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Effect of Botanicals in Inhibiting Growth and Infection of *Lasiodiplodia theobromae*, the Causal Organism of Rotting and Immature Nut Fall of Coconut

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INTRODUCTION

Recently immature nut fall of coconut due to rotting has become a serious problem in many localities, especially southern part of Kerala State with well-distributed rainfall. With wide spread occurrence of eriophyid mite (*Aceria guerreronis* Keifer), severe incidence of nutfall caused by *Lasiodiplodia theobromae* (Pat.). Griffon and Maub. has been observed throughout the year with varying intensities *L. theobromae* was found to be the predominant and major pathogen causing rotting of mite infested nuts (Venugopal and ChandraMohanan, 2006). Infection always started from the white and soft tissue beneath perianth, the tissue infested by mite. When nearly mature or mature nuts were infected by *L. theobromae*, the infection continued to spread internally without any external lesion or with a small lesion on the nut surface beneath the perianth. *L. theobromae* being a wound pathogen enters the tissue through the wound mainly caused by eriophyid mite in the soft tissue beneath the perianth. Studies on the diversity of the pathogen revealed marked variability within *L. throbromae* (Venugoapl *et al.*, 2008).

Considering the economic importance of the problem, detailed studies were undertaken to find out the botanicals which are effective in inhibiting growth of virulent isolate of *L. theobromae* as well as the infection caused by it. The extracts of 52 plants species belonging to 30 families and products of 10 plants belonging to 8 families were tested for fungitoxicity against *L.theobromae*. The details of the plants used in the study are given in Table 38.1. Most of the plants and plant products were selected based on the earlier reports

on their efficacy in inhibiting growth and spore germination of other fungi as well as their availability in and around the place of study/local market. To study the effect of plant extracts/products, three virulent isolates of *L. theobromae* viz., dark gray (APA/L-172), grayish black (EKM/L-307) and white type (TPM/L-38) were selected. These three isolates were earlier identified from 3 main groups of *L. theobromae* isolates based on the detailed studies on diversity of *L. theobromae* causing nut rot and fall in different locations of disease endemic areas (Venugopal *et al.*, 2008).

All plants extracts were made out in 99% acetone. Fresh and healthy garlic and onion bulb of uniform maturity were obtained from local market and outer dry scales of bulb were removed. Fresh plant parts viz., leaves/bulbs/ rhizomes/ entire plant without roots as mentioned in the Table 38.1 were used for extrzction and the extract was tested for their effect on *L. theobromae* isolates by poisoned food technique (Nene, 1971).

Table 38.1. The details of plants used for the studies on the effect of plant extracts on *in vitro* mycelial growth of *L. theobromae* isolates

Sl. No.	*Botanical name*	*Vernacular/ Malayalam name*	*Family*	*Parts tested*
1.	*Acacia auriculiformis* A. Cunn	Acacia(E)	Mimosae	L
2.	*Adathoda vasica.* Nees	Adalodakam(M)	Acanthaceae	L
3.	*Adenocalymma allicea* L.	Garlic climber(E)	Bignoniaceae	L
4.	*Aegle marmelos* Corr	Koovalam(M)	Rutaceae	L
5.	*Aerva lanata* (Linn.) Juss.	Cherula(M)	Amaranthaecae	EP
6.	*Ageratum conyzoides* L	Mudiyumam(M)	Compositae	L
7.	*Allium cepa* L.	Onion(E)	Lilliaceae	B
8.	*Allium sativum.* L	Garlic(E)	Lilliaceae	B
9.	*Anacardium occidentale* L.	Cashew(E)	Anacardiaceae	L
10.	*Areca catechu* L.	Kamuku(M)	Palmae	L
11.	*Azadirachta indica* A.Juss.	Vep (M)	Meliaceae	L
12.	*Boerhavia diffusa* L.	Thazhuthama(M)	Nyctaginaceae	EP
13.	*Calotropis gigantea* R.Br.	Erukku (M)	Asclepiadaceae	L
14.	*Clerodendrum infortunatum* L.	Peruvelum(M)	Verbenaceae	L
15.	*Cinnamomum zeylanicum* Blume.	Cinnamon(E)	Lauraceae	L
16.	*Citrus aurantium* L .	Lemon(E)	Rutaceae	L
17.	*Citrus aurentifolia* Risso.	Orange(E)	Rutaceae	L
18.	*Coffea arabica* L.	Coffee(E)	Rutaceae	L
19.	*Coriandrum sativum* L.	Coriander(E)	Umbelliferae	EP
20.	*Costus* species	Insulin plant(E)	Zingiberaceae	L
21.	*Cocos nucifera* L.	Coconut(E)	Palmae	L
22.	*Curcuma longa* L.	Manjal(M)	Zingiberaceae	R
23.	*Elettaria cardamomum* (L).Maton.	Cardamum(E)	Zingiberaceae	L
24.	*Eucalyptus globulus* Labill.	Eucalyptus(E)	Myrtaceae	L
25.	*Eupatorium odoratum* L.	Communist pacha(M)	Asteraceae	L
26.	*Glycosmis pentaphylla* Corr.	Panal(M)	Rutaceae	L

