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Management of Eumusae leaf spot disease of banana caused by *Mycosphaerella eumusae* with Zimmu (*Allium sativum* × *Allium cepa*) leaf extract

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ABSTRACT

Among extracts of 33 plant species screened against *Mycosphaerella eumusae*, the causal agent of Eumusae leaf spot disease of banana, water extract of *Cassia senna*, Zimmu (Interspecific hybrid between *Allium cepa* × *Allium sativum*) and *Rhincanthus nasutus* provided 100% inhibition of spore germination and 1.7–2.0 cm zone of inhibition of mycelial growth under *in vitro* conditions. The maximum efficacy of mycelial inhibition was observed with Zimmu leaf extract. When Zimmu leaf extract was tested at different concentrations (5, 10, 25, 50 and 100% w/v), all tested concentrations provided complete inhibition of mycelial growth of the pathogen. The field evaluation of Zimmu leaf extract at different concentrations in cv. Grand Naine showed that the application of the water extract of Zimmu leaf at 50% concentration (w/v) provided 55% reduction of disease severity compared to the unsprayed control. Besides, the application of Zimmu leaf extract increased the value of youngest leaf spotted-0 (up to 60.5%) as well as increased the yield of banana (up to 46.8%) as compared to control. The effect of Zimmu in increasing the value of YLS-0 and the bunch yield was comparable with the chemical fungicide Propiconazole 25% EC (0.1%). Thin layer chromatography (TLC) analysis showed that among different major compounds, two lipid compounds (LP-B1 and LP-B2) extracted using methanol had the ability to inhibit *M. eumusae* growth (0.7–1.5 cm zone of inhibition). The gas chromatography–mass spectrometry (GC–MS) analysis of lipid bands revealed the presence of six different lipid compounds, which may be responsible for the growth inhibition of the pathogen. Since the application of water extract of Zimmu was found to be not only effective in controlling the leaf spot disease severity but also increased the number of green leaves and yield of banana fruits, the Zimmu extract can be used effectively in integrated disease management of Eumusae leaf spot disease for enhancing banana production in an ecologically sustainable manner.

1. Introduction

Leaf spot caused by *Mycosphaerella* spp. is considered as a serious threat of banana production worldwide (Arzanlou et al., 2008). This foliar disease causes major economic losses (Ploetz, 2000) and occurs in almost all banana growing regions in the world. In India, the disease has a serious impact in major banana growing states like Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, Maharashtra (Jalgaon district), Gujarat, West Bengal and North-eastern hilly states of India (Anonymous, 2011a). Although previously the causal agent of leaf spot disease was not confirmed in India, recent studies using both morphological and molecular approaches revealed that the majority of 107 leaf spot infected samples (>99%) collected from different varieties and geographic locations showed the presence of *M. eumusae* Crous & X. Mour, which is known as Eumusae leaf spot disease (Anonymous, 2011b). Carlier et al. (2000) has also confirmed the presence of *M. eumusae* causing Septoria leaf spot disease in the Cavendish cv. Grand Naine in southern India.

The disease attacks leaves, resulting in the reduction of the leaf area, thus decreasing the photosynthetic capacity and affecting the growth and productivity of the plants (Arzanlou et al., 2008). This disease affects not only the leaves, but also bunch weight and fruit quality due to premature bunch maturation (Corbana, 1996) and results in yield losses estimated at 40–76% (Meredith, 1970; Ploetz, 2000; Ngongo, 2002).

Eumusae leaf spot disease can be managed effectively by the application of fungicides such as dithiocarbamates, benzimidazoles, azoles and strobilurins. But the use of these fungicides had increased the production cost up to 40–45% (Ngongo, 2002), which has highly affected small and medium banana producers (Cordeiro et al., 1998).

