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Evaluation of new Molecule Fungicides against Finger Millet (*Elusine coracana* (L.) Gaertn.) blast (*Pyricularia grisae*) (Cooke) Sacc.

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ABSTRACT: Finger millet (*Elusine coracana* (L.) Gaertn.) plays an important role in the dietary habits and economy of semiarid tropic farmers. Production was affected by some economically important disease like blast caused by *Pyricularia grisea,* that drastically reduce its production and causing considerable losses every year. The pathogen has breakdown of resistance within short period by develop new pathogenic races causes'. Thus, attempts have been made to manage blast disease in different crops by using different new molecule fungicide groups (eleven fungicides) belonging to two different concentrations (0.1 and 0.2%) were tested their efficacy under *in vitro* condition against blast pathogen using poison food technique. Tricyclazole 75%WP @ of 0.1% effectively inhibited the *Pyricularia grisea* mycelial growth to an extent of 76.67% over control.

The field experiments were conducted based on the *in vitro* studies during *rabi,* 2020 and *rabi,* 2021. Among the 13 treatments, Tricyclazole 75%WP (first spray at the time of blast incidence, second spray 10 to15 days after first spray @ 1 g/lit) recorded less incidence of leaf, neck and finger blasts also recorded the B:C ratio as 1:2.00 in both the seasons.

Keywords: Finger millet, fungicide, antibiotics, *Pyricularia grisea,* Tricyclazole.

INTRODUCTION

Finger millet was introduced from Ethiopian highlands to India around four thousand years ago as original native (Anon., 2012). India is the primary producer of finger millet, which is primarily grown in the states of Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Uttar Pradesh, Bihar, Orissa and Gujarat. It account together for 98% and 95% of the total production and cultivation area of finger millet in the country (Sonnad, 2005) respectively. Finger millet is having rich calcium source, around 10 folds that of wheat or rice. Unlike wheat and rice that require important inputs in terms of soil fertility and water, millets grow well in dry state as rain-fed crops (Michaelraj and Shanmugam 2013).

Generally diseases are the major limitations in production of finger millet. Totally, 25 fungal, 4 viral, 5 bacterial and 6 nematode pathogens have been recorded on this crop (Mundhe, 2005). The most important constraint in the production of finger millet in all the millet-growing regions of the world is blast disease caused by the fungus *Magnaporthe oryzae* (*Magnaporthe oryzae*) B. Couch (anamorph: *Pyricularia oryzae* Cavara); synonym *Magnaporthe grisea* (Hebert Barr) (Zhang *et al*., 2016) and causes yield losses around 28 % (Vishwanath and Seetharam 1989), however in a conducive climate it may go higher to 80 - 90 % (Ramappa *et al*., 2002).

The most capable, feasible, eco-friendly and low cost method to control the plant diseases is grow the resistance variety. Patro *et al*. (2018) understand the inheritance of resistance to *Pyricularia grisea* by attempts are being made to develop resistance finger millet lines. However, host plant resistance is the key factor to manage the rice blast disease. *Pyricularia grisea* has breakdown of resistance within few years by develop new pathogenic races causes' (Ahn, 1994). Thus, attempts have been made to manage blast disease in different crops by using fungicides (Pagani *et al*., 2014). While, old generation fungicides like carbendazim, ediphenphos etc., were found to be effective against blast diseases however, the advanced and new molecule fungicides spray at the time of incidence was lacking. Considering these facts in view, *in vitro* studies and field trials were conducted to manage all the three types of blasts (leaf, neck and finger) of finger millet by using new molecule of fungicides.

MATERIALS AND METHODS

Isolation of Pathogen (*Pyricularia grisea*). To isolate the pathogens from symptomised parts of the leaves were cut in to 2mm size pieces with sterilized scissors. Those pieces were surface sterilized by using 1% sodium hypochloride for 1 minute, followed by two successive cleaning with sterilized distilled water. Then they were kept in clean sterile petridish containing three layers of moistened blotting paper. The samples were then incubated at 25 to 26°C for one day. From the sporulating lesions on the leaf sample, single conidia were transferred to separate sterilized culture tubes of agar slants. Spreading conidia from the discrete lesions on 4% water agar with the help of aseptic inoculating needle under stereomicroscope to get single spore isolates. Transfer the germinating conidia aseptically to agar plate. The plate was incubated at 25± 2°C for 72-96 hours under incubator.

