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In vitro Evaluation of Potential Bio Agents against *Pyricularia setariae* causing Blast of Foxtail Millet

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ABSTRACT: Next to finger millet, foxtail millet is the most significant millet crop. The crop is prone to many biotic and abiotic stresses among them, leaf blast caused by *Pyricularia setariae* is an important disease causing considerable yield loss. The method of managing the disease with chemical fungicides has various drawbacks, such as the emergence of resistance, contamination of the environment, impact on non-target creatures, and potential health risks to humans. An attempt was made to evaluate potential fungal and bacterial bio agents collected from different places against *Pyricularia setariae* under laboratory conditions. Among them, Tv3 strain of *Trichoderma viride* showed highest mycelial growth inhibition (67.19 per cent). Next in the order was Th56 strain of *T. harzianum* with 66.79 per cent inhibition. Among the fungal bio agents, Th41, ThGJ16B, Th4B strain of *T. harzianum* were on par. Least inhibition of mycelial growth was observed in ThB2 strain of *T. harzianum* (56.91 per cent). Among the bacterial bio control agents evaluated, P42 strain of *Bacillus velezensis* exhibited highest percent of mycelial growth inhibition (33.72 per cent). Next in the order was P42 strain of *Pseudomonas fluorescens* (27.16 per cent) and least inhibition was observed in *B. pumilis* (21.07 per cent).

Keywords: Foxtail millet, Blast, *Pyricularia setariae*, Bio agents.

INTRODUCTION

Foxtail millet {*Setaria italica* (L.) Beauv.} is next to the finger millet, as an important small millet crop. Foxtail millet which belongs to family *Poaceae*, is one of the earliest cereal crops and a significant economic crop (Rajesha *et al.*, 2021). It is indigenous to China. (Vavilov, 1926). It is having other names *viz.*, Italian millet, German millet, Chinese millet and Hungarian millet (Baker, 2003). Currently ranking second in the world for millet production, it continues to be a major player in agriculture worldwide, feeding millions of people on roughly six million tons of grain each year, mostly on marginal or poor soils in Southern Europe and temperate, subtropical, and tropical Asia (Marathee, 1993). About one million hectares are used to grow foxtail millet in India; these areas are primarily in northern Karnataka, along with portions of coastal Andhra Pradesh, Uttarakhand, Tamil Nadu, and several northeastern provinces (Mallikarjuna *et al.* (2020b). Many diseases such as blast (*Pyricularia setariae*), brown spot (*Drechslera setariae*), rust (*Uromyces setariae*), downy mildew (*Sclerospora graminicola*) and smut (*Ustilago crameri*) challenge the production of foxtail millet. Blast is the most destructive disease among them. On leaf blades, the disease's symptoms manifest as circular spots with straw-colored centres. The specks have a dark brown edge surrounding them and range in size from 2 to 5 mm. They are tiny and dispersed. When the disease manifests itself in a serious manner in humid weather, in particular, the leaves dry out and wither in a dense plant stand (Mallikarjuna *et al.*, 2020a). It causes severe yield losses of up to 30-40 per cent (Nagaraja *et al.*, 2007).

Farmers have been using chemicals alone to control insect pests and diseases, because of quick and effective control (Prajapati *et al.*, 2020). Apart from advantages, several disadvantages prevail with which such method include development of resistance, environment pollution, effect on non target organisms and health hazards in human beings. Besides, farmers are using unscrupulous materials and practicing unscientific usages, indiscriminate doses and fall short in timely application which is a dangerous scenario. Food products that are healthy and environmentally friendly are in high demand these days. In addition to reducing the need for fungicides, which can lower fungicide contamination and simultaneously encourage plant growth, biocontrol agents will open the door to

