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EFFICACY OF FUNGICIDES AND BIO-CONTROL AGENTS AGAINST *EXSEROHILUM MONOCERAS* CAUSING LEAF SPOT DISEASE BARNYARD MILLET

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ABSTRACT

Barnyard millet (*Echinochloa frumentacea*) is the fastest growing millet .It is used for patients in tolerate to gluten causing celiac disease. Revered for their resilience, adaptability and rich nutritional profile, millets hold a special place in sustainable agriculture and food security. Despite its strengths, Barnyard millet remains underutilized in modern agriculture and its productivity is significantly constrained by biotic stresses. Among these, Leaf spot disease, caused by the fungus *Exerohilum monoceras*, is the most widespread and destructive. Cultural studies revealed that among the solid media tested, highest radial growth of the fungus was recorded on PDA, Czapek's Dox agar media and Oat meal agar followed by Yeast extract potato agar, whereas Corn meal agar had least growth. In, *in vitro* evaluation of six bio-agents, *Trichoderma harzianum* was emerged as the potential antagonist of *Exserohilum monoceras* which showed 77.03% inhibition of the fungus and was followed by *Trichoderma viride* 71.50%. Among the six fungicides was assessed *in vitro* poisoned food technique which revealed that, all the fungicides evaluated showed significant inhibition of pathogen., Carbendazim 12% + Mancozeb 63% WP and Azoxystrobin 18.2% + Difenconazole 11.4% SC, were the most effective fungicide which showed complete inhibition of the mycelial growth of the pathogen.
***Keywords*:** Barnyard millet (*Echinochloa frumentacea*), *Exerohilum monoceras, Trichoderma harzianum,* Leaf Spot disease.

Introduction

Barnyard millet or Japanese millet and other is *Echinochloa frumentacea* which is Indian Barnyard millet. The Indian barnyard millet is also known as Billion Dollar Grass (USDA NCRS. 2002). *Echinochloa frumentacea* is belongs to the family Poaceae and it is self-pollinated crop. *Echinochloa frumentacea* is the hardest millet and commonly known by several names viz.; Sanwa and Jhangor (Hindi), Shyama (Sanskrit), Oodalu (Kannada), Kuthiravaali (Tamil),Udalu and Kodisama (Telgu), Shamul (Marathi), Sama (Gujarati), Shamula (Bengali) and Swank (Punjabi).

Although this crop is hardy, the crop is challenged by many fungal diseases like leaf spot (*Exserohilum monoceras*), blast (*Pyricularia grisea*), grain smut (*Ustilago panici-frumentacei* Bref.) and head smut (*Ustilago crusgalli*) (Prabu *et al*., 2020). Among these diseases, leaf spot is one of the most destructive diseases after smut, which if occurs at early stages as blight, reduces the crop yield drastically. Larger cultivation of kuthiraivali in climate changes the *Helminthosporium* (*Exserohilum monoceras*) leaf spot incidence was high (47.80%), Leaf spot or blight is incited by the fungus *Exserohilum monoceras* (Drechsler) Leonard & Suggs [Syns. *Helminthosporium monoceras* (Drechsler); *Helminthosporium crusgalli* (Nisikado and Miyake); *Bipolaris monoceras* (Drechs.) Shoemaker; *Drechslera monoceras* (Drechs.) Subram and Jain; and *Luttrellia monoceras* (Dreschler) Chochryakov]. Morita *et al.* (2013) described the perfect stage of the fungus as *Setosphaeria monoceras*. Leonard and Suggs (1974) established the genus *Exserohilum* for *Helminthosporium* species and differentiated from its former group.

