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Biohardening of Tissue Cultured Banana Plantlets of cv. Ney Poovan for the Management of Fusarium wilt of Banana with *Bacillus amyloliquefaciens* (VB7) Triggers Defence Gene Products and Growth Promotion

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Authors' contributions

This work was done in collaboration among all the three authors. Authors KS and SN designed the study. Author CYSU performed the experiment, statistical analysis and wrote the first draft of the manuscript. Authors KS and SN supervised the study and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Fusarium wilt of banana is the most devastating disease caused by *Fusarium oxysporum* f.sp. *cubense* (Foc). In order to combat the early onset of Fusarium wilt disease, an experiment was carried out on biohardening of tissue cultured plantlets. Six bacterial endophytes were observed to inhibit the growth of Foc *in vitro.* Among the six bacterial endophytes, *Bacillus amyloliquefaciens* (VB7) inhibited mycelial growth of Foc to an extent of 70.58% over control. Further, these bacterial endophytes were used for biohardening of the tissue cultured banana plantlets

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cv. Ney Poovan (AB) during primary and secondary hardening stages. Among the bacterial endophytes, *B. amyloliquefaciens* was found to significantly enhance plant height, leaf production, root numbers and root length compared to untreated control. Activity of defense enzymes were also enhanced and such increase in activity was observed to be to an extent of 93.67% in peroxidase, 92.39% polyphenol oxidase, 97.60% phenylalanine ammonia lyase and 26.23% in β-1, 3-glucanase defence enzymes in plants biohardened with *B. amyloliquefaciens* (VB7) over untreated control after inoculation of Foc. Tissue cultured plants of Ney Poovan biohardened with *B. amyloliquefaciens* (VB7), *B. paraconglomeratum* (YEB PT2) and *S. maltophilia* (YEB RH2) were completely free from wilt incidence symptoms upto planting stage when challenged with Foc inoculum under pot culture conditions. As among these three endophytes, *B. amyloliquefaciens* (VB7) also influenced favourable growth promotion, it can serve as a potential biocontrol agent for management of Fusarium wilt of banana.

Keywords: Fusarium wilt; banana; bacterial endophytes; biohardening.

1. INTRODUCTION

Banana is the second important crop next to Mango in India. India ranks first in global production of banana with an estimated annual production of 31.75 million metric tonnes from an area of 0.88 million hectares contributing 31.63% of total fruit production in India [1]. Banana cultivation across the different regions in the world including India is facing challenge to the devastating disease, Panama wilt or Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). Foc can survive persistently in the subsoil for more than 30 years and gains entry through roots, colonize in vascular tissues, blocks and results in wilting [2,3]. In India, Fusarium wilt results in yield reduction from 50 - 70%.

Three major races of FOC are found to affect cultivated bananas. For the past many decades the FOC races 1 and 3 are predominatly observed to affect most of the cultivated commercial varieties, except Cavendish group (*Musa,* AAA). The major varieties severly affected by FOC in India are Amrithapani, Karpuravalli, Monthan, Ney Poovan, Rasthali and Virupakshi [4,5].The rising incidence of FOC race 4 in Cavendish group of bananas and other varieties in recent years is now a grave concern among the growers and the scientists across the different parts of the world including India. The variety chosen for the study, Ney Poovan (*Musa*, AB) is a diploid and is appreciated for the fruit quality in the southern states of India, especially in Karnataka, Kerala and Tamil Nadu. It also feteches premium price in the market but suffers easily due to incidenecs of Fusarial wilt.

At present disease free quality banana planting material is commercially propagated through tissue culture [6], but in the process of *in vitro* propagation most of the beneficial endophytic bacteria are also eliminated. Hence, the tissue cultured plantlets are highly and easily susceptible to Fusarium wilt in early stages if proper control measures are not taken up as compared to conventional planting material [7]. Among the different strategies and approaches towards the management of Foc, it is imperative that, sustainable solution such as developing resistant cultivars and identification of antagonist microbes to reduce disease severity is presently needed. Identification and selective use of endophytes has been reported to result in improved yield [8-10], phytoremediation of organic pollutants [11], or heavy metals [12], disease resistance [13] and stress tolerance [14]. Biohardening approach by allowing the selected bacterial endophytes in the plant root/corms either through root feeding or by soil drenching during the hardening stages of *in vitro* grown plants has been documented to result in enhanced tolerance to soil borne and foliar plant pathogens [15]. As a consequence of biohardening, plant growth promotion, uniform seedling stand and induced systemic resistance were reported to be activated through specific defense pathways in plants. Many bacterial antagonists has been successfully exploited earlier for the management of plant pathogens [16-20]. In the present study, biohardening of tissue culture banana plantlets with endophytic bacteria was attempted to manage Fusarium wilt caused by *Fusarium oxysporum* f.sp. *cubense.*

2. MATERIALS AND METHODS

To investigate the efficacy against Fusarium wilt in banana plantlets of cv. Ney Poovan (*Musa* AB) pot culture experiments were conducted with six different

bacterial endophytes, *viz., Brachybacterium paraconglomeratum* (YEB PT2) (Accession No: MK263736), *Achromobacter xyloxidans* (YEBRH5) (Accession No: MK258170), *Myroides odoratimimis* (YEB RT3) (Accession No: MN082530), *Brucella melitensis* (YEB PS3) (Accession No: MN022548), *Bacillus amyloliquefaciens* (VB7) (Accession No: MH348121), *Stenotrophomonas maltophilia* (YEB RH2) (Accession No: MN082440) were obtained from Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore.

