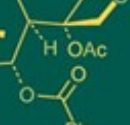
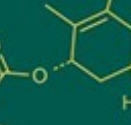
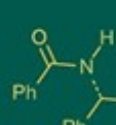


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Ayush A Kale

PG Research Scholar, Plant Pathology Section, College of Agriculture, Nagpur, Maharashtra, India

Dr. SB Bramhankar

Professor (CAS), Plant Pathology Section, Post Graduate Institute, Dr. PDKV, Akola, Maharashtra, India

Dr. Tini S Pillai

Professor, Plant Pathology Section, College of Agriculture, Nagpur, Maharashtra, India

Dr. AG Gathe

Assistant Professor, Plant Pathology Section, College of Agriculture, Nagpur, Maharashtra, India

Dr. PD Peshattiwar

Assistant Professor, Agricultural Botany Section, Shri Shivaji Agriculture College, Amravati, Maharashtra, India

Gita V Shinde

PG Research Scholar, Plant Pathology Section, College of Agriculture, Nagpur, Maharashtra, India

Management of seed borne mycoflora of proso millet by using fungicides and bioagents

Ayush A Kale¹, SB Bramhankar², Tini S Pillai³, AG Gathe⁴, PD Peshattiwar⁵, Gita V Shinde⁶

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ABSTRACT

The present investigation was conducted to study the management of seed borne mycoflora of proso millet using fungicides and bioagents. The fungal pathogens isolated from proso millet seeds were *Fusarium moniliforme*, *Bipolaris panici-miliacei*, *Curvularia lunata*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus* and *Cladosporium cladosporioides*. Among the fungicide tested carboxin 37.5% + thiram 37.5% DS was most effective showing complete mycelial inhibition 100% against *Fusarium moniliforme* at 0.30%, carboxin 37.5% + thiram 37.5% DS and prochloraz 34.8% + propiconazole 7.8% EC showed complete mycelial inhibition against *Bipolaris panici-miliacei*, *curvularia lunata* and *Aspergillus niger*, carbendazim 25% + mancozeb 50% WS showed complete inhibition against *Aspergillus ochraceus*. *Trichoderma asperellum* showed maximum mycelial inhibition against *Fusarium moniliforme* (75.71%), *Bipolaris panici-miliacea* (80.74%) and *Curvularia lunata* (73.07%), *Trichoderma harzianum* showed highest mycelial inhibition against *Aspergillus niger* (74.14%) and *Bacillus subtilis* achieved complete mycelial inhibition against *Aspergillus ochraceus* (79.26%).

Keywords: Proso millet, Seed borne mycoflora, Fungicides, Bioagents, *in vitro*

Introduction

Proso millet (*Panicum miliaceum* L.) classified within the tribe *Paniceae* under family *Poaceae* (*Graminae*) with subfamily *Panicoideae*. It is a fourploid species having four sets of chromosome number totaling 36. (Rajasekaran *et al.*, 2023) [1] The crop is extensively cultivated across several countries, including India, China, Japan, Arabia, Egypt, and regions of Western Europe. In India, it is predominantly cultivated in Andhra Pradesh, Bihar, eastern Uttar Pradesh, Karnataka, Madhya Pradesh, Maharashtra, and Tamil Nadu. Small millets are primarily grown in semi-arid regions like India as rainfed crops. Despite its beneficial qualities small millet production makes up less than 2% of global cereal production. The genetic potential of small millets is significantly constrained by both biotic and abiotic stresses, causing significant yield and economic loss. Among biotic stress factors, diseases caused by fungal pathogens such as blast, leaf spot, leaf blight, rusts and smuts are major challenges in small millet growing areas. (Ramesh *et al.*, 2024) [2]. Pathogen free seeds serve as a prerequisite for successful crop establishment, fostering uniform germination, healthy seedling emergence, and ultimately greater agricultural output. The seed borne mycoflora of proso millet comprises multiple fungi, including *Fusarium moniliforme*, *Bipolaris panici-miliacei*, *Curvularia lunata*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, and *Cladosporium cladosporioides*. These pathogens are critical contributors to poor germination and yield losses. Treating seeds with fungicides, bio-control agents, and other appropriate methods is an effective strategy for managing seed borne fungal pathogens, improving seed health, enhancing crop performance and ultimately increasing yield. Fungicides remain one of the most widely used approaches for controlling fungal diseases, while biological control agents offer environmentally friendly alternatives by suppressing the growth of pathogenic fungi. (Chaudhari *et al.*, 2017) [3]. Therefore the present investigation was conducted to study the management of seed borne mycoflora of proso millet using fungicides and bioagents.

