

Original Research Article

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Cultural, Morphological, Physiological and Biochemical Characteristics of *Magnaporthe grisea* Isolates of Finger Millet

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ABSTRACT

A total of 24 blast infected samples were collected from major finger millet growing areas of Tamil Nadu and also from All India Coordinated Small Millets Improvement Project Centres (ICAR) in India. Pathogenicity test with *Magnaporthe grisea* isolates showed highly significant differences in virulence on the variety KM 252 in the severity of leaf blast. Among the five different media tested, the Oat Meal Agar medium (OMA) was found to be best for the growth of *M. grisea* isolates and all the isolates produced white colony colour. The mean mycelial growth was maximum in OMA medium with 80.12 mm. The temperature 25 °C and pH 6.5 was found to be ideal for growth, sporulation and mycelial dry weight of *M. grisea* isolates. Of the different carbon sources, Richard's medium with supplemented dextrose recorded the maximum mean mycelial growth of *M. grisea* isolates with 83.74 mm. Among the nitrogen sources, the Richard's medium supplemented with NaNO₃ recorded the maximum mean mycelial growth of 85.87 mm. This study was conducted to isolate, identify and characterize the pathogen using cultural, morphological, physiological and biochemical methods.

Keywords

Finger millet, Blast disease, *Magnaporthe grisea*, Characterization

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Introduction

Finger millet (*Eleusine coracana* L.) is one of the most important millet crops belonging to the family Poaceae and sub family Chloridoidea (Dida *et al.*, 2008). Among the small millets, finger millet is widely grown

traditional grain cereal cultivated in semi-arid areas of East and Southern Africa and South Asia as a staple food for millions of poor population. Finger millet blast caused by *Magnaporthe grisea* (Hebert) Barr. (anamorph *Pyricularia grisea* (Cooke) Sacc. is a heterothallic, filamentous fungus, one of the

major destructive disease causing excessive damage to this crop from seedling to ear head forming stages. The disease occurs during all growing seasons and on almost all finger millet varieties cultivated. *M. grisea* parasitizes over 50 grasses, including economically important crops like wheat, rice, barley and millet (Ou, 1985). Yield loss due to blast can be as high as 50% when the disease occurs in epidemic proportions. The fungus appears to overwinter as mycelia in the infected living leaves or dead plant debris in the soil (Uddin, 2000). High temperature, high relative humidity and leaf wetness are critical environmental factors in disease development (Ruiz, 2003). The morphological studies of *P. grisea* isolates of finger millet were carried out and the isolates showed considerable variation in mycelial growth, pigmentation and conidia production in culture (Gatachew *et al.*, 2014). Asfaha *et al.*, (2015) reported that rice blast *P. oryzae* isolates were characterized and identified based on their growth parameters into six isolates *viz.*, Po12, Po28, Po41, Po55, Po72 and Po85. Among the four culture media (oat meal agar, rice flour agar, malt extract agar and potato agar), the *P. oryzae* isolates showed optimum growth and good sporulation in oat meal agar followed by rice flour agar. Hence, this study was initiated to understand the cultural, morphological, physiological and biochemical requirements for the growth and development of the pathogen, which could serve as an input in disease management to minimize yield loss in finger millet. This study was also undertaken to investigate the effects of growth factors on mycelial growth of *M. grisea* isolates.

Materials and Methods

Collection and isolation of *M. grisea*

Field survey was conducted during Kharif in major finger millets growing regions of Tamil Nadu and the blast infected finger millet plant

parts *viz.*, either leaf or neck or finger blast infected samples were taken based on the crop stage available at the time of survey. In addition, the blast infected finger millet samples were also received from the All India Coordinated Small Millet Improvement Project (AICSMIP) centres in India. The collected samples were air dried, separately bagged and stored under refrigerated condition at 4°C for the isolation of the pathogen. The blast pathogen (*M. grisea*) from different plant samples collected was isolated by using the standard tissue isolation method [Tuite, 1969]. Blast infected plant tissues were cut into small pieces and washed in sterile water twice and surface sterilized with 0.1 per cent mercuric chloride solution for 30 sec. followed by rinsing in sterilized water twice and transferred to plates containing Oat Meal Agar Medium (OMA). After 4 days for obtaining monoconidial isolate, a dilute spore suspension was prepared in sterilized distilled water and plated onto 0.8% water agar in Petri plates. After 15 days of incubation at $26 \pm 1^\circ\text{C}$, single germinating conidium was marked under a microscope and transferred to fresh Petri dish containing OMA medium and then the plates were incubated at $26 \pm 1^\circ\text{C}$ for 10 days to get monoconidial isolates (Shanmugapackiam *et al.*, 2017).

Pathogenicity of *M. grisea*

Pathogenicity tests were performed with field collected isolates of *M. grisea* on finger millet susceptible variety KM252 maintained in pots. Highly significant differences were observed among the finger millet isolates based on leaf blast severity scored on a 1–9 scale for recording leaf blast severity developed for Rice blast of IRRI - International Rice Research Institute, Philippines. The scoring for finger blast incidence was carried out at physiological maturity and at harvest. Neck blast incidence was also recorded at physiological maturity and at harvesting. Neck

and finger infections were observed at the milky or dough stage and expressed as per cent finger and neck blast incidence. Were the incidence of neck blast was recorded by counting the number of peduncles infected in a total number of plants. Finger blast was scored on the basis of percentage of finger infection (number of fingers infected out of the total fingers). Neck blast was recorded by counting the number of peduncles infected from 25 panicles. Finger blast was scored on the basis of percentage of finger infection from the number of fingers infected from 25 panicles (Mackill and Bonman, 1992). Among these, the most virulent pathogenic isolates from leaf, neck and finger blast were chosen for further studies.

