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Morphology and Influence of Abiotic Factors on *Magnaporthe grisea*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study entitled "Studies on *Magnaporthe grisea* incitant of blast disease of pearl millet [*Pennisetum glaucum* (L.) R. Br.]" was conducted during the *kharif* 2019 at research farm, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar. Pearl millet blast disease is a devastating fungal disease causing considerable yield losses. Blast of pearl millet incited by *Magnaporthe grisea* is the most widespread and destructive disease of pearl millet in India and other pearl millet growing area of the world. This disease is a major factor limiting full exploitation of high yield potential hybrids in India. In the present investigation, culture medium plays an important role in growth and sporulation of fungus. Among 5 media tested, rice meal agar was found most effective for all the 3 isolates of pearl millet blast were tested. Combined effect of temperature and relative humidity also play an important role in growth and spore germination of fungus. Among five combination of temperature and relative humidity, 30℃ temperature with 100 % relative humidity was found most effective for spore germination.

Keywords: Magnaporthe grisea; blast disease; rice meal agar and spore germination.

1. INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br)] belonging to family *Poaceae* is one of the assured *Kharif* crops domesticated in an area with the annual rainfall of 150 mm to 1000 mm in India. Pearl millet is a rainfed crop which can survive well in the rainfall as 250 mm on

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relatively poor soils. It is a highly cross-pollinated small-seeded cereal crop which is protogynous in nature. Bajra is cultivated in over 30 countries of Africa, America and Asia where dry land system is possible. India and Africa are together occupying 90 % area of total pearl millet producing area in the world [1]. India is the largest producer of pearl millet as largest area also in the world, Rajasthan being the largest producer of pearl millet in India.

In pearl millet, several diseases caused by fungi, bacteria, viruses and nematodes have been recorded. Economically important are only a few that include blast, downy mildew, ergot, smut and rust [2]. In India 60 per cent [3] or more pearl millet is sown with single-cross hybrids that are particularly vulnerable to Pyricularia leaf spot disease or blast disease of pearl millet caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*) and downy mildew disease caused by *Sclerospora graminicola*. In recent years the incidence of blast has increased tremendously and being noticed over the varieties and commercially grown hybrids in the bajra growing regions of the country.

In India, blast disease was first reported during 1953 on a few cultivars of pearl millet and later recorded sporadically on many hybrids and varieties during 1980s. The disease appears as greyish, water-soaked foliar lesions that enlarge and become necrotic, resulting in premature drying of young leave [4]. Depending on the resistance level of the host cultivar, the lesion size varies from small, roundish, elliptical, diamond shaped to elongated, measuring 1-2 mm to 20 mm. Lesions grow and coalesce to cover large surface areas and cause necrosis of tissues. In case of a susceptible cultivar the entire foliage gives a burnt appearance, though not much scientific work has been done in this concern.

M. grisea is a heterothallic filamentous ascos which can reproduce both sexually and asexually. The fungus is highly variable, but highly specialized in their host range. Thus P. grisea strains from rice do not infect pearl millet and vice versa. Mycelium of M. grisea infecting pearl millet is observed in cultures as aerial or submerged, hyaline or olivaceous, 1.5 – 6.0 µm in width, septate branched, conidiophores one to many, fasciculate, simple or rarely branched not or slightly constricted at septa. Asexual conidia of M. grisea are pyriform, hyaline and are mostly three-celled with a small appendage on the basal cell. Conidia measure approximately 17-31 x 6-9 µm and germinate by producing appressorium [5]. M. grisea produce light, inconspicuous, grey to greenish growth on large lesions on leaf, consisting of short delicate conidiophores carrying clusters of conidia at their tip. Conidia are typically obpyriform, hyaline, 2-septate, with protuberant hilum.

Weather variables, particularly relative humidity, leaf wetness duration and temperature play a major role in influencing infection and disease development in any host-pathogen systems. Blast disease has the potential to cause severe crop losses in pearl millet when environmental conditions are favorable for disease development. The pathogen sporulates profusely in the lesions on foliage under favourable conditions and the conidia can be easily dispersed by the wind and splashing rain to reinfect the host. These spores can overwinter in stubble and can infect the next following year crop.

2. MATERIALS AND METHODS

The present investigation entitled, "**Studies on *Magnaporthe grisea* incitant of blast disease of pearl millet [*Pennisetum glaucum* (L.) R. Br.]**" was conducted in the Laboratory and Research Area, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar during *Kharif* 2019.

2.1 Isolation, Purification and Maintenances of different Isolates of *M. grisea*

The pathogen (*Pyricularia grisea*) from the blast infected leaf samples collected from different areas of Haryana were isolated separately by following tissue isolation technique.

