ComparativeEvaluation of Different Biocontrol Agentsagainst Anthracnose of NendranBanana Caused by *Colletotrichum musae*

ABSTRACT

India is the largestproducer of banana in the world. Nendran, a French plantain, is the most important banana cultivar in Kerala. It is mainly affected by many diseases both in the field and after harvest. Being a freshly consumed fruit crop there are a lot of limitations in using chemicals for its disease control. Keeping this view, a study was conducted at the Department of Plant Pathology, College of Agriculture, Vellayani, to control anthracnose, a serious post harvest disease under tropical conditions. In this study comparative evaluation of three fungal biocontrol agents namely [*Trichoderma* sp. (KAU), *Trichoderma* sp. (TRPN-3), *Trichoderma* sp. (TRKR-2)] and bacterial biocontrol agents [*Pseudomonas fluorescens* (PN026), *Bacillus* sp. (B15), *Bacillus* sp. (B17)] were tested against *Colletotrichum musae*. Among the fungal biocontrol agents maximum mycelial growth inhibition was recorded with *Trichoderma* sp. (KAU) (77.44%) followed by *Trichoderma* sp. (TRKR-2) (77.11%). Minimum mycelial growth inhibition was recorded in *P.fluorescens*(PN026) (55.33%). Based on the results reveals that *Trichoderma* sp. (KAU) is a highlypromising biocontrol agent for managing anthracnose of banana.

Key words: Anthracnose, Biocontrol, *Colletotrichum musae*, Banana,*Trichoderma*sp.,*Pseudomonas fluorescens*, *Bacillus* sp.

1. INTRODUCTION

Banana is the second most important fruit crop in India next to mango. Its year round availability, affordability, varietal range, taste, nutritive and medicinal value makes it the favourite fruit among all classes of people. Besides India, other major banana producing countries are China, Philippines, Ecuador, Brazil and Indonesia[1]. India produces a total of 23 per cent of the entire world production of banana [2].

The area of banana cultivation in Kerala estimated about 60678 ha, with a production of 548425 tonnes [3].Nendran, a French plantain cultivar belonging to Musa AAB group is the most important banana variety in Kerala. Owing to its unique taste and flavour it has great suitability for crispy chips. Being a climacteric fruit, postharvest loss in banana is huge and it is about 21.67% [4].

The genus *Colletotrichum* includes fungal species that are responsible for anthracnose diseases in different vegetable and fruit crops worldwide, including bananas [5,6].Anthracnose of banana is a major catastrophic post harvest disease in India [7].Symptoms of anthracnose in bananas caused by *C. musae* include blacksunken lesions and the presence of salmon-colored conidia [8].In India, postharvest losses to disease, including anthracnose, have been estimated at 30-40% [9].Anthracnose caused by *C.musae*, is a major constraint in the marketing of fruit intended for local as well as distant markets. It causes lesions on the fruit peel after ripening, as well as finger and crown rots. It affects fruit quality severely, resulting in significant economic losses in many parts of the tropics [10].

To increase the product yield, pesticides are overused and misused in spite of health concerns, environmental contamination and export bans [11].Buildup of resistance to pathogens and severe environmental pollution, however, are some of the drawbacks from the prolonged application of chemical fungicides such as benomyl, prochloraz and azoxystrobin. A major concern for pesticidetreated fruit is the issue of food safety that has received increased attention from the consumers in recent years. Hence, most overseas markets are no longer permitting chemical treatments for fruit entering their countries [12].There is an urgent need to find alternative ways to replace or reduce the use of synthetic chemicals.

Biological control is a crop treatment which potentially can be used individually or in consortium with other compatible microbes, organic amendments to reduce losses caused by plant pathogens [13]. Fungal biocontrol agents, *Trichoderma* spp. are widely studied for its ability to produce mycoparasitic enzymes like chitinase, β-1,3-glucanase, pectinase, and cellulase, saprophytic ability, competition for space and nutrition, induction of systemic resistance in host helps in ecofriendly management of plant diseases [14]. Bacterial biocontrol agents including *Pseudomonas* spp., *Bacillus* spp., and *Streptomycetes* are mainly used for plant growth promotion and disease management and it includes various effective combination methods to control fruit decay[15].Biological control has been recommended as an alternative method for controlling postharvest pathogens[16,17].In this study, comparative evaluationof fungal and bacterial biocontrol agents efficacy against *C.musae*, causing anthracnose of nendran banana.