***In vitro* evaluation of fungicide against finger millet blast.** Various chemical groups of eleven fungicides at two different concentrations (0.1 and 0.2%) were tested for their efficacy under *in vitro* condition against *Pyricularia grisea* by using poison food technique (Nene and Thapliyal 1979). The fungicides concentrations taken were those of active ingredients present in commercial formulation. The required quantities of each test fungicides were incorporated in a 250 ml conical flask containing 100 ml of molten finger millet leaf extract agar (FLEA) medium so as to get required concentration in per cent (%). The poisoned medium was well shaken and poured in to sterilized petriplates in 20 ml each. On solidification of the medium, the plates were inoculated in the centre by placing 5 mm diameter mycelial disc cut by the help of cork borer from 15 days old actively growing *P. grisea* grown on FLEA medium. Each concentration of respective fungicide were maintaining three repetitions and incubated at 25±10°C temperature under B.O.D. The observations on mycelia growth of fungus were recorded at 24 hours interval up to full growth reached in control petriplate. Vincent (1927) illustrated the per cent growth inhibition (PGI) of the pathogen over control was worked out by using following formula,

$$PGI = 100\ (DC - DT)/\ DC$$

Where, PGI = Per cent growth inhibition

DC = Average mycelial diameter growth in control plate (mm)

DT = Average mycelial diameter growth in fungicide treated plate (mm).

Management of *Pyricularia grisea* under field condition. Based on the results of *in vivo* studies, the field experiments were conducted during the *rabi*, 2019 and *rabi*, 2020 at Centre of Excellence in Millets, Athiyandal (12° 23′N, 70°02′E, 280 m asl) against *Pyricularia grisea.* The finger millet variety CO (Ra) 14 was sown with standard plot size of 5 × 3 m, implementing the recommended spacing and dosage of fertilizers.

Treatment details

T. No.	Treatments	Method of application
T_1	*Bacillus subtilis*	Seed treatment and first spray at the time of blast incidence, second spray 10-15 days after first spray @ 0.6 %
T_2	Tebuconazole 50% + Trifloxystrobin 25 W	First spray at the time of blast incidence, second spray 10-15 days after first spray @ 0.1%
T_3	Tricyclazole 75%WP	First spray at the time of disease incidence, second spray 10-15 days after first spray @ 0.1%
T_4	Tricyclazole + Mancozeb 62%WP	First spray at the time of disease incidence, second spray 10-15 days after first spray @ 0.1%
T_5	Isoprothiolane 40% EC	First spray at the time of disease incidence, second spray 10-15 days after first spray @ 1 ml/lit
T_6	Azoxystrobin + Difenconazole	First spray at the time of disease incidence, second spray 10-15 days after first spray @ 1 ml/lit
T_7	Propiconazole	First spray at the time of disease incidence, second spray 10-15 days after first spray @ 1 ml/lit
T_8	Carbendazim + Mancozeb	First spray at the time of disease incidence, second spray 10-15 days after first spray @ 2 g/lit
T_9	Carbendazim 50%WP	First spray at the time of disease incidence, second spray 10-15 days after first spray @ 1 g/lit
T_{10}	Kasugamycin	First spray at the time of disease incidence, second spray 10-15 days after first spray @ 2 ml/lit
T_{11}	Blasticidin	First spray at the time of disease incidence, second spray 10-15 days after first spray @ 2 ml/lit
T_{12}	Aureofunginsol	First spray at the time of disease incidence, second spray 10-15 days after first spray @ 2 ml/lit
T_{13}	Control	

