

## ***In vitro* Evaluation of Bio-agents and Fungicides Against Leaf Blast (*Pyricularia setariae*) in Foxtail Millet [*Setaria italica* (L.) Beauv.]**

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Various bio agents and fungicides were tested *in vitro* against *Pyricularia setariae* which causes leaf blast in foxtail millet. Among fungal antagonists, all the *Trichoderma* spp tested overgrew on the pathogen and showed cent per cent of mycelia inhibition. Among the bacterial antagonists, *Bacillus cereus* showed 81.14 per cent mycelia inhibition followed by *B. subtilis* (80.26%). Among the contact and combi-products cent per cent inhibition was observed in mancozeb, carbendazim + mancozeb, carboxin + thiram and tebuconazole + trifloxystrobin at 500, 1000 and 2000 ppm. Whereas, in systemic fungicides, Cent per cent inhibition of mycelia growth was recorded in hexaconazole, penconazole, propiconazole and tebuconazole at all the concentrations tested (50, 100 and 200 ppm)

**Keywords:** *Pyricularia setariae*, Fungicides, Bio-agents, Foxtail millet.

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Currently, the area and production of traditional crops are showing a declining trend in most developing countries. Yet, in many parts of the world, these traditional crops play a major role in both the dietary needs and incomes of many rural households. One such traditional group of cereal crops is the minor coarse cereals (small millets). Among the small millets, foxtail millet [*Setaria italica* (L.) Beauv.] is one of the oldest cultivated cereal crops after finger millet. It is native to China<sup>1</sup> and introduced, annual, warm-season crop. It is a staple food and fodder crop and generates income for millions of poor people. It is widely grown throughout China, India, Russia, Africa, and the United States. In India, it is cultivated widely in Andhra Pradesh, Karnataka, Tamil Nadu and Uttarakhand. It is a rich source of carbohydrate,

protein and essential amino acid, leucine. It is very good food for heart and diabetic patients because it contains magnesium<sup>2</sup>. It has medicinal value as it is used for curing rheumatism and is a popular domestic remedy for alleviating pains during pasturation and is also given to the patients having jaundice and measles<sup>3</sup>. It has been suggested that foxtail millet protein be used as a food component to fight type 2 diabetes and cardiovascular diseases<sup>4</sup>.

However this crop is challenged by many fungal diseases like leaf blast, brown spot, rust, downy mildew, udbatta and bacterial diseases like bacterial streak. Among these diseases, leaf blast is one of the most destructive diseases. It causes up to 30 per cent yield loss in its severe form<sup>5</sup>. Therefore, investigations were undertaken to manage *Pyricularia setariae* by different bio agents and fungicides *in vitro* by dual plate and poisoned food technique respectively.

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## MATERIALS AND METHODS

### Bioagents

*In vitro* evaluation was carried out with bioagents listed in Table 1 against *Pyricularia setariae* through dual culture technique.

In dual culture technique, twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates. Fungal antagonists were evaluated by inoculating the pathogen on one side of Petri plate and the antagonist on the opposite side of the same plate by leaving 3-4 cm gap. But for bacterial antagonists, a fungal disc was placed at centre after which bacterial antagonist was streaked around it. Each treatment was replicated three times. After required period of incubation when control plate reached 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was worked out according to the equation<sup>6</sup>.

$$I = \frac{(C-T)}{C} \times 100$$

Where,

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

### Fungicides

Different contact fungicides *viz.*, mancozeb, chlorothalonil, captan and copper oxy chloride and combi-product fungicides *viz.*, carbendazim 12% + mancozeb 63 % WP, carboxin + thiram and tebuconazole 50% + trifloxystrobin 25% WG were evaluated at different concentrations of 500 ppm, 1000 ppm and 2000 ppm and systemic fungicides *viz.*, azoxystrobin, carbendazim, difenconazole, hexaconazole, propiconazole, tebuconazole, thifluzamide and trifloxystrobin were evaluated at different concentrations of 50 ppm, 100 ppm and 200 ppm.