2.1.1 Hyphal tip isolation

Hyphal tip isolation was done on water agar plates. Spore suspension (10-20 spores/ml) was prepared in sterile distilled water and one drop of such suspension was transferred to two per cent water agar plates. Such plates were incubated at 25±1°C and periodically observed for germination of spores under the low power objective (10x) of compound microscope. Hyphal initials/threads emerging from center was traced and marked with the ink. Then tip of the hyphae was cut with sterile cork borer and transferred to the potato dextrose agar slants and incubated at 25 ±1°C for 15 days.

2.1.2 Effect of culture media on growth and sporulation

To understand the morphology of *M.*1*grisea*, the growth characters, i.e. radial growth and sporulation of *P. grisea* were studied on five solid media viz., oat meal agar, potato dextrose agar, rice meal agar, bajra meal agar and Richards's agar. The chemical composition of each medium was as follows:

1. Richards's agar:

Sucrose ($C_{12}H_{22}O_{11}$): 50.00 g
Potassium dihydrogen ortho phosphate (KH_2PO_4): 5.00 g
Potassium nitrate (KNO_3): 10.00 g
Magnesium sulphate ($MgSO_4$): 2.50 g
Ferric chloride ($FeCl_3$): 0.02 g
Agar-agar: 20.00 g
Distilled water:1000 ml (to make up)

All the ingredients were dissolved one by one in 450 ml distilled water and agar was dissolved separately in 500 ml distilled water and mixed with the above solution and the volume was made up to one litre before sterilization.

2. Potato dextrose agar:

Peeled potato: 200 g
Dextrose: 20 g
Agar-agar: 20 g
Distilled water: 1000 ml (to make up)

3. Oat meal agar:
Oat flakes: 30 g
Agar-agar: 20 g
Distilled water: 1000 ml (to make up)

First oat flakes were boiled with 500 ml of distilled water for 30 min and filtered through muslin cloth. Agar-agar was melted in 500 ml water separately. Both the solutions were mixed thoroughly and sterilized.

4. Rice meal agar:

Rice kernel: 30 g
Agar-agar: 20 g
Distilled water: 1000 ml (to make up)

First rice kernels were boiled with 500 ml of distilled water for 30 min and filtered through muslin cloth. Agar-agar was melted in 500 ml water separately. Both the solutions were mixed thoroughly and sterilized.

5. Bajra meal agar:

Bajra grains: 30 g
Agar-agar: 20 g
Distilled water: 1000 ml (to make up)

2.2 Sporulation

One drop of fine spore solution was taken and the conidia were counted by using a haemocytometer. The sporulation was rated as follows.

2.3 Effect of Temperature and Relative Humidity on Growth and Spore Germination

To understand the morphology of *M*. *grisea*, laboratory experiments were conducted to know the effects of various abiotic factors viz., temperature and relative humidity on growth and spore germination of test pathogen. The treatment detail is as follows:

Treatments: **T1**. Temperature (20^0C, 25^0C, 30^0C and 35^0C)
T2. Relative humidity (60%, 70%, 80%, 90% and 100%)

List 1. Rate of sporulation

Rate of sporulation (Rating)	No. of spores / microscopic field
Excellent (++++)	> 40
Good (+++)	21-40
Fair (++)	11-20
Poor (+)	1-10
Nil (-)	0

3. RESULTS

3.1 Collection, Isolation and Purification of *Magnaporthe grisea* Isolates

Pearl millet blast disease samples were collected from different districts of Haryana. Pearl millet blast samples were collected from major pearl millet growing districts of Haryana *viz.,* Hisar, Bhiwani and Mohindergarh during *Kharif* 2018. The pathogen was isolated. Further, monoconidial isolation was also done to obtain pure culture of (*Magnaporthe grisea*) all isolates. The fungus was sub-cultured on potato dextrose agar slants and stored in a refrigerator at 4°C.

3.2 Effect of Different Culture Media on the Mycelial Growth and Sporulation of *Magnaporthe grisea* Isolates

The Hisar, Bhiwani and Mohindergarh isolates were grown on five different solid media *viz.* potato dextrose agar (PDA), rice meal agar, bajra meal agar, oat meal agar and Richard's agar media. The data on radial growth of Hisar isolate presented in Table 1 revealed that amongst all five media, the Petri plate completely filled within 192 h in rice meal agar followed by oat meal agar (8.27 cm), potato dextrose agar (8.23 cm), bajra meal agar (8.23 cm) and it was significantly lower in Richard's agar media (5.90 cm) after 192 h of inoculation. Rice meal agar was found to be best suited medium for fungus growth with mean radial growth of (4.96cm) which was significantly higher than other media. At the same time, data on radial growth of Bhiwani isolates revealed that amongst all five media tested the Petri plate completely filled within 192 h in rice meal agar followed by bajra meal agar (8.20 cm), potato dextrose agar (8.13 cm) and oat meal agar and was significantly lower in Richard's agar media (5.60 cm) after 192 h of inoculation.

