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# Suppression of Fusarium wilt disease of banana by Zimmu (*Allium cepa* L. x *Allium sativum* L.) leaf extract

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**Among 33 botanicals screened against *Fusarium oxysporum* f. sp. *cubense* (*Foc*), the causal agent of Fusarium wilt disease of banana, only the Zimmu leaf extract recorded 100% inhibition of both mycelial growth and spore germination of the *Foc* pathogen. Further evaluation of Zimmu leaf extract at different concentrations under greenhouse conditions in cv. Grand Naine (Cavendish-AAA) showed complete suppression of Fusarium wilt disease (disease score of 1.0 which means healthy) at 50 and 100% concentration in both application methods viz., drenching as well as dipping the banana roots in Zimmu leaf extract. Soil application of Zimmu leaf extract completely inhibited the germination of *Foc* propagules within 20 days of treatment under micro pot conditions. The field evaluation conducted in cv. Grand Naine (Cavendish -AAA) in *Foc*-sick field also demonstrated that drenching with Zimmu leaf extract (50% concentration w/v) recorded very low internal wilt disease scores of 1.5 when compared with control plants which recorded 5.8 on a disease scale of 1 to 6. In addition to significantly increasing plant growth parameters such as plant height, girth and number of leaves, the Zimmu leaf extract treatment also increased the yield parameters in particular bunch weight to 80.8% as compared to untreated control plants. The principle compound involved has been identified as a lipid. This is the first report on the suppressive effect of Zimmu leaf extract on banana Fusarium wilt disease and the finding of this study would ultimately be useful for the sustainable and farmer friendly management of the disease worldwide.**

**Key words:** Fusarium wilt, banana, *Fusarium oxysporum* f.sp. *cubense*, Zimmu, plant extract.

## INTRODUCTION

Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is regarded as one of the most destructive diseases to banana production worldwide (Ploetz, 2005). It is a serious problem not only in India but also in many parts of the world like Malaysia, Brazil, Australia, Vietnam, East Africa and Thailand (Hwang and Ko, 2004). The pathogen (*Foc*) survives in soil, penetrates roots and spreads slowly to the corm. The fungus invades the vascular tissues resulting in pseudostem splitting, leaf chlorosis and necrosis and shows signs

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of vascular discoloration, which are more distinct in the corm tissue. As the disease progresses, the crown and pseudostem also collapse resulting in death of the plant (Nelson, 1981).

The various control strategies currently practiced include field sanitation, soil treatments with fumigants, flood fallowing, addition of organic amendments and crop rotation were not effective in controlling the Fusarium wilt disease (Thangavelu and Mustaffa, 2012). Similarly, the use of fungicides, which are frequently recommended for plant disease management, may negatively impact the environment and non-target organisms (Brimner and Boland, 2003). Planting resistant varieties (Deacon, 1984; Ploetz and Pegg, 2000) also cannot be effectively implemented because of consumer preference (Viljoen, 2002) and occurrence of mutation in *Fusarium* spp. which overcome existing resistance genes (Fravel et al., 2003).

Under these circumstances, exploitation of natural plant products in controlling plant diseases is a promising and environmental friendly strategy (Daayf et al., 1995). Botanicals have the ability to produce natural antimicrobial metabolites, which will be a sustainable and desirable method for the management of disease (Kagale et al., 2004; Rai and Carpinella, 2006). Natural plant products can act directly as pesticides or may provide pesticidal discovery (Duke et al., 2000). Masoko et al. (2007) studied the antifungal activities of leaf extracts of 24 South African *Combretum* species and found that methanolic extracts of *Combretum moggii* and *Combretum petrophilum* were very effective against many fungal species. Kagale et al. (2004) reported that the methanolic extract of *Datura metel* exhibited 85% reduction of the mycelial growth of *Rhizoctonia solani*. Therefore, the objective of our present study was to evaluate various botanical leaf extracts for their efficacy against Fusarium wilt disease of banana (*Foc*-race1) under both *in vitro* and *in vivo* conditions.

## MATERIALS AND METHODS

### Collection and extraction of botanical leaf extracts

Fresh leaf samples from 33 medicinal plants (as listed in Table 1) maintained at the Horticulture College and Research Institute, Periakulam, Tamil Nadu, India were collected. One gram of leaves from each botanical was washed thoroughly under running tap water, dried with blotting paper, cut into smaller pieces and ground using a sterile mortar and pestle by adding 10 ml of sterile distilled water. Finally, it was filtered through two layers of cheesecloth and the extract was then centrifuged at 10,000 rpm for 15 min and the supernatant alone was transferred to a fresh tube. The extract was then sterilized using 0.2 µm disposable syringe filters for further use (Tiwari and Singh, 2005).

### Isolation and maintenance of *Foc* pathogen

The *Foc* pathogen is a member of VCG 0124 of race 1 from cv. Cavendish (Thangavelu and Mustaffa, 2010) which was isolated and pure *Foc* culture in filter paper was prepared and stored at 4°C until used.

### *In vitro* screening of botanical leaf extract against Foc pathogen (VCG-0124)

#### *Spore germination assay*

Thirty microliters of conidial suspension ($4x10^6$ spores $ml^{-1}$) of *Foc* and 70 µl of leaf extract of each botanical were placed in individual cavity slides and the cavity slides were kept in Petri dishes on a glass bridge chamber and incubated at 25°C. The spore suspension in sterile distilled water alone served as the control. The germination of spores was observed for 96 h at 24 h intervals and the percent inhibition of spore germination by the leaf extract was calculated. Three replications were maintained to obtain an average percent inhibition of germination. The percent inhibition of spore germination was calculated for each treatment using the formula:

$I = (C-T) /C \times 100$

Where, I = percent inhibition; C = number of spores not germinated in the control; T = number of spores not germinated in each treatment

#### *Growth inhibition assay*

The six botanical leaf extracts that showed 100% spore germination inhibition under *in vitro* conditions were tested for their antifungal activity using the poisoned food technique (Nene and Thapliyal, 2000). Five different concentrations (1, 5, 10, 25 and 50%) of each botanical leaf extract were mixed individually with PDA medium and poured onto sterile Petri dishes. A 10 mm diameter mycelial disc was cut from a 7 day old culture of *Foc* pathogen and placed at the center of the medium. The plates were then incubated at 28 ± 2°C for 7 days. The PDA medium without the amendment of plant extract was maintained as control and three replicates for each treatment were maintained. The radial growth of *Foc* pathogen was measured after seven days and the percent of mycelial growth inhibition as compared to the control was calculated for each treatment using the formula:

$I = (C-T) /C \times 100$

Where, I = percent inhibition; C = radius of the mycelial growth in the control; T = radius of the mycelial growth for each treatment.

