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Chemical composition and antifungal activity of essential oils against die-back of hippeastrum

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ABSTRACT

Die-back is an important foliar disease infecting leaves of hippeastrum plants. The present investigation is based on the efficacy of 17 essential oils which were analyzed for their antifungal activity against *Lasiodiplodia theobromae* causing die-back of hippeastrum. Then the effective essential oil was further subjected to GC-MS for the identification of active chemical compounds. Of these, lemon grass oil was found to be more effective and caused complete growth inhibition of pathogen even at 0.005% concentration compared to other oils tested. Further, the chemical compounds were isolated from lemon grass oil through GC-MS identified 12 compounds. These compounds may be responsible for the inhibition of pathogen.

Key words: *Bio-control*, *Lasiodiplodia theobromae*, essential oils, Gas Chromatography Mass Spectroscopy analysis.

INTRODUCTION

Hippeastrum (*Amarillis* spp.) is one of the most important ornamental bulbous plants. It belongs to the family Amaryllidaceae and it is native to tropical and sub-tropical regions of the America. It has large beautiful flowers in colours of white, blood-red and crimson bloom. In greek it is called as horseman's star/knight's star.

This crop is being subjected to infection by number of fungal and viral diseases. Among the fungal diseases, die-back caused by *Lasiodiplodia theobromae* (Pat.) Griff. and Maubl. is a serious disease. The main characteristics symptoms are starting with drying at the leaf tip. Then drying extends towards the entire leaf. Finally the infected leaves are hang over along the plant. It will lead to reduce flowering and yield. This disease can be controlled by using fungicides. But the indiscriminate use of fungicides overtime to control *L. theobromae* resulted in the accumulation of residual toxicity in soil, environmental pollution, altered biological balance in the soil by killing the non-targeted microorganisms and development of resistance to the pathogen (Bharathi *et al*., 5). Recent efforts have focused on developing environmentally safe, long lasting and effective plant oils for the management of plant diseases. Use of plant oils for the control of plant disease is desirable. Volatile compounds from plants, especially essential oils, have antimicrobial activity against several plant pathogens (Isman, 10). Several workers reported that essential oils were found (Bouchra *et al*., 7; Bhardwaj and Laura, 6; Sukatta *et al*., 18) to possess antifungal activity. Hence, the present study was undertaken.

MATERIALS AND METHODS

Diseased leaves with die-back symptom (Fig. 1) were collected and washed in running tap water, cut into pieces (1 cm long). Then surface-disinfested in 0.5% NaOCl for 3 min. and placed on potato dextrose agar (PDA) in 9-cm Petri plates (Huang and Lin, 9), and incubated at room temperature (28 ± 2°C) for 7 days. Stock cultures were obtained using the hyphal tip transfer procedure, and maintained in tube slants of PDA at 10°C (Ainsworth, 2). The culture identification was done at Indian Type Culture Collection (ITCC), IARI, New Delhi (Reference No. 7677.10). The culture was identified as *Lasiodiplodia theobromae* (Fig. 2). Koch's postulates were demonstrated for the pathogen isolates.

Seventeen plant oils, *viz*., castor (*Ricinus communis*); citrodara (*Eucalyptus citriodora*); citronella (*Cymbopogon nardus*); coconut (*Cocos nucifera*); curry leaf (*Murraya koenigii*); eucalyptus (*Eucalyptus globules*); geranium (*Pelargonium graveolens*); gingelly (*Sesamum indicum*); groundnut (*Arachis hypogea*); lemon grass (*Cymbopogon citratus*); mustard (*Brassica nigra*); neem (*Azadirachta indica*); olive (*Olea europaea*); palmarosa (*Cymbopogon martinii*); pungam (*Pongamia pinnata*); thulasi (*Ocimum sanctum*) and turmeric (*Curcuma aromatica*) oils were obtained from S.R. Biotech International Limited, Attur.

The inhibitory effects of essential oils were evaluated by measuring the *in vitro* linear growth of *L. theobromae.* All the plant oils were tested at 0.005-0.1% concentration. They were added individually to conical

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Fig. 1. Symptoms of die-back of hippeastrum.

