



## Management of bakanae disease using fungicides and their effect on disease symptomatology

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Received: 9 July 2021; Accepted: 13 July 2022

### ABSTRACT

Bakanae disease is an emerging problem of Basmati rice (*Oryza sativa* L.) in India and most of the basmati rice varieties are susceptible to this disease. Present study was carried out to evaluate and identify effective fungicide against bakanae disease of rice for which 12 fungicides (single and in combination) were evaluated against the bakanae disease as seedling treatments during the rainy (*kharif*) season of the year 2019 and 2020. Minimum disease incidence was observed with the fungicides Carbendazim 50% WP (19.47%), and Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG (25.71%) during the years of evaluation with maximum yield. Area under disease progress curve was minimum (621.58) with the treatment of Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG followed by Carbendazim 50% WP (671.30). Differences in bakanae disease symptoms were observed in different treatments. Among treatments, elongated tillers as major symptoms were observed in Mancozeb 75% WP, Zineb 68% + Hexaconazole 4% and Azoxystrobin 23% SC treated plants. Whereas, tiller elongation with rotting was prominent in Kitazin 48% EC treated and inoculated control plants. In case of Carbendazim 50% WP, Tebuconazole 8% + Captan 32% SC treatments, detachment of tillers was prominent. Therefore, seedling treatment with fungicides was observed to be effective in reducing bakanae disease incidence with low AUDPC and high yield compared to inoculated control.

**Keywords:** Bakanae, Fungicides, Management, Seedling dip, Symptoms

Bakanae disease of rice (*Oryza sativa* L.), also known as foolish seedling disease, is caused by the ascomycetous fungus, *Fusarium fujikuroi* Nirenberg (teleomorph: *Gibberella fujikuroi* (Sawada) Wollenw.). The losses in yield have been reported from rice growing regions across the world ranging from 20–50% (Ou 1985, Desjardins *et al.* 2000). The increasing occurrence and severity of bakanae disease have been reported in South and East Asian countries including Pakistan (Khan *et al.* 2000), Bangladesh (Haq *et al.* 2011), South Korea (Kim *et al.* 2014) and India (Bashyal *et al.* 2016a). In India, high disease incidence was reported from Punjab, Haryana, western Uttar Pradesh, Jammu and Kashmir and Uttarakhand in basmati rice (Bashyal *et al.* 2014, 2016a). Therefore, bakanae disease is one of the major bottle-necks in getting higher yield with quality seed/grain of basmati rice.

*Fusarium fujikuroi* produces different types of symptoms on diseased plant, which include elongation of rice tillers,

crown rot, stunting, empty panicles and grain discoloration (Sun and Snyder 1981, Ou 1985, Webster and Gunnell 1992, Bashyal 2018, Desjardins *et al.* 2000). However, effect of management practices on bakanae disease symptoms is still not known. Bakanae disease is reported to be primarily seed-borne in nature (Webster and Gunnell 1992, Sunani *et al.* 2019). Pathogen's survival in soil for 3–4 months is also reported by various authors (Kanjanasoon 1965, Ou 1985). Sun and Snyder (1981) reported that healthy rice seedlings may be infected sporadically after transplanting in the field. Once disease is established, it is difficult to manage through foliar sprays (Hossain *et al.* 2015). Therefore, seed treatment alone fails to prevent soil-borne infection after transplanting and control bakanae disease under field conditions (Bagga *et al.* 2006). Further, Carbendazim is effective as seed treatment against the bakanae disease (Ou 1985, Titone *et al.* 2004) and identifying alternative chemical will help to develop holistic approach for the disease management with environmental sustainability. Therefore, present study was undertaken to evaluate and identify the effective fungicides as seedling treatment against bakanae disease of rice.

### MATERIALS AND METHODS

*Source of pathogen and rice cultivars:* The virulent

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*Fusarium fujikuroi* isolate “F250” (GenBank Accession no. KM50526; MBPO00000000) isolated from symptomatic bakanae infected rice plant from Hisar, Haryana was used for the study (Bashyal *et al.* 2016a, 2017, 2020). Seeds of rice cultivar Pusa Basmati 1121 were obtained from Division of Genetics, ICAR-IARI, New Delhi. The experiments were conducted at the research farm of ICAR-IARI, New Delhi (located at 320 N latitude and 770 E longitude at 1900 m amsl) during the rainy (*kharif*) season of 2019 and 2020. Rice-wheat cropping system is practiced at the research site since many years.

