Research Paper :

Cultural and physiological studies on *Curvularia lunata,* a casual agent of grain discolouration in rice

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SUMMARY

Many fungi have been isolated from discolored grain, *Curvularia lunata* (Wakker) Boed. was found as a dominant pathogen. Cultural studies revealed that *C. lunata* gave maximum dry mycelial weight (225.00 mg) at 13 th day after incubation. Among the solid media, Potato dextrose agar, Sabouraud's agar and Host extract agar were best for the growth and sporulation of *C. lunata*. Among the various liquid media used, maximum dry mycelial weight was observed in Sabouraud's medium (458.00 mg). The diverse response of *C. lunata* to different temperature levels of 15, 20, 25, 30, 35 and 40^0 C and different pH levels *viz.,* 4, 6, 7, 8 and 10 and also different reletive humidity levels 100, 90, 80, 70 and 60 per cent was tested. Temperature of 25^0 C showed good growth and excellent sporulation. At 90 to 100 per cent of relative humidity and pH 6 were found congenial for growth and sporulation of *C. lunata* under *in vitro* conditions.

Key words : Rice, Grain discolouration, *Curvularia lunata,* Cultural, Physiological studies

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Rice (*Oryza sativa* L.) is an important food crop belonging to family Poaceae and is the staple food of more than half of the world's population. About 90 per cent of the world's rice is produced and consumed in Asia alone. Majority of the fungi, *viz., Curvularia lunata, Alternaria alternata, Fusarium moniliforme, Heliminthosporium oryzae* responsible for causing grain discolouration are reported to be seed borne in nature (Ou, 1985; Mew *et al.,* 1988; Agarwal *et al.*, 1989 and Singh, 1993). Grain discolouration now has assumed great importance in recent years because of the changes of cropping practices into intensive system like increased fertilizer application and developed more rice seasons yearly but not too many resistant varieties combined with good yield characters are available for cultivation. Rice is mostly grown in the wet season, when high humidity and high temperature prevail. These conditions are congenial for infection of seed with number of fungi.

MATERIALS AND METHODS

The investigation on studies on grain discolouration in rice was carried out during 2006-2007 at the Department of Plant Pathology, College of Agriculture, Raichur, University of Agricultural Sciences Dharwad. Raichur is situated in North Eastern Dry Zone (Zone 2) of Karnataka State at 16^o 12$^{'}$ N latitude/ 77^0 21$^{'}$ E longitude with an altitude of 389.37 m above mean sea level.

Discoloured grains were collected from different rice growing areas of Raichur, Gulbarga, Koppal and Dharwad districts. The collected samples were packed in cloth bags and stored at room temperature (25 ± 2^0 C) for further investigation. Fungi were isolated and identified based on the morphological characteristics. Mostly from both unsterilized and sterilized seeds on Potato-dextrose agar and each colony arising separately on the seeds was picked up into PDA slant and the percentage occurrence of each fungal species was calculated from the total isolates.

Growth phase of *C.lunata:*

Twenty ml of Potato dextrose broth (PDB) was added into each of 100 ml conical flasks and sterilized. The flasks were then inoculated with five mm disc of fungal culture and incubated at 28 ± 1^0 C for different intervals. Three flasks were harvested at a time, starting from the third day onwards up to 21st day by leaving a gap of two days between the two successive harvests. The cultures were filtered through previously weighed Whatman number 42 filter paper of nine centimeter diameter, which were dried to a constant weight at 60^0 C in a hot air oven prior to filtration. The mycelial mat on the filter paper was thoroughly washed

with sterile distilled water to get rid of the salts associated with the mycelial mat. The filter paper along with the mycelial mat was dried to a constant weight at 60^0 C and weighed on an analytical balance. The differences between final and initial weight of filter discs were taken as the weight of the mycelia. The data were analyzed statistically.

Cultural characters on solid and liquid media:

The different synthetic media *viz.,* Czapeck's agar, Richards's agar, Martin's rose Bengal agar (MRBA) and also semi-synthetic media including Potato dextrose agar (PDA),Host extract agar, Sabouraud's agar, Oat meal agar, Malt extract agar and Yeast extract agar were used to find out the best medium for the growth of *C.lunata*.Twenty ml of each medium listed above was poured into 90 mm diameter Petri plates. After solidification, 5mm disc of the *C.lunata* were selected from actively growing culture using a cork borer and a single disc was placed at the centre of Petri dish. Thirty ml of each broth was added into each of 150 ml conical flasks and sterilized. The flasks were then inoculated with 5 mm disc of *C. lunata* culture. Each set of experiment was replicated thrice and they were incubated at $28^0 \pm 1^0$ C for ten days respectively..

