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In- vitro Bioassay of Fungicides, Bioagents, Botanicals and Its Against *Pyricularia grisea* (Cooke) Sacc.- Incitant of Pearl Millet Blast

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ABSTRACT

Twenty fungicides, six bioagents, six botanicals and five ITK's were evaluated against blast of pearl millet caused by Pyricularia grisea. Among the non-systemic fungicides evaluated mancozeb 75% WP inhibited cent percent mycelial growth of the pathogen, in systemic fungicides tricyclazole 75%WP (100%) gave maximum inhibition of the mycelial growth of the pathogen. followed by difenconazole 25% EC (94%), hexaconozole 5E (93%) and propiconazole 25% EC (88%). SAAF (Carbendazim+ Mancozeb) @ 0.05% and Nativo (Tebuconazole50% + Trifloxystrobin 25%) among combi product fungicides gave maximum inhibition (100%) of the mycelia growth of the pathogen. Among the bioagents Trichoderma harzianum + Bacillus subtilis (93%) followed by Trichoderma harzianum + Pseudomonas fluorescens (90%) gave maximum inhibition. Among commercially available botanicals evaluated, Agro neem (75%) gave maximum inhibition followed by Neem gold (58%). ITKs at 5 per cent concentration, maximum inhibition was noticed in panchagavy (75%) followed by butter milk (58%). Significantly least inhibition was noticed in cow milk (40%). Hence these effective fungicides, bioagents, botanicals and ITKs can be used as one of the component in the Integrated Management of blast of pearl millet.

Key words: *pearl millet, blast, Pyricularia grisea, fungicide, Bioagents, Botanicals, ITK's*

INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.)R.Br.] a staple cereal grown in India having the largest area of 7.95 m ha distributed almost over entire country with production of 8.79 mt of grains and productivity of 1106 kg ha^{-1}[1]. In Karnataka, it occupies an area of about 3.1 lakh hectares with a production of 2.4 lakh tonnes and productivity of 772 kg ha^{-1}[1]. It is called as a *poor man's food grain;* grown as a nutrient-rich food source for human as well as a forage/fodder crop for livestock. Pearl millet encounters number of diseases which attack the crop during its growth, cause low yield and economic loss to the peasant and finally to the nation as a whole.

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Among several diseases that affect pearl millet such as; downy mildew or green ear disease (*Sclerospora graminicola*) a major problem of pearl millet hybrids, rust (*Puccinia substriata* var. *indica*), smut (*Tolyposporium penicillariae*) and sugary disease or ergot of bajra (*Claviceps fusiformis*) have been major concern to cultivator and researchers alike. However, leaf spots due to several pathogens like; *Pyricularia grisea* (Cooke) Sacc., *Bipolaris setariae*(Sawada) Shoemaker, *Cercospora penniseti* (Chupp), *Curvularia penniseti* (Mitra) Boedijn, *Drechslera dematioidea* (Bubak & Wrobl.) Subram. & B.L.Jain. And*Exserohilum rostratum* (Drechs.) K.J. Leonard & E.G. Suggsetc. are also taking toll on crop destroying foliage and thus reducing yield and yield attributes.The blast also referred as leaf spot caused by *Pyricularia grisea* (Cooke) Sacc. [teleomorph: *Magnaporthe grisea* (Herbert) Barr.] has emerged as a serious disease affecting both forage and grain production in pearl millet. The disease has been considered serious in southern coastal plains of the USA where infection from this disease has been found to have significant adverse effects on green forage yield and digestible dry matter[11]. The disease was first recorded in Uganda in 1933[5]. The disease has geographic distribution in India, Singapore on Napier grass and the United States[4]. In India, the disease was first reported in 1942 from Kanpur, Uttar Pradesh[7]. Once considered a minor disease of pearl millet, incidence of blast disease caused by *Pyricularia grisea*, has increased at an alarming rate in the recent past, particularly on commercial hybrids in several states of India. In view of this, *in vitro* bioassay of different fungicide bioagents, botanicals and ITK's formulations was undertaken to find out their bioefficacy against *Pyricularia grisea*.