2.MATERIALS AND METHODS

2.1 Sampling and isolation of pathogen

The diseased Nendran banana samples were collected from Kuzhalmannam(10°43'17.1"N 76°35'47.0"E)Palakkad district of Kerala, India. After the symptomatology studies, the

diseased fruits were subjected to pathogen isolation.The diseased fruits were thoroughly washed in tap water, followed by washing with sterile water and allowed to dry. Inside the laminar air flow chamber, small bits (6 sq. mm area) of fruit peel were cut out at the margin where the diseased and healthy tissues met. The bits were placed in 0.1 per cent mercuric chloride for60 seconds for surface sterilization and subsequently were transferred to sterile water and rinsed three times to remove residue of the chemical. The bits were allowed to dry over a sterile blotting paper. These bits were then transferred to Petriplates with solidified Potato Dextrose Agar (PDA) medium[18].The plates were wrapped using cling film and kept for incubation at room temperature (28±1°C).When mycelial growth initiation from the bits commenced, pure culturing was done. The hypal tip method was used to obtain pure culture from incubated plates,the tip of the mycelia in the media was cut out using an inoculation needle or a cork borer and was inoculated into sterile petri plates with solidified PDA medium. The Petri plates were kept at 28±1°C. When pure cultures were obtained, the fungi were also transferred into PDA slants and kept at 4 °Cfurther studies.

2.2 Morphological characterization

Morphological features were used to select isolates resembling *Colletotrichummusae*. The selected isolate were cultured on PDA plates at 28±1 °C for7days. 9 mm mycelial disc from colony margins were placed on the centre of 90mm diameterPetri plate and incubated at 28±1°C. The colony colour and culture diameters (two perpendicular directions) were recorded at regular interval. Colony diameters were used to calculate hyphal growth rate (mm/day) and the length and width of conidia were measured.

2.3.Screening of different biocontrol agents against *C.musae*

Antagonistic activity of different fungal and bacterial biocontrol agents against anthracnose of banana pathogen *C.musae* was done by dual culture plate method[19].In dual culture, for fungal bio-agents, fifteen ml of PDA medium was poured into sterilized Petri plate and allowed for solidification. Nine mm diameter discs from actively growing colony of pathogen was cut with a sterile cork borer and placed near the periphery of PDA plate. Similarly, bio-agents were placed on the other side *i.e*, at an angle of 180°. Petri platewith pathogen served as control. The plates were incubated at 28±1°C for seven days. For each treatment, three replications were maintained. The extent antagonistic activity by bio-agents were recorded after incubation period of 7 days by measuring the growth of the test pathogen

in dual culture and in control plates. For bacterial bio-agents nutrient agar medium was used. Per cent inhibition over control was calculated according to formula given by[20].

$$\text{Percentage of inhibition (PI)} = \frac{C-T}{C} \times 100$$

Where,

PI = Per cent inhibition of mycelial growth

C = Mycelial growth of pathogen in control (mm.)

T = Mycelial growth of pathogen in treatment (mm.)

2.4. Statistical analysis

Experiments were performed in triplicates, and collected data were analysed by ANOVA using GRAPES (General R based Analysis Platform Empowered by Statistics)[21].Experiment design was completely randomised design (CRD) for *in vitro* experiment.

3. RESULTS AND DISCUSSION

3.1. Sampling and Isolation

Anthracnose infected banana samples were collected from Kuzhalmannam, Palakkad district of Kerala (Fig 1).*Colletotrichummusae*, the pathogen responsible for anthracnose disease was isolated from the collected sample and pure cultured .The culture were salmon in colour in Petriplateafter seven days of growth and the cylindrical shaped conidia of the pathogen was observed under microscope (Fig. 2,3) .

Fig 1. Symptom of Anthracnose of Nendran Banana collected from Kuzhalmannam, Palakkad district of Kerala

3.2. Morphological characterization

The *C.musae* colonies typically growed rapidly on PDA. Initially, colonies appeared light pink colour and, later turned into salmon colour. The reverse side of culture plate appeared light saffron colour. In Petriplate there is clear visible of black dots *i.e.*, acervuli, which are visible to the naked eye.Conidia are typically hyaline (translucent) and single-celled. Shape of conidia is in cylindricalandsize is 15.82×4.85 µm.