2.1 Antifungal Efficacy of Bacterial Endophytes against Foc

In-vitro dual plate assay was carried out [21] to confirm the antogonism of the bacterial endophytes *viz., B. paraconglomeratum* (YEB PT2), *A. xyloxidans* (YEB RH5), *M. odoratimimis* (YEB RT3), *B. melitensis* (YEB PS3), *B. amyloliquefaciens* (VB7),*S. maltophilia* (YEB RH2) against the pathogenic Foc (Accession Number: MK981549). The efficacy was assessed by measuring the zone of inhibition (mm) and inhibition percentage of the fungal mycelia over pathogen inoculated control. Each treatment were replicated thrice with 10 petri plates per replication.

2.2 Evaluation of Bacterial Isolates against Fusarium Wilt of Banana

Tissue culture plants of banana cv. Ney Poovan produced through shoot tip culture were used for biohardening. For every treatment, plantlets were inoculated with 100 ml of bacterial suspension containing 3×10^{10} CFU $ml^{-1}$of log phase culture of bacterial endophytes by drenching the banana root during both, primary and secondary hardening stages. The bacterial suspension were drenched once in root zone during primary and secondary hardening stages. The plantlets were planted in polybags containing well-decomposed sterilized coir pith, red soil and sand. The plantlets were kept under greenhouse for acclimatization. At the end of secondary hardening stage, the plant height, number of leaves and roots were assessed followed by challenge with macroconidia and microconidia of Foc@10^6CFU ml^{-1}inoculation. The experiment was carried out in completely randomized design. Three replications were maintained for each treatment with 10 plants per replication, and Foc alone-inoculated plants were used as untreated control. Activity of defense related enzymes were estimated 48 hours after challenging with Foc.

2.3 Assay of Defense Related Enzymes

2.3.1 Assay of total phenol

The total phenol content was estimated following the protocol of Folin Ciocalteu extraction and assay mentioned by Zieslin and Ben-Zaken [22]. 500 mg of root bits was transferred to test tube with 5 ml of 80% ethanol. The test tubes were kept in water bath for 10 minutes and cooled to room temperature (28±2ºC). Then the samples were macerated with another 5 ml of 80% ethanol and centrifuged at 5000 rpm for 10 minutes. The supernatant was made up to volume of 25 ml with sterile distilled water. 1 ml of supernatant, 2 ml of 20% sodium carbonate and 1 ml of folin reagent were added together and left for 10 minutes to develop colour and measured at 660nm. Catechol was used as standard. The phenol content was expressed as catechol equivalents g^{-1} fresh weight of roots.

2.3.2 Assay of peroxidase activity (PO)

One part of root sample was macerated with five parts of 0.1 M phosphate buffer at a pH of 7.0 in pre-cooled pestle and mortar. The sample was centrifuged at 3000 g for 15 min at 0 - 5° C. The supernatant was used as an enzyme source. The reaction mixture comprised of 3 ml of 0.05 M pyrogallol, 0.1 ml of enzyme extract and 0.5 ml of 1% H_2O_2. The variation in absorbance of the reaction mixture was documented at 430 nm in an UV-visible spectrophotometer at 30 sec interval for 3 min from zero second of incubation. The enzyme activity was expressed as change in absorbance at 470 nm $min^{-1}g^{-1}$ of fresh root tissue [23].

2.3.3 Assay of polyphenol oxidase (PPO)

One gram of root sample was normalized in 2ml of 0.1 M sodium phosphate buffer (pH 6.5) at 4°C. The homogenate sample was centrifuged at 20,000 rpm for 15 min at 4°C. The supernatant aided as enzyme source and polyphenol oxidase activity was assessed as per Mayer et al. [24]. The reaction mixture comprised of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. In order to initiate

reaction, 200 µl of 0 01 M catechol was supplemented and the activity was converted as change in absorbance at 470 nm $min^{-1}g^{-1}$ of fresh root tissue.

2.3.4 Assay of phenylalanine ammonia lyase (PAL)

One gram of root sample was homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0, enclosing 1.4 mM of 2- Mercaptoethanol and 50 mg of insoluble Polyvinyl Pyrrolydine (PVP). The resulting extracts were sieved through cheese cloth and the filtrate was centrifuged at 20,000 rpm for 15 minutes at 4°C and the supernatant was used as an enzyme source. The PAL activity was resoluted as the rate of conversion of L- Phenylalaline to trans-cinnamic acid at 290 nm given by Ross and Sederoff [25]. Sample comprising 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L- Phenylalaline in the same buffer for 30 minutes at 30°C. The enzyme activity was calculated on fresh weight basis as nmol of trans-cinnamic acid min^{-1} g^{-1} fresh weight of roots.

2.3.5 Assay of β-1,3- glucanase

The enzyme activity was assayed following the colorimetric protocol validated by Pan et al. [26]. The crude enzyme extract of 62.5 µl was added to 62.5 µl of 4% laminarin (4 g Laminarin in 40 ml water) and then incubated at 40 ºC for 10 min. The reaction was stopped by adding 375 µl of dinitrosalicylic acid (DNS) and heated for 5 min on boiling water bath. DNS was prepared by adding 300 ml of 4.5% NaOH to 880 ml containing 8.8 g of DNS and 22.5 g potassium sodium tartarate. The resulting coloured solutions were diluted with distilled water, vortexed and the absorbance was read at 500 nm. The crude extract preparation mixed with laminarin with zero-time incubation served as blank. The enzyme activity was expressed as glucose released min^{-1} g^{-1} fresh weight of roots.