Cultural and morphology characteristics of *M. grisea*

Effect of different culture media on the mycelial growth of *M. grisea*

Cultural characters of different isolates collected were studied using five different media viz., Oat Meal Agar (OMA), Richard's Agar Medium, Potato Dextrose Agar (PDA), Host seed extract +2% sucrose agar and V8 juice agar.

The medium were melted separately and kept for cooling. Just before solidification the medium was transferred to Petri dishes. Three replications were maintained.

After solidification, the Petri dishes were inoculated with 9 mm dia circular discs of fungal mat under aseptically in an inoculation chamber sterilized with UV radiation.

The inoculated agar plates were incubated at 25 ± 1 °C for 15 days. Observations on colony colour, margin, pigmentation, surface texture, mycelial growth and sporulation etc., were recorded.

Influence of various temperature on the mycelial growth and sporulation of

M. grisea

The growth and sporulation of seven virulent isolates of *M. grisea* were studied at various temperatures (15, 20, 25, 30 and 35 °C) on OMA medium (Kumar and Singh, 1995) with three replications. A nine mm mycelial disc was cut from the margin of a ten days old culture and placed aseptically at the centre of each Petri dish 90 mm containing 20 ml OMA medium and incubated at given temperatures for 15 days. The colony diameter was recorded at 15 days after incubation. Four discs of 9 mm were scooped out from each replication and transferred to 5 ml sterilized distilled water in a test tube. The test tubes were agitated to detach the conidia from the mycelial surface and filtered through a cheese cloth. The quantification of conidia in a given suspension was done using a haemocytometer.

Influence of pH on biomass of *M. grisea*

Fifty ml of Richard's broth was transferred to 250 ml conical flasks to study the influence of pH on the mycelial biomass of *M. grisea* isolates. The pH of the medium as adjusted to different pH viz., 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 by adding dihydrogen phosphate citrate acid buffer (Vogel, 1951). Flasks were sterilized at 15 psi for 20 min. After cooling, mycelial disc from seven virulent isolates with 9 mm diameter was taken from the edge of the 10 days old culture grown separately on OMA and placed in the conical flask aseptically and incubated for 20 days at 25 ± 1 °C. After incubation, the individual broth cultures were filtered through pre-weight Whatman No. 1 filter paper. For assessment of the biomass (mycelial dry weight), the broth culture was filtered through muslin cloth and the mat collected as transferred on pre-weighed filter paper, dried

at 40 °C for 2 h and then reweighed. The variation in weight was recorded (Hall and Bell, 1961).

Influence of different carbon and nitrogen sources on the mycelial growth of *M. grisea*

The *M. grisea* isolates were tested for carbohydrate and nitrogen utilization using Richards's basal medium [Sucrose (C₁₂H₂₂O₁₁) 50.00 g, Potassium dihydrogen phosphate (KH₂PO₄) 5.00 g, Potassium nitrate (KNO₃) 10.00 g, Magnesium sulphate (MgSO₄ · 7H₂O) 2.50 g, Ferric chloride (FeCl₃ · 6H₂O) 0.02 g, agar 20.0 g and distilled water (to make up) 1000ml] by replacing the carbon sources with dextrose, maltose, D- fructose, sucrose, glucose and galactose and replacing the nitrogen sources with L- glycine, KNO₃, L- arginine, NaNO₃, asparagine and peptone by each isolate of *M. grisea* (Otsuka *et al.*, 1957). All the carbon and nitrogen sources were dissolved separately and sterilized at 15 lbs pressure for 20 minutes. By using a sterile cork borer, mycelial disc of 9 mm diameter was taken from the periphery of actively growing 10 days old pure culture and inoculated in to the Petri plates containing the Richard's medium supplemented with different carbon, nitrogen sources and the plates were incubated at 25 ± 1 °C for 15 days. Colony diameter for each *M. grisea* isolate grown on plates was measured after 15 days of incubation. Colony diameters of each *M. grisea* isolate on plates were measured as described above.

Statistical analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). Prior to statistical analysis of variance (ANOVA) the percentage values of the disease index were transformed to arcsine.

Data were subjected to analysis of variance (ANOVA) at two significant levels (P< 0.05 and P< 0.01) and means were compared by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Collection and isolation of *M. grisea*

A total of 24 blast infected samples (leaf blast - 9, Neck blast - 13 and Finger blast - 2) were collected from major finger millet growing areas of Tamil Nadu and also from AICSMIP centres of ICAR during Kharif season 2012 and 2013 (Table 1). The pathogen was isolated from the infected leaf, neck and finger portion of the plant based on the infected plant part collected. The pathogen was purified based on single spore isolation method and maintained in Oat Meal Agar (OMA) slants at 4 °C under refrigerator condition.