Each plant extract at 1, 2 and 4 % concentration when added to medium was tested for the efficacy. The per cent inhibition of growth of *L. theobromae* in treatment was calculated by the equation given by Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

where,

I = Inhibition of fungal growth
C = Growth in control
T = Growth in treatment.

The nature of fungitoxicity was also tested in cases where no fungal growth was observed.

The most promising plant extracts were selected based on their efficacy in inhibiting the mycelial growth of *L. theobromae* isolates under *in vitro* condition. The extracts of *Allium sativum* (Garlic), *Syzygium aromaticum* (clove leaf), *Pimenta dioica* (All spice) at four per cent concentration completely inhibited the mycelial growth of *L.theobromae*. Therefore, these three plant extracts were prepared and evaluated.

The acetone extracts of plants which showed 70 % and more inhibition were selected (Table 38.2) and studied for their *in vitro* effect on growth of selected *L.theobromae* isolates viz., dark gray, grayish black and white types.

Table 38.2. The details of plant products used in *in vitro* studies against *L. theobromae* isolates

Sl. No	*Plant products*	*Botanical name of the source plant*	*Family*
1.	Clove oil	*Syzygium aromaticum* (L.) Merr.et Perry	Myristicaceae
2.	Camphor +Soap	*Cinnamomum camphora* L.	Lauraceae
3.	Eucalyptus oil	*Eucalyptus globulus.* Labill	Myristicaceae
4.	Eco neem plus (botanical Pesticide) 10000 ppm	*Azadirachta indica* A. Juss	Meliaceae
5.	Neem oil	*Azadirachta indica* A. Juss	Meliaceae
6.	Hydnocarpous oil +Soap	*Hydnocarpus wightiana* Gaertn.	Bixaceae
7.	Thespesia oil + Soap	*Thespesia populnea.*(L)	Malvaceae
8.	Neem oil + Garlic + Soap*	*Azadirachta indica* A. Juss, *Allium sativum* L.	Meliaceae Lillaceae
9.	Turmocure(Turmeric extract)	*Curcuma longa* L.	Zingiberaceae
10.	Turpentine	*Pinus* species	Pinaceae

*Natural turmeric extract manufactured by Narmada products, Bhavanishankar temple compound, Puttur, Karnataka

The effect of plant extracts and plant products on *L. theobromae* infection of detached coconut was studied using about three months old coconuts of WCT cultivar. Nuts at this stage were found to be convenient in making cavities with cork borer and to fill plant extract/ plant product solution.

The fungitoxicity of plant extracts against *L.theobromae* varied with the concentration. (Table 38.3) In general the response of dark grey (APA/L-172) and greyish black (EKM/ L-307) isolates to different concentrations of plant extracts did not vary. The inhibition of mycelial growth of white type (TPM/L-36) isolate was more than the other two isolates in the presence of plant extract. All isolates exhibited a significant reduction in growth on first day after inoculation. The inhibition of growth is decreased with duration of incubation in most cases. The growth of the three isolates varied with plant extracts and their concentrations.

Among 52 plant extracts tested *in vitro*, the extracts of *A.sativum* (Garlic), *S. aromaticum* (Clove leaf) and *P. dioica* (All spice) at 4% concentration were found to be very effective in inhibiting the growth of selected isolates. The extract of garlic bulb at 1 and 2% concentration showed more than 70% of inhibition of all isolates on first day after inoculation. The growth of white type isolate was completely inhibited in the presence of *A. sativum* extract at2% concentration. The inhibition of growth of this isolate was more than 80% even at 1% concentration of *A. sativum* extract. There were significant variations in growth inhibition on 2nd day after inoculation. Dark grey isolate showed cent per cent inhibition with *A. sativum* 2% on 1st day after inoculation whereas greyish black isolate exhibited only 72.14% inhibition. Percentage of inhibition decreased with duration of incubation in these two isolates. The extract of *P. dioica* at 1 and 2% concentration was found significantly effective than *A. sativum.* Even at lower concentrations it effectively checked the growth of all the selected isolates. White type isolates showed 100% inhibition of growth in the presence of *P. dioica* extract (all concentrations) on first day after inoculation. The inhibition of growth decreased with duration of incubation. This isolate exhibited total inhibition of growth even at 2% concentration of *P. dioica* extract. Dark grey and grayish black isolates showed almost equal growth response with all concentrations of *P. dioica* extract. All the isolates exhibited total inhibition of growth in the presence of *P. dioica* extract at 4% concentration.