MATERIAL AND METHODS

The experiment was conducted at Department of Plant Pathology, College of Agriculture, Dharwad during 2015-16. The bioassay of fungicides, bioagents, botanicals and ITKs was evaluated under *in vitro* condition against *P. grisea*

Twenty fungicides consisting of four non systemic, seven systemic and nine combi product fungicides evaluated for their efficacy against *P. grisea.* under *in vitro* conditions. The systemic fungicides at 0.025, 0.05 and 0.1per cent concentrations whereas non-systemic fungicides at 0.1, 0.2 and 0.25 per cent concentrations and combi product fungicides were evaluated at 0.05, 0.1 and 0.2 per cent concentrations. The poisoned food technique was adopted for *in vitro* testing of fungicides. The calculated quantities of fungicides were thoroughly mixed in the medium before pouring into Petridishes so as to get the desired concentration of active ingredient of each fungicide separately. Twenty ml of fungicide amended medium was poured in each of 90 mm sterilised Petridishes and allowed to solidify. The plates were inoculated centrally with 8 mm disc of 10 days old young sporulating culture of *P. grisea.* Controls devoid of fungicides were also maintained. The experiment was conducted in completely randomised block design (CRBD) with three replications in each treatment. The inoculated Petridishes were incubated at room temperature 28°C±1°C in the laboratory. The colony diameters were measured after 10 days when the control plates were full of fungal growth. Per cent inhibition of growth was calculated by using formula given by Vincent[10].

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

where,

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment (fungicide)

Antagonistic microorganisms like *Bacillus subtilis, Pseudomonas fluorescens, T. harzianum* were evaluated singly and in combination for their antagonistic properties against *P.grisea* by dual culture technique. Culture discs (8 mm) each of the fungal antagonist and the pathogen were taken from

the margin of the actively growing cultures and transferred to potato dextrose agar (PDA) medium contained in 90 mm Petridishes on opposite sides approximately at one cm from the wall of the plate. Simillary, bacteria were streaked on the opposite sides of the pathogen. A check having the test pathogen only was kept for comparison. The Petridishes were subsequently incubated at 25±1°C till the control plate was completely covered by *P. grisea*. Each treatment was replicated thrice. Colony diameter of the test fungus as well as each antagonist up to the zone of inhibition was recorded and the per cent growth inhibition of the test pathogen over control was calculated according to the formula given by Vincent[10].

The anti- fungal activities of some of the commercially available botanicals were evaluated by poison food technique. All the plant products will be evaluated at 0.25%, 0.5% and 1.0% concentration then incorporated into PDA media by transferring two ml of each type of plant extract in to a Petridish containing 20 ml melted warm PDA medium and gently shaken for thorough mixing of the extract. The PDA plates containing the plant extracts were inoculated aseptically with *P. grisea by* transferring eight mm diameter agar disc of 10 days old culture of the pathogen to the centre of PDA medium in Petridish. Three replications were maintained for each treatment. The basal medium (PDA) without any phyto extract served as control. All the inoculated Petridishes were incubated at 25±1°C. The radial growth of the test fungus in the treated plates was measured in all treatments when the pathogen growth touched the periphery in the control Petridishes. The per cent inhibition of fungal growth was estimated by using the formula given by Vincent[10].ITK'S such as cow urine, panchagavya and vermiwash will be evaluated *in vitro* condition against inhibition of spore germination of *P. grisea* at 5, 10 and 20 per cent concentrations.

