



Short communication

Evaluation of fungicides for the management of pearl millet [*Pennisetum glaucum* (L.)] blast caused by *Magnaporthe grisea*

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ARTICLE INFO

Keywords:
Disease control
Blast
Pearl millet
Fungicides

ABSTRACT

Blast disease caused by *Magnaporthe grisea* has emerged as a serious threat to pearl millet cultivation in India. Most of the hybrids being grown in India are susceptible to blast as not much efforts have been made to breed for blast resistance in pearl millet. In the absence of host plant resistance, the disease can be effectively managed with chemical fungicides. Therefore, nine fungicides, chlorothalonil, tricyclazole, hexaconazole, kasugamycin, benomyl, carbendazim, tebuconazole + trifloxystrobin, propiconazole and metalaxyl + mancozeb were tested for their efficacy to manage blast disease on a blast susceptible pearl millet line ICMB 95444. Different combinations of seed treatment and foliar sprays were tested: seed treatment alone, seed treatment + one spray, seed treatment + two sprays, seed treatment + three sprays. None of the fungicides was found effective when used as seed treatment. Results of this study clearly demonstrated that the disease can be effectively managed with three sprays of tebuconazole + trifloxystrobin (Nativo) or propiconazole (Tilt).

1. Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important cereal crop grown on a 30 million ha area in the arid and semi-arid tropics (SAT) of Asia and Africa (Yadav and Rai, 2013). Due to the tolerance to drought, it is cultivated as a major crop in most countries of the world (Bidinger et al., 1987). In India, pearl millet is the third most important rainfed cereal crop grown over 9 million ha with an annual production of 9.5 million tonnes, mainly in the states of Haryana, Gujarat, Maharashtra, Rajasthan and Uttar Pradesh (Yadav and Rai, 2013; Yadav et al., 2012). The grains are highly nutritious with high levels of energy and protein, and high densities of iron and zinc (Rai et al., 2008).

Among the diseases of pearl millet, blast caused by *Pyricularia grisea* (Cooke) Sacc. [Teleomorph: *Magnaporthe grisea* (Herbert) Barr], a disease of minor importance in past years, has gained status of major constraint to pearl millet production in India (Lukose et al., 2007). *Magnaporthe grisea* is externally seed borne and also survives as chlamydospores or as free saprophytic mycelium in the soil/leaf debris which serves as a source of primary inoculum (Singh and Pavg, 1977). The disease also appears in several countries in west and central Africa such as Senegal, Mali, Burkina Faso, Niger, Nigeria and Chad. The disease appears in severe form in forage crops in the southern coastal plains of the USA (Wilson and Gates, 1993). It has been observed on various hybrids and local cultivars being grown in the major pearl millet growing states, Rajasthan, Gujarat, Uttar Pradesh, Haryana and

Maharashtra in India with various levels of disease severity (AICPMIP, 2011–12).

Deployment of resistant varieties is considered as the most economical and ecofriendly method of management of plant diseases. Efforts are being made to understand inheritance of resistance to *M. grisea* and pathogenic variation in the pathogen so as to develop pearl millet parental lines and hybrids resistant to blast (Gupta et al., 2012; Sharma et al., 2013). Although blast disease in rice (*Oryza sativa*) is primarily managed through host plant resistance, the pathogen has the ability to develop new pathogenic races leading to breakdown of resistance within few years (Ahn, 1994). Hence, attempts have been made to manage blast disease in different crops using fungicides (Varier et al., 1993; Lukose et al., 2007; Narayana Swamy et al., 2009; Netam et al., 2014; Pagani et al., 2014). Though host plant resistance is the most economical and viable disease management strategy to control pearl millet blast, most of the commercial hybrids being grown in India are susceptible to blast. In the absence of blast-resistant cultivars, the disease can be best managed with chemical fungicides. *In vitro* studies have shown the inhibition of radial growth of pearl millet isolate of *M. grisea* with fungicides such as chlorothalonil, tricyclazole, hexaconazole, carbendazim and propiconazole (Kumar and Singh, 1995; Bhojya Naik and Jamadar, 2014). These fungicides have also been found to be effective in providing protection to the rice crop against blast disease (Sood and Kapoor, 1997; Prajapati et al., 2004; Dutta et al., 2012). Carbendazim and tricyclazole have been reported to be effective against

