati rice is produced in India, where it contributes 2.3 billion US$ per year in the rice export (Singh *et al.* 2018). Rice production is affected by different diseases of fungal, bacterial, viral or nematode origin. However, in India most of the released cultivars of basmati rice are moderately to highly susceptible to bakanae disease (Bashyal *et al*. 2016a). This disease (caused by *Fusarium fujikuroi*) is a threat to rice cultivation in India causing up to 40% losses in the yield (Ou

1985; Bashyal and Aggarwal 2013; Gupta *et al*. 2014; 2015; Bashyal *et al*. 2016a). It was first identified in the year 1828 in Japan. Presently, the disease is reported in all of the major rice cultivation areas of the world. In India, bakanae disease had been considered as of minor importance earlier and work has been carried out only on resistance evaluation and fungicidal management (Sharma *et al*. 1998; Pannu *et al*. 2009). However, recently high incidence (10 to 40%) of bakanae disease has been observed in basmati rice cultivars namely, Pusa Basmati 1121, Pusa Basmati 1509, Pusa 2511, CSR 30, Dehradun Basmati and Pakistani Basmati (Bashyal *et al*. 2016a) in important rice growing states of India *viz*., Punjab, Haryana and Uttar Pradesh (Bashyal *et al*. 2014), which signifies its importance in rice cultivation in near future.

The typical symptoms of bakanae disease are slender, chlorotic, and abnormally elongated primary leaves with tall lanky tillers. There is also crown rot, resulting in stunted rice plants. Infected plants usually have small numbers of tillers, and plants die in a few weeks. The pathogen is primarily seed borne in nature (Bashyal *et al*. 2020). Therefore, the most common and widely accepted practice for the management of bakanae disease is the seed treatment with fungicides. Fungicidal seed treatment with thiram, benomyl, thiram + carboxin, thiram + carbendazim, thiophanate-methyl, mancozeb, fludioxonil, prochloraz, iprodione + triticonazole, ipconazole, *etc*., 1 to 2% of seed weight is effective (Ou 1987; Tateishi *et al*. 1998; Bagga *et al*. 2007; Ghazanfar *et al*. 2009; Karov *et al*. 2009; Ora *et al*. 2011). Seedling dip treatments and nursery drenching with benzimidazole fungicides such as benlate, carbendazim, and topsin were found effective against the disease (Javed *et al*. 1996; Bhalli *et al*. 2001). Seed treatment of 0.05% propiconazole 25 EC is effective but phytotoxic, as it reduces the plant height and grain yield infield conditions (Singh *et al*. 2012). Seed dressings are the most common way of controlling rice bakanae disease. In China and Japan, the seeds are dressed by dipping them in an aqueous suspension containing fungicides (prochloraz, carbendazim, triflumizole, thiram, *etc.*). However, individual seed or seedling treatments are not sufficient, and disease appears regularly in the field conditions of India (Bashyal and Aggarwal 2013). Despite the considerable economic impact of rice bakanae disease, effective management practices are not available.

Moreover, seed dressing chemicals against bakanae disease are limited and have negative environmental impact also. The fungicides are responsible for residue problems, resistance development in pathogens and different health hazards to human beings and other living organisms. Therefore, alternative methods of control including biological seed treatments need to be integrated with fungicidal seed treatment against the disease. Several antagonistic microorganisms isolated from different sources were tested against *F. fujikuroi*, and effectiveness of biocontrol agents including *Trichoderma* spp. has been established (Halgekar *et al*. 2014). *Talaromyces* spp. proved to be broad spectrum biofungicides against different rice diseases including bakanae (Tateishi *et al*. 2006). Bacteria including *Bacillus oryzicola* YC7007 (Hossain *et al*. 2016) and yeast *Pichia guilliermondii* (Matic *et al*. 2014) are effective against the different rice diseases. Although individual modules for the disease management is present, integrated/sustainable management options against bakanae disease are not developed yet. The integration of disease management strategies could reduce disease severity, resulting in higher grain yield, increased crop productivity, and greater food security. Therefore, the present study aimed to evaluate the control alternatives against bakanae disease under the field conditions and to integrate them for the effective management of the disease and to assess their impact on grain yield and other yield attributing characters of basmati rice under severe artificial inoculum pressure conditions of India.

