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# Biological control of wilt disease of hill banana incited by *Fusarium oxysporum f.sp. cubense*

**I Yesu Raja and EG Ebenezar**

**Abstract**
An intensive survey on incidence of wilt disease of hill banana was conducted at fourteen locations of major hill banana growing areas of Dindigul district and fourteen isolates of pathogen, *Fusarium oxysporum f.sp. cubense* was obtained. The mycelial growth of the isolates was observed and the virulent isolate which was collected from Adaloor village was used for further studies. Fourteen isolates of *Trichoderma viride, Pseudomonas flourescens* and *Bacillus subtilis* were isolated. The *invitro* efficacy of fourteen isolates *Trichoderma viride, Pseudomonas flourescens* and *Bacillus subtilis* were tested against the pathogen by dual plate technique. Among the *Trichoderma viride* isolates tested, Pandrimalai isolate significantly exerted highest per cent mycelial growth inhibition (72.55%) over control. The isolate from KC Patty village showed the least mycelial growth inhibition of 57.52 per cent. Among the *Pseudomonas flourescens* isolates tested, Perumparai isolate significantly exerted highest per cent mycelia growth inhibition of 82.64 per cent over control. The isolate from Manjalparappu village showed the least mycelial growth inhibition of 67.17 per cent. Among the *Bacillus subtilis* isolates tested, Poolathur isolate significantly showed highest per cent mycelial growth inhibition (59.69%) over control. The effective biocontrol agent was evaluated under *in vivo* condition. Among the treatments tested, the treatment viz., rhizome dipping of *Pseudomonas flourescens* (Perumparai isolate) @ 0.2% plus soil application of *Pseudomonas flourescens* (Perumparai isolate) @2.5kg talc based formulation / ha at 30, 60, 90 and 120 DAP recorded the lowest disease incidence of 10.67 per cent and showed the maximum disease reduction of 79.39 per cent over control.

**Keywords:** hill banana, *Fusarium oxysporum f.sp. cubense, Pseudomonas fluorescens, Trichodema viride, Bacillus subtilis*

## Introduction
Bananas, the world's most important fruit in terms of production volume and trade (FAOSTAT, 2017) [2]. It is a staple food for nearly 400 million people worldwide. India produces 27 million tonnes annually, with average productivity levels ranging from 20 to 36 tonnes per hectare. Poor soil health, nutrients imbalance, diseases and nematodes are major production constraints affecting productivity. Among the diseases, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* is recognised as one of the most widespread and destructive banana diseases, and a major production constraint to banana worldwide.

In Asia, the disease was first recorded in 1911 in West Bengal, India, and the disease now is widespread and destructive in almost all the banana-growing states in India, causing disease incidence of 30 per cent in the plant crop and up to 85 per cent in the ratoon crop. The cultivars Rasthali, Karpuravalli and Virupakshi (syn. 'Hill Banana', AAB, Pome) are severely affected by wilt (Mustaffa and Thangavelu, 2009) [4]. In Karnataka, cultivation of the local cultivar 'Nanjangod Rasabale' has been reduced from 500 ha to less than 50 ha (Narendrappa and Gowda, 1995) [5] due to severe incidence of Fusarium wilt. In Bihar, more than 55 per cent of the area under susceptible cultivars was severely infected and yield reduction in these areas was estimated at 50-70 per cent. In Tamil Nadu, it is becoming a major threat, with disease severity as high as 80-90 per cent (Sivamani, 1987) [10]. Cavendish cultivars were also recently affected in Tamil Nadu. In Andhra Pradesh, farmers have abandoned the cultivation of the most susceptible cultivar 'Amrithapani' for more than 15 years due to Fusarium wilt incidence. Since the introduction of the wilt tolerant 'Martamon' (AAB, an ecotype of 'Rasthali' from West Bengal), the cultivation has been revived. However, wilt incidence of up to 2 per cent was noticed in this cultivar also.

Management of disease by using chemicals is known to result in problems like environmental pollution, residual toxicity, short-lived efficacy and expensive nature. Therefore, the present study was undertaken to isolate the rhizosphere antagonists from hill banana growing areas of Dindigul district and to develop effective eco-friendly management practices against the disease by using biological entities.