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Besides, the overuse of fungicides results in high accumulation of chemical residues in banana plants and in the environment, which is not only a serious public concern but may also cause development of resistance to fungicides (Bharathi et al., 2004; Hermanto et al., 2010). Therefore, it is imperative to develop eco-friendly methods for reducing fungicide usage in the management of banana leaf spot diseases and also for sustainable and cost-effective banana production. Among the non-chemical and environmentally friendly methods available, the use of natural plant products in controlling plant diseases is a promising strategy (Daayf et al., 1995). Several authors have reported the use of plant extracts for the management of diseases in crops (Kagale et al., 2004). Besides, these plant extracts have other added advantages such as target specificity, biodegradability and contain many active ingredients in low concentrations, thus possess bio-pesticidal activity against several insect pests and pathogens (Kalaycioglu et al., 1997; Harish et al., 2008). Kagale et al. (2004) reported that the methanolic extract of *Datura metel* exhibited 85% reduction of mycelial growth of *Rhizoctonia solani*.

In this study, we have assessed the ability to manage this serious leaf spot disease using various plant extracts, as an alternative non-chemical integrated disease management (IDM) method. The main objectives of this study were i) to screen various plant extracts against *M. eumusae* under *in vitro* conditions, ii) to study the effectiveness of leaf extracts in reducing the leaf spot disease severity and increasing the yield parameters of banana under field conditions and iii) to identify the antifungal constituents present in the effective leaf extract using thin layer chromatography (TLC) and gas chromatography–mass spectrometry (GC–MS) analyses.

2. Materials and methods

2.1. Isolation and maintenance of fungal culture

The pathogenic fungus, *M. eumusae* was isolated from the leaf spot affected samples collected from cv. Grand Naine using potato dextrose agar (PDA) medium using the spore pick method under laboratory conditions. The culture plates were maintained at 25 °C for seven days. Mycelial culture from the plate was then transferred to PDA slants and maintained at 25 °C (Ainsworth, 1971) till further use.

2.2. Collection and preparation of plant material for in vitro evaluation

In total 33 plant species were collected from the herbal garden maintained at the Horticulture College and Research Institute, Periakulam, Tamil Nadu, India (Table 1). The fresh leaf samples collected from all these 33 plant species were brought to the laboratory immediately, washed in running tap water to remove dust if any, ground in a sterile mortar and pestle by adding 10 ml of sterile distilled water for every 10 g of leaf tissue (100% concentration) and finally filtered through two layers of cheesecloth. The extract was then centrifuged at 10,000 × *g* for 20 min and the supernatant was transferred to a fresh tube. The extract was then sterilized using 0.2 μm disposable syringe filters for further use.

2.3. Spore germination assay

A conidial suspension of *M. eumusae* was prepared by grinding 15-day-old culture with distilled water using a mortar and pestle. The spore suspension was then filtered through two layers of cheesecloth and the filtrates containing the spores were adjusted to 4×10^6 spores ml^{-1} using a haemocytometer. A 100 μl volume of conidial suspension of the pathogen and 100 μl of various leaf extracts were placed individually in the concavity glass slides and mixed thoroughly. The concavity glass slides were kept in separate Petri dishes on a glass bridge chamber and incubated at 25 °C. The spore suspension of the pathogen in sterile distilled water alone was served as a control. For each plant extract three replications were maintained and the experiment was conducted in a completely randomized block design. The germination of the spores was observed after 96 h. Conidia were considered to have germinated if the germ tubes were equal to or longer than the length of the conidia of the respective pathogen (Khan et al., 2001).

The percent of inhibition was calculated using the formula: Percent inhibition $= C - T/C \times 100$, where, $C =$ number of spores germinated in control (average of 10 microscopic fields); $T =$ number of spores germinated in treated (average of 10 microscopic fields).

Table 1
Effect of plant extracts on the inhibition of spore germination and mycelial growth of *M. eumusae*.

Plant species (100% conc.)	Percent inhibition of spore germination	Zone of inhibition of mycelial growth of *M. eumusae* (mm)
Polyagala sinensis	20h	0a
Aadathoda vassica	23j	0a
Pisonia macrophylla	32n	0a
Cassia senna	100r	18c
Boerhaevia diffusa	15d	0a
Alpinia galanga	17e	0a
Caesalpinia sappan	28l	0a
Vitex negundo	19g	0a
Eclipta prostata	38q	0a
Rhinacanthus nasutus	100r	17b
Occimum sanctum	19g	0a
Andrographis paniculata	35p	0a
Tephrosia purpurea	18f	0a
Plumbaga zeglnica	27k	0a
Centella asiatica	0 a	0a
Eclipta prostrata	0a	0a
Hibiscus rosasinensis	6b	0a
Cathranthes roseus	0 a	0a
Allium cepa L. ×	100r	20d
Allium sativum L. (Zimmu)	0a	0a
Muccna pruriens	0a	0a
Costus igneus	21i	0a
Ocimum tenuiflorum	0a	0a
Acalypha indica	0a	0a
Bacopa monnieri	7c	0a
Lippia nodiflora	0a	0a
Gymnema sylvestre	0a	0a
Cissus quandrangularis	30m	0a
Acalypha spp.	0a	0a
Lawsonia mermis	0a	0a
Vitex spp.	34o	0a
Withania sominifera	0a	0a
Cassia alata	0a	0a
Vitex spp.	0a	0a
Control (water alone)	–	–