EXPERIMENTAL

Source of Pathogen, Biocontrol Agents and Rice Cultivars

The virulent *Fusarium fujikuroi* isolate "F250" (GenBank Accession no. KM50526; MBPO00000000) isolated from symptomatic bakanae infected rice plant in Hisar, Haryana (India) was used for the study (Bashyal *et al*. 2016a; 2017; 2020). The pathogen was sub-cultured on Potato Dextrose Agar (potato 200 g, dextrose 20 g, agar 20 g, and water 1000 mL) medium. The cultured plates were incubated at 25±1 °C in the dark for 8 days to allow for growth and sporulation (Bashyal *et al.* 2016a; 2017; 2020). Biocontrol agents *Talaromyces flavus* Tf2 (Rawat *et al*. 2022), *Chaetomium globosum* Cg2 (Aggarwal *et al*. 2004); *Trichoderma harzianum* Th3 (Sharma *et al*. 2012), *Pseudomonas fluorescence* DTPF3 (Singh *et al*. 2017) were taken from Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi. Seeds of rice cultivar 'Pusa Basmati 1121' were procured from Division of Genetics, IARI, New Delhi and seeds of 'Pusa 1176' were taken from IARI, Pusa Bihar. Individual treatments were evaluated in rice cultivar "Pusa Basmati 1121" during the year 2018 and management modules were designed. The modules were evaluated at the research fields of ICAR-IARI, New Delhi (located at 320 N latitude and 770 E longitudes at an altitude of 1900 M) and IARI, Pusa Bihar (located between 25.980 N latitude and 85.670 E longitudes and altitude of 52.0 m) during the *Kharif* season (June-November) of years 2019 and 2020. For which inoculated rice seeds were sown on 20.06.2019 and 02.07.2020 at the IARI, New Delhi, whereas, it was sown on 26.06.2019 and 10.07.2020 at the IARI, Pusa Bihar. The rice-wheat cropping system has been practiced at the research sites since many years. The rice-wheat rotation is the principal cropping system in south Asian countries. It occupies an area of 9.77 million hectares (Yadav 1996), and it dominates agricultural systems in India, mostly in Punjab, Bihar, Haryana, Uttar Pradesh and Madhya Pradesh.

Evaluation of Selected Fungicides and Biocontrol Agents for Seed Treatments

The *F. fujikuroi* isolate "F250" was inoculated for multiplication in sterilized sorghum grains and incubated at 25 °C for 15 days. A spore suspension was prepared with sterile distilled water using 15 days old culture of *F. fujikuroi* and filtered through two layers of sterile muslin cloth and brought to a final concentration of 10^6 spores mL^{-1} for the seed inoculation. The seeds of susceptible cultivar "Pusa Basmati 1121" were first surface sterilized with 1% (v/v) sodium hypochlorite solution, followed by washing with sterile distilled water and soaked into the *F. fujikuroi* inoculum suspension for 18 hours at room temperature (25 °C). Seeds were washed with sterilized water and dipped 0.2% (2.0 g L^{-1} of water) suspension of fungicides *viz*., carbendazim (50% WP), carbendazim 12% + mancozeb 63% WP, captan 50% WP, tricyclazole 75% WP, thiram 75% WP and mancozeb 75% WP separately for 24 h. Similarly, 15 days old cultures of biocontrol agents *viz*., *T. flavus* (Tf2), *C. globosum* (Cg2), *T. harzianum* (Th3), and *P. fluorescence* (DTPF3) were multiplied in sterilized sorghum grains taken for seed treatment. Spore suspensions of biocontrol agents prepared separately and filtered through double layered muslin cloth and concentration of spores were adjusted to 1×10^7 spores mL^{-1}. Seeds inoculated with pathogen were dipped in biocontrol suspension for 24 h. Treated seeds were further sown in nursery bed prepared in glasshouse. Twenty-one days old nursery of the "Pusa Basmati 1121" treated with different fungicides and biocontrol agents were uprooted carefully and transplanted at experimental field of ICAR-IARI. The normal agronomical practices with recommended doses of fertilizers (N: P_2O_5: K_2O: 120: 60: 60 kg ha^{-1}) were followed. Plots were kept weeds free through manual weeding. Bakanae disease observations were taken at 15 days interval until harvesting. Eighty plants were transplanted in 2 x 2 m^2 plots all the treatments and number of plants were the same for all replications. Final disease incidence was calculated as number of plants infected/total number of plants transplanted × 100.

Evaluation of Selected Fungicides and Biocontrol Agents for Seedling Treatment

Healthy surface sterilized seeds of susceptible cultivar "Pusa Basmati 1121" were sown in sterilized soil under glasshouse conditions for seedling rising. Twenty-one days old seedlings were uprooted carefully and washed with sterilized water. Further, root tips (approx. 2 cm in length) of rice seedlings were cut with sterilized scissors and kept in *F. fujikuroi* spore suspension for 12 h. Thereafter, seedlings were dipped in fungicidal suspensions *viz*., 0.1% (1g L^{-1} of water) of carbendazim 50% WP, 0.2% of carbendazim 12% + mancozeb 63% WP, 0.2% of tricyclazole 75% WP, 0.1 % of propiconazole 25% EC, 0.2% of hexaconazole 5% SC, and 0.3% of copper oxychloride 50%WP separately for 12 h. Seedlings inoculated with pathogen only were taken as the control. Inoculated seedlings were transplanted under field conditions. Similarly, biocontrol agents *T. flavus*, *C. globosum*, *T. harzianum*, and *P. fluorescence* (Tf2, Cg2, Th3 and DTPF3) were taken for the seedling treatment. Seedlings already inoculated with spore suspension of *F. fujikuroi* as described above were dipped in spore suspension of biocontrol agents at the concentration of $1x10^7$ spores mL^{-1} for 12 h and transplanted under field conditions. Observations on percent bakanae affected plants were recorded at 15 days interval until harvesting as described above.

Effect of Nursery Drenching on Bakanae Disease

Seeds of susceptible rice cultivar "Pusa Basmati 1121" were inoculated with virulent isolate of *F. fujikuroi* and sown in nursery beds of 2×2 m^2. The different concentration suspensions (0.1%, 0.15%, 0.2%, 0.25%, and 0.3%) of carbendazim 50% WP were prepared and drenched in pre-inoculated nursery (1 L fungicidal suspension was used for 1 m^2) at 7, 5 and 3 days before uprooting. Further, seedlings were uprooted carefully and transplanted at IARI field. Observations for the bakanae disease incidence were recorded. Seeds harvested from drenching treatment *i.e.*, 0.25% carbendazim 50% WP were sent to the pesticide residue analysis laboratory, Division of Agricultural Chemicals, ICAR-IARI, New Delhi.