## Materials and Methods

### Survey and collection of wilt infected hill banana root and rhizome samples

A survey was conducted in hill banana growing areas of Dindigul district for the incidence of fusarial wilt disease. The infected root and rhizome samples were collected from fourteen villages *viz*., Kanalkadu, Mangalakombu, Perumparai, Thandikudi, Kuppammalpatti, KC Patti, Pandrimalai, Adaloor, Pannaikadu, Perumalmalai. Manjalparappu, Poolathur, Kongapatti and Solaikadu for isolation of pathogen. The disease incidence was calculated by counting the total number of infected plants in each field. The percent disease incidence of wilt was calculated using the formula given by (Rajendran, 1995) [7].

$$\text{Disease Incidence} = \frac{\text{Total number of plants infected}}{\text{Total number of plants observed}} \times 100$$

### Isolation of pathogen

The wilt infected roots and rhizomes were collected and used for the isolation of the pathogen. The rotten tissues were washed in sterile distilled water and cut into several bits of three mm size using a sterilized scalpel. The washed bits were surface sterilized with mercuric chloride (0.1%) for 30 seconds and washed repeatedly using sterile water for two times under aseptic conditions. After washing, the tissues were dried using a sterile tissue paper and three bits were placed in Petri plate containing PDA medium amended with streptomycin and incubated at 28±2 ºC for 7 days. The mycelial growth of each isolate was observed separately after seven days of incubation. The hyphal tips of fungus was transferred to the PDA medium under aseptic conditions and the pure culture was maintained at 25 ºC.

### Isolation of bio control agents from the rhizosphere region of hill banana

The bio control agents viz., *Trichoderma viride*, *Pseudomonas flourescens* and *Bacillus subtilis* were isolated from the rhizosphere soil taken from the healthy banana plants. For the isolation of rhizosphere antagonists, ten gram of rhizosphere soil sample was transferred into 250 ml conical flask containing 100 ml of sterilized distilled water. Using the serial dilution technique, the biocontrol agents were isolated (Pramer and Schmidt, 1964) [6]. Then the soil suspension was serially diluted from $10^{-1}$ to $10^{-7}$. One ml of aliquot taken from $10^{-3}$ and $10^{-4}$ dilutions was poured into sterile Petri plates containing *Trichoderma* selective medium by spread plate method and the aliquot from $10^{-5}$ and $10^{-6}$ dilutions to Petri plates containing in Nutrient Agar medium and King's B medium, respectively. For *Trichoderma viride*, the plates were incubated for seven days and in case of bacteria incubate for two days. The morphologically distinct bacterial colonies were isolated separately by using a streak plate method and in case of fungi the colonies were sub cultured into Petri plates to obtain a pure colony.

### *Invito* efficacy of biocontrol agents against pathogen

Fourteen isolates of *Trichoderma viride*, *Pseudomonas flourescens* and *Bacillus subtilis* were screened against *Fusarium oxysporum* f.sp. *cubense* by dual culture method (Dennis and Webster, 1971) [1]. A nine mm mycelial disc of *Fusarium oxysporum* f.sp. *cubense* and the test antagonists were placed opposite to each other near the periphery of the Petri plate separately and incubated at room temperature (28±2 ºC). The Petri plates containing the medium inoculated with the pathogen alone were served as control. Each treatment was replicated three times. When the control plate showed full growth of the fungus the radial growth of the pathogen was measured in all the other treatments. The percent inhibition over control was calculated by using a formula proposed by Vincent (1947) [13].

$$I = \frac{100\ (C - T)}{C}$$

Where,
I = Percent inhibition over control
C = Growth in control
T= Growth in treatment

### Effect of effective antagonist on disease incidence under pot culture conditions

A pot culture experiment with nine treatments was conducted using the promising *Pseudomonas flourescens* (Perumparai isolate) in the pot culture experiment at Horticultural Research Station, Thadiyankudisai. The following treatments were replicated three times in a completely randomized design.

