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To evaluate the *in vitro* efficacy of different bioagents against *Pyricularia grisea* (Cooke) Sacc.

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Abstract
Biotic and abiotic stresses are major constraints in the production of Pearl millet. Among biotic stresses apart from bacterial and viral diseases, many fungal diseases are of economic importance. Blast was the most important and destructive disease of Pearl millet. Blast caused by *Pyricularia grisea* a serious threat to successful cultivation of Pearl millet. For the management of Blast of bajra, an experiment was conducted to study the efficacy of antagonistic organism against *Pyricularia grisea*. The bio-agents i.e. *Trichoderma viride, T harzianum, T hamatum, T.gliocladium, T. konigii, Peudomonas fluorescens* and *Bacillus subtilis* etc. were evaluated *in vitro*, found antifungal to *Pyricularia grisea*. However, *P. fluorescens* was found most significant with highest mycelial growth inhibition (64.61%) of the test pathogen. The second and third inhibitoriest antagonists found were *T. asperllum* and *T. harzianum* with mycelial growth inhibition of 62.39 and 57.03%, respectively.

Keywords: Biocontrol, *Claviceps fusiformis*, *in vitro*, inhibition

Introduction
Pearl Millet [*Pennisetum glaucum* (L.) R. Br)] is one of the assured *Kharif* crop under environment domesticated in the annual rainfall of 150 mm to 1000 mm in India. It has variety of uses for the consumption of human being such as chapatti, breads, snacks, cakes, beverages and predigested weaning food besides poultry feed and fodder for cattle. India and Africa are together occupying 90 per cent area of total pearl millet production in the world (Yadav *et al*., 2012) [9]. It's grain is chiefly served as a food, because of high protein (27 to 32%), higher concentration of essential amino acids, twice the extract (fat) and higher gross energy than maize (Ejeta *et al.*, 1987; Davis *et al.*, 2003) [3, 2]. Number of pathogens attack Pearl Millet during its crop growth period, cause low yield and economic loss to the present growers. Downy mildew, blast, ergot, smut and rust are the major diseases of the crop.
Blast incited by *Pyricularia grisea* (Cooke) Sacc. Was first recorded in Uganda in 1933 (Emechebe, 1975) [4]. The disease has geographic distribution in India, Singapore on Napier grass and the United States (Buckley and Allen, 1951) [1]. Blast incited by *Pyricularia grisea* was first reported in India from Kanpur 1953 (Mehta *et al*., 1953) [5] and remain as minor disease till the end of 20th century but from last one decade the disease has occupied a key position among the Pearl Millet diseases particularly in Maharashtra. The disease appears as grayish, water-soaked foliar lesions that enlarge and become necrotic, resulting in extensive chlorosis and pre mature drying of young leaves (Wilson *et al.*, 1989) [8].
Management of disease through resistant cultivars is the most economical and relevant way of controlling pearl millet blast mainly by resource poor and marginal farmers, who cannot afford to control blast disease by the application of chemical. Chemical control of plant pathogens is most effective and may cause hazardous effects on the environment and their residues on grain and fodder may also cause the harmful effect on human being, animals and birds. Hence in search of an alternative to the chemicals there is a need to test the bio - agents and others for the management of the disease. Keeping the above points in mind the present experiments have been carried out.
Use of resistant cultivars is the best alternative to overcome yield losses caused by *Pyricularia grisea*. It has the ability to overcome resistance within two to three years after the release of resistant variety and made breeding for resistance a constant the pathogen since major studies were carried out on rice blast pathosystems (Sere *et al*., 2007) [6]. However such studies are very limited with the pearl millet blast pathosystem (Sharma *et al*., 2013) [7]. Severe infection of the disease reduced the yield particularly fodder value hence there is a need to work on the line of management of the disease.