Pathogenicity test for *M. grisea* isolates

Pathogenicity tests revealed that *M. grisea* isolates were able to infect all accessions of finger millet. Among the 24 finger millet blast isolates obtained from eleven locations, the highest leaf blast severity was recorded on KM252 designated as TNLB1 which showed 8.76 grade followed by the isolates TNNB8 (8.68), BIFB13 (8.41), JANB18 (8.16), JALB9 (8.12), MANB23 (8.12) and TNNB7 (8.02). The least severity of 4.77 scale was recorded in ODNB17 followed by TNNB5 (4.87), MANB19 (5.40) and BINB12 (5.43) (Table 2). Among the 24 isolates, seven isolates which showed higher leaf blast severity (more than 8.0 grade) were used for further studies. Similar with the earlier report of Maharaja (2012) who isolated the *M. grisea* isolates from leaf, neck and finger of ragi plants collected from different places. A similar method was also adopted by Srinivasachary *et al.*, (2002) to prove the pathogenicity of *P. grisea* on rice. The

isolation technique used in the present study was in concordance with the earlier work of Nagendran (2011) who also used the same technique for the identification of *P. grisea*. The Koch's postulates were also proved in this study as per the procedure followed by Ou (1985).

Cultural and Morphology characters of *M. grisea* isolates

Colony characteristics on different culture media

Significant variation among the colony characteristics of the different isolates were observed in culture plates which were collected from different localities. All the seven isolates grown on five different media were observed for the colony colour, margin, pigmentation, surface texture, mycelial growth and sporulation of *M. grisea* isolates and the results are presented in Table 3. On the OMA medium, the isolates *viz.*, TNLB1, TNNB7, TNNB8, JALB9 and BIFB13 showed white colour whereas the isolates *viz.*, JANB18 and MANB23 recorded light grey and white colour respectively. Among the media tested, the PDA media resulted irregular margin growth in the isolates *viz.*, TNNB7, TNNB8, BIFB13 and MANB23 with brown and dark brown pigmentation. In addition, the host seed extract +2.0 per cent sucrose agar medium showed the same irregular growth in TNNB8 isolate. The Richards agar medium showed grey colour with entire growth and black pigmentation for all the isolates of *M. grisea*. The isolate TNLB1 was velvety in surface texture whereas the other six isolates *viz.*, TNNB7, TNNB8, JALB9, BIFB13, JANB18 and MANB23 were cottony surface texture. The colony of the isolates *viz.*, TNLB1, TNNB8, JALB9, BIFB13 and MANB23 showed good growth whereas other two isolates *viz.*, TNNB7 and JANB18 recorded medium growth. In PDA medium, the colony

of the isolates *viz.*, TNLB1, TNNB7, TNNB8, BIFB13, JANB18 and MANB23 was grey colour whereas the isolate JALB9 was white colour. Five isolates *viz.*, TNNB7, TNNB8, JALB9, JANB18 and MANB23 showed black colour pigmentation whereas other two isolates *viz.*, TNLB1 and BIFB13 were brown in colour. The isolates *viz.*, TNNB7, TNNB8, JANB18 and MANB23 were cottony in surface texture whereas the other isolates *viz.*, TNLB1, JALB9 and BIFB13 were velvety in surface texture. Five isolates *viz.*, TNLB1, TNNB7, TNNB8, JANB18 and MANB23 showed medium growth whereas the other two isolates JALB9 and BIFB13 recorded poor growth. Host seed extract + 2.0 per cent sucrose agar medium showed olive grey colour growth for the isolates *viz.*, TNNB8, BIFB13, JANB18 and MANB23. Two isolates *viz.*, TNNB7 and JALB9 showed light grey colour and TNLB1 showed white colony growth. There was entire growth of margin were observed with black pigmentation for all the isolates. The isolates *viz.*, TNNB7, TNNB8, JANB18 and MANB23 were cottony in surface texture whereas, TNLB1, JALB9 and BIFB13 showed velvety in surface texture. All the isolates of *M. grisea* recorded good growth except three isolates *viz.*, TNLB1, JALB9 and BIFB13 showed medium growth. In v8 juice agar medium, the colony of the isolates *viz.*, TNNB8, JALB9, BIFB13 and JANB18 showed light grey in colour whereas, the isolates *viz.*, TNLB1, TNNB7 and MANB23 were grey in colour. The colony of all the isolates was entire in margin, black in pigmentation. The colony of isolates *viz.*, TNLB1, TNNB8, BIFB13, JANB18 and MANB23 was cottony in surface texture whereas the isolates *viz.*, TNNB7 and JALB9 were velvety in surface texture. Five isolates *viz.*, TNLB1, TNNB7, JALB9, BIFB13 and MANB23 showed good growth and other two isolates namely TNNB8 and MANB18 recorded medium growth. This result was in agreement with the earlier work of Meena

(2005) who studied the mycelial growth and colour of the colony on oat meal agar, host seed extract +2% sucrose agar, potato dextrose agar and richard's agar medium. They reported the colony colour of *P. grisea* isolates of rice was buff with good growth, greyish black with medium growth, raised mycelial growth with smooth colony margin and raised mycelium with concentric ring pattern. Mijan Hossain (2000) reported the same kind of results on the growth and colour of the colony of *P. grisea* isolates from finger millet and rice. Similar variations on cultural characters on *P. grisea* isolates were reported by Ou (1985). The colour difference observed in this study between the segments might be due to the stages of the spores on the different patterns of growth. Getachew *et al.*, 2014 reported that the isolates *M. grisea* showed considerable variation in mycelial growth, pigmentation and conidia production.