2. Materials and Methods

The investigation was conducted under *in vitro* laboratory condition during 2025-26 at the Department of Plant Pathology, College of Agriculture, Nagpur, Maharashtra, India. Seeds of proso millet varieties TNAU-202 and TNAU-145 were used for the detection of seed borne mycoflora. Seed borne mycoflora associated with proso millet seeds were detected using method prescribed by (ISTA, 1976) [4] namely, standard blotter paper method and standard agar plate method. Seven different fungicides were evaluated under *in vitro* against test pathogen by adopting poison food technique. The treatments evaluated include carbendazim 25% + mancozeb 50% WS, penflufen 13.28% + trifloxystrobin 13.28% FS, carboxin 37.5% + thiram 37.5%

Corresponding Author:**Ayush A Kale**

PG Research Scholar, Plant Pathology Section, College of Agriculture, Nagpur, Maharashtra, India

DS, prochloraz 34.8% + propiconazole 7.8% EC, tebuconazole 5.4% FS, fluxapyroxad 333 g/l FS, metalaxyl 8% + mancozeb 64% WP and an untreated control. Potato dextrose agar (PDA) medium was prepared and sterilized in autoclave. Requisite quantity of each of the fungicide (as per concentration) was added in sterilized, melted PDA separately to obtain desired concentration. Flask containing poisoned medium was shaken well to have even and uniform distribution of fungicides. About 20 ml of melted poisoned PDA was poured in each sterilized petri plate and allow to solidify. The petri plates were inoculated separately by test fungus. Five mm disc of one week old fungus culture was cut with sterilized cork borer, lifted and transferred aseptically in the centre of petri plate containing the medium poisoned with test fungicide. The control plates were kept the culture disc grown in same condition on PDA without fungicides. Treated plates were incubated at room temperature ($26 \pm 2^\circ\text{C}$) for a period of seven days. Colony diameter was recorded in mm as per Vincent's formula based on the average colony diameter. The data was subjected to statistical analysis wherever necessary.

Four bioagents were evaluated under *in vitro* condition against test pathogen by adopting dual culture method. The treatments include *Trichoderma asperellum*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, and an untreated control. PDA was autoclaved and poured into 90 mm petri plate, where it solidified. Then, on one end of the plate a 5 mm disc of test species was set and on the other end, an antagonistic disc and striking bacterial culture on both sides where a 5 mm disc of test pathogen is placed in centre. Along with the control, the plates were incubated at room temperature ($26 \pm 2^\circ\text{C}$) for seven days. The data was subjected to statistical analysis wherever necessary. The data recorded were analyzed statistically by Completely Randomized Design for all the experiment *in vitro* and critical difference (CD) were calculated at 1 per cent probability.

3. Result and Discussion

Proso millet seed samples were examined using method prescribed by (ISTA, 1976) [4] namely standard blotter paper method and standard agar plate method. Based on morphological and microscopic observation the fungi observed viz., *Fusarium moniliforme*, *Bipolaris panici-miliacei*, *Curvularia lunata*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus* and *Cladosporium cladosporioides*.

Poisoned food technique

The efficacy of seven fungicides was evaluated *in vitro* at their respective concentration against seed borne mycoflora of proso millet. The data is presented and tabulated in Table 1.

1. ***Fusarium moniliforme*:** Among the seven fungicides tested, carboxin 37.5% + thiram 37.5% DS achieved complete mycelial growth inhibition of 100% against *Fusarium moniliforme* followed by carbendazim 25% + mancozeb 50% WS with 90.58%, prochloraz 34.8% + propiconazole 7.8% EC recorded 84.31%, tebuconazole 5.4% FS recorded 84.11%, penflufen 13.28% + trifloxystrobin 13.28% FS recorded 80.20% and fluxapyroxad 333 g/l FS recorded 62.35%. Metalaxyl 8% + mancozeb 64% WP was the least effective with mycelial growth inhibition of 47.05%. The highest growth of *Fusarium moniliforme* was recorded in the control. The findings of present investigation agree with, Meghavathu *et al.* (2025) [5] and Tammineni *et al.* (2025) [6].