In the present investigation the treatments are framed to test the efficacy of new molecule fungicides (Tebuconazole 50% + Trifloxystrobin 25 W, Tricyclazole + Mancozeb 62% WP, Isoprothiolane 40% EC, Azoxystrobin + Difenconazole, Propiconazole) as it is unique combination of systemic and contact fungicide were tested with standard checks (Tricyclazole 75%WP, Carbendazim 50%WP and Carbendazim + Mancozeb) with bacterial antagonist (*Bacillus subtilis*) and antibiotics (Kasugamycin, Blasticidin and Aureofunginsol) were used to comparison studies under field condition. The trial is design as randomized block design (RBD) with three replications to find out the management of blast in finger millet under field condition. After observing the leaf blast incidence, treatment spray was carried out and second spray 10-15 days after first spray. The leaf blast (50 DAS), neck blast (Flowering stage) and finger blast (Maturity stage) disease incidence and grain yield were recorded.

Blast disease assessment: The occurrences of leaf blast in individual leaves were recorded by using 1–9 scale Standard Evaluation System (SES). The neck blast and finger blast severity (%) were enumerated across all the panicles in each replication and treatment. Total number of infected neck and finger were counted and disease incidence % was worked out by using the following formula as followed in All India Co-ordinated Research Project on Small Millets (AICRP-SM) 27th Annual Group Meeting, 2016 (Patro *et al*., 2020).

Finger millet Leaf blast Standard Evaluation System (SES)

Score	Description
1	Small brown specks of pinhead size without sporulating centre.
2	Small roundish to slightly elongated, necrotic grey spots, about 1-2 mm in diameter with a distinct brown margin and lesions are mostly found on the lower leaves.
3	Lesion type is the same as in scale 2, but significant numbers of lesions are on the upper leaves.
4	Typical sporulating blast lesions, 3 mm or longer, infecting less than 2% of the leaf area.
5	Typical blast lesions infection in 2-10% of the leaf area.
6	Blast lesions infecting 11-25% leaf area.
7	Blast lesions infecting 26-50% leaf area.
8	Blast lesions infecting 51-75% leaf area.
9	More than 75% leaf area affected

$$\text{a) Neck blast (\%)} = \frac{\text{Number of infected panicles}}{\text{Total number of panicles}} \times 100$$

$$\text{b) Finger blast (\%)} \quad \frac{\text{Number of infected fingers}}{\text{Average no. of fingers per plant X Total number of panicles}} \times 100$$

Economic appraisal (B:C ratio) of treatments. Economic analyses of each treatment were worked out on input costs and returns basis. Total returns were calculated by marketable yields of grain and fodder obtained in each treatment. The cost of bio-control agent and fungicides used per treatment and spraying cost of fungicides were estimated. The increase in grain and fodder yield over control was assumed to be exclusively due to the treatments effect. For that reason, partial budgeting was used to magnify the profit per hectare for each treatment. The profit was worked by deducting the treatment cost from additional income derived from yield increase above control (Untreated). Costs of land preparation, sowing, weeding, fertilizer application, irrigation and harvesting were incorporated in the partial budgeting. Benefit-cost ratio, was calculated as

$$\text{Benefit - Cost ratio } = \frac{\text{Net Return (Rs.)}}{\text{Total cost (Rs.)}} \times 100$$

Statistical analysis of the experiment. The experimental data statistical analysis was carried out by adopting the standard method (Gomez and Gomez, 1984). The spray treatments impact was observed by analysis of variance (ANOVA) of randomized block design (RBD). Data of neck blast and finger blast were arcsine transformed before analysis.

RESULTS AND DISCUSSION

***In vitro* studies.** The results indicated that Tricyclazole 75%WP @ of 0.1% effectively controlled the *Pyricularia grisea* mycelial growth to an extent of 76.67% over control. Followed by Tricyclazole + Mancozeb 62%WP @ of 0.1 per cent treatment inhibited the mycelial growth to an extent of 75.56% over control (Table 1). Both treatments are on-bar with each other; however Tricyclazole as the effective component of the fungicide to control the blast pathogen under *in vitro* condition.

Table 1: Management of finger millet blast disease under *in vitro* condition (Poison food technique).

