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Morphological and Cultural studies of *Pyricularia grisea* on Small Millets and their management through bio-control agents

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Abstract
18 monoconidial isolates of *P. grisea* were obtained from four different Small millets sowing characteristic symptom on foliage. Among them, only four isolates were selected and designated as Pg1, Pg2, Pg3 and Pg4 based on their growth characteristics and respective host for intensive characterization. Variability among the isolates of *P. grisea* was also determined on the basis of their sensitivity on six different plant extract/ botanicals and an herbal disease controller under laboratory conditions. In the present study, the commercial herbal plant extract Zuki at 5 and 10 per cent concentrations recorded maximum growth inhibition of all isolates and was found to be the most effective treatment. Among plant extract/botanicals bulb extract of *Allium cepa* (onion) recorded maximum growth inhibition of Pg1 and Pg3 isolates at 5% & 10% concentration whereas leaf extract of *Oxalis latifolia* (Teenpatia) obtained maximum growth inhibition of Pg2 and Pg4 respectively. In present study, there was significant difference among the bio-control agents evaluated against different isolates of *P. grisea*. The bio-control agent *T. viride* inhibit maximum mycelial growth (81.81%) of isolate of finger millet (Pg1) and (79.08%) of isolate of foxtail millet (Pg4) whereas the bio-control agent *P. fluorescens* inhibited the maximum mycelial growth (80.16%) of isolate of little millet(Pg2) and (95.61%) growth of isolate of proso millet (Pg3). Eco-friendly management through botanical and bio-control agents resulted that seed treatment with *T. viride* @ 4g/kg + 1st foliar spray with Zuki @ 0.1% + two foliar spray with *P. fluorescens* @ 0.6% recorded maximum diseases control 55.55 per cent and 61.42 per cent in neck blast and finger blast respectively, with highest grain yield 1530.87 kg/ha.

Keywords: Small Millets, *Pyricularia grisea*, Eco-friendly management

Introduction
Small millets, is a group of six crops comprising of finger millet (*Eleusine coracana*), kodo millet (*Paspalum scrobiculatum),* foxtail millet *(Setaria italica),* little millet *(Panicum sumatrense),* barnyard millet *(Echinochloa frumentacea*) and proso millet *(Panicum miliaceum)* which are mostly cultivated as rain-fed crops on marginal soil under poor to neglected management practices. Though these crops were less prone to diseases and pests but under vulnerable condition, blast incited by the fungus *Pyricularia grisea* Sacc. (Perfect stage = *Magnaporthe grisea* [Hebert]) cause widespread losses in each season. The pathogen attacks all stages of crop development (vegetative and productive stages) although the leaves, panicles (necks) and fingers are the most commonly affected. In these millets, Leaf infection reduces the photosynthetic area of the plant whereas panicle and finger infection reduces the yield. The blast fungus *Pyricularia grisea* (telemorph, *Magnaporthe grisea*) has a wide host range and is known to infect almost 40 species of *Gramineae* (Asuyama, 1965). Study of the host range and its eco-friendly has become an important aspect of the disease management. In the present investigation, four different isolates of *P. grisea* were collected from finger millet, little millet, proso millet and foxtail millet and morphological variation among the isolates were examined and characterized according to their conidial structure (size, shape, colour and septation of

Conidia) and cultural variation according to their radial growth, colony colour and colony texture. Management of *P. grisea* isolates through bio-control agents under laboratory conditions in the department of Plant Pathology, Birsa Agricultural University during 2016 -2017.

Material and Methods

To study the morphological and cultural variation, four different host of small millets *Eleusine coracana* (finger millet), *Panicum sumatrense* (little millet), *Panicum miliaceum* (proso millet) *and Setaria italica* (foxtail millet) were studied.

For morphological studies, conidiophores and conidia of *P. grisea* of different isolates were measured from the infected host tissue mounted in lactophenol on a clean slide. Spores were mixed with lactophenol thoroughly so that, a uniform spread is obtained and then a cover slip was placed over it. Conidia were measured under high power objective lens (45X) using ocular and stage micrometer. The average size and shape of conidia was determined and number of conidium on conidiophores was recorded. Microphotographs were taken to show the typical spore morphology of the pathogen.

