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Biological Management of Banana Wilt

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Banana is the most important tropical fruit crop which holds second position in terms of significance among fruit crops in India following mango. In India, there has been a tremendous rise in acreage, production and productivity during the past two decades.Several biotic constraints impact banana production in India. Among them, Panama wilt is the most devastating and lethal disease of banana.Total nine antagonists were screened to check bio-efficacy against twelve *Fusarium oxysporum*f.sp. *cubense* isolates by dual culture technique and pot condition.Among various bioagents,*Trichoderma viride* NAU was found the most effective in inhibiting the mycelial growth inhibition as well as significantly the lowest (40.00%) banana fusarial wilt incidence was recorded in *T. viride* NAU (10 g/pot/10kg) + *P. fluorescens* NAU (10 g/pot/10kg) under pot condition.

Keywords : Banana wilt, *Trichoderma*, *Fusarium*

INTRODUCTION

Banana (*Musa* spp.) is the most important fruit crop in tropical and subtropical region and staple food originating in South East Asia, mainly in the Philippines and Malaysia (Gundaniya *et al*. 2024). It is known as the 'poor man's apple' due to its low cost and high nutritional value and is popular due to its year-round availability as compared to seasonal availability of other fruits (Somu *et al*.2013). The carbohydrate, protein and moisture content of bananas vary with variety, genotype and climatic conditions (Afzal *et al*. 2022).

Banana is a major fruit crop with annual world production estimated at 135 MT (Anon. 2024a). The global banana trade is worth USD 13.88 billion per year making the commodity one of the most important in the world (FAOSTAT, 2024).

Management of banana wilt
In India, there has been a tremendous rise in acreage, production and productivity during the past two decades. India is the world's greatest producer of bananas, producing 36.61 MT in 2024 from 966 thousand hectares area with a productivity of 35,173 kg/ha (Anon, 2024b).In Gujarat approximately 59,684 hectare of land is under banana cultivation. The banana production was estimated around 40.10 lakh tons during 2023-24 with productivity of 67,200 kg/ha (Anon, 2024c). Banana is susceptible to a variety of illnesses, the most well-known of which is Fusarium wilt disease (Panama disease) which is recognized as one of the most serious dangers to global banana production.The disease has been identified as the leading cause of limited banana cultivation globally. The pathogen infects the roots, block water and nutrient supply through vascular colonization. As a result, the oldest leaves die first and turn yellow. The plants eventually wilt and collapse, leading to significant crop losses The fungus *Fusarium oxysporum* f.sp. *cubense*(*Foc*) causes this disease which has been recorded in all banana-growing regions worldwide including Australia, Asia, Africaand Central and South America (Dong and Wang, 2011).The pathogen is classified into four races based on the banana varieties it affects. These

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are*Foc* race 1 (R1), *Foc* race 2 (R2), *Foc* race 3 (R3), and *Foc* race 4 (R4). *Foc* race 4 is further divided into subtropical race 4 (STR4) and tropical race 4 (TR4) depending on the climatic conditions where Cavendish bananas are affected (Thangavelu *et al.* 2019).

The injudicious application of chemicals resulted health hazards problems to end users. There is need for alternative approaches which have minimal deleterious effects, more ecofriendly and will contribute to the goal of sustainability in agriculture (Chauhan *et al*., 2021 and Waghunde *et al*. 2016). Looking at the significance of crop and threatening by the disease, there is urgent need to develop eco-friendly disease management strategies.

MATERIALS AND METHODS

Isolation, purification and pathogenicity

The Fusarial banana wilt infected samples were collected from banana growing farmer's of Bharuch, Surat and Narmada district during September-2024 to Feburary-2025 and subjected for isolation of pathogen causing banana wilt. The pathogen was isolated by tissue isolation method and purified for further study. The pathogenicity was proved as per method given by Haware (1980). The *in vitro* and pot experiment is conducted at Department of Plant pathology, CoA, NAU, Bharuch during 2025. The cultural and morphological characteristics of *Fusarium* pure cultures were studied.