2.4 Evaluation of Biohardened Plantlets against Fusarium Wilt

Biohardened banana cv. Ney Poovan (AB) plantlets were tested for disease incidence and severity after 30 days of Foc inoculation. The external and internal scoring was followed as recommended by Dita et al. [7].

a. Scoring for external symptoms:

S.No	Symptom	Scale
1.	No symptoms	0
2.	Yellowing of lower leaves at initial stage	1
3.	Yellowing of all the lower leaves with some discoloration of younger leaves	2
4.	All leaves with intense yellowing or plant dead	3

b. Scoring for internal symptoms:

S.No	Symptom	Scale
1.	No symptoms	0
2.	Initial rhizome discoloration (1-20%)	1
3.	Slight rhizome discoloration along the whole vascular system (21-40%)	2
4.	Rhizome with most of the internal tissues showing necrosis (> 40%)	3

2.5 Analysis of Variance

The experiment was taken up with three replications in completely randomized design (CRD). Data were subjected to the standard analysis of variance procedure using SPSS statistical package version 22.

3. RESULTS AND DISCUSSION

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) in banana can survive in soil for several years as chlamydospores and intrude into the host plant through roots. It is important to identify an antagonist against the pathogen, which can live in the host endophytically. Many attempts are being targeted to identify, isolate and exploit endophytic bacteria having antagonistic effect of Foc. The use of different species of *Trichoderma*, *Pseudomonas*, *Streptomyces*, or non-pathogenic *Fusarium* (npFo), of both rhizospheric and endophytic origin have been reported against Fusarium wilt under both glass-house and field conditions [27-33]. However, none of these studies have reported complete suppression of Fusarium wilt by employing the antagonistic microbes. Although the structure and composition of bacterial community are different in plant tissues

and soil environments [34], similar functional species can be observed or isolated in plant as well as soil [10,35]. Hence, in the present study, attempts were made to identify endophytes, which could be antagonistic to Fusarium wilt pathogen along with beneficial plant growth promotion.

3.1 *In vitro* Antagonism of Bacterial Endophytes against Foc

Six bacterial endophytes were screened against Foc to test their efficacy. The results indicated a significant reduction in mycelial growth in terms of per cent inhibition and zones of inhibition. Among the six bacterial isolates *B. amyloliquefaciens* (VB7), the maximum inhibition of 70.58% of mycelial growth was observed followed by *A. xyloxidans* (59.14%). The inhibition zone produced by *B. amyloliquefaciens* (VB7) was to the extent of 20.67 mm and was followed by *B. paraconglomeratum* (18.67 mm) (Table 1, Plate 1). Panda et al. [36] reported that out of the thirty-eight bacterial endophytes from the banana cultivar 'Yangambi km5', twelve isolates exhibited more than or equal to 50.00% mycelium inhibition *in vitro* against Foc. This could be due to direct action by production of antimicrobial pepdides [19,20], lytic enzymes such as chitinase and protease. The inhibition of mycelia growth and formation of inhibition zone in dual plate screening might be due to the antifungal metabolites produced by the bacterial antagonists [37].

3.2 Influence of Bacterial Endophytes on Plant Growth Promotion

Biohardened tissue culture plantlets applied with endophytes in the present study were observed to be capable of promoting growth in banana cv. Ney Poovan (AB). In the present investigation, soil application of endophytes promoted plant growth parameters. *B. amyloliquefaciens* (VB7) significantly increased the plant height (23.30 cm), number of leaves (8.13), number of roots (19.60) and root length (30.26 cm) at secondary hardening stage. This was followed by plantlets biohardened with *B. paraconglomeratum* (Table 2, Plate 2). Improved plant growth after *in vitro* bacterization against Fusarium wilt of banana was reported earlier [38]. This could be due to combined effects of suppression of Fusarium wilt by different biocontrol activities and plant growth promoting characters. Similarly, Yuan et al. [39] evaluated the ability of *B. amyloliquefaciens* to promote the growth of banana plants and reported that this species provided significant increase in plant height, stem diameter and dry weight. Yuan et al. [40] reported that *B.amyloliquefaciens* strain YL-25 when used as bio-organic fertilizer, significantly improved the growth of banana plants and decreased the incidence of Fusarium wilt compared to organic fertilizer and chemical fertilizer due to the production of phytohormones including IAA and GA_3 and stable antifungal compounds under pot culture conditions. Synthesis of IAA, GA_3 and ACC deaminase by *Bacillus* was earlier reported and attributed in regulation of the intracellular phytohormone metabolism leading to enhanced plant stress tolerance and improved plant growth [41].

3.3 Induction of Defense Compounds

In the present study higher activities of defense related enzymes and phenol were observed in endophytic bacteria treated plantlets when compared with untreated control. Pathogen inoculation in untreated control plants also stimulated the defense compounds, but the level was comparatively less than the plants treated with endophytic bacteria. The total phenol content in the roots of biohardened plants ranged from 355.00 catechol equivalents g^{-1} fresh weight of roots to 478.33. The maximum accumulation of total phenolic content was observed in plants treated with *B. amyloliquefaciens* VB7 (478.33 catechol equivalents g^{-1} fresh weight of roots). After challenge inoculation with Foc the total phenolic content increased by 33.45 percent than untreated control (Table 3). Similar trend was also observed in all the other treatments. Upon pathogenic infection, the phenols may get accumulated due to excess hydrogen peroxide. Increase in phenolic compounds and peroxidase activity in banana were positively correlated with Fusarium wilt resistance [42]. Phenols like phytoalexins get accumulated in the plant cells upon pathogenic infection and these are considered to have antimicrobial and antioxidative properties [43]. Anthony et al. [44] inferred that the accumulation of phytoalexins in the plant cells in response to Foc infection, lead to the increase of total phenolic contents.

