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Effect of Plant Oils and Plant Extracts for the Management of Crown Rot of Banana Caused by Lasiodiplodia Theobromae

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Abstract

Banana (*Musa paradisiaca* L.) the major fruit crop across the world is affected by post-harvest losses of the fruit up to 35% due to various reasons. Among them banana crown rot caused by *Lasiodiplodia theobromae* is considered as a major problem. Presently, fungicides are widely used to manage the post-harvest diseases of banana, however the use of these fungicides resulted in development of resistant strains of the pathogen and also leaves the toxic residues in the fruits which causes indirect effect on human health. So as an alternate method, plant oils and plant extracts which are eco-friendly are tested for the management of crown rot disease of banana under *in vitro* conditions. Among the plant oils tested, thyme oil at 0.05%, basil oil at 0.07%, lemon grass and citrodara oil at 0.09% completely inhibited the mycelial growth and Spore germination of *L.theobromae.* Among the various plant extracts tested *in vitro*, maximum mycelial inhibition of *L. theobromae* was observed at 50% Neem leaf extract (94% reduction over control) followed by 50% thulsi leaf extract (93.3% reduction over control). When these plant extracts and plant oils were tested as post-harvest fruit dipping, thyme oil at 0.05% showed significant reduction of crown rot incidence up to 84.73 % at 12 Days after treatment and also increased the self-life of the fruit.

Keywords: Crown rot, thyme oil, post harvest fruit dipping, Neem extract

Introduction:

Banana is the fourth important fruit crop of the world and delivers an important source of starch, particularly in Asia and Africa (Muhammad Ather et al. 2018). Several fungal diseases reduce the quality and post harvest shelf life of this fruit crop (Win et al. 2007). Post harvest diseases cause a wide loss and damage to all the fruit crops and vegetable crops. Among the post harvest diseases in

banana, the most common one is Crown rot of banana caused by Laseodiplodia theobromae (Rattanakreetakul 2013). Extensive damage caused by this disease remains a potential problem to the native exporters.

Management of this post harvest disease remains important to extend the shelf life of the fruit. Though chemical management is available for this disease, the use of chemical is known to cause undesirable effects to the human health because of its toxicity. So it is important to develop an alternate strategy for the control of crown rot disease in banana which should be environment friendly and cheaply available to the farming community.

The use of plant oils and plant extracts paves the way for developing the alternate way for use of chemical fungicides. Because of the presence of certain antifungal compounds in the plant oils and extracts, they inhibit the growth of the pathogen and it also a better management practice as it is eco-friendly.

With this background the present study was conducted (i) to identify the effective plant oil against L. theobromae in vitro (ii) to identify the effective plant extract against L. theobromae in vitro (iii) to screen the effective plant oil and extract against L. theobromae by post harvest dipping of fruits.

Materials and methods:

Collection of plant oils

The plant oil *viz.,* Basil oil, Thyme oil, Lemon grass oil, Citrodara oil, Eucalyptus oil, Lavender oil, Geranium oil were purchased from the manufacturer of plant oils, ACFC tribal welfare society, Marayoor and Tegraj & co., Coimbatore and confirmed that these oils were extracted by hydro distillation process. The efficacy of the above mentioned plant oils were tested against *L. theobromae.*

Plant oils	**Scientific name**	**Famil y**
Basil oil	*Ocimum sanctum*	*Labiatae*
Thyme oil	*Thymus vulgaris*	*Laminaceae*
Lemon grass oil	*Cymbopogan nardus*	*Graminae*

Citrodara oil	*Monarda citrodara*	*Laminaceae*
Eucalyptus oil	*Eucalyptus globules*	*Myrtaceae*
Lavender oil	*Lavendula stoechas*	*Laminaceae*
Geranium oil	*Pelargonium graveolens*	*Geraniaceae*

Evaluation of antifungal activity of essential oils against *L. theobromae* by Poisoned food technique

Fungistatic and antifungal activities of different plant oils were assessed against the *L. theobromae* by the radial growth assay which is followed by poisoned food technique. The plant oils were tested in the concentration range between 0.01 to 0.1 percent (v/v). To 50 ml of sterilized PDA medium different concentration of plant oils were mixed separately and dispensed into the Petri plates. The oils which did not show control below the 0.01% concentration was tested in higher concentration of 0.1%. All the plates were rotated evenly for the dispersal of oil. Plates with normal PDA medium were taken as a control plate. An 8mm culture disc of *L. theobromae* from 7 days old culture was placed in each plate and they were incubated at 28±2°C for 7 days. Three replications for each concentration were maintained. After seven days, the colony diameter was measured and the minimum inhibitory concentration was determined.

