

Original Research Article

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Invitro Evaluation of Bio Control Agents against Blast of Foxtail Millet Caused by *Pyricularia setariae*

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ABSTRACT

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Foxtail millet is a highly nutritious crop affected by several diseases, of which leaf blast is an important disease, hindering productivity. The present study was conducted to evaluate the *in vitro* efficacy of different bio control agents. Fourteen bio control agents which includes five bacterial, five bacterial endophytes and four fungal are evaluated under *in vitro* condition by using dual culture and volatile method. Among these P₄₂ strain of *Bacillus velezensis* showed cent per cent inhibition of mycelial growth followed by bacterial endophyte *Enterobacter cloacae* (56.80 %) in dual culture method whereas Th14 strain of *T. harzianum* shows highest mycelial growth inhibition (77.6 %) followed by Th55 strain (76.81 %) in volatile method.

Introduction

Foxtail millet [*Setaria italica* (L.) Beauv.] is an ancient cultivated crop and most economically important species of the genus *Setaria* belongs to family poaceae and Native to China (Vavilov, 1926). This crop has high importance as it is a rich source of nutrients and grown for both food and fodder purpose. It is also known by several other names such as German millet, Italian millet, Chinese millet and Hungarian millet (Baker, 2003). It ranks 2nd in the total world production of

millet and continues to have an important place in the world agriculture providing around six million tons of food to millions of world population, mainly on marginal or poor soils in Southern Europe and in temperate, subtropical and tropical Asia (Marathee, 1993). It is widely grown throughout Africa, China, India, Russia, and the United States.

In India foxtail millet is grown on about 1 million ha, mainly in northern Karnataka, parts of costal Andhra Pradesh, Uttarakhand, Tamil Nadu, and some parts of the

northeastern states. The grain is used as both food and fodder. It is a good source of carbohydrate, protein and essential amino acids and it is a very good dietary component for diabetic and heart patients because it contains magnesium (Marathee, 1993). The grains are good source of protein, minerals (calcium, iron, potassium, magnesium and zinc) and vitamins (Rai, 2002). It is widely used as an energy source for pregnant, lactating women, sick people and children (Sema and Sarita, 2002). It has got medicinal value as it is used as curative for rheumatism and measles (Wright and Finch, 1962) and also it has been suggested that foxtail millet is used as a food component to cardiovascular diseases and type 2 diabetes (Choi *et al.*, 2005).

Although foxtail millet has high nutritional importance and grown for both food and fodder purpose, the crop is affected by several biotic and abiotic constraints. Among the biotic constraints fungal diseases like leaf blast, brownspot, rust, downy mildew and bacterial diseases like bacterial streak are limiting the production of the crop. Among these diseases, blast caused by the fungus *Pyricularia setariae* Sacc. (teleomorph: *Magnaporthe setariae*) is the most destructive disease and affects both forage and grain production of foxtail millet. Symptoms of the disease appear as circular spots with straw colored centers on leaf blades.

The spots are small and scattered, 2 to 5 mm in diameter and surrounded by a dark brown margin. When the disease appears in severe form during humid weather conditions, especially with a dense plant stand, the leaves wither and dry. Plants are infected at all growth stages of crop growth (Gaikwad, and D' Souza, 1987); lower leaves are the most severely affected. Recognizing the importance of foxtail millet and the constraint caused by

the leaf blast disease, the present study was planned to evaluate different bio control agents under *in vitro* condition to generate primary data on effective bio control agents against *P. setariae* and identified potential bio control agents will be used in designing Integrated Disease Management strategies for management of blast there by production and productivity of the crop will be enhanced.

Materials and Methods

In vitro evaluation was carried out with bio agents (Table.1) through dual culture and volatile method.

Dual culture technique

In the dual culture technique, sterilized PDA media was cooled and about 20 mL of media was poured into sterile Petri plates. Fungal antagonists were evaluated by inoculating the *P. setariae* at one peripheral end of Petri plate and the antagonist on the opposite peripheral end of the same plate by leaving 3 to 4 cm gap. Similarly, for bacterial antagonists fungal disc was placed at one peripheral end of Petri plate and bacterial antagonists were streaked at other end of same plate. Each treatment was replicated three times. After 10 days of incubation period the radial growth of pathogen was measured. Per cent inhibition over control was worked out according to the equation of Vincent (1947).

