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A Sudha
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Kavitha PS
Tapioca and castor Research station, Yethapur, Salem, Tamil Nadu, India

Senthilkumar M
Tapioca and castor Research station, Yethapur, Salem, Tamil Nadu, India

Rajesh M
Centre of Excellence for Millets, Athiyandal, Tiruvannamalai, Tamil Nadu, India

Correspondence
A Sudha
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Effective approach to identify a potential phyto extract on *Lasiodiplodia theobromae* in Coconut

A Sudha, Kavitha PS, Senthilkumar M and Rajesh M

Abstract
Coconut palm (*Cocos nucifera* L.) is one of the most important perennial tropical crops. Stem-end rot is the major postharvest disease of coconut in Brazil. The fungus Lasiodiplodia theobromae is the only species that has been reported to be associated with this disease. The aims of this research were to study about the pathogen and management through botanical. In the present study, a total of thirty phyto extracts were screened against L. theobromae. Among the 30 plant extracts, the leaf extract of *A.cepa* X *A.sativum* (zimmu) at 10 per cent, leaf extract of *A. alliaceum* 10 per cent, bulb extract of *A.sativum* 5 per cent and leaf extract of *A. indica* 10 per cent were highly effective in inhibiting the radial mycelial growth of *B.theobromae*, under *in vitro* condition.

Keywords: potential phyto, *Lasiodiplodia theobromae*, Coconut palm

Introduction
Coconut (*Cocos nucifera* L.) is referred to as 'Tree of Heaven', 'Tree of Abundance' and 'Kalpavriksha'. Cocos nucifera L. is a member of the family Arecaceae and one of the most important perennial tropical crops. Every part of the coconut palm is used by mankind in one way or other and testimonializes its importance as a crop of multifarious use. It was considered to be one among the twenty most vital crops in the world (Bhat, 2002) [3]. There are several biotic factors responsible for the drastic reduction in coconut outputs. Coconut production is limited by several factors, among which diseases play a major role. Stem-end rot is the major postharvest disease of coconut causing significant losses (Viana *et al*. 2007) [25]. The fungus Lasiodiplodia theobromae (Pat.) Griffon & Maubl. is the only species that has been reported to be associated with this disease (Dingley *et al*. 1981; Mendes *et al*. 1998; Piepenbring 2006; Taylor and Hyde 2003) [5, 11, 13, 24]. In Southern parts of India coconut farmers are suffering from root wilt and basal stem-rot diseases. Situation grim as more than lakhs hectares of land under coconut is affected by these diseases. Now coconut farmers are dire-straits following a new wave of pest attack which threaten to reduce coconut output by more than
50 per cent. In India, the malady due to pest attack was first reported in southern parts of Kerala. Ramaraju *et al.* (2000) [17] reported that in Tamil Nadu about more than 70% of palms were severely affected, resulted in both quantitative and qualitative losses.

Recently, Lakshmanan and Jagadeesan (2004) [8] reported that the malady on the nuts of coconut palms might be due to the interaction of eriophyid mite (*Aceria guerreronis)* and the fungus *Botryodiplodia theobromae.* They reported that this fungus produced typical symptoms like malformation, cracking and hardiness in the presence of eriophyid mite infestation. Normal sized with slight cracking symptoms was reported due to eriophyid mite infestation alone. They concluded that the alarming damage might be due to the secondary infection of *B. theobromae.*

L. theobromae (Botryosphaeriaceae: Botryosphaeriales: Dothideomycetes: Ascomycota) is cosmopolitan in distribution; however, this species is more commonly found in tropical and subtropical regions (Marques *et al*. 2013; Punithalingam 1980) [10, 16]. This fungus has a wide host range, including gymnosperms and angiosperms, and can occur in nature as a parasite, saprophyte, or endophyte (Alves *et al*. 2008; Machado *et al*. 2014b; Slippers and Wingfield 2007) [1, 9, 22]. The main characteristics that distinguish Lasiodiplodia from the other related genera are the presence of paraphyses within the pycnidia, and conidia that are initially hyaline and aseptate but become brown and oneseptate with age, forming longitudinal striations due to the deposition of melanin granules on the inner surface of the wall (Hyde *et al*. 2014; Phillips *et al*. 2013; Sutton 1980) [7, 12, 23].

The emergence of fungicide-resistant strains of these fungi and the potential oncogenic risks to the consumer from the fungicidal residues on sprayed fruits, have necessitated the development of non-chemical methods of control. Therefore, management of this malady is of immense importance to realize the potential yield of coconut with a view to increase the production and productivity in the state and the country as a whole.