Colony diameter of the test fungus in the treatment in comparison with that of check gave growth inhibition by the following formula

Per cent inhibition (I) = $I=\frac{C-T}{C}\times 100$

Where,

I = Per cent inhibition

C= Radial growth of the pathogen in control

T= Radial growth of the pathogen in treatment

Collection of plant extracts

Five plant species *viz.*, Neem, Adathoda, Turmeric, Nochi, Tulasi were collected from the field in and around Cuddalore district of Tamil nadu. One gram of leaves were cut from each plant and they were washed in water to remove dust and ground in 1 ml of 0.1 M sodium phosphate buffer (pH

7.0) using a pestle and mortar on ice. The solution was centrifuged at 10000 rpm for 20 min at 4°C and the supernatant collected was filter-sterilized through a 0.22 µm Millipore filter (Thangavelu et al. 2004). The efficacy of the above mentioned plant extracts were tested against *L. theobromae.*

Plant extracts	Scientific name	Family
Neem	*Azadirachta indica*	*Mahogany*
Adathoda	*Justicia adathoda*	*Acanthaceae*
Turmeric	*Curcuma longa*	*Zingiberaceae*
Nochi	*Vitex negundo*	*Laminaceae*
Tulasi	*Ocimum sanctum*	*Labiatae*

Evaluation of antifungal activity of plant extracts against *L. theobromae* by Poisoned food technique:

Fungistatic and antifungal activities of different plant extracts were assessed against the *L. theobromae* by the radial growth assay which is followed by poisoned food technique. The plant extracts were tested in the concentration range between 10 to 50 per cent (w/v). The plant extracts mentioned earlier were added to the conical flasks containing previously sterilized and cooled PDA medium so as to obtain final concentration of the extracts. After thorough mixing, 15 ml of the medium was dispensed into the Petri plates. All the plates were rotated evenly for the dispersal of medium. Plates with PDA medium alone was taken as a control plate. An 8mm culture disc of *L. theobromae* from 7 days old culture was placed in each plate and they were incubated at 28±2°C for seven days. Three replications for each concentration were maintained. After seven days, the colony diameter was measured and the minimum inhibitory concentration was determined (Thangavelu et al. 2004). Colony diameter of the test fungus in the treatment in comparison with that of check gave growth inhibition by the following formula:

Per cent inhibition (I) = $I = \frac{C-T}{C} \times 100$

Where,

I = Per cent inhibition

C= Radial growth of the pathogen in control

T= Radial growth of the pathogen in treatment

Evaluation of post harvest dipping of fruits in plant oils and plant extracts against crown rot incidence

To test the efficacy of the plant oils and plant extracts against the crown rot incidence, post harvest treatments with oils and extracts were done. For this evaluation, healthy and uniform sized fruits were selected. The fruits were washed in tap water and then air dried for half an hour. Then the fruits were dipped in respective oils and plant extracts at selected concentration for five min and then dried in air for 12 hrs. The air dried fruits were wounded superficially with sterilized pins (pin-prick method) and they were inoculated by smearing the conidial suspension (1×10^5 spores/ml) of each isolate. The inoculated fruits were wrapped in perforated polythene bags and were incubated at 28±2°C. The disease severity was assessed based on the score chart (Akhtar et al. 2002). On the 12^{th} day and 25^{th} day disease assessment was made and the disease severity (1-9 scale) was determined.

Treatment schedule

T_1 : Basil oil (0.07%)

T_2 : Lemon grass oil (0.09%)

T_3 : Citrodara oil (0.09%)

T_4 : Thyme oil (0.05%)

T_5 : Lavender oil (0.1%)

T_6 : Neem extract (60%) + Tulasi extract (50%)

T_7 : Propicanazole (0.025%)

T_8 : Control

Result and Discussion:

***In vitro* evaluation of different plant oils at different concentration against *L. theobromae* (Lt_8) by poison food technique**

Among the tested plant oils, Thyme oil completely inhibited the mycelial growth of the pathogen at 0.05% concentration followed by basil oil at 0.07%, lemon grass and citroderra at 0.09% concentration oil at 0.09% concentration completely inhibited the mycelial growth of the pathogen, Whereas Geranium oil showed the least inhibition. It inhibited the mycelia growth only upto 83.5%. Similarly maximum reduction in spore germination also found in thyme oil 0.05%. Vijya P. Rabari et al. (2018) reported that *C. cassia* essential oil was inhibitory to the pathogen *C. gleosporoides* which was followed by *C. zeylanicum, S. aromaticum, C. sativus, J. sambac* and *C. deodara* essential oils. The maximum activity was found in *C. cassia* oil (72.66 mm). C. zelynicum was also found to be active, showing zone of inhibition (65.33 mm). Kishore and Pande (2004) reported that

essential oils are odorous and volatile products of plant secondary metabolisms which are rich sources of broad spectrum antifungal agents that inhibit both fungal growth and production of toxic metabolites. Achour Amiri et al. (2008) reported that integrated treatment of heated eugenol oil with lecithin significantly reduced the effect of four apple post harvest pathogens *viz.*, *B. cinerea, M. fructigena, P. expansum, P. vagabunda*. Similarly, Abd-alla et al. (2013) reported that the orange oil at all tested concentrations significantly reduced the fungal linear growth when compared with other tested essential oils.