RESULTS AND DISCUSSION

Among the non-systemic fungicides evaluated against *P. grisea*, mancozeb 75% WP inhibited cent percent mycelial growth of the pathogen and was significantly superior over the other fungicides, least inhibition of (76.4%) of mycelia growth was observed in zineb of the seven systemic fungicides evaluated against *P. grisea*, tricyclazole 75%WP (100%) gave maximum inhibition of the mycelial growth of the pathogen. followed by hexaconozole 5E (98%), difenconazole 25% EC (95.4%) and propiconazole 25% EC (94.1%) and were found to be on par with each other as well as significantly superior over tridemefon 25% WP (66.3%) which was found to be least efficient in inhibiting mycelial growth of the pathogen. SAAF (Carbendazim 12%+ Mancozeb 63%) 75 WP @ 0.05% and Nativo (Tebuconazole50% + Trifloxystrobin 25%) among combi product fungicides gave maximum inhibition (100%) of the mycelia growth of the pathogen, however acrobat (54.6%) was found least effective in inhibition of mycelia growth. These studies were in accordance with Barnwal *et al*[2]., evaluated six new fungicide formulations to control rice blast in separate field trial with susceptible variety CO 39. Three sprays of RIL 0.13 SDC (Fenoxanil + Iso prothiolane) at 0.2 per cent was found most effective in controlling disease with leaf blast severity of 8.8 per cent and neck blast incidence of 4.7 per cent This fungicide was followed by three sprays of Beam 70WP (Tricyclazole) at 0.06 per cent reducing leaf blast disease severity to 11.9 per cent and neck blast incidence to 6.2 per cent with grain yield of 24.8 q/ha.Bhojanayak[3] reported that among systemic fungicides evaluated against *P. grisea*, tricyclazole 75WP gave maximum inhibition of the mycelial growth (87.78%) of the pathogen followed by difenconazole 25EC (86.91%), hexaconozole 5E (85.33%) and propiconazole 25EC (75.92%) and were found to be on par with

each other as well as significantly superior over carbendazim 50WP (54.23%) which was found to be the least efficient in inhibiting mycelial growth of the pathogen.It was noticed that among bio agents maximum inhibition of mycelia growth of the pathogen was observed in combination of *T. harzianum* + *B. subtilis* (94.1%) followed by *T. harzianum* + *P. fluorescens* (92.2%) and were significantly superior over other bioagents. The inhibitory effect of these bio-agents was probably due to competition and / or antibiosis. The antagonism of *T. harzianum, B. subtilis and P. fluorescens* observed in the present studies is in tune with the findings of Muthaiyan[8] also reported effectiveness of *P. fluorescens* in the control of rice blast disease caused by *P. oryzae.and* similarly Jamaluddin *et al*[6]., evaluated six biocontrol agents *viz*., *Trichoderma harzianum, T. polysporum, T. pseudokoningii, Gliocladium virens, Paecilomyces variotii* and *P. lilacinus* under *invitro* condition and observed maximum mycelial inhibition of *P. oryzae* by *P.lilacinus* followed by *Trichoderma* spp.

Among commercially available botanicals evaluated against inhibition of radial growth of *P. grisea,* Agro neem gave maximum (82.1%) inhibition and significantly superior over other botanicals and least (4.2%) inhibition was observed in Drischeck. Similar results on antifungal activity of a extracts of different plants has been reported by Bhojanayak[3] reported highest inhibition of mycelial growth of *P. grisea* by commercial plant product Soldier and it was significantly superior over onion and chilli leaf extracts (54.95%) followed by neem leaves or seed or oil (45.84%) and garlic extract (42.15%)The effect of ITK's on spore germination was significantly superior over control. All the five ITKs tested reduced the inhibition of spore germination of *P. grisea* at 5 %, 10 %, and 20 % concentrations. At 5 per cent concentration, maximum inhibition was noticed in panchagav (75%) followed by butter milk (58%). Significantly least inhibition was noticed in cow milk (40%). These results are in confirmation with the work of Sumangala and Patil[9] found the antifungal activity of panchagavya against *Curvularia lunata* in rice. It resulted in 86.30 per cent inhibition of mycelial growth and 95.9 per cent of spore germination of *C. lunata*. Seed treatment with panchagavya further enhanced the seed germination with 90.7 per cent and vigour index of 1036. Similarly, it was also reported in Yadav and Lourduraja[12] studied the effect of organic manures and panchagavya spray on rice (*Oryza sativa* L.) quality. Foliar spray of panchagavya recorded significantly higher physical characteristics like grain size, 1000 grain weight and milling quality as well as cooking quality.