sustainable agriculture (Chakraborty *et al.*, 2021). So, now the scientists are looking towards use of bio control agents to manage diseases. Certainly, bio control agents have advantages over chemical method of application. They are safer to non target organisms, no resistance development, ecofriendly, no health hazards, economical and no residue problems. Therefore, bio control agents are the best alternative for management of diseases. Hence, extensive research is being carried out to manage blast disease in different crops using biocontrol agents (Rawat *et al.*, 2022; Patro *et al.*, 2020; Sobanbabu *et al.*, 2020; Kumar *et al.*, 2017). Despite the fact that the disease has become more severe recently and has resulted in a significant loss in yield, nothing is understood about the biology of the pathogen and how to manage it, particularly with regard to use of different fungal and bacterial bio control agents for management of leaf blast disease. By keeping these things in mind, the present investigations were undertaken by using different fungal and bacterial biocontrol agents to manage *Pyricularia setariae* under laboratory conditions.

MATERIAL AND METHODS

A. In vitro evaluation of bio-agents against Pyricularia setariae causing leaf blast of foxtail millet

By using the dual culture method, an *in vitro* assessment of the bio agents was conducted against the pathogen (Table 1). In the dual culture technique, 20 mL of sterilized and cooled PDA was poured into sterile Petri plates. By putting the pathogen on one side of a Petri plate and the antagonist on the other, with a space of three to four centimetres, fungal antagonists were assessed. A similar procedure was used to identify bacterial antagonists: a fungal disc was placed on one end of a Petri plate, and bacterial antagonistic was streaked on the other. Three duplicates of each treatment were conducted. The pathogen's radial growth was assessed ten days after the incubation period. Using Vincent's equation (1947), the percentage inhibition over control was calculated

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

Where, I = Per cent inhibition of mycelium
C= Growth of mycelium in control
T = Growth of mycelium in treatment

Table 1: List of bio agents used for *in vitro* evaluation against foxtail millet leaf blast pathogen.

Sr. No.	Bio-agents	Strain No.	Source
Fungal bio-agents			
1	*Trichoderma viride*	Tv3	Plant Pathology lab, UAS, GKVK, Bengaluru
2	*T. viride*	Tv8	Plant Pathology lab, IIHR, Bengaluru
3	*T. harzianum*	Th14	Plant Pathology lab, UAS, GKVK, Bengaluru
4	*T. harzianum*	Th41	Plant Pathology lab, UAS, GKVK, Bengaluru
5	*T. harzianum*	Th56	Plant Pathology lab, UAS, GKVK, Bengaluru
6	*T. harzianum*	ThB2	Plant Pathology lab, UAS, GKVK, Bengaluru
7	*T. harzianum*	Th4B	Plant Pathology lab, IIHR, Bengaluru
8	*T. harzianum*	ThGJ 16B	Plant Pathology lab, IIHR, Bengaluru
Bacterial bio-agents			
9	*Pseudomonas fluorescens*	-	Bacteriology lab, UAS, GKVK, Bengaluru
10	*Bacillus velezensis*	P42	Bacteriology lab, UAS, GKVK, Bengaluru
11	*P. fluorescens*	P42	Dept. of Agril. Microbiology, UAS, GKVK, Bengaluru
12	*B. pumilus*	-	Dept. of Agril. Microbiology, UAS, GKVK, Bengaluru

RESULTS AND DISCUSSION

A. In vitro evaluation of bio agents against *Pyricularia setariae*

The eight different fungal antagonists *viz.*, *T. harzianum*, *T. viride* and their strains were collected from Department of Plant Pathology, GKVK and Plant Pathology lab IIHR. The bio effectiveness of these biocontrol agents was assessed *in vitro* against *P. Setariae* using the dual culture method. Among all *T. viride* strain Tv3 exhibited the greatest suppression of mycelial development (67.19%). Next in the order was Th56 strain of *T. harzianum* with 66.79 per cent inhibition. Among these Th41, ThGJ16B, Th4B strain of *T. harzianum* were found on par with each other with respect to mycelial growth inhibition. ThB2 strain of *T. harzianum* showed the least amount of suppression of mycelial development (56.91%).

