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Antimicrobial activity of medicinal plants and induction of defense related compounds in banana fruits cv. Robusta against crown rot pathogens

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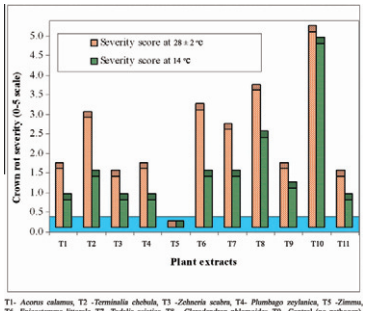
HIGHLIGHTS

- Aqueous leaf extract of Zimmu exhibited complete inhibition of crown rot pathogens under *in vitro*.
- Zimmu leaf extract resulted in complete control of crown rot disease up to 35 days in cold storage.
- This treatment did not alter the organoleptic properties of banana fruits.
- Bananas treated with Zimmu leaf extract resulted in accumulation of defense related compounds.

GRAPHICAL ABSTRACT



T1- *Acorus calamus*, T2 -*Terminalia chebula*, T3 -*Zehneria scabra*, T4- *Plumbago zeylanica*, T5 -Zimmu, T6- *Enicostemma littorale*, T7 -*Todalia asiatica*, T8 - *Clerodendron phlomoides*, T9 -Control (no pathogen), T10 - pathogen alone (*L. theobromae* + *C. musae*), T11- Benomyl (0.1%).
Crown rot severity (0-5 scale, where 0- apparently no disease. 1, 2, 3, 4-rot progression of 25%, 50%, 75% and 100% respectively and 5- rot extended up to the fruit pedicel).

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ABSTRACT

A total of 72 plant extracts were tested *in vitro* for their ability to inhibit the mycelial growth of *Lasiodiplodia theobromae* and *Colletotrichum musae* the causal agents of crown rot disease of banana. The results showed that the leaf extract of Zimmu (an interspecific hybrid of *Allium cepa* L. × *Allium sativum* L.) and tuber extract of *Zehneria scabra* recorded maximum inhibition of mycelial growth and spore germination of both the test pathogens. The dipping of banana fruits in Zimmu leaf extract at 25% conc. exhibited 100% inhibition of crown rot disease in cold storage (14 °C) up to 35 days and increased the shelf life to 64 days. However, at room storage (28 ± 2 °C), the same treatment exhibited 86% inhibition of crown rot disease up to 12 days. It was found that the treatment of banana fruits with Zimmu leaf extract did not alter the organoleptic properties of banana. The biochemical analysis of banana fruits treated with Zimmu leaf extract showed significant increase in phenylalanine ammonia-lyase (PAL), chitinase and β-1,3-glucanase activities and enhanced accumulation of phenolic compounds compared to other treatments. These findings suggest that the effect of Zimmu leaf extract on crown rot disease may be associated with the direct fungi toxic property against the test pathogens and elicitation of defense related compounds in banana fruits.

1. Introduction

Banana is a perishable fruit and the fruit quality deteriorates very rapidly after harvest. The short storage life is the major problem associated with the export of banana over long distances. Postharvest diseases, especially crown rot caused by complex of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl, *Colletotrichum musae* (Berk and Curtis) Arx and *Fusarium* spp., greatly reduces the storage life of bananas in almost all banana-producing countries (Paull et al., 1998). Crown rot disease symptoms first appear on the cut surface of the crescent-shaped crown and the infected tissues become black and soft. Grayish-white hyphal growth appears on the surface of the decaying tissue. Further, this disease decreases the quality of bananas, with damage and drop of fingers.

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Our earlier study showed that in India, *L. theobromae* and *C. musae* are the major pathogens causing crown rot disease (Thangavelu et al., 2007). Similarly the fungi *L. theobromae* and *C. musae* have been reported to be the major pathogens associated with the cause of the crown rot disease in majority of banana producing countries (Anthony et al., 2003; Haque et al., 2003). However, little research has been carried out on the management of crown rot disease caused by this pathogen complex. Crown rot disease is often controlled commercially by postharvest treatment, which involves submerging clusters of banana in solutions of benomyl or thiobendazole (TBZ) or imazalil (Perera and Karunaratne, 2001) These fungicides have been used in postharvest fruit disease control for more than two decades. Due to this intensive practice, the pathogens have developed resistance to these fungicides (Holmes and Eckert, 1999; Mari et al., 2003). Continued use of the fungicides can also cause serious problems in human health (Norman, 1988) and the environment (Bautista-Banos et al., 2006). The United States of America Environmental Protection Agency has classified benomyl as a possible human carcinogen, which can also act as a chronic and as a reproductive toxicant (http://www.pan-uk.org/pestnews/actives/benomyl.htm).

Worldwide 'organically grown' fruit, which has not been treated with fungicide, is becoming popular among consumers (De Costa and Erabadupitiya, 2005). Under these circumstances, an alternative method of controlling crown rot disease without the use of synthetic chemicals is urgently needed. Among the safer alternatives to synthetics, use of plant products has attracted researchers for the management of diseases of several fruits (Bautista-Banos et al., 2002; Win et al., 2007). In our previous study with plant essential oils, treatment with *Ocimum basilicum* oil extended the shelf life of banana to 48 days in cold storage (14 °C) (Sangeetha et al., 2010). Cinnamon extracts have been shown to possess antifungal activity against the anthracnose and crown rot pathogens infecting banana fruits and spraying them on Embul banana prior to storage, controlled crown rot and extended shelf life (Ranasinghe et al., 2003). The active principles present in the plant products may act on the plant pathogen directly, or induce systemic resistance in host plants resulting in reduction of disease development.