The peroxidase activity ranged from 3.52 to 4.62 change in absorbance at 470 nm min^{-1} g^{-1} fresh weight of roots from biohardened plants. The activity of peroxidase was significantly high in plants treated with *S. maltophilia* (4.62 g^{-1} fresh weight of root). The peroxidase activity significantly increased to 93.67% in plantlets

biohardened with *B. amyloliquefaciens* VB7 after challenge inoculation with Foc (7.14 g^{-1} fresh weight of root) (Fig. 1). Peroxidase (PO) condense phenols into lignin and induce hypersensitive reaction towards the ingress of pathogenic [45].

Activity of PPO significantly increased to a tune of 92.39% and PAL activity increased to 97.60% in plantlets biohardened with *B.amyloliquefaciens* after challenge inoculation with Foc (Fig. 1, Fig. 2). The enzymes PO, PPO and PAL have been well known to play a major role in oxidation of phenolic compounds into quinines, synthesis of salicylic acid, contributing to defense mechanisms against plant pathogens [46,47].

Activity of β-1,3-glucanase significantly increased to 26.23% in plantlets biohardened with *B. amyloliquefaciens* VB7 after challenge inoculation with Foc (263.49 nmol of trans-cinnamic acid min^{-1} g^{-1} fresh weight of roots) (Fig. 3). β-1,3-glucanase was strongly stimulated in endophytic bacteria-treated plants challenged with *Foc*, whereas, inoculated control had very low levels of enzyme activity.Similarly, Kavino et al. [48] reported that chitinase and β-1,3-glucanase were stimulated in banana plantlets treated with *Pseudomonas fluorescens* Pf1, CHA0 and *Bacillus subtilis* EPB22 in the nursery. Lian et al. [49] reported that artificial inoculation of banana tissue culture plantlets with indigenous endophytes significantly reduced the incidence of Fusarium wilt besides enhancing the plant growth. Fishal et al. [31] also reported a significant disease suppression in banana cv. Berangan plants pre-inoculated with endophytic *Pseudomonas* strain UPMP3 due to increased accumulation of resistance related enzymes and pathogenesis related proteins.

3.4 Evaluation of Biohardened Plantlets against Fusarium Wilt

Examination of banana plantlets biohardened with different bacterial antagonists challenged with Foc for external and internal disease expression revealed that the complete absence of any symptoms in plantlets biohardened with *B.amyloliquefaciens, B.paraconglomeratum* and *S.maltophilia* (Table 4, Plate 3&4), whereas in pathogen inoculated control wilt the disease score was 3.0 with 100% severity. This could be due to precolonization in rhizosphere by bacterial endophytes. Thangavelu and Gopi [5] reported that *Bacillus sp*. (Klr4) reduced wilt severity to maximum extent (disease score 1.4), and also enhanced plant growth parameters compared to *Foc* alone-inoculated plants. The present study demonstrated that biohardened tissue cultured banana plantlets with the selected bacterial endophytes mediate induction of systemic resistance against pathogens. These endophytic isolates could therefore be used as potential biological control agents for Fusarium wilt disease of tissue culture banana cv. Ney Poovan (AB).

Table 1. Efficacy of bacterial endophytes of banana against Foc

Treatment	Treatment details	% Inhibition of Foc over control (Mean ± SE)	Zone of inhibition (mm) (Mean ± SE)
T1	*Achromobacter xyloxidans*	59.14±0.27^{b}	17.44±0.22^{c}
T2	*Myroides odoratimimis*	33.67±0.36^{f}	12.22±0.29^{e}
T3	*Bacillus amyloliquefaciens*	70.58±0.58^{a}	20.33±0.51^{a}
T4	*Brachybacterium paraconglomeratum*	47.48±0.14^{d}	18.44±0.29^{b}
T5	*Stenotrophomonas maltophilia*	54.65±0.26^{c}	15.67±0.19^{d}
T6	*Brucella melitensis*	38.27±0.43^{e}	10.22±0.29^{f}
T7	Control	00.00±0.00^{g}	0.00±0.00^{g}
	SE(d)	**0.48**	**0.28**
	CD (p = 0.05)	**1.05**	**0.60**

**Values are the mean of three replicates. Means in a column followed by standard error and same letters are not significantly different according to Duncan's multiple range test at p = 0.05*

Table 2. Influence of bacterial endophytes on plant growth promotion of tissue culture banana cv. Ney Poovan at the end of secondary hardening

Treatment	Treatment details	Plant height (cm)	Number of leaves	Number of roots	Root length (cm)
T1	*Achromobacter xyloxidans*	20.47±0.04^{c}	7.73±0.07^{bc}	17.93±0.13^{b}	26.29±0.04^{c}
T2	*Myroides odoratimimis*	15.35±0.04^{e}	7.53±0.18^{c}	14.60±0.23^{c}	23.28±0.05^{e}
T3	*Bacillus amyloliquefaciens*	23.30±0.06^{a}	8.13±0.18^{a}	19.60±0.12^{a}	30.26±0.03^{a}
T4	*Brachybacterium paraconglomeratum*	22.35±0.02^{b}	7.93±0.07^{ab}	18.33±0.18^{b}	28.28±0.10^{b}
T5	*Stenotrophomonas maltophilia*	17.38±0.03^{d}	7.60±0.12^{bc}	17.67±0.18^{b}	24.47±0.04^{d}
T6	*Brucella melitensis*	14.34±0.05^{f}	5.80±0.12^{d}	12.60±0.12^{d}	20.47±0.04^{f}
T7	Control	7.03±0.08g	5.67±0.13^{d}	12.33±0.18^{d}	15.34±0.05^{g}
	SE(d)	0.07	0.18	0.23	0.07
	CD (p = 0.05)	0.14	0.39	0.50	0.16

**Values are the means of three replicates. Means in a column followed by standard error and same letters are not significantly different according to Duncan's multiple range test at p = 0.05*

Table 3. Efficacy of bacterial endophytes on the phenol induction of banana cv. Ney Poovan before and after challenge inoculation with Foc

