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RESEARCH ARTICLE

Impact of management technologies on banana growth and *Fusarium* Wilt mitigation

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Abstract

The study was conducted during 2023-2024 under the Department of Fruit Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The objective of the study was to find out the best technology for the Fusarium wilt management in the banana cultivar Rasthali under pot culture experiment. The experiment was conducted with nine treatments replicated thrice with three plants in each replication in a completely randomized design (CRD). The treatment T7- Bacillus subtilis (10 g/plant) + Salicylic acid 5mM spray showed more root length (71.89 cm) and number of roots (30) than other treatments. The treatment T7- Bacillus subtilis (10 g/plant) + Salicylic acid 5mM spray showed a 33 % improvement in root length over absolute control (T1) and a 53 % increase over inoculated control (T2). Whereas the number of roots was 50% more in T7 than in T1 and 76 % more than in T2. The length of the pseudostem and girth of the pseudostem were 69.40 cm and 25.43 cm. The leaf area was 2.99 m2 and the number of leaves was 12. The physiological parameters such as chlorophyll content (57.03), stomatal resistance (0.521 Scm-1) and transpiration rate (9.572 µg H2O cm2 S-1) of T7 were significant. In soil enzymes, the chitinase activity was 37.50 µmol/min/g and β 1, 3-Glucanase 40.33 µmol/min/g. It showed less disease incidence (DI %) of 18.86 % and a disease severity index (DSI %) of 14.73 %. The absolute control (T1), without the pathogen, had no disease. The inoculated control (T2) showed the highest disease incidence and disease severity index of 100 %. T7- Bacillus subtilis (10 g/plant) + Salicylic acid 5 mM spray showed less disease incidence (DI %) of 18.86 % and a disease severity index (DSI%) of 22.93 % over the absolute control (T1). And 81.14 % and 77.07 % reduced disease incidence (DI %) and disease severity index (DSI %) compared to inoculated control (T2). It is concluded that T7- Bacillus subtilis (10 g/plant) + Salicylic acid 5 mM spray can be used to control Fusarium wilt in cultivar Rasthali.

Keywords

arbuscular mycorrhizal fungi; *Bacillus*; *Fusarium* wilt; Rasthali; salicylic acid; *Trichoderma*

Introduction

Banana (*Musa* spp.) is a globally significant fruit crop, providing food security and income for many, with India leading in production. With 1200 kinds, bananas are the

second most popular fruit crop produced worldwide FAO, 2023 (1). India is reportedly the world's greatest producer of bananas, followed by China, Indonesia and Brazil. According to Indiastat 2023- 2024, 2nd advance estimate in India bananas are produced under an area of 995 million hectares with 37474 million metric tonnes of production and 37.66 MT productivity (2, 3). Tamil Nadu is ranked fourth in India for banana production, with an estimated 3,895.64 million MT, or 10.41 per cent, of the nation's total production (4). The Rasthali banana cultivar is particularly important in states like Tamil Nadu due to its flavor, nutritional value and adaptability to various climates. However, banana production faces serious threats from pathogens, particularly *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*). This fungal disease leads to significant yield losses (5, 6) especially in susceptible cultivars such as Rasthali and is characterized by severe symptoms including leaf yellowing, wilting and vascular discoloration. The pathogen's persistence in soil and varied physiological races complicate management, as different races target specific banana cultivars worldwide. In India, Panama disease is caused by *Fusarium oxysporum* f. sp. *cubense* race 1 strain, resulting in yield losses of 50-70 % and several varieties such as Rasthali, Amirtapani, Karpooravalli, Monthan, Ney Poovan and Virupakshi are affected by this race. Tamil Nadu, one of the major Rasthali-producing areas suggests that *Fusarium* wilt has caused 20- 30% yield loss in affected fields (5).

To combat *Fusarium* wilt, integrated management approaches are essential. Chemical, biological and cultural methods, alongside resistant cultivars, have shown promise (7-9). Recent approaches focus on integrating multiple methods to address these shortcomings, combining antifungal agents, soil amendments and plant resistance inducers to enhance disease control, improve yields and reduce growth periods. These integrated strategies prioritize sustainability, reducing reliance on harmful chemicals, preserving soil health and offering practical, cost-effective solutions for managing Fusarium wilt in banana cultivation. Biological agents like *Bacillus subtilis* and *Trichoderma viride* play key roles in enhancing plant defenses, while biostimulants and biofertilizers such as Salicylic acid, arbuscular mycorrhizal fungi improve plant natural resilience. Using the antagonistic fungi and bacteria lessens the negative impacts of soil-borne phytopathogens on crop yields. Biological control aims to either directly fight phytopathogens or stimulate plant defenses. This results from intricate interactions between plant-pathogen antagonists and the local microbiota (10). According to antagonism is the main biocontrol mechanism. The primary biocontrol mechanism is antagonism. Enzymes that break down cell walls, like glucanase, are the primary antagonistic substances produced by biocontrol bacteria. It was also discovered that the pathogen's metabolic processes were impacted when biocontrol bacteria successfully broke down the pathogen's cell membrane by metabolizing bacteriostatic chemicals (11).

