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Efficacy of fungicides against *Magnaporthe grisea* inciting blast disease of pearl millet *in vitro*

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Abstract
Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of versatile crop grown in arid and semi-arid regions of Africa and Asia. India is the single largest producer of pearl millet and subsistence for food, feed and fodder has been expanding into non-traditional regions of the world. Pearl millet encounter by several diseases during the crop growth, among them Pyricularia leaf spot, caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*) is one of the emerging threats to the production of pearl millet fodder and grain. In the current investigation, *in vitro* efficacy of three fungicides (*Tebuconazole, carbendazim* and propiconazole*)* at six different concentrations were examined against *M. grisea.* Out of which, fungicide propiconazole was found highly effective at 1500 ppm conc. with 100 per cent mycelium growth inhibition, whereas tebuconazole shows 90.74 per cent the mycelium growth inhibition. The maximum growth observed in case of carbendazim with the mean least mycelium growth inhibition (68.21%) against *M. grisea*.

Keywords: Pearl millet, *Tebuconazole, carbendazim*, propiconazole, *in vitro*

Introduction
Pearl millet [*Pennisetum glaucum* (L.) R. Br.] belongs to the family *Poaceae,* which is a staple food for millions of deprived people living in the arid and semi-arid tropical regions of Africa and Asia. It plays a key role in the food and energy security of the rural peoples, particularly in the rainfed areas. The central African countries *viz.*, Burkina Faso, Nigeria, Upper Volta, represent the parts of Africa that are reported to be the centre of origin of pearl millet (Wilson *et al*., 1989) [11]. Pearl millet is more heat tolerant and more efficient in utilization of soil moisture in comparison to maize and sorghum. It is extensively cultivated in more than 30 countries of Africa, Asia and America with great potential where soils have low pH, light in texture, dry, saline and less fertile for other cereal crops. India and Africa together account for 90 per cent area of pearl millet in the world (Yadav *et al.*, 2012) [12].
The pearl millet cultivation for subsistence food, feed and fodder has been expanding into non-traditional regions of the world, where production constraints from biotic factors such as diseases, insect pests, parasitic and non-parasitic weeds assume greater importance. Amongst several constraints in obtaining the high grain yield potential of improved pearl millet cultivars in India and Africa, downy mildew is considered a major problem. Nevertheless, in the last decade, Pyricularia leaf spot, also known as blast disease caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*) has emerged as a very serious threat to the production of pearl millet fodder and grain in the changing climate (Chandra *et al.,* 2017) [2]. Pearl millet blast is a highly destructive and widespread disease in pearl millet growing areas of Africa and Asia. In India, Pearl millet blast disease prevalence has increased at a terrifying rate on dual purpose (fodder and grain) pearl millet commercial hybrids (Thakur *et al.,* 2009) [7]. It infects more than 50 species of grass family including rice, wheat, barley, oats, pearl millet, and finger millet (Tanweer *et al.*, 2015) [6]. The disease has been found to have significant adverse effects on green forage yield and digestible dry matter (Wilson and Gates, 1993) [10] and grain (Timper *et al.*, 2002) [8]. The disease was first recorded in Uganda in 1933 (Emchebe, 1975) [3]. In India, the disease was first reported in 1952 from Kanpur, UP (Mehta *et al.*, 1953) [5] and remained a minor disease for a long time but has recently become a serious threat to pearl millet grain yield production and fodder production. Therefore, an attempt was made to evaluate the systemic fungicides *in vitro* for the control of this emerging threat to pearl millet production.

Material and Methods

Three fungicides *viz*., Tebuconazole, Carbendazim and Propiconazole at different concentrations *i.e.*, 250, 500, 750, 1000, 1250 and 1500 ppm were evaluated with three replications for their chemo-sensitivity to three different *M. grisea* isolates collected from pearl millet growing districts in Haryana under *in vitro* conditions using poisoned food technique (Grover and Moore, 1962). To obtain the desired concentration in parts per million (ppm), the required volumes of each test fungicide were combined in a conical flask containing 100 ml molten OMA medium. The flask containing the poisoned medium was vigorously shaken to ensure an even distribution of fungicides, and 20 ml was poured into each sterilized Petri plate. After the solidification of media in the Petri plates, the plates were inoculated with fungal mycelial disc of 5 mm diameter of actively growing pure culture of each test isolates in the centre. The inoculated Petri plates were incubated in BOD incubator at 25±2 °C temperature.