Although harvested fruit possesses constitutive and inducible defense mechanisms which enable them to ward off infection, this potential has not received enough attention. Generally, upon infection or treatment with elicitors, plant tissues can respond by activating a highly coordinated biochemical and structural defense system that helps to ward off pathogens (Ryalls et al., 1996; Sticher et al., 1997). Chitinase, β-1,3-glucanase and phenylalanine ammonia-lyase (PAL) are known to be involved in plant disease resistance (Anand et al., 2007; Zhao et al., 2008). Mangoes treated with naturally derived chitosan as a postharvest treatment induced higher chitinase and β-1,3-glucanase level than the control and it partially reduced the onset of anthracnose disease in treated mangoes (Jitareerat et al., 2007). However, there are few reports on induction of resistance by plant extracts or on its inhibitory effects against complex fungal pathogens in banana during storage. Moreover, reports on the effective management of crown rot disease with plant products, particularly in Cavendish banana which is the predominant commercial variety worldwide, are also lacking.

In this study, we have attempted to bridge these research gaps, through the following objectives (i) to screen and identify a potential plant extract for the control of both *L. theobromae* and *C. musae* causing crown rot disease in banana fruit; (ii) to investigate the effect of potential plant extracts on banana fruits stored at different temperatures in reducing crown rot disease and on shelf life of banana fruit; (iii) to study the effect of these plant extracts on physicochemical and organoleptic properties of banana fruits and (iv) to study the effect of application of plant extracts on elicitation of defense compounds in banana fruit.

2. Materials and methods

2.1. Fungal isolation and inoculum preparation

The crown rot fungi *L. theobromae* and *C. musae* were isolated from infected banana fruits of cv. Robusta and their identity confirmed based on the colony morphology and spore characters according to Punithalingam (1976) and Sutton and Waterson (1970), respectively. The single spore culture of these fungi were maintained on PDA (Potato Dextrose Agar) media at 4 °C. Inoculum was prepared by using the conidia of two-week old cultures of these fungi. The conidia were dislodged from the surface of the media by flooding with sterile distilled water and gentle rubbing with a sterile glass rod. The suspensions were filtered through cotton wool to remove mycelial fragments and adjusted to 10^5 conidia/mL with a haemocytometer. Following, all three conidial suspensions were mixed prior to use to get a cocktail of spore suspension. This spore suspension was used for inoculation of banana hands in all the experiments (Win et al., 2007).

2.2. In vitro screening of plant extracts for its antimicrobial activity against test pathogens

Aqueous extracts of 72 different medicinal plants were obtained as described by Kurucheve et al. (1997). The plant species used in this study were listed in Table 1. Briefly, 100 g fresh leaves of selected plant species were collected and washed in distilled water and then in sterile water. Leaves were ground with 100 ml of sterile water (1:1 w/v), using pestle and mortar and filtered through double-layered cheesecloth, followed by centrifugation at 5000 rpm for 10 min at 4 °C. Leaf extracts were filter sterilized using a 0.22 μm Millipore filter. Aqueous extracts from tubers, roots, and nuts were similarly prepared and filter sterilized.

Initial screening of plant extracts was carried out by paper discolor assay and inhibition of mycelial growth was measured (Mauch et al., 1988). The plant products exhibiting $\geqslant$0.5 cm diameter zone of inhibition against the test pathogens were selected. Further evaluation of plant extracts at various concentrations (1%, 5%, 10%, 25% and 50% concentration) against *L. theobromae* and *C. musae* was carried out by 'poisoned food technique'. The required concentrations of plant extracts were prepared by mixing the requisite quantity of plant extract from stock and then making up the volume to 100 ml with previously sterilized and cooled PDA medium in 250 ml conical flasks. For preparing 1%, 5%, 10%, 25% and 50% conc. of plant extract, 1, 5, 10, 25, 50 ml of plant extract from stock was added to 99, 95, 90, 75, 50 ml of PDA medium respectively. After thorough mixing, 15 ml of media was poured into sterilized Petri dishes of 9 cm diameter. Using a 0.8 mm cork borer, fungal plugs were removed from the growing margin of one week old pure cultures of each test pathogen and placed at the center of the test plate. PDA medium without plant extract and with either sterile distilled water or benomyl (0.1%) served as controls. The observation on linear growth of the fungus was recorded after 96 h of incubation. Each treatment was replicated three times with five plates per treatment. Data were expressed as growth rate (mm/day) relative to growth of respective pathogen in control plate by plotting mean colony diameter against time (Thangavelu et al., 2004).

Spore germination assay of test pathogens was carried out with various concentration (1%, 5%, 10%, 25% and 50%) of plant extracts. Conidia were considered to have germinated if the germ tubes were equal to or longer than the length of the conidia itself (Khan et al., 2001).

Table 1
List of plant species and its plant parts used in this study.