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pearl millet blast under field conditions (Lukose et al., 2007; Joshi and Gohel, 2015). However, differential sensitivity of the pathogen isolates from rice to tricyclazole and carbendazim has also been reported (Yuan and Yang, 2003; Mohammad et al., 2011). In addition, benomyl, kasugamycin and Nativo (a combination fungicide containing tebuconazole and trifloxystrobin) have also been reported to be effective against rice blast (Narayana Swamy et al., 2009; Ganesh Naik et al., 2012). The present study was planned to evaluate these eight fungicides against pearl millet blast under field conditions to identify promising fungicides for the management of this disease. As major efforts in pearl millet pathology have always been focused on the management of downy mildew, the most important disease of pearl millet, seed treatment with metalaxyl and foliar spray of Ridomil (metalaxyl 8% + mancozeb 64% WP) which is most effective against downy mildew was also included in the present study (Singh et al., 1984; Thakur et al., 2011).

2. Materials and methods

2.1. Field preparation and experiment layout

The field trials were conducted during the rainy seasons of 2012 and 2013 in a research farm at ICRISAT, Patancheru, Hyderabad, India. The trials were laid out in a split plot design with 10 treatments and three replications. Each plot size was 4 m × 2.25 m, and consisted of three rows 0.75 m apart. Main plots and subplots were separated by 1.5 m and 1.0 m, respectively, on each side. The blast susceptible pearl millet hybrid parent line, ICMB 95444 was used in the field trials. Seeds were sown in the plot with a spacing of 75 × 10 cm and uniform number of plants in each plot was maintained by removing the extra seedlings at 15 days after sowing (DAS). The recommended package of practices for pearl millet cultivation was followed (Yadav et al., 2015).

2.2. Fungicides treatment and spray schedule

Nine fungicides (subplot factor), chlorothalonil, tricyclazole, hexaconazole, kasugamycin, benomyl, carbendazim, tebuconazole + trifloxystrobin, propiconazole and metalaxyl [metalaxyl as seed treatment and Ridomil (metalaxyl 8% + mancozeb 64% WP) as foliar spray] were tested for their efficacy against blast disease on the susceptible line, ICMB 95444 (Table 1). Four combinations of seed treatment and foliar sprays (main plot factor) were tested: seed treatment alone (set 0), seed treatment + one spray (set 1), seed treatment + two sprays (set 2), seed treatment + three sprays (set 3). For seed treatment, seeds were

Table 1
Fungicides evaluated against *Magnaporthe grisea* under field conditions during 2012–13.

Treatment	Fungicide	Trade Name	Dose	
			Seed treatment (Kg)	Spray (/L)
T1	Chlorothalonil 75% WP	Kavach	2.5 g	2.5 g
T2	Tricyclazole 75% WP	Baan	0.6 g	0.6 g
T3	Hexaconazole 5% EC	Contaf 5E	0.5 mL	0.5 mL
T4	Kasugamycin 3% SL	KASU-B	2.5 mL	2.5 mL
T5	Benomyl 50% WP	Benofit	2.0 g	1.0 g
T6	Carbendazim 50% WP	Bavistin	2.0 g	0.5 g
T7	Tebuconazole 50% + trifloxystrobin 25% WG	Nativo	0.4 g	0.4 g
T8	Propiconazole 25% EC	Tilt	1.0 mL	1.0 mL
T9	(Metalaxyl 8% + mancozeb 64% WP) for spray/ metalaxyl 35 WS for seed treatment	Ridomil/ Metal	6.0 g	2.5 g
T10	Control			

treated separately with each fungicide with the dose as described in Table 1. The doses of the fungicides found to be effective against blast disease in other crops were selected for this study. The first spray of fungicides was scheduled at seven days before inoculation with *M. grisea* spore suspension. The second spray was applied seven days after inoculation in sets 2 and 3, and the third spray in set 3 was applied 15 days after the second spray.

2.3. Inoculum preparation and inoculation

Magnaporthe grisea isolate Pg 45, collected from pearl millet fields at ICRISAT, Patancheru, India was used in this study. Inoculum of the isolate Pg 45 was prepared according to the procedure described by Sharma et al. (2013). The spore suspension was prepared using sterilised distilled water, adjusted to a desired concentration (1×10^5 spores mL⁻¹) using a haemocytometer (Fisher Scientific) and a drop of surfactant Tween 20 (HiMedia) was added to ensure the uniform dispersal of spores. The crop (30 days old seedlings) was spray inoculated with an aqueous conidial suspension using a hand operated Knapsack sprayer. Perfo-irrigation was provided to the crop twice a day 30 min each in the morning (between 10 and 11 a.m.) and in the afternoon (between 5 and 6 p.m.) on rain-free days to maintain high relative humidity and leaf wetness to facilitate fungal infection and disease development. Weather data during the crop growth (standard meteorological weeks 31–42) in 2012 and 2013 is given in Fig. 1.