Effect of Fungicidal Spray on Bakanae Disease of Rice

Rice seedlings were inoculated with *F. fujikuroi* suspension concentration of 10^3 spores/mL of water (rice seedlings inoculated with 10^6 spores/mL could not survive up to later stages; therefore, a reduced spore concentration of pathogen *i.e.*,1×10^3 spores mL^{-1} of water was taken for this experiment). *F. fujikuroi* inoculated seedlings were transplanted in the field in different plot sizes of 2×2 m^2. Inoculated plants were given the 2 foliar sprays of different fungicides *viz*., 0.3% of copper oxychloride 50% WP, 0.1% of carbendazim 50% WP, 0.1% of propiconazole 25% EC, 0.1% of tebuconazole 50%+ trifloxystrobin 25% w/w (75 WG) at heading stage at 15 days interval. Only *F. fujikuroi* inoculated plants were kept as control. Observations for bakanae disease incidence was taken as described above.

Development of Bakanae Disease Management Modules

Based on individual field experiments most effective fungicide identified was used for the seed and seedling treatments including nursery drenching. *T. flavus* and *P. fluorescens* were selected for seed treatment individually. Six different bakanae disease management modules were developed taking best individual treatments in combination along with inoculated control and tested under field conditions (Table 1). The pathogen, fungicides, and biocontrol agents were inoculated as described above. These modules were evaluated under the field conditions of ICAR-IARI, New Delhi and ICAR-IARI, Pusa Bihar taking the popular cultivars of respective area *i.e*., 'Pusa Basmati 1121' at New Delhi and 'Pusa 1176' at Pusa Bihar, India. Yield attributes such as root length, shoot length, plant height, panicle length, no. of grains per panicle, yield (g m^{-2}) were also evaluated for each module at IARI (in inoculated control maximum plants died before heading. Observations were taken for the surviving plants.

Table 1. Bakanae Disease Management Modules Formulated and Evaluated in the Study

Module (No.)	Treatments
1	Seed treatment (*T. flavus*) + seedling treatment (soil drenching in 0.25% of Carbendazim 50% WP) + foliar spray (0.1% of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)
2	Seed treatment (*P. fluorescence*) + seedling treatment (soil drenching in 0.25% of Carbendazim 50% WP) + foliar spray (0.1% of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)
3	Seed treatment (*T. flavus*) + seedling treatment (root dip in 0.1% of Carbendazim 50% WP) + foliar spray (0.1 % of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)
4	Seed treatment (*P. fluorescence*) + seedling treatment (root dip in 0.1% of Carbendazim 50% WP) + foliar spray (0.1% of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)
5	Seed treatment (0.2% of Carbendazim 50% WP) + seedling treatment (root dip in 0.1% of Carbendazim 50% WP) + foliar spray (0.1% of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)
6.	Seed treatment (0.2% of Carbendazim 50% WP) + seedling treatment (soil drenching in 0.25% of Carbendazim 50% WP) + foliar spray (0.1% of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)
7.	Inoculated control

Statistical Analysis

The experimental data were generated through randomized block design (RBD). Disease incidence was calculated based on 80 plants of each replication. Individual experiments on fungicide and biocontrol evaluation were conducted in three replications for one year, *i.e.*, 2018, and seven modules were tested in five replications as per the availability of resources at the experimental fields. Two years research field data of 2019 and 2020 were used for the data analysis. The statistical tools like basic statistics and ANOVA is used in SAS 9.3 software. The Tukey test was used to compare the means of disease incidence at 5% level of significance. Principal Component Analysis (PCA) was performed on normalized data between seven modules (treatments) and rice parameters in PASTv.3 software to find out the major effect on yield parameter/component associated with the bakanae disease management module.

RESULTS AND DISCUSSION

Effect of Seed and Seedling Treatment with Fungicides on Bakanae Disease

Out of five fungicides used for the seed treatment against bakanae disease, carbendazim 50% WP was observed to be the most effective with minimum disease incidence of 18% (75% disease reduction compared to inoculated control) followed by captan 50% WP (38% disease incidence) and carbendazim 12% + mancozeb 63% WP. (46.5% disease incidence) Mancozeb 75% WP was observed to be least effective followed by thiram 75% WP with the disease incidence of 78.2% and 74.25% respectively (Fig. 1A and Fig. 2). Among six fungicides evaluated as seedling treatment, minimum disease incidence (30%) was observed with carbendazim 50% WP followed by propiconazole 25% EC (disease incidence 40%). Disease incidence of 70.2% was observed with tricyclazole 75% WP and it was 64.5% when treated with copper oxychloride 50% WP (Fig. 1B).

Fungicide seed treatments that were user friendly and having lower impact on non-target animals with recommended concentrations were selected for disease management experiments. Among different fungicides evaluated against the bakanae disease carbendazim was proved to be significantly the most effective as seed treatment, seedling treatment (Bagga and Sharma 2006) and soil drenching (Hossain *et al*. 2015). However, it could not control the disease completely. This may be due to complex survival nature of the *F. fujikuroi* in rice seed. Sunani *et al* (2019) reported *F. fujikuroi* can survive in seed coat, glumes, and embryo of rice seed. Therefore, only seed treatment with carbendazim may not be sufficient to penetrate the embryo of rice seed.

