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RESEARCH ARTICLE

Efficacy and mode of actions of biocontrol agents against *Alternaria alternata* causing leaf blight disease in *Anthurium*

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Abstract

For the eco-friendly management of the Alternaria leaf blight disease, bacterial and fungal biocontrol agents isolated from the rhizosphere of *Anthurium* were compared for their efficacy based on the mechanisms of biocontrol. The effective isolates were formulated and tested against leaf blight of anthurium. Seven isolates of fluorescent pseudomonads, 6 isolates of *Bacillus* spp. and 4 isolates of *Trichoderma viride* was tested against *Alternaria alternata*. Among the biocontrol agents tested, isolates Pf1, BsM3 and Tv2 was found to be highly antagonistic to *A. alternata in vitro*. Among 13 isolates, Pf1, BsM3 and Tv2 showed the strongest *in vitro* antagonism. Molecular characterisation confirmed the identity of rhizobacterial isolates. Isolates of *Pseudomonas fluorescens*, *Bacillus subtilis* and *T. viride* produced siderophore *in vitro*. The isolates CFP1 and Pf1 were found to produce salicylic acid (SA), indole-3 acetic acid (IAA) and ammonia. In pot and field experiments, soil application of coir pith-vermicompost formulation of Pf1 (250 g/plant) followed by 3 foliar sprays of the liquid formulation (0.2 %) reduced disease severity by approximately 55 % (pot culture) and 48 % (field) relative to the control. The results demonstrate the potential of Pf1-based formulations as effective and eco-friendly options for managing Alternaria leaf blight in *Anthurium*.

Keywords: alternaria leaf blight; biocontrol agents; *in vitro;* rhizobacteria

Introduction

Anthurium (*Anthurium andraeanum* Linden Ex André) is a slow-growing perennial that requires shady, humid conditions as found in tropical forests. *Anthurium* belongs to the family Araceae and is native of tropical zones of Central and South America. The name *Anthurium* is derived from the Greek word 'anthos' meaning flower and 'oura' meaning tail, referring to the spadix. Diseases appear to be the major constraint to the production of *Anthurium*. Among the diseases, anthracnose or blacknose (spadix rot) caused by *Colletotrichum gloeosporioides* (Penz) Sacc. (1) and bacterial blight caused by *Xanthomonas campestris* pv. *dieffenbachiae* Vauterin (2) are found to be serious worldwide causing economic losses in terms of quality and quantity. Viruses viz., Tomato spotted wilt virus (3) and Dasheen mosaic virus (4) and a leaf spot incited by *Phomopsis anthuri* Henn (5) are the other pathogens recorded in *Anthurium*.

The wide use of fungicides to manage plant diseases is known to cause undesirable effects such as residual toxicity, environmental pollution, health hazards to humans and animals. To modify this condition alternative methods of control are adopted. Management of plant diseases through biological method envisages the use of a few genera of bioagents viz., *Trichoderma, Pseudomonas* and *Bacillus*. Though biocontrol agents are successfully employed in controlling soil borne pathogens, in recent years their commercial formulations are being applied to manage many foliar diseases under greenhouse and field conditions. Biological control of plant pathogens and deleterious microbes through the production of antibiotics, lytic enzymes, hydrogen cyanide (HCN) and siderophore or through competition for nutrient and space can significantly improve plant health and promote growth by increasing seedling emergence, plant vigour and yield (6). Because of these reasons, biological control which has a limited impact on environment is an alternative to chemical control.

Most tests conducted for the identification of bacteria are based on physiology and nutrition. Phenotypic characterisation includes biochemical characterisation, fatty acid profiling and multi-locus enzyme electrophoresis. Biochemical characterisation helps in rapid identification of selective bioagents and tracking of economically important ones. Molecular characterisation of bacterial species includes polymerase chain reaction (PCR) based typing and DNA sequence-based characterisation. Probes using target sequences of 16S rDNA gene have been widely used to estimate relationships among bacteria and to identify an unknown bacterium up to the genus or species level (7).

Antibiotics are microbial toxins at low concentrations can poison other microorganisms and they are effective against plant pathogens and the disease they cause. The most effective biocontrol agents studied appear to antagonise plant pathogens employing several modes of action (8). Therefore, the present study aimed to

(i) isolate and screen efficient antagonists against *Alternaria alternata*; (ii) characterise the effective bacterial isolates through biochemical and molecular approaches; (iii) elucidate the mechanisms underlying pathogen suppression and (iv) develop coir pith–vermicompost and liquid-based formulations of selected antagonists and evaluate their efficacy against anthurium leaf blight under pot culture and field conditions.

Materials and Methods

Isolation of biocontrol agents

Rhizosphere soil samples were collected from anthurium-growing areas. *Trichoderma viride* was isolated using Trichoderma selective medium, fluorescent pseudomonads on King's B medium and *Bacillus* spp. on nutrient agar (NA), following the serial dilution technique (9).

In vitro screening of antagonists

The antagonistic activity of 7 fluorescent pseudomonads, 6 of *Bacillus* spp. and 4 *T. viride* isolates were evaluated against *A. alternata* using the dual culture technique (10). A 5 mm mycelial disc of the pathogen was placed on potato dextrose agar (PDA) and the antagonists were streaked or placed opposite to it. Plates without antagonists served as controls. Mycelial growth inhibition (%) was calculated when control plates reached full growth.

Biochemical characterisation of bacterial isolates

Effective bacterial isolates were characterized using standard diagnostic tests (11). Tests for *P. fluorescens* included Gram staining, KOH solubility, growth at 4 °C and 41 °C, pigment production on King's B medium, levan formation, arginine dihydrolase and gelatin liquefaction. Tests for *B. subtilis* included Gram reaction, catalase, citrate utilisation, starch hydrolysis, anaerobic growth, growth at 45 °C and 4 °C and growth in 7 % NaCl.

Molecular identification of rhizobacteria

Genomic DNA was extracted from *P. fluorescens* and *B. subtilis* using standard phenol–chloroform methods. The PCR amplification of the 16S–23S rDNA intergenic spacer region was performed using ITS1–ITS2 primers for *P. fluorescens* (12) and BCF1–BCF2 primers for *B. subtilis*. The PCR products were resolved on agarose gels and compared with known band sizes (13).

Compatibility among antagonists

Compatibility among effective isolates of *P. fluorescens, B. subtilis and T. viride* was assessed following the perpendicular streak method (14). Absence of growth inhibition at the interaction zones was recorded as compatibility.