Values are the mean of 3 replications. Values followed by same letter in the column do not differ statistically ($P < 0.05$; Duncan's test).

2.4. Fungal growth inhibition assay

Antifungal activity of leaf extracts against *M. eumusae* was determined by fungal growth inhibition assay (agar well diffusion method) under sterile conditions. A pathogen seeded medium was prepared by adding five milliliters of spore suspension (4×10^6 spores ml^{-1}) to 150 ml of molten PDA medium supplemented with 100 μg ml^{-1} streptomycin. The media was then poured into Petri dishes. A 7 mm diameter well was cut at the centre of the plate containing seeded medium using a cork borer. Two hundred microlitres of undiluted filter sterilized leaf extracts was added to each well and incubated at room temperature (25 ± 2 °C). PDA medium containing the pathogen alone served as control. The zone of mycelial inhibition was measured

after 15 days of incubation and inhibition percentage of fungal growth in relation to the control treatment was calculated.

Further, the Zimmu leaf extract, which was found very effective against *M. eumusae* pathogen compared to other plant extracts, was tested at various concentrations (5, 10, 25, 50 and 100% w/v) by poison food technique (Nene and Thapilyal, 2000). The Zimmu extract prepared as above was added to 15 ml of molten PDA medium to a final concentration of 5, 10, 25, 50 and 100% (v/v) poured into sterile Petri plates and allowed to solidify. For proper solidification of the medium, the water required for the medium preparation was adjusted depending on the amount of leaf extract added. One hundred microlitre of spore suspension (4×10^6 spores ml^{-1}) was added to each plate and spread evenly over the medium using sterile glass rods. The plates were then incubated at 25 °C for 15 days before being observed for mycelial growth.

2.5. Field evaluation of Zimmu leaf extract against Eumusae leaf spot disease

A field experiment was conducted in a known hot spot area in the Theni district of Tamil Nadu, India on cv. Grand Naine to test the efficacy of Zimmu leaf extract against Eumusae leaf spot disease. Zimmu leaf extract was prepared at concentrations of 10%, 25% and 50% (w/v) in water and sprayed four times at 20 days interval over the banana plants immediately after the onset of the disease during the vegetative stage of the crop. The fungicide Propiconazole 25% EC (0.1%) and water sprays (untreated control) were also included for comparison. The experiment was conducted in a randomized block design (RBD). For each treatment, totally 10 replicates of 20 plants/replicate were used. The disease severity index was calculated as per Gauhl's modification of Stover's severity scoring system (Gauhl et al., 1995; Carlier et al., 2002) using the formula: Infection index $= \sum nb/(N-1)T \times 100$ where, $n =$ number of leaves in each grade, $b =$ grade, $N =$ number of grades used in the scale and $T =$ total number of leaves scored.

2.6. Isolation and characterization of principle compounds

Ten grams of Zimmu leaves were homogenized in 100 ml of methanol and the leaf homogenate filtered through two layers of cheesecloth. The filtrate was centrifuged at 7500 × *g* for 20 min and the clear supernatant retained. The methanol was evaporated using Laborota 4000 Heidolph at 40 °C and the residue was dissolved in 2 ml of distilled water.