Botanical Name	Family	Plant part used
Abutilon indicum G. Don.	Malvaceae	Leaf
Acalypha indica L.	Euphorbiaceae	Leaf
Achyranthes aspera L.	Amaranthaceae	Leaf
Acorus calamus L.	Araceae	Rhizome
Adhatoda vasica Nees.	Acanthaceae	Leaf
Aegle marmelos Corr.	Rutaceae	Leaf
Aerva lanata (L.) Juss.ex Schult.	Amaranthaceae	Leaf
Allium cepa L.	Liliaceae	Bulb
Allium cepa L. × *Allium sativum* L.	Liliaceae	Leaf
Allium sativum L.	Liliaceae	Bulb
Alpinia officinarum Hance	Zingiberaceae	Leaf
Annona sqaumosa L.	Annonaceae	Leaf
Azadirachta indica A. Juss.	Meliaceae	Leaf
Cadaba indica Lam.	Capparaceae	Leaf
Cassia auriculata L.	Caesalpinoideae	Flower
Cassia tora L.	Caesalpiniaceae	Leaf
Catharanthus roseus (L.) G. Don	Apocynaceae	Leaf
Centella asiatica (L.) Urban	Apiaceae	Leaf
Cissus quadrangularis L.	Lauraceae	Stem
Citrus aurantifolia Swingle.	Rutaceae	Leaf
Cleome gynandra L.	Cleomaceae	Leaf
Clerodendron inerme (L.) Gaertn	Verbenaceae	Leaf
Clerodendron phlomoides L.F.	Verbenaceae	Leaf
Clitoria ternatea L.	Fabaceae	Leaf
Colacasia antiquoram Schott.	Araceae	Leaf
Coleus aromaticus Benth.	Labiatae	Leaf
Crossandra undulaefolia Salisb.	Acantahceae	Leaf
Cyanodon dactylon Pers.	Poaceae	Leaf
Cyperus rotundus L.	Cyperaceae	Rhizomes
Datura metel L.	Solanaceae	Leaf
Eclipta alba HassK.	Asteraceae	Leaf
Enicostemma littorale Blume	Gentiaceae	Leaf
Eucalyptus globulus Labill	Myrtaceae	Leaf
Evolvulus alsinoides L.	Solanaceae	Leaf
Gossypium hirsutum L	Malvaceae	Leaf
Heliotropium indicum L.	Boraginaceae	Leaf
Hemidesmus indicus R.Br.	Ascelpiadaceae	Leaf
Hibiscus abelmoschus L.	Malvaeceae	Leaf
Jatropha glandulifera Roxb.	Euphorbiaceae	Leaf
Launaea sarmentosa L.	Compositae	Leaf
Lawsonia inermis L.	Lythraceae	Leaf
Lippia nodiflora Mich.	Verbenaceae	Leaf
Mentha arvensis L.	Labiatae	Leaf
Morinda tinctoria Roxb. *Var. tomentosa*	Rubiaceae	Leaf
Mukia maderaspatana(L.)M.Roemr	Cucurbitaceae	Leaf
Myristica fragrans Houtt.	Myristicaceae	Nut
Ocimum basilicum L.	Labiatae	Leaf
Ocimum canum Sims.	Labiatae	Leaf
Ocimum sanctum L.	Labiatae	Leaf
Passiflora foetida L.	Passifloraceae	Leaf
Phyllanthus emblica L.	Euphorbiaceae	Fruit
Piper betle L.	Piperaceae	Leaf
Plumbago zeylanica L.	Plumbaginaceae	Leaf
Pongamia glabra Vent.	Papilionaceae	Leaf
Rhinacanthus communis Nees.	Acanthaceae	Leaf
Ricinus communis L.	Euphorbiaceae	Leaf
Sesbania cannabina Pers.	Papilionaceae	Leaf
Sesbania grandiflora Pers.	Papilionaceae	Leaf
Solanum melongena L.	Solanaceae	Leaf
Solanum nigrum L.	Solanaceae	Leaf
Solanum pubescens Heyne.Ex Walp.	Solanaceae	Leaf
Solanum torvum Swartz	Solanaceae	Leaf
Solanum trilobatum L.	Solanaceae	Leaf
Solanum xanthocarpum Schard & Wendl.	Solanaceae	Leaf
Terminalia chebula Retz& Willd.	Combretaceae	Nut
Toddalia asiatica (L.) Lam	Rutaceae	Leaf
Tribulus terrestris L.	Zygophyllaceae	Leaf
Tridax procumbens L.	Astraceae	Leaf
Typhonium trilobatum Schott.	Araceae	Leaf
Vetiveria zizanioides Nash.	Graminae	Root
Zehneria scabra(L.f.) Sonder.	Cucurbitaceae	Tuber
Zingiber officinalis Roxb.	Zingiberaceae	Rhizome

2.3. Fruit preparation

Banana cv. Robusta (AAA group) was harvested at 75–80% maturity from a commercial plantation in Thiruchirapalli district, Tamil Nadu, India. Banana hands free of visual defects, with uniform shape and weight were used for all the experiments. The fruits were de-handed along with the crown portion using a sharp knife (surface sterilized with 70% ethanol) and kept until the latex stopped flowing. The hands were thoroughly washed in running tap water to remove dusts; surface sterilized with 70% ethanol and allowed to dry for 6 h at room temperature for use in all experiments. The crown surface of each hand was re-cut using a sterilized knife to make a fresh wound surface for artificial inoculation. The hands were then randomly allocated to treatment groups. Treatments were applied within 24 h of harvest.

2.4. Effect of selected plant extracts on the incidence of crown rot disease of banana

The effect of plant extracts on crown rot disease and total shelf life of banana was evaluated at room temperature (28 ± 2 °C and 72% RH) and in low temperature storage (14 °C and 90% RH) conditions. Among various aqueous plant extracts tested, those which showed effectiveness in suppressing the growth of both test pathogens under *in vitro* conditions were taken to assess their effect on banana fruits in reducing crown rot disease. The plant species used in the *in vivo* study were *Acorus calamus* (root), *Terminalia chebula* (nuts), *Zehneria scabra (tuber), Plumbago zeylanica* (leaf), Zimmu (leaf), *Enicostemma littorale* (leaf), *Toddalia asiatica* (leaf) and *Clerodendron phlomoides* (leaf). Banana hands were artificially inoculated with a cocktail of test pathogens before treatment with plant extracts. For this, a small cavity was made on the crown portion of the banana hand and inoculated with 200 μL cocktail of spore suspension of crown rot pathogens (100 μL of L. *theobromae* and 100 μL of *C. musae*, both at 1×10^5 spores ml^{-1}). The inoculated crown portion was then covered with moist cotton for 6 h to ensure initial germination of pathogens.

The banana fruits were then dipped in individual plant extracts (at 25% concentration) for 5 min and allowed to air dry for 6 h. Banana hands dipped in chemical benomyl (0.1%) served as standard check. Dipping of hands in sterile distilled water alone was kept as an uninoculated control. Another set of fruits inoculated with test pathogens alone (with out any treatment) served as an inoculated control. Finally, the inoculated fruits were wrapped in perforated polythene bags and divided into two groups. One group was incubated at room temperature (28 ± 2 °C) and another group was held in low temperature storage (14 °C and 90% RH) conditions. Four replicates for each treatment and for each storage regime were maintained in a completely randomized block design and all the experiments were repeated twice. Each replicate consisted of one hand with 10 fingers each (Thangavelu et al., 2007).