Material and Method

In dual culture technique, twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates. Fungal antagonists were evaluated by inoculating the pathogen at one side of Petri plate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3 cm gap. For this actively growing cultures were used. In case of bacterial antagonist evaluation, two mycelial discs of pathogen was inoculated and bacterial antagonist was streaked in the center of the plate. Each treatment was replicated three times. After required period of incubation i.e., in the control plate growth reached 90 mm diameter, the radial growth of pathogen was measured.

Per cent inhibition over control was calculated by applying the formula given.

$$R = \{(C - T) /C\} \times 100$$

Where
R= Per cent inhibition,
C = Radial growth of pathogen colony in control and
T = Radial growth of pathogen colony in treatment

Details of experiment

Design: CRD
Replications: Three
Treatments: Eight

Treatment details

In vitro* evaluation of bioagents against *Pyricularia grisea

Treatment details

Treatment	Bioagents
T_1	*Pseudomonas fluorescens*
T_2	*Trichoderma harzianum*
T_3	*Trichoderma viride*
T_4	*Trichoderma gliocladium*
T_5	*Trichoderma hamatum*
T_6	*Trichoderma koningi*
T_7	*Bacillus subtilis*
T_8	Control

Observations on radial mycelial growth of the test fungus and bio control were recorded at 24 hrs interval and were continued till growth of the test pathogen in untreated control plate was fully covered. Per cent inhibition of the test pathogen was calculated by applying formula given.

$$R = \{(C - T) / C\} \times 100$$

Where,
R= Per cent inhibition
C= growth of the test pathogen in untreated Control plates
T= growth of the test pathogen a in treated plates

Results

In this study, pure culture of bio-agents *Pseudomonas fluorescens Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma* (*Gliocladium*) *virens*, *Trichoderma hamatum*, *Trichoderma koningi* and *Bacillus subtilis* obtained from Department of Plant Pathology, VNMKV, Parbhani, (Maharashtra). In the present study, the effects of six known antagonists were tested against *P. grisea* by dual culture.

Table 1: *In vitro* efficacy of bioagents against *Pyricularia grisea*

Sr. No	Treatments	Colony Dia*(mm)	% Inhibition
T_1	*Pseudomonas fluorescens*	32.00	64.61 (53.49)
T_2	*Trichoderma harzianum*	38.67	57.03 (49.04)
T_3	*Trichoderma viride*	34.00	62.39 (52.17)
T_4	*Trichoderma gloicladium*	71.33	20.74 (27.09)
T_5	*Trichoderma hamatum*	42.67	52.59 (46.48)
T_6	*Trichoderma koningi*	39.00	56.66 (48.83)
T_7	*Bacillus subtilis*	47.33	47.40 (43.51)
T_8	Control	90.00	0.00 (0.00)
S.E(m) ±		0.80	0.89
C.D (P=0.01)		2.42	2.70

*Mean of three replication, Dia: Diameter
Figures in parenthesis are Aresin transformed value

The data presented in (Table No 1, Fig. 1 and plate 1) revealed that all the antagonists were found significantly superior in checking the growth of the pathogen over control. Out of seven antagonists tested, significantly least growth of the pathogen was recorded in *Pseudomonas fluorescens* (32.00 mm). However the efficiency of *Trichoderma viride* (34.00 mm) was statistically at par with that of *Pseudomonas fluorescens*. Next best in order of efficiency were *Trichoderma harzianum* (38.67 mm), *Trichoderma koningi* (39.00 mm), *Trichoderma hamatum* (42.67 mm) and *Trichoderma* (*Gliocladium*) *virens* (71.33 mm).

It is evident from these studies that among all the antagonists evaluated by dual culture technique, *Pseudomonas fluorescens* (64.61%) showed maximum growth inhibition as an antagonistic and *Trichoderma viride* (62.39%) was found statistically at par with the *Pseudomonas fluorescens* followed by *Trichoderma harzianum* (57.03%), *Trichoderma koningi* (56.66%), *Trichoderma hamatum* (52.59%) *and Trichoderma (Gliocladium) virens*, (20.74%) *inhibit growth of P. grisea.* (Table No1, Fig 2 1and plateV1).Similar work carried out by Gnanamanickam *et al*., (1990) when he experimently found that *Pseudomonas fluorescens* strain was most effective against *P. oryzae* Cav. as it produced fluorescent antiblast antibiotics in culture which inhibited the conidial germination of *P. oryzae* Cav. *in vitro*.