2.6.1. Thin layer chromatography

Thin layer chromatography analysis was carried out on silica gel (20 × 20 cm; 0.25 mm thick; E-Merck). The plates were activated at 100 °C for 30 min and cooled prior to spotting with 5 ml of methanol extract of Zimmu. Samples were loaded at 1.5 cm intervals approximately 2 cm from the bottom of the plate. In TLC, different compounds such as phenols, amino acids and lipids were separated, developed and detected using respective solvent system and developer and the Rf values were calculated (Sadasivam and Manickam, 1992). Following compounds showing different Rf values were gently separated from the silica gel plate and eluted in their respective solvents before testing for their ability to inhibit the growth of *M. eumusae* under *in vitro* conditions. In this *in vitro* test, for each band, five replications were maintained and the experiment was conducted in a completely randomized block design.

2.6.2. In vitro testing of principle compounds

The compounds extracted from TLC plates were tested individually against *M. eumusae* by the filter paper disc method. For this, four sterilized filter paper discs of 3 mm diameter were spotted with 200 µl of compounds of Zimmu leaf extract and placed at the centre of the Petri plates containing PDA medium seeded with *M. eumusae* pathogen and incubated at room temperature (25 °C) for 15 days. Sterilized filter paper discs spotted with respective solvents alone served as control. The efficacy of the compounds was assessed by measuring the inhibition zone and the percent reduction over the control was calculated.

2.6.3. Analysis of antifungal compound through gas chromatography–mass spectrometry

The lipid constituents of Zimmu leaf extract which have only shown mycelial inhibition of the pathogen were determined using a GC Clarus 500 Perkin Elmer Gas chromatography which was equipped with a mass detector – Turbo mass gold containing a Elite-

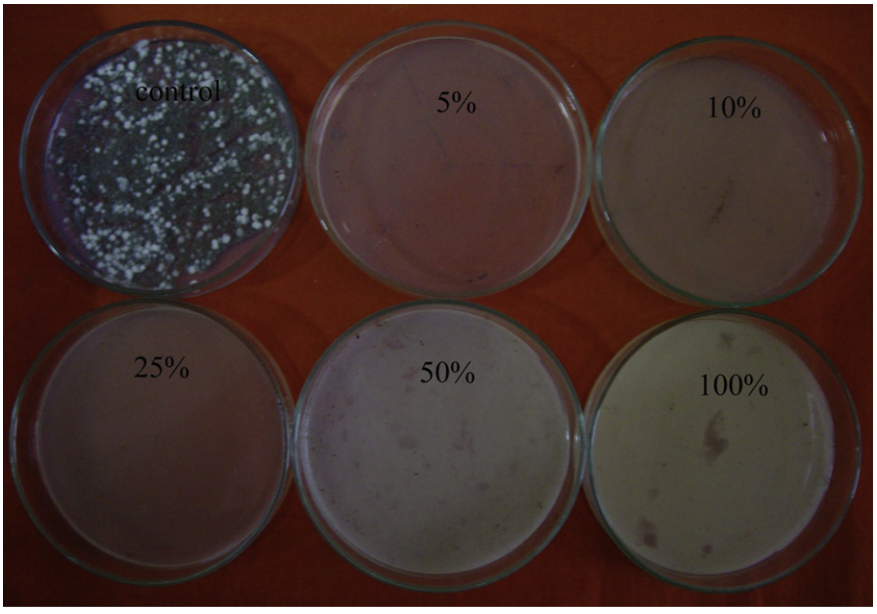

Fig. 1. Effect of various concentrations of Zimmu leaf extract on the mycelial growth of *M. eumusae* under *in vitro* condition (Poison food technique).

Table 2
Field evaluation of Zimmu leaf extract at various concentrations against Eumusae leaf spot disease of banana in cv. Grand Naine.

Treatments	Vegetative stage		Flowering stage		Harvesting stage	
	Disease severity	Reduction over control (%)	Disease severity	Reduction over control (%)	Disease severity	Reduction over control (%)
Zimmu 10%	41.3b	17.9	56.3b	9.8	53.0bc	24.4
Zimmu 25%	30.6c	39.2	50.8c	18.6	54.9b	21.7
Zimmu 50%	22.7d	54.9	32.9d	47.3	49.0c	30.1
Propiconazole 25% EC (0.1%)	13.8e	72.6	34.7d	44.4	38.0d	45.8
Control	50.3a	–	62.4a	–	70.1a	–

Values are the mean of 10 replications. Values followed by same letter in the column do not differ statistically ($P < 0.05$; Duncan's test).