Rice meal agar was best suited medium for the growth of fungus with the mean radial growth of (5.45 cm) which was significantly higher than other media.

Table 1. Effect of different culture media on the mycelial growth of different *Magnaporthe grisea* isolates

Name of isolate	Name of Medium	Colony diameter (cm) at different time intervals								
		24h	48h	72h	96h	120h	144h	168h	192h	Mean
Hisar	Potato dextrose agar	1.46	2.33	3.23	4.13	5.13	6.03	7.06	8.23	4.70
	Rice meal agar	1.33	2.13	3.03	4.13	5.33	6.76	8.00	9.00	4.96
	Bajra meal agar	1.23	2.10	2.96	4.00	4.90	5.93	7.10	8.23	4.55
	Oat meal agar	1.43	2.33	3.23	4.23	5.16	6.16	7.16	8.27	4.75
	Richard's medium	0.76	1.40	2.03	2.70	3.50	4.26	5.00	5.90	3.19
	Mean	1.24	2.06	2.90	3.84	4.80	5.83	6.86	7.92	
Bhiwani	Potato dextrose agar	1.23	2.03	2.83	3.70	4.66	5.83	7.00	8.13	4.42
	Rice meal agar	1.63	2.63	3.76	4.96	6.06	7.20	8.36	9.00	5.45
	Bajra meal agar	1.36	2.23	3.10	3.96	4.93	5.96	7.10	8.20	4.60
	Oat meal agar	1.43	2.26	3.10	3.96	4.96	5.93	6.96	8.10	4.59
	Richard's medium	0.90	1.50	2.13	2.66	3.33	4.06	4.73	5.60	3.11
	Mean	1.31	2.13	2.98	3.85	4.79	5.80	6.83	7.80	
M. garh	Potato dextrose agar	1.13	1.96	2.83	3.73	4.73	5.80	7.00	8.13	4.41
	Rice meal agar	1.46	2.50	3.50	4.60	5.73	6.96	8.23	9.00	5.25
	Bajra meal agar	1.26	2.03	2.86	3.90	4.93	5.96	7.13	8.26	4.54
	Oat meal agar	1.26	2.10	2.93	3.83	4.83	5.90	7.00	8.23	4.51
	Richard's medium	0.76	1.53	2.16	2.83	3.53	4.33	5.16	6.03	3.29
	Mean	1.18	2.02	2.86	3.78	4.75	5.79	6.90	7.93	
		Hisar			Bhiwani			Mohindergarh		
CD (P=0.05)	Medium	0.13			0.10			0.08		
	Time	0.16			0.12			0.11		
	Medium x Time	0.36			0.28			0.24		

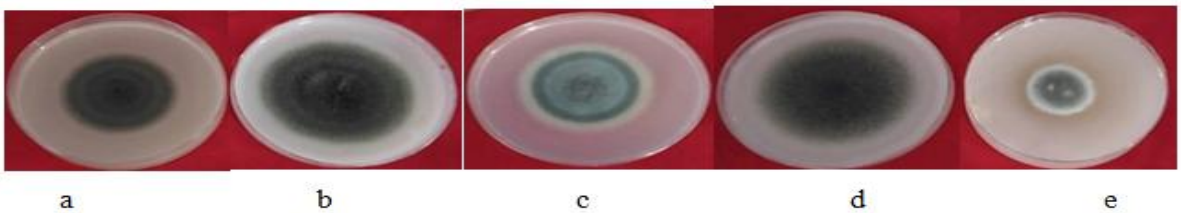

Plate 1. Culture of *Magnaporthe grisea* of Hisar isolate on 5 different media (a) Bajra meal agar (b) Oat meal agar (c) Potato dextrose agar (d) Rice meal agar (e) Richards agar

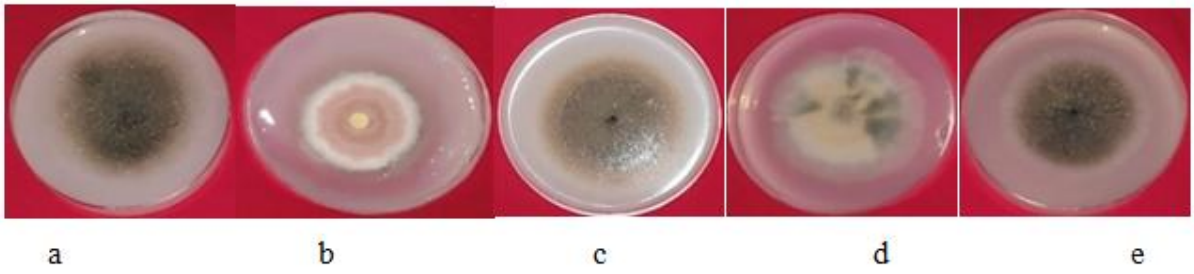

Plate 2. Culture of *Magnaporthe grisea* of Bhiwani isolate on 5 different media (a) Rice meal agar (b) Richards agar (c) Oat meal agar (d) Potato dextrose agar (e) Bajra meal agar