### Pot culture evaluation of botanical leaf extracts for the suppression of Fusarium wilt disease

All the six botanical leaf extracts which were effective against *Foc* race -1 (VCG 0124) *in vitro* were further evaluated on plants in greenhouse conditions for the suppression of Fusarium wilt disease in cv. Grand Naine (Cavendish-AAA). Plants of Grand Naine were obtained as tissue-cultured banana plants from Jain Irrigation Pvt. Ltd. Udumalpet, Tamil Nadu, India. The treatments were given to the plants by three different methods, i) inoculation of *Foc* in the pots at the time of planting and drenching with 250 ml/plant of plant extract after 10 days of pathogen inoculation, ii) drenching with 250 ml/plant of plant extract immediately after planting and inoculation with 15 g *Foc* sand maize culture (Saravanan and Muthusamy 2006)/plant 10 days after drenching and iii) dipping the roots of banana plants in the botanical leaf extracts for 90 min before planting and inoculation with 15 g of *Foc* plant in the pots 10

**Table 1.** Effect of botanicals on the inhibition of spore germination and mycelial growth of *Foc* pathogen-VCG 0124.

| Scientific name | Percent inhibition of spore germination | Percent inhibition of mycelial growth |
|---|---|---|
| *Polyagala sinensis* | 0[a] | 0[a] |
| *Aadathoda vassica* | 0[a] | 0[a] |
| *Pisonia macrophylla* | 0[a] | 0[a] |
| *Cassia senna* | 0[a] | 0[a] |
| *Boerhaevia diffusa* | 0[a] | 0[a] |
| *Alpinia galanga* | 100[b] | 63.4[e] |
| *Caesalpinia sappan* | 0[a] | 0[a] |
| *Vitex negundo* | 0[a] | 0[a] |
| *Eclipta prostata* | 0[a] | 0[a] |
| *Rhinacanthus nasutus* | 100[b] | 51.2[c] |
| *Occimum sanctum* | 0[a] | 0[a] |
| *Andrographis paniculata* | 0[a] | 0[a] |
| *Tephrosia purpurea* | 0[a] | 0[a] |
| *Plumbaga zeglnica* | 0[a] | 0[a] |
| *Centella asiatica* | 0[a] | 0[a] |
| *Eclipta prostrata* | 0[a] | 0[a] |
| *Hibiscus rosasinensis* | 100[b] | 53.6[d] |
| *Cathranthes roseus* | 0[a] | 0[a] |
| *Allium sepa L. X Allium sativum L.* | 100[b] | 100[g] |
| *Muccna pruriens* | 0[a] | 0[a] |
| *Costus igneus* | 0[a] | 0[a] |
| *Ocimum tenuiflorum* | 100[b] | 82.9[f] |
| *Acalypha indica* | 0[a] | 0[a] |
| *Bacopa monnieri* | 0[a] | 0[a] |
| *Lippia nodiflora* | 0[a] | 0[a] |
| *Gymnema sylvestre* | 0[a] | 0[a] |
| *Cissus quandrangularis* | 0[a] | 0[a] |
| *Acalypha* spp. | 0[a] | 0[a] |
| *Lawsonia mermis* | 0[a] | 0[a] |
| *Withania sominifera* | 0[a] | 0[a] |
| *Vitex altissima* | 0[a] | 0[a] |
| *Cassia alata* | 0[a] | 0[a] |
| *Vitex trifolia* | 100[b] | 48.7[b] |
| Control | 0[a] | 0[a] |

Values are the mean of three replications. Values followed by same letter in the column do not differ statistically (P<0.05; Duncan's test).

days after planting. Six months after planting, the observation on various plant growth parameters such as plant height, girth, number of leaves, leaf area and number of roots were recorded and the intensity of vascular discoloration was calculated using a 1 to 6 scale (Orjeda, 1998) where 1 = corm completely clean, 2 = isolated points of discoloration in vascular tissue, 3 = discoloration up to 1/3 of vascular tissue, 4 = discoloration between 1/3 and 2/3 of vascular tissues, 5 = discoloration greater than 2/3 of vascular tissues, and 6 = total discoloration of vascular tissues.

**Pot culture evaluation of Zimmu leaf extract at various concentrations against Foc race-1 (VCG 0124)**

The Zimmu leaf extract was evaluated at various concentrations against *Foc* race 1 (VCG 0124) under pot culture conditions. The treatments were given to the plants by three different methods as described previously. The Zimmu leaf extract was mixed with water and the treatment was given at 5, 10, 25, 50 and 100% concentrations. Six months after planting observations on various plant growth parameters such as height, girth, number of leaves and leaf area, number of roots and the intensity of vascular discoloration were recorded as stated earlier.

**Field evaluation of Zimmu leaf extract for the suppression of Fusarium wilt disease**

A field experiment was conducted in a known wilt-sick field in the Theni district of Tamil Nadu, India with cv. Grand Naine to test the

efficacy of Zimmu leaf extract for the suppression of Fusarium wilt disease. The field (the soil is red loamy (amorphous) has 6.2 pH. The average annual rainfall was 1000mmm with average temperature of 20.3 to 38.6°C and RH of 37 to 80%) which was abandoned for commercial production due to severe wilt incidence (70%) and was selected for this purpose. The tissue-cultured plants obtained from Jain Irrigation Pvt. Ltd. were planted (August 19, 2012) in the field with 6 x 6 feet spacing. The Zimmu leaf extract (50% w/v) was prepared in tap water and applied @ 1000 ml plant$^{-1}$ at the time of planting. Control plants were treated with equal volumes of water. Twenty replications per treatment were maintained. Timely applications of fertilizers, manures, water and other intercultural operations were followed according to standard production practices. The observations on plant height, girth, total number of leaves, leaf area, percentage plants yielded saleable bunches, total number of hands, number of fingers per hand, bunch weight, total number of plants free from wilt disease and internal wilt disease score (as described earlier) were taken at the time of harvest.

### Effect of Zimmu leaf extract on the population density of *Foc* pathogen (race-1 VCG 0124) in soil under micro pot conditions

The Zimmu leaf extract, which had shown effectiveness under *in vivo* conditions, was further evaluated at different concentrations for their effect on the population density of *Foc* in micro pots under laboratory conditions. Five grams of sand maize culture of *Foc* ($4\times10^{10}$ cfu g$^{-1}$) was inoculated in a sterile paper cup containing about 350 g of sterile soil. 10 ml of Zimmu leaf extract was added to the soil and mixed thoroughly. The paper cup was covered with a double layer of aluminium foil and incubated at 25°C. Soil with only sterile distilled water served as the control. Three replicates were maintained for each treatment. The population density of *Foc* was determined at 0, 10 and 20$^{th}$ day after inoculation by following a serial dilution technique (Bowers and Locke, 2000).