Using the dual culture approach, four distinct bacterial bio control agents were evaluated against *P. setariae*. The *Bacillus velezensis* P42 strain exhibited the highest percentage of inhibition of mycelial growth (33.72%) when compared to other bacterial biocontrol agents. Next in the order was P_{42}strain of *Pseudomonas fluorescens* which inhibited mycelial growth of 27.16 per cent and the minimum inhibition of growth observed in *Bacillus pumilus* (21.07%) (Table 2, Fig. 1 and Plate 1).

The use of bioagents in IDM methods to manage diseases is a key strategy that is now being utilized in the organic farming system. Thus, under *in vitro* conditions, various *Trichoderma* spp. and bacterial biocontrol agents were assessed against *P. setariae* in the current study. It was discovered that *Trichoderma* spp. was efficient against the fungus and that the pathogen's hyphae were coiling as a result of *Trichoderma*'s action to impede mycelial growth (Ali and Nadarajah 2014). The fungal cell wall may be broken down by the strong endo chitinase activity of the cell wall-degrading enzyme generated by

Trichoderma spp. as reported by Kalaivani *et al.* (2014). Similarly, Somashekhar Konda *et al.* (2016); reported that *Trichoderma species* worked well to combat *P. setariae.*

Table 2: *In vitro* evaluation of bio agents against *P. setariae* causing blast of foxtail millet.

Sr. No.	Bio control agents	Strain	Per cent inhibition of mycelial growth
1.	*Trichoderma harzianum*	Th41	61.97 (51.93) *
2.	*T. harzianum*	Th14	58.64 (49.96)
3.	*Trichoderma viride*	Tv8	59.17 (50.28)
4.	*T. harzianum*	ThGJ 16B	61.23 (51.47)
5.	*T. harzianum*	ThB2	56.91 (48.95)
6.	*T. harzianum*	Th56	66.79 (54.79)
7.	*T. harzianum*	Th4B	61.35 (51.55)
8.	*T. viride*	Tv3	67.19 (55.08)
9.	*Pseudomonas fluorescens*	-	23.70 (29.10)
10.	*Bacillus velezensis*	P42	33.72 (35.16)
11.	*P. fluorescens*	P42	27.16 (31.38)
12.	*B. pumilus*	-	21.07 (31.38)
	S.Em±		2.25
	CD (p 0.01)		6.56

Note: *Figures in the parenthesis are arc sine transformed values

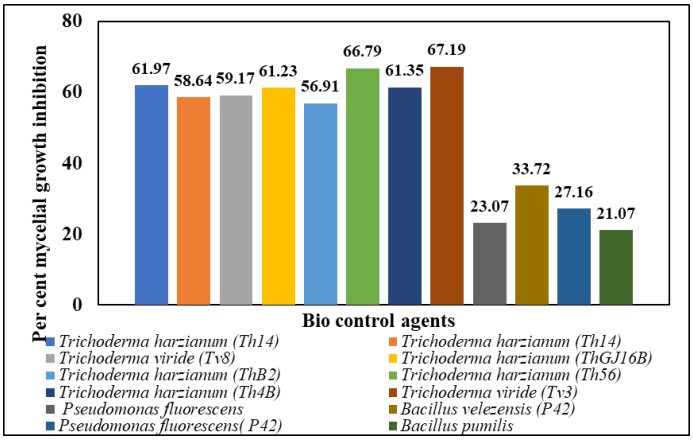
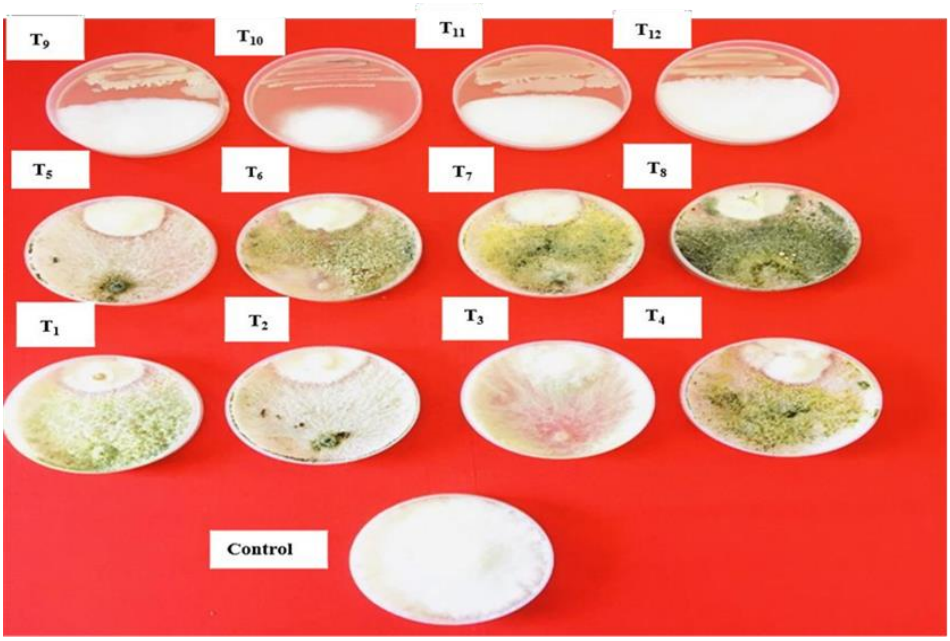