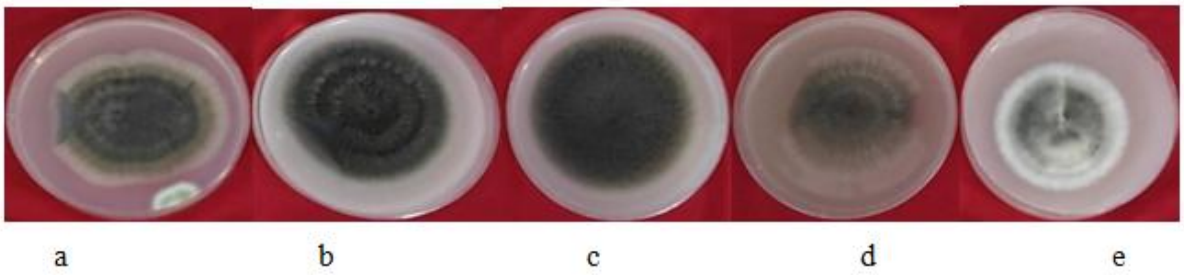

Plate 3. Culture of *Magnaporthe grisea* of Mohindergarh isolate on 5 different media (a) Potato dextrose agar (b) Oat meal agar (c) Rice meal agar (d) Bajra meal agar (e) Richards agar

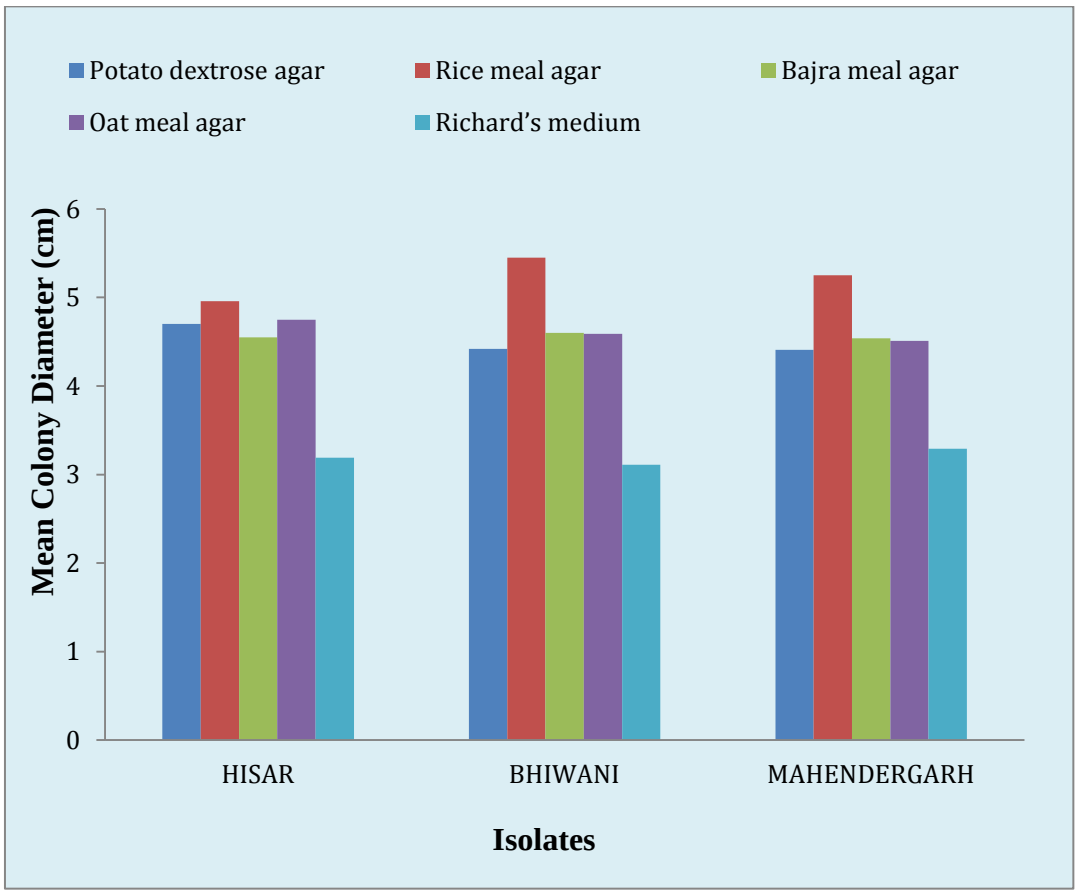

Fig. 1. Mycelial growth of *Magnaporthe grisea* isolates on different culture media