### Purification of antimicrobial compounds using TLC

About 10 g of Zimmu leaf tissue was homogenized in 100 ml of methanol and the homogenate was filtered through two layers of cheesecloth. The filtrate was then centrifuged at 7500 *g* for 20 min and the clear supernatant was removed. The methanol was evaporated using a Laborota 4000 Heidolph at 40°C and the residue was dissolved in 2 ml of distilled water and this sample was used for further analysis (Thangavelu et al., 2013). On a silica gel TLC plate, 5 ml of the Zimmu methanol extract was spotted at 1.5 cm intervals and different compounds were separated, developed and detected using different spray reagents (Developer) (Table 5) and the $R_f$ values were calculated (Sadasivam and Manickam, 1992). The individual compound showing different $R_f$ values was gently removed from the silica gel plate and eluted in acetic acid/chloroform (1:9) for phenolic compounds, n-Butanol/Acetic Acid/Water (8:2:2) for amino acids and chloroform/methanol/water (65:25:4) for lipids and tested for ability to inhibit the growth of *Foc* under *in vitro* conditions.

### Bio-assay of principle compounds

The compounds extracted were tested individually against *Foc* using the filter paper disc method. Sterilized filter paper discs of 3 mm diameter were placed at the center of the PDA plate containing *Foc* and spotted with 200 µL of compounds of Zimmu leaf extract (Thangavelu et al., 2013). Sterilized filter paper discs spotted with water alone served as control. Then the plates were incubated at room temperature (27±2°C) for 15 days and the inhibition zone was measured.

### Analysis of principle compound by mass spectroscopy (GC-MS)

The lipid constituents of Zimmu leaf extract which showed mycelial inhibition of the pathogen were determined using a GCClarus-500PerkinElmerGas chromatograph which was equipped with a mass detector-Turbo mass gold containing a Elite-1 (100% Dimethyl Poly Siloxane), 30 m x 0.25 mm ID x 1 µM df. The following conditions were employed such as carrier gas - helium (1mL/min), oven temperature program- 110°C (2 min) to 280°C (9 min); injector temperature (250°C); total GC time (36 min) for GC-MS analysis.

The water extract was injected into the chromatograph in 2.0 ml aliquots. The major constituents were identified with the aid of a computer-driven algorithm and then by matching the mass spectrum of the analysis with that of a library (NIST Version 2.0, 2005). Software used for gas chromatography mass spectroscopy (GC-MS) was Turbo mass-5.1. This work was carried out at the Indian Institute of Crop Processing Technology (IICPT), Thanjavur, Tamil Nadu, India.

### Statistical analysis

The data on effect of treatments on the mycelial growth, inhibition of spore germination, percent of plants yielding saleable bunches, number of plants free from wilt disease, internal wilt disease score, growth and yield parameters were analyzed by analysis of variance (ANOVA) and treatment means were compared by Duncan's multiple range test (DMRT) and by least significance difference (LSD) at P=0.05. The data on inhibition of spore germination, mycelial growth, the percent of saleable bunches and percent of plants free from the disease were arcsine transformed and the data on the effect of Zimmu leaf extract on population density of *Foc* were log transformed before undergoing statistical analysis (Gomez and Gomez, 1984). The package used for analysis was IBM SPSS Statistics Version 21 developed by the International Business Machines Corporation.

## RESULTS

### *In vitro* screening of botanical leaf extracts against *Foc* pathogen

Out of 33 botanical leaf extracts screened against *Foc* pathogen by spore germination assay, only six viz., *Alpinia galanga, Rhinacanthus nasutus, Hibiscus rosasinensis, Allium cepa L.* x *Allium sativum L.* (Zimmu), *Ocimum tenuiflorum and Vitex* spp. showed 100% inhibition of spore germination. When all these six botanical extracts evaluated for the inhibition of mycelial growth by the poison food technique, only the Zimmu leaf extract recorded complete inhibition (100%) of mycelial growth of *Foc* pathogen (Table 1 and Plate 1).

### Pot culture evaluation of botanical leaf extract against Fusarium wilt disease

The evaluation of six different leaf extracts (which was

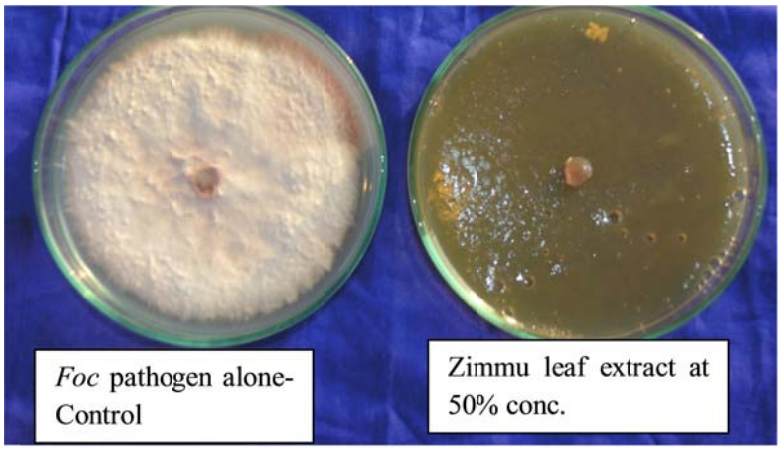


**Plate 1.** Effect of Zimmu leaf extract (50% conc.) on the mycelia growth of *Foc* pathogen under *in vitro* condition. Note that there is no mycelial growth of *Foc* in the plate added 50% concentration of Zimmu leaf extract.

**Table 2.** Effect of dipping the Zimmu leaf extract for 90 min after planting and application of *Foc* -VCG 0124 (15 g/plant) after 10 days of dipping on the plant growth parameters, root numbers and wilt disease score in cv. Grand Naine.