During the storage period, crown rot severity was evaluated using a scale developed by Finlay and Brown (1993) based on crown rot severity (0–5 scale, where 0 – no disease, 1–4 – rot progression of 25%, 50%, 75% and 100%, respectively and 5–rot extended up to the pedicel), crown color (1–7 scale, where 1 = Green; 2 = Green/Yellow; 3 = Yellow/Green, 4 = Yellow; 5 = Yellow/black; 6 = black/Yellow; 7 = black) and crown texture (0–4 scale, where 0 = Hard, 1 = 0–25%, 2 = 25–50%, 3 = 50–75%, 4 = 75–100% of the crown soft). In addition, the total shelf life of banana fruit (green life and yellow life) was also counted in days from the same set of banana fruits used in above mentioned study. Disease assessment was made on 12th day after treatment for the fruits

incubated at room temperature and on 35th day for the fruits incubated at low temperature storage condition.

2.5. Fruit quality

Peel color, flavor, texture and overall acceptability of banana fruits treated with plant extracts were assessed by a panel of ten people. Five hands of banana were allocated for measurement of fruit quality. The treated fruits were transferred from one month of storage at 14 °C to room temperature for one week, for quality assessment. Each quality parameter mentioned above was scored (Excellent = 9–10; Good = 6–8; Fair = 4–5; Poor = 1–3) as mentioned by Hewage (1996).

2.6. Physico-chemical properties

For assay of weight loss, banana hands were weighed before and after treatment and the difference was expressed as percentage weight loss. Total soluble solids were analyzed from fruit pulp using hand-held refractometer (Brix; 0–32%). Each reading was multiplied by a dilution factor to calculate the actual TSS content (°Brix) of the pulp (Somogyi, 1952).

2.7. Assay for phenol and induced defense-related proteins

The enzyme extract from crown tissue of banana was prepared as described by Hwang et al. (1997). The samples from the crown portion of banana fruits treated with plant extracts of Zimmu, *Z. scabra*, Benomyl and challenge inoculated with *L. theobromae* and *C. musae*, uninoculated control (sterile water alone) and inoculated control (inoculated with test pathogens alone) were collected at various time intervals (0, 1, 2, 3 and 4 days after pathogen inoculation) and quickly frozen in liquid nitrogen and stored at −70° C.

To estimate the phenolic content, 1 g of crown tissue was homogenized with 10 ml of 80% methanol and agitated for 15 min at 70 °C (Zieslin and Ben-Zaken (1993). To one milliliter of this methanolic extract, 5 ml of distilled water and 250 ml of Folin–Ciocalteau reagent (1 N) were added and the solution was kept at 25° C. The intensity of blue color was determined by measuring the absorbance at 725 nm in spectrophotometer (Remi, India).

To assay the chitinase activity, one gram of crown tissue was ground using a pre-chilled pestle and mortar with 0.1 M sodium citrate buffer (pH 5.0) at 4 °C. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was used as a crude enzyme extract for assaying chitinase activity. Similarly, for the extracts of β-1, 3-glucanase and phenylalanine ammonia-lyase (PAL) enzymes, 0. 05 M sodium acetate buffer (pH 5.0) and 0.1 M sodium borate buffer (pH 7.0) at 4° C respectively were used. The changes in the chitinase activity were determined by colorimetric assay described by Boller and Mauch (1988). The PAL activity was determined as the rate of conversion of L-phenylalanine to *trans*-cinnamic acid at 290 nm as described by Dickerson et al. (1984) and β-1, 3-glucanase activity was assayed by the laminarin-dinitrosalicylic acid method (Pan et al., 1991). The results from these assays were graphed using Microsoft Excel.

2.8. Statistical analysis

The data on the effect of treatments on the growth of pathogens, rot severity scores, total shelf life period and activity of defense enzymes were subjected to analysis of variance (ANOVA) and treatment means were compared by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984). The package used for analysis of results was IRRISTAT version 92-1 developed by the International Rice Research Institute, Biometrics Unit, The Philippines.

3. Results

3.1. Effect of plant extracts on the growth of L. theobromae and C. musae

The aqueous extracts (leaves, roots, nuts, tubers) of 72 plant species, belonging to different families were selected and evaluated for their antimicrobial activity against *L. theobromae* and *C. musae* (Table 1). In the paper disc assay study with *L. theobromae*, among 72 plant extracts, the leaf extract of Zimmu, tuber extract of *Z. scabra* (L.f.) Sonder., root extract of *A. calamus* L. and nut extract of *T. chebula* Retz and Willd exhibited maximum inhibition of mycelial growth with 1.8, 1.4, 1.2, 0.7 cm diameter zone of inhibition, respectively, as against 2.0 cm diameter zone of inhibition in benomyl (0.1%) treatment (data not shown). Further evaluation of all botanicals by poisoned food technique revealed that among the botanicals, Zimmu at 25% concentration and *A. calamus*, *Z. scabra* and *T. chebula* at 50% concentration recorded complete inhibition of mycelial growth of *L. thoebromae.* Similarly, when these plant extracts were tested for inhibition of spore germination of *L. theobromae*. Zimmu extract recorded 100% inhibition of spore germination at 25% concentration (Fig 1B); where as the other three plant extracts showed the same result effect at 50% concentration.

However, when these botanicals were tested against *C. musae*, leaf extract of Zimmu, tuber extract of *Z. scabra,* root extract of *E. littorale* Blume, leaf extracts of *P. zeylanica* L., *C. phlomoides* L.F. and *T. asiatica* (L.) Lam showed maximum inhibition of mycelial growth [2.0, 1.2, 1.0, 0.9, 0.8 and 0.5 cm dia. zone of inhibition respectively as against 2.0 cm dia. zone of inhibition in Benomyl (0.1%) treatment (data not shown)]. The further evaluation of these botanicals by poisoned food technique showed that leaf extract of Zimmu and tuber extract of *Z. scabra* at 25% concentration and *E. littorale, P. zeylanica* and *C. phlomoides* at 50% concentration recorded complete inhibition of mycelial growth of *C. musae* (Fig 2A).

The above said six plant extracts from Zimmu, *Z. scabra, E. littorale, P. zeylanica, C. phlomoides* and *T. asiatica* were also tested for their ability to inhibit spore germination of C. *musae*. The results indicated that the leaf extract of Zimmu and tuber extract of *Z. scabra* at 25% concentration were found to completely inhibit spore germination of *C. musae*, while none of the other plant extracts were found to have any inhibitory affect at this concentraion (Fig 2B). However, the leaf extract of *P. zeylanica* exhibited complete inhibition of spore germination only at its 50% conc. compared to other plant extracts.