2.4. Disease assessment and analysis

The blast severity was measured visually as percent infected foliage at seven days after inoculation, and further measurement was done up to 35 days at seven days intervals. The disease severity values at each recording were used to calculate the area under the disease progress curve (AUDPC). The AUDPC was calculated using the formula:

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

where “t” is the time of each reading, “y” is percent disease severity at each reading and “n” is the number of readings.

Disease severity recorded at 35 days after inoculation was used to calculate percent disease reduction (PDR) in the fungicide treatments over control using the formula:

$$PDR = \frac{\text{Disease severity in control} - \text{disease severity in treatment}}{\text{Disease severity in control}} \times 100$$

GENSTAT statistical package version 10.1 (Rothamsted Experiment Station, Harpenden, Herts AL52JQ, UK) was used for randomization of treatments and analysis of variance (ANOVA) for the arcsine transformed values of percent disease reduction over control for the comparison of treatments.

3. Results

The disease symptoms were clearly visible seven days after inoculation in the untreated control. Hence, the first observation was made at seven days after inoculation. Significant differences ($P < 0.05$) for percent disease reduction over the control were observed among fungicides and different sets of treatments (Table 2). This indicated differences in the effectiveness of fungicides against blast. None of the fungicides provided an adequate level of disease control through seed treatment alone (Set 0) and the fungicide treatments were comparable to the untreated control (Table 3). AUDPC values were also high in Set 0 and were comparable to untreated control (Fig. 2).

Disease severity increased substantially 14 days after inoculation both during 2012 and 2013 in the untreated control and other treatment plots except propiconazole and tebuconazole + trifloxystrobin

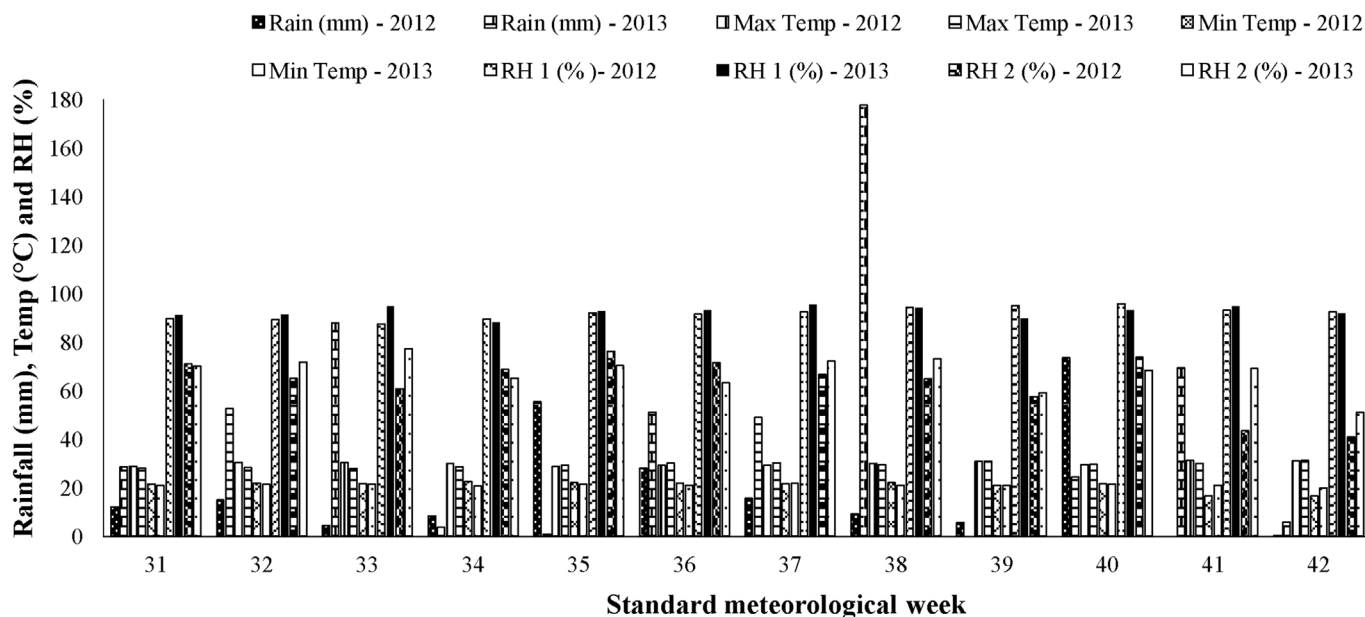


Fig. 1. Relative humidity (RH) (%), maximum and minimum temperature (°C) and rainfall (mm) in the standard meteorological weeks 31–42 during 2012 and 2013. RH 1, Relative humidity recorded at 0717 h; RH 2, Relative humidity recorded at 1417 h.