| Concentration of Zimmu leaf extract (%) | Height (cm) | Girth (cm) | Leaf area ($cm^2$) | Total no. of leaves | Total no. of roots (cm) | Wilt disease score (1-6 scale) |
|---|---|---|---|---|---|---|
| 5 | 22.2$^{c}$ (50.00) | 9.0$^{b}$ (40.63) | 293.71$^{c}$ (139.67) | 6.0$^{b}$ (30.43) | 22.8$^{bc}$ (34.12) | 1.4$^{a}$ |
| 10 | 27.6$^{ab}$ (86.49) | 10.0$^{bc}$ (56.25) | 377.46$^{ab}$ (208.00) | 6.4$^{b}$ (39.13) | 24.0$^{c}$d (41.18) | 2.0$^{b}$ |
| 25 | 29.4$^{a}$ (98.65) | 11.0$^{c}$ (71.88) | 419.09$^{a}$ (241.97) | 6.0$^{b}$ (30.43) | 27.6de (62.35) | 1.2$^{a}$ |
| 50 | 25.0$^{bc}$ (68.92) | 10.2$^{c}$ (59.38) | 331.45$^{bc}$ (170.46) | 6.0$^{b}$ (30.43) | 29.0ef (70.59) | 1.0$^{a}$ |
| 100 | 25.4$^{bc}$ (71.62) | 10.0$^{bc}$ (56.25) | 342.31$^{bc}$ (179.32) | 6.4$^{b}$ (39.13) | 32.0f (88.24) | 1.0$^{a}$ |
| *Foc* alone | 14.8d (0.00) | 6.4$^{a}$ (0.00) | 122.55d (0.00) | 4.6$^{a}$ (0.00) | 17.0$^{a}$ (0.00) | 4.4$^{c}$ |

Values are the mean of five replications. Values followed by same letter in the column do not differ statistically (P<0.05; Duncan's test).

effective under *in vitro* condition) for the suppression of Fusarium wilt disease by three different methods under pot culture condition indicated that among different plant extracts, only the Zimmu leaf extract treatment showed complete suppression of Fusarium wilt disease (disease score of 1.0) in two different application methods: i) drenching of Zimmu leaf extract immediately after planting and inoculation of *Foc* after 10 days and ii) dipping of banana roots in Zimmu leaf extract for 90 min before planting and inoculation of *Foc* after 10 days of dipping. The *Foc* alone-inoculated plants recorded a disease score of 4.4. It was also observed that the application of all the six plant extracts increased the plant growth parameters such as plant height (up to 100%), girth (up to 54.6%), number of leaves (up to 30.4%), leaf area (up to 241.3%) and total number of roots (up to 78.8%) significantly as compared to *Foc* alone inoculated control plants (data not shown).

### Effect of Zimmu leaf extract at various concentrations against Fusarium wilt disease

The application of five different concentrations (5, 10, 25, 50 and 100%) of Zimmu leaf extract for the suppression of Fusarium wilt disease indicated that the Zimmu leaf extract application at 50 and 100% concentrations completely suppressed the Fusarium wilt disease (disease score of 1.0) in two application methods such as dipping the banana roots in Zimmu leaf extract (Table 2) and drenching of banana plants with Zimmu leaf extract

**Table 3.** Effect of drenching of Zimmu leaf extract (at 250 ml/plant) immediately after planting and application of *Foc* -VCG 0124 (at 15 g/plant) after 10 days of drenching on the plant growth parameters, root numbers and wilt disease score in cv. Grand Naine.

| Concentration of Zimmu leaf extract (%) | Height (cm) | Girth (cm) | Leaf area ($cm^2$) | Total no. of leaves | Total no. of roots | Wilt disease score (1-6 scale) |
|---|---|---|---|---|---|---|
| 5 | 19.0$^{b}$ (28.38) | 9.2$^{b}$ (43.75) | 357.05$^{ab}$ (191.35) | 6.4e (39.13) | 23.6$^{bc}$ (38.82) | 1.6$^{b}$ |
| 10 | 20.2$^{b}$ (36.49) | 9.0$^{b}$ (40.63) | 320.11$^{b}$ (161.21) | 5.4$^{bc}$ (17.39) | 20.4$^{ab}$ (20.00) | 1.6$^{b}$ |
| 25 | 21.2$^{b}$ (43.24) | 10.0$^{b}$ (56.25) | 429.3$^{a}$ (250.31) | 5.2$^{abc}$ (13.04) | 23.4$^{bc}$ (37.65) | 1.4$^{ab}$ |
| 50 | 21.0$^{b}$ (41.89) | 9.2$^{b}$ (43.75) | 322.4$^{b}$ (163.08) | 5.6$^{c}$d (21.74) | 26.7$^{c}$ (57.06) | 1.0$^{a}$ |
| 100 | 19.5$^{b}$ (31.76) | 10.0$^{b}$ (56.25) | 295.49$^{b}$ (141.12) | 6.2de (34.78) | 24.2$^{bc}$ (42.35) | 1.0$^{a}$ |
| *Foc*alone | 14.8$^{a}$ (0.00) | 6.4$^{a}$ (0.00) | 122.55$^{c}$ (0.00) | 4.6$^{a}$ (0.00) | 17.0$^{a}$ (0.00) | 4.4$^{c}$ |

Values are the mean of five replications. Values followed by same letter in the column do not differ statistically (P<0.05; Duncan's test).


**Plate 2.** Effect of Zimmu leaf extract on the Fusarium wilt disease severity in cv. Grand Naine (from left) 1,2: *Foc* alone inoculated control plants, 3-6: Plants treated with 50% Zimmu leaf extract (note the complete absence of vascular discolouration in the corm of Zimmu leaf extract treated plants).

(Table 3) when compared with *Foc* alone inoculated plants which recorded a disease score of 4.4 (Plate 2). Besides, the application of Zimmu leaf extract also increased the plant growth parameters such as plant height (up to 98.6%), girth (up to 71.8%), total number of leaves (up to 63%), leaf area (up to 254.5%) and total number of roots (up to 88.2%) significantly as compared to *Foc* alone inoculated control plants (Table 2 and 3).

## Field evaluation of Zimmu leaf extract for the suppression of Fusarium wilt disease

The analysis of field evaluation data shows that the soil drenching of banana plants with Zimmu leaf extract recorded a minimum internal wilt disease score of only 1.5 when compared with control plants, which recorded a disease score of 5.8 on a disease scale of 1-6 where 1 is corm completely clean and 6 is total discoloration of vascular tissues in the corm (Figure 1 and Plate 3). It was also observed that in the Zimmu leaf extract treatment, about 65% of the plants were free from the symptoms of wilt disease as against zero in the case of untreated control plants.