Effect of different solid media on the mycelial growth of *M. grisea* isolates

The results revealed that there is a considerable variation among the colony diameter of the *M. grisea* isolates on different solid media (Table 4). The mean of radial growth of various isolates on different solid media revealed that OMA recorded the maximum mean mycelial growth of 80.12 mm followed by Richards's agar with 76.11 mm, Host seed extract + 2.0 per cent sucrose agar with 75.87 mm, V8 juice agar with 75.10 mm and PDA with 72.52 mm. In OMA, among the seven isolates tested the maximum mycelial growth was observed in TNNB8 with 86.00 mm followed by the isolates *viz.*, TNLB1 and JALB9 with 85.00 mm and 83.00 mm respectively. In case of Richard's agar medium, the isolate TNNB8 recorded the maximum mycelial growth of 81.80 mm followed by TNLB1 and MANB23 with 80.20 mm and 79.80 mm respectively. In Host seed extract + 2.0 per cent sucrose agar medium

maximum mycelial growth was observed in TNNB8 with 84.20 mm followed by JALB9, TNLB1 and JANB18 with 81.80, 81.20 and 75.00 mm respectively. In V8 juice agar medium, maximum mycelial growth was observed in TNLB1 with 84.30 mm followed by JALB9, JANB18 and TNNB8 with 84.20, 78.00 and 77.60 mm respectively. In PDA medium maximum growth was observed in TNLB1 with 82.60 mm followed by TNNB8 and JALB9 with 81.70 mm and 81.00 mm mycelial growth, respectively. Similarly, Susan and Ambika (2011) reported that the vegetative mycelial growth of *P. grisea* isolates was high in V8 agar (V8A), potato dextrose agar (PDA) and oat meal agar (OMA). Similar results were also reported by Afshana *et al.*, (2011) and indicated that the OMA was suitable for growth of *P. oryzae*. Subsequently, Khadka *et al.*, (2012) reported that the peptone agar and OMA media were found to be the effective media for mycelial growth and sporulation of *M. grisea* isolates of rice and finger millet. This is also in accordance with Getachew *et al.*, (2014) observed that the OMA medium favoured the growth of *P. grisea* much more than PDA medium. Recent findings have also confirmed that the oat meal agar was the best medium for the growth and sporulation of *P. oryzae* (Asfaha *et al.*, 2015).

Spore morphology of *M. grisea* isolates

The morphological characteristics of seven isolates of *M. grisea* on OMA were observed. The results revealed that all the conidia in each isolate were pyriform in shape, hyaline to pale olive colour. The number of spores varied from $1 \times 10^{+5}$ to $6.0 \times 10^{+5}$ conidia ml^{-1} . The length and width of the spore also varied in different isolates of *M. grisea*. Maximum mean spore size of $21.91 \times 7.42 \mu\text{m}$ was observed in the isolate TNNB8 followed by TNLB1 and JANB18 with $19.45 \times 7.60 \mu\text{m}$ and $18.32 \times 6.49 \mu\text{m}$ respectively (Table 5).

Table.1 Collection of isolates of *M. grisea* from different regions of India

S. No.	Isolates	Location	States	Plant parts	Variety
1	TNLB1	Krishnagiri	Tamil Nadu	Leaf	CO14
2	TNLB2	Krishnagiri	Tamil Nadu	Leaf	CO14
3	TNLB3	Dharmapuri	Tamil Nadu	Leaf	CO14
4	TNLB4	Coimbatore	Tamil Nadu	Leaf	CO14
5	TNNB5	Krishnagiri	Tamil Nadu	Neck	GPU28
6	TNNB6	Krishnagiri	Tamil Nadu	Neck	GPU28
7	TNNB7	Dharmapuri	Tamil Nadu	Neck	GPU28
8	TNNB8	Coimbatore	Tamil Nadu	Neck	KM252
9	JALB9	Ranchi	Jharkhand	Leaf	RAU8
10	JANB10	Ranchi	Jharkhand	Neck	RAU8
11	BILB11	Dholi	Bihar	Leaf	PR202
12	BINB12	Dholi	Bihar	Neck	PR202
13	BIFB13	Dholi	Bihar	Finger	PR202
14	ANNB14	Vizianagaram	Andhra Pradesh	Neck	VR 708
15	MANB15	Dindori	Madhya Pradesh	Neck	GPU28
16	ODNB16	Berhampur	Odisha	Neck	KM252
17	ODNB17	Berhampur	Odisha	Neck	KM252
18	JANB18	Sisai	Jharkhand	Neck	Local
19	JANB19	Sisai	Jharkhand	Neck	Local
20	JAFB20	Sisai	Jharkhand	Finger	Local
21	MALB21	Rewa	Madhya Pradesh	Leaf	Local
22	MALB22	Rewa	Madhya Pradesh	Leaf	Local
23	MANB23	Rewa	Madhya Pradesh	Neck	Local
24	KALB24	Bangalore	Karnataka	Leaf	GPU64