Calibration

In order to calibrate the microscope, the ocular micro meter was first placed inside the eye piece of 10x magnification and then objective lens of 40x magnification was focused. The stage micro meter was brought into clear focus under objective and ocular division of ocular. Mycelium, conidia and conidiophores used in measurement were obtained from ten days old culture grown on PDA medium. A small piece of inoculums was taken on the slide and the fungal structures were measured with the help of stage micro meter in micro. (100 division of stage micro meter = 1 mm, 1 division of stage micro meter = 0.01 = 10µm, supposing 65 divisions of ocular coincided with 100 divisions of stage micro meter, 1 division of ocular = 1.538 divisions of stage micro meter).

For cultural studies, the growth characters of all the isolates were studied on four solid media *viz*., Potato dextrose agar medium, Corn meal agar medium, Czapek's Dox agar medium and Richard's agar medium. Fifteen ml of each of the medium was poured into each of sterilized Petri plates. Inoculation was made by transferring the five mm disc of mycelial mat, taken from the periphery of 10 days old culture of each of the twelve isolates. Each treatment was replicated thrice. The plates were incubated at 28 ±1˚C. Observation on colony radial growth was taken when the maximum growth was attained in any one of the media tested. Other cultural characters *viz*., texture, type of margin and colony colour were also recorded.

The bio-efficacy of three bio-control agents *viz., Trichoderma viride*, *Trichoderma harzianum*, and *Pseudomonas fluorescens* were tested against *P. grisea* isolates through Dual culture technique. Twenty ml of sterilized and cooled PDA media was poured into sterile petri plates. Three cm disc of test fungus and bio-control agents were placed at opposite sides to each other in Petri dishes that containing sterilized PDA medium. Fungal antagonists were evaluated by inoculating the pathogen at one side of Petri plate and the antagonist on the opposite side of the same plate by leaving 3-4 cm gap. But for bacterial antagonists, it was streaked in the centre of the plate after which a fungal disc was placed near it. Each treatment was replicated three times. After required period of incubation i.e. after the growth in the control plate reached 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was worked out. The growth inhibition in per cent was calculated by following the formula given by Vincent (1927).

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

Where,

I = Percentage of growth inhibition
C = Average growth (mm) in control
T = Average growth (mm) in the treatment.

Results and Discussion

Morphological variation

Morphological variation among the isolates were examined and characterized according to their conidial structure (size, shape, colour and septation of conidia).The mycelia of the isolates were highly branched, septate, superficial and bearing conidia at the tip or side of the conidiophores. In all isolates, the conidia were two septate, three celled and pyriform in shape except Pg3 formed sub pyriform conidia. The base of the conidium was broad with variable apex. The apex of Pg2 and Pg4 was narrow whereas Pg1 have pointed apex and Pg3 formed slightly constricted apex. The conidiophores of all the isolates were simple, hyaline bearing 2 conidia (Pg1), 2-3 conidia (Pg4), 6 conidia (Pg2) and (Pg3) at their tip (Table 1). In past investigation, the causal agent of rice blast *P. grisea* has been described by Shirai (1896) [6], Sawada (1917) and Nishikado (1926) [7]. They reported that the mycelium in culture media was aerial, hyaline or olivaceous, 1.5-6.0 µm in width, septate and branched. The conidiophores are one to many, fasciculate, simple or rarely branched, 2 to 4 septate and slightly constricted at septa, olivaceous to fuliginous, base swollen, dark coloured and becoming lighter in colour towards the apex. Conidia are variable in size and shape, terminal, pyriform to obclavate with broad base, apex narrowed and generally 2 septate, rarely 1 to 3 septate with or without constriction at septa. The conidia are almost hyaline to pale olive, 14-40 × 6-13 µm in size with small basal appendage.