Mode of action of biocontrol agents

Antibiotic extraction and activity

Antibiotic metabolites were extracted from 5-day-old cultures grown in pigment production broth, acidified to pH 2.0 and extracted with benzene. The dried residue was dissolved in 0.1 N NaOH (15). Antifungal activity against *A. alternata* was evaluated by filter paper disc assay (16).

Non-volatile and volatile metabolites *Trichoderma*

Non-volatile metabolites were tested using the cellophane overlay method (10). Volatile metabolite activity was assessed by paired plate technique, where plates containing the pathogen were inverted over plates inoculated with antagonists and sealed (17).

Hydrogen cyanide production

Qualitative assay

Hydrogen cyanide production was assayed qualitatively using picrate-impregnated filter paper and quantitatively in glycine-amended King's B (KB) Medium broth. Colour change intensity was measured at 625 nm (18).

Siderophore production

Qualitative assay

Siderophore production was evaluated qualitatively using Chrome Azurol S (CAS) agar and quantitatively using Hathway reagent (19). Absorbance was read at 700 nm and siderophore concentration was expressed using standard DHBA calibration.

Production salicylic acid (SA), indole-3 acetic acid (IAA) and ammonia

Salicylic acid (SA): Quantified from acidified culture supernatant extracted with chloroform and measured at 527 nm (20). Indole-3 acetic acid (IAA): Measured from Salkowski reagent reaction at 530 nm using IAA standards (21). Ammonia: Detected in peptone water using Nessler's reagent (22).

Development of liquid formulations

Liquid formulations of *P. fluorescens* and *B. subtilis* were developed in nutrient broth supplemented with 10 mM trehalose or 10 mM glycerol to enhance shelf life (23). Viable cell counts were monitored monthly using serial dilution and spot plating on KB or NA.

Pot culture experiment

A completely randomised design with 9 treatments and 3 replications was used. *Anthurium* seedlings were planted in sterile coconut husk medium. Coir pith–vermicompost formulations (250 g/pot at 30 days after planting (DAP)) were followed by 3 foliar sprays (60, 90, 120 DAP) of liquid formulations (0.2 %). Pathogen inoculation was done using *A. alternata* spore suspension (6×10^4 spores/mL). Disease severity (0–9 scale), percent disease index (PDI), plant height and number of leaves were recorded (24).

Field evaluation

Field trials were conducted at Kodai nursery, Thadiyankudisai, using a randomized block design with 9 treatments and 3 replications. Biocontrol formulations and mancozeb (0.2 %) were applied at 30, 60, 90 and 120 DAP. Disease severity (24), PDI, plant growth parameters and flower yield were assessed.

Statistical analysis

Data were analysed using IRRISTAT. Means were compared using Duncan's multiple range test (DMRT) (25). Percentage data were arcsine-transformed before analysis.

Results and Discussion

Isolation and screening of biocontrol agents

Fluorescent pseudomonads, *Bacillus* spp. and *T. viride* were successfully isolated from the *Anthurium* rhizosphere. Preliminary *in vitro* screening revealed that three *P. fluorescens* isolates (Pf1, CFP1 and MFP3) inhibited the mycelial growth of *A. alternata* significantly, with Pf1 exhibiting the highest reduction (56.20 %). These results corroborate earlier reports of Pf1 suppressing *A. alternata* on chilli and noni (26). Among *Bacillus* isolates, BsM3 showed maximum inhibition (55.50 %), consistent with previous observations of

Bacillus spp. inhibiting *A. porri*. All *T. viride* isolates overgrew the pathogen (Table 1 & Fig 1–3) indicating strong mycoparasitism, as previously documented against *Alternaria* spp. on potato, amla and chilli (27).

Molecular identification of bacterial antagonists

Biochemical profiling confirmed the identity of *P. fluorescens* isolates based on fluorescence, growth responses and enzyme activities. Similarly, *B. subtilis* isolates were identified using diagnostic tests including catalase activity, citrate utilisation and growth under high salinity (Fig 4, 5). Molecular characterisation strengthened these observations (Fig 6, 7). Amplification of the 16S–23S rDNA intergenic region confirmed 7 isolates as *P. fluorescens* (560 bp) and the remaining 6 isolates as *B. subtilis* (546 bp). These results are comparable with earlier studies where molecular markers provided greater discriminatory power than biochemical tests for *Bacillus* and *Pseudomonas* identification (28, 29).

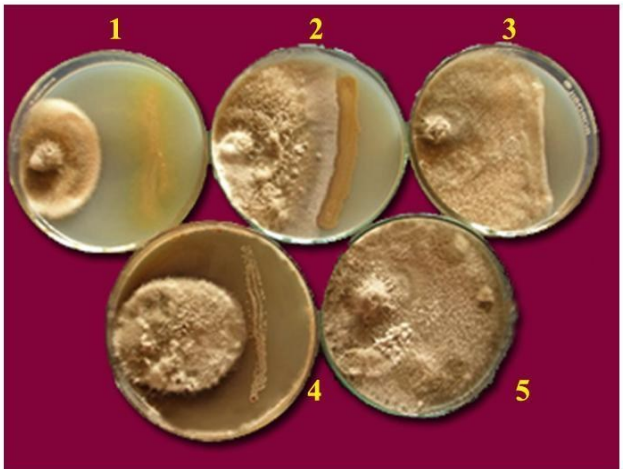
Compatibility among biocontrol agents

All tested isolates of *P. fluorescens*, *B. subtilis* and *T. viride* were mutually compatible, indicating their potential for combined application (Fig. 8). Similar compatibility patterns have been reported for *Pseudomonas*, *Bacillus* and *Trichoderma* mixtures used for disease suppression in various crops (30, 31).