Volatile metabolites

The efficacy of all fourteen bio control agents were evaluated against *P. setariae* on Potato dextrose agar medium to test inhibitory effect of volatile compounds under *in vitro* conditions.

For this study 20 mL of PDA was poured into each Petri dish. A 5 mm diameter agar disc from the two days old pure culture of each of

fungus bio control agent culture was placed at centre of each agar plate. Similarly, bacterial bio control agents were streaked on previously poured Petri dish containing PDA. Afterwards the disk of same size was taken from *P. setariae* culture and placed in another agar plate. The lids were removed and test fungus culture plate was immediately placed over each of bio control agents plates held together with adhesive tapes.

The space prevented any physical contact between pathogen and bio control agent, so that the volatile compounds were formed and confined to the interior atmosphere of the two plates. For the control plate, only *P. setariae* culture placed on PDA plate. Each treatment was replicated three times. After 10 days of incubation period the radial growth of pathogen was measured. Per cent inhibition over control was worked out according to the equation of Vincent (1947).

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

Where, I = Per cent inhibition of mycelium

C= Growth of mycelium in control

T = Growth of mycelium in treatment

Statistical analysis

The data generated by different experiments were analyzed using the WASP software developed by ICAR- Central Coastal Agricultural Research Institute, Goa and the inferences were made with a probability of one per cent.

Results and Discussion

The fungal antagonists such *Trichoderma harzianum*, *T. viride* and their strains were collected from Mycology lab Department of Plant Pathology, GKVK. These bio control agents were evaluated against *P. setariae*

under *in vitro* condition for their efficacy by using dual culture technique as described in material and methods. Among these *Th14* strain of *T. harzianum* showed highest mycelial growth inhibition (57.70 %) followed by *ThB5* strain of *T. harzianum* (42.82 %) and least mycelial growth inhibition was observed in *Th55* strain. *T. harzianum*.

Ten different bacterial bio control agents were tested against *P. setariae* by using dual culture method. Among these bacterial bio control agents *P₄₂* strain of *Bacillus velezensis* showed cent per cent inhibition of mycelial growth followed by bacterial endophyte *Enterobacter cloacae* (56.80 %) and least inhibition of mycelial growth observed in *Bacillus megatorium* which accounted 2.07 per cent (Table 2, plate 1, fig 1).

Volatile Organic Compounds (VOC) produced by microorganisms played an important role during their evolution while interaction with other organisms. Such interaction results in functional response by the organism which involved in coincidental disadvantage to other microorganisms. Hence the effect of volatile compounds produced by all fourteen bio control agents was tested against *P. setariae* by using volatile method as described in material and methods (section 3.5.1B). Among these bio control agents highest mycelial growth inhibition recorded in *Th14* strain of *T. harzianum* (77.6 %) followed by *Th55* strain (76.81 %) and least inhibition of mycelia was observed in *Bacillus megatorium* (38.45 %) (Table 2, plate 2, fig 2).

Utilization of bio control agents for the management of diseases is the important strategy in IDM practices and currently has important role in the organic farming system. So, in the present study, different *Trichoderma* spp and bacterial bio control

agents were evaluated against *P. setariae* under *in vitro* conditions. The *Trichoderma* spp. were found more effective against the fungus and it is reported that inhibition of the mycelial growth was due to coiling of hyphae of the pathogen by *Trichoderma* (Ali and Nadarajah 2014).

It may also due to the cell wall degrading enzyme produced by *Trichoderma* spp. which has high endochitinase action that results in breakdown of the cell wall (Chitin) as reported by Kalaivani *et al.*, (2014). Fuji *et al.*, (1978) and Vinale *et al.*, (2008) he also

reported that secondary metabolites produced by *Trichoderma* spp. such as antibiotics (6-pentyl-alpha-pyrone (6pp), isocyanide derivatives), acids (heptelidic and koningic acid) and peptaibols that are resulted in the inhibition of radial growth of numerous plant pathogenic fungi. Similarly, Somashekhar Konda (2015) reported that *Trichoderma* spp. were effective against *P. setariae*. Several other workers (Watanabe, 1985; Gouramanis, 1997; Hajano *et al.*, 2012 and Arumugam *et al.*, 2013) also described that *Trichoderma* spp. were very effective against *P. oryzae*.