Similarly, in the Zimmu leaf extract treatment, all (100%) the banana plants yielded saleable bunches, whereas it was only 25% in the control treatment. Besides, the same Zimmu leaf extract treatment also

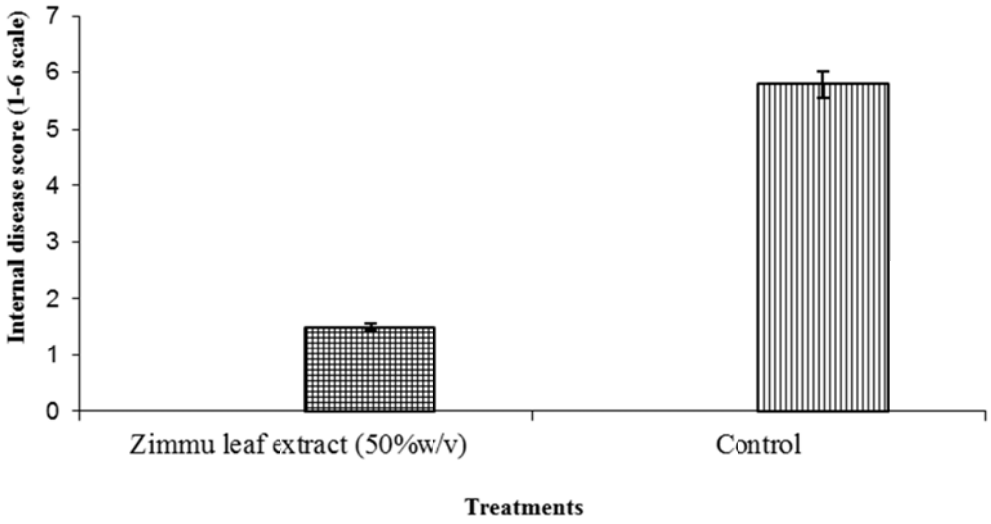


**Figure 1.** Suppression of Fusarium wilt disease in banana due to drenching of Zimmu leaf extract at 50% concentration (w/v) in cv. Grand Naine (Cavendish-AAA) under field condition. Values are mean of 20 replicates and bar represents SD value of treatments.

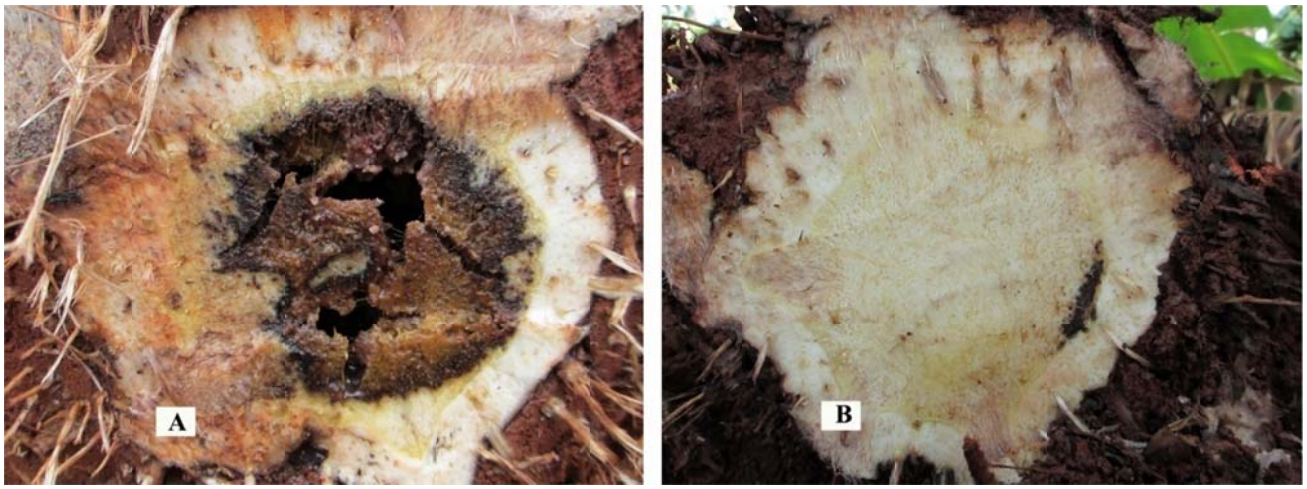


**Plate 3.** Effect of drenching of Zimmu leaf extract at 50% concentration (w/v) on the vascular browning of corm tissues in cv. Grand Naine (Cavendish-AAA) under field condition. A- Untreated control plants, B- treatment with Zimmu leaf extract.

increased the plant growth parameters such as plant height (36%), girth (35.7%), total number of leaves (39.3%), leaf area (66%) and yield parameters such as number of hands per bunch (53.6%), number of fingers per hand (57.9%) and the bunch weight (80.8%) (Plate 4) significantly as compared to the control plants (Table 4).

## Effect of Zimmu leaf extract on the population density of Foc pathogen in soil

The soil application of 50% concentration of Zimmu leaf extract on the population density of *Foc* pathogen in the soil at 0, 10 and 20 days after application indicated that the initial population of 11.7 x $10^7$ cfu/g of soil of *Foc* was drastically reduced to 1x $10^7$ cfu/g of soil 10 days after treatment and 20 days after inoculation no colony of *Foc* was seen where the Zimmu leaf extract was applied to the micro pot, whereas in the untreated control, the *Foc* population remained as 6.3x$10^7$ cfu/g of soil. This study indicated that the soil application of Zimmu leaf extract had completely inhibited the germination of *Foc* pathogen within 20 days after treatment (Figure 2).

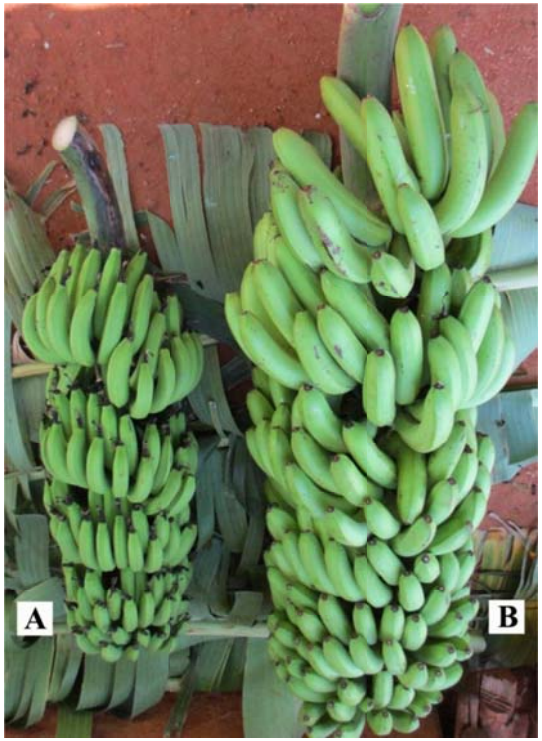


**Plate 4.** Effect of Zimmu leaf extract treatment on the bunch weight of banana plants. A- untreated control B- bunch from Zimmu leaf extract drenched banana plant cv. Grand Naine (AAA).