The fungicides were tested against *Pyricularia setariae* by adopting 'Poisoned food technique'. Molten sterilized potato dextrose agar was prepared and autoclaved. The medium was cooled to 40 °C. The fungicides were dissolved in sterilized water to make stock solution. Then appropriate quantity of stock solution was added to PDA, so as to get a required concentration and the flasks were agitated gently so as to disperse the fungicidal solution thoroughly into agar medium.

About 15 to 20 ml of poisoned PDA was poured into 90 mm Petri plates and allowed to solidify. The plates were rotated in clockwise direction to aid in uniform distribution of the medium. The actively growing peripheral growth of seven days old culture of fungus was carefully cut under aseptic condition by a gel cutter and transferred to centre of each Petri plates containing the poisoned medium. Suitable control was maintained in which the fungal pathogen was grown under similar conditions on PDA without poisoning the medium. Inoculated plates were incubated at 27±1°C for seven days and the colony diameter was recorded after seven days of incubation by measuring the radial growth of the fungus in two directions at right angle to each other and average diameter was calculated. The per cent inhibition of growth over control was determined<sup>6</sup> as mentioned earlier.

## RESULTS AND DISCUSSION

The fungal antagonists like *Trichoderma asperillum*, *T. harzianum* and *T. viride* and bacterial bioagents *viz.*, *Pseudomonas fluorescens* and *Bacillus subtilis* secured from different sources were tested on *Pyricularia setariae* by dual plate technique and data is presented in Table 2, Plate 1.

All the fungal antagonists, showed hundred per cent inhibition of mycelial growth by overgrew on the test pathogen *Pyricularia setariae*. Among the bacterial bio agents *Bacillus cereus* and *B. subtilis* were effective by inhibiting 81.14 per cent and 80.26 per cent of the mycelial growth of the test pathogen.

Species of *Trichoderma* showed more mycelial inhibition of organisms compared to bacterial antagonists. This can be attributed to higher competitive ability of the *Trichoderma* spp. either by mycoparasitism, antibiosis or siderophore production. Inhibition of mycelial growth was due to coiling of hyphae of the pathogen by *Trichoderma* as reported<sup>7</sup>. It may also be due to the production of cell wall degrading enzyme (CWDE) which has high endochitinase activity that can break down the cell wall of the fungus as reported by<sup>8, 9, 10</sup> reported that *Trichoderma* spp. produced secondary metabolites such as antibiotics (6-pentyl-alpha-pyrone (6pp), isocyanide derivatives), acids (heptelidic and koningic acid), peptaibols and CWDE that are implicated in the inhibition of radial

growth of many phytopathogenic fungi. Several other workers<sup>11-14</sup> also reported that *Trichoderma* spp. were very effective in inhibiting the mycelial growth of *P. oryzae*. Likewise, among the different bacterial antagonists, maximum mycelial growth inhibition (81.14 %) was showed by *Bacillus cereus* (GKVK, Bengaluru) followed by *B. subtilis* (GKVK, Bengaluru) which showed 80.26 per cent of inhibition. In this study, the mechanism of inhibition of bacterial isolates against *P. setariae* presumably due to the activity of antibiotic like substance. *Bacillus* produces variety of antibiotic that are effective against many fungi such as zwittermycin-A<sup>15</sup>, kanamycin and lipopeptida of it urin, surfatin and fengycin<sup>16</sup>. Also,<sup>17</sup> reported chitinolytic activity of *Bacillus cereus* II.14.<sup>18</sup> <sup>19</sup> also found 60 per cent inhibition of *P. grisea* by *B. subtilis* strain NSRS 89-24 in dual culture.

Similarly,<sup>20</sup> also found that the growth of *P. setariae* was 2.5 and 3.0 cm due to inhibition of *Bacillus polymyxa* VLB-17 and *Pseudomonas fluorescens* Pf-52 respectively.