Table.2 Pathogenicity potential of *M. grisea* isolates of finger millet

S. NO.	Isolates	Leaf blast (1-9 scale)*
1	TNLB1	8.76
2	TNLB2	6.55
3	TNLB3	6.24
4	TNLB4	7.17
5	TNNB5	4.87
6	TNNB6	6.13
7	TNNB7	8.02
8	TNNB8	8.68
9	JALB9	8.12
10	JANB10	6.79
11	BILB11	7.50
12	BINB12	5.43
13	BIFB13	8.41
14	ANNB14	6.12
15	MANB15	7.83
16	ODNB16	7.82
17	ODNB17	4.77
18	JANB18	8.16
19	JANB19	5.40
20	JAFB20	7.65
21	MALB21	6.00
22	MALB22	7.89
23	MANB23	8.12
24	KALB24	6.95

*Mean of three replication

Table.3 Colony characters of *M. grisea* isolates on different solid media

Isolates	Media	Colony characteristics					
		Colony colour	Margin	Pigmentation	Surface texture	Mycelial growth	Sporulation
TNLB1	OMA	Off white	Entire	Black	Cottony	Good growth	Good
	Richard's agar	Grey	Entire	Black	Velvety	Good growth	Fair
	PDA	Grey	Entire	Brown	Velvety	Medium growth	Poor
	Host seed extract +2% sucrose agar	Grey	Entire	Black	Velvety	Medium growth	Fair
	V8 juice agar	Light grey	Entire	Black	Cottony	Medium growth	Fair
TNNB7	OMA	Off white	Entire	Black	Cottony	Good growth	Good
	Richard's agar	Grey	Entire	Black	Cottony	Medium growth	Good
	PDA	Grey	Irregular	Dark brown	Cottony	Medium growth	Fair
	Host seed extract +2% sucrose agar	Light grey	Entire	Black	Cottony	Good growth	Good
	V8 juice agar	Light grey	Entire	Black	Velvety	Medium growth	Poor
TNNB8	OMA	Off white	Entire	Black	Cottony	Good growth	Good
	Richard's agar	Grey	Entire	Brown	Cottony	Good growth	Fair
	PDA	Grey	Irregular	Dark brown	Cottony	Medium growth	Poor
	Host seed extract +2% sucrose agar	Olive grey	Irregular	Black	Cottony	Good growth	Good
	V8 juice agar	Grey	Entire	Black	Cottony	Good growth	Fair
JALB9	OMA	Off white	Entire	Black	Cottony	Good growth	Good
	Richard's agar	Grey	Entire	Black	Cottony	Good growth	Fair
	PDA	White	Entire	Dark brown	Velvety	Poor growth	Poor
	Host seed extract +2% sucrose agar	Light grey	Entire	Black	Velvety	Medium growth	Poor
	V8 juice agar	Grey	Entire	Black	Velvety	Medium growth	Fair
BIFB13	OMA	Off white	Entire	Black	Cottony	Good growth	Good
	Richard's agar	Grey	Entire	Black	Cottony	Good growth	Fair
	PDA	Grey	Irregular	Brown	Velvety	Poor growth	Poor

	Host seed extract +2% sucrose agar	Olive grey	Entire	Black	Velvety	Medium growth	poor
JANB18	V8 juice agar	Grey	Entire	Black	Cottony	Medium growth	Poor
	OMA	Light grey	entire	Black	Cottony	Good growth	Good
	Richard's agar	Grey	Entire	Black	Cottony	Medium growth	Fair
	PDA	Grey	Entre	Dark brown	Cottony	Medium growth	Poor
	Host seed extract +2% sucrose agar	Olive grey	Entire	Black	Cottony	Good growth	Fair
	V8 juice agar	Grey	Entire	Black	Cottony	Good growth	Poor
MANB23	OMA	white	Entire	Black	Cottony	Good growth	Good
	Richard's agar	Grey	Entire	Black	Cottony	Good growth	Fair
	PDA	Grey	Irregular	Dark brown	Cottony	Medium growth	Poor
	Host seed extract +2% sucrose agar	Olive grey	Entire	Black	Cottony	Good growth	Fair
	V8 juice agar	Light grey	Entire	Black	Cottony	Medium growth	Poor

Table.4 Effect of different solid media on the mycelial growth of *M. grisea* isolates

Isolates	Colony diameter (mm)				
	Oat Meal Agar	Richards's Agar	Potato Dextrose Agar	Host seed extract +2% sucrose Agar	V8 juice Agar
TNLB1	85.00 ^a	80.20 ^a	82.60 ^a	81.20 ^{ab}	84.30 ^a
TNNB7	76.10 ^{bc}	70.80 ^{bc}	70.80 ^b	73.40 ^c	68.00 ^b
TNNB8	86.00 ^a	81.80 ^a	81.70 ^a	84.20 ^a	77.60 ^a
JALB9	83.00 ^{ab}	78.00 ^{ab}	81.00 ^a	81.80 ^{ab}	84.20 ^a
BIFB13	79.20 ^{abc}	75.00 ^{ab}	65.40 ^{bc}	67.40 ^c	66.40 ^b
JANB18	79.00 ^{abc}	67.20 ^c	62.20 ^c	75.00 ^{bc}	78.00 ^a
MANB23	72.60 ^c	79.80 ^a	64.00 ^{bc}	68.10 ^c	67.20 ^b
Mean	80.12	76.11	72.52	75.87	75.10

In a column, means followed by the same letter (s) are not statistically different (p=0.05) by DMRT