*Evaluation of selected fungicides for seedling treatment:* Healthy surface sterilized seeds of susceptible cultivar Pusa Basmati 1121 were sown in sterilized soil under glasshouse conditions for raising of seedlings. 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 carefully cut with sterilized scissors and kept in *F. fujikuroi* spore suspension ( $1 \times 10^6$ ) for 12 h. Thereafter, seedlings were dipped in fungicidal suspensions, viz. 0.2% of Tebuconazole 8% + Captan 32% SC, 0.2% of Mancozeb 75% WP, 0.2% of Azoxystrobin 23% SC, 0.1% Tebuconazole + Trifloxystrobin 75 WG (50% + 25 w/w), 0.3% Validamycin 3% L, 0.2% of Kitazin 48% EC, 0.2% of Propineb 70% WP, 0.1% of Tebuconazole 250 EC (25.9% w/w), 0.3% of Copper oxychloride 50% WP, 0.1% Propiconazole 25% EC, 0.2% of Zineb 68% + Hexaconazole 4%, 0.1% of Carbendazim 50% WP separately for 12 h (Bashyal *et al.* 2016a). Seedlings inoculated with pathogen only were taken as the control. Inoculated seedlings were transplanted under field conditions. Observations on disease incidence (%) were recorded at 15 days interval until harvesting as described by Bashyal *et al.* (2016a).

$$\text{Disease incidence (\%)} = \frac{\text{Total no. of infected plants}}{\text{Total no. of plants in } 1 \text{ m}^2 \text{ area}} \times 100$$

*Assessment of different plant growth parameters:* Growth parameters like root and shoot length (cm), yield/m<sup>2</sup>, no. of tillers/plant were recorded for each treatment along with inoculated control. Experiment was conducted in randomized block design. Seven plants were selected as one replication and 3 replications were taken for the study.

*Area Under the Disease Progress Curve (AUDPC):* The area under the disease progress curve (AUDPC) was calculated from disease severities (Shaner and Finney 1977). Plants were considered infected when the symptoms of elongation/stunting/rotting appeared and disease severity was assessed as described above. For the AUDPC, disease incidence is considered to be equal to the disease severity and severity data of different time intervals was used based on the formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} (y_{(i+1)} + y_i / 2) (t_{(i+1)} - t_i),$$

where n, the number of assessment times; y, disease incidence and t = time (15 days).

*Statistical analysis:* The experimental data were generated through randomized block design (RBD).

Disease incidence was calculated based on 80 plants of each replication. Two years research field data of 2019 and 2020 were used for the data analysis. Pooled ANOVA was used to check the existence of treatment  $\times$  environment interactions. Homogeneity of variances was tested through Bartlett's Chi-square test using OPSTATS.

## RESULTS AND DISCUSSION

*Effect of fungicides on bakanae disease and its symptoms:* Significant differences in disease incidence were observed among fungicides for the management of bakanae disease (Table 1). Out of 12 fungicides evaluated during the years 2019 and 2020 as seedlings treatment for the bakanae disease management, minimum disease incidence was observed with Carbendazim 50% WP (19.47%) followed by Tebuconazole 50% + Trifloxystrobin 25% 75 WG (25.71%) and Tebuconazole 250 EC (32.53%). The maximum disease incidence was observed in Copper oxychloride 50% WP treatment (68.23%) followed by Mancozeb 75% WP (66.25%).

Various management practices including fungicidal seed treatment were evaluated for bakanae disease by different workers (Hossain *et al.* 2007). However, reports on fungicidal seedling treatments are not much available. We have selected fungicides recommended for rice diseases for the experiment and evaluated them for the seedling treatment. Carbendazim 50% WP and Tebuconazole 50% + Trifloxystrobin 25% w/w (75 WG) were identified as the most effective as disease incidence was minimum when rice seedlings were treated with these fungicides. Carbendazim is reported as effective fungicide for the seed treatment by earlier workers (Hossain *et al.* 2007, 2015). Hossain *et al.* (2015) reported seed treatment with Carbendazim, mix application @1 g/m<sup>2</sup> in nursery beds along with seedling dip for 3 h in 0.1% Carbendazim is effective. Bagga and Sharma (2006) reported seedling treatment with Carbendazim or benlate (0.1%) for 6 and 8 h reduced the disease incidence significantly and improved the grain yield also. Tilt 25 EC @ 0.05% was also observed effective, but it was phytotoxic as it reduced plant height. Here seedling treatment with Carbendazim 50% WP and Tebuconazole 50% + Trifloxystrobin 25% w/w (75 WG) were observed to be effective when seedlings were treated for 12 h.