Fig. 1. *In vitro* evaluation of bio-agents against *P. setariae* by dual culture technique.



T_1 - *Trichoderma harzianum (Th41);* T_2 - *Trichoderma harzianum (Th14);* T_3 - *Trichoderma viride (Tv8;);* T_4 - *Trichoderma harzianum (ThGJ 16B);* T_5 - *Trichoderma harzianum (ThB2);* T_6 - *Trichoderma harzianum (Th56)* ; T_7 - *Trichoderma harzianum (Th48)*; T_8 - *Trichoderma viride (Tv3)*; T_9 - *Pseudomonas fluorescens;* T_{10} - *Bacillus velezensis (P42);* T_{11} - *Pseudomonas fluorescens (P42)* ; T_{12} - *Bacillus pumilis*

Plate 1. *In vitro* evaluation of bio agents against *P. setariae* by dual culture technique.

Arumugam *et al.* (2013); Hajano *et al*. (2012) were among the other researchers who reported that *Trichoderma* spp. was highly effective against *P. oryzae*. Additionally, Karthikeyan and Gnanamanickam (2008) discovered that *P. setariae* growth was inhibited by *Bacillus polymyxa* VLB-17 and *P. fluorescens* Pf-52, resulting in 2.5 and 3.0 cm of growth, respectively. Bacterial endophytes of *Bacillus* spp. were discovered to be effective against *P. setariae* in this study.

CONCLUSIONS

By using the dual culture method, the biocontrol agents' effectiveness against *P. setariae* was assessed *in vitro*. The Tv3 strain of *Trichoderma viride* exhibited the highest percentage of suppression of mycelial growth (67.19%). Next in the order was Th56 strain of *T. harzianum* with 66.79 per cent inhibition. Among these Th41, ThGJ16B, Th4B strain of *T. harzianum* were on par with each other. ThB2 strain of *T. harzianum* showed the least amount of suppression of mycelial development (56.91 percent).

The dual culture approach was used to test four distinct bacterial bio control agents against *P. setariae*. The P42 strain of *Bacillus velezensis* exhibited the highest percentage of suppression of mycelial growth (33.72%) of all the bacterial biocontrol agents that were examined. The P42 strain of *Pseudomonas fluorescens* (27.16 percent) came next in the ranking, whereas *B. pumilis* (21.07 percent) showed the least inhibition of mycelial growth.

FUTURE SCOPE

The results obtained from the present laboratory studies, helps in choosing the potential fungal and bacterial bio control agents to use in field conditions for the management of blast of foxtail millet. Further, integrated disease management of blast of foxtail millet will be formulated by using novel fungicides, bio agents and botanicals for the management of *P. setariae* under field condition.

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