2. ***Bipolaris panici-miliacei*:** Among the seven fungicides, carboxin 37.5% + thiram 37.5% DS and prochloraz 34.8% + propiconazole 7.8% EC achieved complete mycelial growth inhibition of 100% followed by fluxapyroxad 333 g/l FS recorded 81.53%, tebuconazole 5.4% FS recorded 80.76%, metalaxyl 8% + mancozeb 64% WP recorded 76.92% and carbendazim 25% + mancozeb 50% WS recorded 76.15%. Penflufen 13.28% + trifloxystrobin 13.28% FS was the least effective with mycelial growth inhibition of 68.21%. The highest growth of *Bipolaris panici-miliacei* was recorded in the control. The findings of present investigation in agreement with Gupta *et al.* (2025) [7].
3. ***Curvularia lunata*:** Among the seven fungicides, carboxin 37.5% + thiram 37.5% DS and prochloraz 34.8% + propiconazole 7.8% EC achieved complete mycelial growth inhibition of 100% against *Curvularia lunata* followed by carbendazim 25% + mancozeb 50% recorded 91.81%, metalaxyl 8% + mancozeb 64% WP recorded 88.76%, penflufen 13.28% + trifloxystrobin 13.28% FS recorded 88.19%, and fluxapyroxad 333 g/l FS recorded 87.62%. Tebuconazole 5.4% FS was the least effective with mycelial growth inhibition of 64.57%. The highest growth of *Curvularia lunata* was recorded in the control. The findings of present investigation are in agreement with Siren *et al.* (2022)
4. ***Aspergillus niger*:** Among the seven fungicides, carbendazim 25% + mancozeb 50% WS, carboxin 37.5% + thiram 37.5% DS, prochloraz 34.8% + propiconazole 7.8% EC and fluxapyroxad 333 g/l FS recorded 100% mycelial growth inhibition against *Aspergillus niger*, followed by penflufen 13.28% + trifloxystrobin 13.28% FS recorded 88.10%, tebuconazole 5.4% FS recorded 87.14% and metalaxyl 8% + mancozeb 64% WP recorded 67.14%. The highest growth of *Aspergillus niger* was recorded in the control. The findings of present investigation are in agreement with Sutar *et al.* (2024) [8].
5. ***Aspergillus ochraceus*:** Among the seven fungicides, carbendazim 25% + mancozeb 50% WS recorded 100% mycelial growth inhibition against *Aspergillus ochraceus*, followed by prochloraz 34.8% + propiconazole 7.8% EC recorded 91.85%, carboxin 37.5% + thiram 37.5% DS recorded 90.85%, metalaxyl 8% + mancozeb 64% WP recorded 84.26%, tebuconazole 5.4% FS recorded 83.15%, fluxapyroxad 333 g/l FS recorded 52.41% and penflufen 13.28% + trifloxystrobin 13.28% FS recorded 00.00%. The highest growth of *Aspergillus ochraceus* was recorded in the control. The findings of present investigation are in agreement with Sutar *et al.* (2024) [8].

Dual culture method

The efficacy of four biocontrol agents viz., *Trichoderma asperellum*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis*, was evaluated against seed borne mycoflora of proso millet by dual culture method. Results obtained are tabulated in Table 2.

1. ***Fusarium moniliforme*:** Maximum mycelial growth inhibition of *Fusarium moniliforme* was observed in *Trichoderma asperellum* (75.71%) followed by *Trichoderma harzianum* (68.92%) and *Bacillus subtilis* (61.42%). Minimum mycelial growth inhibition was observed in *Pseudomonas fluorescens* (36.61%). The findings of present investigation are in agreement with Meghavathu *et al.* (2025) [5] and Tammineni *et al.* (2025) [6].
2. ***Bipolaris panici-miliacei*:** Maximum mycelial growth inhibition of *Bipolaris panici-miliacei* was observed in *Trichoderma asperellum* (80.74%) followed by *Trichoderma harzianum* (66.17%) and *Bacillus subtilis* (57.21%). Minimum mycelial growth inhibition was observed in *Pseudomonas fluorescens* (50.74%). The findings of present investigation are in agreement with Gupta *et al.* (2025) [7].

3. **Curvularia lunata:** Maximum mycelial growth inhibition of *Curvularia lunata* was observed in *Trichoderma asperellum* (73.07%) followed by *Bacillus subtilis* (69.23%), *Trichoderma harzianum* (65.38%). Minimum mycelial growth inhibition was observed in *Pseudomonas fluorescens* (56.15%). The finding of present investigation in agreement with Tekade *et al.* (2017) [9].
4. **Aspergillus niger:** Maximum mycelial growth inhibition of *Aspergillus niger* was observed in *Trichoderma harzianum* (74.14%) followed by *Trichoderma asperellum* (57.95%), *Bacillus subtilis* (44.31%). Minimum mycelial growth inhibition was observed in *Pseudomonas fluorescens* (03.52%). The present investigation in agreement with Sutar *et al.* (2024) [8].
5. **Aspergillus ochraceus:** Maximum mycelial growth inhibition of *Aspergillus ochraceus* was observed in *Bacillus subtilis* (79.26%) followed by *Trichoderma asperellum* (65.05%), and *Trichoderma harzianum* (60.79%). Minimum mycelial growth inhibition was observed in *Pseudomonas fluorescens* (25.28%). The findings of present investigation are in agreement with Sutar *et al.* (2024) [8].