Materials and Methods
Effect on radial mycelial growth
The efficacy of plant extract was tested against the mycelial growth of *B. theobromae* by poisoned food technique (Schmitt *et al.*, 2001). Twenty ml of this mixture was poured into each sterilized Petri dish under aseptic condition and allowed to set. A nine mm actively growing PDA culture disc of *B. theobromae* cut out by means of a sterilized cork borer was placed onto the centre of the medium. Three replications were maintained. The plates were incubated at room temperature (28±2º C) for 12 days. PDA without plant extract served as control. Mancozeb (0.25%) served as check. The radial mycelial growth was measured daily. The results were expressed as per cent growth inhibition.

Table 1: *In vitro* assay of plant extract against radial mycelial growth and spore germination of *B.theobromae*

Sl.No.	Botanical Name	Radial mycelial growth* (mm)	Disease Reduction over control (%)
1	*Acalypha indica* L	75.10	16.56
2	*Acorus calamus*. L	66.50	26.12
3	*Adenocalyma alliaceum*	42.22	53.09
4	*Allium sativum* L.	47.20	47.56
5	*Allium cepa x Allium sativum*	20.50	77.22
6	*Aloevera Mill*	62.50	30.56
7	*Alpinia galanga* (Linn) willd	60.90	32.34
8	*Azadirachta indica* A. Juss	48.30	46.34
9	*Bougainvillea spectabilis* Willd	86.33	4.11
10	*Catheranthus roseus* (L.) G. Don	62.50	3.56
11	*Coleus aromaticus* Benth	50.30	44.20
12	*Curcuma longa* L.	86.00	4.45
13	*Delonix regia*	85.67	4.88
14	*Datura stramonium* L.	71.30	20.78
15	*Eucalyptus globulus* Labill	84.67	6.00
16	*Gliricidia maculata Stewd*	74.30	17.45
17	*Lawsonia inermis* L.	54.00	40.00
18	*Murraya koengii* (Lim)Spreng	66.30	26.34
19	*Nerium oleander* L.	82.00	8.80
20	*Ocimum bacilicum* L	50.50	43.89
21	*Ocimum sanctum* L.	84.67	6.00
22	*Phyllanthus niruri* L.	61.70	31.45
23	*Polyalthia longifolia* Benth	75.53	16.30
24	*Pongamia glabra* Vent.	85.33	5.22
25	*Prosophis juliflora* (SW) DC	85.00	5.55
26	*Solanum trilobatum L*	62.50	30.56
27	*Thevetia peruviana* (Pers) Merr.	69.60	22.67
28	*Tridax procumbans* L.	85.00	5.55
29	*Vitiveria zizoniodes* (L.) Nash	52.60	41.56
30	*Vitex negundo* (L.)	71.33	20.74
31	Mancozeb	8.42	90.64
32	Control	90.00	

*Mean of three replications

Results
Symptomatology and fungal isolation. In symptomatic fruit, black rot begins on the peduncle below the bracts and progresses to the whole fruit, including the endosperm. In older lesions, numerous cirri form on the pycnidia (Fig. 1).

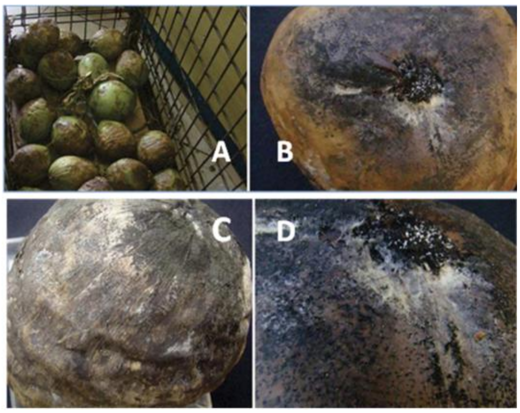

Fig 1: Symptoms of stem-end rot in coconut fruit. A, Fruit presenting stem-end rot symptoms for sale in a local market; B and C, symptomatic fruit, with the disease beginning on the peduncle and progressing to the whole fruit; D, fruit with numerous cirri formed on the pycnidia

In vitro* effect of plant extract on *B. theobromae

In vitro effects of thirty plant extracts were studied on radial mycelial growth, spore germination and the results were presented in table 1. The result indicated that among the extract, maximum inhibition (77.22 per cent) of radial mycelial growth of *B. theobromae* was recorded by *Allium cepa* x *Allium sativum* leaf extracts. This was followed by *Adenocalyma alliaceum* (53.00 per cent), *Allim sativum* (47.56), *Azadirachta indica* (46.37 per cent), *Coleus aromaticua* (44.20 per cent) and *Ocimum bacilicum* (43.89 per cent). Maximum reduction in spore germination (66.62 per cent) was also recorded by zimmu extract. This was followed by *Vitiveria zizoniodes* (55.11 per cent), *A. indica* (50.50 per cent), O.*bacilicum* (48.33) and *A. alliaceum* (45.60).