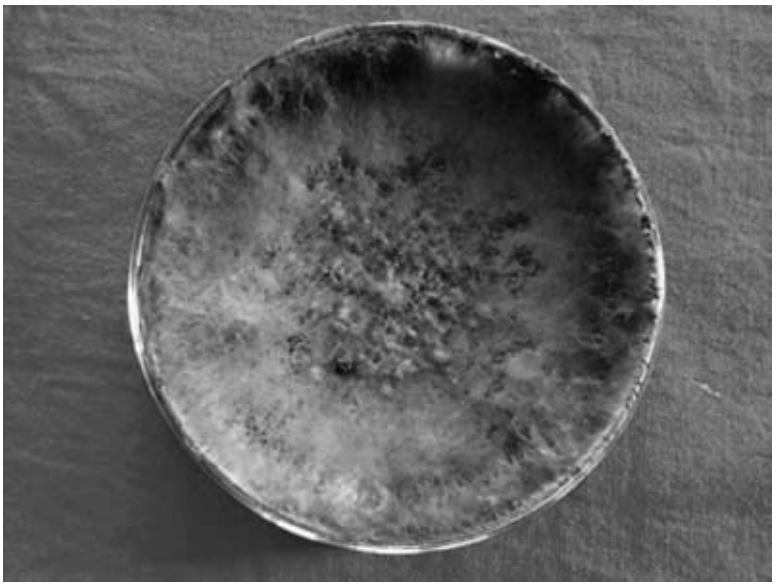

Fig. 2. Axenic culture of *Lasiodiplodia theobromae.*

flasks containing sterilized PDA medium before its solidification to obtain these concentrations (Nehal and El-Mougy, 13). The supplemented media were poured into Petri plates nearly 20 ml per each. Mycelial discs (6 mm) taken from the periphery of an actively growing PDA culture of *L. theobromae* were placed at the centre of the prepared petri plates, then incubated for 3 days at room temperature (28 ± 2°C). The medium without oil was served as control. Four replicates were used for each treatment. The diameter of mycelial growth of pathogen was measured after the incubation period.

Based on the growth inhibition studies lemongrass oil was selected and chemical constituents were determined with a GC Clarus 500 Perkin Elmer Gas chromatography equipped with a mass detector-Turbo mass gold-Perkin Elmer containing a Elite-1 (100% Dimethyl Poly Siloxane), 30 m × 0.25 mm ID × 1 µm df. Conditions employed were the following: Carrier gas, helium (1 ml/min.; Split-10:1); oven temperature program-110°C (2 min.) to 280°C (9 min.); injector temperature (250°C); total GC time (36 min.).

The extract was injected into the chromatograph in 2.0 µl aliquots. The major constituents were identified with the aid of a computer-driven algorithm and then by matching the mass spectrum analysis with that of a library used (NIST Version. 2.0, year-2005). Software used for GC-MS is Turbo mass-5.1. This work was conducted at Indian Institute of Crop Processing Technology (IICPT), Thanjure.

All the experiments were of completely randomized design (CRD) and repeated twice. The data were analyzed using the IRRISTAT version 92-1 program developed by the Biometrics Unit, International Rice Research Institute, Manila, the Philippines. Data were subjected to analysis of variance (ANOVA). The treatment means were compared with Duncan's multiple range test (DMRT) at the 5% significance level (Gomez and Gomez, 8).

RESULTS AND DISCUSSION

The idea of a suitable agricultural practice and environmental protection enhance the importance of plant oils. The adoption of a sustainable agricultural practice, using strategies that are environmentally friendly, less dependent on agricultural chemicals is gaining worldwide recognition. One of the key elements of such sustainable agriculture is the application of plant derived oils. The present study addresses the effect of plant oils against *L. theobromae* causing die-back of hippeastrum. Of the 17 plant oils tested for their effectiveness against mycelial growth of *L. theobromae*, lemon grass oil was found to be more effective and caused complete growth inhibition of pathogen even at 0.005% concentration (Table 1). The concentration of 0.006% required complete growth inhibition of pathogen by citronella, geranium and palmarosa oil, while concentrations of citrodara and thulasi oils required 0.007 and 0.006% concentration for the complete inhibition on the growth of pathogen. At 0.04% concentration, *Eucalyptus* and turmeric oil caused 100% mycelial growth inhibition of pathogen. The concentrations of 0.5% required complete growth inhibition of pathogen by curry leaf and olive oil. At 1.0% concentration mustard, neem, castor, gingelly, coconut, pungam and groundnut oil caused 100% growth inhibition of pathogen.