Mode of action

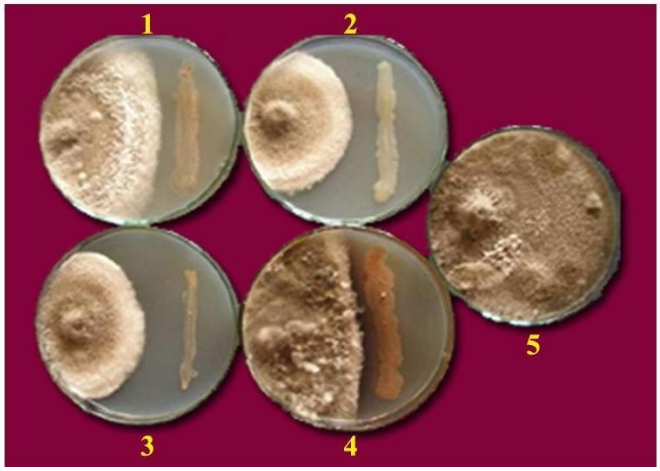
Antibiotic-mediated suppression

Fluorescent *pseudomonas* also synthesises a variety of volatile organic chemicals, including several types of molecules involved in antagonistic interactions with other species and in inducing systemic reactions in plants (32). Crude antibiotic extracts of Pf1 showed the strongest inhibition of *A. alternata* (78.65 %), followed by MFP3 (77.53 %) and CFP1 (76.40 %) (Fig. 9 a, b). Fluorescent pseudomonads are known to produce multiple antifungal antibiotics including phenazines, pyrrolnitrin and polyketides (33, 34). Phenazine-like compounds were also detected. Crude antibiotics from *B. subtilis* isolate Bs10 markedly inhibited fungal growth (61.80 %), aligning with previous findings showing spore malformation and germ tube abnormalities caused by *Bacillus* metabolites (35).



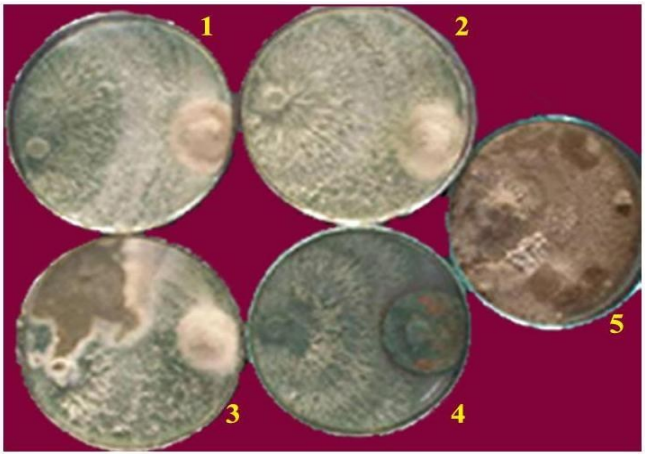
Pf1 2. CFP1 3. FP7 4. MFP3 5. Control

Fig. 1. *In vitro* inhibition of *Alternaria alternata* by fluorescent pseudomonads.



BsC1 2. BsM3 3. BsW2 4. Bs10 5. Control

Fig. 2. *In vitro* inhibition of *Alternaria alternata* by *Bacillus* spp.



1. Tv1 2. Tv2 3. Tv3 4. Tv4 5. Control

Fig. 3. *In vitro* inhibition of *Alternaria alternata* by *Trichoderma viride*.

Volatile and non-volatile Metabolites

Non-volatile metabolites of *T. viride* Tv2 showed strong suppression (85.39 %) of fungal growth (Fig. 10), while volatiles released by CFP1 and Tv1 substantially reduced pathogen growth of 85.14 and 79.43 % respectively (Fig. 11). Similar antifungal effects of *Trichoderma* volatiles and bacterial volatile organic compounds

Table 1. Inhibitory effect of antagonists on the growth of *Alternaria alternata*

Isolates	Fluorescent pseudomonads*							*Bacillus* spp.*					*Trichoderma viride***					
	Pf1	CFP1	WFP2	MFP3	TFP4	YFP5	FP7	BsC1	BsW2	BsM3	BT1	BY1	Bs10	Tv1	Tv2	Tv3	Tv4	Control
*Mycelial radial Growth (mm)	39.0	51.0	62.0	54.0	56.0	57.0	63.0	55.8	41.4	39.6	54.00	57.0	51.9	26.0	21.0	28.0	34.0	89.0
Inhibition over Control (%)	56.2	42.7	30.3	39.3	37.1	35.9	29.2	46.1	53.5	55.5	39.3	35.9	41.6	70.8	76.4	68.5	61.8	-
Inhibition zone (mm)	17.0 (4.1)	2.0 (1.4)	0.0 (0.7)	4.0 (2.0)	0.0 (0.7)	5.0 (2.2)	3.0 (1.7)	5.0 (2.2)	11.0 (3.3)	5.0 (2.2)	0.0 (0.70)	0.0 (3.3)	15.0 (3.8)	over grown				0.0

Mean of 3 replications. In a column, means followed by the same letter do not differ significantly ($p < 0.05$) according to DMRT (Duncan's Multiple Range Test). Figures in parentheses are square-root–transformed values.

** Mean of 4 replications.

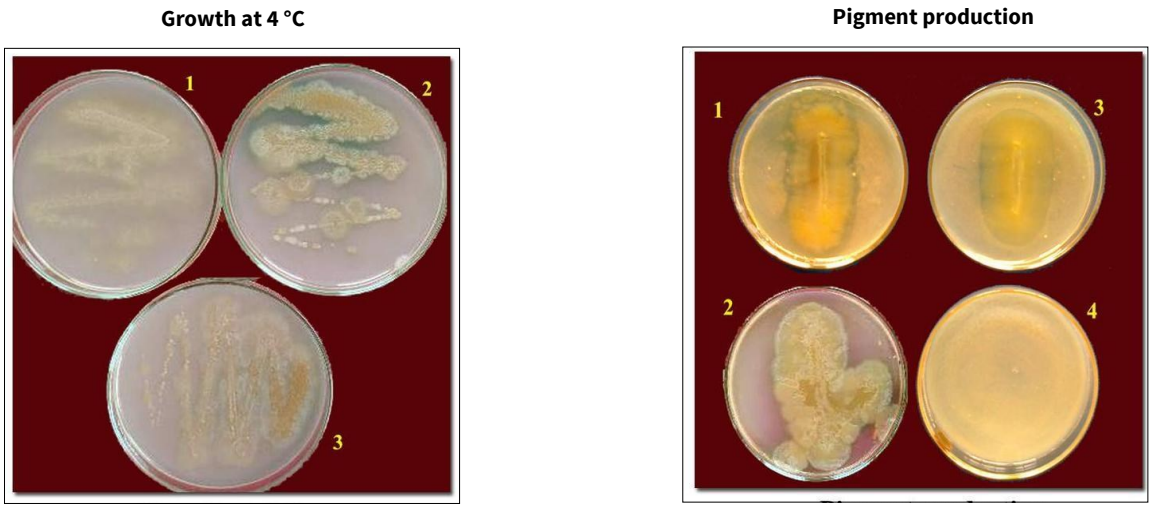


Fig. 4. Biochemical characterisation of fluorescent pseudomonads.