Discussion and Conclusion

A number of plant species have been reported to possess some natural substances in their leaves/ bulbs/Roots/ Rhizome which are toxic to many fungal pathogens (Singh *et al*., 1984; Singh *et al*., 1991; Biswas *et al*., 1995) [20, 21, 4]. *A. sativum* contains different antimicrobial components like allicin, E-and Z-ajoene, iso-E-10-devinylajoene, which are effective against phytopathogenic fungi. (Prithiviraj *et al*., 1998; Yoshida *et al*., 1999) [15, 27]. The antifungal activity of *A. indica* has been reported by Singh *et al*. (1984) [20]. The chemical basis of this antifungal activity has been attributed with the presence of oil in the plant parts of *A.indica*. The ethanol extracts from *A.sativum* bulb inhibited the spore germination of *B. theobromae* in tea. (Saha *et al*., 2005) [19]. *Ocimum basilicum* oil found effective against banana crown not caused by *B. theobromae O. basilicum* oil demonstrated synergistic action during both in-vivo bioassays (Anthony *et al*. 2004) [2]. Neem oil extracted using 90 per cent methanol (MeoH) was concentrated to dryness M/s Vacuo at 45º C used against *B. theobromae* (Govindachari *et al*. 1998) [6] *Lawsonia alba* and *Cantheranthus roseus* proved to be efficient in reducing growth of *B. theobromae* and disease development in papaya (Pramod, 1999) [14]. The presence of antifungal compounds in higher plants has long been recognized as a key factor in disease resistance (Mahadevan, 1982). Such compounds, being biodegradable and selective in their toxicity, are considered valuable in controlling several plant diseases (Singh and Dwivedi, 1987). Exploitation of naturally available chemicals that have the tendency to retard the growth and or reproduction of plant pathogenic fungi would be a more realistic and ecologically sound method of integrated plant disease management. The ban of some synthetic pesticides has stimulated research into novel control strategies of pests and diseases. The need to minimize pesticide residues in the marketable products has saddled researchers and chemical companies with the responsibility of developing biologically active plant derived pesticides for crop protection (Yanar *et al*., 2011) [26].