The efficacy of four contact fungicides and four combi-products were tested against *Pyricularia setariae* at three concentrations i.e., 500, 1000 and 2000 ppm by poisoned food technique.

Contact fungicide mancozeb alone showed complete inhibition (100.00 %) of mycelial growth at all the three concentrations tested (500, 1000 and 2000 ppm). Among the different combi-product fungicides, carbendazim + mancozeb, carboxin + thiram and tebuconazole + trifloxystrobin showed cent per cent of mycelial inhibition at 500, 1000 and 2000 ppm concentrations whereas least growth inhibition was observed in chlorothalonil (Table 3,

**Table 1.** List of bioagents used for *in vitro* evaluation against *P. setariae*

S No.	Bioagents	Source / isolate
1	<i>Trichoderma harzianum</i>	NBAIR, Bengaluru
2	<i>T.viride</i>	NBAIR, Bengaluru
3	<i>T. harzianum</i>	Microbiology lab, UAS, GKVK, Bengaluru
4	<i>T.asperellum</i>	Bacteriology lab, UAS, GKVK, Bengaluru
5	<i>T.harzianum</i>	Bacteriology lab, UAS, GKVK, Bengaluru
6	<i>T. harzianum-6</i>	IIHR, Bengaluru
7	<i>Pseudomonas fluorescens</i>	Bacteriology lab, UAS, GKVK, Bengaluru
8	<i>P.fluorescens</i>	NBAIR, Bengaluru
9	<i>Bacillus subtilis</i>	Bacteriology lab, UAS, GKVK, Bengaluru
10	<i>B. subtilis</i>	NBAIR, Bengaluru
11	<i>B. cereus</i>	Bacteriology lab, UAS, GKVK, Bengaluru

**Table 2.** Effect of bio agents on the growth of *Pyricularia setariae* *in vitro*

S. No.	<i>Trichoderma</i> isolate	Percent inhibition of mycelial growth
1	<i>T. harzianum</i> 1 (IIHR)	100.00 (90.00)
2	<i>T. viride</i> (GKVK)	100.00 (90.00)
3	<i>T. harzianum</i> 6 (IIHR)	100.00 (90.00)
4	<i>T. viride</i> (NBAIR)	100.00 (90.00)
5	<i>T. harzianum</i> (NBAIR)	100.00 (90.00)
6	<i>T. asperellum</i> (GKVK)	100.00 (90.00)
7	<i>Bacillus cereus</i> (GKVK)	81.14 (64.27)
8	<i>B. subtilis</i> (NBAIR)	72.40 (58.30)
9	<i>B. subtilis</i> (GKVK)	80.26 (63.60)
10	<i>Pseudomonas fluorescens</i> (NBAIR)	75.80 (60.54)
11	<i>P. fluorescens</i> (GKVK)	78.25 (62.18)
	S.Em±	0.64
	CD (P 0.01)	1.90
	CV(%)	1.44

Note: Figures in the parenthesis are arc sine transformed values

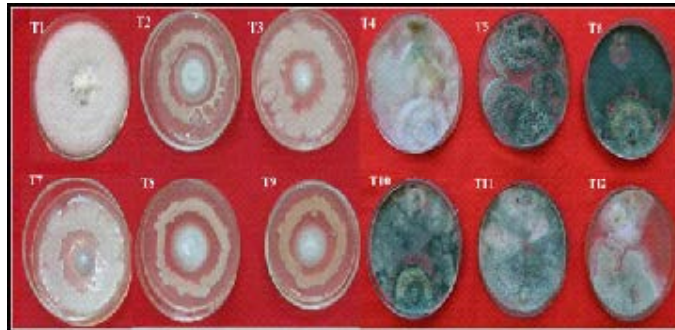
**Table 3.** *In vitro* efficacy of contact fungicides and combi-products on *P. setariae*