Materials and Methodology

Isolation of the pathogen

The fungus was isolated by tissue isolation technique. The diseased tissues along with some healthy leaf portion (1-2 mm2) were cut with a sterilized razor blade at the margins of the diseased spots and surface sterilized in 0.1 per cent mercuric chloride solution for 30 seconds. The segments were then rinsed thrice in sterilized distilled water to remove the traces of mercuric chloride solution, blotted dry and placed on acidified potato dextrose agar (PDA) medium (pH adjusted to 6.5 with N/10 HCl) in sterilized petri plates under aseptic conditions. The inoculated PDA plates were incubated at 25±1 ºC for 7 days and sub cultured onto fresh PDA medium at the same temperature for 15 days.

Purification by single spore technique

Exserohilum turcicum being a sporulating fungus was purified by single spore technique (Johnston and Booth, 1983). Dilute spore suspension of the pathogen was prepared in sterilized distilled water containing 8 to 10 spores per microscopic field at low power from 15 days old culture.

One ml of such suspension was spread uniformly on 2 per cent solidified water agar plates and incubated at 25±1 ºC for 12 hours. The plates were examined under stereoscopic microscope, single spores were marked with a marker, allowed to germinate and finally picked up using cork borer and transferred aseptically to potato dextrose agar medium in sterilized petri plates for further growth in the incubator at 25±1 ºC. The pure culture thus obtained, was used for further studies.

Cultural characteristics of *E. monoceras* on different culture media

Pure culture of isolate was used to study the variation in growth of the pathogen on different nutrient media. Seven solid media viz., potato dextrose agar (PDA), malt extract agar (MEA), corn meal agar (CMA), Richard's agar (RA), Czpeck's dox agar (CDA), oatmeal extract agar and yeast media were used to study the best nutrient medium for the radial growth of the test pathogen. Media were prepared and sterilized by autoclaving at 1.05 kg/cm² pressure (121.6°C) for 20 minutes. Each medium was poured in petri dishes containing equal quantity and were inoculated with 5 mm culture discs taken from the 7 days old culture of the pathogen. Three replications were kept and the plates were incubated at 25+1°C. Radial mycelial growth of the colonies obtained after 7 days of inoculation on each culture medium were recorded and analyzed statistically to know the most suitable solid media for the growth of fungus. The colony characters of pathogen *viz.,* shape and colour on each media were recorded.

In vitro evaluation of bio-control agents against *Exserohilum monoceras*

Bio-control agents were evaluated for their antagonistic potential against pathogen using dual culture technique (Morton and Stroube, 1955). Twenty ml of PDA per petri plate was poured and allowed to solidify. Five mm discs of *E. monoceras* taken from seven days old culture was placed at one end of petri plate and respective antagonistic organism (5mm) was inoculated at the opposite side of the petri plate. The test pathogen being relatively slow growing was incubated 48 hours prior to *Trichoderma* spp. to cope up with the fast growth of *Trichoderma* spp. In case of bacterial antagonist *E. monoceras* was placed at the one end of petri plate and the bacterial culture was streaked at the other half of the petri plate. Three replications were maintained for each treatment. Control having test pathogen only was kept for comparison. Petri plates were incubated for seven days at 25±1°C. The activity of antagonistic organisms was recorded after 96 hours by measuring colony diameter of test pathogen i.e. *E. monoceras* in each treatment and compared with control. Per cent growth inhibition of the test pathogen over control was calculated according to the formula given by Vincent (1927).

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

Where.

I = Per cent inhibition

C =Colony diameter in control

T =Colony diameter in treatment

In vitro evaluation of fungicides against *Exserohilum monocera*

In fungicides consisting of viz., Propiconazole 25% EC, Difenconazole 25% EC, Azoxystrobin 18.2% + Difenconazole 11.4% SC, Mancozeb 63% + Carbendazim 12% WP, Hexaconazole 5% EC, Pyraclostrobin 25% + Epoxiconazole 10% SC were assayed for their efficacy against *E. monocera* under in vitro condition. Ten days old young sporulating culture of *E. monoceras*. Control plates without fungicides were also maintained. For each treatment three replications were maintained. The inoculated petri plates were incubated at 25±1°C in the laboratory. The colony diameter was measured when fungus touched the periphery in control plates. Per cent inhibition of

growth were calculated by using formula given by Vincent (1927).