The radial growth of mycelium was recorded when there is 90 mm growth in check plates at 25±2 °C and per cent growth inhibition will be estimated by using the formula given by Vincent (1927) [9].

$$\text{Growth Inhibition (\%)} = \frac{\text{Growth in control} - \text{growth in treatment}}{\text{Growth in control}} \times 100$$

Result and Discussion

The chemo sensitivity of three fungicides *viz*., Tebuconazole, Carbendazim and Propiconazole at six different concentrations *viz*., 250, 500, 750, 1000, 1250 and 1500 ppm were evaluated against three *M. grisea* isolates. All the fungicides evaluated against *M. grisea* were found significantly superior in inhibiting the mycelial growth over control. Among the fungicides evaluated propiconazole reported minimum (7.67 mm) mean mycelium growth and had maximum inhibition of the mycelial growth (91.48%) of the fungus followed by Tebuconazole as it showed 21.44 mm mean mycelial growth and 76.17 per cent growth inhibition. The both the chemicals were significantly superior over carbendazim (68.21%) which was found to be the least effective in inhibiting mycelial growth of the fungus. Similarl, Bhojyanaik (2013) who reported that maximum inhibition of fungal growth was observed in tricyclazole 75WP (87.78%) followed by difenconazole 25EC (86.91%), hexaconozole 5E (85.33%) and propiconazole 25EC (75.92%) whereas, carbendazim 50WP found least effective in inhibiting fungal growth (54.23%). Similarly, Joshi and Gohel (2015) resulted that propiconazole, mancozeb, tricyclazole and carbendazim (12%) + mancozeb (63%) were completely inhibited the fungal growth at all the three concentrations used. Carbendazim 50WP @250 ppm found least effective in inhibiting the mycelial growth. The similar trend was followed in inhibiting the mycelial growth of *M. grisea* as propiconazole reported most effective in inhibiting the mycelial growth in all the three isolates followed by tebuconazole and carbendazim. Triazole fungicides such as propiconazole, difenoconazole, tebuconazole, and penconazole are effective because they interfere with the synthesis of fungal sterols and hinder the formation of ergosterol. Ergosterol is essential for the construction of the cell wall in many fungi, and its absence causes irreversible damage to the cell wall, resulting in the death of the fungal cell.

Table 1: Effect of fungicides on *Magnaporthe grisea in vitro*

Fungicide	Mycelial growth (mm)						Mean	Growth inhibition (%)						Mean
	Concentrations (ppm)							Concentrations (ppm)						
	250	500	750	1000	1250	1500		250	500	750	1000	1250	1500	
Tebuconazole	35.67 (36.65)	31.33 (34.02)	22.33 (28.16)	18.33 (25.34)	12.67 (20.84)	8.33 (16.77)	21.44 (26.97)	60.37 (50.97)	65.19 (58.82)	75.18 (60.10)	79.63 (63.15)	85.93 (67.95)	90.74 (72.26)	76.17 (61.37)
Carbendazim	43.33 (41.15)	35.67 (36.66)	33.00 (35.05)	25.33 (30.21)	21.33 (27.50)	13.00 (21.12)	28.61 (31.95)	51.85 (46.04)	60.37 (50.97)	63.33 (52.71)	71.85 (57.93)	76.30 (60.85)	85.56 (67.65)	68.21 (56.02)
Propiconazole	20.67 (27.03)	17.67 (24.84)	7.67 (16.06)	0.01 (0.57)	0.01 (0.57)	0.01 (0.57)	7.67 (11.61)	77.04 (61.35)	80.37 (63.68)	91.48 (73.01)	99.99 (89.39)	99.99 (89.39)	99.99 (89.39)	91.48 (77.70)
Control	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	47.42 (44.09)	43.67 (41.76)	38.25 (37.71)	33.42 (31.91)	31.00 (30.11)	27.84 (27.50)		47.32 (39.59)	51.48 (42.12)	57.50 (46.46)	62.87 (52.62)	65.56 (54.55)	69.07 (57.33)	
CD (P=0.05)	Conc.			(0.31)				(0.33)						
	fungicide			(0.38)				(0.41)						
	Conc. × fungicide			(0.76)				(0.82)						
SE±	Conc.			(0.11)				(0.12)						
	fungicide			(0.13)				(0.14)						
	Conc. × fungicide			(0.27)				(0.29)						