3.2. Effect of selected plant extracts on crown rot disease of banana friut

Since the experiments were repeated twice with no significant difference ($P > 0.05$) between the experiments, the results were combined and analyzed as a single experiment.

The results of the analyses of the experiments showed that dipping of banana fruits in aqueous leaf extract of Zimmu greatly reduced the crown rot disease of banana under both room and cold storage conditions compared to control and other treatments (Table 2). The reduction of crown rot severity was 86 and 100% at room storage (up to12 days of incubation) and cold storage (up to 35 days of incubation) conditions respectively. It was interesting to note that, the effect of Zimmu in reducing the rot severity

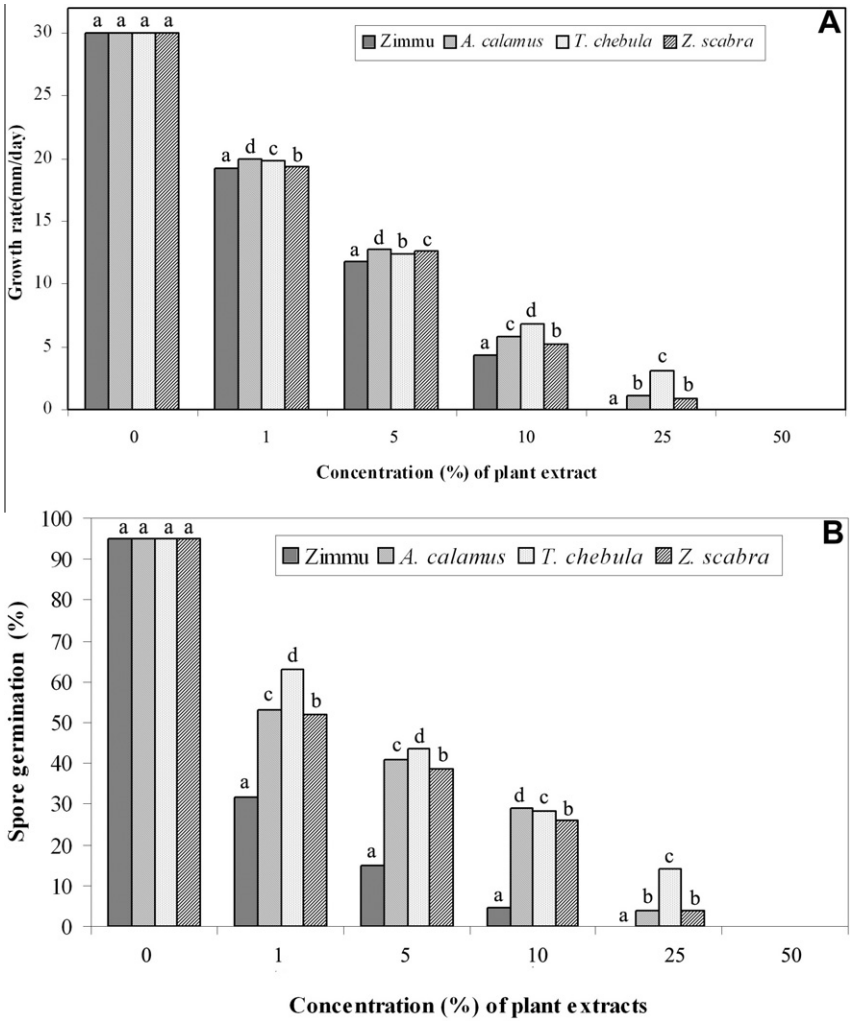

Fig. 1. Effect of various plant extracts at different concentration on mycelial growth (A) and spore germination (B) of *L. thoebromae in vitro* at room temperature (28 ± 2 °C). Colony diameter was measured daily and each column represents the mean of five replicates. Above each column means followed by a common letter are not significantly different at 5% level by DMRT.

of banana under room storage was better than the chemical benomyl (0.1%). Extracts of *A. calamus*, *Z. scabra* and *P. zeylanica* also recorded significant reduction of crown rot disease up to 75% at room temperature (12 days of incubation) and up to 85% at cold storage (35 days of incubation) conditions. In general, the color and texture of crowns from banana hands treated with plant extracts (Zimmu, *A. calamus*, *P. zeylanica* and *Z. scabra*) were similar to that of benomyl treated banana hands under room storage condition.

Next to Zimmu, the effect of *A. calamus*, *Z. scabra* and *P. zeylanica* in reducing the rot severity in cold storage were on par with the effect of benomyl (with the rot score of 0.7) at 35 days after treatment. This was followed by *T. chebula*, *E. littorale* and *T. asiatica* with the crown rot score of 1.3 against the crown rot severity score of 4.7 in pathogen inoculated control. In cold storage, the color of crown in Zimmu, *A. calamus* and *P. zeylanica* treated banana hands were similar to that of benomyl treated hands (score of 4.5), followed by *Z. scabra* with the score of 5.0. However, banana fruits treated with Zimmu and chemical benomyl alone exhibited normal texture of crown followed by *A. calamus* and *Z. scabra* compared to all other plant extracts. In cold storage, considering rot score, color and texture of crown of banana fruit, Zimmu performed best of all the plant extracts. In pathogen alone inoculated banana hands, on 12th and 35th day of observation (room and cold storage respectively) there was complete rotting and blackening of crown region and rotting was extended up to pedicel of the fruit.

3.3. Effect of plant extracts in increasing the total shelf life of banana

Generally application of all the plant extracts significantly extended the total shelf life of banana fruits under both storage conditions, which was ranged from 12.0 to 16.7 d in room temperature storage and 44.3 to 64.0 d in cold storage (Table 3). In room storage, treating the banana fruits with Zimmu leaf extract increased the shelf life of banana fruits to 16–17 days compared to 8.7 d in pathogen only inoculated control while in cold storage it was 64 d compared to 34 d in pathogen alone inoculated control. Under room temperature storage conditions, the effect of Zimmu on increasing the shelf life of banana was on par with fungicide benomyl, however, under cold storage, Zimmu leaf extract performed significantly better than the benomyl. Among the plant extracts, *T. asiatica*, *E. littorale*, and *C. phlomoides* extracts were found to be least effective in extending the shelf life of banana in both the storage conditions. However, treatment of banana fruit with *T. chebula* increased the shelf life to 13.3 d in room storage and 45 d in cold storage condition compared to pathogen alone inoculated control (8.7 and 34 d, respectively).