References

1. Alves A, Crous PW, Correia A, Phillips AJL. Morphological and molecular data reveal cryptic speciation in Lasiodiplodia theobromae. Fungal Divers. 2008; 28:1-13.
2. Anthony S, Abeywickrama K, Dayananda R, Wijeratnam SW, Arambewela L. Fungal pathogen associated with banana fruit in Sri Lanka, and their treatment with essential oils. Mycopathologia. 2004; 157:91-97.
3. Bhat SG. Industrial uses of coconut products. Indian Coconut J. 2002; 33:8-12.
4. Biswas S, Das NK, Qadri SMH, Sarathandra B. Evaluating different plant extracts against three major diseases of mulberry. Indian Phytopath. 1995; 48:342-346.
5. Dingley JM, Fullerton RA, McKenzie EHC. Records of fungi, bacteria, algae and angiosperms pathogenic on plants in Cook Islands, Fiji, Kiribati, Niue, Tonga, Tuvalu and Western Samoa. In: Survey of Agricultural Pests and Diseases, Technical Report, 1981, 2. FAO, Rome.
6. Govindachari TR, Suresh G, Gopalakrishnan G, Banumathy B, Masilamani S. Identification of antifungal compounds from the seed oil of *Azadirachta indica*. Phytoparasitica. 1998; 26:1-8.
7. Hyde KD, Nilsson RH, Alias SA, Ariyawansa HA, Blair JE, Cai L *et al*. One stop shop: Backbones trees for important phytopathogenic genera: I. Fungal Divers. 2014; 67:21-125.
8. Lakshmanan P, Jagadeesan R. Malformation and cracking of nuts in coconut palms (*Cocos nucifera* L.) due to the interaction of the eriophyid mite *Aceria guerreronis* and *Botryodiplodia* in Tamil nadu, India. Journal of Plant Diseases and Protection. 2004; 111:206-207.
9. Machado AR, Pinho DB, Pereira OL. Phylogeny, identification and pathogenicity of the Botryosphaeriaceae associated with collar and root rot of the biofuel plant Jatropha curcas in Brazil, with a description of new species of Lasiodiplodia. Fungal Divers. 2014b; 67:231-247
10. Marques MW, Lima NB, Morais MA, Jr. Barbosa MAG, Souza BO, Michereff SJ *et al*. Species of ˆ Lasiodiplodia associated with mango in Brazil. Fungal Divers. 2013; 61:181-193.
11. Mendes MAS, Silva VL, Dianese JC, Ferreira MASV, Santos CEN, Gomes Neto E *et al*. Fungos em plantas no Brasil. Embrapa-SP/Embrapa-Cenargen, Bras´ılia, Brazil, 1998.
12. Phillips AJL, Alves A, Abdollahzadeh J, Slippers B, Wingfield MJ, Groenewald JZ *et al*. The Botryosphaeriaceae: Genera and species known from culture. Stud. Mycol. 2013; 76:51-167.
13. Piepenbring M. Checklist of fungi in Panama. Preliminary version. Puente Biol. 2006; 1:1-190.
14. Pramod G, Studies on post-harvest diseases of papaya (*Carica papaya* L.), M.Sc. Thesis. Tamil Nadu Agricultural University, Coimbatore. 1999, 146.
15. Prithiviraj B, Singh UP, Singh KP, Plank-Schumacher K. Field evaluation of ajoene, a constituent of garlic (*Allium sativum*) and neemazal, a product of neem (*Azadirachta indica*) for the control of powdery mildew (*Erysiphe pisi*) of pea (*Pisum sativum*). J Plant Dis. Protection. 1998; 105:274-278.
16. Punithalingam E. Plant Diseases Attributed to Botryodiplodia theobromae. Pat. In: Bibliotheca Mycologica Series, no 71, J. Cramer, Vaduz, Liechtenstein, 1980.
17. Ramaraju K, Natarajan K, Sundrababu PC, Palanisamy S, Rabindra RJ. Studies on coconut eriophyid mite, *Aceria guerreronis* Keifer in Tamil Nadu, India. In: Proceedings international workshop on coconut mite, CRI, Sri Lanka, 2000, 13-31.
18. Rosado AWC, Machado AR, Freire FCO, Pereira OL.

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19. Saha D, Dasgupta S, Saha A. Antifungal activity of some plant extracts against fungal pathogens of Tea *(Camellia sinensis).* Pharmaceutical Biology. 2005; 43:87-91.
20. Singh UP, Singh HB, Chauhan VB. Effect of some plant extracts and oil on inoculum density of different nodal leaves of pea (*Pisum sativum)*.Z. Pflanzenkr Pfanzenschutz. 1984; 91:20-26.
21. Singh UP, Srivastava BP, Singh KP, Mishra GD. Control of powdery mildew of pea by ginger extract. Indian Phytopathol. 1991; 44:55-59.
22. Slippers B, Wingfield MJ. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: Diversity, ecology and impact. Fungal Biol. Rev. 2007; 21:90-106.
23. Sutton BC. The Coelomycetes: Fungi Imperfecti with Acervuli, Pycnidia and Stromata. Commonwealth Mycological Institute, Kew, UK, 1980.
24. Taylor JE, Hyde KD. Microfungi of Tropical and Temperate Palms. Fungal Diversity Research Series 12. Fungal Diversity Press, Hong Kong, 2003.
25. Viana FMP, Uchoa CN, Freire FCO, Vieira IGP, Mendes FNP, Saraiva HAO. Tratamento do coco verde para exportação com enfase no controle da podridão-basal-p ˆ os-colheita. In: Boletim de pesquisa e ´ desenvolvimento 29, Embrapa Agroindustria Tropical, Fortaleza, Cear ´ a, Brazil, 2007.
26. Yanar D, Kadioglu I, Gokce A. Acaricidal effects of different plant parts extracts on two spotted spider mite (Tetranychus urticae Koch). Afr. J Biotechnol. 2011; 10(55):11745-11750.
27. Yoshida H, Katsuzaki H, Onhta R, Ishikawa K, Fukuda H, Fujino T *et al*. An organosulphur compound isolated from oil-macerated garlic extract and its antimicrobial effect. Biosci. Biotechnol. Biochem. 1999; 63:588-590.