Treatment	Treatment details	Total phenol content prior to challenge inoculation with *Foc*	Total phenol content after challenge inoculation with *Foc*	% Change of total phenol content over control
T1	*Achromobacter xyloxidans*	428.33±3.33 ^{c}	533.33±4.41^{c}	24.51
T2	*Myroides odoratimimis*	371.67±3.33 ^{e}	435.00±2.89^{e}	17.04
T3	*Bacillus amyloliquefaciens*	478.33±4.41 ^{a}	638.33±4.41^{a}	33.45
T4	*Brachybacterium paraconglomeratum*	451.67±4.41 ^{b}	590.00±5.77^{b}	30.63
T5	*Stenotrophomonas maltophilia*	383.33±4.41 ^{d}	471.67±4.41^{d}	23.04
T6	*Brucella melitensis*	355.00±2.89 ^{f}	408.33±4.41^{f}	15.02
T7	Control	325.00±2.89 ^{g}	365.00±2.89^{g}	12.31
	SE(d)	5.27	6.04	
	CD (p = 0.05)	11.41	13.09	

**Values are the means of three replicates. Means in a column followed by standard error and same letters are not significantly different according to Duncan's multiple range test at p = 0.05.Total Phenol Content expressed as catechol equivalents g^{-1}fresh weight of roots*

Table 4. Influence of select bacterial endophytes on Fusarium wilt disease severity index in cv. Ney Poovan

Treatment	Treatment details	External Score	External DSI	Internal Score	Internal DSI
T1	*Achromobacter xyloxidans*	1.00	11.11 (3.10)^{bc}	1.33	14.81 (3.45)^{b}
T2	*Myroides odoratimimis*	0.33	3.70 (1.83)^{b}	1.00	11.11 (3.48)^{b}
T3	*Bacillus amyloliquefaciens*	0.00	0.00 (1.00)^{a}	0.00	0.00 (1.00)^{a}
T4	*Brachybacterium paraconglomeratum*	0.00	0.00 (1.00)^{a}	0.00	0.00 (1.00)^{a}
T5	*Stenotrophomonas maltophilia*	0.00	0.00 (1.00)^{a}	0.00	0.00 (1.00)^{a}
T6	*Brucella melitensis*	1.00	11.11 (3.10)^{bc}	2.33	25.92 (5.07)^{b}
T7	Control	3.00	100.00 (10.05)^{c}	3.00	100.00 (10.05)^{c}
	SE(d)		0.95		0.86
	CD (p = 0.05)		2.07		1.87

**Values are the means of three replicates. Means in a column followed by values in parenthesis are square root transformed values and same letters are not significantly different according to Duncan's multiple range test at p = 0.05*