In banana plants, endophytic *Bacillus subtilis* has improved resistance to *Fusarium* wilt. The treatment shortened the growth time in the field, improved yields and managed the illness. Mycosubtilin, a lipopeptide with potent antifungal effects, is produced by *Bacillus subtilis*. Mycosubtilin interacts with cell membranes and compromises their integrity to show action against various fungi, including *Fusarium oxysporum (12).*

The study helps evaluate these strategies' effectiveness, specifically Focusing on *Fusarium* wilt in Rasthali bananas. The objectives include studying the morphological, physiological, biochemical, soil and disease parameters, thus identifying the most effective approach for controlling *Fusarium* wilt under pot culture conditions (13).

Materials and Methods

Tissue culture-derived Banana cv. Rasthali plants were collected from Southern Petrochemical Industries Corporation Ltd. (SPIC), Agri Biotech Coimbatore, Tamil Nadu 641018 and brought to Tamil Nadu Agricultural University, Coimbatore. The plants were kept in a shade net house with panchagavya spray rich in beneficial microorganisms (e.g., *Lactobacillus* and *actinomycetes*) that suppress soil-borne pathogens like *Fusarium oxysporum*. The plants were inoculated with *Fusarium oxysporum* f. sp. *cubense* Race 1 culture, obtained from the Department of Plant Pathology, TNAU, Coimbatore. The roots of the banana plants were gently crushed before inoculation to create wounds. The roots and rhizomes were dipped for 30 minutes in 200 mL of *Foc* spore suspension (10^6 conidia/ml) contained in a 500 mL sterile beaker. The absolute control plants underwent the same process but were treated with sterile distilled water. A second inoculation with the same *Foc* spore suspension was carried out 20 days after the initial treatment with *Fusarium.*

The experiment was conducted as a pot culture study due to several practical and experimental advantages that allow for controlled, reproducible and precise observations, particularly for the evaluation of *Fusarium* wilt management strategies and requires less time to observe disease progression and treatment efficacy compared to field trials, where environmental factors and larger areas may slow observations. It was conducted in a Complete Randomized Design (CRD) with nine treatments including control. The treatment details are as follows, **T_1**- Absolute control (without pathogen); **T_2**- Inoculated control (with pathogen); **T_3**- *Bacillus subtilis* @ 10 g/plant soil application; **T_4**- *Trichoderma viride* @ 10 g/plant soil application; **T_5**- AM fungi 20 g/plant soil application; **T_6**- Salicylic acid 5 mM spray; **T_7**- *Bacillus subtilis* @ 10 g/plant + Salicylic acid 5 mM spray; **T_8**- *Trichoderma viride* @ 10 g/plant + Salicylic acid 5 mM spray; **T_9**- Arbuscular mycorrhizal Fungi 20 g/plant + Salicylic acid 5 mM spray. Each treatment was replicated thrice with three plants per replication, resulting in 72 plants. The effect of the treatments ensures that environmental conditions are uniform across all experimental units including control, enhancing the validity and reliability of the study's findings. The treatments were applied on 0, 30 and 60 days of post-inoculation. The observations were recorded on morphological, physiological, biochemical and soil parameters.

Morphological parameters

Root length

The length of the longest root was measured using a tape and noted in cm.

Number of roots

The number of roots was observed and counted by uprooting banana plants.

Pseudostem height

Using a measuring tape, the height of the banana plants was determined from the base of the plant to the surface to their first internode, expressed in cm.

Pseudostem girth

The girth of the pseudostem was measured at a height of 15 cm from the ground.

Number of leaves

The number of viable leaves on the pseudostem was counted and the mean values were calculated.

Total leaf area

The functional leaf area in cm^2 was calculated by multiplying the leaf length and leaf breadth of the third leaf by the factor 0.8 (k_1).

Physiological parameters

Chlorophyll content

The amount of chlorophyll was estimated using the portable chlorophyll meter SPAD-502, resulting in arbitrary units.

Stomatal resistance

The resistance offered by stomata to exit the water from the leaves' surface was measured using a Steady-State Porometer (LI-1600, LICOR, Inc., Nebraska and USA) and expressed in Scm^{-1}.

Transpiration rate

Water transpiration loss was measured with a Steady-State Porometer (LI1600, LICOR, Inc., Nebraska and USA) and expressed in µg H_2O cm^2s^{-1}.