5MS (5% Diphenyl/95% Dimethyl Poly Siloxane), 30 × 0.25 mm ID × 0.25 μm df. The following conditions were employed such as Carrier gas – helium (1 ml min^{-1}), 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 μl 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, year-2005). Software used for GC–MS was Turbo mass-5.2. This work was carried out at Indian Institute of Crop Processing Technology (IICPT), Thanjavur, Tamil Nadu, India.

2.7. Statistical analysis

The data of effect of treatments on the mycelial growth and inhibition of spore germination of pathogen, disease severity and yield parameters were analysed 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 and the leaf spot disease severity index were arcsine transformed before undergoing statistical analysis (Gomez and Gomez, 1984). The package used for analysis was IRRISTAT Version 92.1 developed by the Biometrics Unit of the International Rice Research Institute, The Philippines.

3. Results

3.1. Spore germination and fungal growth inhibition assay

In total, 33 plant species were collected and evaluated for the inhibition of spore germination and fungal growth. Of these, plant extracts of *Cassia senna*, Zimmu and *Rhincanthus nasutus* provided 100% inhibition of conidial germination whereas the remaining plant extracts and control did not inhibit conidial germination. For the fungal growth inhibition assay of all the plant extracts, Zimmu leaf extract produced the largest zone of inhibition of 20 mm followed by *C. senna* (18 mm) and *R. nasutus* (17 mm). The remaining plant extracts and control did not produce any zone of inhibition (Table 1). Zimmu was alone further evaluated at different concentrations (5, 10, 25, 50 and 100% w/v) for the inhibition of mycelial growth using the poison food technique. The result showed that Zimmu leaf extract completely inhibited the mycelial growth of *M. eumusae* at all concentrations tested (Fig. 1).

3.2. Field evaluation of Zimmu leaf extract against Eumusae leaf spot disease

Field evaluation of Zimmu leaf extract at 5, 10, 25 and 50% concentrations with Propiconazole 25% EC (0.1%) was carried out in cv. Grand Naine. Disease severity index observed at three different stages of crop growth specifically vegetative, flowering and harvest stages. The results indicated that the maximum reduction of leaf spot disease severity was recorded in the Propiconazole 25% EC (0.1%) sprayed plants (up to 72.6%), followed by Zimmu leaf extract sprayed plants which produced 54.9% reduction at 50% concentration, as compared to untreated control plants (Table 2). Similarly, the observation on youngest leaf spotted (YLS)–0 indicated that the maximum increase of 84.2% was obtained with Propiconazole 25% EC (0.1%) treated banana plants followed by 60.5% increase in the Zimmu leaf extract (at 50% concentration) sprayed plants when compared to untreated control plants. However, the effect of fungicide on the reduction of disease severity and increase in YLS-0 value was on par with the spraying of Zimmu leaf extract at 50% concentration at flowering stage (Table 3). It was also observed that, the spraying of fungicide Propiconazole 25% EC (0.1%) and Zimmu leaf extract at different concentrations increased the yield parameters such as number of fingers (up to 25.7%), number of hands (up to 28.6%) and bunch weight (up to 46.8%) significantly as compared to control plants. It was interesting to note that the effect of the fungicide in increasing the yield parameters was comparable with the effect of Zimmu leaf extract at 50% concentration (Table 4).

3.3. Isolation, purification and identification of principle compounds through TLC and GC–MS analysis

Since the Zimmu leaf extract alone had recorded the highest antifungal activity and also had the ability to control Eumusae leaf spot disease severity, further studies were performed to determine the nature of inhibitory compound present. In TLC, three different compounds, specifically phenols, amino acids and lipids, were separated individually resulting in the extraction of seven different compounds of different Rf values. *In vitro* bioassay carried out against *M. eumusae* by filter paper disc method indicated that only two lipid compounds (LP-B1 and LP-B2) with the Rf value 0.83 & 0.90 showed maximum inhibition of mycelial growth of the pathogen (zone of inhibition of 0.7 cm and 1.0 cm respectively) (Table 5). Further, the GC–MS analysis of these two lipid compounds revealed the presence of six and five different compounds, respectively (Table 6).