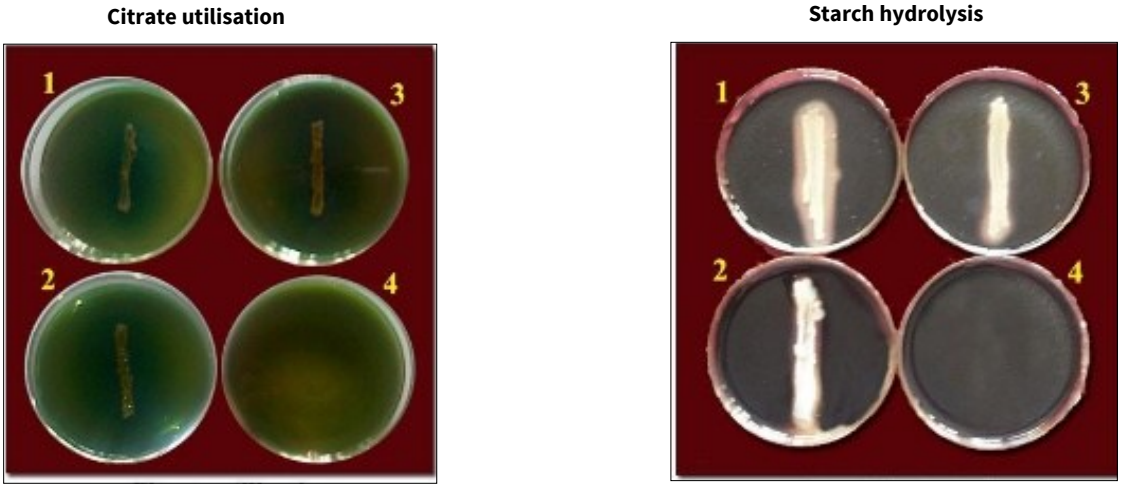


Fig. 5. Biochemical characterisation of *Bacillus subtilis*.

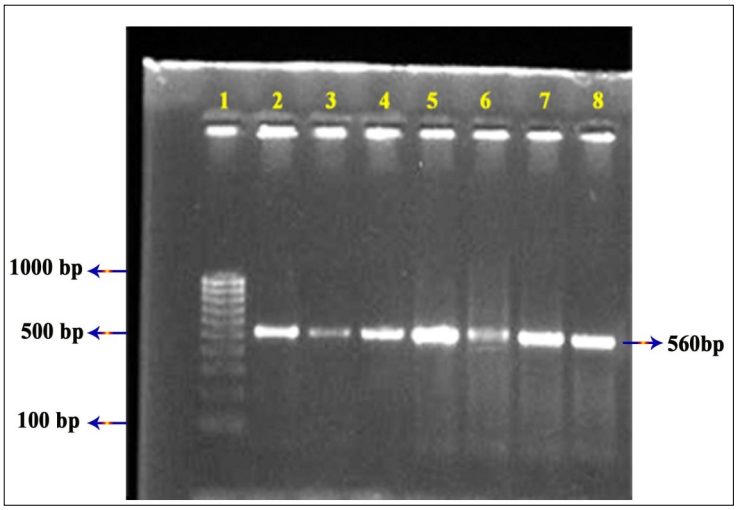
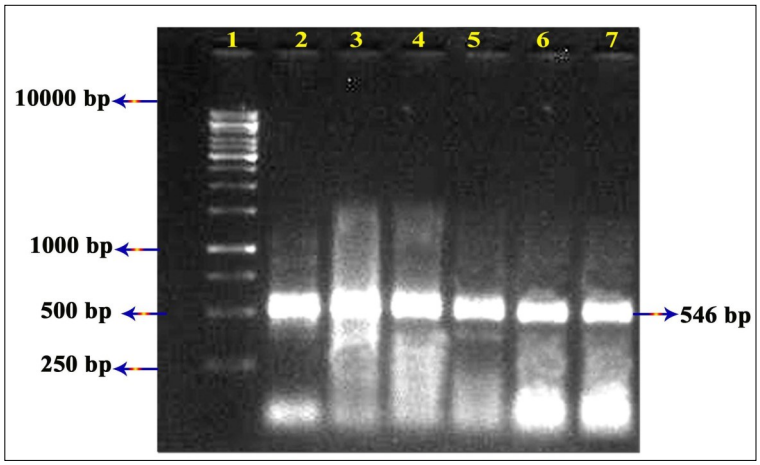
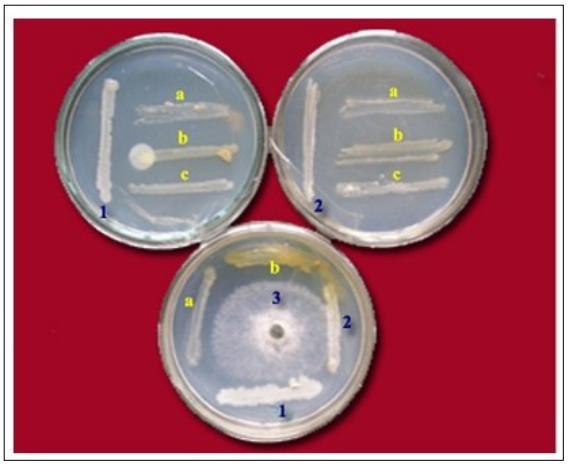


Fig. 6. Molecular identification of *Pseudomonas fluorescens*.



1 kb DNA ladder 2. BsC1 3. BsW2 4. BsM3 5. BT1 6. BY1 7. Bs10

Fig. 7. Molecular identification of *Bacillus subtilis*.



BsW2 2. BsM3 3. Tv1 4 a. Pf1 b. CFP1 c. MFP3

Fig. 8. Compatibility among the effective bacterial and fungal antagonists.

a.

1. Pf1 2. CFP1 3. FP7 4. MFP3 5. Control

a.

1. BsC1 2. BsW2 3. BsM3 4. Bs10 5. Control

Fig. 9. Antifungal activity of crude antibiotic extracts of *Pseudomonas fluorescens* and *Bacillus subtilis* against *Alternaria alternata* in disc-diffusion assay: (a) *Pseudomonas fluorescens*; (b) *Bacillus subtilis*.