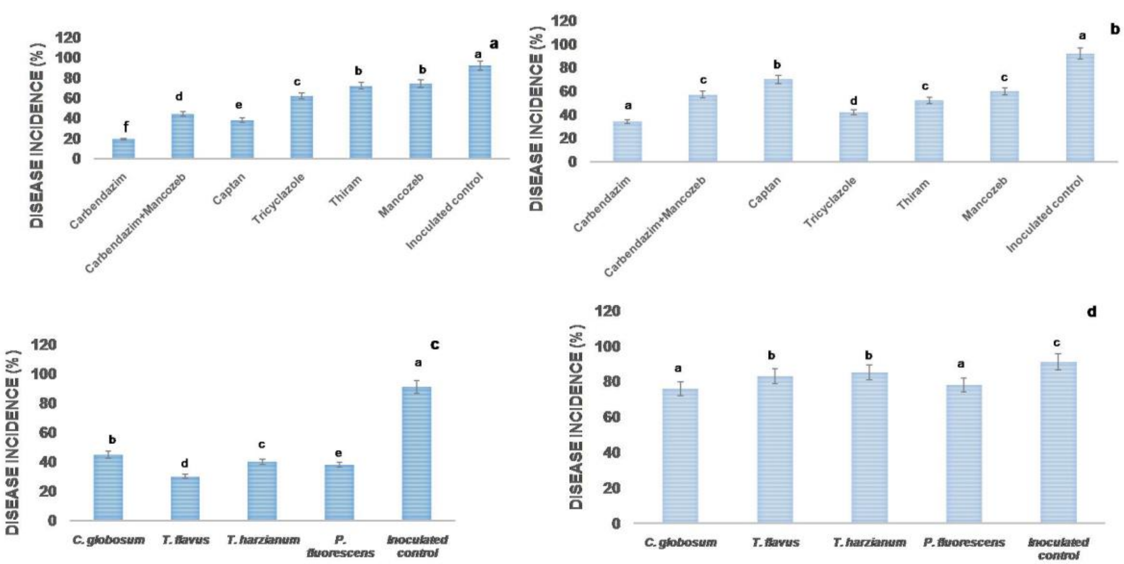

Fig. 1. Effect of different treatments on bakanae disease incidence A) Fungicides evaluated as seed treatment; B) Fungicides evaluated as seedling treatment; C) Biocontrol evaluated as seed treatment; D) Biocontrol evaluated as seedling treatment with significance level ($p<0.05$).

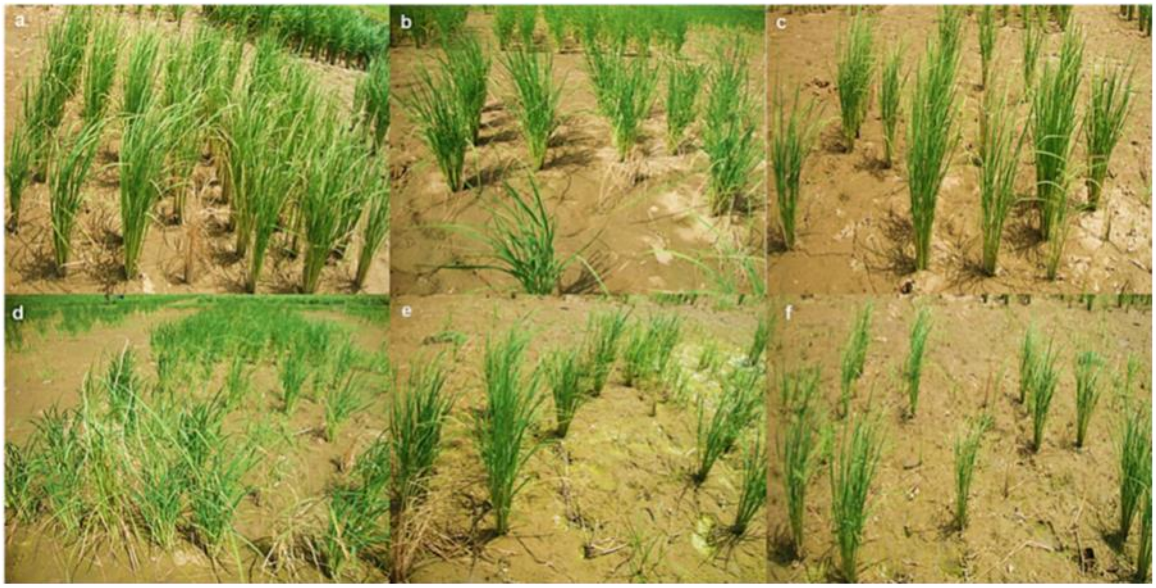

Fig. 2. Effect of seed treatment with different fungicides on bakanae disease incidence under field conditions **A**: Carbendazim; **B**: Carbendazim + Mancozeb; **C**: Captan; **D**: Tricyclazole; **E**: Thiram; **F**: Mancozeb

Effect of Seed and Seedling Treatments with Biocontrol Agents on Bakanae Disease

Out of five biocontrol agents tested as seed treatment against the bakanae disease, minimum disease incidence (31%) was observed with *T. flavus,* followed by *P. fluorescens, T. harzianum*, and *C. globosum* with disease incidence of 38%, 40% and 45%, respectively (Fig. 1C). Differences in bakanae disease incidence were observed when seedlings were treated with biocontrol agents (Fig. 1D). Minimum disease incidence was observed when seedlings were treated with *P. fluorescens* (78.14%) followed by *T. flavus* (83%) and *T. harzianum* (85%). Overall, results indicated that bakanae disease incidence was more when seedlings were treated with biocontrol agents compared to fungicidal treatments.