Table 3
Field evaluation of Zimmu leaf extract at various concentrations on youngest leaf spotted (YLS-0) in cv. Grand Naine.

Treatments	Vegetative stage		Flowering stage		Harvesting stage	
	YLS 0%	Increase over control (%)	YLS 0%	Increase over control (%)	YLS 0%	Increase over control (%)
Zimmu 10%	7.8a	2.6	7.5c	10.3	6.5c	1.6
Zimmu 25%	10.0bc	31.6	7.6c	11.8	7.2b	12.5
Zimmu 50%	12.2b	60.5	9.8a	44.1	7.3b	14.1
Propiconazole 25% EC (0.1%)	14.0d	84.2	8.9b	30.9	8.7a	35.9
Control	7.6c	–	6.8c	–	6.4c	–

Values are the mean of 10 replications. Values followed by same letter in the column do not differ statistically ($P < 0.05$; Duncan's test).

Table 4
Effect of Zimmu leaf extract on the different yield parameters of banana in cv. Grand Naine.

Treatments	No. of fingers	Increase over control (%)	No. of hands	Increase over control (%)	Bunch weight (kg)	Increase over control (%)
Zimmu 10%	14.8bc	5.7	7.6b	8.6	23.8bc	1.3
Zimmu 25%	15.1bc	7.9	8.0b	14.3	28.0ab	19.2
Zimmu 50%	17.6a	25.7	8.8a	25.7	34.5a	46.8
Propiconazole 25% EC (0.1%)	16.6ab	18.6	9.0a	28.6	34.0a	44.7
Control	14.0c	–	7.0b	–	23.5c	–

Values are the mean of 10 replications. Values followed by same letter in the column do not differ statistically ($P < 0.05$; Duncan's test).

4. Discussion

The dependence on fungicides for controlling banana leaf spot disease has led to a problem of fungicide resistance. The fungi *Mycosphaerella fijiensis* (Smith, 1988) and *Mycosphaerella musicola* (Knight et al., 2002) have become resistant to fungicides such as benimidazoles and strobilurins (azoxystrobin) which were previously reported as highly effective in suppressing the leaf spot diseases (Smith, 1988; Knight et al., 2002). Also, overuse of these fungicides might result in leaving chemical residues in banana plants and their environments, which will become a serious public concern. Therefore, for the sustainable banana production, it is necessary to develop eco-friendly methods, which will reduce fungicide usage drastically in the management of banana leaf spot diseases. Among different non-chemical methods of disease management, the use of natural plant products in controlling plant diseases is a promising environmentally friendly strategy (Daayf et al., 1995; Akila et al., 2011). Therefore, in the present study, we made an attempt to find an effective plant species for the management of Eumusae leaf spot disease of banana, which is becoming very serious in different banana growing states of India.