Table.5 Conidial characteristics of *M. grisea* isolates

Isolates	Conidial characteristics						
	Conidial size (µm)				Conidial shape	Conidial colour	Sporulation (x10 ⁻⁵ conidia ml ⁻¹)
	Length		Width				
	Range	Mean	Range	Mean			
TNLB1	18.77-21.75	19.45	7.45-7.82	7.60	Pyriform	hyaline to pale olive	6
TNNB7	17.07-20.18	17.82	6.86-5.80	6.25	Pyriform	hyaline to pale olive	2
TNNB8	21.52-23.66	21.91	7.82-7.03	7.42	Pyriform	hyaline to pale olive	4
JALB9	16.00-18.23	16.54	5.68-4.97	5.29	Pyriform	hyaline to pale olive	3
BIFB13	16.21-21.02	17.88	6.13-5.78	5.92	Pyriform	hyaline to pale olive	4
JANB18	17.32-20.19	18.32	6.73-6.32	6.49	Pyriform	hyaline to pale olive	2
MANB23	15.98-18.78	16.65	5.37-5.00	5.16	Pyriform	hyaline to pale olive	1

Table.6 Influence of temperature on the radial growth of *M. grisea* isolates

Isolates	Radial growth (mm) at different temperature*				
	15 °C	20 °C	25 °C	30 °C	35 °C
TNLB1	14.90 ^a	51.60 ^b	78.90 ^a	64.10 ^{abc}	9.20 ^a
TNNB7	13.50 ^b	43.80 ^c	72.40 ^{ab}	68.40 ^{ab}	3.80 ^{cd}
TNNB8	15.70 ^a	56.10 ^a	73.70 ^{ab}	61.30 ^c	4.10 ^c
JALB9	15.20 ^a	59.30 ^a	77.80 ^a	70.10 ^a	6.00 ^b
BIFB13	13.20 ^b	40.20 ^c	75.40 ^{ab}	63.20 ^{bc}	3.40 ^{de}
JANB18	12.80 ^b	50.40 ^b	70.30 ^b	65.80 ^{abc}	3.00 ^e
MANB23	13.10 ^b	43.60 ^c	74.60 ^{ab}	60.30 ^c	2.30 ^f
Mean	14.05	49.28	74.73	64.74	4.54

*Mean of three replications

In a column, means followed by the same letter (s) are not statistically different (p=0.05) by DMRT

Table.7 Influence of temperature on the sporulation of *M. grisea* isolates

Isolates	Sporulation ($\times 10^{-5}$ conidia ml ⁻¹) at different temperature*				
	15 °C	20 °C	25 °C	30 °C	35 °C
TNLB1	0.0	1.8 ^a	3.6 ^a	2.3 ^a	0.0
TNNB7	0.0	0.7 ^d	1.8 ^c	1.3 ^d	0.0
TNNB8	0.0	1.6 ^b	2.2 ^b	1.2 ^d	0.0
JALB9	0.0	1.8 ^a	2.4 ^b	2.1 ^b	0.0
BIFB13	0.0	1.5 ^b	1.9 ^c	2.0 ^b	0.0
JANB18	0.0	1.4 ^{bc}	2.1 ^{bc}	1.7 ^c	0.0
MANB23	0.0	1.2 ^c	1.6 ^d	1.6 ^c	0.0
Mean	0.0	1.4	2.2	1.7	0.0

*Mean of three replications

In a column, means followed by the same letter (s) are not statistically different (p=0.05) by DMRT

Table.8 Influence of pH level on the dry mycelial weight of *M. grisea* isolates

Isolates	Dry mycelial weight (mg) at different pH Level*									
	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5
TNLB1	10.26 ^a	22.12 ^c	53.38 ^b	70.70 ^b	99.20 ^a	102.33 ^b	164.56 ^a	230.45 ^a	146.40 ^a	101.67 ^{ab}
TNNB7	4.20 ^{de}	18.63 ^d	22.21 ^d	72.02 ^b	92.81 ^{ab}	106.43 ^{ab}	147.88 ^b	226.12 ^b	139.00 ^b	98.67 ^{ab}
TNNB8	3.73 ^e	22.21 ^c	22.98 ^d	71.95 ^b	94.93 ^a	106.88 ^{ab}	150.53 ^{ab}	222.88 ^{bc}	129.63 ^d	94.19 ^{bc}
JALB9	10.31 ^a	39.72 ^b	50.80 ^{bc}	72.21 ^b	84.99 ^{bc}	102.97 ^b	167.43 ^a	219.67 ^c	146.40 ^a	105.67 ^a
BIFB13	9.43 ^b	42.23 ^a	68.10 ^a	92.81 ^a	83.77 ^c	105.43 ^{ab}	149.00 ^b	205.46 ^c	135.30 ^c	51.56 ^e
JANB18	8.35 ^c	10.44 ^e	48.17 ^c	72.32 ^b	97.45 ^a	111.32 ^a	155.16 ^{ab}	217.20 ^d	120.17 ^e	73.00 ^d
MANB23	4.56 ^d	10.33 ^e	26.24 ^d	51.47 ^c	77.79 ^c	109.10 ^{ab}	160.34 ^{ab}	211.08 ^f	118.65 ^f	87.50 ^c
Mean	7.26	23.66	41.69	71.92	90.13	105.61	156.98	216..54	141.77	87.46

*Mean of three replications

In a column, means followed by the same letter (s) are not statistically different (p=0.05) by DMRT

Table.9 Effect of different carbon sources on the mycelial growth of *M. grisea* isolates