Fig 2. PDA culture plate showing 7 days old culture of *C. musae*

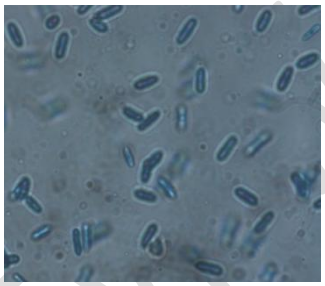

Fig 3. Microscopic view of *C. musae* (40X)

3.3. Screening of different biocontrol agents against *C.musae*

The present study was focused on comparative evaluation of fungal bio agents *viz*., *Trichoderma* sp. (KAU), *Trichoderma* sp. (TRPN-3), *Trichoderma* sp.(TRKR-2) and bacterial biocontrol agents includes *P. fluorescens* (PN026), *Bacillus* sp.(B15) and *Bacillus*sp.(B17)were tested *in vitro* against growth of *C.musae*(Table 1) (figure 4, 5).Maximum mycelial growth inhibition of the pathogen was recorded with *Trichoderma* sp. (KAU)(77.44 per cent)followed by *Trichoderma* sp. (TRKR-2) (77.11 per cent)by using fungal biocontrol agents. In case of screening with bacterial biocontrol agents maximum mycelial growth inhibition of the pathogen was recorded with *Bacillus* sp. (B17)(55.55 per cent)followed by *Bacillus* sp. (B15)(55.44 per cent). In mango the evaluationof six biocontrol agents *viz., T. hamatum, T. harzianum, T. koningii, T. longibrachiatum, T. pseudokoningii* and *T. viride* against *Colletotrichum gleosporioidesin vitro* by dual culture technique. Among these isolates *T. harzianum* was the most effective, reducing of pathogen growth by 42per cent. *T. viride, T. harzianum* and *T. koningii* were efficient in inhibiting the growth of *C. musae*[22,23].Antagonistic effect of *T. longibrachiatum, T. viride, T. asperellum* and *T. harzianum* against *C. gloeosporioides* in dual culture[24].Similarly treatment with each *Trichoderma* species reduced radial growth of *C. gloeosporioides* in plates by 53–56per cent in papaya.*Trichoderma harzianum* strain DGA01 has been reported to be effective in managing postharvest pathogens in banana, namely *Lasiodiplodiatheobromae, C.musae,*

Thielaviopsis paradoxa and *Fusarium verticillioides* during *in vitro* and *in vivo*evaluations [25].Many species from *Trichoderma* are widely studied against destructive pathogens including *Alternaria sesami* causing leaf spot in sesame, stem rot of groundnut caused by *Sclerotium rolfsii*[26,27].Recently, 11 species of native *Trichoderma* from 16 crop rhizhosphere have been identified and their antagonistic potential were evaluated for the management of *Sclerotium rolfsi* and *Rhizoctonia solani*[28]. To develop alternatives to chemical fungicides is triggered by consumersdemand for food safety, environmental concern and increasing resistance of pathogens. The use of antagonistic microorganisms offers a sustainable and promising eco-friendlyalternative to chemicals like the genus *Trichoderma* spp. that manages major postharvest decays of fruits[29].

Table 1. *In vitro*screening of different biocontrol agents against *C.musae*

S.No	Treatments	Mean Radial Growth (mm)	Per Cent Inhibition over Control (%)
T1	*P.fluorescens* (PN026)	40.20*****	55.33(48.06)******
T2	*Trichoderma* sp. (KAU)	20.30	77.44(61.64)
T3	*Trichoderma* sp. (TRPN-3)	20.80	76.88(61.26)
T4	*Trichoderma* sp. (TRKR-2)	20.60	77.11(61.42)
T5	*Bacillus* sp. (B15)	40.10	55.44(48.12)
T6	*Bacillus* sp. (B17)	40.00	55.55(48.19)
T7	Pathogen inoculated control	90.00	0.00(0.00)
T8	Absolute control	0.00	0.00(0.00)
	CD 1%		1.406
	SEM		0.469