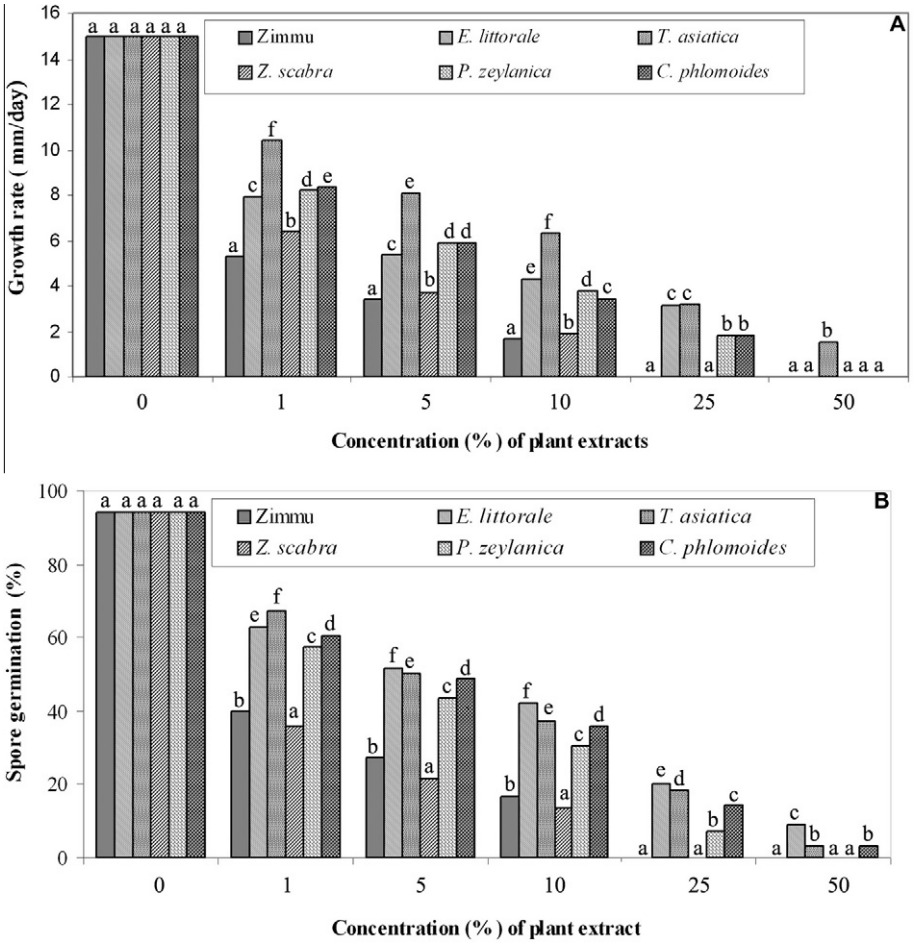

Fig. 2. Effect of various plant extracts at different concentration on mycelial growth (A) and spore germination (B) of *C. musae in vitro* at room temperature (28 ± 2 °C). Colony diameter was measured daily and each is the mean of five replicates. Above each column means followed by a common letter are not significantly different at 5% level by DMRT.

Table 2
Evaluation of medicinal plant extracts against crown rot disease complex caused by *L. theobromae* and *C. musae* in banana cv. Robusta (AAA).

Plant extracts	Severity score at 28 ± 2 °C on 12th day*			Severity score at 14 °C on 35th day*		
	Rot (0–5)	Color (1–7)	Texture (0–4)	Rot (0–5)	Color (1–7)	Texture (0–4)
Acorus calamus	1.5 b	5.0 ab	1.5 ab	0.7 ab	4.5 a	1.0 b
Terminalia chebula	2.8 cd	5.5 bc	2.3 c	1.3 b	5.5 bc	2.5 cd
Zehneria scabra	1.3 b	5.0 ab	2.0 bc	0.7 ab	5.0 ab	1.0 b
Plumbago zeylanica	1.50 b	5.0 ab	1.3 a	0.7 ab	4.5 a	2.3 c
Zimmu	0. 7 a	4.5 a	1.3 a	0.0 a	4.5 a	0.0 a
Enicostemma littorale	3.0 de	5.5 bc	4.0 d	1.3 b	5.8 c	3.0 d
Toddalia asiatica	2.5 c	5.5 bc	2.5 c	1.3 b	5.5 bc	2.5 cd
Clerodendron phlomoides	3.5 ef	6.0 c	2.5 c	2.3 c	5.5 bc	3.0 d
Control (no pathogen)	1.5 b	5.0 ab	1.5 ab	1.0 b	4.5 a	2.5 cd
Pathogen alone (*L. theobromae* + *C. musae*)	5.0 f	6.8 d	4.0 d	4.7 d	6.5 d	3.8 e
Benomyl (0.1%)	1.3 b	4.8 a	1.5 ab	0.7 ab	4.5 a	0.0 a

In a column means followed by a common letter are not significantly different at the 5% level by DMRT.
* Mean of four replications.

3.4. Effect of plant extracts on the organoleptic properties of banana fruits

Dipping of banana fruits in the effective plant extracts viz., Zimmu, tuber *Z. scabra* and chemical benomyl (0.1%) did not alter the organoleptic properties of banana. The overall, the quality of banana hands was improved as a result of these plant extract treatment (Table 4). However, treatment of banana fruits with root extract of *A. calamus* and leaf extract of *P. zeylanica* caused a mild to strong odor to develop which persisted for one month in cold storage. In all these treatments, the fruits remained green in color for up to one month of cold storage and ripened normally under room temperature condition (data not shown), but in the case of pathogen-alone inoculated control, the color broke from green to yellow in a month of cold storage.