Table 2. Effect of different culture media on the sporulation of *Magnaporthe grisea*

Isolate	Different media	Sporulation	Rating
	Potato dextrose agar	Good	+++
	Rice meal agar	Excellent	++++
Hisar	Bajra meal agar	Excellent	++++
	Oat meal agar	Good	+++
	Richard's medium	Poor	-
	Potato dextrose agar	Fair	++
	Rice meal agar	Excellent	++++
Bhiwani	Bajra meal agar	Good	+++
	Oat meal agar	Excellent	++++
	Richard's medium	Poor	-
	Potato dextrose agar	Fair	++
	Rice meal agar	Excellent	++++
Mohindergarh	Bajra meal agar	Good	+++
	Oat meal agar	Excellent	++++
	Richard's medium	Poor	-

The data on radial growth of Mohindergarh isolate presented in Table 1 revealed that among all five media tested the Petri plate completely filled within 192 h in rice meal agar followed by bajra meal agar (8.26 cm), oat meal agar (8.23 cm),potato dextrose agar (8.13 cm), and it was significantly lower in Richard's agar media (6.03 cm) after 192 h of inoculation. Rice meal agar was best suited medium for the growth of fungus with the mean radial growth of (5.25 cm) which was significantly higher than the other media.

Sporulation was excellent in rice meal agar and poor in Richard's agar of all the 3 isolates tested whereas, it was also excellent in oat meal agar and bajra meal agar of Bhiwani, Mohindergarh and Hisar isolates respectively.

3.3 Effect of Different Combination of Temperature and Relative Humidity Regimes on the Mycelial Growth of *Magnaporthe grisea* Isolates

Among four temperature level tested on the Hisar isolate, the highest mean radial growth (6.03 cm) was observed at 30°C T & 90% RH which was significantly higher than other temperature levels whereas, it was significantly lower (2.07 cm) at 20°C T& 60% RH. The Petri plate was completely filled within 192 h only in case of 30°C at all the RH levels except at 25°C & 80% RH. The radial growth showed gradual decline on either side of 30°C T at all relative humidity levels. Highest mean radial growth of Bhiwani isolate was found (5.79 cm) at 30°C T& 90% RH which was significantly higher than other temperature levels whereas, it was significantly lower (2.23 cm) at 20°C& 60% RH. As the data presented in Table 5, highest mean radial growth of Mohindergarh isolate was found (5.76 cm) at 30°C T & 90% RH which was significantly higher than other temperature levels whereas, it was significantly lower (1.96 cm) at 20°C & 60% RH. The radial growth showed gradual decline on either side of 30°C T at all the relative humidity levels except at two RH levels (80% and 90%) of 25°C and at 90% of 35°C temperature. Finally, it was found common from this study that radial growth of all the three isolates was significantly higher at 30°C temperature with 90% relative humidity level and with increase in level of RH at all the temperature range, radial growth also increases proportionally. Petri plate was also completely filled within 192 h only at 30°C temperature at all the relative humidity levels.

3.4 Effect of Different Combination of Temperature and Relative Humidity Regimes on the Spore Germination of *Magnaporthe grisea* Isolates

Spore germination was recorded in every 12 hours upto 36 hours and data of all the 3 isolates is presented in Tables 6. It is clear from the data presented in Table 6 that among the four temperature level tested on the Hisar, Bhiwani and Mohindergarh isolate, the highest mean spore germination was observed at 30°C T & 100% RH which was significantly higher than other temperature levels whereas, it was significantly lower at 20°C T & 60% RH in all the 3 isolates. The spore germination showed gradual decline on either side of 30°C T at all the relative humidity levels. It was also found that

Table 3. Effect of different combination of temperature and relative humidity on the mycelial growth of Hisar isolate of *Magnaporthe grisea*

