Research Paper :

Evaluavation of bio-agents against *Curvulara lunata*, a causal agent of grain discolouration in rice

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SUMMARY

In the present studies, the antagonistic microorganisms were tested for their efficacy in inhibition of growth of *C. lunata*. Many fungi have been isolated from discoloured grain, *Curvularia lunata* (Wakker) Boedign, was found dominant pathogen (35.30%) of grain discoloration of rice in rice growing tracts Tungabhadra Project Area and Upper Krishna Project Area of Karnataka state in India comprising Raichur, Koppal and Gulberga districts during 2007. Among the different bio-agents, *Bacillus subtilis* (97.77%) followed by fungal bio-agent, *Trichoderma viride* (96.44%) and *T. harzianum* (93.50%) were found to be effective. Seed treatment with *T. viride* was found to inhibit *C. lunata* association with grain discolouration with 90.05 per cent germination and 1170.00 vigour index followed by *Bacillus subtilis* 87.99% germination and 989.11 vigour index

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Rice (*Oryzae sativa* L.) is most widely grown cereal crop of the world. Majority of the fungi, *viz., Curvularia lunata, Alternaria alternata, Fusarium moniliformae* and *Heliminthosporium oryzae* are responsible for causing grain discolouration are reported to be seed borne in nature (Ou, 1985; Mew *et al.,* 1988; Singh, 1993). Grain discolouration has assumed to be great importance in recent years because of the changes in cropping practices; intensive system like increased fertilizer application and yearly more rice seasons. But not too many resistant varieties combined with good yield characters are available for cultivation.

In the present investigation, an attempt has been made to identify the best bio-agent for the inhibition of mycelial growth of *C. lunata* and so also to get the higher seed germination and seedling vigour through seed treatment.

MATERIALS AND METHODS

The 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° 12' N latitude/ 77° 21' E longitude with an altitude of 389.37 m above mean sea level.

Collection of the samples:

Discoloured rice 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° C) for further investigation. Fungi were isolated and identified based on the morphological characteristics.

Dual culture test:

Bioagents obtained indigenously and Tamil Nadu Agricultural University (TNAU), Coimbatore were tested for their efficacy under *in vitro* using dual culture technique as well as seed treatment.

Bioagents were evaluated for their efficacy through dual culture technique. The

bioagents and the test fungus were inoculated side by side on a single Petridish containing solidified PDA medium. Three replications were maintained for each treatment with one control by maintaining only pathogen and bio-agent, separately. They were incubated for eight days. The diameter of the colony of both bioagents and the pathogen was measured in two directions and average was recorded. Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1927).

Seed treatment with biocontrol agent:

The efficacy of biocontrol agents were studied. The seeds of paddy were soaked in culture of fungal and bacterial bio-agents for 4 hrs followed by 30 minute shade drying and the seeds were inoculated with spore suspensions of (1 x 10^5 spores/ ml) *C.lunata*. The seeds treated with the fungus alone was (control inoculated) and distilled water (control uninoculated) were maintained for comparison. Fourty seeds were sown separately in pots having sterilised soil for each treatment. The seed germination and seedling vigour was calculated 21 days after sowing, as per the method of Abdulbaki and Anderson (1976).

Seedling vigour was measured by calculating vigour index. The vigour index was calculated by using the following formula given by Abdulbaki and Anderson (1976).

Seedling vigour = (Mean shoot length + Mean root length) x germination(%)

RESULTS AND DISCUSSION

The frequency of mycoflora has to be detected to get better control measure for them. It is evident from earlier reports and present investigation that, 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 including *viz., H.oryzae, P.grisea*, *Aspergillus, Penicillium, Rhizopus* spp. and other unidentified organisms (Table 1). Similar results were reported by Babo and Lokesh (1996).