The following treatments were replicated three times in a completely randomized design.

| | |
|---|---|
| T1 | The incitant fungus alone |
| T2 | Rhizome dipping of effective antagonist *P. fluorescens* - 0.2% + T1 |
| T3 | Soil application of *P. fluorescens* (2.5kg/ha) at the time of planting + T1 |
| T4 | Soil application of *P. fluorescens* (2.5kg/ha) at 30 DAP + T2 |
| T5 | Soil application of *P. fluorescens* (2.5kg/ha) at 30 and 60 DAP + T2 |
| T6 | Soil application of *P. fluorescens* (2.5kg/ha) at 30, 60 and 90 DAP + T2 |
| T7 | Soil application of *P. fluorescens* (2.5kg/ha) at 30, 60, 90 and 120 DAP + T2 |
| T8 | Std control(Carbendazim 0.1% drenching + T1) |
| T9 | Uninoculated control |

### Statistical analysis

The pot culture and laboratory experiments will be conducted by following Completely Randomized Design (CRD). The analysis was done by the AGRES software (The means were compared using Duncan's Multiple Range Test (DMRT). The values in percentage were transformed into arc sine values and standard error and critical differences were calculated at 5 per cent significant levels.

## Results

### Survey on the incidence of wilt disease of hill banana in Dindigul district

An intensive survey to assess the incidence of wilt disease of hill banana was conducted in major hill banana growing areas of Dindigul district. The results of the studies were presented in table 1. The survey indicated that the wilt incidence was recorded with the ranges of 3.37 to 16.99 per cent. Severe

incidence of 16.99 per cent was recorded in Manjalparappu village followed by Adaloor village which recorded 13.27 per cent. The lowest incidence of 3.37 per cent was recorded in Perumparai village (Table 1)

**Isolation of different isolates of pathogen in different hill banana growing areas of Dindigul district**

Wilt infected root and rhizome samples were collected from the surveyed areas (14 places) for isolation of pathogen and fourteen isolates of the pathogen *Fusarium oxysporum f.sp. cubense* were isolated. The vigour of the mycelial growth was tested for these isolates. The results were presented in table 2. Among the isolates, the pathogen isolated from Adaloor was recorded the maximum mycelial growth of 87.33 mm followed by Manjalparappu village which recorded the mycelia growth of 84.67 mm. The Adaloor isolate was selected for further studies.

**Isolation and screening of different isolates of antagonistic micro flora under *in-vitro***

The rhizosphere soil samples were collected from different hill banana growing areas of Dindigul district (14 places) for isolation of antagonistic microflora. Fourteen isolates of *Trichoderma viride*, *Pseudomonas flourescens* and *Bacillus subtilis* were isolated. The *invitro* efficacy of fourteen isolates *Trichoderma viride*, *Pseudomonas flourescens* and *Bacillus subtilis* were tested against the pathogen by dual plate technique. Among the *Trichoderma viride* isolates tested, Pandrimalai isolate significantly exerted the highest per cent mycelial growth inhibition (72.55%) over control. The isolate from KC Patti village showed the least mycelial growth inhibition of 57.52 per cent (Table 3). Among the *Pseudomonas flourescens* isolates tested, Perumparai isolate significantly exerted the highest per cent mycelia growth inhibition of 82.64 per cent over control. The isolate from Manjalparappu village showed the least mycelial growth inhibition of 67.17 per cent (Table 4).Among the *Bacillus subtilis* isolates tested, Poolathur isolate significantly exerted highest per cent mycelial growth inhibition (59.69%) over control (Table 5).

**Management of wilt disease of hill banana using promising antagonist under pot culture condition**

In the pot culture experiment, among the treatments, the treatment viz., rhizome dipping of *Pseudomonas flourescens* (Perumparai isolate) @ 0.2% plus soil application of *Pseudomonas flourescens* (Perumparai isolate) @2.5kg talc based formulation / ha at 30,60,90 and 120 DAP recorded the lowest disease incidence of 10.67% and showed the maximum disease reduction of 79.39 per cent over control (Table 6).