Disease parameters

Disease incidence (DI %)

One of the symptoms of *Fusarium* wilt disease that has been seen and recorded in banana plants is the wilting or yellowing of the leaves. The following formula was used to calculate the percentage of DI (Eqn. 1).

$$\text{Distance incidence (\%)} = \frac{\text{Number of Fusarium infected plants}}{\text{Total number of plants assessed}} \times 100$$

Disease severity index (DSI %)

Using a modified disease rating scale on leaf yellowing by (14) the disease severity was examined. The disease-symptom evaluation and infection counts were used to calculate the DSI index (Eqn. 2).

$$\text{Disease severity index (\%)} = \frac{\Sigma(\text{Number of plants in the scale category} \times \text{specific scale category})}{\text{Total number of plants assessed} \times \text{Highest scale category}} \times 100$$

Scoring for external symptoms:

Symptoms	Scale
No symptoms	0
Lower leaves exhibit yellowing and necrotic signs.	1
Older and few younger leaves are yellowish in color or exhibit necrosis	2
All the leaves are yellowish or have necrosis on them or the plant is dead	3

Biochemical parameters

Peroxidase

1 mL of enzyme extract, 1 mL of o-Dianisidine (1 mg/mL) and 3 ml of phosphate buffer (0.2 M) were added into a cuvette within a spectrophotometer. The reaction was initiated by adding 0.2 mL of hydrogen peroxide (0.1 M). The enzyme kinetics were monitored at 430 nm for three minutes at 30 s intervals. For each minute, a linear rise in absorbance was plotted against time to assess the peroxidase activity. (15). It was denoted in ΔA min/g (Eqn. 3).

$$\text{Peroxidase } (\Delta A/\text{min/g}) = \frac{\Delta A/\text{min}}{2} \times \frac{10}{2} \times 1$$

Polyphenol oxidase

0.1 M Phosphate buffer (10ml) of PH 6.00 was prepared and used to macerate one gram of root tissue powder of banana and centrifuged to obtain an aliquot further used for the estimation as the enzyme source. It was added to the cuvette which contains 1.5 ml of 0.1 M Phosphate buffer and 0.2 ml of enzyme extract. Then 200 µl of 0.001 M Catechol was added to start the reaction. The catechol oxidation was measured at 495 nm for about 5 minutes at an interval of 30 seconds and expressed as ΔA/min/g.

Phenylalanine ammonia-lyase

For enzyme extraction, 0.5 g of root sample was homogenized with 5 mL of cold (4°C) borate HCl (25 mM, pH 8.8) with 5 mM mercaptoethanol employed as the buffer. 0.2 mL of the enzyme extract, 0.5 mL of the buffer and 1.3 mL of water were taken into the test tube. Subsequently, 0.1 M L- phenylalanine was added and incubated at 32 °C for 30 to 60 min. Then, the reaction was stopped by adding 0.5 mL of IM Trichloroacetic acid (TCA). On the other hand, phenylalanine was introduced after the TCA as a control. Several amounts of trans-cinnamic acid were used in the standard's creation. (16). PAL activity was measured spectrophotometrically by monitoring the synthesis of trans-cinnamic acid, which shows an increase in absorbance at 290 nm. The value that was obtained was expressed in µg/min/g (17) (Eqn. 4)

$$\text{PAL } (\mu g/\text{min/g}) = \frac{\text{Graph value} \times 5 \times 1}{0.2 \times 0.5 \times 30}$$

Soil properties

Chitinase

In Soil Extraction add 10 g of soil to 50 mL phosphate buffer (0.1 M, pH 7.0) and shake for 30-60 min. Centrifuge at 10,000 rpm for 15 minutes and collect the supernatant as the enzyme extract. For the substrate preparation colloidal chitin is suspended in a Phosphate buffer for enzyme assay. Incubation is done by mixing 1 mL of the soil enzyme extract with 1 mL of substrate solution and it is incubated at 37 °C for 1-2 hrs. Then measure at absorbance 540 nm using a spectrophotometer and denoted as µmol/min/g.

β-1,3-Glucanase

To extract the enzymes, take 10 g soil in 50 mL Phosphate buffer (0.1 M, pH 7.0), shake, then centrifuge. Dissolve laminarin (typically 0.5 % solution) in the buffer for the preparation of substrate. Combine 1 mL enzyme extract with 1 mL substrate

solution. Incubate at 37 °C for one to two hours. Add 1 mL dinitro salicylic acid (DNS) reagent, then heat at 100 °C for 5-10 min for colorimetric detection. Measure the absorbance at 540 nm using a spectrophotometer and the activity was expressed in µmol/min/g.