Generally, loss of weight of fruits increased in all treatments throughout the storage and there were no significant difference in weight loss by treatment of banana hands with plant extracts

Table 3
Evaluation of plant extracts on total shelf life of banana cv. Robusta (AAA) infected with crown rot pathogens *L. theobromae* and *C. musae*.

Plant extracts	Shelf life at* 28 ± 2 °C (d)	Percent increase in shelf life over pathogen inoculated fruits	Shelf life at* 14 °C (d)	Percent increase in shelf life over pathogen inoculated fruits
A. calamus	14.7b	40.8	50.3d	32.4
T. chebula	13.3cd	34.6	45.0e	24.4
Z. scabra	14.7b	40.8	53.0c	35.8
P. zeylanica	13.7c	36.5	50.0d	32.0
Zimmu	16.7a	47.9	64.0a	46.9
E. littorale	12.3ef	29.2	44.3 fg	23.2
T. asiatica	12.0f	27.5	44.7ef	23.9
C. phlomoidis	12.7def	31.4	44.7ef	23.9
Control (no pathogen)	13.0cde	33.1	44.0 g	22.7
Pathogen alone (*L. theobromae* + *C. musae*)	8.7 g	–	34.0 h	–
Benomyl (0.1%)	16.3a	46.6	60.7b	43.9

In a column means followed by a common letter are not significantly different at the 5% level by DMRT.
* Mean of four replications.

(data not shown). The total soluble solids (TSS) have increased as ripening progressed. The TSS values of all treatments were within the range of 4.9–5.3 (°Brix) after cold storage and before subjected to natural ripening (data not shown).

3.5. Activity of defense-related metabolites of banana fruit treated with extracts of Zimmu and Z. scabra

Phenolic compounds, PAL, chitinase and β-1, 3-glucanase are inducible defense compounds that are involved in plant defense system against invading pathogen. The study on the changes in phenolic content due to the plant extract treatment indicated that the content of phenolics increased gradually up to 2 days after treatment and reached its peak on 3rd day. In general, the phenolic content was significantly higher in Zimmu leaf extract treated banana fruits compared to all other treatments (Fig. 3A). As that of Zimmu, *Z. scabra* treated banana hands had significantly higher phenolic content from 2 to 4 days after treatment compared to benomyl and pathogen alone inoculated control treatments. However, on fourth day, in all treatments including banana hands treated with Zimmu, there was decline in phenolic content.

The PAL activity also increased significantly in banana hands treated with Zimmu from first to fourth day after treatment compared to other treatments. The PAL activity reached its maximum on the third day following treatment and then declined on 4th day (Fig. 3B). In pathogen alone inoculated control, there was a slight increase in the accumulation of phenolic compounds and PAL compared to uninoculated control.

The accumulation of PR-proteins such as chitinase (up to 2.6-fold) and β-1,3-glucanase (up to 2.5-fold) was significantly higher in banana hands treated with leaf extract of Zimmu compared to other treatments (Fig 3C and Fig 3D). Similar to PAL, the activity of chitinase and β-1, 3-glucanase also gradually increased from first day after treatment and reached their peak at the 3rd day after treatment before declining.

4. Discussion

In the present study, we evaluated the antimicrobial activity of leaf extracts of 72 non-host plant species commonly available in South India against crown rot pathogens. Among them, leaf extract of Zimmu alone had completely inhibited the mycelial growth and spore germination of both *L. theobromae* and *C. musae* under *in vitro* conditions at a minimum concentration of 25% compared to other plant extracts. The tuber extract of *Z. scabra* at 25% concentration completely inhibited the mycelial growth and spore germination of *C. musae* but not *L. theobromae*. The other plant extracts viz., *A. calamus, T. chebula*, *E. littorale*, *T. asiatica, P. zeylanica*, *C. phlomoides* showed complete inhibition against any one of the test pathogen even at 50% concentration.

Several earlier reports showed the antifungal and antibacterial effect of Zimmu against various plant pathogens. Satya et al. (2005) demonstrated the antifungal activity of Zimmu leaf extract and snake wood (*Strychnos nux-vomica* Linn.) against *Rhizoctonia solani*. Similarly, Muthukumar et al. (2010) reported that the aqueous leaf extract of Zimmu at 10% concentration gave maximum control of black arm of cotton and chili damping-off respectively. Karthikeyan et al. (2007) also reported that the foliar application of Zimmu formulation significantly reduced the incidence of grain mold and also resulted in significant reduction of aflatoxin B1 content in sorghum grains. The antifungal activity of Zimmu might be due phenolic compounds (Satya et al., 2006).

It has been well documented that antimicrobial compounds are abundantly present in medicinal plants (Fiori et al., 2000; Ghosh et al., 2002). These compounds are thought to be involved in defense of plants against microbial pathogens in addition to their

Table 4
Organoleptic properties of banana cv. Robutsa treated with effective plant extracts.

Plant extracts	Peel color* (1–9)	Flavor* (1–9)	Texture* (1–9)	Over all acceptability* (1–9)
A. calamus	5.3c	4.5c	5.3c	5.0c
Z. scabra	7.8a	7.2a	7.2a	7.1a
P. zeylanica	6.5b	5.5b	6.5b	6.2b
Zimmu	7.8a	7.7a	7.2a	7.5a
Benomyl (0.1%)	7.8a	7.7a	7.3a	7.5a
Control	6.5b	6.5a	6.5b	6.0b
A. calamus	5.3c	4.5c	5.3c	5.0c

In a column means followed by a common letter are not significantly different at the 5% level by DMRT.
* Each value is mean of five replications.