**Discussion**

In the present investigation, among the *Trichoderma viride* isolates tested, Pandrimalai isolate significantly recorded the highest per cent mycelial growth inhibition (72.55%) over control which was followed by Thandikudi isolate with 69.17 per cent growth reduction over control. The results are also in accordance with the findings of Kamala and Devi (2012) [3] who have tested 114 *Trichoderma* isolates against *Fusarium oxysporum* under dual culture techniques. The findings of Rajendran and Ranganathan (1996) [8] were similar with the results that the fungal antagonists *T. viride, T. harzianum, T. hamatum, T. koningii, T. pseudokoningii* were effective against onion basal rot pathogen under *in vitro* conditions. Thiruvudainambi *et al*., (2010) [12] reported that MNT 7 isolate of *Trichoderma viride* significantly reduced the mycelial growth of *S. rolfsii* to an extent of 76.30 per cent over control under *in vitro* conditions. Among the *Pseudomonas flourescens* isolates tested, Perumparai isolate significantly showed the highest per cent mycelial growth inhibition of 82.64 per cent over control and followed by Pannaikadu isolate which recorded 81.51 per cent growth reduction over control. Thangavelu *et al*. (2001) [11] reported that **s**everal strains of *Pseudomonas fluorescens* were isolated from the rhizosphere of banana (Musa sp.). These isolates were tested for their antagonistic effect towards the banana Fusarium wilt pathogen, *Fusarium oxysporum f. sp. cúbense in vitro*. Among the 11 isolates tested, PflO was the most effective in inhibiting the mycelial growth of *Fusarium oxysporum f. sp. cúbense*. Saravanan *et al*. (2003) [9] also reported that the Pfm strain of *Pseudomonas fluorescens* showed the maximum growth inhibition of the pathogen, *Fusarium oxysporum f. sp. cúbense.*

In the pot culture experiment, rhizome dipping of *Pseudomonas flourescens* (Perumparai isolate) @ 0.2% plus soil application of *Pseudomonas flourescens* (Perumparai isolate) @2.5kg talc based formulation / ha at 30,60,90 and 120 DAP recorded the lowest disease incidence of 10.67 per cent and showed the maximum disease reduction of 79.39 per cent over control. Saravanan *et al*. (2003) [9] reported that basal application of neem cake at 0.5 kg/plant plus sucker dipping in spore suspension of *P. fluorescens* for 15 min plus soil application of *P. fluorescens* at 10 g/plant at 3.5 and 7 months after planting showed the greatest suppression of fusarial wilt disease in banana and this was on par with basal application of neem cake at 0.5 kg/plant plus application of *P. fluorescens* at 10 g/plant at 3.5 and 7 months after planting.

**Table 1:** Survey on the incidence of wilt disease of hill banana

| Sl. No. | Locations | Disease incidence (%)* |
|---|---|---|
| 1. | Kanalkadu | 5.68 (13.78)$^{f}$ |
| 2. | Mangalakombu | 7.44 (15.81)$^{de}$ |
| 3. | Perumparai | 3.37 (10.56)$^{g}$ |
| 4. | Thandikudi | 5.66 (13.75)$^{f}$ |
| 5. | Kuppammalpatti | 8.26 (16.71)$^{d}$ |
| 6. | K C Patti | 11.23 (19.58)$^{c}$ |
| 7. | Pandrimalai | 11.74 (20.03)$^{c}$ |
| 8. | Adaloor | 13.27 (21.36)$^{b}$ |
| 9. | Pannaikadu | 5.27 (13.27)$^{f}$ |
| 10. | Perumalmalai | 3.71 (11.09)$^{g}$ |
| 11. | Manjalparappu | 16.99 (24.33)$^{a}$ |
| 12. | Poolathur | 11.44 (19.85)$^{c}$ |
| 13. | Kongapatti | 11.92 (20.19)$^{c}$ |
| 14. | Solaikadu | 6.86 (15.17)$^{e}$ |
| CD (p= 0.05) | | 0.97 |

* Mean of four replications

Figures in parentheses are arc sine transformed values.