The extracts of *S. aromaticum* at2% concentration completely inhibited the growth of white type isolate. On the other hand, 2z% concentration of *S. aromaticum* extract exhibited only 61.46% and 72.96% inhibition of dark gray and grayish black isolates respectively. This extract at 4% concentration was found fungistatic to all the three isolates.

The extract of *G. pentaphylla* (all the concentrations) had stimulatory effects on growth of dark gray and grayish black isolates. The extract obtained from *A. occidentale* (all the concentrations) also promoted the growth of dark gray isolate. The extracts of *C. infortunatum* (at 1%) and *C. nucifera* (2 and 4 %) had stimulatory effect on the growth of grayish black isolate. *V. rosea* (1% concentration) and *M. pudica* (1 and 2% concentrations) had no effect on any of the three isolates.

Among 52 plants screened for fungitoxic properties against selected isolates of *L. theobromae*, the extracts (at 4% conc.) from *H. wightiana*, *L. inermis* and *S. nux-vomica*

showed more than 70% of inhibition of growth of all the three isolates. The extracts of *A. auriculoformis, E. globules, A. marmelos, A. conyzoides, C. longa and Z. officinale* at 4% concentration showed 50% inhibition of growth of white isolate after 24 h of inoculation. Thus, among the 52 plant species screened for fungitoxic properties, extracts of 20 plant species exhibited 60% inhibition of dark grey isolate. There was 60% inhibition of growth of both greyish black and white isolates in the presence of extracts of 17 plant species.

Table 38.3. Percentage inhibition of growth of selected isolates of *L. theobromae* after 2 days of inoculation at different concentrations of plant extracts

Sl. No	*Plant extracts*	*Conc.%*	**Per cent inhibition after 2 days of inoculation L. theobromae isolates*		
			Dark grey type	*Greyish black type*	*White type*
1.	*Acacia auriculiformis*	4	72.59 (58.44) -	61.85(51.83) +++	5.41(13.39) -
2.	*Aegle marmelos*	4	71.85(57.93) -	72.96(58.64) +++	34.90(36.19) -
3.	*Aerva lanata*	4	60.37(50.97) -	71.11(57.46) +++	36.86(37.29) -
4.	*Ageratum conyzoides*	4	40.00(39.20) -	72.96(58.64) +	57.09(49.05) -
5.	*Allium cepa*	4	7.40(12.97) -	30.37(33.41) +++	39.49(38.89) -
6.	*Allium sativum*	2	64.44(53.37) -	54.44(47.53) +	100.00(90.00) -
7.	*Calotropis gigantia*	4	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
8.	*Curcuma longa*	4	74.81(59.85) -	72.96(58.64) ++	55.01(47.85) -
9.	*Eucalyptus globulus*	4	70.74(57.23) +	71.85(57.93) +	71.02(57.42) -
10.	*Hydnocarpus* species	4	74.81(59.88) -	72.96(58.64) +++	61.75(51.77) -
11.	*Pimenta dioica*	4	71.85(57.94) -	85.18(67.34) ++	100.00(90.00) -
12.	*Piper nigrum.*	1	70.37(57.00)-	61.11(51.40)+	64.29(53.30) -
		2	83.33(65.93) -	81.48(64.49) -	100.00(90.00) -
		4	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
13.	*Psidium guajava*	4	75.18(60.10) -	67.78(55.39) +++	57.12(49.07) -
14.	*Syzygium aromaticum*	1	62.96(52.49) +++	58.15(49.67) +++	82.43(65.19) -
15.	*Strychnos nux- vomica*	2	68.89(56.07) +++	54.07(47.32) +++	82.43(65.19) -
		4	77.04(61.35) +++	39.26(38.76) +++	85.00(67.19) -
16.	*Tamarindus indicus*	1	100.00(90.00) -	100.00(90.00)-	100.00(90.00) -
		2	72.59(58.42) -	70.18(56.88) +++	76.21(60.79) -
17.	*Zingiber officinale*	4	76.66(61.10) -	74.07(59.38) +++	79.32(62.93) -
		4	81.48(64.56) -	75.37(60.24) +++	100.00(90.00) -
		4	70.74(57.24) -	45.18(42.21) ++	16.24(23.67) +
			70.74(57.23)	22.59(28.35)	62.20(52.07) -
	C.D (P = 0.05)		3.73	2.60	4.12

Results of only those plant extracts that gave higher percentage inhibition of growth of *L.theobrome* are given

*Mean of three replications; Figures in parenthesis are angular transformed values

Degree of sporulation after 14 days of inoculation +++ Abundant, ++ Moderate, + Poor, - Nil

When there was complete inhibition of growth, the nature of fungitoxicity was further tested. Extracts of *A. sativum*, *P. dioica* and *S. aromaticum* at 4% concentration were found fungistatic to all the three isolates. Therefore higher concentrations such as 5, 6, 8 and 10% of these extracts were tested. *A. sativum* at 10% and *P. dioica* and *S. aromaticum* each at 8% concentration were found fungicidal to all the three isolates of *L.theobromae*.

