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Assessment of bioagents for the management of Fusarium wilt disease in banana

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ABSTRACT

Banana is cultivated predominately as a sole crop and also as an intercrop in coconut, rubber and spices-based cropping systems. Fusarium wilt of banana a lethal fungal disease causing heavy yield loss to the growers. On-farm testing (OFT) on Fusarium wilt management in banana with were evaluated and found that Trichoderma viride NRCB 1 soil treatment at 25 g/plant followed by soil application during 2, 4, 6th month after planting recorded the less incidence of Fusarium wilt disease (3%) followed by Pseudomoans fluorescens soil treatment at 2nd, 4th and 6th month after planting (5.8%) with higher yield of 450 quintal/ hectare (q/ha) in technology option 2 followed by 441.41 q/ha in technology option 1 and lowest in Farmers practice 389q/ha. Front-line demonstration (FLD) was conducted with soil application of Trichoderma viride and Paecilomyces lilacinus 25g / plant. The results revealed that in demonstration plots recorded the 26.72 percent reduction in yellowing symptoms and 18.52 percent reduction in corm infection by Fusarium wilt and 15.99 percent reduction in nematode root necrosis index. Demo plots recorded a higher yield of 315.10 q/ha whereas farmers' practice recorded yield of 292.70 q/ha respectively which is 7.65 percent increase in the yield over Farmer's practice with benefit cost ratio (BCR) of 2.36 in the demo field and 2.13 in farmers practice

Keywords: *Banana, Fusarium wilt, Biocontrol, Trichoderma viride, Pseudomoans fluorescens, Paecilomyces lilacinus, nematode gall index, management*

Introduction

The banana ranks second in area and first in production among all fruit crops in India. India produces 28.6 million tons of bananas yearly [1]. Bananas are so common and well-liked in India. The availability of fruits throughout the year, in contrast to the periodic availability of other tree fruits, has made them a need for all purposes in any Indian home.

In the Kanyakumari district, bananas are cultivated on an area of 8,500 hectares as a pure crop, as an intercrop, or as a mixed crop in coconut, rubber, and spice-based cropping systems. Ineffective farming methods, nutritional imbalances, and widespread insect and disease issues are the main causes of the low yield and profitability. The fungal disease fusarium wilt of banana is a soil-borne caused by *Fusarium oxysporum* f. sp. *cubense* and causes significant yield losses for farmers. Yellowing of the lower leaves that progresses upward until only the heart leaf retains green color is one of the disease's symptoms. Around the pseudostem, the leaves droop and hang down. Pseudostem longitudinal splitting, vascular discoloration, and reddish brown radiating mycelial threads in the corms' cross-section are the typical symptoms.

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A typical vascular wilt disease is fusarium wilt. Through the roots, the fungus enters the vascular tissue, causing discoloration and wilting before destroying the plant. The progress of the internal symptoms can influence the first appearance of the external symptoms. The fruit do not exhibit any symptom. The fungus spreads via sucker and soil particles clinging to farm equipment, footwear, clothing, pets, and cars. Its spread is greatly aided by rain and irrigation water. Currently, there is no effective chemical control available, and once established, the fungal propagules can survive for years in the soil. If control measures are not taken, the disease may spread to new locations. The use of fungicides to control the fusarium wilt of bananas is costly, ineffective, and not eco-friendly to the environment. Therefore, effective and efficient control strategies are required to eradicate the disease. Crop rotation, rhizobacteria boosting plant growth, and biological control are some of the techniques for effective management.

In vitro co-culturing of banana plants with *Pseudomonas fluorescens* Pf1, *Bacillus subtilis* EPB 10, and EPB 56 resulted in increased plant biomass and yield with successful control of fusarium wilt of banana under field conditions [2]. Endophyte and rhizobacteria strains led to 78% control of fusarium wilt with significantly greater bunch yield [3]. Under greenhouse conditions when banana plants were treated with endophytes, a significantly reduction in wilt disease (67%) by Foc R4 and growth promotion was observed [4]. Plant development was significantly accelerated in banana tissue culture plantlets treated with *Burkholderia cenocepacia* 869T2 and showed a 86 percent reduction in disease incidence caused by Foc TR4 [5].