The treatment means are compared using Duncan Multiple Range Test (DMRT). In a column, mean followed by a common letter(s) are not significantly different (p=0.05)

**Table 2:** Mycelial growth of different isolates of pathogen *Fusarium oxysporum cubense*

| Isolate No. | Locations | Diameter of mycelial growth (mm)* |
|---|---|---|
| I1. | Kanalkadu | 71.00$^{de}$ |
| I2. | Mangalakombu | 73.33$^{d}$ |
| I3. | Perumparai | 68.00$^{f}$ |
| I4. | Thandikudi | 71.33$^{de}$ |
| I5. | Kuppammalpatti | 73.67$^{d}$ |
| I6. | K C Patti | 78.33$^{e}$ |
| I7. | Pandrimalai | 78.67$^{bc}$ |
| I8. | Adaloor | 87.33$^{a}$ |
| I9. | Pannaikadu | 69.67$^{ef}$ |
| I10. | Perumalmalai | 63.33$^{g}$ |
| I11. | Manjalparappu | 84.67$^{a}$ |
| I12. | Poolathur | 79.33$^{bc}$ |
| I13. | Kongapatti | 81.33$^{b}$ |
| I14. | Solaikadu | 72.00$^{de}$ |
| CD (p= 0.05) | | 2.82 |

* Mean of four replications

The treatment means are compared using Duncan Multiple Range Test (DMRT). In a column, mean followed by a common letter(s) are not significantly different (p=0.05)

**Table 3:** *In vitro* efficacy of different isolates of *Trichoderma viride* against wilt pathogen

| Isolate No. | Locations | Diameter of mycelial growth (mm)* | Per cent inhibition over control* |
|---|---|---|---|
| Tv1. | Kanalkadu | 35.67 | 59.77(50.64)$^{de}$ |
| Tv2. | Mangalakombu | 32.33 | 63.53(52.85)$^{c}$ |
| Tv3. | Perumparai | 33.33 | 62.40(52.18)$^{cd}$ |
| Tv4. | Thandikudi | 27.33 | 69.17(56.27)$^{b}$ |
| Tv5. | Kuppammalpatti | 36.33 | 59.02(50.19)$^{ef}$ |
| Tv6. | K C Patti | 37.67 | 57.52(49.32)$^{ef}$ |
| Tv7. | Pandrimalai | 24.33 | 72.55(58.51)$^{a}$ |
| Tv8. | Adaloor | 29.33 | 66.92(54.89)$^{b}$ |
| Tv9. | Pannaikadu | 29.00 | 67.29(55.18)$^{b}$ |
| Tv10. | Perumalmalai | 38.33 | 66.77(48.88)$^{f}$ |
| Tv11. | Manjalparappu | 32.33 | 63.53(52.85)$^{c}$ |
| Tv12. | Poolathur | 33.67 | 62.02(51.96)$^{cd}$ |
| Tv13. | Kongapatti | 28.33 | 68.03(55.59)$^{b}$ |
| Tv14. | Solaikadu | 33.33 | 62.40(52.18)$^{cd}$ |
| | Control | 88.67 | 0.00(0.286)$^{g}$ |
| CD (p=0.05) | | | 1.58 |

* Mean of four replications
Figures in parentheses are arc sine transformed values.

The treatment means are compared using Duncan Multiple Range Test (DMRT). In a column, mean followed by a common letter(s) are not significantly different (p=0.05)

**Table 4:** *In vitro* efficacy of different isolates of *Pseudomonas fluorescens* against wilt pathogen

| Isolate No. | Locations | Diameter of mycelial growth (mm)* | Per cent inhibition over control* |
|---|---|---|---|
| Pf1. | Kanalkadu | 18.67 | 78.87(62.64)$^{cd}$ |
| Pf 2. | Mangalakombu | 19.33 | 78.12(62.11)$^{cd}$ |
| Pf 3. | Perumparai | 15.33 | 82.64(65.38)$^{a}$ |
| Pf 4. | Thandikudi | 18.33 | 79.25(62.90)$^{cd}$ |
| Pf 5. | Kuppammalpatti | 23.33 | 73.59(59.07)$^{e}$ |
| Pf 6. | K C Patti | 22.33 | 74.71(59.81)$^{e}$ |
| Pf 7. | Pandrimalai | 23.33 | 73.59(59.07)$^{e}$ |
| Pf 8. | Adaloor | 20.00 | 77.35(61.60)$^{d}$ |
| Pf 9. | Pannaikadu | 16.33 | 81.51(64.53)$^{ab}$ |
| Pf 10. | Perumalmalai | 18.00 | 79.62(63.18)$^{bc}$ |
| Pf 11. | Manjalparappu | 29.00 | 67.17(55.04)$^{f}$ |
| Pf 12. | Poolathur | 23.33 | 73.59(59.07)$^{e}$ |
| Pf 13. | Kongapatti | 23.33 | 73.59(59.07)$^{e}$ |
| Pf 14. | Solaikadu | 19.33 | 78.11(62.10)$^{cd}$ |
| | Control | 88.33 | 0.00(0.286)$^{g}$ |
| CD (p=0.05) | | | 1.47 |

* Mean of four replications
Figures in parentheses are arc sine transformed values.