Among the extracts tested, 22 totally inhibited the sporulation in dark grey isolate. Different concentrations of remaining 30 plant extracts reduced the degree of sporulation to moderate level. Most of the extracts did not inhibit the sporulation of greyish black isolate. The extracts like *A. auriculoformis* (4%), *A. lanata* (4%), *A. sativum,* (1%) *C. gigantia* (2 and 4%), *C. zeylanicum* (2 and 4%), *C. arabica* (2 and 4%), *C. sativum* (1%), *C. longa,* (1%), *H. wightiana* (4%), *T. indica* (2 and 4%) and *Z. officinale* (1%) reduced the sporulation of greyish black isolate to moderate level. *Z. officinale* (2 and 4%), *S. aromaticum* (1%), *P. dioica* (1%), *C. longa* (2 and 4%), *Allium sativum* (2%), *and A. conyzoides* (4%) exhibited marked reduction in the degree of sporulation and thus there was only poor sporulation in the presence of these extracts. There was moderate sporulation in white type isolate in the presence of extracts of *A. allicea* (1%), *A. indica* (all conc.), and *M. koenigii* (all conc.). The extracts of 29 species completely inhibited the sporulation of white isolate even at 1% concentration.

Out of 52 plant species tested, the extracts of *A. sativum, S. aromaticum* and *P. dioeca* were the most promising in inhibiting the growth of all the three isolates. The sporulation of all the three isolates of *L.theobromae* was also completely inhibited by 4% concentation of *A. sativum* and 2% concentration of the other two plant extracts.

Fresh plant extracts of *A. sativum*, *S. aromaticum* and *P. dioica* without autoclaving were found to be the most promising in inhibiting the growth of *L.theobromae* under *in vitro* condition. The mycelial growth of *L.theobromae* in the presence of autoclaved extracts of *A. sativum* (4% conc.), *S. aromaticum* (4% conc.) and *P. dioica* (4% conc.) varied significantly among the three isolates as well as within the isolates with respect to different concentrations (Table 38.4).

The study also revealed that *S. aromaticum* was superior to all other extracts tested followed by *P. dioica*. Out of three extracts tested, *S. aromaticum* inhibited growth of dark gray and grayish black isolates one day after inoculation. The fungitoxicity varied with concentration of plant extracts and days of incubation. White isolate exhibited cent percent growth inhibition at 4% concentration of *S. aromaticum* and *P. dioica*. The autoclaved extracts of *A. sativum* inhibited the growth of white and greyish black isolates below 50 % level on first day whereas in dark grey it was 37.80%. When the extract of *A. sativum* at 4% concentration without autoclaving was incorporated into sterilized and cooled medium, it inhibited fungal growth completely. But autoclaving the extract decreased its antifungal property.

Table 38.4. Percentage of inhibition of growth of selected isolates of *L. theobromae* on 2nd day after inoculation at different concentrations of autoclaved plant extracts

Sl.No	Plant extract	Conc.%	*Percent inhibition on 2nd day after inoculation L.theobromae isolates		
			Dark grey type	Greyish black type	White type
1.	*Allium sativum*	1	0.00(0.00)	0.00(0.00)	12.49(20.24)
		2	0.00(0.00)	0.00(0.00)	16.27(23.54)
		4	0.00(0.00)	0.00(0.00)	19.99(26.52)
2.	*Pimenta dioica*	1	24.07(29.34)	16.29(23.63)	39.73(39.05)
		2	55.18((47.95)	57.03(49.03)	80.27(63.62)
		4	78.52(62.38)	75.92(60.69)	100.00(90.00)
3.	*Syzygium aromaticum*	1	18.52(25.42)	29..26(32.72)	49.32(44.59)
		2	49.26(44.55)	54.44(47.53)	76.53(61.00)
		4	72.22(58.21)	83.33(66.16)	100.00(90.00)
	Gen. Mean		29.76	31.09	50.96
	C.D(P=0.05)		2.94	5.04	4.72

The acetone extracts of plants which showed 70% and above inhibition were selected and the aqueous extract of this plant species were tested to find out their inhibitory effects (Table 38.5). All isolates showed significant variation in growth in the presence of the selected plant extracts. Acetone extracts were found superior in inhibiting growth than aqueous extract.