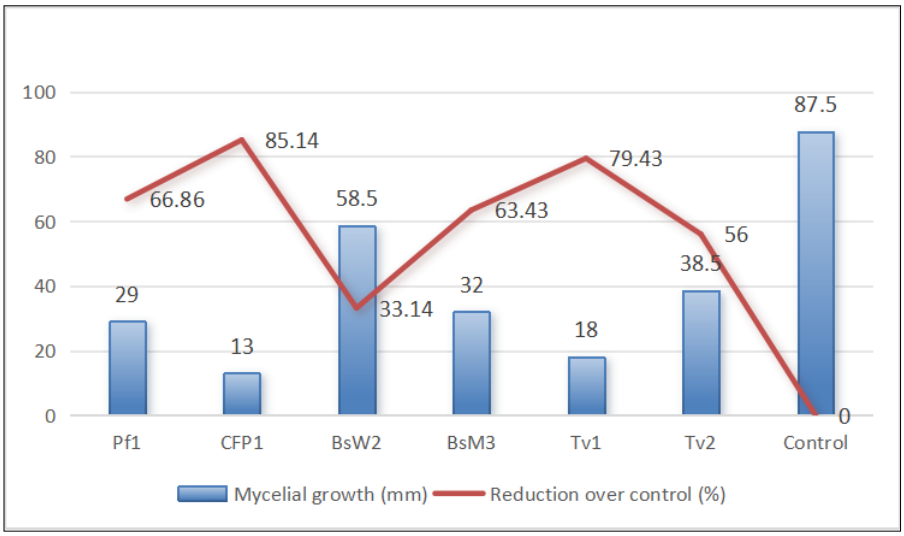


Fig. 10. Effect of volatiles of antagonists on the growth of *Alternaria alternata*.

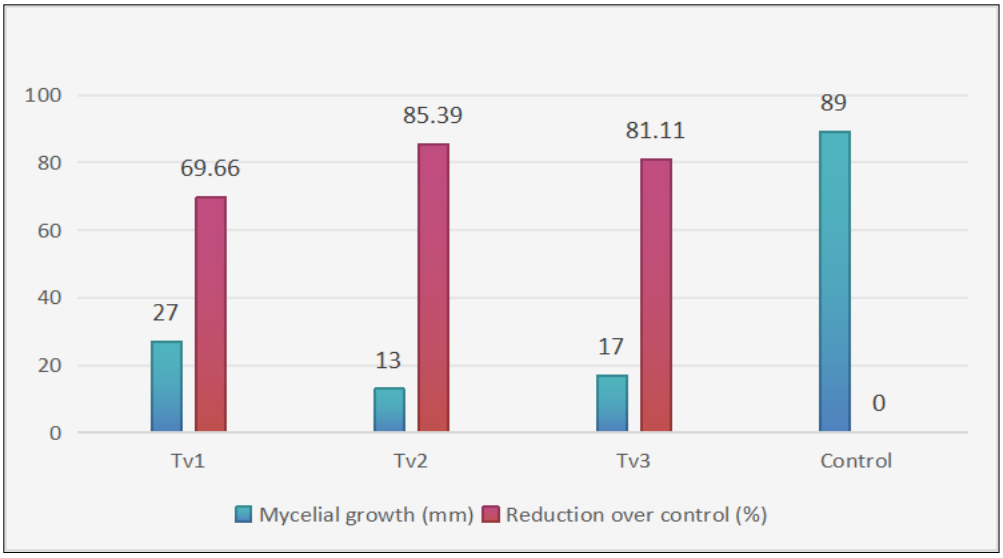


Fig. 11. Effect of non-volatile metabolites of *Trichoderma viride* on the growth of *Alternaria alternata*.

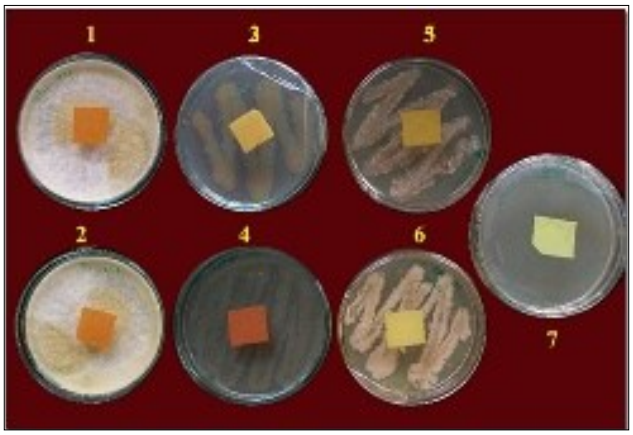
(VOCs) have been documented for *Alternaria* diseases in watermelon (36).

Hydrogen cyanide and production

Hydrogen cyanide production was strongest in CFP1, Tv1 and Tv2, followed by Pf1 (Fig. 12). Hydrogen cyanide disrupts cytochrome oxidase activity and interferes with fungal energy metabolism. Reports of hcnBC-governed HCN synthesis in *Pseudomonas* strains support its role as a key antifungal trait (37). Fluorescent *pseudomonas* produce varying amounts of HCN in the rhizosphere, depending on environmental variables (38).

Siderophore production

Siderophore production was pronounced in Pf1 and CFP1, both qualitatively and quantitatively (Table 2 & Fig. 13a). Siderophores restrict pathogen growth by competitively chelating iron. *Bacillus* isolates (except BsW2) also produced appreciable siderophore levels (Table 2 & Fig. 13b). Although siderophore production in *Trichoderma* spp. is less commonly studied, all 3 isolates in this study were positive (Table 2 & Fig. 13c), consistent with earlier reports



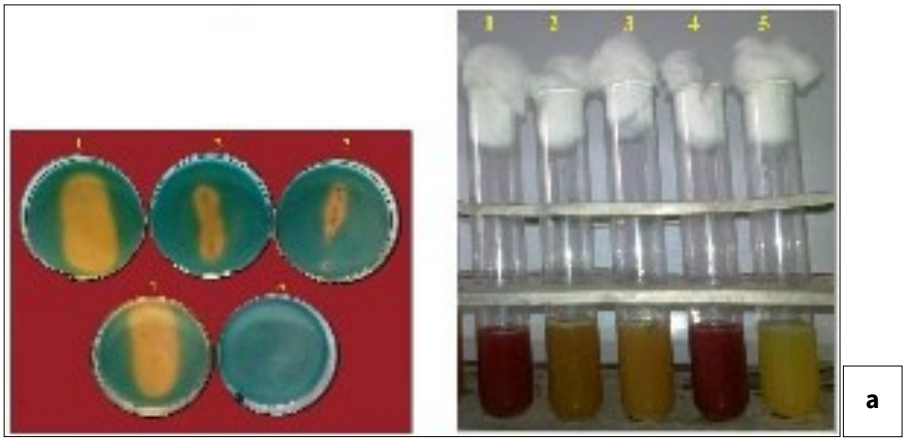
1.Tv1 2. Tv2 3. Pf1 4. CFP1 5. BsW2 6. BsM3 7. Control

Fig. 12. Production of hydrogen cyanide by antagonists.