Table.1 Bio agents used for *in vitro* evaluation against the *P. setariae*

SI No.	Bio agents	Strain/ Isolate	Source
1	<i>Bacillus subtilis</i>	-	Bacteriology lab, UAS, GKVK, Bengaluru
2	<i>B. megatarium</i>	-	Microbiology lab, UAS, GKVK, Bengaluru
3	<i>Pseudomonas fluorescens</i>	-	Bacteriology lab, UAS, GKVK, Bengaluru
4	<i>B. velezensis</i>	P ₄₂	Bacteriology lab, UAS, GKVK, Bengaluru
5	<i>B. velezensis</i>	A ₆	Bacteriology lab, UAS, GKVK, Bengaluru
6	<i>B. cereus</i>	GPUR-10	Bacteriology lab, UAS, GKVK, Bengaluru
7	<i>Bacillus spp.</i>	GPUR-12	Bacteriology lab, UAS, GKVK, Bengaluru
8	<i>Enterobacter cloacae</i>	GPUL-19	Bacteriology lab, UAS, GKVK, Bengaluru
9	<i>Pennibacillus polymyxa</i>	GPUS-13	Bacteriology lab, UAS, GKVK, Bengaluru
10.	<i>B. mojarensis</i>	UMR-9	Bacteriology lab , UAS, GKVK, Bengaluru
11	<i>Trichoderma viride</i>	Tv ₁	Mycology lab, UAS, GKVK, Bengaluru
12	<i>T. harzianum</i>	Th ₁₄	Mycology lab, UAS, GKVK, Bengaluru
13	<i>T. harzianum</i>	ThB ₅	Mycology lab, UAS, GKVK, Bengaluru
14	<i>T. harzianum</i>	Th ₅₅	Mycology lab, UAS, GKVK, Bengaluru

Table.2 *In vitro* efficacy of bio agents against *P. setariae*

Sl No.	Bio control agents	Per cent. inhibition of mycelial growth	
		Dual culture method	Volatile method
1	<i>Bacillus subtilis</i>	16.26 (23.77)	56.74 (48.87)
2	<i>B. megatarium</i>	2.07 (8.26)	38.45 (38.32)
3	<i>B. velezensis</i> (A ₆)	48.31 (44.03)	62.47 (52.22)
4	<i>Pseudomonas fluorescens</i>	22.72 (28.46)	61.88 (51.87)
5	<i>B. velezensis</i> (P ₄₂)	100.00 (90.00)	61.13 (51.43)
6	<i>Bacillus spp.</i> (GPUR-12)	55.56 (48.19)	58.93 (50.14)
7	<i>B. cereus</i> (GPUR-10)	45.22 (42.25)	53.14 (46.80)
8	<i>B. mojarensis</i> (UMR-9)	50.94 (45.54)	54.59 (47.63)
9	<i>Enterobacter cloacae</i> (GPUL-19)	56.80 (48.90)	50.88 (45.50)
10	<i>Pennibacillus polymyxa</i> (GPUS-13)	38.46 (38.32)	61.31 (51.54)
11	<i>Trichoderma viride</i> (Tv1)	38.63 (38.43)	64.65 (53.52)
12	<i>T. harzianum</i> (Th14)	57.70 (49.43)	77.6 (61.75)
13	<i>T. harzianum</i> (ThB5)	42.82 (40.60)	66.65 (54.73)
14	<i>T. harzianum</i> (Th55)	38.08 (38.10)	76.81 (61.21)
	Control	0.0	0.0
	SEm±	2.01	0.21
	CD(0.01)	6.02	0.69