During the experiments, different types of bakanae disease symptoms were observed in rice variety Pusa Basmati 1121 which included elongation of few tillers of a hill (Fig 1a-b), rotting of whole plant (Fig 1c), detachment of elongated tillers from main hill (Fig 1d), empty panicles with dried tillers (Fig 1e), empty panicles with green tillers (Fig 1f), and blackening of nodes and white mycelia growth in nodal and internodal areas (Fig 1g-h). Differences were observed among treatments for the bakanae symptoms. In treatments, Mancozeb 75% WP, Zineb 68% + Hexaconazole 4%, Azoxystrobin 23% SC major symptom produced was tiller elongation. Whereas, tiller elongation with rotting was prominent in Kitazin 48% EC and inoculated control. Tiller detachment was prominently observed in many

Table 1 Effect of seedling treatment on bakanae disease of rice

Fungicide	Disease incidence % (2019)	Disease incidence % (2020)	Mean	Major symptom produced
Tebuconazole 8% + Captan 32% sc	53.08	51.75	52.41	Elongation and detached tillers
Mancozeb 75% WP	63.08	69.43	66.25	Elongation, rotting, detached tillers
Azoxystrobin 23% sc	50.00	51.89	50.95	Elongation and detached tillers
Tebuconazole + Trifloxystrobin 75 WG (50% + 25 w/w)	26.92	24.50	25.71	Partial elongation, rotting
Validamycin 3% L	46.92	46.16	46.54	Elongation, rotting, detached tillers
Kitazin 48% EC	60.00	58.48	59.24	Elongation-rotting
Propineb 70% WP	56.92	53.704	55.31	Elongation, rotting
Tebuconazole 250 EC (25.9% w/w)	31.54	33.52	32.53	Elongation
Copper oxychloride 50% WP	67.69	68.78	68.23	Elongation
Propiconazole 25% EC	43.85	41.37	42.61	Elongation
Zineb 68% + Hexaconazole 4%	39.23	38.73	38.98	Elongation
Carbendazim 50% WP	20.77	18.17	19.47	Detached tillers, elongation
Control	85.38	95.44	90.41	Rotting, elongation
CD for Years		9.85		
CD for Treatments		11.78		
CD for Years × Treatments		19.43		

CD (P=0.05)

treatments such as Carbendazim 50% WP and Tebuconazole 8% + Captan 32% sc (Table 1).

Distinct bakanae disease symptoms were observed in some treatments compared to others. Bashyal *et al.* (2016b) concluded pathogenicity of *F. fujikuroi* on rice may depend on the balance of toxins and growth regulators, which were affected by the pathogen isolate, environment and the nutritional status of the plant. Symptoms like detachment of infected tillers from the hill may be the defence response of plant against the disease as an indirect effect of fungicide. The strobilurin fungicides inhibit the growth of fungi by blocking the electron transport at the cytochrome-*bc*<sub>1</sub> complex during the respiration in mitochondria. They also prompt a positive effect on plant growth and physiology by interacting with the transfer of electrons in mitochondria (Diaz-Espejo *et al.* 2012).

*Effect of fungicides on plant growth parameters:* Root length was maximum in Propineb 70% WP treatment followed by Zineb 68% + Hexaconazole 4% treatments (15 cm). Shoot length was maximum in Azoxystrobin 23% sc (52.20 cm) followed by Tebuconazole +Trifloxystrobin 75 WG. Plant weight was observed maximum (163.33 g) in Zineb 68% + Hexaconazole 4% treated plants followed by Carbendazim 50% WP (158.33g) and Tebuconazole 50%