Similarly, Sangeetha *et al*. (15) reported that out of 14 plant oils tested, *Cymbopogon citratus*, *C. martini*,

Table 1. Minimum inhibitory concentration of essential oils on the mycelial growth of *L. theobromae.*

Plant oil	Mycelial growth of pathogen in mm*						
Conc. (%)	0.005	0.006	0.007	0.04	0.5	0.6	1.0
Castor	9.0f	9.0d	9.0c	9.0b	7.3c	6.7bc	0.0a
Citrodara	3.8d	1.3b	0.0a	0.0a	0.0a	0.0a	0.0a
Citronella	1.5b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Coconut	9.0f	9.0d	9.0c	9.0b	7.7cd	6.3b	0.0a
Curry leaf	9.0f	9.0d	9.0c	9.0b	0.0a	0.0a	0.0a
Eucalyptus	7.1e	6.4c	6.1b	0.0a	0.0a	0.0a	0.0a
Geranium	1.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Gingelly	9.0f	9.0d	9.0c	9.0b	7.3c	6.0b	0.0a
Groundnut	9.0f	9.0d	9.0c	9.0b	7.7cd	6.3b	0.0a
Lemon grass	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Mustard	9.0f	9.0d	9.0c	9.0b	7.3c	6.7bc	0.0a
Neem	9.0f	9.0d	9.0c	9.0b	7.0c	6.0b	0.0a
Olive	9.0f	9.0d	9.0c	9.0b	2.3b	0.0a	0.0a
Palmarosa	2.0c	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Pungam	9.0f	9.0d	9.0c	9.0b	7.3c	6.3b	0.0a
Thulasi	2.7c	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Turmeric	7.0e	6.4c	6.1b	0.0a	0.0a	0.0a	0.0a

* Mean of four replications; 0.0 = 100% growth inhibition.

In a column, means followed by a common letters are not significantly different at 5 per cent level by DMRT.

C. nardus and *Pelargonium graveolens* oils completely arrested the mycelial growth of *L. theobromae* and *C. musae* (crown rot of banana) at their lowest concentration compared to other oils tested. Tripathi and Shukla (19) revealed that geranium, mint, palmarosa and thyme oils at 200,100,100 and 50 ppm were found to exhibit absolute fungitoxic activity against *B. theobromae* causing stem end rot of mango. Lambat *et al*. (12) reported that the concentrations of lemongrass oil required for the complete growth inhibition of *C. gloeosporioides* at 0.03-0.66% concentration. Abeywickrama *et al*. (1) observed that *Cympopogon nardus*, *Ocimum basilicum*, *Eucalyptus citriodara* and *Elettaria cardamomum* oils were found to be effective against *L. theobromae* and *C. musae* at 0.03-0.665%. The plant oils having antifungal compounds which might be responsible for the strong inhibition of pathogen (Singh *et al*., 16; Wang *et al*., 20). The results of this study confirmed that activity of oils was found to be concentration dependent, while microorganisms showed differential sensitivity to the different oils tested.

On the basis of performance of plant oils in the preceding *in vitro* studies, lemon grass oil was selected to determine the nature of chemical compound by GC-MS analysis. The results revealed that 12 compounds were identified from lemon grass oil. The molecular weight, name of the compound, chemical formula, retention time and peak area percentage was given in Table 2. However there is no report is available on the identification of chemical constituents of lemon grass oil through GC-MS and tested for its efficacy against *L. theobromae*. Various workers reported that the chemical compounds present in the lemon grass oil were analysed through GC-MS (Kasali *et al*., 11; Saleem *et al*., 12; Sridhar *et al*., 17; Barbosa *et al*., 4; Anaruma *et al*., 3).

In conclusion, plant oils such as lemon grass, citronella, geranium and palmarosa were more effective and caused complete growth inhibition of die-back pathogen at minimum concentration. Further, the chemical compounds were detected from lemongrass oil through GC-MS analysis identified 12 compounds. In future, the purification of particular compound must be tested against pathogen and that particular compound might be responsible for the inhibition of pathogen.

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Table 2. Major chemical compounds detected in the lemon grass oil used in this study.

RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
8.98	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethynone	$C_{13}H_{18}O_2$	206	0.33
10.00	Sucrose	$C_{12}H_{22}O_{11}$	342	3.71
10.96	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.62
13.07	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	67.18
14.41	3,7,11,15-Tetramethyl-2-hexadecan-1-ol	$C_{20}H_{40}O$	296	3.39
16.16	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.62
18.44	Phytol	$C_{20}H_{40}O$	296	0.74
18.76	9,12-Octadecadienoic acid (z,z)	$C_{18}H_{32}O_2$	280	1.45
18.86	9,12,15-Octadecatrienoic acid, (z,z,z)	$C_{18}H_{30}O_2$	278	7.07
19.18	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.60
24.18	Phenol, 2,4-bis(1-phenylethyl)	$C_{22}H_{22}O$	302	0.60
24.78	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	4.69

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