Table 1: Conidial characteristics of the test pathogen isolates

	Characteristics features	
	Conidiophore	**Conidia**
Pg1	Simple, hyaline with two conidium born at the tip of conidiophores	2 septation, 3 celled, pyriform with broad base and pointed apex
Pg2	Simple, hyaline with six conidium born at the tip of conidiophores	2 septation, 3 celled, pyriform with broad base and narrow apex
Pg3	Simple, hyaline with six conidium born at the tip of conidiophores	2 septation, 3 celled, sub-pyriform with broad base and slightly constrict apex
Pg4	Simple, hyaline with two or three conidium born at the tip of conidiophores	2 septation, 3 celled, pyriform with broad base and narrow apex

Length and width of the spores of different isolates of *P. grisea* (µm)

Variations in the size of conidia of *P. grisea* were noticed among the isolates from different host. Size of the conidia (LxB) varied from 32.98 × 9.49 to 20.75× 7.23µm with the largest conidia (32.98 × 9.49 µm) observed in the isolate Pg 3 followed by Pg 4 (27.38 × 7.45 µm), and Pg 2 (25.27 × 7.29 µm). Smallest conidia of 20.75× 7.23 µm were observed in the isolate Pg1 (Table 2).

Table 2: Length and width of the spores of different isolates of *P. grisea* (µm)

Isolates	Length (µm)	Breadth (µm)	Average (µm)
Pg1	17.50 – 24	6.46 – 8	20.75 × 7.23
Pg2	20.95 – 29.6	6.38 – 8.2	25.27 × 7.29
Pg3	27.56 – 38.4	8.08 – 10.9	32.98 × 9.49
Pg4	22.96 – 31.81	6.32 – 8.58	27.38 × 7.45

*Observation of 50 conidia

Cultural variation

Radial growth of *P. grisea* isolates on different media

The radial growth and mycelial growth of all the isolate of *P. grisea* were carried out in four different solid media. Among all solid media, maximum mycelium growth of all isolates were obtained in PDA media (89.80 mm, 50.72 mm, 84.33 mm and 89.98 mm) followed by corn meal agar media (89.56mm, 41.28 mm, 82.21 mm and 89.96 mm) whereas, minimum radial growth was obtained in Richard's agar media (77.52 mm, 30.58 mm, 59.53 mm and 80.32 mm) after 72 hrs. Of inoculation. Among the isolates maximum radial growth was recorded in Pg4 (89.98 mm, 89.96 mm, 89.15 mm and 80.32 mm) followed by Pg1 (89.80 mm, 89.56 mm, 86.69 mm and 77.52) respectively. whereas, the isolates Pg2 recorded minimum radial growth (50.72mm, 41.28mm, 36.05mm, 30.58 mm) (Table 3). In past studies, Hajano *et al*. (2013) [3] recorded maximum colony growth of *M. oryzae* on Potato dextrose agar (PDA) followed by Potato carrot agar (PCA), whereas Oat meal agar (OMA) obtained minimum growth under laboratory conditions.

Vanraj *et al*. (2013) tested five natural media (Oat meal agar; Rice agar; Rice polish agar; Malt extract agar; Potato dextrose agar) for isolation of *P. oryzae* and resulted that the mycelium growth of pathogen was fast on PDA and malt extract agar while slow on Rice agar and Rice polish agar.

Table 3: Radial growth (mm) of *P. grisea* isolates on different media after 72 hrs

Isolates	potato dextrose agar medium (mm)	Corn meal agar media (mm)	C'zapek Dox agar media (mm)	Richard's agar media (mm)
Pg1	89.80	89.56	86.69	77.52
Pg2	50.72	41.28	36.05	30.58
Pg3	84.33	82.21	63.56	59.53
Pg4	89.98	89.96	89.15	80.32
SE(m) ±	0.69	1.09	1.20	1.22
CD at 5%	2.28	2.57	4.00	4.28
CV%	1.51	1.77	3.04	3.61