4. Conclusion

The study identified *Fusarium moniliforme*, *Bipolaris panici-miliacei*, *Curvularia lunata*, *Aspergillus niger*, *Aspergillus ochraceus*, as predominant seed borne fungi associated with seed of proso millet. The *in vitro* evaluation of fungicide has clearly shown that carboxin 37.5% + thiram 37.5% DS @0.3% and prochloraz 34.8% + propiconazole 7.8% EC @0.2% achieved complete mycelial inhibition against *Fusarium moniliforme*, *Bipolaris panici-miliacei*, *Curvularia lunata* and *Aspergillus niger*. Carbendazim 25% + mancozeb 50% WS @0.2% showed 100% mycelial inhibition against *Aspergillus niger* and *Aspergillus ochraceus*. The *in vitro* evaluation of bioagents shows *Trichoderma asperellum* showed maximum mycelial growth inhibition against *Fusarium moniliforme* (75.71%), *Bipolaris panici-miliacei* (80.74%) and *Curvularia lunata* (73.07%). *Trichoderma harzianum* showed maximum mycelial inhibition against *Aspergillus niger* (74.14%), while *Bacillus subtilis* showed maximum mycelial inhibition against *Aspergillus ochraceus* (79.26%).

Table 1. Efficacy of fungicides against seed borne mycoflora of proso millet *in vitro*

Tr. No	Treatment details	Conc. Per cent	<i>Fusarium moniliforme</i>		<i>Bipolaris panici-miliacei</i>		<i>Curvularia lunata</i>		<i>Aspergillus niger</i>		<i>Aspergillus ochraceus</i>	
			MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)
T ₁	Carbendazim25%+ Mancozeb 50% WS	0.20%	08.00	90.58	15.50	76.15	07.16	91.81	00.00	100	00.00	100
T ₂	Penflufen13.28%+ Trifloxystrobin 13.28% FS	0.10%	16.83	80.20	20.66	68.21	10.33	88.19	08.33	88.10	90.00	00.00
T ₃	Carboxin 37.5%+Thiram 37.5 DS	0.30%	00.00	100	00.00	100	00.00	100	00.00	100	08.66	90.37
T ₄	Prochloraz34.8%+Propiconazole7.8 % EC	0.20%	13.33	84.31	00.00	100	00.00	100	00.00	100	07.33	91.85
T ₅	Tebuconazole 5.4%FS	0.10%	13.50	84.11	12.50	80.76	31.00	64.57	00.00	87.14	15.16	83.15
T ₆	Fluxapyroxad 333 g/l FS	0.10%	32.00	62.35	12.00	81.53	10.83	87.62	09.00	100	42.83	52.41
T ₇	Metalaxyl 8%+ Mancozeb 64%WP	0.25%	45.00	47.05	15.00	76.92	09.83	88.76	23.00	67.14	14.16	84.26
T ₈	Control	--	85.00	--	65.00	--	87.50	--	70.00	--	90.00	--
	SE(m)±	--	0.76	--	0.50	--	0.58	--	0.42	--	0.65	--
	CD(P = 0.01)	--	3.145	--	2.079	--	2.397	--	1.755	--	2.721	--

MCD: Mean colony diameter, PGI: Per cent growth inhibition

Table 2. Efficacy of bioagents against seed borne mycoflora of proso millet *in vitro*

Tr.No.	Treatment details	<i>Fusarium moniliforme</i>		<i>Bipolaris panici-miliacei</i>		<i>Curvularia lunata</i>		<i>Aspergillus niger</i>		<i>Aspergillus ochraceus</i>	
		MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)	MCD (mm)	PGI (%)
T ₁	<i>Trichoderma asperellum</i>	17.00	75.71	16.37	80.74	17.75	73.07	37.00	57.95	30.75	65.05
T ₂	<i>Trichoderma harzianum</i>	21.75	68.92	28.75	66.17	22.50	65.38	22.75	74.14	34.50	60.79
T ₃	<i>Pseudomonas fluorescens</i>	44.37	36.61	41.37	50.74	28.50	56.15	85.00	03.52	65.75	25.28
T ₄	<i>Bacillus subtilis</i>	27.00	61.42	36.37	57.21	20.00	69.23	49.00	44.31	18.25	79.26
T ₅	Control	70.00	--	85.00	--	65.00	--	88.00	--	88.00	--
	SE(m)±	0.84	--	0.91	--	0.67	--	0.78	--	0.85	--
	CD(P = 0.01)	3.51	--	3.81	--	2.808	--	3.26	--	3.57	--

MCD – Mean colony diameter PGI – Per cent growth inhibition

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