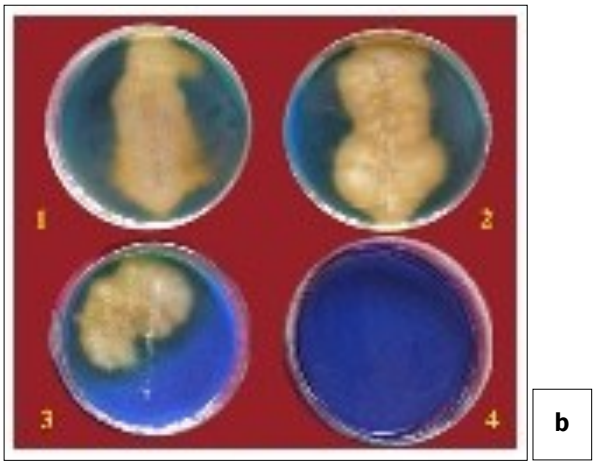
Table 2. Production of siderophore by antagonists

Isolates	Qualitative	Area of production (mm)	Quantitative (µg mL^{-1})	Nature of siderophore	
				Hydroxymate	Carboxylate
Pf1	+++	40.50	2.03	+++	-
CFP1	+++	30.00	2.04	++	-
MFP3	++	18.00	1.79	+	-
FP7	++	18.00	1.52	+++	-
BsC1	++	45.00	1.72	*	*
BsW2	-	-	0.09	*	*
BsM3	++	42.80	1.63	*	*
Bs10	+++	52.00	1.75	*	*
Tv1	+++	72.80	*	*	*
Tv2	++	57.10	*	*	*
Tv3	+++	72.80	*	*	*
Control	-	-	0.00	-	

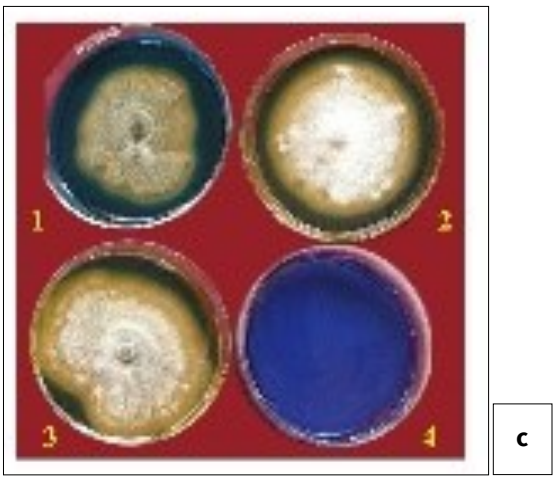
Siderophore production: +++ strong; ++ moderate; - negative; * Not tested.



1.Pf1 2. MFP3 3. FP7 4. CFP1 5. Control



1. BsC1 2. BsM3 3. Bs10 4. Control



TV1 2. Tv2 3. Tv3 4. Control

Fig. 13. Production of siderophore by antagonists: (a) *Pseudomonas fluorescens*; (b) *Bacillus subtilis*; (c) *Trichoderma viride.*

detecting coprogen derivatives (39, 40).

Plant growth-promoting metabolites (IAA, SA and Ammonia)

The IAA production was highest in CFP1, followed by FP7 and Pf1. Salicylic acid production was greatest in Pf1, indicating its possible role in induced systemic resistance. The Pf1 and CFP1 also produced higher quantities of ammonia, a trait linked to nutrient mineralisation and disease reduction (41). The SA acts as a signal molecule in disease resistance thereby inducing systemic resistance in plants. In the present study, SA produced by Pf1 of *P. fluorescens* was maximum whereas it was lower in *B. subtilis* isolate BsC1 (Fig. 14). Foliar spray of SA stimulated the activities of chitinase, β, 1-3 glucanase, Peroxidase (PO), Polyphenol oxidase (PPO) and Phenylalanine ammonia-lyase (PAL) in groundnut leaves and Induced Systemic Resistance (ISR) against late leaf spot incited by *C. personata.* Among the 4 isolates of *B. subtilis* and *P. fluorescens,* production of SA was significantly higher in *P. fluorescens* isolate PfC6 (35).

In the present investigation, among the 4 isolates of *P. fluorescens* tested, Pf1 and CFP1 released more ammonia than MFP3 and FP7 (Fig. 15). The involvement of ammonia released by Plant growth-promoting rhizobacteria (PGPR) in plant growth and disease suppression has been ascertained by earlier research (41).

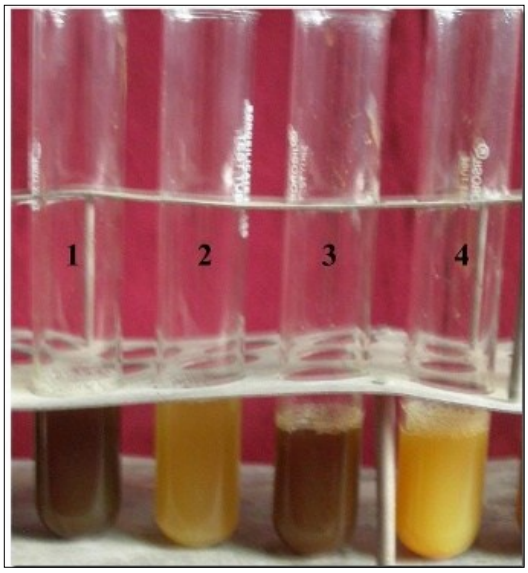
Survival of liquid bioformulations

Glycerol-amended nutrient broth supported the long-term survival of *P. fluorescens* up to 6 months at room temperature (Table 3). For *B. subtilis*, trehalose-amended broth showed the highest survival (Table 4). These findings agree with previous studies highlighting trehalose and glycerol as effective osmoprotectants enhancing microbial stability (42, 43).