Among the different bacterial bio control agents, maximum mycelial growth inhibition (100 %) was showed by P₄₂ stain of *Bacillus velezensis* followed by bacterial endophyte *Enterobacter cloacae* (56.80 %). Similarly, Karthikeyan and Gnanamanickam (2008) also found that the growth of *P. setariae* was 3.0 and 2.5 cm due to inhibition of *Pseudomonas fluorescens* and Pf-52 *Bacillus polymyxa* VLB-17 respectively. In this study, bacterial endophytes of *Bacillus species* were also

found effective against *P. setariae* this was due to the activity of antibiotic like substance. *Bacillus* produces different antibiotic substances that are effective against many fungi such as Zwittermycin-A (He *et al.*, 1994), Kanamycin, lipopeptida and fengycin (Stabb *et al.*, 1994). Mubariket *et al.*, (2010) reported chitinase activity of *Bacillus cereus*. Harman (2000) and Leelasuphakul *et al.*, (2006) also found 60 per. cent inhibition of *P. grisea* by *B. subtilis* strain NSRS 89-24i.

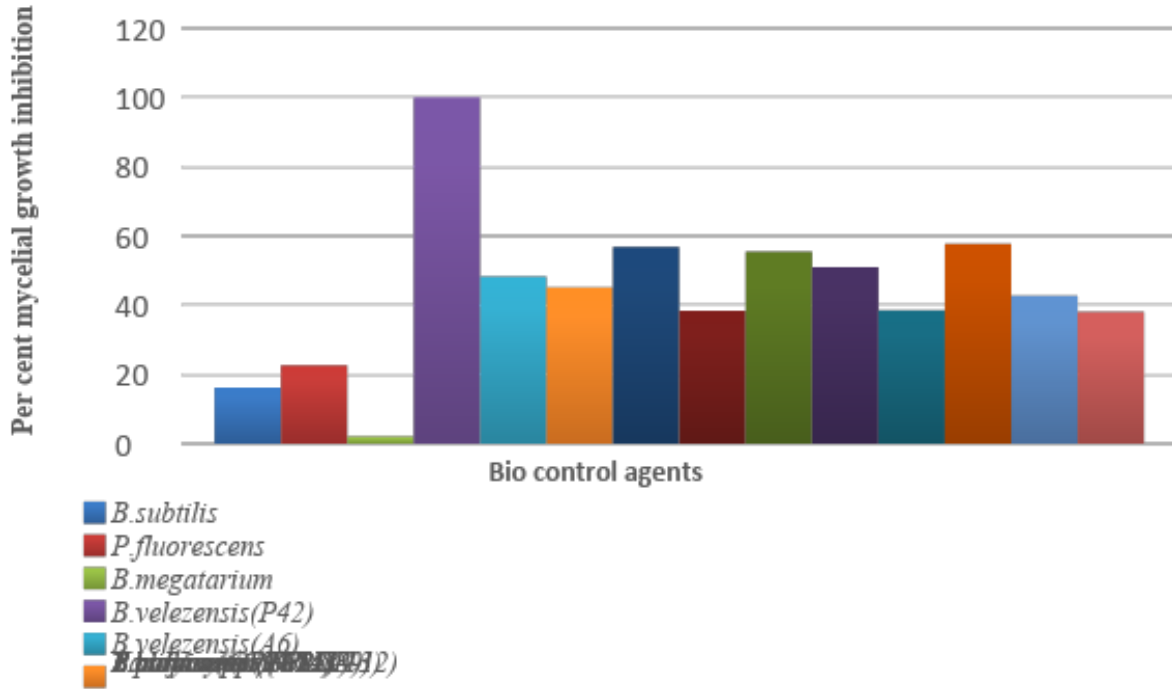


Fig.1 Effect of bio agents on the mycelial growth inhibition of *P.setariae* (Dual culture technique)

