JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2016. Vol. **10**(1), p. 489-495

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

Somashekhar Konda[1]*, A. Nagaraja[2], Gowdra Nagamma[1], P.S. Sangeetha[1], Suresh Patil[2], Devanshu Dev[1] and Syeda Samina Anjum[1]

[1]Department of Plant Pathology, UAS, GKVK, Bengaluru - 560 065, India.
[2]Project Coordinating Unit (Small Millets), ICAR, GKVK, Bengaluru - 560 065, India.

(Received: 13 November 2015; accepted: 10 January 2016)

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.

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[1] 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[2].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[3]. It has been suggested that foxtail millet protein be used as a food component to fight type 2 diabetes and cardiovascular diseases[4].

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[5]. Therefore, investigations were undertaken to manage *Pyricularia setariae* by different bio agents and fungicides *in vitro* by dual plate and poisoned food technique respectively.

* To whom all correspondence should be addressed.
E-mail:somukonda9@gmail.com; anagaraja60@gmail.com

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 at 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[6].

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

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 [6] as mentioned earlier.

RESULTS AND DISCUSSION

The fungal antagonists like *Trichoderma asperullum*, *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[7]. 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 [8]. [9, 10] 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 [11-14] 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 [15], kanamycin and lipopeptida of it urin, surfatin and fengycin [16]. Also, [17] reported chitinolytic activity of *Bacillus cereus* II.14. [18, 19] also found 60 per cent inhibition of *P. grisea* by *B. subtilis* strain NSRS 89-24 in dual culture. Similarly, [20] 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-

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

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

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

S. No.	*Trichoderma* isolate	Percent inhibition of mycelial growth
1	*T. harzianum* 1 (IIHR)	100.00 (90.00)
2	*T. viride*(GKVK)	100.00 (90.00)
3	*T. harzianum*6 (IIHR)	100.00 (90.00)
4	*T. viride(*NBAIR)	100.00 (90.00)
5	*T. harzianum*(NBAIR)	100.00 (90.00)
6	*T. asperellum*(GKVK)	100.00 (90.00)
7	*Bacillus cereus* (GKVK)	81.14 (64.27)
8	*B. subtilis* (NBAIR)	72.40 (58.30)
9	*B. subtilis* (GKVK)	80.26 (63.60)
10	*Pseudomonas fluorescens*(NBAIR)	75.80 (60.54)
11	*P. fluorescens*(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			

Note: Figures in the parenthesis are arc sine transformed values

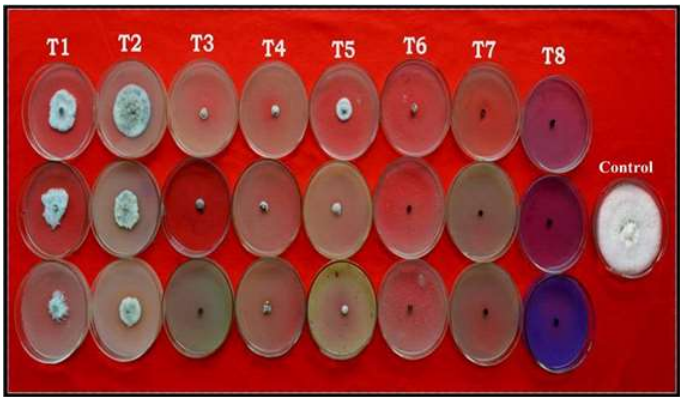
Table 4. *In vitro* efficacy of systemic fungicides on *Pyricularia setariae*

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
Thifluzamide	17.96 (25.05)	27.03 31.31)	44.44 (41.75)	29.81
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)	F × 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