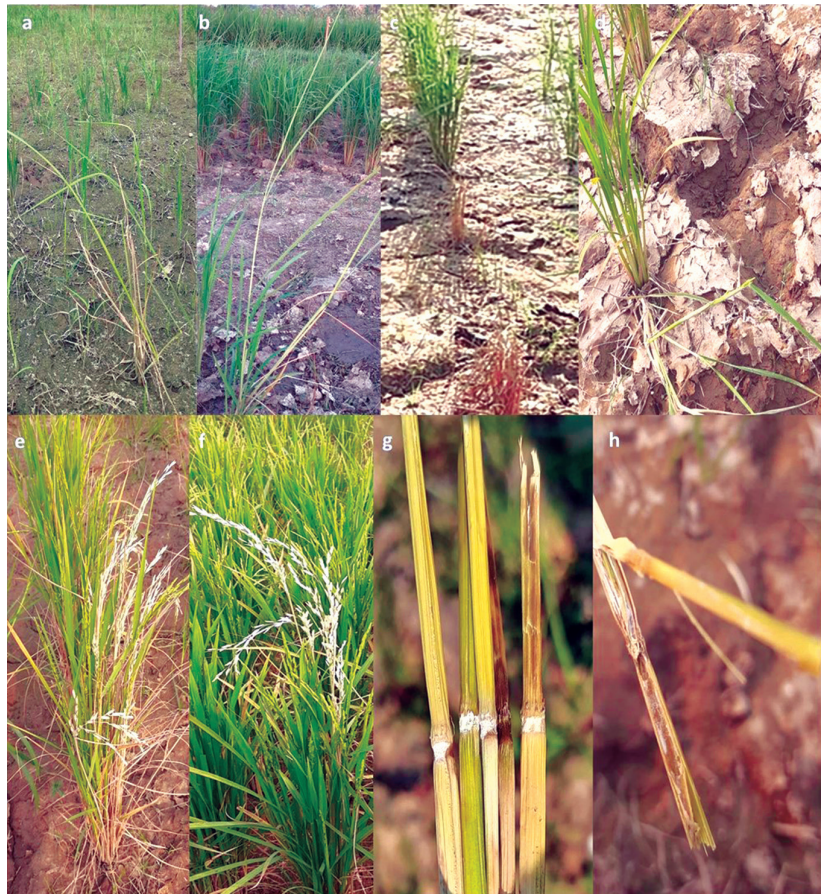


Fig 1 Bakanae disease symptoms observed in Pusa Basmati 1121. (a-b), elongation of few tillers of a hill; (c), rotting of whole plant; (d), detachment of elongated tillers from main hill; (e), empty panicles with dried tillers (f); empty panicles with green tillers; (g-h), blackening of nodes and white mycelia growth in nodal and intermodal areas.

Table 2 Effect of seedling treatments on different yield parameters of rice

Fungicide	Root length (g)	Total plant weight (g)	Shoot length (cm)	No. of tillers/plant	Yield g/m <sup>2</sup>
Tebuconazole 8% + Captan 32% SC	14.83	106.67	43.30	22.80	121.50
Mancozeb 75% WP	14.83	147.33	47.60	18.40	91.50
Azoxystrobin 23% SC	13.83	140.00	52.20	16.80	95.83
Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG	14.83	150.00	51.00	20.80	195.83
Validamycin 3% L	13.66	103.33	44.40	14.40	115.00
Kitazin 48% EC	13.00	136.66	45.80	17.60	52.50
Propineb 70% WP	16.33	113.33	45.20	17.80	105.00
Tebuconazole 250 EC (25.9% w/w)	14.50	100.00	47.70	19.00	100.50
Copper oxychloride 50% WP	12.16	116.67	44.30	18.20	69.17
Propiconazole 25% EC	14.16	113.33	50.20	16.80	75.00
Zineb 68% + Hexaconazole 4%	15.00	163.33	43.00	16.60	75.00
Carbendazim 50% WP	14.16	158.33	47.30	24.20	210.00
Control	17.34	146.66	44.40	15.80	47.50
CD for Years	1.10	2.982	2.80	1.84	20.64
CD for Treatments	3.20	16.22	5.46	5.52	40.61
CD for Years × Treatments	4.47	30.66	9.75	7.81	63.22

CD (P=0.05)

+ Trifloxystrobin 25% 75 WG. Significant differences were observed in different treatments for tiller numbers. Maximum no. of tillers were observed with Carbendazim 50% WP (24.20) followed by Tebuconazole 8% + Captan 32% SC (22.80) and Tebuconazole 50% + Trifloxystrobin 25% 75 WG (20.80). Tillers were minimum for the Validamycin 3% L (14.40). Significant differences were observed for the yield (g/m<sup>2</sup>) when seedlings were treated with fungicides compared to control (Table 2). Maximum yield (210 g/m<sup>2</sup>) was observed for Carbendazim 75% WP followed by Tebuconazole 50% + Trifloxystrobin 25% 75 WG (195.83 g/m<sup>2</sup>).