*****Average of three replications

******Values in parentheses are angular transformed data

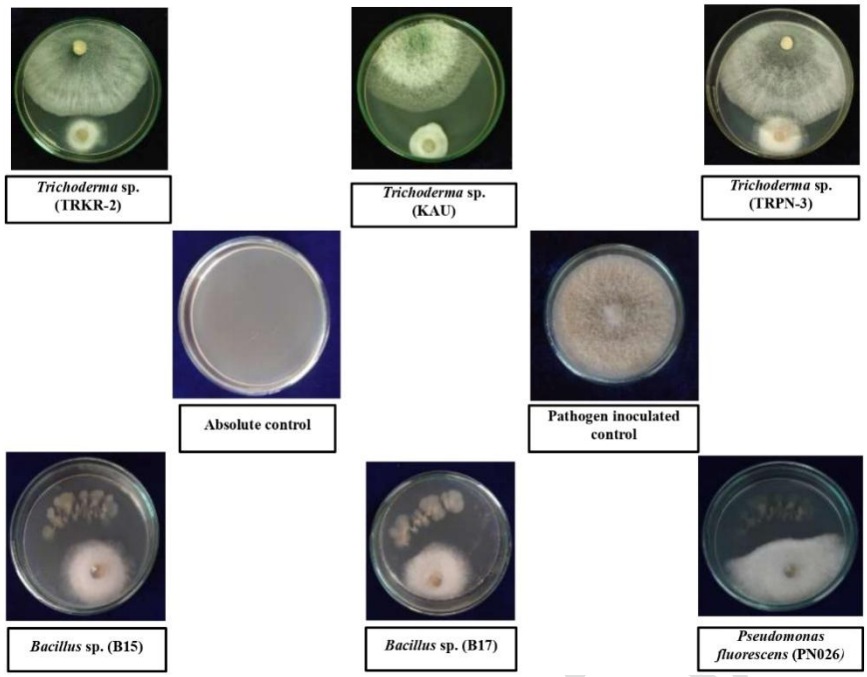

Fig4.*In vitro*screening of different biocontrol agents against *C.musae*

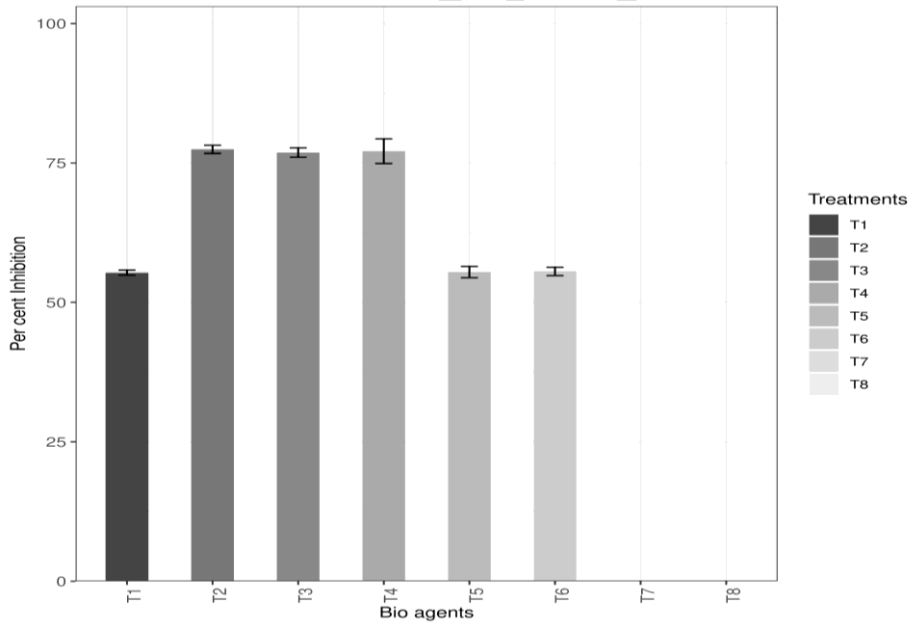

Fig 5. Evaluation of different bio control agents T1- *P. fluorescens*,T2-*Trichoderma* sp. (KAU), T3-*Trichoderma* sp. (TRPN-3), T4-*Trichoderma* sp. (TRKR-2),T5-*Bacillus* sp. (B15), T6-*Bacillus* sp. (B17) T7-Pathogen inoculated control T8-Absolute control, against *C.musae*

4. CONCLUSION

Based on our present study, out of six biocontrol agents, *Trichoderma* sp. (KAU) was found most effective in inhibiting mycelial growth of *C.musae* followed by *Trichoderma* sp. (TRKR-2) and *Trichoderma* sp. (TRPN-3).*P.fluorescens* (PN026) was least effective in *in vitro* (Dual culture method) condition.

FUTURE SCOPE

Based on the obtained results from present study indicated that, *Trichoderma* sp. (KAU) exhibiting excellent mycelial growth control of anthracnose pathogen *C.musae* in *in vitro* condition could be utilized in *in vivo* conditions for the management of anthracnose of nendran banana.

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