Results

Morphological parameters

The data given in Table 1 showed significant differences among the combination treatment of *Bacillus subtilis* and 5mM salicylic acid spray (T_7) with the longest root length at 90 dpi, measuring 71.89 cm compared to the absolute control (T_1) with a root length of 53.69 cm, whereas the inoculated control (T_2), with a shorter root length of 46.84 cm, indicating the impact of disease on root development. The combination of *Bacillus subtilis* (10 g/plant) with a 5mM salicylic acid spray (T_7) yielded significance with a number root count of 30 roots, making it the most effective treatment in promoting root growth. The absolute control (T_1) produced 20 roots, while the inoculated control (T_2) had fewer roots, with only 17, indicating that disease presence negatively impacted root development. This suggests that the treatment with *Bacillus subtilis* and salicylic acid had a notable positive effect on root growth, potentially due to enhanced resistance to Fusarium or improved plant vitality. More pseudostem height was observed in the *Bacillus subtilis* and salicylic acid spray (T_7), which reached 69.40 cm, making it the most effective in promoting stem growth. The absolute control (T_1) showed a height of 54.02 cm, while the inoculated control (T_2) had a lower height of 51.01 cm, reflecting the adverse impact of the disease. Greater pseudostem girth was observed in the (T_7) *Bacillus subtilis* and salicylic acid spray treatment, achieving 25.43 cm. Absolute control T_1 had a girth of 11.33 cm and inoculated control T_2measured 10.23 cm, indicating reduced stem thickness in diseased, untreated plants. The best result was achieved by the *Bacillus subtilis* (10 g/plant) combined with a 5mM salicylic acid spray (T_7), which reached a significantly larger leaf area of 2.999 cm^2. For leaf area, the absolute control (T_1) measured 0.787 cm^2, while the inoculated control (T_2) showed a lesser leaf area of 0.613 cm^2, highlighting the negative impact of disease. The T_7 *Bacillus subtilis* and salicylic acid spray produced 12 leaves per plant, which was the highest count. This was followed by T_8 and T_9, each producing 11 leaves. T_1 absolute control resulted in 9 leaves, whereas T_2 inoculated control had 8 leaves, showing the disease's impact on leaf production.

Physiological parameters

In Table 1 chlorophyll content, the best-performing treatment was *Bacillus subtilis* (10 g/plant) combined with salicylic acid spray (T_7), which reached a higher chlorophyll content of 57.03 SPAD units, whereas absolute control (T_1) showed 42.00 SPAD units and the inoculated control (T_2) 35.00 SPAD units respectively. Significant differences were obtained in stomatal resistance during 90 days of post-inoculation. T_7 *Bacillus subtilis* with salicylic acid spray showed the least results, with a resistance of 0.521 Scm^{-1}, indicating a lower resistance compared to the inoculated control (T_2) at 1.021 Scm^{-1}, which is associated with increased stress levels. The absolute control (T_1) had a stomatal resistance of 0.870 Scm^{-1}. The transpiration rate of banana cv. Rasthali at 90 days post-inoculation (dpi), was highest with the *Bacillus subtilis* (10 g/plant) + salicylic acid spray (T_7), which recorded a transpiration rate of 9.572 µg H_2O cm^2 S^{-1}. This treatment significantly outperformed both the absolute control (T_1) at 8.008 µg H_2O cm^2 S^{-1} and the inoculated control (T_2) at 6.732 µg H_2O cm^2 S^{-1}.

Disease parameters

Disease incidence (DI %)

The analysis of data given in Table 2, aimed to assess the effectiveness of different management strategies in reducing *Fusarium* wilt incidence in banana plants (cv. Rasthali). There was a significant difference at 90 dpi among the treatments, the combination of *Bacillus subtilis* with salicylic acid spray (T_7) demonstrated a significant result lowest DI of 18.86 %. The absolute control group (T_1) showed no disease throughout, while the inoculated control group (T_2) reached 100 % DI by 90 dpi, indicating severe infection under untreated conditions. These results highlight the superior performance of *Bacillus subtilis* combined with salicylic acid in reducing disease incidence, suggesting its potential as an effective, integrated approach for managing *Fusarium* wilt in banana cultivation.

Disease severity index (DSI %)

Table 2 on the disease severity index (DSI %) of *Fusarium* wilt in banana plants (cv. Rasthali) at 90 days post-inoculation (dpi) inferred that there was a significant difference among the treatments. The absolute control (T_1) showed no disease symptoms, maintaining a DSI of 0.00%. In contrast, the inoculated control (T_2) exhibited the highest severity with a DSI of 100.00 %. Among the treatments, the combination of *Bacillus subtilis* and salicylic acid spray (T_7) proved to be the most effective, with a DSI of 14.73 % at 90 dpi. This was significantly lower than other treatments.