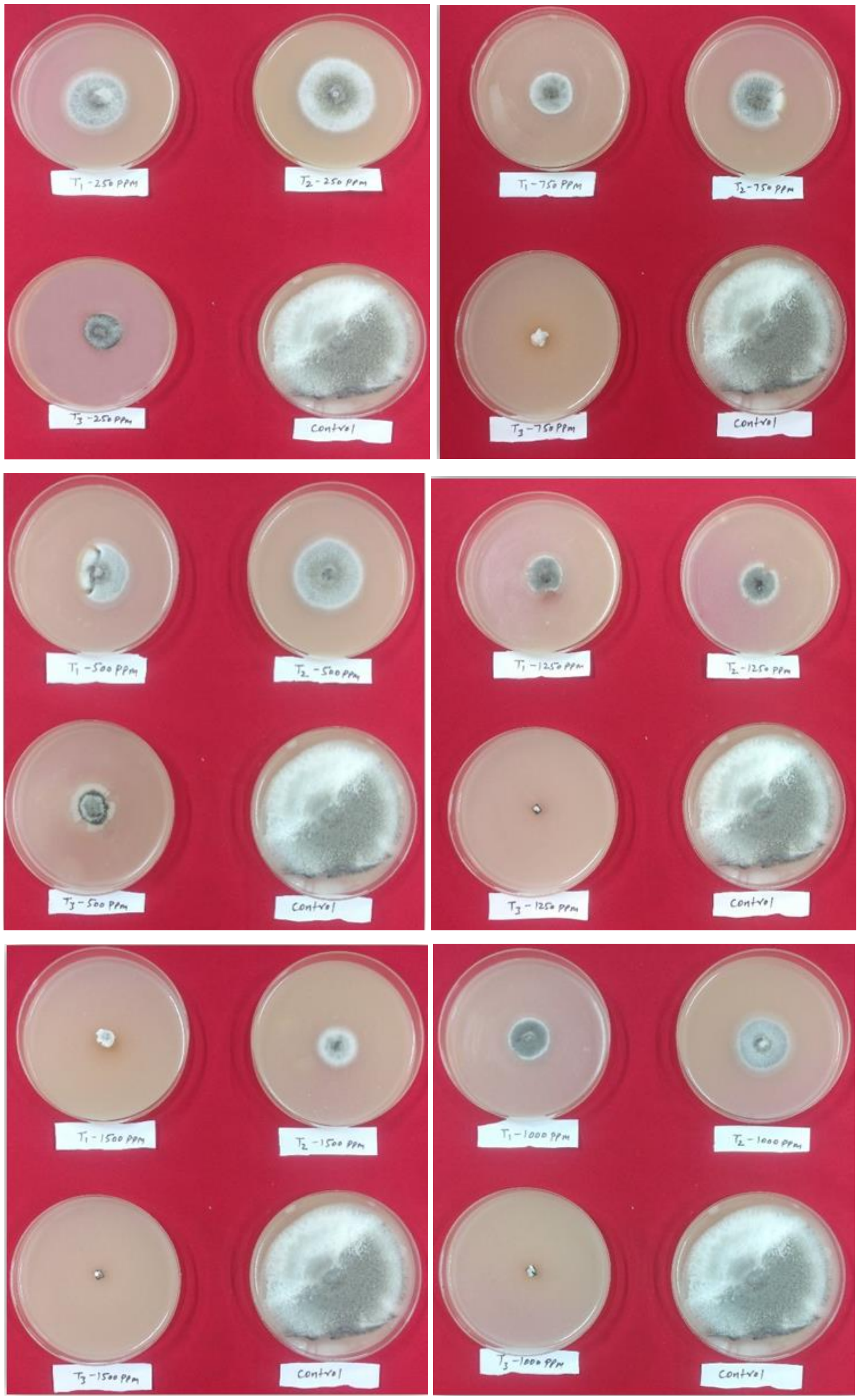

Plate: Growth inhibition of *Magnaporthe grisea* by fungicides

Conclusion

Among the fungicides evaluated propiconazole reported minimum (7.67 mm) mean mycelium growth and had maximum inhibition of the mycelial growth (91.48%) of the fungus followed by *Tebuconazole* as it showed 21.44 mm mean mycelial growth and 76.17 per cent growth inhibition. The both the chemicals were significantly superior over carbendazim (68.21%) which was found to be the least effective in inhibiting mycelial growth of the fungus.

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