S. No.	Isolates	Colony diameter (mm)*					
		Dextrose	Maltose	D- fructose	Sucrose	Glucose	Galactose
1	TNLB1	88.60 ^a	82.60 ^{ab}	84.00 ^a	81.70 ^a	84.50 ^a	79.30 ^a
2	TNNB7	81.60 ^{ab}	86.30 ^a	74.50 ^{bc}	70.50 ^c	79.40 ^{ab}	84.80 ^a
3	TNNB8	84.20 ^{ab}	78.20 ^b	68.90 ^c	74.50 ^{bc}	80.20 ^{ab}	71.80 ^b
4	JALB9	83.90 ^{ab}	82.10 ^{ab}	80.10 ^{ab}	78.20 ^{ab}	82.00 ^{ab}	78.90 ^a
5	BIFB13	80.10 ^b	79.50 ^{ab}	78.70 ^{ab}	70.10 ^c	74.70 ^b	69.00 ^b
6	JANB18	85.20 ^{ab}	82.10 ^{ab}	80.10 ^{ab}	70.90 ^c	84.20 ^a	81.70 ^a
7	MANB23	82.60 ^{ab}	80.20 ^{ab}	78.40 ^{ab}	80.60 ^{ab}	80.90 ^{ab}	80.00 ^a
Mean		83.74	81.57	77.81	75.21	80.84	77.93

*Mean of three replications

In a column, means followed by the same letter (s) are not statistically different (p=0.05) by DMRT

Table.10 Effect of different nitrogen sources on the mycelial growth of *M. grisea* isolates

S. No.	Isolates	Colony diameter (mm)*					
		L-glycine	KNO ₃	L - arginine	NaNO ₃	Asparagine	Peptone
1	TNLB1	86.70 ^a	85.20 ^{ab}	87.90 ^a	88.40 ^a	76.50 ^{ab}	81.40 ^a
2	TNNB7	74.10 ^c	80.10 ^{abc}	76.30 ^b	81.30 ^a	82.10 ^a	76.10 ^a
3	TNNB8	80.90 ^{abc}	78.20 ^{bc}	77.60 ^b	85.60 ^a	82.80 ^a	79.30 ^a
4	JALB9	84.30 ^{ab}	87.10 ^a	88.20 ^a	87.90 ^a	70.40 ^b	79.40 ^a
5	BIFB13	78.40 ^{bc}	73.20 ^c	75.20 ^b	87.00 ^a	76.30 ^{ab}	83.10 ^a
6	JANB18	80.50 ^{abc}	78.30 ^{bc}	81.10 ^{ab}	86.50 ^a	77.80 ^a	76.30 ^a
7	MANB23	81.10 ^{abc}	74.10 ^c	82.30 ^{ab}	84.40 ^a	80.30 ^a	79.73 ^a
Mean		80.86	79.45	81.23	85.87	78.03	79.33

*Mean of three replications

In a column, means followed by the same letter (s) are not statistically different (p=0.05) by DMRT

Mijan Hossain (2000) who observed that the mycelium in cultures was first hyaline in colour then changed to olivaceous, 1 – 5.2 µm in width, septate and branched. The aerial mycelium was white to grey, as it was observed by Getachew *et al.*, (2013). Getachew *et al.*, (2014) reported that the shape of the conidia was typically pyriform with rounded base, apex narrowed, 2-3 septate, 2-4 celled and middle cells were swollen. Recently, Asfaha *et al.*, (2015) showed that *P. grisea* conidia was pyriform in shape, base rounded, apex narrowed, two septate with three celled, hyaline to pale olive colors.

Physiological studies

Influence of temperature on the mycelial growth and sporulation of *M. grisea*

Temperature is one of the most important physical environmental factors for regulating the growth and reproduction of *M. grisea* isolates. All the isolates showed different responses to the different incubation temperatures (Table 6). There was maximum mean radial mycelial growth occurred at 25 °C and 30 °C with 74.73 mm and 64.74 mm respectively. The lesser mean radial mycelial growth at 15, 20 and 35 °C with 14.05, 49.28

and 4.54 mm. However the temperature 25 °C was found optimum for the growth of all the isolates. All the isolates produced the maximum mean sporulation at 20, 25 and 30 °C with 1.4, 2.2 and 1.7 x10⁵ conidia ml⁻¹ respectively, but did not sporulate at 15 and 35 °C (Table 7). Among the isolates of *M. grisea* at temperature 25 °C, maximum sporulation was recorded in TNLB1, JALB9, TNNB8 and JANB18 with 3.6, 2.4, 2.2 and 2.1x10⁵ conidia ml⁻¹ respectively. This is in agreement with the earlier work of Arunkumar and Singh (1995) who studied the differential response of *P. grisea* isolates from rice, finger millet and pear millet to temperature and reported that the maximum growth at 30°C. The results of the present study was in corroboration with Netam *et al.*, (2013); Getachew *et al.*, 2013; 2014 have recorded the highest mycelium growth and sporulation of *P. grisea* isolate of finger millet at 25 – 30°C. The most suitable temperature for mycelial growth was observed as 25°C which was reported by Fu *et al.*, (2013); Asfaha *et al.*, (2015) and the similar result was also obtained in the present result.