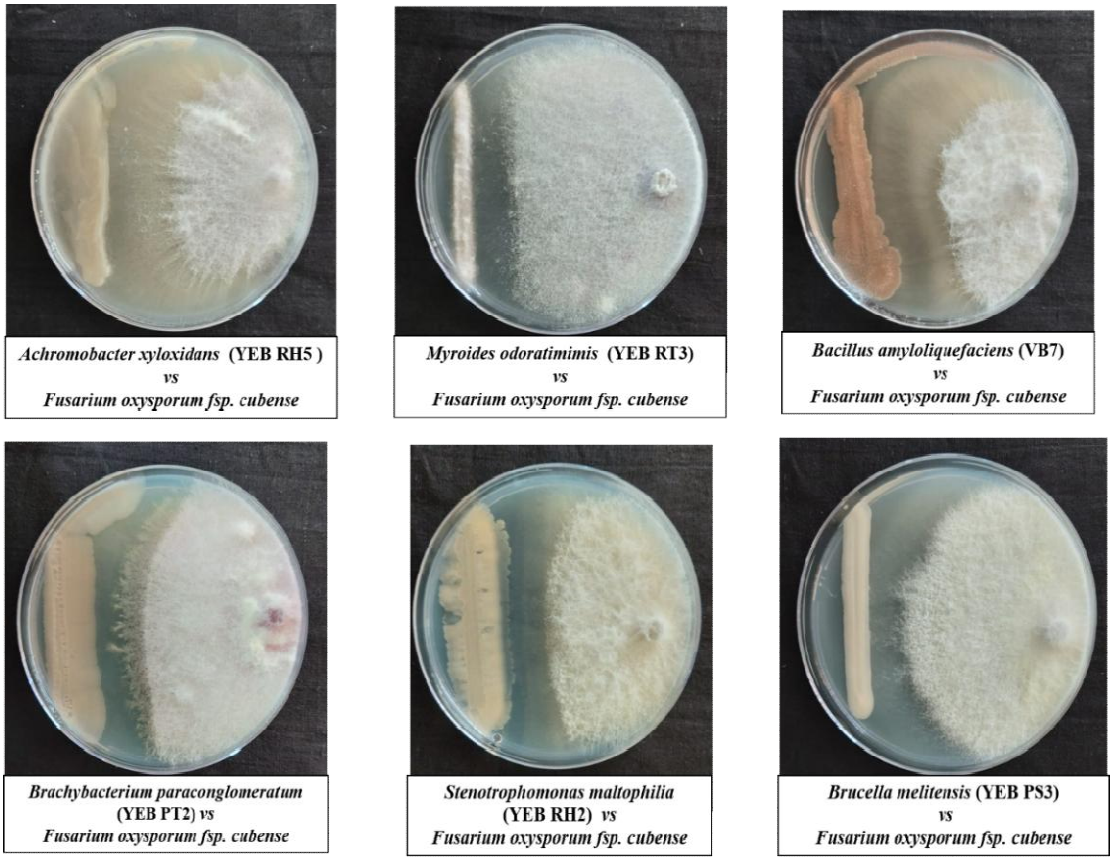

Plate 1. *In Vitro* antagonism of bacterial endophytes against Foc

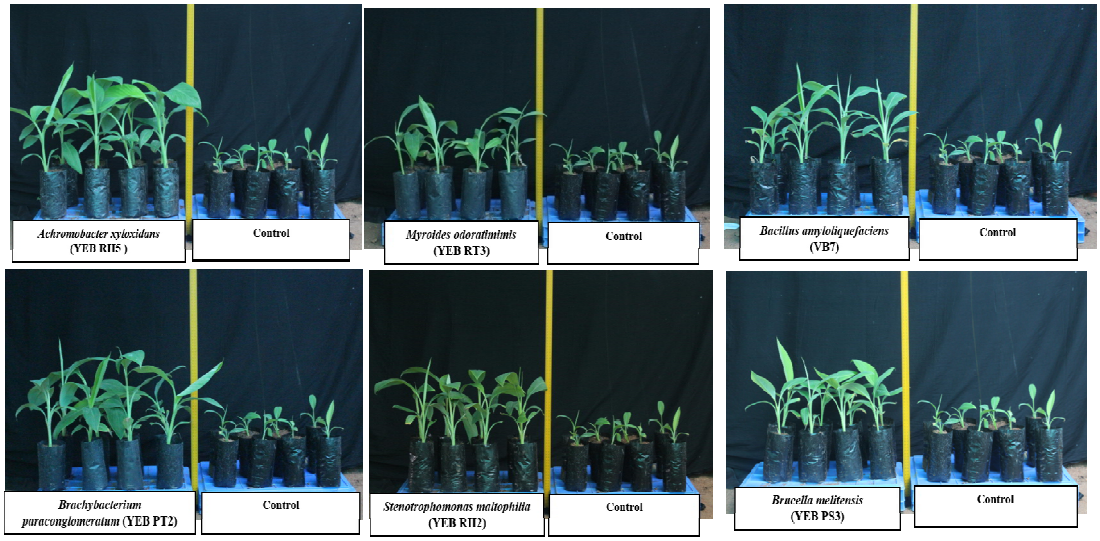

Plate 2. Effect of endophytes on plant growth promotion of TC plants of Banana cv. Ney Poovan

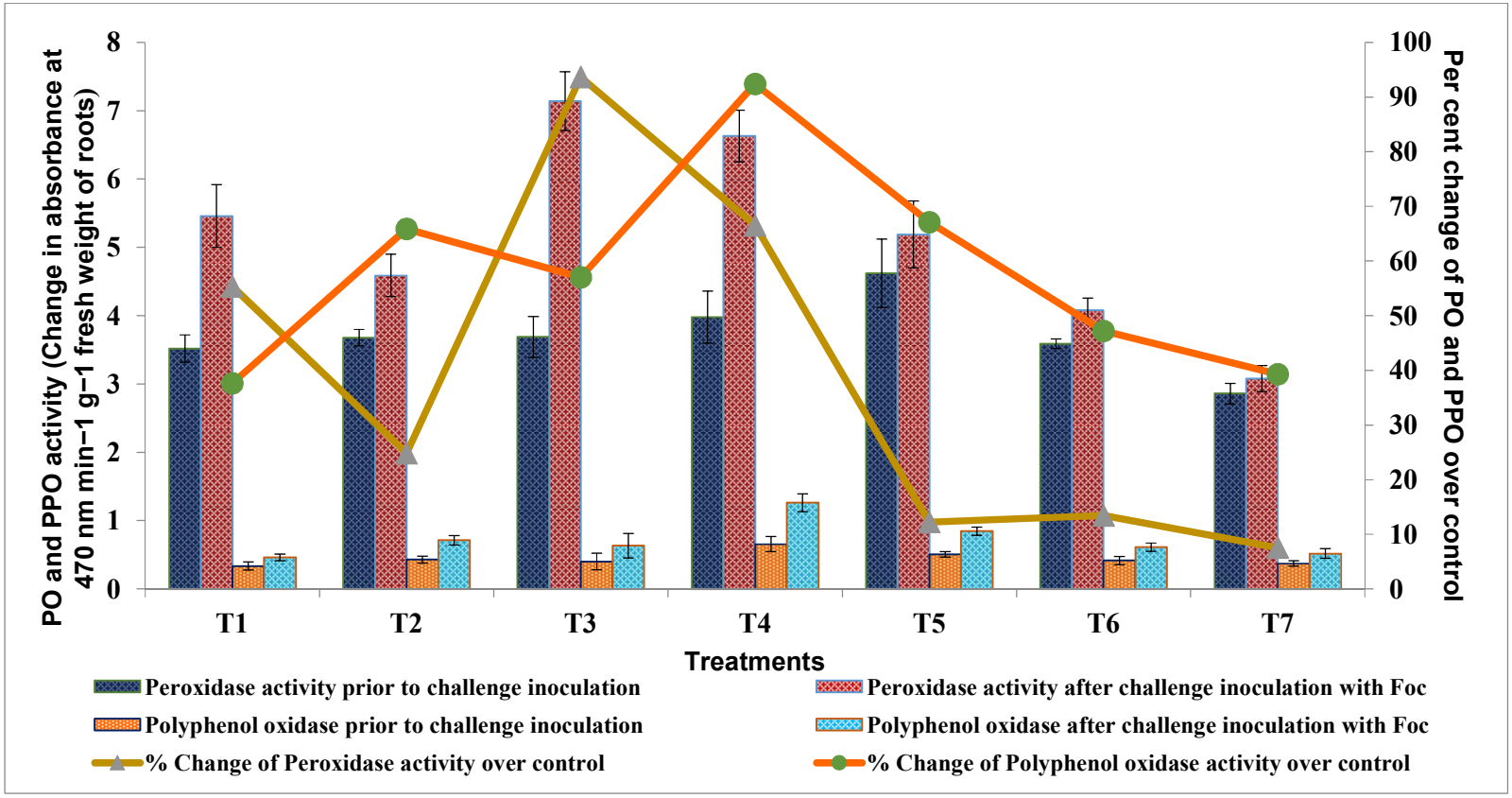

Fig. 1. Efficacy of bacterial endophytes on peroxidase and polyphenol activity in roots of banana cv.Ney Poovan against Foc