Physiological studies:

The growth of fungi was tested at 15, 20, 25, 30 and 40° C. Potato dextrose agar was poured into 90 mm diameter petriplates. After solidification, 5 mm from actively growing culture were cut and inoculated to the media containing Petriplates and incubated for fifteen days in the incubators adjusted to required temperature levels. Each treatment was replicated thrice. The pH of the medium was adjusted before autoclaving with the help of HCl (0.1 N) and NaOH (0.1 N) using pH meter/ litmus paper. After autoclaving, the medium with 4, 6, 7, 8 and 10 pH was poured in sterilized plates and inoculated with the organism. Three replications were maintained, observation recorded for growth and sporulation. At different RH levels *viz.,* 60, 70, 80, 90 and 100 per cent, Potato dextrose agar was poured into 90 mm diameter Petriplates described earlier. The different levels of relative humidity were created by using different concentrations of sulphuric acid (H_2SO_4). The desiccators were kept at room temperature with three replications.

RESULTS AND DISCUSSION

The major fungi isolated from the discoloured rice samples from North Eastern Karnataka were *C. lunata* (35.30 %) followed by *A.alternata* (20.60 %), *F.moniliforme* (18.20 %) with other organisms included *H.oryzae, P.grisea*, *Aspergillus, Penicillium, Rhizopus* spp. and other unidentified organisms (Table 1). As it was evident from observation that *C. lunata* was found more when compared to other organisms irrespective of crop stage and locations, therefore. *C. lunata* was selected as dominant fungal pathogen and further used for all experiment purposes. Similar results were reported by Babo and Lokesh (1996).

Table 1 : Mycoflora of discoloured grains of rice detected by Potato dextrose agar plate method

Sr. No.	Fungi	Per cent incidence	
		Unsterilized	Sterilized
1.	*Curvularia lunata*	35.30	8.53
2.	*Alternaria alternata*	20.60	--
3.	*Fusarium moniliforme*	18.20	10.50
4.	*Aspergillus* spp.	7.20	--
5.	*Penicilium* spp.	6.50	--
6.	*Helminthosporium oryzae*	5.10	--
7.	*Pyricularia grisea*	--	1.20
8.	*Rhizopus* spp.	1.50	--

Maximum dry mycelial weight (225.00 mg) of *C.lunata* was obtained on 13th day of incubation after seeding in Potato dextrose broth and it was taken as the optimum period for the growth of the fungus for all future studies. Thereafter, the growth of the fungus declined gradually with the increase in number of days of incubation (Table 2). This may be due to autolysis of the mycelium and exhuastation of nutrients in the medium after incubation for optimum number of days. This is not an

Table 2 : Growth phase of *C. lunata* on Potato dextrose broth at different incubation periods

Sr. No.	Incubation period (days)	Dry mycelial weight (mg)
1.	3	91.00
2.	5	94.00
3.	7	110.33
4.	9	161.00
5.	11	206.00
6.	13	225.00
7.	15	178.00
8.	17	132.00
9.	19	99.00
10.	21	94.00
	S.E.±	1.35
	C.D. (P=0.01)	5.50

Table 3 : Growth characters of *C. lunata* on different solid media

Sr. No.	Media	Mycelium			Radial growth (mm)	Sporulation
		Colour	Growth	Type of margin		
1.	Czapeck's agar	Light brownish colour	Flat	Irregular	68.00	++
2.	Martin's Rose Bengal agar	Dark greenish colour	Flat	Regular	58.50	+
3.	Richard's agar	Dark greenish colour	Flat	Irregular	43.53	+
1.	Potato dextrose agar	Dark greenish colour	Raised fluffy growth	Smooth, circular regular	90.00	++++
2.	Host extract agar	Dark greenish colour	Raised fluffy growth	Smooth, circular regular	90.00	++++
3.	Sabouraud's agar	Dark greenish colour	Raised fluffy growth	Smooth, circular regular	90.00	++++
4.	Yeast extract agar	Light brownish colour	Flat	Smooth/ Regular	54.25	+
5.	Malt extract agar	Dark greenish colour	Flat	Irregular	78.50	+
6.	Oat meal agar	Dark greenish colour	Raised fluffy growth	Smooth circular regular	84.25	+++
	S.E.±				1.61	
	C.D. (P=0.01)				6.38	