Table 2
Analysis of variance (ANNOVA) for percent disease reduction (arcsine transformation) over control.

Source of variation	df	MS	F-value
Main plot (Set)	3	3361.48	72.82*
Subplot (Treatment)	8	3393.6	73.52*
Year	1	1275.62	27.64*
Set × Treatment	24	546.11	11.83*
Set × Year	3	191.41	4.15*
Treatment × Year	8	121.75	2.64*
Set × Treatment × Year	24	90.2	1.95*
Residual	123	46.16	
Total	196		

*Significant at ($P < 0.05$).

treated plots. It was noted that the disease progress was less in plants sprayed with propiconazole and tebuconazole + trifloxystrobin compared to the control and other fungicides. As significant differences were observed for the years (Table 2), data on percent disease reduction over the control in different treatments for both 2012 and 2013 are presented in Table 3. Prophylactic spray of propiconazole and tebuconazole + trifloxystrobin showed 31.2 and 39.1 percent reduction of disease over the control, respectively, during 2012; whereas in 2013, the disease reduction was 56.4 and 57.3 percent with the foliar spray of propiconazole and tebuconazole + trifloxystrobin, respectively (Table 3). In other treatments, a maximum of 23.2% (2012) and 14.6% (2013) reduction of disease over the control was observed with tricyclazole and hexaconazole treatment, respectively (Table 3). The AUDPC values for propiconazole and tebuconazole + trifloxystrobin treated plots were significantly lower than the untreated control and other fungicides (Fig. 2).

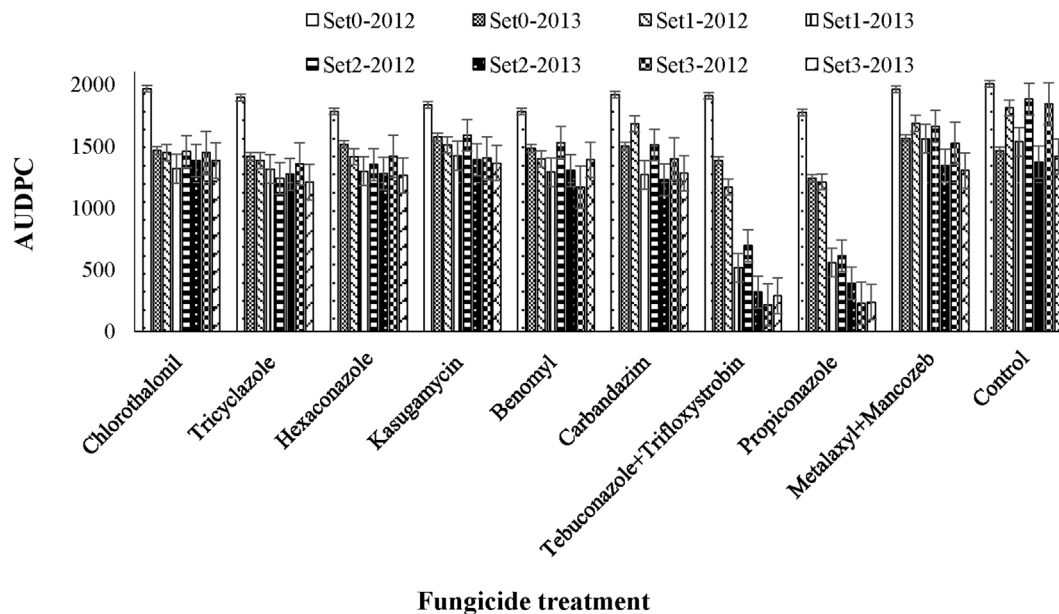


Fig. 2. Area under the blast disease progress curve in response to different fungicide treatments.

Table 3
Effect of fungicide treatments on blast severity in pearl millet line ICMB 95444.