Sr. No.	Plants	Concentration (%)	Mean mycelial growth (cm)*	Per cent decrease over control
1.	Tebuconazole 50% + Trifloxystrobin 25 W	0.1	3.10	65.56 (54.07)
2.		0.2	3.25	63.89 (53.06)
3.	Tricyclazole 75%WP	0.1	2.10	76.67 (61.12)
4.		0.2	2.25	75.00 (60.00)
5.	Tricyclazole + Mancozeb 62%WP	0.1	2.20	75.56 (60.37)
6.		0.2	2.60	71.11 (57.49)
7.	Isoprothiolane 40% EC	0.1	3.65	59.44 (50.44)
8.		0.2	3.95	56.11 (48.51)
9.	Azoxystrobin + Difenconazole	0.1	2.90	67.78 (55.42)
10.		0.2	4.00	55.56 (48.19)
11.	Propiconazole	0.1	3.15	65.00 (53.73)
12.		0.2	3.65	59.44 (50.44)
13.	Carbendazim + Mancozeb	0.1	3.75	58.33 (49.80)
14.		0.2	4.05	55.00(47.87)
15.	Carbendazim 50%WP	0.1	3.95	56.11(48.51)
16.		0.2	4.10	54.44 (47.55)
17.	Kasugamycin	0.1	4.80	46.67 (43.09)
18.		0.2	5.15	42.78 (40.85)
19.	Blasticidin	0.1	4.90	45.56 (42.45)
20.		0.2	5.25	41.67 (40.20)
21.	Aureofunginsol	0.1	4.95	45.00 (42.13)
22.		0.2	5.00	44.44 (41.81)
23.	Control	-	9.00	00.00 (00.72)
	S.Em ±	-	-	0.99
	CD at 5%	-	-	2.07

* Mean of three replications

The fungicides evaluation under i*n vitro* condition against rice blast pathogen, *Pyricularia grisea* showed that tricyclazole + tebuconazole (36% SC), tebuconazole 25% SC, hexaconazole 5% EC, zineb 68% + hexaconazole 4% WP and tebuconazole 50% + trifloxystrobin 25% WG inhibited completely the growth of fungus and germination of fungal spores in all concentration (Kavanashree *et al.*, 2019). Neelkanth *et al*. (2017) revealed that carbendazim, tricyclazole and trifloxystrobulin + tebuconazole of all concentrations were found to be effective against blast pathogen showing 100% inhibition of mycelial growth under *in vitro* condition.

Field studies. In the Active Tillering stage (30-35 DAS), leaf blast incidence occurred in all the plot (up to 8 grade). At the time of incidence the spray treatment were carried out and observations taken on flowering stage or 50 DAS. Among the 13 treatments, including the new molecule of fungicides, Tricyclazole 75%WP (T_3 - first spray at the time of disease incidence, second spray 10-15 days later @ 1 g/lit) recorded less incidence of leaf, neck and finger blasts in both the trials. It reflected in high grain yield during *rabi* 2019 and 2020. Followed by Tricyclazole + Mancozeb 62%WP spray (T_4) treatment recorded the lesser incidences of finger millet blasts. Antibiotics and bio-control agents are showed the least recovery of blast incidences under field conditions (Table 2&4).

The Tricyclazole, sole and also as constituent new molecule fungicides are effectively controlled the finger millet blast pathogen under field conditions. Both the treatments are on-bar with each other. In this condition the B:C ratio analyzed through partial budgeting method. All the new molecule of fungicides recorded considerable yield increase than bio-control agent and antibiotics (1:1.40 to 1:1.50). Tricyclazole alone (T_3) recorded as 1:2.00 and combined with Mancozeb (T_4) recorded as 1:1.98 during *rabi,* 2019 and 2020 (Table 3&5 Chart 1).