Isolation and purification

The soil samples and infected root samples were collected from the banana growing farmers of Bharuch, Narmada and Surat district. The soil samples were subjected for nematode confirmation and protocol was taken from Patel *et al*. (2024) for nematode isolation. The infected root samples were subjected for tissue isolation method. Among collected various root sample, six pure cultures of pathogen causing banana wilt were purified and used further for the investigation. Microscopic study of fungus done under the microscope for primary identification.

Pathogenicity

Pathogenicity of six pure culture of pathogen causing banana wilt was confirmed by growing banana plants in pot containing pathogen-infested soil as per Koch's postulates. Firstly, the soil was sterilized by using formalin solution (2.0%) then the mass multiplied fungal inoculums (3×10^4/ml sporulation) was mixed separately @ 10ml/kg with sterilized soil then the inoculated soil was filled in sterilized pots. The pots were filled with inoculated soil and kept in green house for five days and sterile water was given to allow the establishment of pathogen. Cavendish banana (G-9) tissue plantlet was used for pathogenicity. Three replications were maintained and three pots as un-inoculated were kept as a control. The pots were observed for the wilt symptoms. The re-isolation of the pathogen was done to confirm the Koch's postulates.

Dual culture

The antifungal efficacy of six pure cultures (Banana: 1 to 6) of pathogen causing banana wilt was tested *in vitro* by dual culture techniqueby *Trichoderma* spp. The four native *Trichoderma* spp. (*Trichoderma viride*, *T. longibrachiatum*, *T. koningii* and *T. harzianum*) of NAU along with five other SAU *Trichoderma* spp. were tested by dual culture method and mycelial growth was recorded 7days after incubation. The observation on mycelial growth (mm) was recorded and Per cent Growth Inhibition (PGI) of pathogen in each treatment was calculated by following formula. The pure cultures of *Trichoderma viride*, *T. longibrachiatum*, *T. koningii* and *T. harzianum, Pseudomonas fluorescens* cultures were brought from the NMCA, NAU, Navsari.

$$PGI\ \% = \frac{C-T}{C} x100$$

C- Control and T- Treatment

Pot Study

The efficacy of various bioagents and fungicides as per Table1 was tested against *Fusarium oxysporum*f.sp.*cubense*under pot condition against pathogencausing banana wilt. The mass

culture of pathogen was prepared as per method described by Haware (1980) i.e. pure culture was mixed in sterilized soil separately @50g/kg soil having 10 kg sterile soil/pots. One kg of well decomposed FYM was added in the pot. Cavendish banana (G-9) tissue plantlet was used during pot study. The bioagent was applied in pathogen inoculated pots @ 10g/pot and drenching of fungicide as per dose mentioned in (Table1) was applied after 5 days of planting of G-9. Pot without pathogen inoculation was considered as control. The experiment was conducted in CRD. Each treatment was replicated five times independently. The disease incidence (%) and per cent wilt index was recorded using scale suggested by AICRP on Banana upto 60 days at 15 days interval.

$$\text{PDI \%} = \frac{\text{Number of plants infected}}{\text{Total Number of plants evaluated}} \times 100$$

External symptoms (% of wilt index, 1-5 scale):

Category	Reaction
1	Healthy
2	Slight chlorosis and wilting with no petiole buckling
3	Moderate chlorosis and wilting with some petiole bucking and or splitting of leaf base
4	Severe chlorosis, severe wilting, petiole bucking and dwarfing of the newly emerged leaf
5	Dead

$$\text{\% Wilt index} = \frac{\text{Sum of all numerical rating}}{\text{Number of Plants observed} \times \text{Maximum grade}} \times 100$$

RESULTS AND DISCUSSION

Isolation and purification

The soil and infected root samples were collected and subjected for nematode confirmation but not a single sample was found positive in nematode activity. The infected root samples were subjected for tissue isolation method. Six pure fungal cultures (Banana:1 to 6) causing banana wilt were isolated and purified from root samples collected from various location which were further used for the investigation. The microscopic study of these five pure cultures was done and fungal pathogen revealed presence of micro/macro conidia and chlamydospore (Fig.1). The microscopic study revealed that based on morphological characters pathogen was identified as *Fusarium oxysporum*.