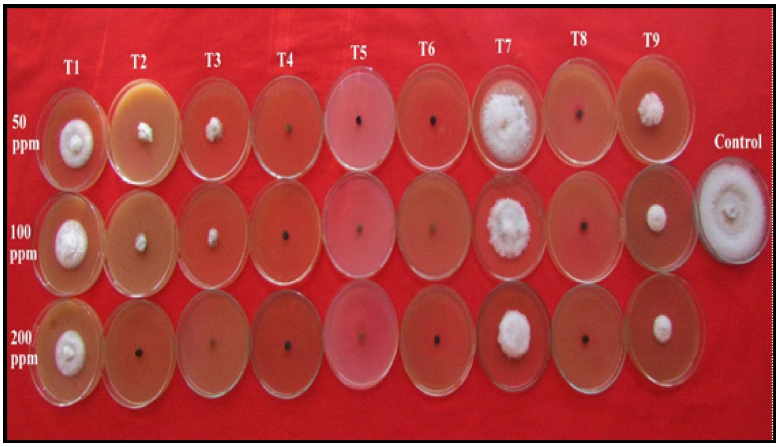
Treatment details: T_1= Control, T_2= *B. subtilis,* T_3= *B. subtilis*(GKVK), T_4= *T. harzianum*(GKVK), T_5= *T. harzianum*(Microbiology, GKVK), T_6= *T. harzianum*- 6 (IIHR), T_7= *B. cereus,*T_8= *P. fluroscens*(NBAIR), T_9= *P. fluroscens*(GKVK), T_{10}= *T. viride* (NBAIR), T_{11}= *T. harzianum*(NBAIR), T_{12}= *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 combiproducts 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***

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, 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[13] who reported that among the fungicides tested only mancozeb was the highly effective fungicide restricting the complete mycelia growth of *P. oryzae*. [22]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 [23].

Similarly, [24]evaluated different fungicides against *P. grisea* and found tebuconazole, propiconazole, difenconazole, tricyclazole and azoxystrobin + difenconazoleas significantly effective over others. Several other workers [25, 26, 27] also found both contact and systemic fungicides as effective in inhibiting the growth of *Pyricularia*.

REFFERENCES

1. *Vavilov, N. I., Studies on the origin of cultivated plants. *Appl. Bot. Plant Breed*., 1926; **26**: 1–248.
2. Marathee, J.P., Structure and characteristics of the world millet economy. pp. 159–178. In: K.W. Riley, S.C. Gupta, A. Seetharam, and J.N. Mushonga (Ed.), Advances in small millets. Oxford and IBH Publ.Co. Pvt. Ltd., 66 Janpath, New Delhi, 1993.
3. Wright, W. G. and Finch, R. C., Firm seeds in the foxtail millets. *Proc. Assoc. off. Seed. Ann. North. America.,* 1962; **52:** 109-111.
4. Choi, Y., Osada, K., Ito, Y., Nagasawa, T., Choi, M. and Nishizawa, N., Effects of dietary protein of Korean foxtail millet on plasma adinopectin, HDL-cholesterol and insulin levels in genetically type 2 diabetic mice. *Biosci. Biotechnol. Biochem*., 2005; **69**:31-37.
5. Nagaraja, A., Kumar, J., Jain, A. K., Narasimhadu, Y., Raghuchander, T., Kumar, B. and Gowda, B. H., Compendium of small millets diseases. Project Coordinator Cell, All India Coordinated Small Millets Improvement Project, UAS, GKVK Campus, Bengaluru. 2007; 80.
6. *Vincent, J. M., Distribution of fungal hyphae in the presence of certain inhibitors. *Nature*, 1947; **159**: 850.
7. Ali, H. and Nadarajah, K., Evaluating the efficacy of *Trichoderma* spp and *Bacillus substilis* as biocontrol agents against *Magnaporthe grisea* in rice. *Aus. J. Crop. Sci.,* 2014; **8**(9): 1324-1335.
8. Kalaivani, N., Hamdia, Z. A. and Nurfarahana, S. O., The isolation and characterization of an endochitinase gene from a Malaysian isolate of *Trichoderma* sp. *Aust. J. Crop Sci.,* 2014; **8**(5): 711-721.
9. Fuji, K., Fujita, E., Takaishi, Y., Fujita, T., Arita, I., Komatsu,M. and Hiratsuka, N., New antibiotics, trichopolyns A and B: Isolation and biological activity. *Experientia*., 1978; **34**: 237-239.
10. Vinale, F., Sivasithamparam, K., Ghisalberti, E.,

Marra, R., Woo, S. and Lorito, M., *Trichoderma*-plant– pathogen interactions. *Soil Biol. Biochem.,* 2008; **40**(1): 1-10.