New generation chemical, Tricyclazole can offered effective management against rice blast pathogen (Singh *et al*., 2000). Similar report of Raj and Pannu (2017) also showed the managing rice blast pathogen by Tricyclazole and Propiconazole under field condition and Mohiddin *et al*. (2021) reported that the Tricyclazole was most effective against rice blast and recorded a leaf blast incidence of only 8.41%. Neelkanth *et al*. (2017) found that tricyclazole, was found drastically controlling the pathogen with the lowest PDI (Per cent Disease Index), in addition significant increase in the yield was observed in tricyclazole sprayed plots as compared to other fungicides. In rice ecosystem, fungicides proved very effective control against *Pyricularia oryzae* (Dutta *et al*., 2012; Prajapati *et al*., 2004; Sood and Kapoor, 1997). For pearl millet blast, Carbendazim and Tricyclazole showed effective control under *in vivo* conditions (Joshi and Gohel 2015; Lukose *et al.*, 2007). On the other hand, rice blast pathogen isolates showed differential sensitivity to Tricyclazole and Carbendazim (Yuan and Yang, 2003; Mohammad *et al*., 2011).

Table 2: Finger millet blast disease management under *in vivo* condition during *rabi* 2019.

Trt. No.	Leaf blast (G) 30 DAS	Leaf blast (G) 50 DAS	Neck blast (%)	Finger blast (%)	Grain yield (kg/ha)	Fodder yield (kg/ha)	Yield increase over control (%)
T_1	7.33	6.33	14.00 (21.96)	12.00 (20.26)	1880	4710	1.71 (7.51)
T_2	6.67	5.33	6.17 (14.38)	5.97 (14.14)	2606	5693	22.93 (28.60)
T_3	7.33	3.67	2.83 (9.68)	3.17 (10.25)	2718	5937	28.18 (32.05)
T_4	7.33	4.00	3.55 (10.86)	4.73 (12.56)	2681	5740	25.95 (30.61)
T_5	7.67	5.67	7.95 (16.37)	7.57 (15.96)	2488	5620	21.38 (27.53)
T_6	7.67	5.67	6.17 (14.38)	6.05 (14.23)	2595	5575	20.36 (26.81)
T_7	7.33	5.33	7.33 (15.70)	6.28 (14.51)	2525	5477	18.25 (25.28)
T_8	7.33	6.00	10.18 (18.60)	7.35 (15.72)	2345	5270	13.80 (21.80)
T_9	7.67	5.67	9.40 (17.85)	6.07 (14.26)	2500	5350	15.53 (23.20)
T_{10}	7.00	6.33	12.00 (20.26)	10.00 (18.43)	1910	4730	2.14 (8.41)
T_{11}	7.67	6.00	11.50 (19.82)	9.50 (17.94)	1925	4710	1.71 (7.51)
T_{12}	7.33	6.00	12.50 (20.70)	10.50 (18.90)	1940	4750	2.57 (9.22)
T_{13}	7.67	7.33	21.50 (27.61)	19.50 (26.19)	1810	4632	0.00 (0.72)
S. Em ±	0.46	0.58	1.34	0.96	59.74	78.06	1.63
CD at 5%	0.97	1.22	2.79	2.00	124.24	162.34	3.38

Figures in the parentheses are arcsine transformed values

Table 3: Calculation of cost benefit ratio of treatments (Partial budgeting method).

Treatment	Fixed cost (Rs.)	Treatment (Chemical + Spray) cost (Rs.)	Total cost (Rs.)	Grain Yield (kg/ha)	Straw Yield (kg/ha)	Gross Return (Rs.)	Net Return (Rs.)	B:C ratio
T_1	47500	960	48460	1880	4710	69333	20873	1:1.43
T_2	47500	1600	49100	2606	5693	95480	46380	1:1.94
T_3	47500	1550	49050	2778	5936	99582	50532	1:2.00
T_4	47500	1925	49425	2631	5740	96390	46965	1:1.98
T_5	47500	1250	48750	2488	5621	91296	42546	1:1.87
T_6	47500	2450	49950	2595	5574	95006	45056	1:1.90
T_7	47500	1850	49350	2525	5476	92482	43132	1:1.87
T_8	47500	1650	49150	2345	5270	86028	36878	1:1.75
T_9	47500	1200	48700	2500	5350	91513	42813	1:1.87
T_{10}	47500	1650	49150	1910	4730	70398	21248	1:1.43
T_{11}	47500	1625	49125	1925	4710	70908	21783	1:1.44
T_{12}	47500	1850	49350	1940	4750	71463	22113	1:1.44
T_{13}	47500	-	47500	1810	4631	66823	19323	1:1.40