Many species of the genus *Bacillus* have been examined as BCAs of various *Fusarium*-induced plant diseases since they are frequently detected in the rhizosphere of banana plants [6]. The antagonistic effect of *Bacillus subtilis* against several bacterial and fungal plant diseases is well established. The synthesis of antibiotics is primarily responsible for its biocontrol activity [7]. Banana plants pre-treated with bio-organic fertilizer colonized by *B. amyloliquefaciens* NJN-6 showed a decreased disease incidence by 68.5%, with higher yield [8].
Various rhizospheric strains of *P. fluorescens* were evaluated and found that strain Pfm has highest *in vitro* inhibitory impact on Foc R1[9]. Vascular discolouration of banana rhizome was significantly reduced when talc based formulation of *P. fluorescens* strain Pfms was applied during greenhouse trials [10]. Banana fusarium wilt was reduced up to 75–80% by treating with rhizospheric strain of *T. viride* NRCB1 [11]. The bioformulation prepared from rice chaffy grains had better efficacy than talc powder formulation. Treatment with endophytic *Trichodema asperellum* prr2 and the rhizospheric *Trichoderma* sp. NRCB3 reduced the fusarium wilt incidence of bananas by 47% and increased the yield by 45% [12].
The objective of this present investigation is to study the efficacy of bioagents against Fusarium wilt disease through On farm testing and technology demonstration through Frontline demonstrations in a farmers field.

Materials and Methods

Krishi Vigyan Kendra is doing on-farm testing to test and assess the research findings of Research Stations at the farmer's field as well as to improve and adapt the technologies, if necessary, for greater uptake by farmers. It serves as a confirmation of previously established research findings in a practical farming setting. Given the prior knowledge, it is vital to engage farmers in participatory research testing on their farms. In addition to persuading them, there is a chance to polish or change the advice to fit the needs of the farmers in a particular farming environment. Participatory Rural Appraisal (PRA) was used to determine the issues that farmers were facing, and based on the severity of those issues, OFT was proposed. Technologies that are both operationally and economically practical and meet farmers' needs are found to address their issues.
ICAR-Krishi Vigyan Kendra, Kanyakumari has conducted on-farm testing experiment on the assessment of fusarium wilt disease management technologies in banana with technological options developed by Tamil Nadu Agricultural University, Coimbatore and National Research Centre for Banana, Thiruchirapalli to evaluate suitable bioagents for the management of Fusarium wilt disease in Kanyakumari district. The following treatments were imposed in banana variety Rasthali
Technology Option1: *Pseudomans fluorescens* liquid formulation @ 4 lit /ha at planting, 2nd, 4th and 6th month after planting.
Technology Option 2: Soil application of *Trichoderma viride* NRCB 1 @ 25 g/plant as basal + 2, 4, 6th month after planting
Technology Option3 : Farmers practice untreated control
The experimental trial was conducted under a randomized block design with 7 replications in five farmers' fields during the Kharif season at Kalkurichi village of Thuckalay block of Kanyakumari district. The plants were externally inspected and the percentage of yellowing/wilting leaves was scored following a 0–5 scale [13]. The biometric parameters viz., plant height, stem girth, number of fingers, and bunch weight were recorded to study the effect of bioagents on plant growth promotion.

Front Line Demonstrations (FLDs) are an innovative method for establishing a direct line of communication between scientists and farmers because the scientists are directly involved in the planning, execution, and monitoring of the demonstrations for the technologies they have developed and receive direct input from the farmers' fields. In FLDs, the scientists plan the demonstrations on technology. Researchers and extension workers can thus use FLDs to better understand farmer resources and needs to fine-tune and/or change technologies for simple adaption at farmers' fields. During 2020-21 ICAR-Krishi Vigyan Kendra, Kanyakumari has conducted FLD on Demonstration of Fusarium wilt and nematode management in Bananas using bioagents at Killiyoor block during Kharif 2020 in 10 farmers field in banana variety Rasthali in an area of 4 ha. The following technologies were demonstrated viz., soil application of *Trichoderma viride* and *Paecilomyces lilacinus* 25g / plant (at planting; 2nd , 4 th and 6th month after planting) and was compared with farmers practice on application of fungicides and insecticides.
For Fusarium wilt disease, the plants were externally inspected and percentage of yellowing/wilting leaves were scored following a 0–5 scale [13].

Score Individual Proportion of leaves
0 Healthy 0
1 Yellowing < 50%
2 Yellowing or yellowing & tip wilting > 50%
3 Yellowing or yellowing & tip wilting & total leaf wilting < 50%
4 Yellowing or yellowing & tip wilting & total leaf wilting > 50%
5 Stem base split > 50%

For internal symptom evaluation, plants were uprooted, cleaned, and cut longitudinally at the rhizome (corm) of each plant. Disease severity was visually assessed following a 1–6 scale where
1 = No discoloration in the corm,
2 = < 5% discoloration in the corm
3 = 5 - 30% discoloration in the corm
4 = 30 50% discoloration in the corm
5 = 50 - 90% discoloration in the corm
6 = < 90% discoloration in the corm
The root necrosis index was used to determine root rotting on a 0 to 4 scale [14] to calculate the nematode-root necrosis index
0 = no damage; = <25% of total root cortex with necrosis
2= 26–50% of the total root cortex with necrosis
3 = 51–75% of total root cortex with necrosis
4 = >75% of total root cortex with necrosis