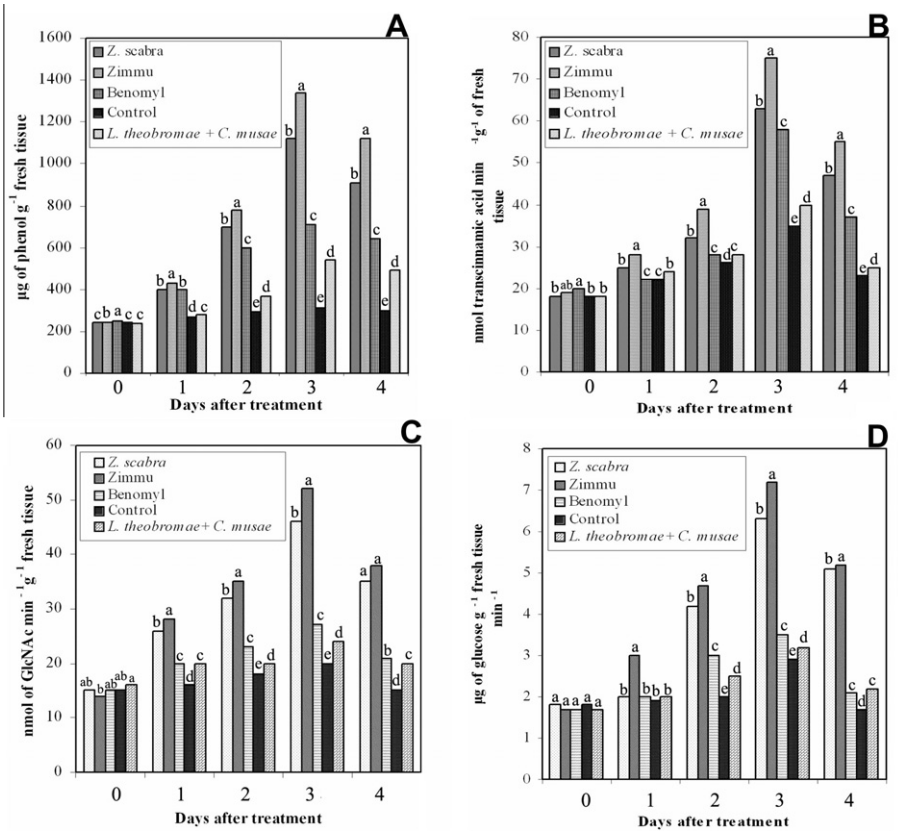

Fig. 3. Changes in Phenol (A) and induction of phenylalanine ammonia-lyase (B), chitinase (C) and β-l, 3-glucanase (D) activities in banana fruits cv. Robusta treated with plant extracts against the crown rot pathogens viz., *L. thoebromae* and *C. musae*. Above each column means followed by a common letter are not significantly different at 5% level by DMRT.

direct antimicrobial activity. Ranasinghe et al. (2003) reported that spraying of cinnamon extracts on Embul banana prior to storage, controlled anthracnose and crown rot diseases and also extended the shelf-life of banana fruit. Similarly, Thangavelu et al. (2004) reported that extract from *Solanum torvum* effectively controlled anthracnose disease and increased the shelf-life of bananas significantly compared to control. In a similar study, Win et al. (2007) reported the efficacy of cinnamon extract, chitosan and hot water treatment in reducing the crown rot disease of banana on a commercial scale.

In the present study, the aqueous leaf extract of Zimmu was found to be effective in reducing the crown rot disease of banana; this effect was on par with the fungicide benomyl under cold storage conditions and an improvement over benomyl under room storage conditions. Similarly, treatment of banana fruits with Zimmu leaf extract had resulted in extension of shelf life of banana to 16–17 d and 64 d under room and cold storage conditions respectively.

The present study also showed that treatment of banana fruits with extracts of Zimmu and *Z. scabra* had no adverse effect on the peel color, flavor or consumer acceptability. Similarly, Sanguansin (2002) reported that there were no significant adverse effects on quality of mango when it was treated with plant extracts. In our earlier study also we have reported the effectiveness of *Ocimum sanctum* oil in controlling the crown rot disease of banana without affecting the organoleptic properties of the fruit (Sangeetha et al., 2010). However, treatment of banana fruits with root extracts of *A. calamus* and leaf extract of *P. zeylanica* had some odor on the fruit.

The activity of natural plant products on the host tissue may involve direct interaction with the pathogen or induction of host resistance (Capdeville et al., 2002; Porat et al., 2002). Previously, several workers have reported the association between higher levels of chitinase and β-l, 3-glucanase and greater disease resistance against postharvest diseases. Similarly, increase in PAL activity is associated with biosynthesis of active metabolites in plant defense pathways (Milosevic and Slusarenko, 1996). A higher level of phenolic compounds at the site of pathogen invasion could restrict or slow the growth of the pathogen (Reimers and Leach, 1999). In the present study, it was demonstrated that treatment of banana fruit with Zimmu leaf extract effectively enhanced PAL, β-1, 3-glucanase and chitinase activities compared to other treatments. Similarly, the level of phenolic compounds in banana fruit was also enhanced by treatment with Zimmu leaf extract. These results suggest that the enhanced activity of defense enzymes observed in this study might have played role in enhancing the resistance of banana fruits against postharvest pathogens. Tian et al. (2006) also reported that when pear fruit was treated with different elicitors, the activity of β-1, 3-glucanase, PAL, PO and PPO were significantly enhanced and the disease incidence caused by *Alternaria alternata* also decreased. Earlier, Sangeetha et al. (2010) reported that bananas treated with mixture of antagonistic bacteria induced resistance by enhancing the activities of β-1, 3-glucanase, Chitinase, PAL, PO and PPO in harvested banana fruits and effectively controlled crown rot disease of banana. Although the induction of defense related enzymes such as β-1, 3-glucanase, chitinase and PAL is correlated with induced resistance of fruits, other mechanisms also might have been involved in disease control.

In conclusion, the present study showed that among the botanicals screened under *in vitro* and *in vivo* experiments, the leaf extract of Zimmu alone exhibited maximum inhibitory effect against crown rot and anthracnose diseases of banana caused by *L. theobromae* and *C. musae* when applied at 25% concentration. In addition, Zimmu leaf extract also extended the shelf life of banana fruits to 64 days without affecting the organoleptic properties of banana. Therefore, dipping of banana hands in aqueous leaf extract of Zimmu will be an ecofriendly technology substituting fungicides in the control of crown rot disease of banana. Further studies on the identification of principle compounds responsible for the control of these two diseases are warranted. Besides, safety tests are to be conducted for Zimmu leaf extract before recommending its use on commercial scale.

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