Among the aqueous plant extract tested, *A. sativum* at 4% concentration showed above 50% of inhibition of dark grey and greyish black isolates on first and second day after inoculation. White isolate was found more sensitive and its growth was completely inhibited by aqueous extracts of *A. sativum* (4%) on first day. Aqueous extract of *S. aromaticum* at 4% concentration exhibited above 65% inhibition of growth of dark grey and white isolates. On the other hand there was only 55.34% inhibition of grayish black isolates in the presence of this extract on first day.

Among the three isolates screened, dark grey(APA/L-172) and greyish black (EKM/ L-307) isolates showed almost similar response in growth in the presence of different plant products whereas isolate of white colony(TPM/L-36) group exhibited more response than other two groups (Table 38.6). Analysis of variance showed that there was significant difference among the plant products and their concentrations in inhibiting the mycelial growth. All isolates exhibited significant reduction in mycelial growth one day after inoculation on PDA incorporated with plant products when compared to control. The inhibition of growth decreased with duration of incubation in most cases.

Table 38.5. Percentage of inhibition of growth of selected isolates of *L. theobromae* on 2nd day after inoculation at different concentrations of aqueous plant extracts

Sl. No	Plant extract	Conc.%	*Percent inhibition on 2nd day after inoculation L. theobromae isolates*		
			Dark grey type	Greyish black type	White type
1.	*Allium sativum*	1	5.18(13.09)	4.81(12.65)	45.11(42.18)
		2	39.63(38.99)	42.59(40.70)	69.39(56.39)
		4	56.29(48.59)	59.63(50.54)	79.95(63.37)
2.	*Hydnocarpus wightiana*	1	0.00(0.00)	45.55(42.42)	17.12(24.33)
		2	0.00(0.00)	37.77(37.89)	22.94(28.53)
		4	0.00(0.00)	52.22(46.25)	47.74(43.69)
3.	*Lawsonia inermis*	1	0.00(0.00)	21.48(27.48)	37.26(37.52)
		2	0.00(0.00)	32.96(35.02)	43.01(40.96)
		4	0.00(0.00)	32.22(34.56)	49.84(44.89)
4.	*Pimenta dioica*	1	0.00(0.00)	0.00(0.00)	0.00(0.00)
		2	0.00(0.00)	0.00(0.00)	0.00(0.00)
		4	0.00(0.00)	12.96(21.08)	0.00(0.00)
5.	*Strychynos nux- vomica*	1	0.00(0.00)	0.00(0.00)	0.00(0.00)
		2	0.00(0.00)	0.00(0.00)	0.00(0.00)
		4	0.00(0.00)	13.33(21.32)	17.12(24.33)
6.	*Syzygium aromaticum*	1	68.14(55.63)	0.00(0.00)	55.16(47.95)
		2	75.55(60.34)	0.00(0.00)	65.66(54.13)
		4	83.33(65.88)	17.03(23.98)	72.03(58.05)
	Gen. Mean		15.70	21.88	16.01
	C D (P = 0.05)		1.10	3.94	2.22

*Mean of three replications
Figures in parentheses are angular transformed values

Among 10 plant products screened in *vitro*, clove oil (2% and above), eucalyptus oil (1% and above), Eco-neem plus (commercial product) (1% and above) and neem oil + garlic + soap mixture (5% and above) were found very effective in inhibiting the growth of selected isolates. Eco neem at 8% concentration and neem oil + garlic + soap at 10% concentration completely inhibited the growth of all isolates and were found fungitoxic to all isolates. The response of dark grey and greyish black isolates to eco neem plus at 0.25 and 0.5% concentration was almost similar whereas white type isolate showed a significant reduction of growth at these concentrations. Neem oil (all con.), *Hydnocarpus* oil + soap (1 and 2%) and *Thespesia* oil + soap (1 and 2%) promoted the growth of dark grey isolate. All the three concentrations of turmocure (turmeric extract) significantly reduced the growth of all the three isolates. Dark grey and greyish black isolates at all the three concentrations of turpentine oil showed significant reduction in growth over the control. On the other

hand, white types isolate showed total inhibition of growth even at 1% concentration of this plant product. The plant products significantly inhibited the sporulation in all isolates.

The effect of plant extracts and plant products which exhibited 100% inhibition of mycelial growth of selected isolate of *L.theobromae* (EKM/L-307) under *in vitro* condition was tested by inoculating detached coconuts with this isolate.

The bulb extract of *A. sativum* (10%) and leaf extract of *S. aromaticum* (8%) were found to be effective and showed a significant reduction in lesion size over control. The extracts of *A. sativum* (10%) and *S. aromaticum* (8%) inhibited the lesion development for 3 days after inoculation. The lesion size on 5th day was 12.66 mm × 10.33 mm and 11mm × 10.33 mm respectively (Table 38.7).