Pathogenicity

Pathogenicity of six pure cultures (Banana:1 to 6) causing banana wilt was confirmed by as per Koch's postulates. After inoculation of pathogen suspension, the leaves became yellow which extends from leaf margins towards the midrib, turgidity of leaves become poor, eventual collapse at the petiole and longitudinal splitting of the pseudostem result into brown and blackening of vascular system. The yellowing of leaves starts after 15 days of inoculation and complete wilting of plants was observed after 25 days in Banana-1 and 2 isolates as compare to others. The marginal wilting of leaves were observed in Banana-3 while in Banana-4 and 5 drooping down of leaves with wilting appearance observed after 35 days of inoculation.

Dual culture

The six pure cultures of *Fusarium* were tested *in vitro* against nine *Trichoderma* spp. available in the Department of Plant Pathology, CoA, NAU, Bharuch by dual culture technique. The radial growth of pathogen was recorded on 7days after incubation and PGI was calculated and mentioned in (Table2). The significantly highest (59.25, 55.67, 59.25, 66.75, 21.07 and 62.58%) per cent growth inhibition of pathogen was recorded in *Trichoderma viride* NAU against all six pathogen causing banana wilt, respectively as compared to other tested *Trichoderma* spp. The next best *Trichoderma* in growth inhibition wasSAU-1 which was effective against Banana-1 & 3 (57.25%) and SAU-4 against Banana-6 (61.61%) than other *Trichoderma* spp. *Trichoderma longibrachiatum* NAU was effective against Banana-2 (39.00%), Banana-4 (37.50%) and Banana-5 (19.20%).

Table 1: Evaluation of bioagents and fungicideagainst banana wilt under potcondition

Tr. No.	Treatment
1	Pathogen inoculated (3×10^6/ml spore)
2	*Trichoderma viride* NAU (10 g/pot/10kg) 1.5 WP ($2x10^6$ cfu/g)
3	*P. fluorescens* NAU (10 g/pot/10kg) 1.5 LF($1x10^8$ cfu/ml)
4	*T. viride* NAU (10 g/pot/10kg) +*P. fluorescens* NAU (10 g/pot/10kg)
5	Carbendazim 50WP (1g/lit)
6	Propiconazole 25EC (1ml/lit)
7	Control (Water only)

Table 2 : Per cent Growth Inhibition (PGI) of pathogen causing banana fusarial wilt by *Trichoderma* spp.

Tr. No.	Treatment details	Percent wilt incidence	Percent wilt index	First appearance of wilt symptom(days)
1	Pathogen inoculated (3×10^6/ml spore)	100.00	100.00	15
2	*Trichoderma viride* NAU (10 g/pot/10kg) (1×10^6/ml spore)	60.00	60.40	22
3	*P. fluorescens* NAU (10 g/pot/10kg) (1×10^8/ml spore)	80.00	72.00	20
4	*T. viride* NAU (10 g/pot/10kg) + *P. fluorescens* NAU (10 g/pot/10kg)	40.00	50.40	35
5	Carbendazim 50WP (1g/lit)	80.00	72.80	20
6	Propiconazole 25EC (1ml/lit)	80.00	66.00	25
7	Control (Water only)	10.00	8.40	
	CD at 1%	4.10	3.75	
	S.Em. (±)	1.31	1.20	
	C.V. (%)	6.63	6.54	

Table 3: Effect of various treatments on banana fusarial wilt

Tr. No.	Treatment details	Percent wilt incidence	Percent wilt index	First appearance of wilt symptom(days)
1	Pathogen inoculated (3×10^6/ml spore)	100.00	100.00	15
2	*Trichoderma viride* NAU (10 g/pot/10kg) (1×10^6/ml spore)	60.00	60.40	22
3	*P. fluorescens* NAU (10 g/pot/10kg) (1×10^8/ml spore)	80.00	72.00	20
4	*T. viride* NAU (10 g/pot/10kg) + *P. fluorescens* NAU (10 g/pot/10kg)	40.00	50.40	35
5	Carbendazim 50WP (1g/lit)	80.00	72.80	20
6	Propiconazole 25EC (1ml/lit)	80.00	66.00	25
7	Control (Water only)	10.00	8.40	
	CD at 1%	4.10	3.75	
	S.Em. (±)	1.31	1.20	
	C.V. (%)	6.63	6.54	