Efficacy of against alternaria leaf blight

Pot culture

The PGPR affect plant growth and development directly or indirectly, either by releasing plant growth regulators or other biologically active substances and uptake of nutrients through fixation and mobilisation, reducing harmful effects of pathogenic microorganisms on plants and by employing multiple mechanisms of action. Besides they play an important role in soil fertility (33). Based on the results of experiments *in vitro*, 6 biocontrol agents were selected for pot culture studies. Among the biocontrol agents, soil application followed by foliar spray of Pf1 reduced the severity of Alternaria leaf blight (55.46 %) and enhanced plant growth (Table 5).



1.Pf1 2. MFP3 3. CFP1 4. FP7

Fig. 15. Production of ammonia by bacterial antagonists.

Though the treatment mancozeb was ranked first in reducing the severity of the disease, enhanced plant growth parameters were significant with Pf1. Soil application followed by foliar sprays of *T. viride* reduced fruit rot incidence in chilli incited by *A. alternata* and *A. capsici* (44). In glasshouse studies, the use of biocontrol agents in combination (Seed treatment + Soil application with (PfC5 + Tv3) + Foliar spray with PfC5) significantly recorded maximum (72.50) per cent reduction of head rot disease in cabbage and increase the yield (45).

Field trial

The efficacy of the biocontrol agents evaluated in pot culture against *A. alternata* was tested in the field. Soil application (30 DAP) followed by three foliar sprays (60, 90 and 120 DAP) with biocontrol agents was found to reduce the severity of leaf blight (14.40 %) and enhanced the plant growth and yield when compared to control (27.90 %). However, mancozeb 0.2 % was superior to others (Table 6 & Fig. 16). Management of plant diseases by different biocontrol agents through various formulations has been reported by earlier research (33). Foliar application with liquid formulation of *P. fluorescens* (Pf1) significantly reduced the severity of tomato leaf blight caused by *A. solani* under glasshouse and field conditions (42). Combined application of seed treatement and foliar spray with *P. fluorescens* (Pf1) and *B. subtilis* (Bs1) as biocontrol agents

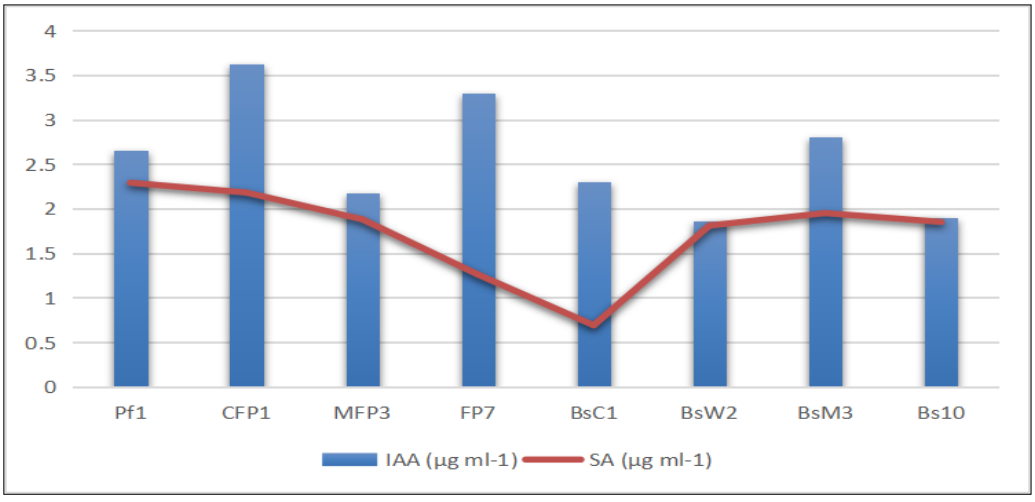


Fig. 14. Production of plant growth promoting metaboloites (Indole-3-acetic acid (IAA) and salicylic acid (SA)) by bacterial antagonists.

Table 3. Survival of *Pseudomonas fluorescens* in nutrient broth supplemented with different chemical amendments

Days	Pf1			CFP1		
	Population (cfu/mL)			Population (cfu/mL)		
	Glycerol (10 mM)	Trehalose (10 mM)	Broth alone	Glycerol (10 mM)	Trehalose (10 mM)	Broth alone
0th day	3.95×10^{10}	3.60×10^{10}	3.35×10^{10}	4.20×10^{10}	4.05×10^{10}	3.80×10^{10}
2nd day	6.80×10^{10}	6.65×10^{10}	5.35×10^{10}	7.15×10^{10}	7.20×10^{10}	5.75×10^{10}
5th day	4.75×10^{12}	2.30×10^{12}	1.70×10^{12}	5.80×10^{12}	2.95×10^{12}	1.96×10^{12}
10th day	2.85×10^{12}	1.15×10^{12}	0.65×10^{12}	3.25×10^{12}	1.45×10^{12}	0.80×10^{12}
15th day	1.70×10^{12}	0.80×10^{12}	3.05×10^{8}	2.05×10^{12}	0.95×10^{12}	3.25×10^{8}
30th day	0.90×10^{12}	0.75×10^{12}	1.55×10^{5}	1.15×10^{12}	0.90×10^{12}	1.95×10^{5}
45th day	0.80×10^{11}	0.95×10^{11}	2.55×10^{3}	1.05×10^{11}	0.85×10^{11}	2.80×10^{4}
60th day	0.75×10^{10}	6.75×10^{9}	1.95×10^{2}	0.90×10^{10}	7.15×10^{9}	2.25×10^{2}
90th day	8.55×10^{9}	6.55×10^{8}	0.0	9.00×10^{9}	6.95×10^{8}	0.0
120th day	7.90×10^{8}	4.10×10^{8}	0.0	8.15×10^{8}	4.80×10^{8}	0.0
150th day	6.15×10^{7}	3.80×10^{7}	0.0	6.70×10^{7}	4.10×10^{7}	0.0
180th day	7.05×10^{7}	0.75×10^{5}	0.0	7.45×10^{7}	0.85×10^{5}	0.0

Table 4. Survival of *Bacillus subtilis* in nutrient broth supplemented with different chemical amendments

