Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP1: 2287-2289

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Cross inoculation studies of *Pyricularia grisea* on Small Millets and their management through botanicals

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Abstract
Small millets 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)* are the poor man's food that ensures nutritional security to the people living in harsh and difficult terrains. Though the crop is less prone to diseases and pests but under vulnerable condition, blast caused by *Pyricularia grisea* (Cooke) Sacc. Telemorph of a heterothallic ascomycetes, *Magnaporthe grisea* (Hebert) Barr. [Anamorph:] had wide host range of *Gramineae* and took heavy toll of the crop in various ecological zones. To study the pathogenic variability of isolates of *P.grisea* cross inoculation test were carried out on their respective host plant viz., finger millet, foxtail millet, proso millet and little millet. The studies revealed that all isolates Pg1, Pg2, Pg3 and Pg4 were found to be pathogenic in their own host. The isolate of Finger millet (Pg1) and isolate of Foxtail millet (Pg4) were found to be cross infective with each other whereas, the isolate of proso millet (Pg3) and little millet (Pg2) were non-infective on other hosts. Among botanicals the extracts of onion (*Allium cepa*), garlic (*Allium sativum* L.), neem (*Azadirachta indica* L.) amla (*Emblica officinalis*), teenpatia (*Oxalis latifolia*), babool (*Vachellia nilotica)* and herbal plant extract (Zuki) when used against *P.grisea* by poisoned food technique, only the herbal plant extract (Zuki) inhibited the maximum mycelial growth of all the test pathogens at 5& 10 % conc.

Keywords: Cross inoculation, *Pyricularia grisea, Small* Millets

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 were cross inoculated with their respective host to study their pathogenic variability and sensitivity of *P. grisea* isolates to different plant extract/ botanicals under laboratory conditions in the department of Plant Pathology, Birsa Agricultural University during 2016 -2017.

Material and Methods
To test the pathogenic 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 tested with four different *P. grisea* isolates. The isolate Pg1was isolated from finger millet, Pg2 from little millet, Pg3 from proso millet and Pg4 from foxtail millet. Each hosts with their highly susceptible variety Udru mellege for finger millet, Gujarat vari-1 for little millet, TNAU 103 for proso millet and GPUP-24 for foxtail millet were grown in 15 cm diameter pots (10 seeds/pot) filled with sterilized soil-sand-FYM mix (2:1:1) with three replications. The plants were irrigated properly 48 hours before inoculation. The leaves of

thirty five days old seedling were injured at several places by a sterile fine needle and inoculated with spore suspension obtained from the culture grown on PDA medium. The seedlings after spray inoculation were kept in green house condition with water sprayed regularly both during morning and evening hours to maintain relative humidity and covered with polythene bags. After 48 hours, the polythene bags were removed. Periodical observations were made for the development of typical blast symptom on the inoculated plants.

In order to find out the inhibitory effect on mycelia growth of test fungus *P. grisea*, extracts of seven botanicals *viz.*, Neem (*Azadirachta indica*), Garlic (*Allium sativum*), Onion (*Allium sepa*), Teenpatia (*Oxalis latifolia*), Babool (*Vachellia nilotica*), Amla (*Emblica officinalis*), Herbal diseases controller (Zuki, composition: plant extract/derivatives- 55%, emulsifier- 5%, soya oil- q.s.) were tested against the pathogen. These extracts (1:1w/v) were obtained from thoroughly washed fresh materials by grinding in sterilized cold water in a mixer grinder and filtered. The extracts obtained were considered as pure and 10 per cent of each extract were added to PDA and sterilized at 15 lbs/cm^2 pressure for 20 minutes. The medium containing extracts was poured into 90 mm pre-sterilized Petri dishes keeping three replications for each treatment and allowed to solidify. Suitable controls were also maintained side by side. All the Petri dishes were inoculated with 3 mm mycelial disc of pathogen in centre and incubated at room temperature (28±2°C) for ten days. The radial growth of the fungus was recorded for each replication individually. 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

Cross inoculation test

Cross inoculation studies of the *P. grisea* isolates showed that all the isolates were host-specific. The isolate of finger millet (Pg1) and isolate of foxtail millet (Pg4) were found to be cross infective with each other whereas the isolate of proso millet (Pg3) and the isolate of little millet (Pg2) was non cross infective on other millets (Table 1.). The present study showed that isolates of *Pyricularia* from foxtail millet and finger millet are closely related. According to Thompson (1941) and Ramkrishnan (1948), *Pyricularia* from finger millet and the fungus from *Setaria italica* were capable of infecting both of their own hosts when they were cross inoculated. The findings of pathogenicity tests done by Viji *et al.* (2000) in India showed that the blast fungus from the two hosts, rice and finger millet did not cross-infect, nor did the two forms cross in the laboratory. The results confirm that the rice and millet-infecting *M. grisea* populations in India were distinct. It has also been found that the pathogenicity of the fungus and cross inoculation reaction among different species may be changed according to the time and condition because of the genetic modification and crossing between the different isolates (Kato 1978).Similar results were also observed by Khadka *et al*. (2012) during cross inoculation studies.