Pot study

A pot experiment was conducted during 2025 to find effective treatment against banana fusarial wilt management.The tissue plantlets (G-9) were used for planting during pot experiment. Significantly the lowest (40.00%) banana fusarial wilt incidence was recorded in *T. viride* NAU (10

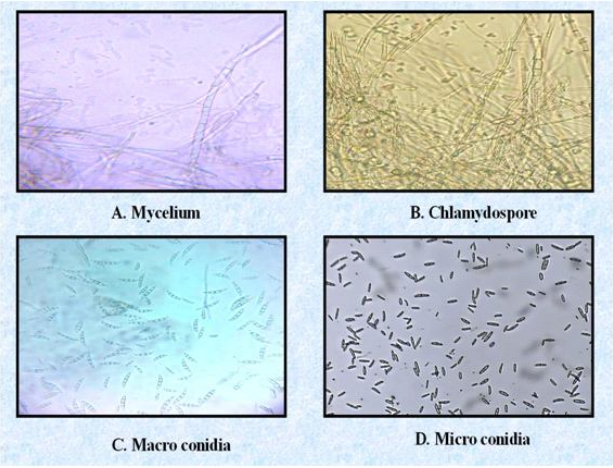

Fig. 1: Microscopic photographs of*Fusarium*

g/pot/10kg) + *P. fluorescens* NAU (10 g/pot/10kg) and first appearance of disease was observed 35 days after planting as compared to other treatments. *Pseudomonas fluorescens* NAU,Carbendazim 50WP (1g/lit) and Propiconazole 25EC (1ml/lit) recorded 80% wilt incidence and first appearance of wilting symptoms were recorded in 20, 20 and 25 days after planting, respectively. The bioagent, *T. viride* NAU (10 g/pot/10kg) recorded 60% wilt incidence and wilt initiation started 22 days after planting, respectively. The maximum percent disease incidence (100%) was recorded in pathogen inoculation only treatment and first appearance of wilting symptoms was observed after 15 days of planting.

Significantly the lowest percent wilt index (50.40%) was recorded in *T. viride* NAU (10 g/ pot/10kg) + *P. fluorescens* NAU (10 g/pot/10kg) as compared to other treatments followed by *T. viride* NAU (10 g/pot/10kg) (60.40%). The next best treatment in order of lower wilt index was Propiconazole 25%EC (1ml/lit) (66.00%), *P. fluorescens* NAU (10 g/pot/10kg) (72.00%) and Carbendazim 50%WP (1g/lit) (72.80%). The highest percent wilt index (100%) was recorded in pathogen inoculation only treatment.

Similar result also found by Savani *et al.* (2021) who found that the maximum mycelial growth inhibition was recorded in *T. reesei*(72.33%) followed by *R. vinctus*(70.00%) and *S. tabacisoli*(65.50%). Moreover, Pant*et al.* (2023) observed that *T. viride* and *T. harzianum* were effective in reducing banana wilt incidence under pot conditions. Current research finding is also in accordance with Shukla *et al.* (2024) who screened the native and commercial isolates of *T. viride* and *T. harzianum* under *in vitro* and pot conditions. *T. viride* (native) recorded maximum mycelial growth inhibition (64.60%) and reduce disease suppression (62.10%) under pot condition.

CONCLUSION

The pathogen causing banana wilt was isolated from various farmers' field and based on morphological study, it was identify as *Fusarium oxysporum*. Significantly the highest (59.25, 55.67, 59.25, 66.75, 21.07 and 62.58%) per cent growth inhibition of pathogen was recorded by *Trichoderma viride* NAU against all six pathogen causing banana wilt. The significantly lowest (40.00%) disease incidence and percent wilt index (50.40%) was recorded in *T. viride*NAU (10 g/pot/ 10kg) + *P. fluorescens* NAU (10 g/pot/10kg) treatment against banana fusarial wilt under pot condition.

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest

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