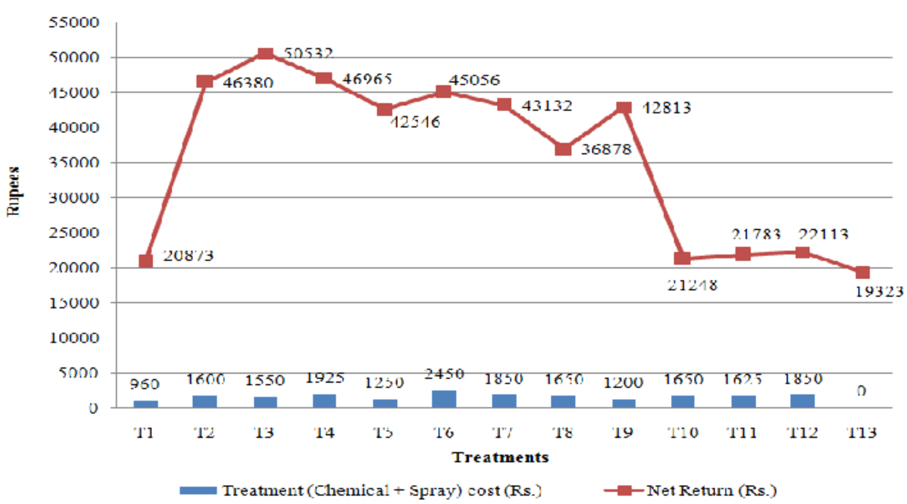

Chart 1. Calculation of cost benefit ratio of treatments.

Table 4: Finger millet blast disease management of under *in vivo* condition during *Rabi* 2020.

Trt. No.	Leaf blast (G) 30 DAS	Leaf blast (G) 50 DAS	Neck blast (%)	Finger blast (%)	Grain yield (kg/ha)	Fodder yield (kg/ha)	Yield increase over control (%)
T_1	7.67	6.67	16.00 (23.57)	11.00 (19.36)	1760	4720	1.94 (8.00)
T_2	7.67	6.33	16.10 (23.65)	15.20 (22.94)	2500	5690	22.89 (28.57)
T_3	8.00	3.33	2.00 (8.13)	4.00 (11.53)	2680	5940	28.29 (32.12)
T_4	7.67	4.33	5.50 (13.56)	6.75 (15.05)	2605	5745	24.08 (29.38)
T_5	8.00	6.67	17.50 (24.72)	12.57 (20.76)	2377	5620	21.38 (27.53)
T_6	7.67	6.33	16.75 (24.15)	11.00 (19.36)	2490	5570	20.30 (26.77)
T_7	7.67	6.33	17.25 (24.53)	14.25 (22.17)	2420	5470	18.14 (25.20)
T_8	8.33	6.33	12.80 (20.95)	16.33 (23.83)	2245	5275	13.93 (21.91)
T_9	8.00	6.67	16.40 (23.88)	13.07 (21.19)	2410	5357	15.68 (23.32)
T_{10}	7.67	6.67	12.00 (20.26)	16.00 (23.57)	1980	4735	2.27 (8.66)
T_{11}	7.67	6.67	14.50 (22.37)	14.50 (22.37)	2010	4715	1.84 (7.79)
T_{12}	8.33	6.33	16.50 (23.96)	16.50 (23.96)	1990	4757	2.72 (9.49)
T_{13}	8.00	8.67	20.50 (26.91)	21.50 (27.61)	1690	4630	0.00 (0.72)
S. Em ±	0.60	0.54	1.15	1.28	75.98	78.38	1.42
CD at 5%	1.25	1.13	2.41	2.67	158.01	163.01	2. 97

Figures in the parentheses are arcsine transformed values

Table 5: Calculation of cost benefit ratio of treatments (Partial budgeting method).