All the selected biocontrol agents could reduce the disease incidence as seed treatment. Maximum disease reduction was observed for the *T. flavus* and *P. fluorescens*. According to Kato *et al*. (2012) and Miyake *et al*. (2012) *Talaromyces sp.* occupies the same region of coleoptiles and roots as *F. fujikuroi* parasitize the pathogen directly reducing the disease incidence. *P. fluorescens* also produces secondary metabolites and promotes the growth of the rice plants (Kazempour and Elaninia 2007). However, mode of action of *T. flavus* (Tf2) and *P. fluorescens* used in the present investigation needs to be explored. For seedling treatment *P. fluorescens* was most effective against the bakanae disease. However, disease incidence for the seedling treatment was higher compared to seed treatment and it was less effective in comparison to fungicides used. Therefore, biocontrol agents were used only for the seed treatment in the developed modules.

Effect of Nursery Drenching against Bakanae Disease

Nurseries of paddy seedlings were drenched before uprooting with different concentrations of carbendazim 50% (WP) to observe their effect on bakanae disease incidence. A significant difference in bakanae disease incidence was observed when soil was drenched with carbendazim 50% (WP) 5 days before the nursery uprooting (Table 2).

Minimum disease incidence was recorded at the dose of 0.3% (disease incidence 13.16%) followed by 0.25% of carbendazim 50% WP (disease incidence 15.14%) with nursery drenching at 7 days before uprooting.

Table 2. Effect of Nursery Drenching with Carbendazim 50% WP on Bakanae Disease Incidence of Rice Cultivar 'Pusa Basmati 1121'

Carbendazim concentration (g/L of water)	Disease incidence (%) (Fungicide applied days before nursery uprooting)			Carbendazim residue in seeds* (mg/kg)
	3 days	5 days	7 days	
1.0 (0.1%)	60.5 ± 1.63^{b}	56 ± 0.81^{c}	55 ± 2.44^{cd}	0.05^{b}
1.5 (0.15%)	52 ± 1.63^{de}	50 ± 0.07^{e}	49.16 ± 0.84^{e}	0.09^{c}
2.0 (0.2%)	40 ± 1.65 ^{f}	27.53 ± 0.84 ^{h}	26.83 ± 0.94 ^{h}	0.09^{c}
2.5 (0.25%)	33 ± 2.44^{i}	17.53 ± 2.04^{ij}	15.16 ± 0.47^{ij}	0.15^{d}
3.0 (0.3%)	27 ± 1.63^{h}	15 ± 0.81^{j}	13.16 ± 0.84 ^{ij}	0.17^{e}
Control	75.33±0.81^{a}	76±1.24^{a}	75.16±0.86^{a}	0.00^{a}

where*: Harvested seeds evaluated for the carbendazim (2.5g/L) drenching at 5 days before uprooting treatment; ±: standard deviation. Alphabets letter showed the significance ($p<0.05$) difference between the treatments at different days 3, 5 and 7 days respectively.

Similarly, minimum disease incidence 15% and 27% was observed with 0.3% carbendazim 50% WP when applied at 3 and 5 days before uprooting, respectively. Since the disease incidence was non-significant at 5 days and 7 days treatments; harvested seeds received from drenching before 5 days of uprooting treatment were evaluated for the fungicide residue analysis. Carbendazim residue was estimated at par with 0.05 to 0.09 mg kg^{-1} of seed in 1.0, 1.5, and 2.0 g L^{-1} of water treatments; however, it differed significantly and showed concentration of 0.15 and 0.17 mg/kg of seed in 2.5 and 3 g L^{-1} treatments, respectively (Table 2).

Bakanae disease can appear throughout the crop growth stages in nursery, transplanted paddy field up to panicle emergence stage and affect grain filling also. Therefore, along with seed treatment the effect of seedling dip and nursery drenching were also evaluated in present study. Carbendazim was also identified as effective fungicide for the seedling treatment. However, rice seedling treatment is difficult for the resource limited farmers. Therefore, nursery drenching could be good alternative option for the bakanae disease management. Carbendazim was reported with limited mobility in soil (0-5 cm layer) both in dry and wet soil conditions with 60 to 80% of fungicide degradation at 4 weeks (Solel *et al.* 1979). Therefore, carbendazim was selected and in the present investigation nursery drenching was optimized. In the present investigation nursery drenching was optimized with 0.25% of 50% carbendazim WP before 5 days of uprooting which could reduce the disease at maximum level.

Effect of Fungicidal Spray against Bakanae Disease

Inoculated plants were given the two sprays of different fungicides at 15 days interval which reduced the bakanae disease incidence in the field (Table 3). Minimum disease incidence (50%) was observed with the tebuconazole 50% + trifloxystrobin 25% w/w (75 WG) followed by carbendazim 50% WP (disease incidence 55%). The effect of fungicide spray(s) on seed infection after harvest was also analyzed through agar plate method of International Seed Testing Association (ISTA). Significant difference was observed for the seed infection when fungicidal spray was given. Seed infections were minimum (7.17%) with the foliar spray of tebuconazole 50% + trifloxystrobin 25% w/w (75 WG) followed by carbendazim 50% WP (17%) and propiconazole 25% EC (18.06%).