*Mean of three replications

Colony colour and colony texture of *P. grisea* isolates on different media after fourteen days

On Potato Dextrose Agar (PDA) all the isolates produced greyish white to milky white colonies having light grey centre in Pg1, Pg2 and Pg3 except isolate Pg4 exhibited dark grey centre. On Corn meal agar media, isolate of finger millet Pg1 and foxtail millet Pg4 formed greyish black colonies with smooth margin whereas isolate of little millet Pg2 and proso millet Pg3 produced buff coloured colonies with irregular margin. On C'zapek Dox agar media, all isolate formed dull to buff white colonies with light grey centre. All isolates of *P. grisea* formed greyish white centre with buff white margin in on Richard's agar media (Table 4).

Table 4: Colony colour of *P. grisea* isolates on different media after fourteen days

Isolates	Potato dextrose agar medium	Corn meal agar media	C'zapek Dox agar media	Richard's agar media
Pg1	Light Grey in the centre and greyish white at margins	Greyish black with smooth margin	Dull white with smooth margin	Greyish white centre with buff white margin
Pg2	Light grey in the centre with milky white margin	Buff white with irregular margin	Light grey in centre with dull white margin	Greyish white centre with buff white margin
Pg3	Light grey in the centre with milky white margin	Buff white with smooth margin	Light grey in centre with buff white margin	Greyish white centre with buff margin
Pg4	Dark grey in the centre with grayish white margin	Greyish black with smooth margin	Light grey in centre with buff white margin	Greyish white centre with buff white margin

Colony texture of *P. grisea* isolates on different media after fourteen days

Isolate of finger millet (Pg1) produced raised colony with medium to uniform growth in Potato dextrose agar and Richard's agar medium whereas, smooth colony with uniform growth in Corn meal agar and C'zapek Dox agar medium. Isolate of little millet exhibited Smooth to raised colony with medium growth in PDA, C'zapek Dox agar and Richard's agar media as compare to coarse colony with irregular growth in Corn meal agar media. Isolate of proso millet formed smooth to raised colonies with medium to uniform growth in all four media. Isolate of foxtail millet produced variable texture in all media, In PDA media the colony became aerial and fluppy, smooth and coarse to raise in Corn meal, C'zapek Dox and Richard's agar media with uniform to poor growth (Table 5). In past studied, Anjum *et al*. (2015) [1] evaluated the morphological characteristics of 24 different *P. grisea* isolates for its colony colour, texture and topography, type of margin, size and shape of conidia and its production by growing on the Ragi Yeast Lactose Agar medium. The result indicated that colour of the colony on different media varied from greyish black to whitish grey and grey to white. With respect to colony morphology the pathogen produced aerial mycelium with raised centre and margin on Ragi Yeast Lactose Agar medium, raised centre with sectoring on OMA and Ragi leaf medium, raised mycelia with concentric rings on Mathur's medium and Malt extract medium, raised mycelia in Rose bengal medium, Czapek's dox medium, Richard's medium, Ragi meal medium, Potato dextrose agar medium and Peptone dextrose medium and deep centered with raised surrounding mycelia on host extract + Sucrose medium.

Kulmitra *et al*. (2017) [4] studied the cultural characteristics of seven isolates of *P. oryzae* with respect to colony characters

like type of growth, colour of colony. On PDA medium the isolates exhibited white colonies to slightly greyish white colonies. In Takahashii's medium all isolates developed creamy white colour colonies. In rice leaf extract medium one isolate developed white colony while other developed dark greyish colour colonies. In oat meal agar two isolates developed black colour colonies while other isolates developed white colour colonies.