Fungicide	Percent reduction of blast severity over control											
	Set-0			Set-1			Set-2			Set-3		
	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean
Chlorothalonil 75% WP	2.0 (8.13) ^a	2.8 (9.63)	2.4 (8.91)	18.1 (25.18)	11.7 (20.00)	14.9 (22.71)	19.4 (26.13)	2.0 (8.13)	10.7 (19.09)	17.3 (24.58)	0.7 (4.80)	9.0 (17.46)
Tricyclazole 75% WP	7.7 (16.11)	3.8 (11.24)	5.7 (13.81)	23.2 (28.73)	13.2 (21.30)	18.2 (25.25)	29.1 (32.65)	8.4 (16.85)	18.7 (25.62)	17.7 (24.88)	15.4 (23.11)	16.5 (23.97)
Hexaconazole 5% EC	13.8 (21.81)	0.1 (1.81)	6.9 (15.23)	22.5 (28.25)	14.6 (22.46)	18.5 (25.48)	27.1 (31.37)	9.3 (17.76)	18.2 (25.25)	18.2 (25.25)	7.2 (15.56)	12.7 (20.88)
Kasugamycin 3% SL	10.7 (19.09)	0.0 (0.0)	3.3 (10.47)	18.1 (25.18)	8.1 (16.54)	13.1 (21.22)	14.5 (22.38)	0.4 (3.63)	7.4 (15.79)	22.0 (27.97)	5.2 (13.18)	13.6 (21.64)
Benomyl 50% WP	12.0 (20.27)	4.2 (11.83)	8.1 (16.54)	20.3 (26.78)	10.3 (18.72)	15.3 (23.03)	15.2 (22.95)	3.7 (11.09)	9.5 (17.95)	29.7 (33.02)	0.0 (0.0)	14.7 (22.55)
Carbendazim 50% WP	4.2 (11.68)	0.0 (0.0)	1.7 (7.49)	8.7 (17.16)	8.8 (17.26)	8.8 (17.26)	18.1 (25.18)	7.7 (16.11)	12.9 (21.05)	19.1 (25.92)	8.9 (17.36)	14.0 (21.97)
Tebuconazole 50% + Trifloxystrobin 25% WG	10.1 (18.53)	5.8 (13.94)	8.0 (16.43)	39.1 (38.70)	57.3 (49.14)	48.2 (43.97)	61.8 (51.83)	79.4 (63.01)	70.6 (57.17)	89.1 (70.72)	81.3 (64.38)	85.2 (67.37)
Propiconazole 25% EC	12.6 (20.79)	4.9 (12.79)	8.7 (17.16)	31.2 (33.96)	56.4 (48.62)	43.8 (41.44)	66.0 (54.33)	75.7 (60.47)	70.8 (57.29)	87.9 (69.64)	85.1 (67.29)	86.5 (68.44)
Metalaxyl 8% + Mancozeb 64% WP	8.0 (16.43)	0.0 (0.0)	2.1 (8.33)	9.4 (17.85)	1.4 (6.80)	5.4 (13.44)	12.5 (20.70)	6.4 (14.65)	9.4 (17.85)	16.0 (23.58)	6.2 (14.42)	11.1 (19.46)
Set mean	16.3	12.4	14.4	26.6	26.2	26.4	31.6	26.4	29.0	35.6	26.6	31.1
Control ^b	78.3	76.1	77.2	76.7	79.4	78.1	80.0	71.1	75.6	76.1	71.1	73.6

LSD ($P < 0.05$) for Set = 2.46; Treatment = 3.88; Year = 1.74; Set \times Treatment \times Year interaction = 10.98.

^a Values in parentheses are arcsine transformations.

^b Percent disease severity in control.

Disease progress was restricted over a period of time during both years in propiconazole and tebuconazole + trifloxystrobin treated plots, but a steady increase in disease severity was observed in all other treatments including the control. In terms of percent reduction of disease over control after the second spray, the same fungicides, propiconazole and tebuconazole + trifloxystrobin showed maximum reduction of disease severity during both years, whereas other fungicides achieved < 30 percent and < 10 percent reduction in disease severity during 2012 and 2013, respectively (Table 3). Disease reduction in the case of tebuconazole + trifloxystrobin was 61.8 and 79.4 percent with two sprays, and 89.1 and 81.3 percent following three sprays during 2012 and 2013, respectively. Similarly, two sprays of propiconazole resulted in 66.0 and 75.7 percent disease reduction over the control during 2012 and 2013, respectively, whereas with three sprays, the disease reduction was 87.9 percent in 2012 and 85.1 percent in 2013. The significantly lower AUDPC values in tebuconazole + trifloxystrobin, and propiconazole treated plots compared to other fungicide treatments clearly indicated the superiority of propiconazole and tebuconazole + trifloxystrobin over other fungicides for the management of pearl millet blast (Fig. 2).