Table 3. Effect of Fungicidal Sprays on Bakanae Disease Incidence of Rice Cultivar Pusa Basmati 1121

Fungicide (Doses)	Disease Incidence (%)	Rice Seeds Infected with *Fusarium* spp. (%)
Copper oxychloride 50% WP (0.3%)	60.00 ± 4.20	21.70 ± 1.66
Propiconazole 25% EC (0.1%)	58.00 ± 3.25	18.06 ± 2.50
Carbendazim 50% WP (0.1%)	55.00 ± 3.33	17.00 ± 2.14
Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG (0.1%)	50.00 ± 5.66	7.17 ± 1.05
Inoculated control	75.00 ± 5.35	32.50 ± 1.57

±: Standard deviation

Among different chemical fungicides evaluated as spray application, 0.1% of tebuconazole 50% + trifloxystrobin 25% w/w (75 WG) was comparatively effective, with 33.3% bakanae disease reduction in the field followed by carbendazim. Carbendazim's mode of action is based on the inhibition of *β-tubulin* polymerization and suppressing microtubule assembly during mitosis, which typically leads to cell cycle arrest and apoptosis of affected cells, thereby adversely affecting fungal growth. Tebuconazole is a *demethylase inhibitor* (DMI), which interferes in process of building the structure of fungal cell wall. Finally, it inhibits the reproduction and further growth of fungus. Trifloxystrobin interferes with respiration in plant pathogenic fungi. The data revealed that fungicidal spray application could not considerably reduce the disease incidence in the field, but it proved to be helpful in reducing the bakanae in next season by reducing the seed infection. The fungus produces conidia on diseased plants and its high production coincides with flowering and ripening of rice, when the conidia are able to infect or contaminate the seeds (Ou 1985). Therefore, spray at flowering stage may have reduced the seed infections in the field.

Effect of Management Modules against Bakanae Disease of Rice

Based on the observations of individual treatment against bakanae disease, six different modules were formulated integrating the most effective individual treatment. These modules were evaluated at two different locations *i.e.*, IARI, New Delhi, and IARI, Pusa Bihar taking two different locally popular cultivars *i.e.*, 'Pusa Basmati 1121' and 'Pusa 1176' for the two consecutive rice seasons, 2019 and 2020 (Table 4; Fig. 3). On an average at IARI, New Delhi, minimum disease incidence (7%) was observed with module 5 [Seed treatment (0.2% of carbendazim 50% WP) + seedling treatment (root dip in 0.1% of carbendazim 50% WP) + foliar spray (0.1 % of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)], followed by module 6 (10.75%). At IARI, Pusa Bihar also minimum disease incidence was observed for module 5 (2.41%) followed by module 6 (4%) and module 3 (8%).

Table 4. Effect of Different Management Modules on Bakanae Disease Incidence in Different Rice Genotypes at IARI Delhi and Pusa Bihar

Module No.	Description	Percent disease incidence (cv. 'Pusa 1121') *	Percent disease incidence (cv. 'Pusa 1176') *
M1	Seed treatment (*T. flavus*) + seedling treatment (soil drenching in 0.25% of Carbendazim 50% WP) + foliar spray (0.1 % of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)	22.75±0.50[bc]	11.16±2.01[c]
M2	Seed treatment (*P. fluorescence*) + seedling treatment (soil drenching in 0.25% of Carbendazim 50% WP) + foliar spray (0.1 % of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)	26.083±1.01[b]	14.75±1.50[b]
M3	Seed treatment (*T. flavus*) + seedling treatment (root dip in 0.1% of Carbendazim 50% WP) + foliar spray (0.1 % of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)	21.91±0.54[bc]	8.0±0.87[d]
M4	Seed treatment (*P. fluorescence*) + seedling treatment (root dip in 0.1% of Carbendazim 50% WP) + foliar spray (0.1 % of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)	23.375±1.00[b]	10.0±0.98[cd]
M5	Seed treatment (0.2% of Carbendazim 50% WP) + seedling treatment (root dip in 0.1% of Carbendazim 50% WP) + foliar spray (0.1 % of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)	7±0.50[c]	2.41±0.38[e]
M6	Seed treatment (0.2% of Carbendazim 50% WP) + seedling treatment (soil drenching in 0.25% of Carbendazim 50% WP) + foliar spray (0.1 % of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG)	10.75±0.45[bc]	4.0±0.87[e]
M7	Inoculated control	93.25±1.73[a]	57.01±1.46[a]

*Average ± standard deviation of percentage disease incidence with significance level ($p<0.05$)

Yield attributes *viz*., no. of tillers/plant, total plant height, panicle length, grains/panicle, root length, dry weight, and yield, were also recorded from each replication in different modules developed. For 'Pusa Basmati 1121' maximum plant height was observed in inoculated control (92.16 cm), followed by module 6 (90.33cm), module 2 (88.75 cm), module 3 (86.41 cm), and module 5 (86.08 cm). Maximum nos. of tillers/plant were observed for the module 4 (31) in both the years *i.e.*, 2019 and 2020 followed by module 2 (30) and module 1 (28.50). Panicle length varied between 21.25 cm to 23.41 cm. Maximum length of the panicle was observed for the inoculated control (24.0 cm) whereas, minimum for module 5 (21.83 cm). Maximum yield (351.25 g m^{-2}) was observed for module 5, followed by module 6 (311.66 g m^{-2}) and module 4 (271.66 g m^{-2}).