Table 1. Effect of management technologies on the growth and physiological parameters of banana cv. Rasthali at 90 days of post-inoculation

Treatments	Root length (cm)	Number of roots	Pseudostem height (cm)	Pseudostem girth (cm)	Leaf area (cm^2)	Number of leaves	Chlorophyll content (SPAD meter)	Stomatal resistance (Scm^{-1})	Transpiration rate (µg H_2O cm^2 S^{-1})
T_1	53.69^{e}	20.00^{c}	54.02^{d}	11.33^{d}	0.787^{e}	9.00^{c}	42.00^{d}	0.870^{b}	8.008^{bc}
T_2	46.84^{f}	17.00^{d}	51.01^{e}	10.23^{e}	0.613^{f}	8.00^{d}	35.00^{e}	1.021^{a}	6.732^{d}
T_3	61.78^{c}	23.00^{b}	63.23^{bc}	17.36^{bc}	1.920^{c}	11.00^{a}	48.00^{bc}	0.727^{d}	9.005^{a}
T_4	60.98^{cd}	21.00^{bc}	62.03^{c}	16.36^{c}	1.953^{c}	10.00^{b}	42.00^{d}	0.773^{d}	8.018^{a}
T_5	59.01^{d}	19.00^{c}	61.83^{c}	15.46^{c}	1.097^{d}	10.00^{b}	47.03^{bc}	0.802^{b}	8.090^{b}
T_6	60.23^{cd}	20.00^{c}	60.24^{c}	17.08^{bc}	1.294^{cd}	10.00^{b}	48.21^{bc}	1.301^{a}	8.065^{bc}
T_7	71.89^{a}	30.00^{a}	69.40^{a}	25.43^{a}	2.999^{a}	12.00^{a}	57.03^{a}	0.521^{c}	9.572^{a}
T_8	67.85^{ab}	27.00^{ab}	68.20^{a}	23.23^{b}	2.100^{b}	11.00^{b}	49.00^{b}	0.602^{a}	8.900^{bc}
T_9	63.75^{b}	25.00^{ab}	65.47^{b}	21.30^{b}	2.000^{c}	11.00^{b}	46.00^{c}	0.589	8.751^{c}
SE(d)	**0.983**	**0.582**	**1.270**	**0.562**	**0.043**	**0.293**	**1.206**	**0.043**	**0.227**
CD	**2.084**	**0.898**	**2.692**	**1.192**	**0.090**	**0.621**	**2.556**	**0.870^{b}**	**0.490**

T_1- Absolute control (without pathogen); **T_2**- Inoculated control (with pathogen); **T_3**- *Bacillus subtilis* @ 10 g/plant soil application; **T_4**- *Trichoderma viride* @ 10 g/plant soil application; **T_5**- AM fungi 20 g/plant soil application; **T_6**- Salicylic acid 5 mM spray; **T_7**- *Bacillus subtilis* @ 10 g/plant + Salicylic acid 5 mM spray; **T_8**- *Trichoderma viride* @ 10 g/plant + Salicylic acid 5 mM spray**; T_9**- AM Fungi 20 g/plant + Salicylic acid 5 mM spray. Each value is a mean for three replicates (p = 0.05).

Table 2. Effect of management technologies on the disease incidence and disease severity index on banana cv. Rasthali at 90 days of post-inoculation

Treatments	Disease incidence (%)	External disease severity index (%)
T_1	0.00^{g}	0.00^{e}
T_2	100.00^{a}	100^{a}
T_3	27.88^{d}	20.93^{d}
T_4	30.70^{c}	21.97^{d}
T_5	31.76^{b}	28.80^{c}
T_6	30.44^{cd}	34.97^{b}
T_7	18.86^{f}	22.93^{d}
T_8	20.73^{e}	36.00^{b}
T_9	24.59^{b}	1.325
SE(d)	1.347	1.325
CD	2.856	2.808

T_1- Absolute control (without pathogen); **T_2**- Inoculated control (with pathogen); **T_3**- *Bacillus subtilis* @ 10 g/plant soil application; **T_4**- *Trichoderma viride* @ 10 g/plant soil application; **T_5**- AM fungi 20 g/plant soil application; **T_6**- Salicylic acid 5 mM spray; **T_7**- *Bacillus subtilis* @ 10 g/plant + Salicylic acid 5 mM spray; **T_8**- *Trichoderma viride* @ 10 g/plant + Salicylic acid 5 mM spray**;** **T_9**- AM Fungi 20 g/plant + Salicylic acid 5 mM spray. Each value is a mean for three replicates (p = 0.05).

Biochemical parameters

Peroxidase

The combination of *Bacillus subtilis* with Salicylic acid spray (T_7) exhibited the highest peroxidase activity at 90 dpi, reaching 1.764 ΔA min/g. The absolute control (0.697 ΔA min/g) and inoculated control (0.797 ΔA min/g), demonstrated weaker defense responses as represented in Fig. 1.

Polyphenol oxidase

For polyphenol oxidase activity at 90 dpi (Fig. 1), the best-performing treatment was *Bacillus subtilis* (10 g/plant) with Salicylic acid 5mM spray (T_7), which reached 0.089 ΔA min/g. These combinations demonstrated a clear enhancement in polyphenol oxidase activity compared to the absolute control (0.060 ΔA min/g) and inoculated control (0.050 ΔA min/g), indicating their role in improving the plant's defense mechanisms.