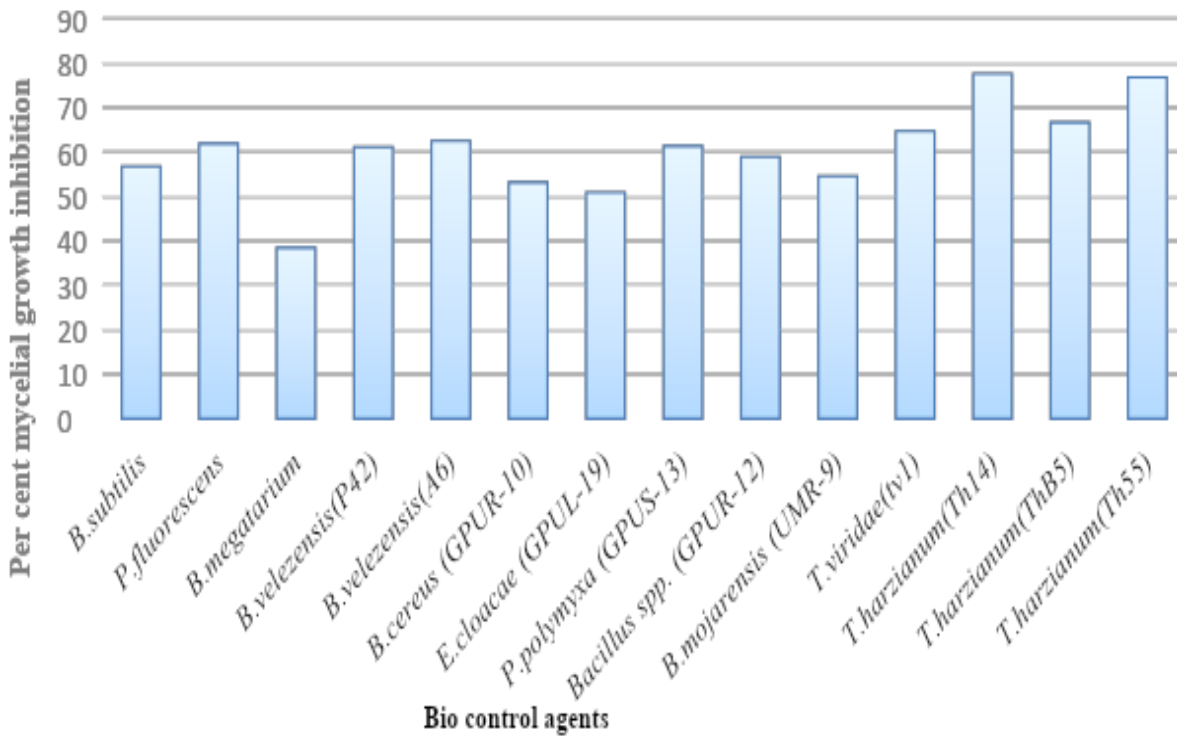


Fig.2 Effect of bio agents on the mycelial growth inhibition of *P.setariae* (Volatile metabolites)

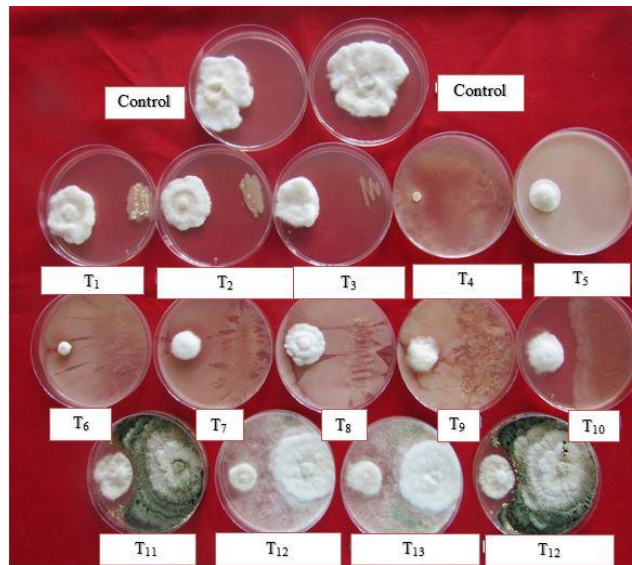


Plate.1 Efficacy of bio agents on inhibition of mycelial growth of *P.setariae* (Dual culture technique)