Treatment details

T1 –*Achromobacter xyloxidans*
T2- *Myroides odoratimimis*
T3 -*Bacillus amyloliquefaciens*
T4 –*Brachybacterium paraconglomeratum*
T5 – *Stenotrophomonas maltophilia*
T6 – *Brucella melitensis*
T7 – *Control*

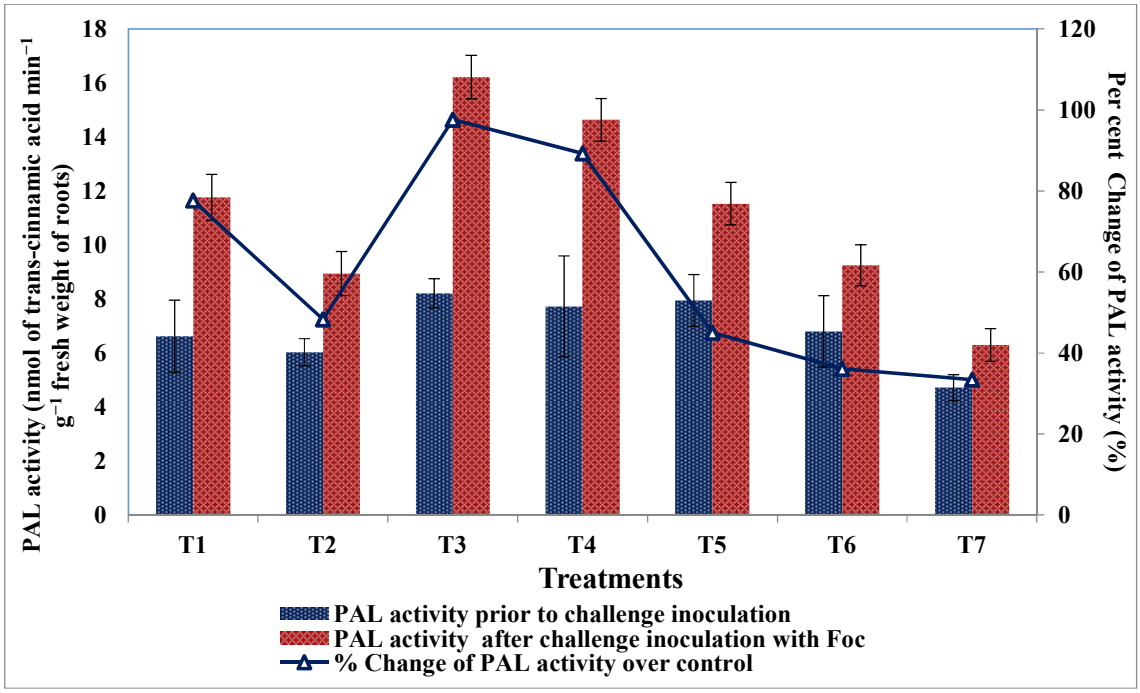

Fig. 2. Efficacy of bacterial endophytes on phenylalanine ammonia lyase activity in roots of banana cv. Ney Poovan against Foc

Treatment details

T1 –*Achromobacter xyloxidans*
T2- *Myroides odoratimimis*
T3 -*Bacillus amyloliquefaciens*
T4 –*Brachybacterium paraconglomeratum*
T5 – *Stenotrophomonas maltophilia*
T6 – *Brucella melitensis*
T7 - Control

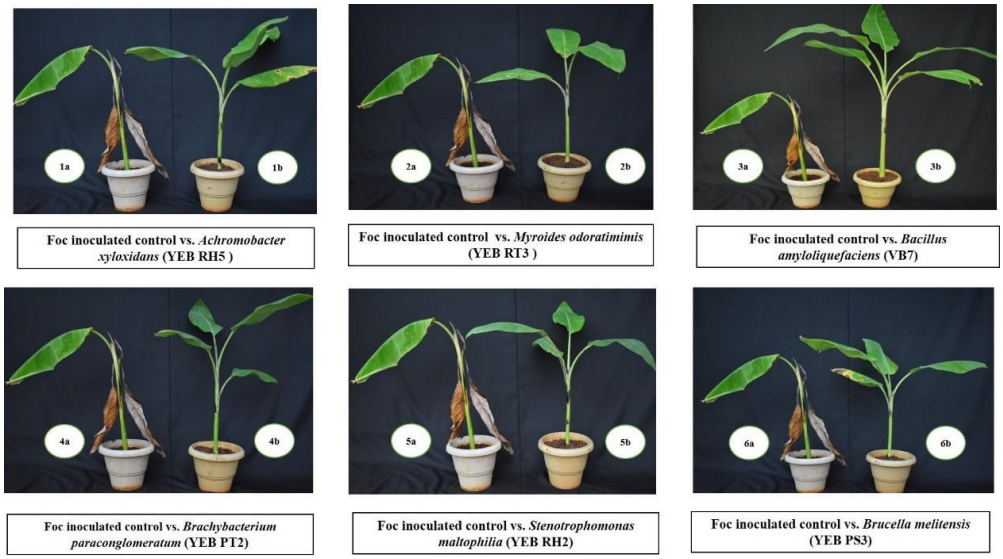

Plate 3. External Fusarium wilt disease severity index of biohardened plants of banana cv. Ney Poovan (AB)