In vitro evaluation of different plant extracts at different concentration against _L. theobromae_ (Lt_8) by poison food technique

Among the various plant extracts tested, neem extract reduced the growth of mycelium of the pathogen at 50% concentration. It showed 94% reduction over control. It was followed by tulasi extract which showed 93.3% reduction over control at 50% concentration. The spore germination of *L. theobromae* was also tested by cavity slide method. The spore germination was also reduced in the same trend with the mycelial growth of the pathogen (Table 2). Similarly Rabeya et al. (2018) reported that the combination of neem leaf extract (40%) and banana pulp extract (40%) is suitable post harvest treatment for prolonging the shelf life by maintaining the better quality in Amarapali mango fruit. Parsa Tabassum et al. (2018) also reported that combined plant extracts such as combination of guava leaf (20%) and lemon extracts (15%) maintained a positive impact on the desired physico- chemical characteristics during storage of sabri banana at ambient conditions. Similarly, Setu Bazie et al. (2014) reported that out of 21 plants tested, *Prosophis julifera* exhibited the highest antifungal activity with inhibition zone of 30.77 mm diameter against *C. musae*. Shazia Parveen et al. (2014) also reported that among all the plant extracts used, *A. absinthium* at highest concentration (S) brought about highest inhibition in the mycelial growth (73.04%) followed by *P. lanceolata* (71.92%), *T. officinale* (69.67%), *R. obtusifolius* (65.18%) and *M. sylvestris* (62.92%). The present study was related to the above results.

Management of crown rot of banana by post harvest dipping of fruits with selected plant oils and extracts

Among all the treatments *viz.*, basil oil @ 0.07%, Lemon grass oil @ 0.09%, citrodara oil @ 0.09%, thyme oil @ 0.05%, lavender oil @ 0.1%, neem extract @ 60% + tulasi extract @ 50%, propicanazole @ 0.1% tested, the fruits treated with thyme oil @ 0.05% recorded the maximum reduction of growth over control about 83.8% which was followed by propicanazole @ 0.1% which has the percent disease reduction over control about 81.1% .Lavender oil was found to be the least effective one on fruit under ambient storage (Table 3). Lokeshwari (2019) reported that basil oil,

thyme oil and citrodara oil completely inhibited the growth of *L. theobromae* at the lowest concentration of about 0.02%. Jenisha (2018) reported that lemon grass oil @ 0.08% completely inhibited the growth of *L. theobromae.* Divya Jagana et al. (2018) also reported that out of five oils evaluated against anthracnose, lemon grass oil @ 2.0 % and 1.0 % concentration and neem oil which exhibited the disease reduction of 91.89 per cent. These were on par with eucalyptus and neem oil @ 1.0 % concentration exhibited the disease reduction of 89.19 per cent. Similarly, Gatto et al. (2011) reported that in nectarines and apricots, brown rot due to *M. laxa* was completely inhibited by *S. minor* extract after 6 days at 15±1°C. More over extracts of *O. crenata* and *B. officinalis* in nectarines, and *O. crenata* and *P. coronopus* in apricots showed 47% and 40%, and 75% and 57% of lesion diameter reduction, respectively, when compared to control. Similarly Fe Dela Cueva and Mark Angelo Balendres (2018) reported that Citronella essential oil (1.25µl/ml) was comparably effective as other synthetic fungicides and superior to biological fungicide. Similarly Mishra and Dubey (1994) and Adegoke and Odesola (1996) reported that lemon grass oil was comparable with the fungicides

Table 1. Effect of selected plant oil against *L. theobromae* by poison food technique

Plant oil	**Concentration (%)**	**Radial growth of pathogen (mm)**	**Reductionover control (%)**	**Spore germination after 24 hours (%)**	**Reduction over control (%)**
Basil oil	0.01	18.6[d]	79.3	26.33[c]	64.9
	0.03	6.7c	92.5	19.77[c]	73.7
	0.05	1.0b	98.8	16.61[b]	77.9
	0.07	0.0a	100	0.0a	100
	0.09	0.0a	100	0.0a	100
Lemongrass oil	0.01	19.6[e]	78.2	27.09[d]	63.9
	0.03	17.5[d]	80.5	24.32[d]	67.6
	0.05	2.8c	96.8	19.50[c]	74.1
	0.07	1.0b	98.8	16.74[b]	77.7
	0.09	0.0[a]	100	0.0[a]	100
Eucalyptusoil	0.01	40.80[e]	54.6	44.52[e]	40.8
	0.03	36.86[d]	59.0	42.12[d]	43.9
	0.05	16.19[c]	82.0	24.54[c]	67.3