Table 1: Cross inoculation studies of different isolates of *P. grisea Sacc.* on different hosts at 30 days after inoculation

	Host Plant (30 days)			
Isolates	**Finger millet**	**Little millet**	**Proso millet**	**Foxtail millet**
Pg 1	+	-	-	+
Pg 2	-	+	-	-
Pg 3	-	-	+	-
Pg 4	+	-	-	+

+ Cross infective - Non Cross infective

Suppression of *Pyricularia grisea* by botanicals

In *in vitro* evaluation, all isolates of *P.grisea* recorded variable reaction among the plant extract/ botanicals. Among seven plant extracts evaluated, a herbal plant extract Zuki have suppressed all the four isolates of *P.grisea* very significantly over control. Plant extract, *Allium cepa* and *Allium sativum* at 10 per cent concentration were found to be the next best treatment to inhibit the mycelial growth of Pg1 (isolate of finger millet) by 75.93 and 70.69 per cent, respectively. The least per cent inhibition of Pg1 was 8.38 per cent recorded in the plant extract of *Vachellia nilotica* at 5 per cent concentration.

Plant extract of *Oxalis latifolia* and *Azadirachta indica* at 10 per cent concentration were found to be the next best treatment to inhibit the mycelial growth of Pg 2 (isolate of little millet) by 71.05 and 61.51 per cent, respectively. However, the least per cent inhibition of 02.22 per cent was recorded in the plant extract of *Allium sativum* at 5 per cent concentration.

The isolate of proso millet Pg3 recorded 95.49 and 91.33 per cent growth inhibition at 10 per cent concentration with *Allium cepa* and *Azadirachta indica* plant extract and were found to be the next best treatment. The least per cent inhibition of 32.91 per cent was recorded in the plant extract of *Vachellia nilotica* at 5 per cent concentration.

In isolate of foxtail millet Pg4 the plant extract *Oxalis latifolia* and *Vachellia nilotica* at 10 per cent concentration were found to be the next best treatment and inhibit the growth of fungal mycelia by 78.74 and 72.44 per cent, respectively. However, the least per cent inhibition of 00.41 per cent was recorded in the plant extract of *Emblica officinalis* at 5 per cent concentration (Table 2 & 3). In past, Thangavelu *et al.* (1995) studied the effect of different neem-based formulations on rice blast disease both *in-vitro* and *in-vivo* conditions and reported that Replin RD 9 a commercially available neem based formulation was found superior among all the test treatments to reduce the per cent germination of spores *in-vitro*. The extraordinary use of chemical fungicides resulted in environmental pollution and ill health to biotic community as a whole. Therefore, the use of botanicals for plant disease management seems to be a better alternative to chemical fungicides in managing the blast disease. The results projected that Herbal diseases controller (Zuki, composition: plant extract/derivatives- 55%, emulsifier- 5%, soya oil- q.s.) has significantly suppressed the all isolate of *P.grisea* which is followed by *Allium cepa, Allium sativum,* and *Oxalis latifolia* in the decreasing order.

Table 2: *In-vitro* evaluation of percentage growth inhibition of isolates *P. grisea* against plant extracts at 5% concentration

Treatments	Plant species	Plant Parts	Pg1	Pg2	Pg3	Pg4
T_1	*Allium cepa* (Onion)	Bulb	66.60	38.18	92.63	05.10
T_2	Herbal disease controller (Zuki)	Plant extract	73.88	71.10	93.82	80.51
T_3	*Allium sativum* (Garlic)	Bulb	53.24	02.22	87.46	32.77
T_4	*Azadirachta indica* (Neem)	Leaves	52.85	50.83	91.85	44.24
T_5	*Emblica officinalis* (Amla)	Leaves	48.32	37.49	72.96	00.41
T_6	*Oxalis latifolia* (Teenpatia)	Leaves	50.33	51.80	66.82	75.30
T_7	*Vachellia nilotica* (Babool)	Leaves	08.38	29.99	32.91	66.46

*Mean of four replication

Table 3: *In-vitro* evaluation of percentage growth inhibition of isolates *P. grisea* against plant extracts at 10% concentration

Treatments	Plant species	Plant Parts	Pg1	Pg2	Pg3	Pg4
T_1	*Allium cepa* (Onion)	Bulb	**75.93**	53.71	**95.49**	10.26
T_2	Herbal disease controller (Zuki)	Plant extract	77.12	75.63	95.77	82.93
T_3	*Allium sativum* (Garlic)	Bulb	70.69	2.30	**88.83**	39.44
T_4	*Azadirachta indica* (Neem	Leaves	67.21	61.51	91.33	49.30
T_5	*Emblica officinalis* (Amla)	Leaves	52.71	51.60	75.24	04.85
T_6	*Oxalis latifolia* (Teenpatia)	Leaves	51.83	**71.05**	68.99	78.74
T_7	*Vachellia nilotica* (Babool)	Leaves	12.44	47.30	35.52	72.44

*Mean of four replications

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