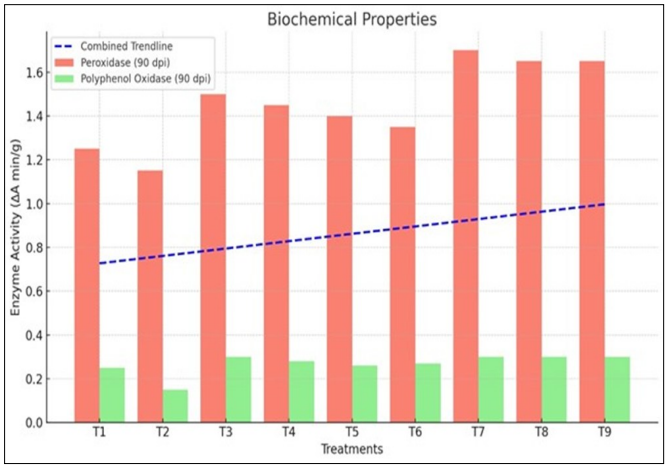

Fig. 1. Effect of management technologies on the peroxidase (ΔA min/g) and polyphenol oxidase (ΔA min/g) of banana cv. Rasthali at 90 days of post-inoculation. **T_1**- Absolute control (without pathogen); **T_2**- Inoculated control (with pathogen); **T_3**- *Bacillus subtilis* @ 10 g/plant soil application; **T_4**- *Trichoderma viride* @ 10 g/plant soil application; **T_5**- AM fungi 20 g/plant soil application; **T_6**- Salicylic acid 5 mM spray; **T_7**- *Bacillus subtilis* @ 10 g/plant + Salicylic acid 5 mM spray; **T_8**-*Trichoderma viride* @ 10 g/plant + Salicylic acid 5 mM spray**;** **T_9**- AM Fungi 20 g/plant + Salicylic acid 5 mM spray. Each value is a mean for three replicates (p = 0.05).

Phenylalanine ammonia-lyase

In Fig. 2 the percentage increase in phenylalanine ammonia-lyase (PAL) activity from the absolute control (8.53 µg/min/g) was highest in the combination of *Bacillus subtilis* + Salicylic acid (T_7), which showed an impressive increase of 51.82 %, reaching 12.95 µg/min/g at 90 dpi. The inoculated control (T_2) showed a smaller increase of 8.21 %, reaching 9.23 µg/min/g. These results demonstrate that the combined treatments, particularly *Bacillus subtilis* with Salicylic acid, significantly enhanced PAL activity compared to the absolute control.

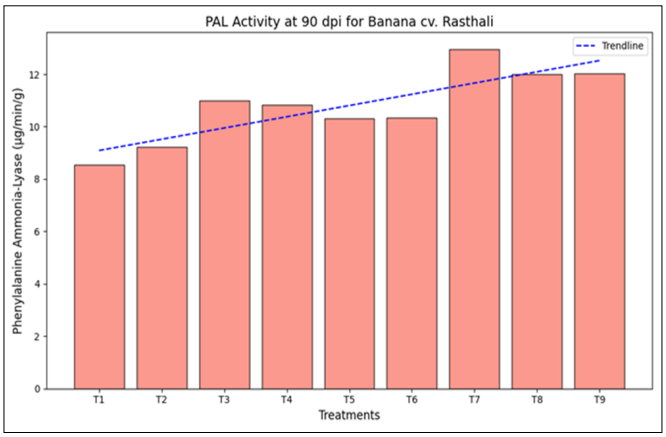

Fig. 2. Effect of management technologies on the phenylalanine ammonia-lyase (µg/min/g) of banana cv. Rasthali at 90 days of post-inoculation. **T_1**- Absolute control (without pathogen); **T_2**- Inoculated control (with pathogen); **T_3**- *Bacillus subtilis* @ 10 g/plant soil application; **T_4**- *Trichoderma viride* @ 10 g/plant soil application; **T_5**- AM fungi 20 g/plant soil application; **T_6**- Salicylic acid 5 mM spray; **T_7**- *Bacillus subtilis* @ 10 g/plant + Salicylic acid 5 mM spray; **T_8**- *Trichoderma viride* @ 10 g/plant + Salicylic acid 5 mM spray**;** **T_9**- AM Fungi 20 g/plant + Salicylic acid 5 mM spray. Each value is a mean for three replicates (p = 0.05).

Soil properties

Chitinase

The data showed in Fig. 3 at 90 dpi, the *Bacillus subtilis* + Salicylic acid (T_7) treatment had the highest chitinase activity with a value of 37.50 µmol/min/g, significantly outperforming all other treatments. In comparison, the inoculated control (T_2) recorded a lower chitinase value of 27.82 µmol/min/g, while the absolute control (T_1) showed the least activity at 22.0 µmol/min/g.