Table 1*:* Effect of non systemic fungicides against *P. grisea*

Sl. No.	Chemicals	Percent inhibition of the mycelial growth of fungus			
		Concentrations (%)			Mean
		0.10	0.20	0.25	
1	Mancozeb (Indofil M-45) 75WP	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	**100.0 (90.0)**
2	Captan (Captaf) 50WP	75.36 (60.38)	84.78 (67.18)	100 (90.0)	**86.71 (72.52)**
3	Chlorothalonil (Kavach) 75WP	36.94 (37.42)	42.01 (40.39)	77.53 (61.80)	**52.16 (46.54)**
4	Zineb (Dithane Z-78) 75WP	63.64 (52.56)	63.02 (52.58)	74.63 (59.79)	**66.89 (54.17)**
	Mean	**68.83 (60.09)**	**72.45 (62.53)**	**88.04 (75.4)**	**76.44 (66.0)**
				S. Em ±	**C.D @ 1%**
			Fungicide (F)	2.38	6.98
			Concentration (C)	2.06	6.04
			FXC	1.37	4.03

* Figures in parenthesis are angular transformations

Table 2*:* Effect of systemic fungicides against *P. grisea*

Sl. No.	Chemicals	Percent inhibition of the mycelial growth of fungus			
		Concentrations (%)			Mean
		0.025	0.050	0.10	
1	Tricyclazol (Baan 75 WP)	100.0 (90)*	100.0 (90.0)	100.0 (90.0)	**100.0 (90.0)**
2	Tricyclazole (Baangold 75 WG)	93.0 (75.61)	100.0 (90.0)	100.0 (90.0)	**97.6 (85.20)**
3	Hexaconazole (Contaf 5E)	94.0 (76.20)	100.0 (90.0)	100.0 (90.0)	**98.0 (85.4)**
4	Propiconazole (Tilt 25EC)	88.27 (69.97)	94.20 (76.20)	100.0 (90.0)	**94.1 (78.7)**
5	Difenconazole (Score 25EC)	93.19 (74.89)	93.19 (74.89)	100.0 (90.0)	**95.4 (83.2)**
6	Carbendazim (Bavistin 50 WP)	63.62 (52.7)	72.10 (58.14)	88.06 (69.8)	**74.5 (60.3)**
7	Tridemefon (Bayleton 25 WP)	45.67 (42.52)	63.37 (52.7)	90.12 (71.77)	**66.3 (55.6)**
	Mean	**82.53 (68.80)**	**88.98 (75.99)**	**96.80 (85.93)**	**89.4 (76.9)**
				S. Em ±	**C.D @ 1%**
			Fungicide (F)	0.53	2.01
			Concentration (C)	0.34	1.31
			FXC	0.91	3.48

* Figures in parenthesis are angular transformations

Table 3: Effect of combi product fungicides against *P. grisea*

Sl. No.	Chemicals	Percent inhibition of the mycelial growth of fungus			
		Concentrations (%)			Mean
		0.05	0.10	0.20	
1	Carbendazim 12% + Mancozeb 63% (SAAF 75% WP)	100.0 (90)*	100.0 (90.0)	100.0 (90.0)	**100.0 (90.0)**
2	Tebuconazole50%+ trifloxystrobin 25% (Nativo 75% WG)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	**100.0 (90.0)**
3	Difenonazole + Propiconazole (TASPA)	83.0 (65.7)	95.1 (78.8)	95.8 (78.5)	**91.3 (74.3)**
4	Fenamidan + Mancozeb (Sectin 60 WG)	55.0 (47.8)	71.9 (58.0)	72.5 (58.3)	**66.4 (54.7)**
5	Hexaconazole 4% + Zineb 68% WP (Avtar 72 %WP)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	**100.0 (90.0)**
6	Tricyclazole + Mancozeb (Merger)	94.8 (77.4)	100.0 (90.0)	100.0 (90.0)	**98.2 (85.8)**
7	Zineb 68+ Hexaconazole 4 (Taqat 75WP)	94.0 (76.3)	100.0 (90.0)	100.0 (90.0)	**98.0 (85.4)**
8	Dimethomorph + Mancozeb (Acrobat 50% WP)	43.7 (41.3)	56.1 (48.5)	64.1 (53.2)	**54.6 (47.6)**
9	Famoxadone 16.6% + Cymoxanil 22.1% SC (Equation pro 38.7% SC)	70.6 (57.2)	71.9 (58.0)	72.5 (58.3)	**71.6 (57.8)**
	Mean	**74.50 (70.63)**	**88.3 (77.0)**	**89.4 (68.5)**	**85.0 (68.2)**
				S. Em ±	**C.D @ 1%**
			Fungicide (F)	0.68	2.54
			Concentration (C)	0.39	1.47
			FXC	1.18	4.41