Temp.	Relative Humidity	Colony diameter (cm) at different time intervals								
		24h	**48h**	**72h**	**96h**	**120h**	**144h**	**168h**	**192h**	**Mean**
	60 %	0	0.40	0.90	1.50	2.16	2.93	3.90	4.80	2.07
	70 %	0	0.37	0.96	1.60	2.40	3.20	4.13	5.10	2.22
20°C	80 %	0	0.57	1.20	2.06	2.93	3.93	5.00	6.10	2.72
	90 %	0	0.60	1.43	2.33	3.23	4.16	5.23	6.33	2.91
	100 %	0	0.40	1.00	1.60	2.40	3.26	4.26	5.13	2.25
	Mean	0	0.47	1.10	1.82	2.62	3.50	4.50	5.49	
	60 %	1.00	1.97	2.93	3.86	4.93	5.93	6.93	8.03	4.45
	70 %	1.07	1.96	2.96	3.90	4.90	5.93	7.03	8.16	4.49
25°C	80 %	1.43	2.50	3.60	4.80	5.93	7.20	8.46	9.00	5.36
	90 %	1.33	2.33	3.33	4.33	5.40	6.53	7.63	8.70	4.95
	100 %	1.07	1.90	2.80	3.83	4.80	5.90	7.10	8.30	4.46
	Mean	1.18	2.13	3.12	4.14	5.19	6.30	7.43	8.44	
	60 %	1.33	2.30	3.33	4.43	5.63	6.86	8.03	9.00	5.11
	70 %	1.47	2.50	3.53	4.66	5.86	7.13	8.36	9.00	5.31
30°C	80 %	1.60	2.73	3.93	5.16	6.46	7.76	9.00	9.00	5.70
	90 %	1.97	3.16	4.40	5.63	6.93	8.20	9.00	9.00	6.03
	100 %	1.60	2.70	3.83	4.96	6.13	7.36	8.53	9.00	5.51
	Mean	1.59	2.68	3.80	4.97	6.20	7.46	8.58	9.00	
	60 %	0.77	1.50	2.16	2.93	3.73	4.53	5.53	6.56	3.46
	70 %	0.83	1.56	2.36	3.26	4.16	5.10	6.03	7.00	3.79
35°C	80 %	1.23	2.03	2.93	3.83	4.83	5.90	6.96	8.06	4.47
	90 %	1.27	2.20	3.13	4.13	5.16	6.20	7.26	8.40	4.72
	100 %	1.47	2.36	3.36	4.36	5.40	6.36	7.40	8.50	4.90
	Mean	1.11	1.93	2.79	3.70	4.66	5.62	6.64	7.70	
			20°C		25°C		30°C			35°C
CD	Relative Humidity	0.07		0.09		0.09		0.07		
(P=0.05)	Time	0.10		0.11		0.11		0.09		
	RH x Time	0.22		0.26		0.25		0.21		

Table 4. Effect of different combination of temperature and relative humidity on the mycelia growth of Bhiwani isolate of *Magnaporthe grisea*

Temp.	Relative Humidity	Colony diameter (cm) at different time intervals								
		24h	**48h**	**72h**	**96h**	**120h**	**144h**	**168h**	**192h**	**Mean**
20°C	60 %	0	0.40	1.00	1.56	2.36	3.23	4.20	5.10	2.23
	70 %	0	0.36	1.03	1.70	2.50	3.36	4.26	5.23	2.30
	80 %	0	0.60	1.40	2.26	3.16	4.16	5.10	6.03	2.84
	90 %	0	0.60	1.36	2.23	3.16	4.20	5.30	6.43	2.91
	100 %	0	0.40	1.10	1.80	2.60	3.50	4.43	5.36	2.40
	Mean	0	0.47	1.18	1.91	2.76	3.69	4.66	5.63	
25°C	60 %	1.00	1.83	2.80	3.73	4.76	5.83	6.93	8.10	4.37
	70 %	1.16	2.03	2.93	3.96	4.93	6.03	7.16	8.33	4.57
	80 %	1.56	2.60	3.66	4.86	6.06	7.33	8.56	9.00	5.45
	90 %	1.60	2.53	3.60	4.76	5.86	7.03	8.20	9.00	5.32
	100 %	1.33	2.23	3.20	4.23	5.30	6.33	7.36	8.53	4.81
	Mean	1.33	2.24	3.24	4.31	5.38	6.51	7.64	8.59	
30°C	60 %	1.33	2.30	3.36	4.36	5.46	6.56	7.70	8.83	4.99
	70 %	1.50	2.46	3.53	4.66	5.80	7.00	8.23	9.00	5.27
	80 %	1.66	2.80	4.03	5.23	6.50	7.73	8.93	9.00	5.73
	90 %	1.70	2.86	4.03	5.30	6.56	7.86	9.00	9.00	5.79
	100 %	1.56	2.60	3.73	4.86	6.03	7.23	8.46	9.00	5.43
	Mean	1.55	2.60	3.74	4.88	6.07	7.28	8.46	8.96	
35°C	60 %	0.80	1.50	2.16	2.96	3.76	4.60	5.53	6.56	3.48
	70 %	0.86	1.53	2.30	3.13	4.00	4.93	5.90	6.93	3.70
	80 %	1.23	2.03	2.93	3.83	4.83	5.90	6.96	8.10	4.47
	90 %	1.26	2.20	3.20	4.23	5.30	6.33	7.36	8.43	4.79
	100 %	1.36	2.33	3.40	4.36	5.43	6.50	7.60	8.66	4.95
	Mean	1.10	1.92	2.80	3.70	4.66	5.65	6.67	7.74	
			20°C		25°C		30°C			35°C
CD (P=0.05)	Relative Humidity	0.04		0.12		0.10		0.07		
	Time	0.06		0.15		0.12		0.09		
	RH x Time	0.13		0.34		0.28		0.21		