Results and Discussion

Effect of different media and cultural characteristics

The cultural and growth characteristics of *Exserohilum monoceras*, the causal organism of leaf spot disease, were studied on nine different solid media. Observations were recorded with respect to colony colour, texture, surface appearance, topography, consistency, margin, lustre, and sporulation.

With respect to cultural characteristics, the colony color varied from grey to black. Mycelial growth varied from fluffy growth to slightly raised colony. The fungus showed greyish colour with irregular margin and slightly raised mycelium on Potato dextrose agar, Yeast extract potato agar, Malt extract agar and also on Richard's agar. Mycelial growth on Oat meal media was whitish gray colour with regular margin. Mycelial growth on Czapeck's Dox agar and Corn agar media showed blackish colour with regular and irregular margin respectively having slightly raised and flattened mycelium respectively. Effect of different solid media on cultural characters of *E. monoceras.*

Previous workers Nataraj (2014) and Rashmi (2015) reported that among different media tested PDA was found effective for growth and development of *E. turcicum*.

Table 1: Cultural characters of *E. monoceras* on different solid media

Sr. No.	Media	Colony character	Margin	Margin color
1.	Czpeck's Dox agar	Excellent growth, blackish, slightly raised growth colony	Regular	Black
2.	Yeast extract potato agar	Good growth, greyish, slightly raised colony	Irregular	Light gray
3.	Malt extract agar	Good growth, greyish, slightly raised cottony growth colony	Irregular	Light gray
4.	Potato dextrose agar	Excellent growth, greyish, slightly raised growth colony	Irregular	Light gray
5.	Corn meal agar	Poor growth, blackish, flattened colony	Irregular	Black
6.	Oat meal agar	Excellent growth, whitish gray, fluffy with raised cottony growth colony	Regular	Gray
7.	Richard's agar	Good growth, greyish, slightly raised cottony growth colony	Irregular	Light gray

Table 2 : Effect of different solid media on the growth of *E. monoceras*

Sr.No.	Medium	Radial mycelial growth (mm)*
1	Czpeck's Dox agar	90.00
2	Yeast extract potato agar	71.80
3	Malt extract agar	51.06
4	Potato dextrose agar	90.00
5	Corn meal agar	39.83
6	Oat meal agar	90.00
7	Richard's agar	49.78
SE ± m		0.55
C.D.@1%		1.69

In vitro Evaluation of Bio-control Agents against *Exserohilum monocera*

Six bio-agents were evaluated against test pathogen by employing standard 'Dual culture technique'. Some bio-agents were showed complete inhibition of test pathogen growth while some were unable to show the complete inhibition. The data are presented in Table, Figure. The findings of *in vitro* experiment conducted to know the most effective bio-control agent against *E. monoceras*.

From the data presented it is revealed that all bio-control agents were significantly superior to inhibit the fungal growth over control. The per cent inhibition ranged from 43.46 to 77.03. The results revealed that

Trichoderma harzianum was most effective antagonist exhibiting 77.03 per cent mycelial inhibition of fungus followed by *Trichoderma viride* (71.50 %). Moreover, *T. harzianum* and *T. hamatum* were at par with each other in inhibiting mycelial growth. Next effective antagonists reported were *T. koningii* and *T. hamatum* which gave mycelial inhibition of 57.78 and 53.79 per cent, respectively. Among all the treatments *Pseudomonas fluorescens* exhibiting least mycelial inhibition (47.45%) with 47.30 mm mean colony diameter of *E. monocras*.