Efficacy of bacterial and fungal bio-agents was studied under *in vitro* condition and the results on inhibition of mycelial growth of *C. lunata* were recorded and presented in Table 2 and Fig. 1. There was a significant difference among the bio-agents tested. Among the fungal bio agents, *T. viride* (96.44%) was found to be significant in inhibiting the mycelial growth of *C. lunata* followed by *T.harzianum* (93.50 %). Among the bacterial bio-agents,

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* sp.	7.20	--
5.	*Penicillium* sp.	6.50	--
6.	*Helminthosporium oryzae*	5.10	--
7.	*Pyricularia grisea*	--	1.20
8.	*Rhizopus* sp.	1.50	--

Table 2 : Efficacy of bio-agents in inhibiting the growth of *C. lunata*

Sr. No.	Bioagents	Per cent inhibition
1.	*Bacillus subtilis* (E)	40.99 (39.21)**
2.	*Bacillus subtilis* (PGPR)	43.00 (41.02)
3.	*Bacillus subtilis*(I)	97.77 (81.25)
4.	*Pseudomonas fluorescens* (E)	41.99 (40.38)
5.	*Pseudomonas fluorescens* (I)	47.44 (44.10)
6.	*Trichoderma viride* (I)	96.44 (72.59)
7.	*Trichoderma harzianum* (I)	93.50 (75.23)
S.E.±		0.92
C.D. (P=0.01)		2.84

1) *Bacillus subtilis* (E)
2) *Bacillus subtilis* (PGPR)
3) *Bacillus subtilis* (I)
4) *Pseudomonas fluorescens* (E)
5) *Pseudomonas fluorescens* (I)
6) *Trichoderma viride* (I)
7) *Trichoderma harzianum* (I)

Fig. 1 : Evaluavation of bio-agents against *Curvulara lunata*

Bacillus subtilis (I) (97.77%) was recorded maximum inhibition which was found superior among all the bio-agents followed by *P. fluorescens* (I) (47.44%) and *Bacillus subtilis* (PGPR) (43.00%) and least per cent inhibition was recorded in *P. fluorescens* (E) (41.99%) which was at par with *B. subtilis*(E) (40.99). Similar studies made by Saleem and Kandasamy (2002) and

Table 3 : Effect of seed treatment with bioagents on seed germination and vigour index

Sr. No.	Treatments	Germination (%)	Mean root length (cm)	Mean shoot length (cm)	Vigour index
1.	*Bacillus subtilis* (E)	70.66 (57.22)**	3.3	4.3	538.00
2.	*Bacillus subtilis* (PGPR)	73.00 (59.75)	3.4	4.7	639.26
3.	*Bacillus subtilis*(I)	87.99 (70.65)	4.9	5.2	989.11
4.	*Pseudomonas fluorescens* (E)	74.66 (59.75)	3.5	4.6	632.12
5.	*Pseudomonas fluorescens* (I)	43.23 (41.10)	3.2	4.4	411.07
6.	*Trichoderma viride* (I)	90.05 (86.45)	5.1	6.5	1170.00
7.	*Trichoderma harzianum* (I)	85.00 (67.25)	4.5	6.1	816.49
8.	Control inoculated	26.00 (31.71)	3.5	4.5	208.00
9.	Control (un inoculated)	87.10 (68.92)	3.0	3.6	512.90
S.E.±		2.20	0.08	0.07	109.61
C.D. (P=0.01)		8.82	0.25	0.22	322.18

** Arcsine values

Sarhan and Shibly (2003) also confirm the findings of present investigations.

Effect of seed treatment with bioagents:

The efficacy of different bio-agents, was tested through seed treatment against *C. lunata* and the results of the experiment are presented in Table 3.

The results indicated that the bio agent *T. viride* (I) gave good germination per cent of 90.05 and vigour index (1170.00) followed by *B. subtilis* (I) recorded germination (87.99%) and vigour index (989.11), respectively and least germination (43.23) and with vigour index (411.70) was recorded in *P. fluorescens* (I), which is in agreement with the result of Das *et al.* (1998).

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