Among the plant products evaluated, Eco neem plus (botanical pesticide) at 8% concentration gave promising result. This plant product completely inhibited lesion development. Neem oil + garlic + soap mixture at 10% concentration produced lesion size of 10 mm × 10 mm on 5th day after inoculation. Eco neem at 8% concentration completely inhibited the lesion size.

The presence of antifungal compounds in higher plants has long been recognized as an important factor to disease resistance (Mahadevan, 1982). Such compounds, being biodegradable and selective in their toxicity, are considered as valuable in controlling some plant diseases (Singh and Dwivedi, 1987). The present study is the first attempt to find out the antifungal activity of plant extract against *L.theobromae*, the causal organism of fruit rot and immature nut fall in coconut. The *in vitro* evaluation of 52 plant extracts extracted in acetone revealed that extracts from bulb of *Allium sativum* (garlic), leaf extracts of *Syzygium aromaticum* (clove) and *Pimenta dioica* (all spice) were superior in inhibiting the mycelial growth of *L .theobromae.*

As observed in the present study the excellent performance of garlic in inhibiting several other fungi was also reported by other workers (Singh *et al.*, 1979; Singh and Singh, 1980). Aqueous and ethnol extracts of five Nigerian spices [*Allium sativum* (garlic), *Zingiber officinale, Afromomum melegueta, Xylopia aetheopica*, and *Monodora myristica*] inhibited the growth of fungi such as *Botryodiplodia theobromae, Aspergillus niger, Aspergillus flavus, Mucor* sp., *Rhizopus stolinifer, Penicillium* sp.and *Fusarium* sp (Ejechi *et al.*, 1997). Chakraborty *et al.* (2006) reported the effective management of dieback of bottle brush (*Callestemon citrinus*) caused by *Botryodiplodia theobromae* using biocides (extracts of *Azadirachta indica, Allium sativum, Allium cepa* and *Eucalyptus* species). The active principle in garlic viz., allicin had been reported to contain antimicrobial property (Cavellito and Bailey, 1944., Rao *et al.,* 1946 and Pereira *et al.,* 2006).

Leaf extracts of *S. aromaticum* at 2% concentration exhibited 61.46 and 72.96% inhibition of growth of dark grey and greyish black isolates respectively and total inhibition of growth of white isolate in the present study. *S. aromaticum* contains high percentage of

eugenol, which has been identified as a compound exhibiting antifungal properties (Garg and Siddigui, 1992). Several workers observed that clove (*S. aromaticum*) promoted total inhibition of mycelial growth of *Penicillium roqueforti* (Pereira *et al.,* 2006; Azzous and Bullerman, 1992 and Farag *et al.*, 1989), *Rhizotonia solani* (Myeong *et al.,* 2003) and *L. theobromae (*Ranasinghe *et al.,* 2002).

The extract of *P. dioica* (all spice) at 1 and 2% concentration was more effective than *A. sativum.* Its lower concentrations effectively checked the growth of all the three isolates. Lima *et al.* (2006) reported eugenol as the active principle of the *Pimenta* sp. A recent study indicated that 1, 8 cineole and eugenol exhibited strong antifungal activity against *Fusarium moniliformae* (Garg and Siddigui, 1992). There are no reports in the literature on antifungal properties of *P. dioica* against *L. theobromae.*

Another interesting aspect in the present study was the effect of certain plant extract promoting the mycelial growth of *L.theobromae*. The leaf extracts of *Glycosmis pentaphylla* (at all the concentrations tested) had stimulatory effects on growth of dark grey and greyish black isolates. The extract obtained from *Anacardium occidentale* (all the concentrations tested) had stimulatory effect on growth of dark gray isolate. Leaf extracts of *C. infortunatum* (at 1%) and *C. nucifera* (2 and 4%) also stimulated the growth of greyish black isolate. Shekhawat and Prasada (1971) reported that the extract of *Ficus bengalensis* stimulated spore germination in *Aspergillus tenuis* and *Helmithsporium* species and the extract of *Xanthium straumarium* stimulated the growth of *Penicillium oxalicum* and *Aspergillus flavus*. The extracts of *Hyptis sauveoleens* had stimulating effect on the growth of *Aspergillus flavus* (Tiwari, 1987).