Table 5. Effect of different combination of temperature and relative humidity on the mycelial growth of Mohindergarh isolate of *Magnaporthe grisea*

Temp.	Relative Humidity	Colony diameter (cm) at different time intervals								
		24h	48h	72h	96h	120h	144h	168h	192h	Mean
	60 %	0	0.40	0.90	1.43	2.00	2.76	3.66	4.53	1.96
	70 %	0	0.43	1.00	1.60	2.40	3.20	4.10	4.93	2.20
20°C	80 %	0	0.56	1.23	2.03	2.90	3.93	5.00	6.10	2.72
	90 %	0	0.56	1.43	2.33	3.26	4.23	5.26	6.33	2.92
	100 %	0	0.46	1.16	1.86	2.63	3.50	4.40	5.30	2.41
	Mean	0	0.48	1.14	1.85	2.64	3.52	4.48	5.44	
	60 %	1.06	1.90	2.70	3.66	4.60	5.63	7.10	7.93	4.32
	70 %	1.16	2.06	3.00	3.96	4.93	6.00	7.13	8.20	4.55
25°C	80 %	1.60	2.63	3.76	4.96	6.10	7.23	8.36	9.00	5.45
	90 %	1.50	2.50	3.56	4.70	5.80	7.03	8.30	9.00	5.30
	100 %	1.26	2.13	3.00	4.03	5.03	6.13	7.26	8.43	4.66
	Mean	1.32	2.24	3.20	4.26	5.29	6.40	7.63	8.51	
	60 %	1.13	2.06	2.96	4.10	5.26	6.46	7.73	9.00	4.84
	70 %	1.40	2.33	3.40	4.50	5.63	6.83	8.06	9.00	5.14
30°C	80 %	1.63	2.73	3.93	5.16	6.43	7.73	8.90	9.00	5.69
	90 %	1.73	2.86	4.10	5.33	6.56	7.70	8.83	9.00	5.76
	100 %	1.46	2.53	3.60	4.70	5.86	7.10	8.30	9.00	5.32
	Mean	1.47	2.50	3.60	4.76	5.95	7.16	8.36	9.00	
	60 %	0.83	1.53	2.30	3.13	4.00	4.96	6.00	6.96	3.71
	70 %	0.90	1.63	2.40	3.26	4.13	5.06	6.00	7.13	3.81
35°C	80 %	1.30	2.20	3.20	4.23	5.26	6.33	7.40	8.46	4.80
	90 %	1.40	2.40	3.40	4.40	5.43	6.56	7.63	8.73	4.99
	100 %	1.40	2.26	3.20	4.06	4.96	5.86	6.90	7.96	4.57
	Mean	1.16	2.00	2.90	3.82	4.76	5.76	6.78	7.85	
			20°C		25°C		30°C			35°C
CD	Relative Humidity	0.04		0.12		0.09		0.06		
(P=0.05)	Time	0.06		0.15		0.11		0.07		
	RH x Time	0.13		0.34		0.26		0.17		

Table 6. Effect of different combination of temperature and relative humidity on the spore germination of *Magnaporthe grisea* isolates