Treatment	Fixed cost (Rs.)	Treatment (Chemical + Spray) cost (Rs.)	Total cost (Rs.)	Grain Yield (kg/ha)	Straw Yield (kg/ha)	Gross Return (Rs.)	Net Return (Rs.)	B:C ratio
T_1	47500	960	48460	1760	4720	65140	16680	1:1.34
T_2	47500	1600	49100	2500	5690	91768	42668	1:1.86
T_3	47500	1550	49050	2680	5940	98582	49532	1:2.00
T_4	47500	1925	49425	2605	5745	95484	46059	1:1.93
T_5	47500	1250	48750	2378	5620	87445	38695	1:1.79
T_6	47500	2450	49950	2490	5570	91328	41378	1:1.82
T_7	47500	1850	49350	2420	5470	88803	39453	1:1.79
T_8	47500	1650	49150	2245	5275	82531	33381	1:1.67
T_9	47500	1200	48700	2410	5356	88367	39667	1:1.81
T_{10}	47500	1650	49150	1980	4735	72851	23701	1:1.48
T_{11}	47500	1625	49125	2010	4715	73886	24761	1:1.50
T_{12}	47500	1850	49350	1990	4756	73217	23867	1:1.48
T_{13}	47500	-	47500	1690	4630	62623	15123	1:1.31

Fig 1. Leaf, Neck and Finger blast symptoms and field view.

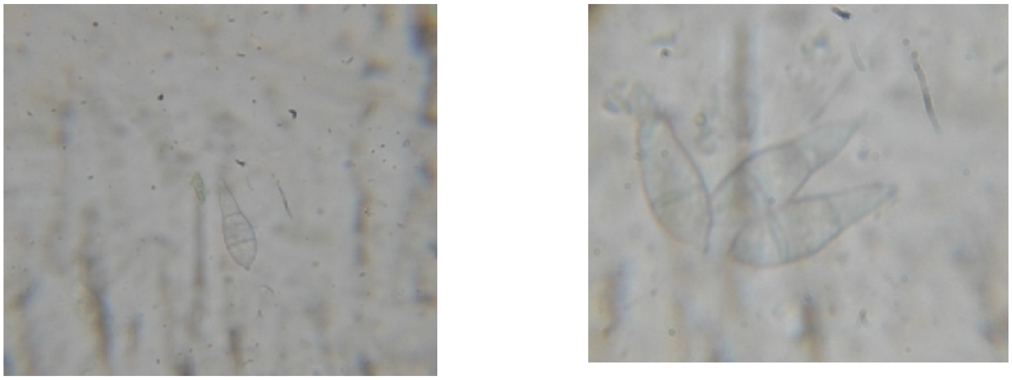

Fig. 2. *Pyricularia grisea* conidia (100 and 400 magnification)

Fig. 3. *In vitro* evaluation of biogents *viz., Trichoderma viride/asperellum* and *Bacillus subtilis* by dual culture technique.

Fig. 4. Field view of integrated management of finger millet blast disease under field condition.

CONCLUSION

All the new molecule of fungicides recorded considerable yield increase. Among that, Tricyclazole is the effective component of the new molecule fungicide to control the blast pathogen in all the cereal crops. Fungicides are effective component for the management of pathogens like *Pyricularia grisea* has the ability to overcome the resistance by developing new pathogenic races within few years or seasons. Generally millets are low value crops, resistant variety, seed treatment, time of sowing; good agricultural practices are the key component to compact the blast diseases, even though certain conditions they may prevail under field conditions. In that situation, the fungicide spray at the time of disease occurrence effectively controls the pathogen in an economic way as indicated present article. Fungicides are one of the unavoidable, essential bricks to build the integrated disease management practices especially pathogens like *Pyricularia*. The component of the integrated management practices may change in future based on the reaction of the pathogen.

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Conflict of Interest. None.

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