The antifungal activity of leaf extract of *L. inermis* was reported by various workers in many pathogenic fungi such as *Drechslera oryzae* (Natarajan and Lalitha Kumari, (1987a; 1987b), *C. gloeosporioides* (Babu and Reddy, 1986), and *Rhizotonia solani* (Kurucheve *et al.,* 1997). Muhamed *et al.* (1996) reported that thirty four other plants including *L. inermis, Mentha arvensis, Mimusops elangi, Ocimum sanctum, Phyllanthus niruri, Piper nigrum, Solanum nigrum* and *Tinospora tuberculata* showed selective antifungal activity against various fungi such as *Colletotrichum capsici, Fusarium palidoroseum, Botryodiplodia theobromae, Alternaria alternata, Penicillium citrinum, Phomopsis caricae-pepayae and Aspergillus niger.* But leaf extract of *L.inermis* at 4% conc. exhibited only 34.9%, 73.3 and 69.47% inhibition of growth of dark grey, greyish black and white type isolates of *L. theobromae* respectively.

Studies on the effect of seven aqueous plant extracts on the mycelial growth of *L. theobromae* revealed that acetone extract was superior to water extract. The result of the present findings is in accordance with the findings of Nwachukwu and Umechuruba (2001). They reported that crude leaf extracts of *A. indica, Vernonia amygdalina, Ocimum basilicum, Carica papaya* and *Cymbopogen citratus* were more effective than aqueous extracts in inhibiting mycelial growth and spore germination of seed borne fungi such as *F. moniliforme, B. theobromae* and *Aspergillus niger.*

Though the extract of *A. sativum* (garlic), *P. dioica* (all spice) and *S. aromaticum* (clove)

inhibited the growth of the three selected *L. theobromae* isolates completely, all these extracts were only fungistatic at 4% concentration. The higher concentrations such as extract of *A. sativum* at 10% and extract of *P. dioica* and *S. aromaticum* each at 8% were found fungicidal. The extracts of *P. dioeca* (all spice) and *S. aromaticum* (clove) retained mycelial inhibitory effects even after autoclaving. This might be due to the thermostable nature of active principle present in these extracts. When garlic was incorporated into sterilized and cooled PDA medium, it inhibited growth of the isolates completely at 4%. But autoclaving of the extract reduced the antifungal property considerably and thus exhibited least inhibition of growth. Parkinson and Clarke (1964) reported the loss of inhibitory effect of onion bulb extract by boiling. They reported that though onion bulb extract totally inhibited the growth of *Pythium apanidermatum* boiling the extract destroyed the inhibitory effect.

It could be concluded that the leaf extracts having fungicidal properties, markedly differed in their fungitoxicity. The difference was also significant between the isolates of *L.theobromae* selected for the study. Thus, the plant extracts in general and garlic and clove leaf extracts in particular, have great potential in inhibiting the growth and sporulation of *L. theobromae* isolates.

Striking differences were also noticed in the response of selected isolates of *L. theobromae* to plant products tested. All the isolates exhibited significant reduction in mycelial growth one day after inoculation compared to control. The rate of growth increased with duration of incubation in most cases. Among 10 plants products screened *in vitro*, eco neem plus at 8% and neem oil + garlic + soap mixture at 10% were found fungicidal to all three isolates. When fresh leaf extracts of *A. indica* was used there was least inhibition of growth of isolates. Clove oil was found effective in inhibiting the growth of various fungal pathogens viz., *Penicillium roqueforti* (Farag *et al.*, 1989; Azzous and Bullerman, 1992; Pereira *et al.*, 2006), *Rhizotonia solani* (Myeong *et al.*, 2003) and *L. theobromae* (Ranasinghe *et al.*, 2002).

When garlic extract at 4% concentration completely inhibited the growth of the isolates, a mixture of neem oil + garlic + soap at 4% did not inhibit the growth completely. Spraying of Eco neem plus and neem oil + garlic + soap at 2% are recommended for effective management of eriophyid mite. But the recommended dose (2%) for mite management did not completely inhibit the growth of *L.theobromae* isolates. Spraying a mixture of neem oil + garlic + soap at 10 % concentration can successfully check mite as well as *L. theobromae* infection.

The effect of plant extract and plant products which exhibited 100% inhibition of growth of the three selected isolates under *in vitro* studies was tested on detached coconut inoculated with the most virulent isolate of *L.theobromae* (greyish black isolate). Eco neem plus (8%) was found to be the most effective treatment when tested on detached nut. There was no lesion development when treated with this plant product. Neem oil + garlic + soap (10%) significantly inhibited the lesion development when compared to control. Garlic bulb extract (10% concentration) and clove leaf extract (8% concentration) inhibited lesion development for 3 days from the day of inoculation. Hence, Eco neem plus (8%), neem oil + garlic + soap mixture (10%), garlic bulb extract (10%) and clove leaf extract (8%) can be

Table 38.6. Percentage inhibition of growth of selected isolates of *L. theobromae* on 2nd day after inoculation at different concentrations of plant products