\- No suporulation, + Poor, ++ Moderate, +++ Slow ++++ Excellent

exception to the finding of Lilly and Barnett (1951) who depicted a definite pattern of growth of fungi depending on species, environmental and nutritional conditions. The study is also supported by Mathur and Sarbhoy (1977).

The effect of different culture media on growth of fungus differed significantly. *C. lunata* recorded maximum growth and sporulation on Potato dextrose agar, Host extract, and Sabouraud's agar with mean colony diameter of 90.0 mm followed by Oat meal agar (84.25 mm). The poor growth and sporulation was in malt extract agar (78.50 mm), Czapeck's agar (68.00 mm), Martin's rose Bengal agar (58.50 mm), and Richards's agar (43.53 mm) (Table 3). Semi- synthetic media supported better growth because of prevalence of some nutrients which are essential for growth and development of organisms.

Among the liquid media (Table 4) The data revealed that, there was significant difference in the growth among liquid media. The maximum growth of *C. lunata* was observed in Sabouraud's broth (458.00 mg) and was significantly superior to all other media tested. Next best was Malt extract broth (325.00 mg) followed by Oatmeal broth (268.00 mg). Least dry mycelial weight was recorded in Martin's rose Bengal broth (98.66 mg). This is in agreement with the observations made by Mathur and Sarbhoy (1977).

Curvularia lunata is the major causative agent of grain discolouration and the factors like temperature and relative humidity play a vital role for infection. Present investigation revealed that, temperature at 25^{0}C recorded maximum growth (86.33 mm) and excellent sporulation, pH 6 was good for growth (87.00mm) and excellent sporulation. 90-100 per cent relative humidity was congenial for excellent growth and sporulation of *C. lunata* (Table 5, 6 and 7). It indicates the similarity of crop requirement for temperature and relative humidity at milky stage of developments (Jiang-Ming *et al*., 2005, Mathur and Sarbhoy, 1977 and Tonapi *et al.,* 2001).

Table 4 : Growth of of *C. lunata* in different liquid media

Sr. No.	Media	Dry mycelial weight (mg)
1.	Potato dextrose broth	259.33
2.	Martin's Rose bengal broth	98.66
3.	Czapeck's broth	125.66
4.	Sabouraud's broth	458.00
5.	Oat meal broth	268.00
6.	Richard's broth	186.00
7.	Yeast extract broth	166.66
8.	Malt extract broth	325.00
9.	Host extract broth	258.00
	S.E.±	1.15
	C.D. (P=0.01)	4.75

Table 5 : Growth of *C. lunata* at different temperatures

Sr. No.	Temperature (^{0} C)	Radial growth (mm)	Sporulation
1.	15	17.00	++
2.	20	32.66	+++
3.	25	86.33	++++
4.	30	83.33	+++
5.	35	72.00	++
6.	40	51.00	+
	S.E.±	0.86	
	C.D. (P=0.01)	3.86	

++++ Excellent +++ Good
++ Medium + Low

Table 6 : Growth of *C. lunata* at different pH levels

Sr. No.	pH	Radial growth (mm)	Sporulation
1.	4	54.00	++
2.	6	88.00	++++
3.	7	85.00	++++
4.	8	71.33	++
5.	10	63.00	+
6.	40	51.00	+
	S.E.±	0.69	
	C.D. (P=0.01)	3.31	

++++ Excellent +++ Good
++ Medium + Low

Table 7 : Growth of *C. lunata* at different relative humidities

Sr. No.	Relative humidity (%)	Radial growth (mm)	Sporulation
1.	100	87.00	++
2.	90	85.00	++++
3.	80	79.66	+++
4.	70	59.00	++
5.	60	52.33	+
	S.E.±	0.96	
	C.D. (P=0.01)	4.55	

++++ Excellent +++ Good
++ Medium + Low

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