	0.07	8.72[b]	90.3	24.51[b]	67.4
	0.09	4.6a	94.8	21.18[a]	71.8
Citrodara oil	0.01	22.61[e]	74.8	30.76[e]	59.0
	0.03	18.93[d]	78.9	24.53[d]	67.3
	0.05	12.45[c]	86.1	22.14[c]	70.5
	0.07	3.87[b]	95.7	19.80[b]	73.6
	0.09	0.0a	100	0.0a	100
Thyme oil	0.01	17.2[c]	80.8	25.97[e]	65.4
	0.03	4.3b	95.2	22.74[b]	69.7
	0.05	0.0[a]	100	0.0[a]	100
	0.07	0.0[a]	100	0.0[a]	100
	0.09	0.0[a]	100	0.0[a]	100
Lavender oil	0.01	27.33[e]	69.6	31.42[e]	58.2
	0.03	21.32[d]	76.3	25.03[d]	66.7
	0.05	14.95[c]	83.3	23.96[c]	68.1
	0.07	8.24[b]	90.8	23.18[b]	69.1
	0.09	3.96[a]	95.6	19.82[a]	73.6
Geranium oil	0.01	68.82[e]	25.3	68.02[e]	9.5
	0.03	61.76[d]	31.3	51.12[d]	32.0
	0.05	33.53[c]	62.7	39.59[c]	47.3
	0.07	21.32[b]	76.3	27.84[b]	62.9
	0.09	14.85[a]	83.5	21.23[a]	71.7
Control		90	100	75.2	100

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (p=0.05)

Table 2. Effect of selected plant extracts against *L. theobromae* by poison food technique

Plant extracts	**Concentration (%)**	**Radial growth of pathogen (mm)**	**Reduction over control (%)**	**Spore germination after 24 hours(%)**	**Reduction over control (%)**
Adathoda	10	21.4[e]	76.2	30.6[e]	60.1

extract	20	15.94[d]	82.2	27.7[d]	63.88
	30	12.36[c]	86.2	24.1[c]	68.57
	40	11.24[b]	87.5	23.9[b]	68.83
	50	6.73[a]	92.5	20.6[a]	73.14
Nochi extract	10	21.62[e]	75.9	31.2[e]	59.32
	20	18.53[d]	79.4	28.5[d]	62.84
	30	13.32[c]	85.2	25.7[c]	66.49
	40	11.72[b]	86.9	24.4[b]	68.18
	50	9.30[a]	89.6	21.9[a]	71.44
Tulasi extract	10	19.6[e]	78.2	29.4[e]	61.66
	20	14.5[d]	83.2	26.6[d]	65.31
	30	11.2[c]	87.5	23.7[c]	69.10
	40	9.8b	89.1	22.6[b]	70.53
	50	6.0a	93.3	19.4[a]	74.70
Neem extract	10	19.27[e]	78.5	29.1[d]	62.06
	20	13.35[d]	85.1	24.1[c]	68.57
	30	10.34[c]	88.5	22.90[b]	70.13
	40	9.30[b]	89.6	21.38[b]	72.12
	50	5.34[a]	94.0	18.41[a]	75.99
Turmeric extract	10	29.4[e]	67.3	41.68[e]	45.65
	20	26.5[d]	70.5	36.26[d]	52.72
	30	22.9[c]	74.5	31.5[c]	58.93
	40	20.35[b]	77.3	27.7[b]	63.88
	50	15.64[a]	82.6	25.24[a]	67.09
Control		90	100	76.7	100

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (p=0.05)

Table 3: Biological management of crown rot disease of banana by post harvest dipping of fruits with selected plant oils and plant extracts

Treatment no.	Treatments	Percent Disease Index (%)					
		Fruits harvested from trees			Disease reduction over control (%)		
		4DAS	8DAS	12DAS	4DAS	8DAS	12DAS
T1	Basil oil (0.07%)	0.0	3.9c	4.89[c]	100	79.6	80.71
T2	Lemon grass oil (0.09%)	0.0	4.7d	5.48[d]	100	75.4	78.38
T3	Citrodara oil (0.09%)	0.0	5.7e	6.04e	100	70.2	76.17
T4	Thyme oil (0.05%)	0.0	3.1a	3.87[a]	100	83.8	84.73
T5	Lavender oil (0.1%)	0.0	7.73[g]	9.38[g]	100	59.6	62.99
T6	Neem extract (60%) + tulasi extract (50%)	0.0	6.20[f]	6.83[f]	100	67.6	73.05
T7	Propicanazole (0.1%)	0.0	3.61[b]	4.32[b]	100	81.1	82.95
T8	Control	3.6	19.17[h]	25.35[h]	-	-	-

DAS : Days after storage

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (p=0.05)

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