		Symptom	*Scale*
1a,2a,3a,4a,5a,6a	:	*All leaves with intense yellowing or plant dead*	*-3*
2b,6b	:	*Yellowing of lower leaves at initial stage*	*-1*
3b,4b,5b	:	*No symptoms*	*-0*

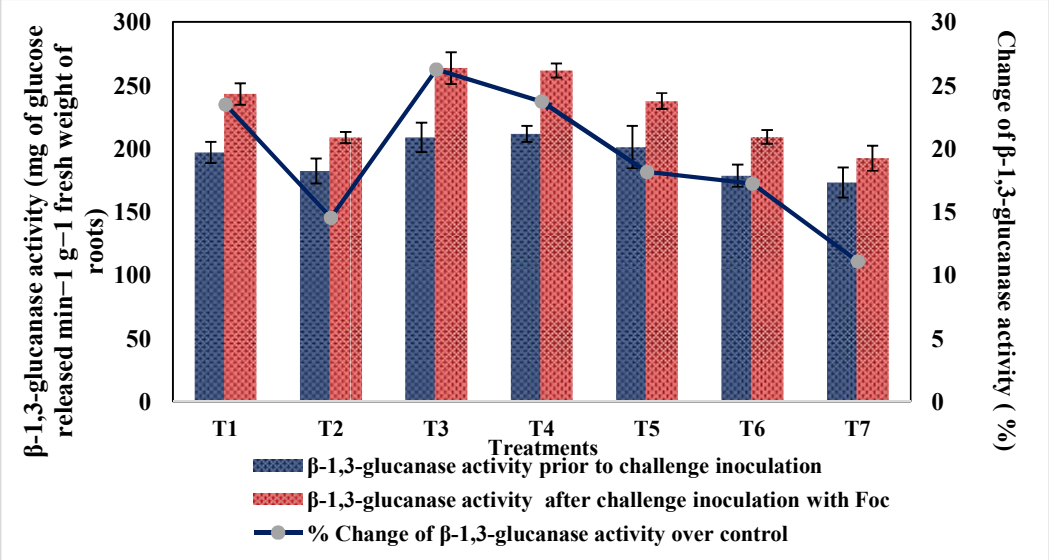

Fig. 3. Efficacy of bacterial endophytes on the activity of β-1,3-glucanase in roots of banana cv. Ney Poovan against Foc

Treatment details

T1 –*Achromobacter xyloxidans*
T2- *Myroides odoratimimis*
T3 -*Bacillus amyloliquefaciens*
T5 – *Stenotrophomonas maltophilia*
T6 – *Brucella melitensis*
T7 -Control T4 –*Brachybacterium paraconglomeratum*

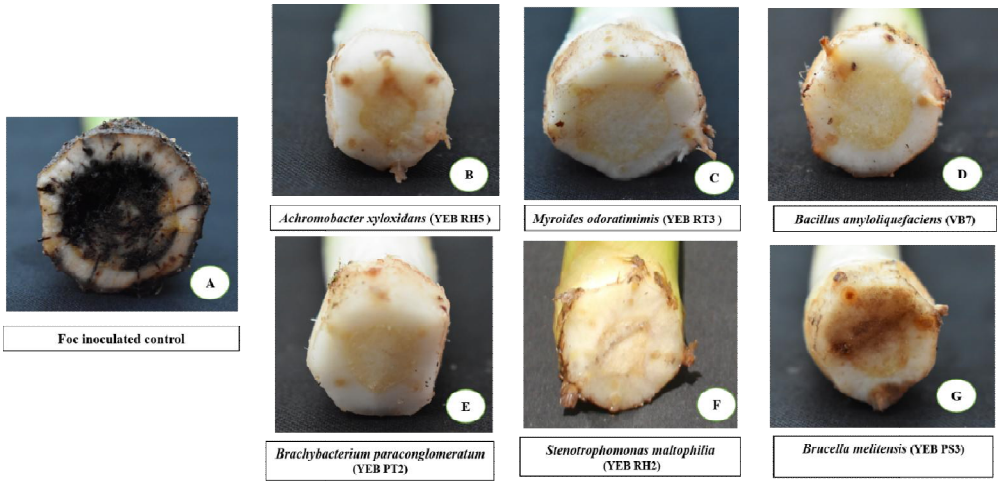

Plate 4. Internal disease severity index of biohardened plants of banana cv. Ney Poovan (AB)

		Symptom	Scale
A	:	*Rhizome with most of the internal tissues showing necrosis (> 40%)*	*-3*
B,C	:	*Initial rhizome discoloration (1-20%)*	*-1*
D,E F	:	*No symptoms*	*-0*
G	:	*Slight rhizome discoloration along the whole vascular system (21-40%)*	*-2*

4. CONCLUSION

The present study demonstrated that tissue culture banana plantlets of cv. Ney Poovan (AB) biohardened with 100 ml of bacterial suspension containing 3×10^{10} CFU ml^{-1} of select bacterial endophytes *viz.*, *Bacillus amyloliquefaciens* (VB7), *Brachybacterium paraconglomeratum* (YEB PT2) and *Stenotrophomonas maltophilia* (YEB RH2) by drenching the banana root during both, primary and secondary hardening stages mediate induction of systemic resistance against

Foc and effectively combat Foc . Among these, as the growth promotion was also better with *Bacillus amyloliquefaciens* (VB7), it can serve as a potential biological control agent for the management of Fusarium wilt in tissue culture banana.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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