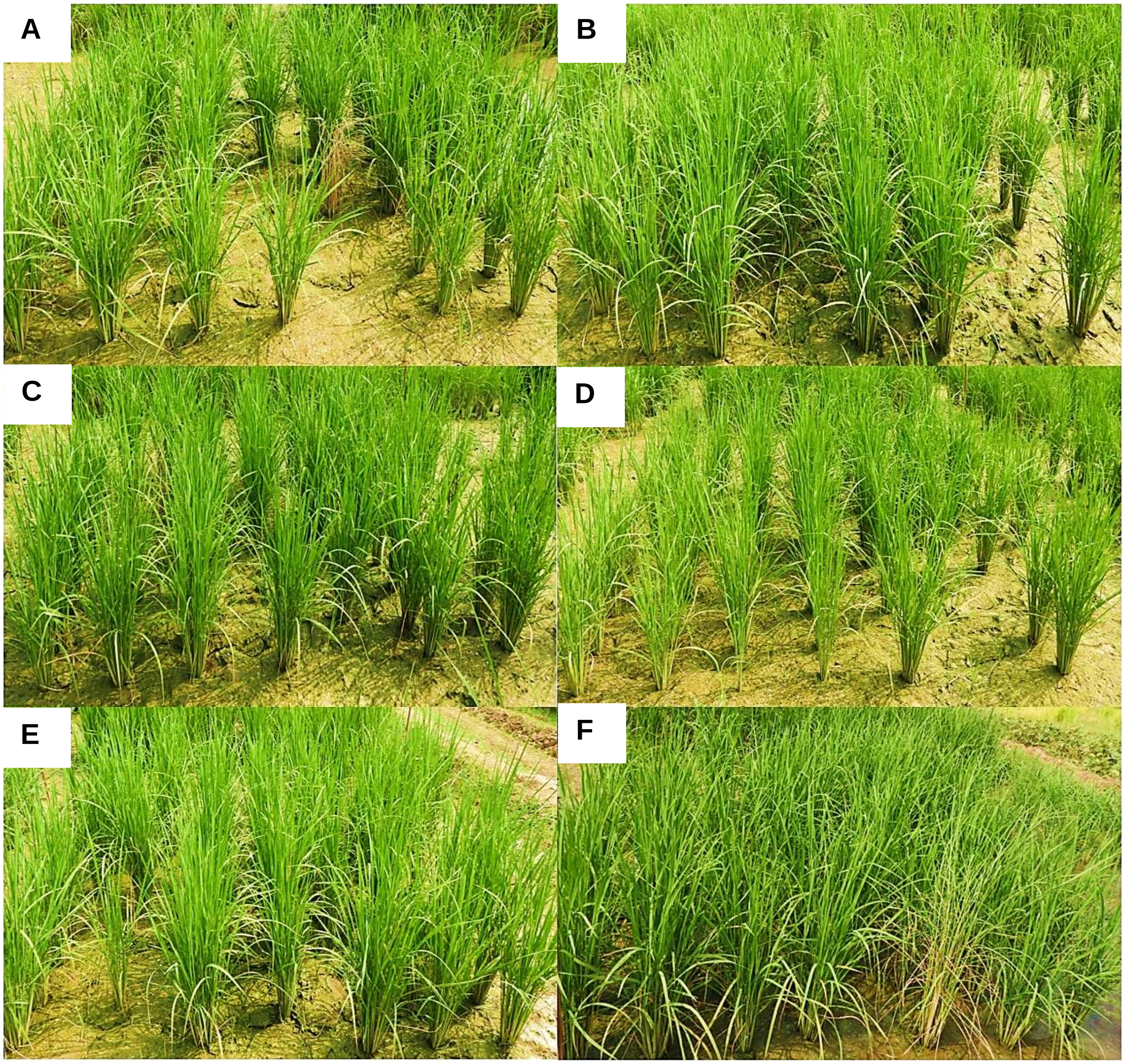

Fig. 3. Effect of different management modules on bakanae disease incidence in the year 2019 at ICAR-IARI, New Delhi; **A**: module 1; **B**: module 2; **C**: module 3; **D**: module 4; **E**: module 5; **F**: Module 6

Principal component analysis (PCA) was performed with the six modules (treatments), including inoculated control taking different parameters (root length, shoot length, plant height, panicle length, grains per panicle, yield, dry weight, and tiller number per plant). Results showed principal component 1 (PC 1) explaining 56.12% and principal component 2 (PC 2) explaining 20.6% variability in the data sets. Module 1, module, and module 7 strongly correlated with PC 1 and Module 5 and Module 6 are strongly correlated with PC 2. Observation from biplot showed that module 1, module 4, module 5 and module 6 influencing tiller numbers per plant, yield, grains per panicle and root length respectively (Fig. 4).