* Figures in parenthesis are angular transformations

Table 4: Effect of bioagents against *P. grisea*

Sl. No.	Bio-agents	Per cent inhibition
1	*Trichoderma harzianum*	90.0 (71.6)*
2	*Pseudomonas fluorescens*	80.4 (63.8)
3	*Bacillus subtilis*	79.6 (63.2)
4	*P. fluorescens + B. subtilis*	88.9 (70.6)
5	*T. harzianum +P. fluorescens*	92.2 (73.9)
6	*T. harzianum +B. subtilis*	94.1 (76.0)
	S.E m ±	0.68
	C.D @ 1%	2.95

* Figures in parenthesis are angular transformation

Table 5: Effect of botanicals against *P. grisea*

Sl. No.	Botanicals	Percent inhibition of the mycelial growth of fungus			
		Concentrations (%)			Mean
		0.25	0.5	1.0	
1	Agronee (Neem oil based herbal pesticide)	74.1 (59.49)*	78.3 (62.36)	93.8 (75.68)	82.1 (38.3)
2	Rawneem oil (Azadirachtin)	56.5 (48.76)	54.7 (47.3)	60.33 (50.99)	57.1 (49.0)
3	Soldier (*Aegl marbelos* (20%),*Ricinus,communis*(20%),*Hygrophila spinosa* (20%), *Laminaria spp*. (20%) *Lantana camera* (20%))	44.1 (41.6)	45.0 (42.1)	57.83 (49.53)	49.0 (44.4)
4	- Discheck :(*Ficus bengalensis* - 0.0001%, *Ficus religiosa* - 0.0001%,*Ficus retus* - 0.0001%, Aqua solvent - 99.99%)	5.50 (13.5)	5.8 (13.9)	1.5 (6.9)	4.2 (11.4)
5	Neemgold (Azadirachtin 0.15%)	57.0 (49.0)	56.8 (48.9)	66.8 (54.8)	60.2 (48.1)
6	Nimbicidine (Azadirachtin 0.03%)	43.8 (42.3)	47.5 (41.6)	67.0 (54.9)	51.8 (46.1)
	Mean	**46.8 (42.3)**	**47.5 (41.6)**	**57.8 (48.8)**	**41.3 (39.8)**

	S.Em. ±	C.D @1%
Botanicals (B)	0.28	1.08
Concentration (C)	0.18	0.71
BXC	0.49	1.87

* Figures in parenthesis are angular transformations

Table 6: Effect of different ITK'S on per cent spore germination of *P. grisea*

	ITK's	Percent inhibition of spore germination			
		Concentrations (%)			Mean
		5	10	25	
1	Panchagavy	75.0 (60.5)	78.0 (62.0)	93.5 (74.8)	**82.0 (65.8)**
2	Vermi wash	54.3 (47.4)	56.0 (48.4)	61.9 (51.9)	**57.4 (49.2)**
3	Cow urine	44.1 (41.6)	44.7 (41.9)	58.1 (49.7)	**49.0 (44.4)**
4	Cow milk	40.7 (39.6)	43.0 (41.0)	55.0 (47.8)	**46.2 (42.8)**
5	Butter milk	58.8 (50.1)	59.4 (50.4)	67.2 (55.1)	61.8 (58.9)
	Mean	**54.5 (47.9)**	**56.2 (48.74)**	**67.0 (61.4)**	**59.2 (52.6)**

	S. Em ±	C.D @ 1%
Fungicide (F)	0.45	1.74
Concentration (C)	0.35	1.35
FXC	0.79	3.02

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