Results

The performance evaluation of bioagents on the management of *Fusarium* wilt in banana are presented in Table 1. The results of OFT revealed that technology option 2 with soil application of *T.viride* NRCB 1 as basal followed by soil application during 2, 4, 6th months recorded lower incidence of wilt incidence with PDI of 56.19 percent yellowing symptom and 65.0 percent PDI of Corm infection which is 32.56 percent and 25.71 percent lower than the farmers practice respectively. Usage of *P. fluorescens* liquid formulation @ 4 lit /ha at planting, 2nd, 4th and 6th MAP recorded lower incidence of wilt incidence with PDI of 63.81 percent yellowing symptom and 72.50 percent PDI of Corm infection which is 23.42 percent and 17.14 percent lower than the farmers practice respectively. Biometric observation on pseudostem height, pseudostem girth, Bunch weight and number of hands per bunch were recorded and presented in Table 2. Soil application of *T.viride* NRCB 1 @ 25 g/plant as basal + 2, 4, 6th MAP significantly increased the height, stem girth, Bunch weight and number of hands per bunch in treated plots when compared to untreated control plants.

A higher yield of 450q/ha was recorded in Technology Option 2- Soil application of *T.viride* NRCB 1 @ 25 g/plant as basal + 2, 4, 6th MAP followed by 441.51 q/ha in Technology Option 1 *P. fluorescens* liquid formulation @ 4 lit /ha at planting, 2nd, 4th and 6th MAP and lowest in Farmers practice 389q/ha (Table 3). The higher net return of Rs. 342600 /ha with BCR of 2.41 was recorded in technology option 2- Soil application of *T.viride* NRCB 1 followed by Rs. 331680/ ha with BCR of 2.37 in technology option 1- *P. fluorescens* liquid formulation whereas farmers practice recorded net return of Rs. 275075/ ha with BCR of 2.19 (Table 3).

The results of OFT was further demonstrated during 2020-21 by conducting Front Line Demonstration(FLD) at Killiyoor block during Kharif 2020. The technology of soil application of *T.viride* NRCB 1 @ 25 g/plant was further demonstrated during 2020-21 with the application of *Paecilomyces lilacinus* 25g / plant (at planting; 2nd , 4th and 6th month after planting) by conducting Front line Demonstration(FLD) at Killiyoor block during Kharif 2020. The results revealed that in demo plots with soil application of *Trichoderma viride* and *Paecilomyces lilacinus* recorded the 26.72 percent reduction in yellowing symptoms and an 18.52 percent reduction in corm infection by *Fusarium* wilt and a 15.99 percent reduction in nematode-root necrosis index(Table 4). Demo plots recorded a higher yield of 315.10 q/ha whereas farmers' practice recorded a yield of 292.70 q/ha respectively which is 7.65 percent increase in the yield over farmer's practice. BCR of 2.36 was recorded in demo field and 2.13 in the Farmers practice (Table 5).

Discussion

Soil-borne pathogen *Fusarium oxysporum* infects fruits and vegetables and causes Fusarium wilt disease. The persistent soil-borne fungus pathogen Foc tropical race 4 (Foc- TR4) is responsible for a pandemic disease on banana (Musa spp.) [15]. Fusarium wilt disease has devastating effects on banana crop, prompting several nations to take action to stop its spread, transmission, infection, and yield loss. Biocontrol is a potential strategy for managing the Fusarium wilt disease. One of the most effective ways to manage disease was by using biocontrol agents (BCAs) [16, 17].

In vitro studies [18] and glasshouse trials [19, 20] were used to investigate and describe the effects of BCAs on the growth of *Fusarium oxy sporum* var *cubense*. *Bacillus* spp. could be used as a biocontrol agent to treat banana Panama disease [8]. Field and glasshouse trials using both rhizospheric and endophytic bacterial strains successfully controlled the Panama disease of bananas [21]. Numerous *Bacillus* spp. strains have been successfully employed as BCAs to combat the Fusarium wilt diseases [22].

Compounds produced by PGPR, such as antibiotics were contributed to the indirect promotion of growth and pathogen control in numerous plants [23] . The secondary metabolites produced by the *B. amyloliquefaciens* NJN-6 strain are antagonistic to several soil-borne pathogens. Because of this, the usage of PGPR strain NJN-6 has improved the growth of banana seedlings while reducing the severity and incidence of Panama disease. Activation of the plants defenses against the Foc pathogen was one of the active molecules produced by NJN-6 [17]. Development of new bioformulation with multiple mechanisms of action such as antagonistic and resistance-inducing PGPR, could lead to more effective biological control and to increase crop production [23].