Influence of pH level on the dry mycelial weight of *M. grisea*

The results on the effect of different pH level on the growth of *M. grisea* isolates in Richard's medium revealed that, the optimum pH for the growth was found to be pH 6.5 with maximum mean dry mycelial weight of 216.54 mg followed by pH 6.0 (156.98 mg) and 7.0 (141.77 mg). The pH of 7.5, 5.5, 5.0, 4.54.0, 3.5 and 3.0 recorded less mean mycelial dry weight of 87.46, 105.61, 90.13, 71.92, 41.69, 23.66 and 7.26 mg, respectively (Table 8). This is in concordance with the finding of Arunkumar and Singh (1995) who opined the best growth at pH 6.5 for the *P. grisea* isolates. Similar result was also reported by Mijan Hossain (2000) who

highlighted that the growth of *P. grisea* isolates increased with increase in pH from 3.5 to 6.5. The result was in accordance with Meena (2005) who reported that the pH 6.5 was significantly superior for the growth *P. grisea* over other treatments. The findings of the present study was also in accordance with Getachew *et al.*, (2014) and Asfaha *et al.*, (2015) stated that the maximum mycelial growth of *P. grisea* was recorded at pH 6.5 followed by pH 6.0 and pH 7.0.

Biochemical studies

Effect of different carbon sources on the mycelial growth of *M. grisea*

The results revealed that the different carbon sources supplemented in the growing medium showed significant difference in utilization of carbon sources by different isolates of *M. grisea* (Table 9). Among the carbon sources, dextrose was found to be the best carbon source by recording the highest mean mycelial growth of 83.74 mm followed by maltose, glucose, galactose, D – fructose and sucrose with mean mycelial growth of 81.57, 80.84, 77.93, 77.81 and 75.21 mm, respectively. In dextrose, maximum mycelial growth was noticed in the isolate TNLB1 with 88.60 mm followed by JANB18 with 85.20 mm. In maltose, maximum mycelial growth was observed in the isolate TNNB7 with 86.30 mm. In glucose, maximum mycelial growth was observed in isolate TNLB1 with 84.50 mm.

In galactose, maximum mycelial growth was observed in isolate TNNB7 with 84.80 mm. In D – fructose, TNLB1 recorded the maximum mycelial growth of 84.00 mm. In sucrose as carbon source, the isolate TNLB1 recorded the maximum mycelial growth of 81.70 mm. This is in corroboration with a result of Gatachew *at el.* (2014) who reported that dextrose as a good carbon sources for

higher mean colony diameter. Hossain (2004) noted considerable variation in the ability to use various carbon sources thereby indicating biochemical differences among the *P. grisea* isolates. Tripathi (2006) has also reported that maltose better used as the carbon source for the mycelial growth and sporulation.

Whereas the fungal pathogen *P. grisea* on pearl millet showed maximum mycelial growth in starch supplemented media followed by sucrose (Mijan Hossain, 2000). Netam *et al.*, (2013) have indicated that among the different carbon sources, glucose supported significantly higher mycelial growth of *M. grisea* followed by sucrose, galactose.

Effect of different nitrogen sources on the mycelial growth of *M. grisea* isolates

Results showed that, variable utilization nitrogen sources *viz.*, L- glycine, KNO₃, L- arginine, NaNO₃, asparagine and peptone by each *M. grisea* isolate. Maximum growth was observed in NaNO₃ with maximum mean mycelial growth of 85.87 mm followed by L - arginine, L – glycine, KNO₃, peptone and asparagine with radial mycelial growth of 81.23, 80.86, 79.45, 79.33 and 78.03 mm respectively.

In NaNO₃, maximum growth was noticed in the isolates *viz.*, TNLB1, JALB9 and BIFB13 with 88.40, 87.90 and 87.00 mm respectively, while in L - arginine the isolate JALB9 recorded the maximum mycelial growth of 88.20 mm. In L – glycine, the isolate TNLB1 recorded the maximum mycelial growth of 86.70 mm.

In KNO₃, the isolate JALB9 recorded the maximum mycelial growth of 87.10 mm. In peptone, the isolate BIFB13 recorded the maximum mycelial growth of 83.10 mm. In asparagine supplemented medium, the isolates

viz., TNNB8 and TNNB7 recorded the maximum mycelial growth of 82.80 and 82.10 mm respectively (Table 10). Earlier work of Mijan Hossain (2000) enunciated that the asparagine was the best nitrogen source for the growth of *M. grisea* which was significantly superior from other nitrogen sources studied in that study. Gatachew *at el.* (2014) reported that NaNO₃ were the most suitable nitrogen sources for mycelial growth of *P. grisea*. Netam *et al.*, (2013) have supported that nitrogen source such as ammonium nitrates favoured the mycelial growth and sporulation of *P. grisea*. Anil Kumar (2015) also reported that among nitrogen sources, *Colletotrichum capsici* preferred potassium nitrate followed by sodium nitrate and asparagines.

The study clearly indicated that the response by *M. grisea* isolates to exogenous supply of nitrogenous compounds was variable among the isolates.

From this study it is clear that the Oat Meal Agar medium (OMA) was found best for the growth of *M. grisea* isolates tested. The temperature 25⁰C and pH 6.5 was found to be ideal for growth, sporulation and mycelial dry weight of *M. grisea* isolates.

Richard's medium with supplemented carbon and nitrogen sources *viz.*, dextrose and NaNo3 supported the maximum mean mycelial growth of *M. grisea* isolates of finger millet.

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