Note: Figures in the parenthesis are arc sine transformed values

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|--|--|--|
| T ₁ - <i>Bacillus subtilis</i> . | T ₂ - <i>Pseudomonas fluorescens</i> | T ₃ - <i>B. megatarium</i> |
| T ₄ - <i>B. velezensis</i> (P ₄₂) | T ₅ - <i>B. velezensis</i> (A6) | T ₆ - <i>Enterobacter cloacae</i> (GPUL-19) |
| T ₇ - <i>B. cereus</i> (GPUR-10) | T ₈ - <i>Pennibacillus polymyxa</i> (GPUS-13) | T ₉ - <i>Bacillus spp.</i> (GPUR-12) |
| T ₁₀ - <i>B. mojarensis</i> (UMR-9) | T ₁₁ - <i>Trichoderma virida</i> (Tv1) | T ₁₂ - <i>T. harzianum</i> (Th14) |
| T ₁₃ - <i>T. harzianum</i> (ThB5) | T ₁₄ - <i>T. harzianum</i> (Th55) | |

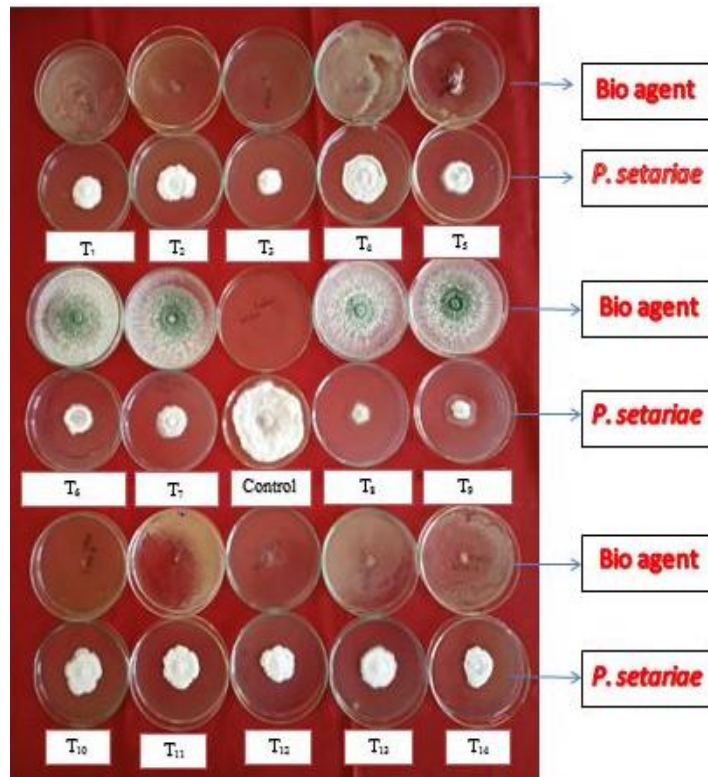


Plate.2 Efficacy of bio agents on inhibition of mycelial growth of *P.setariae* (Volatile method)

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|--------------------------------------|--|--|
| T1- <i>Pseudomonas fluorescens</i> . | T2- <i>Bacillus velezensis</i> (P42) | T3- <i>Bacillus velezensis</i> (A6) |
| T4- <i>B. megatarium</i> | T5- <i>B. subtilis</i> | T6- <i>T. harzianum</i> (ThB5) |
| T7- <i>Trichoderma viridae</i> (Tv1) | T8- <i>T. harzianum</i> (Th14) | T9- <i>T. harzianum</i> (Th55) |
| T10- <i>B. cereus</i> (GPUR-10) | T11- <i>B. mojarensis</i> (UMR-9) | T12- <i>Enterobacter cloacae</i> (GPUL-19) |
| T13- <i>Bacillus spp.</i> (GPUR-12) | T14- <i>Pennibacillus polymyxa</i> (GPUS-13) | |

Among fourteen biocontrol agents evaluated P₄₂ strain of *Bacillus velezensis* showed cent per cent inhibition of mycelial growth followed by bacterial endophyte *Enterobacter cloacae* and least inhibition of mycelial growth observed in *Bacillus megatorium*.

Among fungal antagonists Th14 strain of *Trichoderma harzianum* showed highest mycelial growth inhibition followed by ThB5 strain of *T. harzianum* and least mycelial growth inhibition was noticed in Th55 strain of *T. harzianum*. Identified potential bio control agents will be used in designing Integrated Disease Management strategies for management of blast there by production and productivity of the crop will be enhanced.

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