Sl. No	*Plant products*	*Conc.%*	**Per cent of inhibition on 2nd day after inoculation L. theobromae isolates*		
			Dark grey type	*Greyish black type*	*White type*
1.	Clove oil	1 %	83.33(65.88) +	88.15(69.84) -	100.00(90.00) -
		2 %	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
		4%	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
2.	Camphor + soap	1 %	78.51(62.44) -	85.55(63.31) +	100.00(90.00) -
		2 %	80.37(63.71) -	100.00(90.00) -	73.80(59.19) -
		4 %	100.00(90.00)	100.00(90.00) -	100.00(90.00) -
3.	Eucalyptus oil	0.25 %	-61.48(51.64) -	69.26(56.40)++	100.00(90.00) -
		0.5 %	100.00(90.00) -	100.00(90.00)++	100.00(90.00) -
		1 %	100.00(90.00) -	00.00(90.00) -	100.00(90.00) -
		2 %	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
		4 %	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
		0.25 %	5.55(11.17) -	25.92(30.56) +	100.00(90.00) -
4.	Eco neem plus	0.5 %	4.44(7.13) -	38.14(38.11) -	100.00(90.00) -
	(Botanical Pesticides)	1 %	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
		2%	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
		4 %	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
		6 %	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
		8 %	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
		1 %	0.00(0.00) -	0.00(0.00) -	100.00(90.00) -
5.	Hydnocarpous oil +	2 %	0.00(0.00) -	0.00(0.00) -	0.00(0.00) -
	soap Neem oil	4 %	0.00(0.00) -	0.00(0.00) -	24.08(29.16) -
		1 %	0.00(0.00) +	2.59(7.39) ++	29.80(32.92) -
6.	Thespesia oil+ soap	2 %	0.00(0.00) +	17.78(24.57) ++	3.63(8.98) -
		4 %	23.70(29.08) -	125.92(30.57) ++	14.14(22.05) -
		1 %	0.00(0.00) -	6.67(14.92) ++	27.25(31.42) -
7.	Neem oil+ garlic +soap	2 %	0.00(0.00) -	12.59(20.70) ++	23.56(28.99) -
		4 %	32.22(33.82) -	23.33(28.84) +	17.25(24.43) -
8.	Turmeric extract	2%	0.00(0.00) +	0.00(0.00) +	29.80(32.92) -
		4 %	14.81(22.53) -	27.78(31.79) -	63.33(52.71) -
		5%	100.00(90.00) -	100.00(90.00) -	77.97(62.02) -
		6 %	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
		8 %	100.00(90.00) -	100.00(90.00) -	100.00(90.00) -
9.	Turpentine	10%	64.07(53.16) -	100.00(90.00) -	100.00(90.00) -
		1%	67.78(55.39) -	70.00(56.79) ++	100.00(90.00) -
		2%	79.26(62.88) -	75.37(60.22) -	69.62(56.53) -
		4 %	88.89(70.51) -	82.40(65.17) -	74.88(59.09) -
10.		1 %	90.00(71.53) -	88.52(70.17) -	78.53(62.37) -
		2 %	100.00(90.00) -	89.63(71.19) -	100.00(90.00) -
		4 %			100.00(90.00) -
					100.00(90.00) -
	Gen. Mean		54.47	58.95	66.42
	C.D (P = 0.05)		5.74	3.07	3.53

*Mean of three replications

Figure in parenthesis are angular transformed values

Degree of sporulation after 14 days of inoculation ++ Moderate, + Poor, - Nil

selected for the field trial to find out effective control measure under conditions of natural incidence of the disease. Thus, this method of testing the plant extracts and plant products on detached nuts before field trial was found to be very helpful in selecting the effective concentrations for field trial.

Table 38.7. Effect of plant extracts and plant products on *L. theobromae* infection of detached coconut

Sl.No.	Plant products /Plant extracts	Conc.(%)	No. of days taken for appearance of lesion	Mean lesion diameter (in mm) on 5th day of inoculation	
				length (mm)	breadth (mm)
1.	Plant products Neem oil + garlic + soap	6	3	23.33	17.00
		8	3	14.66	12.00
		10	5	10.00	10.00
2.	Eco neem plus (Botanical Pesticide) Plant extract	4	3	27.66	13.66
		6	3	14.33	12.33
		8	-	0.00	0.00
3.	*Allium sativum* (garlic bulb extract)	6	3	28.00	22.00
		8	3	17.00	15.66
		10	5	12.66	10.33
4.	*Syzygium aromaticum* (clove leaf extract)	4	3	31.33	25.33
		6	3	18.66	16.33
		8	5	11.00	10.33
5.	Control		3	42.66	39.33

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