Temp.	RH	MOHINDERGARH				HISAR				BHIWANI			
		12h	24h	36h	Mean	12h	24h	36h	Mean	12h	24h	36h	Mean
	60 %	12.33	23.33	33.33	23.00	14.66	25.66	35.00	25.11	16.66	27.66	37.33	27.22
	70 %	18.00	32.00	41.00	30.33	20.33	34.00	44.00	32.77	23.00	36.33	46.33	35.22
20°C	80 %	24.33	34.66	47.33	35.44	27.00	37.00	49.66	37.88	29.66	39.33	52.33	40.44
	90 %	27.66	43.33	58.33	43.11	29.66	45.00	59.00	44.55	31.66	47.33	61.66	46.88
	100 %	39.00	54.33	71.33	54.88	40.33	57.00	74.33	57.22	43.00	59.00	76.66	59.55
	Mean	24.26	37.53	50.26		26.40	39.73	52.40		28.80	41.93	54.86	
	60 %	18.33	28.33	47.66	31.44	20.33	30.33	50.33	33.66	22.66	32.66	52.33	35.88
	70 %	23.33	37.33	57.66	39.44	25.33	39.66	60.33	41.77	27.33	42.33	62.33	44.00
25°C	80 %	33.33	47.66	67.33	49.44	35.33	50.33	69.66	51.77	37.33	52.66	71.66	53.88
	90 %	41.00	57.33	77.33	58.55	43.33	59.66	80.33	61.11	45.66	62.00	82.33	63.33
	100 %	47.66	66.33	86.00	66.66	50.33	68.33	87.00	68.55	52.66	70.33	89.00	70.66
	Mean	32.73	47.40	67.20		34.93	49.66	69.53		37.13	52.00	71.53	
	60 %	22.33	37.33	57.66	39.11	24.66	39.66	60.33	41.55	27.00	42.00	62.33	43.77
	70 %	33.00	48.33	67.00	49.44	35.33	50.33	69.66	51.77	37.66	52.66	72.33	54.22
30°C	80 %	42.66	57.33	77.66	59.22	45.66	59.66	80.33	61.88	47.33	61.66	82.33	63.77
	90 %	50.00	67.33	87.66	68.33	52.33	70.33	90.33	71.00	53.00	72.00	92.00	72.33
	100 %	57.66	76.66	92.33	75.55	60.33	79.66	96.66	78.88	62.33	82.00	98.00	80.77
	Mean	41.13	57.40	76.46		43.66	59.93	79.46		45.46	62.06	81.40	
	60 %	15.00	25.00	39.33	26.44	17.33	27.33	42.33	29.00	19.33	29.66	44.66	31.22
	70 %	20.33	34.66	39.66	31.55	22.33	37.33	42.33	34.00	24.66	39.66	44.66	36.33
35°C	80 %	30.00	44.66	65.33	46.66	32.33	47.33	67.33	49.00	34.66	49.66	69.66	51.33
	90 %	37.00	52.33	72.00	53.77	39.66	55.33	75.33	56.77	42.33	57.33	77.33	59.00
	100 %	41.66	58.66	77.33	59.22	45.00	62.33	80.33	62.55	47.33	65.00	82.33	64.88
	Mean	28.80	43.06	58.73		31.33	45.93	61.53		33.66	48.26	63.73	
		20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C
CD (P=0.05)	RH	2.80	2.36	2.30	2.41	3.12	2.54	2.30	2.46	3.05	2.63	2.33	2.44
	Time	2.16	1.82	1.78	1.86	2.41	1.97	1.78	1.91	2.37	2.04	1.81	1.89
	RH x Time	4.85	N/S	N/S	4.18	5.40	N/S	N/S	4.27	5.29	N/S	N/S	4.23

spore germination was increases continuously with increase in relative humidity at all the temperature levels. While spore germination was also increased with increase in temperature from 20°C to 30°C but decrease from 30°C to 35°C.

4. DISCUSSION

In the present investigation five different culture media were used to see mycelial growth and sporulation of *Magnaporthe grisea* isolates of pearl millet. Among all the media and isolates tested the Rice meal agar was best suited medium for the growth of fungus with the mean radial growth of 54.50 mm which was significantly higher than the other. It was followed by Bajra meal agar (46.00 mm) and oat meal agar (45.9 mm) which were found at par with each but significantly higher than Potato dextrose agar (44.20 mm) and least Richard's agar (31.1 mm) medium. These results are in agreement with that [6] and [7] but contradict with that [8] and [9]. The extent of sporulation produced on the given media is in confirmation with the finding of [10] but contradict with that [11] and [12].

Among the four temperature along with five relative humidity level tested, the optimum temperature for the growth and conidial germination of *Magnaporthe grisea* was found to be 30°C along with 90% RH followed by 25°C along with 90% RH whereas, least growth and germination was observed at 20°C along with 60% RH in all the three isolates of Haryana. The results of mycelial growth obtained during the investigation are in more or less agreement with that [13], [14] and [15]. The results of spore germination obtained during the investigation are in more or less agreement with that [16] and [17].

[7] used four culture media for the study of mycelial growth of *P. grisea* and among them PDA media supported maximum mycelial growth followed by Richard's Agar medium after 168 hours of incubation. According to [3], potato dextrose agar supported maximum radial growth (81.24 mm) which was followed by oat meal agar (80.81 mm). Least radial growth was observed in host extract + 2% sucrose agar (62.92 mm). [18] recorded maximum mycelial growth of *M. oryzae* on carrot agar media and potato dextrose agar, whereas Czapek-Dox agar media completely suppressed the growth in rice isolates.

5. CONCLUSION

The *Magnaporthe grisea* exhibited diversified cultural characters, on different solid media. Amongst the different the solid media, maximum radial growth of the fungus was recorded on rice meal agar followed by bajra meal agar and oat meal agar. Sporulation was also observed best on rice meal agar. Among the four temperature and relative humidity level tested, the optimum temperature for the growth of *Magnaporthe grisea* was found to be 30°C followed by 25°C at 90% relative humidity level in each case whereas, least growth was observed at 20°C with 60% relative humidity level combination. While the optimum temperature for conidial germination of *Magnaporthe grisea* was found to be 30°C followed by 25°C at 100% relative humidity level whereas, least growth was observed at 20°C with 60% relative humidity level combination.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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