Treatments	Per cent inhibition of mycelial growth			Mean
	@500ppm	@1000ppm	@2000ppm	
Captan	55.37* (48.07)**	63.70 (52.94)	70.92 (57.37)	63.33
Chlorothalonil	44.97 (41.79)	60.18 (50.86)	71.48 (57.71)	58.88
Copper oxy chloride	87.77 (69.50)	100.00 (90.00)	100.00 (90.00)	95.92
Mancozeb	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
Cymoxanil+ mancozeb	85.00 (67.21)	87.59 (69.36)	100.00 (90.00)	90.86
Tebuconazole + trifloxystrobin	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
Carbendazim+mancozeb	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
Carboxin + thiram	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
Mean	82.75	87.54	91.41	87.23
	Fungicide (F)			
Concentration (C)	F×C			
S. Em±	0.33	0.20	0.57	
CD @ P 0.01	1.18	0.72	2.04	
CV (%)	1.25			

**Table 4.** *In vitro* efficacy of systemic fungicides on *Pyricularia setariae*

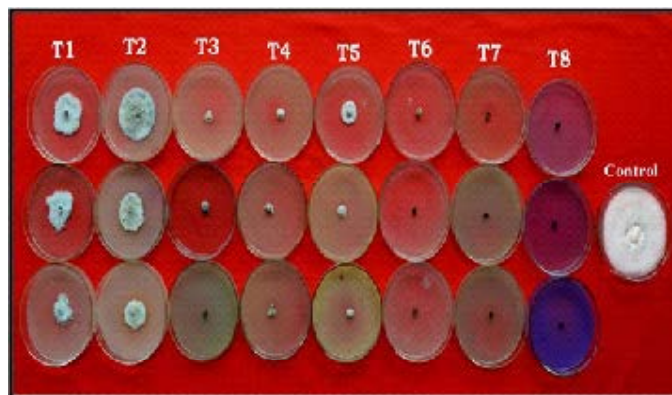
Treatments	Per cent inhibition of mycelial growth			Mean
	@500ppm	@1000ppm	@2000ppm	
Azoxystrobin	49.62* (44.77)**	52.96 (46.68)	56.66 (48.81)	53.08
Carbendazim	56.66 (48.81)	86.85 (68.74)	86.85 (68.74)	85.18
Difenconazole	86.85 (68.74)	88.14 (69.85)	100.00 (90.00)	89.01
Hexaconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
Penconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
Propiconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
	17.96	27.03	44.44	29.81
Thifluzamide	(25.05)	31.31	(41.75)	
Tebuconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
Trifloxystrobin	65.18 (53.82)	70.74 (57.24)	74.81 (59.86)	70.24
Mean	76.91	80.64	84.89	80.10
	Fungicide (F) Concentration (C)			
S. Em±	0.39	0.23	0.68	
CD @ P 0.01	1.46	0.84	2.52	
CV (%)	14.37			

Note: Figures in the parenthesis are arc sine transformed values



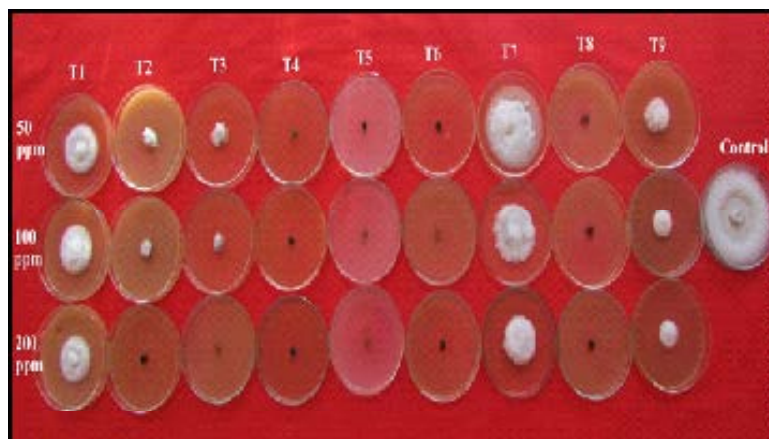
Treatment details: T<sub>1</sub>=Control, T<sub>2</sub>=*B. subtilis*, T<sub>3</sub>=*B. subtilis*(GKVK), T<sub>4</sub>=*T. harzianum*(GKVK), T<sub>5</sub>=*T. harzianum*(Microbiology, GKVK), T<sub>6</sub>=*T. harzianum*-6 (IIHR), T<sub>7</sub>=*B. cereus*, T<sub>8</sub>=*P. fluorescens*(NBAIR), T<sub>9</sub>=*P. fluorescens*(GKVK), T<sub>10</sub>=*T. viride* (NBAIR), T<sub>11</sub>=*T. harzianum*(NBAIR), T<sub>12</sub>=*T. asperellum*