These results are in line with those reported by Manu *et al*. (2017) who reported maximum inhibition of mycelial growth (98.65 %) by *T. harzianum*-2 followed by *T. viride* (98.34 %). Among the bacterial antagonists, *Pseudomonas fluorescence*-1 and *Pseudomonas fluorescence*-2 showed the mycelial inhibition of 95.49% and 94.24%, respectively. In present study bio-control agents were found effective in controlling the growth of test fungus under *in vitro* conditions. Earlier, Harlapur *et al*. (2007) and Khedekar *et al*. (2012) reported that *Trichoderma harzianum* was effective in inhibiting the mycelial growth.

These results are further supported by the results of Singh and Singh (2014). Ramchandra (2000) studied anatagonist against *Exserohilum hawaiiensis in vitro* and reported that *T. viride* and *T. harzianum* decreased the growth and sporulation significantly

Table 3: *In vitro* exploration of bio-agents against *Exerohilum monoceras*

Tr.no.	Treatment	Mean colony dia.*(mm)	% Growth inhibition
T1	*Trichoderma harzianum*	20.67	77.03
T2	*Trichoderma koningii*	38.00	57.78
T3	*Trichoderma viride*	25.65	71.50
T4	*Trichoderma hamatum*	41.59	53.79
T5	*Pseudomonas fluorescens*	47.30	47.45
T6	*Bacillus subtilis*	43.46	51.70
T7	Control	90.00	-
SE ± m		0.68	
C.D. @1%		2.08	

In vitro* evaluation of fungicides against *E. monoceras

The data given in Table revealed that all fungicides were significantly superior in reducing mycelial growth. Among different fungicides, mancozeb 63%+ carbendazim 63% WP was found most effective exhibiting maximum mean mycelial growth inhibiton (100 per cent) and best treatment was Azoxystrobin 18.2% + Difenconazole 11.4 % SC with 100 per cent mycelial growth inhibition. It was followed by pyraclostrobin 25% + epoxiconazole 10%SC (88.33%), propiconazole (86.66%). Moreover, the fungicides pyraclostrobin + epoxiconazole, propiconazole were at par with each other at the recommended concentration in inhibiting mycelial growth of fungus. Least inhibition of mycelial growth was recorded in Hexaconazole 5% EC (66.88%).

Table 4: *In vitro* exploration of bio-agents against *Exserohilum monoceras*

Tr. No.	Chemical	Conc.%	Colony dia. of pathogen*(mm)	% Growth inhibition
T1	Propiconazole 25% EC	0.1%	11.78	86.66
T2	Difenconazole 25% EC	0.1%	25.84	70.70
T3	Hexaconazole 5% EC	0.1%	29.17	66.88
T4	Azoxystrobin 18.2% + Difenconazole 11.4% SC	0.15	00	100
T5	Pyraclostrobin 25% + Epoxiconazole 10% SC	0.1%	10.29	88.33
T6	Mancozeb 63% + Carbendazim 12% WP	0.2%	00	100
T7	Control	-	88.13	-
-		SE ± m	0.45	-
CD @1%		1.38		-

The results obtained during present study are in consonance with the findings of Reddy *et al*. (2013) who evaluated seven fungicides against *E. turcicum* under *in vitro* condition and reported that the lowest per cent disease index (PDI) was in treatment mancozeb @ 0.25 % and combination treatment of carbendazim and mancozeb, @ 0.25 % with per cent disease control of 73.0 and 72.1 %, respectively over control. Kumar *et al*. (2015) found that among the six fungicides tested zineb 75 WP @ 0.25 % concentration was most effactive in inhibiting the growth of *E. turcicum*. Similarly, mancozeb 63 % + carbendazim 12 % @ 0.25 % was equally effective in reducing disease severity which can be used as an alternative to zineb. Many workers like Veerabhadraswamy *et al*. (2014) and Anand *et al*. (2013) reported that mancozeb 63 % + carbendazim 12 and tebuconazole 50% + trifloxystrobin 25% were effective to inhibit growth of fungus at different concentrations. Similar results were reported by Manu *et al.* (2017) showing that amomg systematic fungicides, tebuconazole completely inhibited the pathogen at all the concentrations tested, while among contact fungicides, propineb was highly effective inhibiting 83.89 per cent mycelial growth of *E. turcicum* at 500 ppm. Among combi-products tested against *E. turcicum*, only mancozeb 63 % + carbendazim 12 at 500 ppm showed complete inhibition of mycelial growth of *E. turcicum*.