Days	BsW2			BsM3		
	Population (cfu/mL)			Population (cfu/mL)		
	Glycerol (10 mM)	Trehalose (10 mM)	Broth alone	Glycerol (10 mM)	Trehalose (10 mM)	Broth alone
0th day	9.30×10^{10}	9.30×10^{10}	9.30×10^{10}	9.45×10^{10}	9.69×10^{10}	9.10×10^{10}
2nd day	9.60×10^{10}	9.90×10^{10}	9.60×10^{10}	9.70×10^{10}	9.90×10^{10}	9.46×10^{10}
5th day	8.70×10^{11}	9.60×10^{11}	8.60×10^{11}	7.80×10^{11}	8.10×10^{11}	7.15×10^{11}
10th day	3.10×10^{12}	5.51×10^{12}	2.90×10^{12}	4.67×10^{12}	7.40×10^{12}	2.36×10^{12}
15th day	4.30×10^{12}	7.20×10^{12}	4.40×10^{12}	5.20×10^{12}	7.01×10^{12}	4.01×10^{12}
30th day	3.65×10^{11}	7.45×10^{11}	8.01×10^{10}	3.90×10^{11}	6.30×10^{11}	7.65×10^{10}
45th day	8.30×10^{9}	7.21×10^{10}	9.20×10^{8}	8.65×10^{9}	6.89×10^{10}	8.26×10^{8}
60th day	4.50×10^{9}	8.56×10^{9}	4.20×10^{7}	4.77×10^{9}	8.60×10^{9}	4.10×10^{7}
90th day	6.15×10^{8}	7.01×10^{8}	7.40×10^{6}	6.36×10^{8}	7.26×10^{8}	6.96×10^{6}
120th day	4.02×10^{8}	6.70×10^{8}	4.90×10^{5}	4.02×10^{8}	6.89×10^{8}	4.10×10^{5}
150th day	6.90×10^{7}	6.01×10^{7}	2.30×10^{5}	7.85×10^{7}	6.45×10^{7}	2.10×10^{5}
180th day	1.17×10^{6}	5.40×10^{6}	6.40×10^{4}	1.60×10^{6}	4.69×10^{6}	5.10×10^{4}

Table 5. Efficacy of antagonists on Alternaria blight and plant growth under pot culture

Treatments	Alternaria blight		Growth parameters			
	*PDI	Reduction over control (%)	Plant height (cm)	Increase over control (%)	Number of leaves	Increase over control (%)
Pf1	20.62 (27.00)	55.46	33.66^{a}	66.69	8.5^{a}	64.70
CFP1	26.70 (31.11)	42.33	22.66^{b}	54.98	6.8^{b}	55.88
BsW2	30.26 (33.37)	34.64	16.64^{d}	38.70	5.1^{d}	41.17
BsM3	31.47 (34.21)	32.03	17.51^{d}	41.74	5.3^{d}	48.39
Tv1	27.82 (31.82)	39.91	20.00^{c}	49.00	5.8^{c}	48.27
Tv2	23.27 (24.88)	49.74	21.23^{c}	51.95	6.1^{c}	50.82
Mancozeb (0.2 %)	12.96 (21.10)	72.01	17.46^{d}	41.58	4.6^{e}	34.78
Control (inoculated)	46.30 (42.87)	-	10.20^{f}	-	3.0^{g}	-
Control (non inoculated)	3.30 (10.51)	-	15.36^{e}	-	4.2^{f}	-
CD (0.05)	0.91	-	-	-	-	-

* Mean of 3 replications. In a column, means followed by the same letter do not differ significantly ($p < 0.05$) according to DMRT (Duncan's multiple range test). Figures in parentheses are arcsine-transformed values.

a. Field view

b. Pf1

Fig. 16. Field performance of Pf1 and other antagonists in reducing leaf blight severity in *Anthurium*: (a) Field view; (b) Pf1 treatment.

Table 6. Field efficacy of biocontrol agents against leaf blight

Treatments	Alternaria blight		Growth parameters				Flower yield / year		C:B ratio
	*PDI	Reduction over control (%)	Plant height (cm)	Increase over control (%)	No. of leaves	Increase over control (%)	No. of flowers/ plant	No. of flowers/ plot	
Pf1	14.40 (22.30)	48.39	56.65^{a}	74.10	9.0^{a}	55.55	3.86^{a}	1326.5^{a}	1:3.82
CFP1	17.11 (24.04)	38.67	42.84^{b}	65.75	8.0^{b}	50.00	3.57^{ab}	1249.5^{b}	1:3.56
BsW2	21.35 (27.07)	23.48	17.68^{g}	17.02	5.7^{f}	29.82	2.48^{c}	868.0^{g}	1:1.87
BsM3	23.00 (28.81)	17.56	19.57^{f}	25.03	6.4^{e}	37.50	2.75^{c}	962.5^{f}	1:2.28
Tv1	18.87 (25.05)	32.37	24.25^{d}	39.50	7.6^{c}	47.36	3.26^{b}	1141.0^{d}	1:1.66
Tv2	16.33 (23.45)	41.47	28.00^{c}	47.60	8.0^{b}	50.00	3.17^{b}	1109.5^{e}	1:3.10
Mancozeb (0.2 %)	11.11 (19.46)	60.18	21.34^{e}	31.25	7.0^{d}	42.85	3.41^{b}	1193.5^{c}	1:3.01
Control	27.90 (31.85)	-	14.65^{h}	-	4.0^{g}	-	1.33^{d}	465.5^{h}	-
CD (0.05)	2.26	-	-	-	-	-	-	-	-

* Mean of 3 replications. In a column, means followed by the same letter do not differ significantly ($p < 0.05$) according to DMRT (Duncan's multiple range test). Figures in parentheses are arcsine-transformed values.

effectively reduced the severity of alternaria leaf blight of groundnut and improves yield (46).

Conclusion

This study identified *Pseudomonas fluorescens* Pf1 as the most effective biocontrol agent against *Alternaria alternata* infecting *Anthurium*. The Pf1 and other selected isolates suppressed the pathogen through multiple mechanisms viz., antibiosis, siderophore and hydrogen cyanide production and growth-promoting metabolites like IAA, SA, ammoina and improved plant growth. The Pf1-based formulations significantly reduced disease severity in both pot and field conditions. These findings highlight Pf1 as a promising eco-friendly alternative to chemical fungicides for managing anthurium leaf blight.

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Authors' contributions

ST conceptualised and drafted the manuscript. SM and MD provided expertise and critically reviewed the manuscript. SKM and NI revised and finalised the manuscript. TKSL provided valuable insights. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they do not have any conflict of interest to declare.

Ethical issues: None

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