Plate I: *In vitro* efficacy of various bio-agents against *P. grisea*

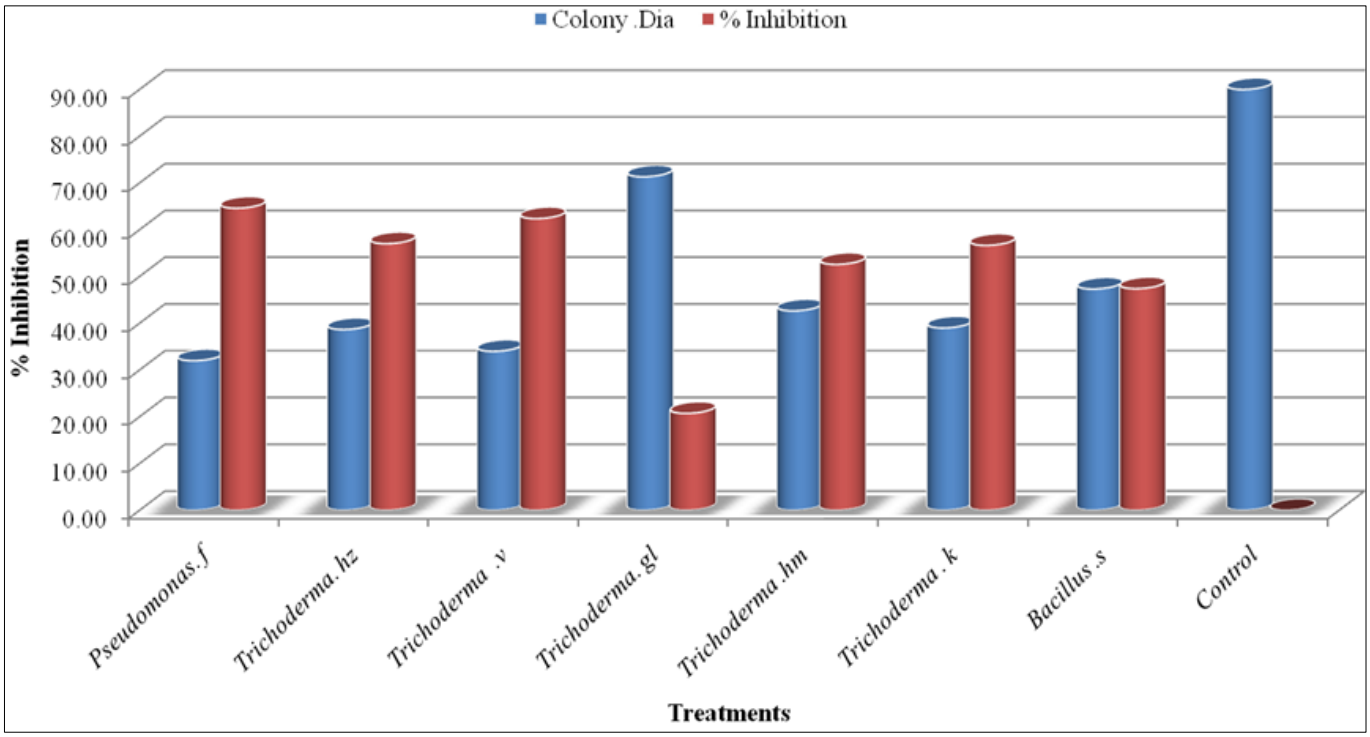

Fig 1: Effect of Bioagents on Mycelial Growth Inhibition of *P.grisea.*

Conclusion

From the present study, it may be concluded that, in biological control, *P. flurosence* was found most significant.

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