The results are similar to the findings of [12,24,25] that prophylactic application of bioagents reduces the *Fusarium* wilt in bananas. The incidence of FWB was decreased by 47% and the bunch weight was increased by 45% as a result of combined treatments of the endophytic *T. asperellum* prr2 and the rhizospheric *Trichoderma* sp. NRCB3 [21]. Endophyte and rhizobacteria strains produced a considerable increase in bunch weight and a 78% FWB control [3]. The outcomes are in line with the findings of Thangavelu and Gopi 2015a which showed that fusarium wilt in bananas can be prevented by applying bioagents on a preventative basis.

The results revealed that prophylactic basal application of *Trichoderma viride* and *Paecilomyces lilacinus* during planting and 2, 4, 6th months could significantly reduce the Fusarium wilt and nematode incidence in bananas with higher yield.

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Table: 1 Evaluation of bioagents for the management of Fusarium wilt in banana

Technology Option	Fusarium (yellowing) PDI (%)	PROC	Fusarium (Corm infection) PDI (%)	PROC
Technology Option 1	63.81 (53.07)^b	23.42	72.50 (60.57)^a	17.14
Technology Option 2- Soil application of *T.viride* NRCB 1 @ 25 g/plant as basal + 2, 4, 6th MAP	56.19 (48.57)^b	32.56	65.00 (59.30)^a	25.71
Control-Farmers Practice	83.33 (66.51)^a		87.50 (55.04)^b	
CD (5%)	5.65		2.19	
CV	8.65		3.22	

* **PROC-Percent reduction over control,** values are mean of seven replications

Figures in the parentheses represent arcsine transformed values. in each treatment numbers followed by same numbers are not significantly different to LSD test at P<0.05%

Table: 2 Effect of bioagents on growth parameters in banana

Technology Option	Pseudostem height (cm)	Pseudostem girth (cm)	Bunch weight (kg)	Number of hands per bunch
Technology Option 1 *P. fluorescens* liquid formulation @ 4 lit /ha at planting, 2nd, 4th and 6th MAP	214.86[b]	59.6[b]	14.03[b]	7.1[b]
Technology Option 2- Soil application of *T.viride* NRCB 1 @ 25 g/plant as basal + 2, 4, 6th MAP	223.50[a]	61.3[a]	14.73[a]	8.2[a]
Control-Farmers Practice	196.64[c]	58.2[c]	12.73[c]	6.2[c]
CD (5%)	5.68	1.10	0.35	0.10
CV	2.30	1.57	2.40	0.09

values are mean of seven replications
Figures in the parentheses represent arcsine transformed values. in each treatment numbers followed by same numbers are not significantly different to LSD test at P<0.05%

Table: 3 Effect of bioagents on yield and economic parameters in banana

Technology Option	Yield (q/ha)	Net returns (Rs./ha)	B:C ratio
Technology Option 1 *P. fluorescens* liquid formulation @ 4 lit /ha at planting, 2nd, 4th and 6th MAP	441.51	331680	2.37
Technology Option 2- Soil application of *T.viride* NRCB 1 @ 25 g/plant as basal + 2, 4, 6th MAP	450.0	342600	2.41
Control-Farmers Practice	389.0	275075	2.19
CD (5%)	16.66		
CV	3.35		

Table: 4 Effect of biocontrol agents against Fusarium wilt and nematode incidence in banana

Treatments	Fusarium (yellowing) PDI (%)	PROC	Fusarium (Corm infection) PDI (%)	PROC	Nematode-Root necrosis index (%)	PROC
Technology demonstrated - soil application of *T. viride* @25 g/ plant and *Paecilomyces lilacinus* 25g / plant (at planting ; 2nd , 4th and 6th month after planting)	45.00	27.12	73.33	18.52	70.00	15.99
Control-Farmers Practice (FP)	61.75	-	90.00	-	83.33	-

** PROC-Percent reduction over control*

Table: 5 Effect of biocontrol agents on yield and economic parameters in banana

Treatments	Yield (q/ha)	% increase over FP	Net returns (Rs./ha)	B:C Ratio
Technology demonstrated - Soil application of *T. viride* @25 g/ plant and *Paecilomyces lilacinus* 25g / plant (at planting ; 2nd , 4th and 6th month after planting)	315.10	7.65	236130.0	2.36
Control-Farmers Practice (FP)	292.70		185988.0	2.13

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