**Table 4.** Effect of drenching of Zimmu leaf extract at 50% concentration (w/v) on the plant growth and yield parameters in banana cv. Grand Naine under field condition.

| Treatment | Plant height(cm) | Girth (cm) | Total no. of leaves | Leaf area ($cm^2$) | No. of hands | Total no. of fingers $hand^{-1}$ | Bunch weight (Kg) | Percent of plants free from disease | Percent of plants yielded saleable bunches |
|---|---|---|---|---|---|---|---|---|---|
| Zimmu leaf extract (at the time of planting) | 275.3[a] (36.0) | 70.0[a] (35.7) | 8.5[a] (39.3) | 13812[a] (66.0) | 10.6[a] (53.6) | 19.3[a] (57.9) | 28.2[a] (80.8) | 65a | 100a |
| Control (untreated) | 202.5[b] (0.0) | 51.6[b] (0.0) | 6.1[b] (0.0) | 8322[b] (0.0) | 6.9[b] (0.0) | 12.2[b] (0.0) | 14.4[b] (0.0) | 0.0b | 25b |

Values are mean of 20 replications. Figures in parentheses are percent increase over control plants. Values followed by same letter in the column do not differ statistically ($P<0.05$; Duncan's test).


**Figure 2.** Effect of Zimmu leaf extract on the population density of *Foc* pathogen race-1 (VCG 0124) under micro pot condition.

**Table 5.** Efficacy of purified compounds of Zimmu leaf extract on the mycelial growth of *Foc* pathogen (VCG 0124) under *in vitro* condition.

| Compound | Solvent system | Spray reagent | Colour of the spot | Zone of inhibition (mm) | |
|---|---|---|---|---|---|
| | | | | Band 1 | Band 2 |
| Phenolic compound | Acetic acid/chloroform (1:9) | Folins-Ciocalteu reagent/water (1:1) followed by spraying with $Na_2Co_3$ | Blue | $0_b$ | $0_b$ |
| Amino acid | n-Butanol/acetic acid/water (8:2:2) | 0.1% Ninhydrin in acetone and heat the plate for 10 min at 100°C | Pink or Purple | $0_b$ | $0_b$ |
| Lipid | Chloroform/methanol/ water (65:25:4) | 50%$H_2SO_4$ and heat the plate for 20 min at 100°C | Violet | $0.8_a$ | $1.1_a$ |

Values are the mean of three replications. Values followed by same letter in the column do not differ statistically (P < 0.05; Duncan's test).

## Isolation and purification of principle compounds from Zimmu leaf extract by TLC and GC-MS analysis

By TLC analysis, eight bands of phenols (2 nos.), amino acids (4 nos.) and lipids (2 nos.) were identified and the same were extracted individually. The bioassay of these bands individually against *Foc* indicated that only the lipid compounds (LP-B1 and LP-B2) had the ability to inhibit the *Foc* mycelial growth by producing the zone of inhibition of 1.1 cm (Table 5). Further analysis of these lipid compounds by GC-MS revealed the presence of six different compounds in lipid B1 and five different compounds in lipid B2. Among these compounds, five compounds were common in both Lipid B1 and B2, and the compound 1-chloro heptacosane was present only in lipid B1 (Table 6).

## DISCUSSION

Generally, botanicals are considered not only as an alternative to chemicals but also as less expensive, easily available and eco-friendly in the management of various plant diseases including Fusarium wilt (Ashwani Tapwal et al., 2011; Akila et al., 2011). Therefore, in the present study, an attempt was made to find out the effective

**Table 6.** Chemicals components detected in the lipid compounds of Zimmu leaf extract by GC-MS analysis.

| Retention time | Name of the component | Presence of component | Molecular formula | MW | Peak area (%) |
|---|---|---|---|---|---|
| 2.65 | Tetradecane | LB1 LB2 | C14H30 | 198 | 22.53 |
| 3.02 | Dodecane, 2,6,10-trimethyl | LB1 LB2 | C15H32 | 212 | 26.37 |
| 4.95 | Hexadecane | LB1 LB2 | C16H34 | 226 | 17.03 |
| 5.54 | Heptadecane, 2,6,10,15-tetramethyl- | LB1 LB2 | C21H44 | 296 | 18.68 |
| 7.63 | Heptadecane, 9-hexyl- | LB1 LB2 | C23H48 | 324 | 8.79 |
| 8.18 | Heptacosane, 1-chloro- | LB1 | C27H55Cl | 414 | 6.59 |

LB1 is the lipid band in TLC having RF value of 0.83 and LB2 is the lipid band in TLC having RF value of 0.90.

botanicals for the management of Fusarium wilt disease of banana, which is becoming a very serious problem in different banana growing regions of the world. In our study, screening of 33 botanical leaf extracts resulted in identification of six different botanical leaf extracts (*A. galanga, R. nasutus, H. rosasinensis, A. cepa L.* x *A. sativum L.* (Zimmu)*, Ocimum tenuiflorum* and *Vitex* spp.) showing effect in inhibition of mycelial growth and spore germination of *Foc* pathogen under *in vitro* condition. However, among these six botanicals, Zimmu leaf extract alone exhibited 100% inhibition of both mycelial growth and spore germination of *Foc*. Similarly, Satya et al. (2007) showed that among the leaf extracts of 39 medicinal plants, the Zimmu (*A. cepa* x *A. sativum*) leaf extract had the highest antifungal activity against *Rhizoctonia solani* under *in vitro* conditions. The evaluation of fungitoxic effects of 66 medicinal plants *in vitro* on *Pythium aphanidermatum*, also indicated that only the Zimmu leaf extract showed the highest inhibition of mycelial growth (Muthukumar et al., 2010). Karthikeyan et al. (2007) reported that the leaf extract of Zimmu exhibited strong antifungal activity against *Aspergillus flavus, Fusarium moniliforme, Curvularia lunata* and *Alternaria alternata* and caused *in vitro* fungal growth inhibition of 73.3, 71.1, 70.0 and 74.4%, respectively. The crude extract of different *Allium* species such as *Allium fistulosum* (Green onion), *A. sativum* (Garlic) and *Allium tuberosum* (Chinese leek) inhibited the spore germination of *Alternaria brassicola* by 100% (Ho et al., 2007). The reason for the effective inhibition of mycelial growth and spore germination of various pathogens by different plant extracts might be due to the presence of antifungal compounds present in the leaf extracts (Ghosh et al., 2002; Kagale et al., 2004).