11. Watanabe, N., Antagonism by various kinds of *Trichoderna* fungi to airborne plant pathogens. *Bull. Faculty Agric.,* Meiji Univ., 1985; **68**: 1-9.
12. Gouramanis, G.D., Biological and chemical control of rice blast disease in North Greece. *Cahiers Options Mediterraneennes*, 1997; **15**: 61-68.
13. Hajano, J., Lodhi, A. M., Mumtaz, A. P,Khanzada, M. A. and Shah, S. G., *In vitro* evaluation of fungicides, plant extracts and bio-control agents against rice blast pathogen *Magnaporthe oryzae* Couch. *Pak. J. Bot*., 2012; **44**(5): 1775-1778.
14. Arumugam, K., Ramalingam, P. and Appu, M., Isolation of *Trichoderma viride* and *Pseudomonas fluorescens* organism from soil and their treatment against rice pathogens. *J. Microbiol. Biotech. Res*., 2013; **3**(6):77-81.
15. *He, H. L. A., Laura, A. S. S., Handelsman, J. and Clardy, J., Zwittermicin A: an antifungal and plant protection agent from *Bacillus cereus*. *Tetrahedron Lett*.,1994; **35**:2499.
16. Stabb, E. V. L. M., Jacobson, J. And Handelsman, L., Zwittermicin A-producing strains of *Bacillus cereus* from diversion soils. *Appl. Environ. Microbiol*., 1994; **60**:4404.
17. *Mubarik, N. R. I., Mahagiani, A. A., Putri, S., Santoso and Rusmana, I., Chitinolytic bacteria isolated from chilirhizosphere: chitinase characterization and application as biocontrol for whitefly (*Bemisia tabaci* Genn.). *Am. J. Agric. Biol. Sci.*, 2010; **5**:430-535.
18. Harman, G. E., Myths and dogmas of biocontrol. *Pl. Dis.*, 2000; **84**(4): 377-393.
19. Leela Suphakul, W., Pranom, S. and Souwalak, P. H., Purification, characterization and synergistic activity of 1,3-glucanase and antibiotic extract from an antagonistic *Bacillus subtilis* NSRS 89-24 against rice blast and sheath blight. *Enzyme Microbial. Technol.*, 2006; **38**: 990-997.
20. Karthikeyan, V., Gnanamanickam, S.s., Biological control of *Setaria* blast (*M. Grisea*) with bacterial strains, *Crop Protection,* 2008; **27**(2): 263-267.
21. Anwar, A., Bhat, G. N. and Singhara, G. N., Management of sheath blight and blast in rice through seed treatment. *Ann.Pl. Protec. Sci*., 2002; **10**: 285-287.
22. Nene, Y. L. and Thapliyal, P. N., *Fungicides in Plant Disease Control.* Oxford and IBH publishing house, New Delhi, 1973; 163.
23. Mohan, C., Amrinder, K. and Sandeep, R., *In vitro* evaluation of different fungicides against *Pyricularia grisea*. *Pl. Dis. Res.,* 2011; **26**(2): 178.
24. Gohel, N. M., Chauhan, H. L. and Mehta, A. N., Bio-efficacy of fungicides against *Pyricularia oryzae* the incitant of rice blast. *J. Pl. Dis*. *Sci*., 2008; **3**(2): 189-192.
25. Bhojyanaik, V. K. and Jamadar, M. M., *In vitro* bioassay of different fungicides against blast of pearl millet caused by *Pyricularia grisea*(Cooke.) Sacc. *Karnataka J. Agric. Sci.,* 2014; **27**(1): pp. 88-90.
26. Netam, R.S., Tiwari, R.K.S., Bahadur, A.N. and Shankar, D., *In vitro* and *in vivo* efficacy of fungicides against *Pyricularia grisea* causing finger millet blast disease. *Int. J. Plant Protec.*, 2014; **7**(1): 137-142.