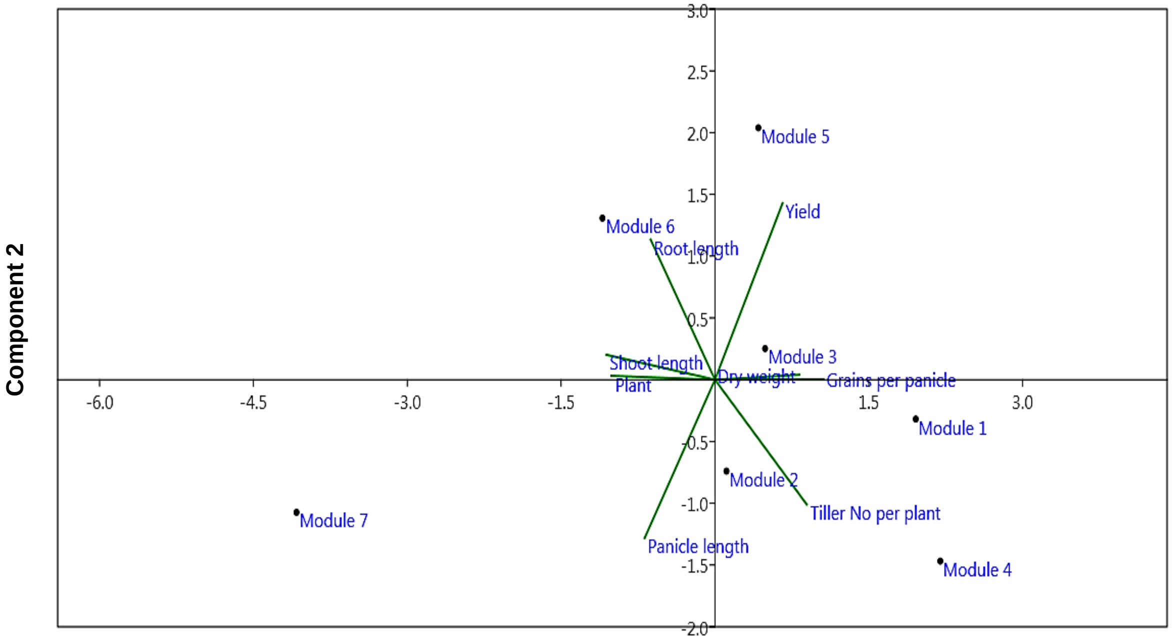

Fig. 4. PCA Biplot between six modules (treatments) along with inoculated control with different rice parameters

Based on the individual observations, the most effective treatments were selected and integrated in bakanae disease management modules. Modules 5 and 6 were most effective for the bakanae disease management. All the developed modules reduced the bakanae disease incidence and improved associated agronomic traits in rice. However, PCA analysis suggested that the main increase in yield is due to increase in number of plants. Disease incidence was low for the modules 5 and 6, for which only chemicals were used for the bakanae disease management compared to modules where biocontrol was used for the seed treatments. However, improved agronomic traits, such as a greater number of tillers, higher dry weight, and filled grains were observed for the modules 1, 2, 3, and 4 where biocontrol was used suggesting the plant growth promoting effect of biocontrol agents. Seedling dip modules were observed to be more effective in disease management compared to nursery drenching. Seedling dips are more targeted to the root surfaces compared to nursery drenching which may have reduced the disease incidence in root dip treatments. The performances of all the modules in term of disease reduction and yield enhancement were at par in both the locations, *i.e*., ICAR-IARI New Delhi and ICAR-IARI Pusa Bihar. However, disease incidence was high in Pusa Basmati 1121 compared to Pusa 1176 although same isolate of pathogen was used for the inoculation. Pusa Basmati 1121 is most susceptible cultivar against bakanae disease of rice (Bashyal *et al*. 2016 a, b) and highest disease incidence was reported mainly in basmati growing hot humid conditions of India. Therefore, growing cv., Pusa Basmati 1121 in hot humid conditions (New Delhi, India) might have supported higher incidence of the bakanae disease. When integrated the management practices, disease reduction percent was increased and modules were found effective against the bakanae disease in ICAR-IARI, New Delhi and Pusa Bihar on susceptible cultivar ‘Pusa basmati 1121’ and ‘Pusa 1176’, respectively. The combined treatments brought the coherence of integrated disease management practices for reduction of bakanae disease and seed infections along with enhanced plant growth, grain yield and its attributes. The farmers of this region should adopt these integrated practices as a part of holistic crop management approach to achieve yield stability and sustainability in rice production under rice-wheat cropping system. However, weather conditions, soil types, soil inoculum, and crop varieties may influence the performance of these modules which is required to be validated with different varieties at different locations.

CONCLUSION

1. Carbendazim (50% WP) was found to be the most effective fungicide as seed and seedling treatment against the bakanae disease of rice. Soil drenching treatment was most effective when 0.25% (2.5 g L^{-1} water) carbendazim (50% WP) was applied at 5 days before the uprooting of nursery.
2. Foliar spray of 0.1% tebuconazole 50%+ trifloxystrobin 25% w/w (75 WG) significantly reduced the disease. Biocontrol agents *T. flavus* (Tf2) and *P. fluorescence* (DTPF3) could reduce the disease incidence effectively as seed treatment.
3. Integrated management module [Seed treatment (0.2% of carbendazim 50% WP) + seedling treatment (root dip in 0.1% of carbendazim 50% WP) + foliar spray (0.1 % of tebuconazole 50% + trifloxystrobin 25% w/w 75 WG)] was most effective for the bakanae disease management.
4. The findings of this investigation elucidate the role of integrated management practices in decreasing the bakanae disease incidence and increasing the agronomic performance of rice in Northern India which needs to be validated in different locations in different varieties.

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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.

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