The usefulness of different species of *Allium* plants for the management of various fungal and bacterial pathogens has been studied in agriculture, food science and medicine extensively (Najjaa et al., 2007; Lazarevic et al., 2011; Chen et al., 2011; Phay et al., 1999). In the present investigation also, among the six different botanical extracts (which have shown effectiveness against *Foc in vitro*), the Zimmu leaf extract particularly 50 and 100% concentrations suppressed the Fusarium wilt disease completely (disease score of 1.0 means healthy) in cv. Grand Naine (Cavendish-AAA) in two different application methods such as drenching and dipping of banana roots in Zimmu leaf extract. Similarly, in the field experiment, under sick field conditions, drenching of Zimmu leaf extract (50% concentration) recorded an internal disease score of 1.5 as compared to 5.8 in the untreated control plants. The Zimmu leaf extract treatment also enhanced both growth and yield parameters significantly as compared to untreated control plants. For example, there was an 80.8% increase in bunch weight upon Zimmu leaf extract application and moreover all the plants (100%), which received Zimmu treatment yielded saleable bunches, whereas, in the case of the control treatment, the amount of salable bunches was only 25%. This might be due to the effective suppression of Fusarium wilt disease in the Zimmu leaf extract treated plants. These are in accordance with the findings of Huang et al. (2012) who have studied the effect of Chinese leek (*Allium tuberosum*) on Fusarium wilt disease incidence and reported 58% reduction of incidence in the banana variety Baxi (AAA) and 79% in the banana variety Guangfen No.1 (ABB) under greenhouse conditions. The foliar application of Zimmu formulation 50 EC at 3 ml $L^{-1}$ (v/v) concentration at 60, 75 and 90 days after sowing significantly reduced the incidence of grain mold and increased the grain weight and grain hardness (Karthikeyan et al., 2007). The combined application of *Trichoderma viride* + *Pseudomonas fluorescens* + Zimmu leaf extract as seed treatment under pot culture studies was superior in reducing the pre and post-emergence chilli damping-off incidence (8.3 and 17.0% respectively) and increasing the plant growth and yield (shoot length and root length of 13.7 and 6.3 cm, 146 g/plant, respectively) of chilli as compared to the control (Muthukumar et al., 2010). In the present study, we also observed that the soil application of Zimmu leaf extract under micro pot conditions completely inhibited the germination of *Foc* propagules in 20 days of treatment. This study, therefore, indicates that the planting of Zimmu as an intercrop in the banana field could reduce the population density of the pathogen propagules in the soil and thus would be highly useful for

the sustainable management of Fusarium wilt disease at the field level**.** Similarly, the treatment of the soil with 10% aqueous emulsions of pepper/mustard, cassia and clove extracts reduced the population density of *F. oxysporum* f.sp. *chrysanthemi* 99.6, 96.1 and 97.5%, respectively, when compared with the untreated control (Bowers and Locke, 2000). Earlier, it was also reported that Zimmu leaf extract at 50% concentration controlled the eumusae leaf spot disease of banana effectively at the field level (Thangavelu et al., 2013). Therefore, by growing Zimmu as an intercrop in the banana field, the banana growers can manage the two deadly diseases of banana such as Fusarium wilt and eumusae leaf spot disease in a sustainable, eco-friendly and an economical way.

Several authors have identified many substances in many plant extracts and were thought to be responsible for the antifungal activity, namely 3-(4-hydroxyphenyl)-2(E)-propenoate isolated from *Costus speciou*s (Bandara et al., 1989); isobutyric acid, butyric acid, valeric acid and caproic acid from *Portulaca oleracea* (Park et al., 1986); tiliacorine from *Tiliacora racemosa* (Tripathi and Dwivedi, 1989); guaianolides from *Chichorium intybus* (Mares et al., 2005) and acetoxychavicold acetate from *Alpinia galanga* (Janssen and Scheffer, 1985). Similarly, in our study, two lipid compounds (LP-B1 and LP-B2), which showed the highest antifungal activity against *Foc* pathogen, were analyzed through GC-MS. The results revealed the presence of six different compounds in Lipid B1 and five different compounds in Lipid B2. This clearly indicated that these compounds might be responsible for the inhibition of *Foc* under *in vitro* and *in vivo* conditions. However, Satya et al. (2005) reported that the compounds of Zimmu leaf extract which showed strong antifungal activity against *R. solani* were phenolic compounds. In addition, Muthukumar et al. (2010) identified the presence of 22 compounds in the Zimmu leaf extract through GC-MS analysis. These studies showed the presence of different compounds with differential in efficacy towards different pathogens including Fusarium wilt pathogen.

In conclusion, the present study identified Zimmu as an effective botanical for the suppression of Fusarium wilt disease of banana cv. Grand Naine (Cavendish -AAA) at the field level. Also, the principle compound involved in the inhibition of *Foc* pathogen has been identified as a lipid. To the best of our knowledge, this is the first report on the use of Zimmu leaf extract for the management of Fusarium wilt disease of banana and this study will definitely give a high scope for all the banana growers for the effective and biological based management of Fusarium wilt disease.

## Conflict of Interests

The authors have not declared any conflict of interests.

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## REFERENCES

Akila R, Rajendran L, Harish S, Saveetha K, Raguchander T, Samiyappan R (2011). Combined application of botanical formulations and biocontrol agents for the management of *Fusarium oxysporum* f.sp. *cubense* (*Foc*) causing Fusarium wilt in banana. Biol. Control 57:175-83.

Ashwani Tapwal, Nisha, Shipra Garg, Nandini Gautam, Rajesh Kumar (2011). *In vitro* antifungal potency of plant extracts against five phytopathogens. Braz. Arch. Biol. Technol. 54:1093-98.

Bandara BMR, Kumar NS, Samaranayake KMS (1989). An antifungal constituent from the stembark of *Butea monosperma*. J. Ethnopharmacol. 25:73-75.

Bowers JH, Locke JC (2000). Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of Fusarium wilt in the greenhouse. Plant Dis. 84:300-305.

Brimner TA, Boland GJ (2003). A review of the non-target effects of fungi used to biologically control plant diseases. Agric. Ecosyst. Environ. 100:3-16.

Chen CH, Chou TW, Cheng LH, Ho CW (2011). *In vitro* anti-adenoviral activity of five *Allium* plants. J. Taiwan Ins. Chem. Eng. 42:228-232.

Daayf F, Schmitt A, Belanger RR (1995). The effects of plant extracts of *Reynoutria sachalinensis* on powdery mildew development and leaf physiology of long English cucumber. Plant Dis. 79: 577-580.

Deacon JW (1984). Panama disease of banana in South Africa. Hortic. Sci.1:29-31.

Duke SO, Dayan FE, Romagni JG, Rimando AM (2000). Biosynthesized products as sources of herbicides: Current status and future trends. Weed Res. 40:99-111.

Fravel DR, Olivan C, Alabouvette C (2003). *Fusarium oxysporum* and its biocontrol. New Phytol. 157:493-502.

Ghosh M, Thangamani D, Thapliyal M, Yasodha R, Gurumurthi K (2002). Purification of a 20 KDa antifungal protein from *Plumbago capensis* a medicinal plant. J. Med. Aromat. Plant Sci. 24:16-18.