During present studies all the tested fungicides at various concentrations significantly inhibited the mycelial growth of *E. monoceras* over untreated control. As the concentration of fungicides was increased, mycelial growth inhibition also increased. Among all the tested fungicides complete inhibition of pathogen was obtained with mancozeb 63 % + carbendazim 12 and Azoxystrobin 18.2% + Difenconazole 11.4 % SC. It was followed by and pyraclostrobin 25% + epoxiconazole 10%SC.

Plate 1: Symptoms showing leaf spot of barnyard millet leaves

Plate 2: Pure culture of fungal isolate *Exserohilum monoceras*

Plate 3: Pure culture of fungal isolate (Slants) *Exserohilum monoceras*

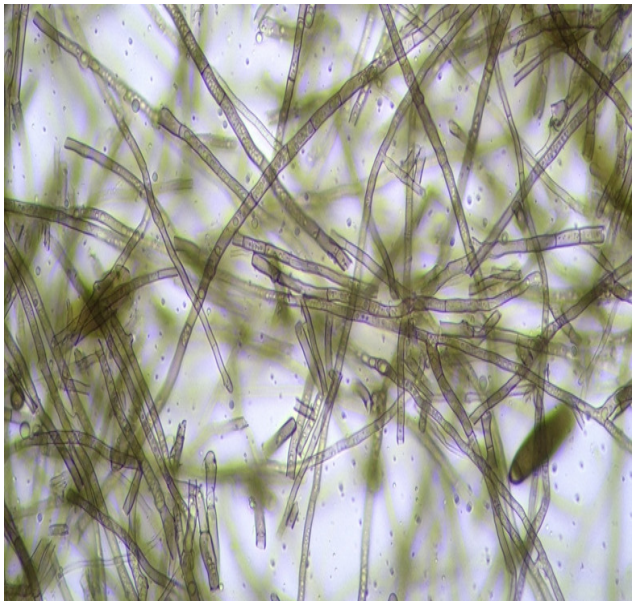


Plate 4: Mycelium of *Exserohilum monoceras*

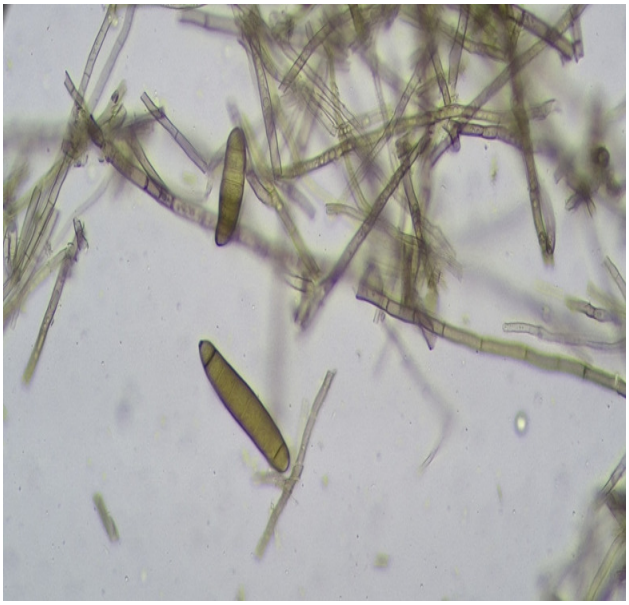


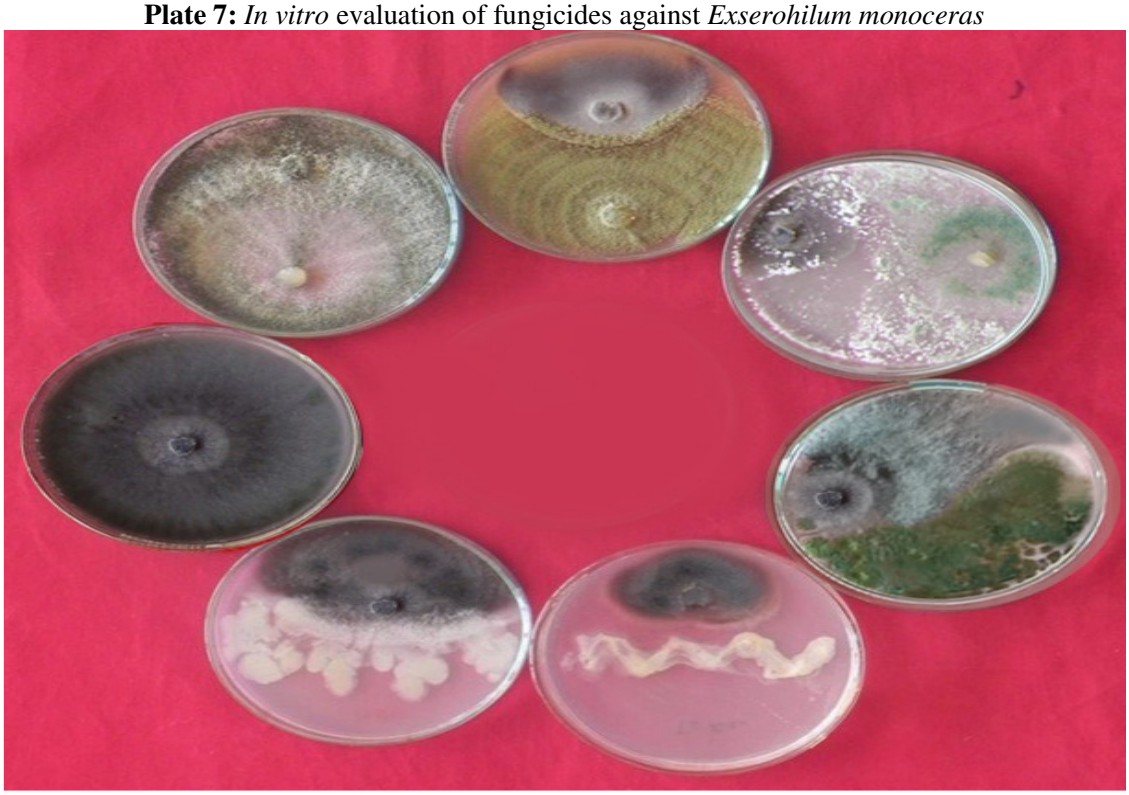
Plate 5: Conidia of *Exserohilum monoceras*

PDA : Potato dextrose agar
CDA : Czpeck's Dox agar
YeEA : Yeast extract potato agar
MEA : Malt extract agar
CMA : Corn meal agar
OMA : Oat meal agar
RA : Richard's agar

Plate 6: *Exserohilum turcicum* colonies grown on different solid media

T1 : Propiconazole 25% EC
T2 : Difenconazole 25% EC
T3 : Hexaconazole 5% EC
T4 : Azoxystrobin 18.2% + Difenconazole 11.4% SC
T5 : Pyraclostrobin 25% + Epoxiconazole 10% SC
T6 : Mancozeb 63% + Carbendazim 12% WP
T7 : Control

Plate 7: *In vitro* evaluation of fungicides against *Exserohilum monoceras*



T1 : *Trichoderma harzianum*
T2 : *Trichoderma koningii*
T3 : *Trichoderma viride*
T4 : *Trichoderma hamatum*
T5 : *Pseudomonas fluorescens*
T6 : *Bacillus subtilis*
T7 : Control

Plate 8 : *In vitro* evaluation of bio-control agents against *Exserohilum monocera*

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