4. Discussion

Seed treatment with metalaxyl and foliar spray of Ridomyl (metalaxyl 8% + mancozeb 64% WP) was ineffective against pearl millet blast in the present study. Metalaxyl has been reported to be very effective against downy mildew, another important disease of pearl millet (Singh et al., 1984; Thakur et al., 2011); hence, it was included in the present study with the idea that if found effective, the same fungicide can be recommended to control both downy mildew and blast of pearl millet. The fungicides chlorothalonil, tricyclazole, hexaconazole, kasugamycin, benomyl, carbendazim, tebuconazole + trifloxystrobin and propiconazole have been reported to effectively manage blast disease in other crops (Hegde et al., 2000; Kumbhar, 2005; Groth, 2006; Lukose et al., 2007; Ghazanfar et al., 2009; Narayana Swamy et al., 2009; Vahid et al., 2011; Dutta et al., 2012; Ganesh Naik et al., 2012; Bhojya Naik and Jamadar, 2014). Though these fungicides have been reported

to be effective against blast, only two of them were effective against pearl millet blast in this study. Maximum disease control was achieved by tebuconazole + trifloxystrobin and propiconazole. Three sprays of Nativo (tebuconazole + trifloxystrobin) (0.4 g/L) or Tilt (propiconazole) (1 mL/L) were most effective in controlling blast of pearl millet. In fact, even two sprays of these fungicides were more effective than the three sprays of other fungicides as evident from the AUDPC values. Three sprays of carbendazim (0.05%) have been reported to be effective against pearl millet blast (Lukose et al., 2007). However, carbendazim when used as a seed treatment and foliar spray (three sprays) in the present study resulted in only 19.1 and 8.9 percent reduction in disease severity over the control during 2012 and 2013, respectively. Development of resistance to carbendazim has been reported in the rice blast fungus *M. grisea* (Mohammad et al., 2011). Therefore, differential sensitivity of pearl millet infecting populations of *M. grisea* from Gujarat (Lukose et al., 2007) and that of the Patancheru isolate used in this study to carbendazim could be attributed to the development of resistance in the pathogen to the fungicide. Similarly, another fungicide, tricyclazole, which is reported to be very effective against rice blast (Sood and Kapoor, 1997; Prajapati et al., 2004; Kunova et al., 2013) and pearl millet blast in Gujarat (Joshi and Gohel, 2015) was also ineffective against pearl millet blast. Differential sensitivity to tricyclazole in the rice blast isolates collected from different areas in China has also been reported (Yuan and Yang, 2003).

Induction of defense response in rice against *M. grisea* by treating seeds with chitosan and hydrolyzed chitosan has been reported (Rodríguez et al., 2007). Seed treatment with pyroquilon fungicide in rice cultivars that differed in their level of resistance suppressed leaf blast until 62 and 47 days after seeding in 1987 and 1988, respectively, when averaged across cultivars (Filippi and Prabhu, 1997). However, treatment of pearl millet seed with metalaxyl in the present study was found ineffective against *M. grisea*. Seed treatment in general is a good insurance against seedling diseases, and is likely to stop any seed transmission of blast. However, it does not prevent infection from wind-borne spores (<http://extension.missouri.edu/p/MP645>). In the present study as well, seed treatment with none of the fungicides was effective when plants were artificially inoculated with spores of *M. grisea*.

This study clearly demonstrated the superiority of Tilt (propiconazole) and Nativo (tebuconazole + trifloxystrobin) over other commonly used fungicides for the control of blast disease. Thus, the disease can be effectively managed in pearl millet with two to three sprays of propiconazole or tebuconazole + trifloxystrobin at 15 day intervals with the first spray at 20–25 days after sowing. The information generated in this study will be helpful in managing the disease especially in seed production plots as many female parents such as ICMB 89111, ICMB 95222 and ICMB 95444 of the commercial pearl millet hybrids being grown in India are highly susceptible to blast (AICPMIP, 2011–12).

Acknowledgements

The authors gratefully acknowledge the funding support from Pearl Millet Hybrid Parents Research Consortium to carry out this research work.

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