The treatment means are compared using Duncan Multiple Range Test (DMRT). In a column, mean followed by a common letter(s) are not significantly different (p=0.05)

**Table 5:** *In vitro* efficacy of different isolates of *Bacillus subtilis* against wilt pathogen

| Isolate No. | Locations | Diameter of mycelial growth (mm)* | Per cent inhibition over control* |
|---|---|---|---|
| Pf1. | Kanalkadu | 40.33 | 54.00(47.29)[bc] |
| Pf 2. | Mangalakombu | 38.33 | 56.27(48.60)[b] |
| Pf 3. | Perumparai | 36.00 | 58.93(50.14)[a] |
| Pf 4. | Thandikudi | 44.00 | 49.82(44.89)[d] |
| Pf 5. | Kuppammalpatti | 35.67 | 59.31(50.36)[a] |
| Pf 6. | K C Patti | 38.33 | 56.26(48.60)[b] |
| Pf 7. | Pandrimalai | 42.33 | 51.71(45.98)[cd] |
| Pf 8. | Adaloor | 43.33 | 50.56(45.32)[d] |
| Pf 9. | Pannaikadu | 44.33 | 49.43(44.67)[de] |
| Pf 10. | Perumalmalai | 49.67 | 43.35(41.17)[g] |
| Pf 11. | Manjalparappu | 46.33 | 47.15(43.36)[ef] |
| Pf 12. | Poolathur | 35.33 | 59.69(50.58)[a] |
| Pf 13. | Kongapatti | 38.33 | 56.27(48.60)[b] |
| Pf 14. | Solaikadu | 47.33 | 46.00(42.69)[f] |
| | Control | 87.67 | 0.00(0.286)[h] |
| CD(p=0.05) | | | 1.41 |

* Mean of four replications
Figures in parentheses are arc sine transformed values.

The treatment means are compared using Duncan Multiple Range Test (DMRT). In a column, mean followed by a common letter(s) are not significantly different (p=0.05)

**Table 6:** Effect of promising antagonist on hill banana wilt disease incidence under pot culture experiment

| T. No. | Treatment Details | Disease Incidence (%)* | Disease reduction over control (%)* |
|---|---|---|---|
| T1 | The incitant fungus alone | 52.67 | 0.00(0.286)[f] |
| T2 | Rhizome dipping of effective antagonist *P. fluorescens* Perumparai isolate - 0.2% + T1 | 35.00 | 53.26(35.11)[e] |
| T3 | Soil application of *P. fluorescens* (2.5kg/ha) at the time of planting + T1 | 27.50 | 47.86(43.77)[d] |
| T4 | Soil application of *P. fluorescens* (2.5kg/ha) at 30 DAP + T2 | 20.83 | 60.37(50.99)[c] |
| T5 | Soil application of *P. fluorescens* (2.5kg/ha) at 30 and 60 DAP + T2 | 15.83 | 69.88(56.73)[b] |
| T6 | Soil application of *P. fluorescens* (2.5kg/ha) at 30, 60 and 90 DAP + T2 | 13.33 | 74.72(59.82)[ab] |
| T7 | Soil application of *P. fluorescens* (2.5kg/ha) at 30, 60, 90 and 120 DAP + T2 | 10.67 | 79.39(63.03)[a] |
| T8 | Std control(Carbendazim 0.1% drenching + T1) | 15.83 | 69.97(56.77)[b] |
| T9 | Uninoculated control | 0.00 | 0.00(0.286)[f] |
| CD(p=0.05) | | | 3.66 |

* Mean of four replications
Figures in parentheses are arc sine transformed values.

The treatment means are compared using Duncan Multiple Range Test (DMRT). In a column, mean followed by a common letter(s) are not significantly different (p=0.05)

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