Among 33 plant species screened, the medicinal plant Zimmu (Interspecific hybrid between *Allium cepa* × *Allium sativum*) showed complete inhibition of mycelial growth and spore germination at all concentrations tested under *in vitro* conditions. Similarly, Satya et al. (2007) reported that the leaf extract of Zimmu was effective in inhibiting the growth of several agronomically important fungal and bacterial pathogens including *Aspergillus flavus*, *Curvularia lunata*, *Alternaria solani*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas campestris* pv. *malvacearum* and *Xanthomonas axonopodis* pv. *citri*. Besides, Satya et al. (2005) also reported that among the plant extracts tested, the leaf extract of Zimmu and Snake wood (*Strychnos nuxvomica* Linn.) showed the highest antifungal activity against *R. solani*. The crude extract of *Eucalyptus citriodora* also inhibited the mycelial growth of *R. solani*, *Sclerotium rolfsii*, *Phytophthora* spp., *Alternaria alternata* and *Colletotrichum graminicola* (Schwan-Estrada et al., 1998). A possible explanation for the effective inhibition of mycelial growth of the above said 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 Zimmu leaf extract, which showed effective inhibition of pathogen *M. eumusae* under *in vitro* conditions, was further evaluated for its efficacy in suppressing Eumusae leaf spot disease under field conditions in cv. Grand Naine. The result of the field study showed that the Zimmu leaf extract sprayed four times at a 20-day interval at the vegetative stage immediately after the onset of the disease resulted in 55% reduction of disease severity under field condition. Besides, the applications also increased the value of YLS-0 (which indicates the number of green leaves indirectly) by 60.5% and the yield by 46.8% as compared to untreated control plants. It is very important to note that the effect of Zimmu leaf extract in suppressing the leaf spot disease severity and increasing the number of green leaves was comparable with the fungicide Propioconazole 25% EC (0.1%) at flowering stage and slightly better than the fungicide Propioconazole 25% EC (0.1%) for yield parameters such as the number of fingers and bunch weight. Several authors have also reported the efficacy of plant products in reducing the disease severity caused by different pathogens in different crops. Satya et al. (2007) have also reported that the application of 10% concentration of Zimmu leaf extract recorded maximum reduction in the number of lesions (73%) in cotton plants affected by bacterial blight caused by *Xanthomonas campesteris* pv. *malvacearum* as compared to untreated control plants. Foliar application of 50 EC formulation of Zimmu extract at 3 ml l^{-1} concentration on 60, 75 and 90 days after sowing significantly increased the grain hardness and reduced the incidence of grain mould under field conditions (Karthikeyan et al., 2007). Similarly, Singh et al. (1995) demonstrated that ajoene, a constituent of garlic (*A. sativum*), significantly controlled powdery mildew of pea under glasshouse conditions. Bowers and Locke (2004) showed that treatment of soil infested with *Phytophthora nicotianae* with 10% aqueous emulsions of pepper extract–mustard oil formulation and cassia extract formulations and synthetic cinnamon oil formulation significantly reduced the population densities of *P. nicotianae* and suppressed Phytophthora blight in Periwinkle up to 96.7% compared with control.

In order to identify the principle compound present in the Zimmu leaf extract, the extract was subject to solvent extraction, purification by TLC followed by GC–MS analysis. From these analyses, two lipid compounds exhibiting significant effect on the mycelial growth and spore germination of the pathogen *M. eumusae* were purified and the GC–MS analysis of these compounds indicated the presence of up to six different components and the further research work on these components is in progress for the wide use of this plant extract. Muthukumar et al. (2010) also reported the presence of 22 compounds in Zimmu leaf extract and opined that among these, the lipid compound n-Hexadecanoic acid may be responsible for the inhibition of the growth of test pathogen *Pythium aphanidermatum*. Conversely, Satya et al. (2005) reported that the phenolic compounds

Table 5
Efficacy of purified compounds of Zimmu leaf extract on the mycelial growth of *M. eumusae*.

Compounds	Solvent system	Spray reagents	Colour of the spot	Zone of inhibition (mm)	
				Band 1	Band 2
Phenolic compounds	Acetic acid/ Chloroform (1:9)	Folins-Ciocalteu reagent/Water (1:1) followed by spraying with Na_2Co_3	Blue	0b	0b
Amino acids	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	0b	0b
Lipids	Chloroform/Methanol/ Water (65:25:4)	50%H_2So_4 and heat the plate for 20 min at 100 °C	Violet	0.7a	1.0a

Values are the mean of 3 replications. Values followed by same letter in the column do not differ statistically ($P < 0.05$; Duncan's test).

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

Retention time	Name of the components	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.

having Rf value of 0.65 and 0.90 eluted from the Zimmu leaf extract by preparative TLC exhibited strong antifungal activity against *R. solani*. The difference in principle compound isolation might be due to the test pathogens involved in the study.

In the present study, an effective plant species Zimmu (*A. cepa* × *A. sativum*) was identified for the management of Eumusae leaf spot disease of banana. The application of Zimmu also increased the value of YLS-0 i.e. the number of green leaves as well as the bunch yield considerably which was comparable with the effect of normally recommended fungicide Propiconazole 25% EC (0.1%). In general, it is well known that for the sustained management of leaf spot diseases of banana, an integrated approach with minimum application of fungicide as well as use of fungicides with different mode of action is very important for the lower cost of control, lower risk of fungicide resistance and lower environmental negative impact. In this context, the Zimmu leaf extract can integrate very well with IDM approaches for the effective and sustained management of Eumusae leaf spot disease, which in turn paves a way for enhancing banana production in an ecologically sustainable manner.

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