β- 1, 3- Glucanase

In Fig. 3, the *Bacillus subtilis* + Salicylic acid (T_7) treatment exhibited the highest β-1,3-glucanase activity with a value of 40.33 µmol/min/g, significantly surpassing all other treatments at ninety days of post-inoculation. In contrast, the inoculated control (T_2) had a lower value of 30.37 µmol/min/g, while the absolute control (T_1) showed the least enzyme activity at 17.38 µmol/min/g.

Statistical analysis

The statistical design used was Completely Randomized Design following (18) standard procedures. The CD values were calculated for a 5 % (0.05) probability and the results were interpreted. The recorded observation was statistically analyzed using the GRAPES software.

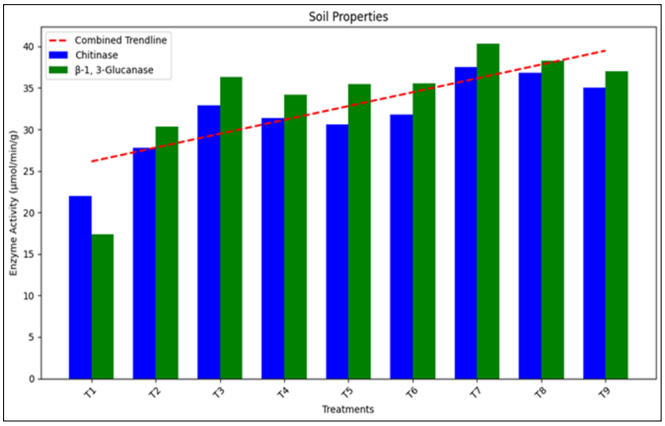

Fig 3. Effect of management technologies on the chitinase (µmol/min/g) and β- 1, 3- Glucanase (µmol/min/g) of banana cv. Rasthali at 90 days of post-inoculation. **T_1**- Absolute control (without pathogen); **T_2**- Inoculated control (with pathogen); **T_3**- *Bacillus subtilis* @ 10 g/plant soil application; **T_4**- *Trichoderma viride* @ 10 g/plant soil application; **T_5**- AM fungi 20 g/plant soil application; **T_6**- Salicylic acid 5 mM spray; **T_7**- *Bacillus subtilis* @ 10 g/plant + Salicylic acid 5 mM spray; **T_8**- *Trichoderma viride* @ 10 g/plant + Salicylic acid 5 mM spray**; T_9**- AM Fungi 20 g/plant + Salicylic acid 5 mM spray. Each value is a mean for three replicates ($p = 0.05$).

Discussion

In several pot culture trials, biocontrol agents and bio-stimulants reduced the severity of the *Fusarium* wilt of bananas. These treatments to a lesser extent decreased the disease, but combinations of either *Bacillus subtilis or Trichoderma viride* or AM fungi with salicylic acid have some of the most promising against the pathogen.

In this study among the different treatments tested, the combination of *Bacillus subtilis* with SA emerged as the most effective. This combination outperformed both individual applications and other treatment pairings, demonstrating enhanced efficacy in promoting plant growth, boosting defense enzyme activity and suppressing *Fusarium* wilt. By increasing root development and nutrient uptake, the *Bacillus*-SA combination strengthens defense and encourages robust plant growth. *Bacillus* solubilizes vital nutrients, increasing their availability to the plant and creates phytohormones such as indole-3-acetic acid (IAA) (19). The plant's ability to use these nutrients more effectively is enhanced by SA priming its stress tolerance systems, which leads to increased root length, leaf growth and general plant vigor (20). This robust growth further supports the plant's ability to withstand and recover from pathogen attacks, contributing to higher productivity and resilience against *Fusarium* wilt. According to the findings of Lastochkina, Phytostimulation (21), *B. subtilis* in a mixture with SA was found to improve growth and tolerance in plants.

The findings of this study align with previous research demonstrating the efficacy of biocontrol agents (BCAs) in managing *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Various studies have highlighted the potential of antagonistic microorganisms such as *Trichoderma* spp., *Bacillus* spp. and other beneficial rhizosphere organisms in suppressing fungal pathogens and improving plant health. The effectiveness of biocontrol agents depends on improving their persistence and activity to minimize the need for repeated applications. Additionally, restoring the functional diversity of the native microbiota and enhancing soil's physical and chemical properties plays a crucial role.