**Plate 1.** Efficacy of bioagents on inhibition of mycelial growth of *P. setariae*



Treatment details: T1 = Captan, T2= Chlorothalonil, T3 = Copper oxychloride, T4 = Mancozeb, T5= Cymoxanil+Mancozeb, T6= Tebuconazole + trifloxystrobin, T7= Carbendazim + mancozeb, T8= Carboxin + thiram

**Plate 2.** *In vitro* efficacy of different contact fungicides and combiproducs against *P. setariae*



Treatment details: T1= Azoxystrobin, T2=Carbendazim, T3=Difenconazole, T4=Hexaconazole, T5=Penconazole, T6= Propiconazole, T7= Thifluzamide, T8= Tebuconazole, T9=Trifloxystrobin

**Plate 3.** *In vitro* efficacy of different systemic fungicides against *P. setariae*

Plate 2).

Among the different concentrations, maximum inhibition of mycelial growth (91.41 %) was recorded at 2000 ppm whereas least inhibition (82.75 %) was recorded at 500 ppm. Among the fungicide and concentration interaction, cent per cent inhibition was recorded in mancozeb, tebuconazole + trifloxystrobin, carbendazim + mancozeb and carboxin + thiram at all the three concentrations as also by copper oxy chloride at 1000 and 2000 ppm and also by cymoxanil + mancozeb only at 2000 ppm concentration. The lowest mycelial inhibition was observed in chlorothalonil at 500 ppm (44.97 %). The findings agree with<sup>13</sup> who reported that among the fungicides tested only mancozeb was the highly effective fungicide restricting the complete mycelia growth of *P. oryzae*.<sup>22</sup> observed that mancozeb exhibited excellent control of rice blast disease caused by *M. oryzae*.

Among systemic fungicides tested, cent per cent inhibition of mycelium was recorded in hexaconazole, penconazole, propiconazole and tebuconazole. The least growth inhibition was observed in thifluzamide (29.81 %) (Table 4; Plate 3).

The complete inhibition (100 %) of mycelial growth was observed at 200 ppm while least inhibition (76.91 %) was observed at 50 ppm. Among the interaction effects of fungicide and concentrations, the complete inhibition of mycelial growth was recorded by hexaconazole, penconazole, propiconazole and tebuconazole at all the three concentrations tested and also by difenconazole only at higher concentration (200 ppm). The least growth inhibition of 17.96 per cent was observed in thifluzamide at 50 ppm concentration.

The effectiveness of the triazole fungicides like propiconazole, difenconazole, tebuconazole and penconazole may be attributed to their interference with the biosynthesis of fungal sterols and inhibition of ergosterol biosynthesis. In many fungi, ergosterol is essential to the structure of cell wall and its absence cause irreparable damage to cell wall leading to death of fungal cell<sup>23</sup>.

Similarly,<sup>24</sup> evaluated different fungicides against *P. grisea* and found tebuconazole, propiconazole, difenconazole, tricyclazole and azoxystrobin + difenconazole as significantly

effective over others. Several other workers<sup>25, 26, 27</sup> also found both contact and systemic fungicides as effective in inhibiting the growth of *Pyricularia*.

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