Significant yield increase was recorded when seedlings were treated with fungicides compared to control. However, differences were observed for the different yield parameters evaluated. Yield of a crop depends on many other parameters and variation on those parameters might be the reason for the yield difference. Mia *et al.* (2021) also could not get significant correlation between yield and yield parameters in bakanae disease. Reports are available for increased grain yield, kernel weight, delay in flag leaf senescence after the application of strobilurins, tebuconazole and carbendazim fungicides (Zhang *et al.* 2010). However, effect of these fungicides on bakanae disease symptoms expression needs to be studied further.

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*Effect of fungicide on Area Under Disease Progress Curve (AUDPC):* Area under disease progress curve (AUDPC) was minimum for the Tebuconazole 50% + Trifloxystrobin 25% 75 WG (621.58) followed by Carbendazim 50% WP (671.30). Whereas, it was maximum in Mancozeb 75% WP (1903.51) treated plants followed by Copper oxychloride 50% WP (1799.42). AUDPC values were relatively higher in the year 2020 compared to the year 2019.

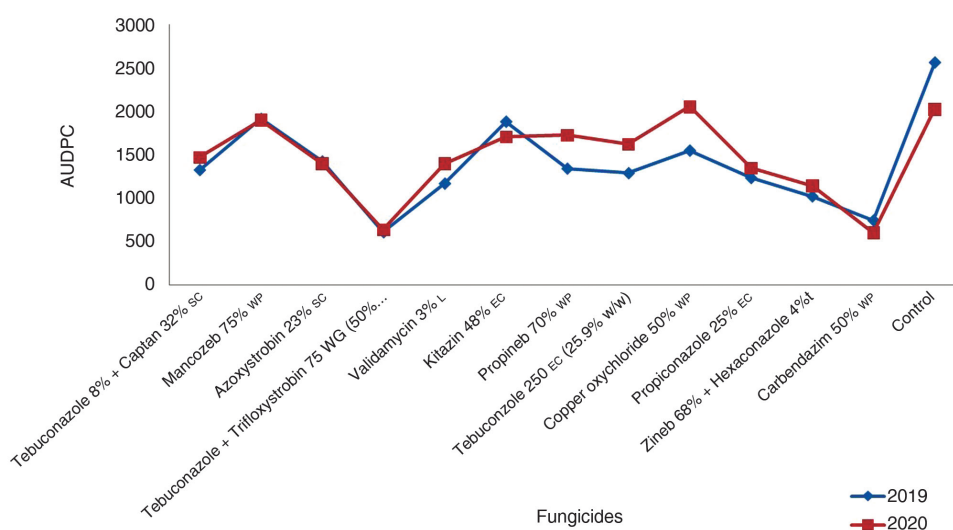


Fig 2 AUDPC recorded during the years 2019 and 2020 in rice plants treated with different fungicides.

Further, AUDPC was observed minimum with the treatment of Tebuconazole 50% + Trifloxystrobin 25% w/w (75 WG) followed by Carbendazim 50% WP, which indicated less disease progress in these fungicides compared to others. AUDPC generally observed to be highly correlated with disease severity in many diseases (Bashyal *et al.* 2010, Burlakoti *et al.* 2012). Here, we also observed the strong correlation (0.8) of AUDPC with bakanae disease of rice (Fig 2).

Based on our study, we suggest to use seedling treatment with Carbendazim 50% WP/Tebuconazole 50% + Trifloxystrobin 25% w/w (75 WG) for the bakanae disease management as they gave minimum disease incidence with higher yield compared to other fungicides. These fungicides have shown different effects on bakanae disease symptoms expression. Therefore, further comprehensive investigations need to be carried out on this aspect. An integrated disease management module needs to be developed and validated under field conditions using identified fungicides.

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