The physiological benefits of the *Bacillus subtilis* and SA combination involve an intricate network of defense activation, resource optimization and stress adaptation. By regulating oxidative stress, enhancing nutrient uptake, reinforcing cell walls and modulating hormonal signaling, this approach ensures comprehensive and robust resistance against pathogens. These physiological mechanisms collectively contribute to healthier and more resilient plants. By leveraging these multifaceted physiological processes, plants are better equipped to withstand pathogen attacks while maintaining growth and productivity. In banana plants afflicted by *Fusarium* wilt, the combination of *Bacillus subtilis* and salicylic acid has been demonstrated to favorably alter stomatal resistance, transpiration rate and chlorophyll content. By improving the physiological responses of the plants, this synergistic impact promotes better development and resistance to disease. *Bacillus subtilis* treatment dramatically raises banana plants' levels of total chlorophyll, which is essential for photosynthesis and general plant health (22). Better growth performance results from the addition of *Bacillus subtilis* to treatments like salicylic acid, which further increases the amount of chlorophyll. Increased leaf gas exchange rates have been associated with *Bacillus subtilis* treatment and this has a direct impact on transpiration rates. Increased transpiration aids in the plant's absorption of nutrients and cooling. Additionally, salicylic acid may alter stomatal conductance, which would affect transpiration dynamics. Salicylic acid and *Bacillus subtilis* together can decrease stomatal resistance, allowing for improved gas exchange and enhancing the plant's capacity to withstand *Fusarium* wilt-induced stress (12). Enhanced root growth and physiological changes induced by *Bacillus subtilis* contribute to lower stomatal resistance, promoting efficient water use and nutrient absorption.

Plants include a large number of defense-related genes that are connected to the induction of defensive enzymes. Fighting fungal infections requires the cooperation of important defensive enzymes as chitinase, peroxidase and phenylalanine ammonia-lyase (PAL). While SA increases enzyme synthesis through SAR signaling, *Bacillus subtilis* primes these enzymes by starting ISR, resulting in greater levels of enzyme activity than would be possible with separate treatments (23). The last enzymatic step in lignin biosynthesis, which entails the conversion of hydroxycinnamyl alcohols into free radical intermediates that are then coupled into the lignin polymer, has been associated with peroxidase, one of the protective enzymes (24).

The chitin in *Fusarium* cell walls is broken down by the high amounts of chitinase, whereas peroxidase fortifies cell walls by producing lignin, forming physical barriers that prevent disease invasion. These enzymes work in concert to disrupt *Fusarium* colonization and stop it from infecting plant tissues. Similarly, Saengchan, Sangpueak (25) found that exogenous SA and *B. subtilis* treatment was characterized by various known defense responses including increased hydrogen peroxide (H_2O_2) and defense enzyme activities (peroxidase, polyphenol oxidase and catalase) against *Fusarium* in potatoes.

Applying SA and *Bacillus* not only benefits the plant but also improves the rhizosphere and surrounding soil, which reduces the growth-promoting conditions for *Fusarium*. *Bacillus* promotes the development of advantageous microbial communities and improves the amount of nutrients that are

available in the soil (26), while SA influences root exudation patterns that selectively favor beneficial microbes (27). Together, they foster a disease-suppressive soil environment, further reducing the risk of *Fusarium* proliferation. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two complementary defensive mechanisms that are simultaneously activated by salicylic acid (SA) and *Bacillus subtilis* to boost the plant's immune system. The production of lipopeptides and other microbial-associated molecular patterns (MAMPs) by *Bacillus* triggers the ethylene and jasmonic acid (JA) pathways in plants, resulting in ISR (28). Meanwhile, SA elicits the SAR pathway, which primarily involves the salicylic acid pathway itself (29).Because of this dual activation, the plant is more immune-ready and can react to pathogenic threats quickly and effectively. In comparison to applying either therapy alone, the combination generates a more complete defense by activating both ISR and SAR, offering better protection against *Fusarium* wilt. The results are in line with the findings of (30) in cucumber was a combination of SA (foliar spray) and *B. subtilis* (soil drench), before fungal infection, exhibited a reduction of fungal infection and increased plant growth. According to the results, SA as a chemical elicitor and *B. subtilis* as a biocontrol agent and plant growth promoter can be integrated for effective protection to reduce the *Fusarium* wilt incidence. This can be applied to the soil with 10g of *Bacillus subtilis* per plant and as a foliar spray with a 5mM concentration of salicylic acid, at the 30th, 60th and 90th days after planting.

Conclusion

Combining *Bacillus subtilis* and salicylic acid strengthens plant defense by activating both induced systemic resistance (ISR) and systemic acquired resistance (SAR). This approach enhances enzyme activity to suppress pathogens, improves soil health, supports beneficial microbes and boosts growth by improving nutrient uptake, root development and resilience. It provides a sustainable and efficient solution for controlling *Fusarium* wilt and promoting robust banana crops. To ensure the robustness and scalability of the findings, it is crucial to explore the long-term effects and evaluate the treatments across diverse environmental conditions.

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Authors' contributions

DS was responsible for the conceptualization, data curation and drafting the original manuscript. PSK contributed to conceptualization, supervision, funding acquisition, reviewing and editing of the manuscript. IM played a key role in conceptualization, reviewing and editing, methodology development, supervision and validation. AS handled the software implementation and formal analysis, while KV provided resources, validation and visualization. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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