Gomez KA, Gomez AA (1984). Statistical Procedure for Agricultural Research. John Wiley and Sons, New York.

Ho WC, Wu TY, Su HJ, Ko WH (2007). Effect of oriental medicinal plant extracts on spore germination of *Alternaria brassicicola* and nature of inhibitory substances from speed weed. Plant Dis. 91:1621-1624.

Huang YH, Wang RC, Li CH, Zuo CW, Wei YR, Zhang L, Yi GJ (2012). Control of Fusarium wilt in banana with Chinese leek. Eur. J. Plant Pathol. 134:87-95.

Hwang SC, Ko WH (2004). Cavendish banana cultivars resistant to Fusarium wilt acquired through somaclonal variation in Taiwan. Plant Dis. 88: 580-588.

Janssen AM, Scheffer JJ (1985). Acetoxychavicol acetate an antifungal component of *Alpinia galanga*. Planta Med. 6:507-511.

Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R (2004). Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and Xoo. Physiol. Mol. Plant Pathol. 65: 91-100.

Karthikeyan M, Sandosskumar R, Radhajeyalakshmi R, Mathiyazhagan S, Khabbaz SE, Ganesamurthy K, Selvi B, Velazhahan R (2007). Effect of formulated zimmu (*Allium cepa* L. × *Allium sativum* L.) extract in the management of grain mold of sorghum. J. Sci. Food Agric. 87: 2495-2501.

Lazarevic JS, Dorđevic AS, Zlatkovic BK, Radulovic NS, Palic RM (2011). Chemical composition and antioxidant and antimicrobial

activities of essential oil of *Allium sphaerocephalon* L. subsp. *sphaerocephalon* (*Liliaceae*) inflorescence. J. Sci. Food Agric. 91:322-329.
Mares D, Romagnoli C, Tosi B, Andreotti E, Chillemi G, Poli F (2005). Chicory extracts from *Cichorum intybus* L. as potential antifungal. Mycopathologia 160: 85-92
Masoko P, Picard J, Eloff JN (2007). The antifungal activity of twenty-four South African *Combretum* species (*Combretaceae*). S. Afr. J. Bot. 73:173-183.
Muthukumar A, Eswaran A, Nakkeeran S, Sangeetha G (2010). Efficacy of plant extracts and biocontrol agents against *Pythium aphanidermatum* inciting chilli damping-off. Crop Prot. 29:1483-1488.
Najjaa H, Neffati M, Zouari S, Ammar E (2007). Essential oil composition and antibacterial activity of different extracts of *Allium roseum* L., a North African endemic species. Comptes Rendus Chimie 10: 820-826.
Nelson PE (1981). Life cycle and epidemiology of *Fusarium oxysporum*. In: Beckmann CH, editor. Fungal wilt diseases of plants. St Paul, Minnesota: APS Press. pp. 51-79.
Nene Y, Thapilyal L (2000). Poisoned food technique of fungicides in plant disease control. 3rd Edn. Oxford and IBH Publishing Company, New Delhi.
Orjeda G (1998). Evaluation of Musa germplasm for resistance to Sigatoka diseases and Fusarium wilt. INIBAP Technical Guidelines 3. International Plant Genetic Resources Institute.
Park JS, Nishimura S, Marumo S, Katayama M (1986). Isolation and identification of antifungal fatty acids from the extract of *Portulaca oleracea* L. Korean J. Plant Pathol. 2:82-88.
Phay N, Higashiyama T, Tsuji M, Matsuura H, Fukushi Y, Yokota A (1999). An antifungal compound from roots of Welsh onion. Phytochemistry 52:271-74.
Ploetz RC (2005). Panama disease, an old enemy rears its ugly head: Parts 1 and 2. In: Plant Health Progress, APSnet: Online doi: 10.1094/PHP-2005-1221-01-RV.
Ploetz RC, Pegg KG (2000). Fusarium wilt. In: Diseases of Banana, Abacá and Enset. D. R. Jones, ed. CABI Publishing, Wallingford, UK. pp. 143-159.
Rai M, Carpinella M (2006). Naturally Occurring Bioactive compounds. Elsevier, Amsterdam: 502 pp.
Sadasivam S, Manikam A (1992). Biochemical methods (Second edition). New Age International (P) Limited Publishers, New Delhi and TNAU, Coimbatore, India. 256p
Saravanan T, Muthusamy M (2006). Influence of *Fusarium oxysporum* f.sp. *cubense* (E.F. Smith) Snyder and Hansen on 2,4-Diacetyl phloroglucinol production by *Psudomonas fluorescens* migula in banana rhizosphere. J. Plant Prot. Res. 46:241-254.
Satya VK, Gayathri S, Bhaskaran R, Paranidharan V, Velazhahan R (2007). Induction of systemic resistance to bacterial blight affected by bacterial blight caused by *Xanthomonas campesteris* pv. *malvacearum* in cotton by leaf extract from a medicinal plant Zimmu (*Allium sativum* L. x *Allium cepa* L.). Arch. Phytopathol. Plant Prot. 40:309-322.
Satya VK, Radhajeyalakshmi R, Kavitha K, Paranidharan V, Bhaskaran R, Velazhahan R (2005). *In vitro* antimicrobial activity of Zimmu (*Allium sativum* L. X *Allium cepa* L.) leaf extract. Arch. Phytopathol. Plant Prot. 38:185-192.
Thangavelu R, Ganga Devi P, Gopi M, Mustaffa MM (2013). Management of Eumusae leaf spot disease of banana caused by *Mycosphaerella eumusae* with Zimmu (*Allium sativum* x *Allium cepa*) leaf extract. Crop Prot. 46:100-105.
Thangavelu R, Mustaffa MM (2010). A Potential isolate of *Trichoderma viride* NRCB1and its mass production for the effective management of Fusarium wilt disease in banana. Tree For. Sci. Biotechnol. 4:76-84.
Thangavelu R, Mustaffa MM (2012). Current advances in the Fusarium wilt disease management in banana with emphasis on biological control. In: C. J. Cumagun (ed.), Plant Pathology. InTech. pp. 273-298.
Tiwari S, Singh A (2005). Possibility of using latex extracts of *Nerium indicum* plant for control of predatory fish, *Channa punctatus*. Asia Fish. Sci. 18:161-73.
Tripathi YC, Dwivedi RK (1989). Antifungal activity of alkaloids of *Tiliacora racemosa*. Natl. Acad. Sci. Let. 12: 69-71
Viljoen A (2002). The status of Fusarium wilt (Panama disease) of banana in South Africa. S. Afr. J. Sci. 98:341-344.