Table 5: Colony textures of *P. grisea* isolates on different media after fourteen days

Isolates	Potato dextrose agar medium	Corn meal agar media	C'zapek Dox agar media	Richard's agar media
Pg1	Raised colony with uniform growth	Smooth colony with uniform growth	Smooth colony with uniform growth	Raised colony with Medium growth
Pg2	Smooth colony with medium growth	Coarse colony with irregular growth	Smooth colony with medium growth	Raised colony with poor growth
Pg3	Raised colony with uniform growth	Smooth colony with uniform growth	Smooth colony with medium growth	Raised colony with medium growth
Pg4	Aerial colony with fluppy growth	Smooth colony with uniform growth	Coarse colony with uniform growth	Raised colony with medium growth

In-vitro evaluation of bio-control agents against *P. grisea* isolates

Efficacy of three bio-control agents were evaluated for their antifungal properties by dual culture as well as monoculture technique as mentioned in "Materials and Methods". Observation on per cent inhibition of mycelial growth of isolates was recorded after 72 hrs. Of incubation and presented in the Table 16 and 17.

The result revealed that, *Trichoderma viride* inhibited maximum mycelial growth of 81.81 per cent in the isolate of finger millet (Pg1) followed by 79.08 per cent growth inhibition in the isolate of foxtail millet (Pg4). Minimum 72.33 per cent inhibition was recorded in the isolate of little millet (Pg2).

In-vitro evaluation of *Trichoderma harzianum* against test pathogen resulted that maximum growth inhibition 73.96 per cent was recorded in isolate of foxtail millet (Pg4) followed by 72.84 per cent growth inhibition in the isolate of proso millet (Pg3). Minimum 56.76 per cent inhibition was recorded in the isolate of little millet (Pg2).

In-vitro evaluation of bacterial Bio-control agent *Pseudomonas fluorescens* showed highest hyphal inhibition of 95.61 per cent in the isolate of proso millet (Pg3) followed by 80.16 per cent growth inhibition in the isolate of little millet (Pg2) whereas, minimum inhibition 68.43 per cent was recorded in the isolate of foxtail millet (Pg4). In past studied, Getachaw *et al*. (2014) observed that *T. viride* inhibited maximum mycelial growth of 77.1 and 74.1 per cent of *P. grisea* isolates Pg41 and Pg22, respectively, whereas the isolate Pg40 *P. fluorescens* showed maximum mycelial growth inhibition of isolates Pg40 (57.2%) followed by Pg26 (56.1%) *in-vitro* condition.

Kulmitra *et al*. (2017) [5] evaluated five different bio-agents *viz., Trichoderma viride*, *T. harzianum*, *T. virens*, *Pseudomonas fluorescens* and *Bacillus subtilis* against *Pyricularia oryzae* causing rice blast at four and eight days after incubation through dual culture technique and recorded highest per cent inhibition of mycelial growth of fungus in *T. virens* i.e. 67 per cent and 70 percent after four and eight days after incubation respectively with mean of 68.5 per cent followed by *Trichoderma viride* with the inhibition of 61 and 63 per cent respectively with mean of 62 per cent. The *Pseudomonas fluorescens* did not show any inhibition of mycelial growth of *P. oryzae as* the pathogen over grew the bio-control agents.

Table 6: Evaluation of bio-control agents on radial growth of *P. grisea* isolates

Bio-control agents	Pg1 (mm)	Pg2 (mm)	Pg3 (mm)	Pg4 (mm)
Trichoderma viride (*Tv*)	16.33	14.03	21.13	18.82
Trichoderma harzianum (*Th*)	29.39	21.93	22.90	23.43
Pseudomonas fluorescens (Pf)	26.56	10.06	03.70	28.40
Control	89.76	50.43	84.60	89.96
SE(m) ±	0.50	0.41	0.33	0.49
CD at 5%	1.79	1.47	1.18	1.74
CV%	3.64	4.58	3.31	3.62

*Mean of three replications

Table 7: Percentage growth inhibition of *P. grisea* isolates against bio-control agents

Bio-control agents	Pg1 (mm)	Pg2 (mm)	Pg3 (mm)	Pg4 (mm)
Trichoderma viride (Tv)	81.81	72.33	75.94	79.08
Trichoderma harzianum(Th)	67.27	56.76	72.84	73.96
Pseudomonas fluorescens (Pf)	70.44	80.16	95.61	68.43

*Mean of three replications

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