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Determination of Antifungal Activity against Phytopathogenic Fungi by Essential Oils Extracted from some Medicinal Plants

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KEY WORDS

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ABSTRACT Antifungal activity of essential oils of the common medicinal plants against phytopathogenic fungi was determined. *Litsea cubeba* was very effective in controlling the growth of phytopathogenic fungi. *L. cubeba* oil at 500 ppm in culture media restricted growth of *Alternaria alternata*. *Mucor hemilis*, *Helminthosporium solani*, and *Humicola grisea* could grow only after 92 h of incubation. Minimum Inhibitory Concentration (MIC) of *L. cubeba* essential oil was 200 ppm for *H. solani* and *B. cinerea*, 300 ppm for *A. alternate*, and *H. grisea*. The MIC for *Lantana camera* and *Piper mullesua* against phytopathogenic fungi was 200 and 400 ppm. In broth culture at 500 ppm treatment of essential oils, the biomass of *A. alternata* ranged from 0 to 81 mg after 288 h of incubation. In case *M. hemilis*, it was between 0 and 50 mg, *H. solani* between 0 and 51 mg, *H. grisea* between 0 and 94 mg and *B. cinerea* 0 and 66 mg after a similar duration. In controls (untreated) biomass of various fungi ranged from 157 to 186 mg. *Ageratum conyzoides* effectively controlled *A. alternata* and *M. hemilis. Lantana camara* was effective against *A. alternata, M. hemilis,* and *Humicola grisea*. Similarly, *P. mullesua* essential oil was found to limit the growth of *Botrytis cinerea, M. hemilis, H. solani,* and *H. grisea*.

INTRODUCTION

Plant research as a source of new bio-molecules for human disease control has increased rapidly over the past few years (Li *et al*., 2020; Zhu *et al*., 2020). Conventionally, plants have been well exploited by humans in treating human diseases (such as Ayurveda), but little is known about the exploitation of plants to control plant diseases, especially against phytopathogenic fungi. Several fungi, which cause significant damage to stored food products, are usually controlled by synthetic chemicals, which are considered effective and efficient (Galvano *et al*., 2001; Valková *et al*., 2021). Continued use of these fungicides in practice began to expose non-biodegradability and is known to have residual toxicity (Pimentel and Levitan, 1986). Therefore, the use of plant metabolites in the control of plant diseases has become an integral part of Integrated Pest Management (Robledo *et al*., 2018), as plant metabolites are eco-friendly. There has been a gradual renewal of interest in using medicinal plants with aromatic plants because the products derived from plants have been reported to be safe and have no side effects (Hanen *et al.*, 2021).

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Essential oils play a key role in protecting plants such as antibacterial, antivirals, antifungals, insecticides, and herbivores by reducing their appetite for such plants (Arasu *et al*., 2019). Essential oils extracted from various aromatic plants represent a necessary part of traditional pharmacopoeia, especially in tropical and subtropical countries (National Pharmacopoeia Commission, 2015). They are unchanging and rare in color, dissolved in lipids, and soluble in natural solvents. They can occur in all fragrant plant parts, that is, leaves, flowers, leaves, stems, shoots, seeds, fruits, roots, wood, or bark, as secondary metabolites stored in secret cells, cavities, trenches, epidermis cells, or gland trichomes (Bakkali *et al*., 2008). A plant with a high content of essential oils may provide alternatives to current regulatory agents because the composition is rich in bioactive chemicals (Yong *et al*., 2021). Literature reveals that essential oils found in plants have antifungal (Yong *et al*., 2021), antimicrobial (Mangalagiri *et al*., 2021; Ghavam *et al*., 2021), and nematicidal properties (Pandey *et al*., 2000). However, their importance in correcting phytoremediation is not widely discussed.

India is heavily dependent on agriculture, and phytopathogenic fungi mainly contribute to crop losses, low productivity, farm degradation, and crop loss/post-harvest losses. *Ageratum conyzoides*, *Lantana camara*, and *Litsea cubeba* are common weeds found in abundance in India. *Piper mullesua* is a perennial plant cultivated by local tribes for commercial purposes in Arunachal Pradesh. Therefore, these plants were selected for evaluation against phytopathogenic fungi that attack vegetables and fruits and many shrubs and trees.

MATERIALS AND METHODS

Collection of Plant Material

Plants with ethnomedicinal properties were selected for the current study. Fresh and healthy leaves of *A. conyzoides*, *L. cubeba*, L. *camara*, and *P. mullesua* were collected, thoroughly rinsed (2-3 times) in running water and finally with clear distilled water. After washing, the leaves were dried in the shade, ground into a powder, and subjected to oil extraction. For future use, a voucher template for these plants was submitted to Department of Botany, Rajiv Gandhi University, Arunachal Pradesh, India.

Extraction of Essential Oils

The dried aerial parts of the plants were hydro distilled in Clevenger apparatus (Borosil) with pure water (1: 3 w/v) for 5 h (Tripathi, 2005). Extraction was done several times to collect the required amount of essential oil. The oil was separated from the water using a disposable syringe (1 mL) and a glass filter tube. The oil was stored in a sealed glass bottle in the refrigerator at 4°C until further use. Oil yields varied from 1 to 2 mL depending on the plant used. Essential oils were not dried and used directly after the required purification.

Phytopathogenic Fungus Collection

In the study, five phytopathogenic fungi *Alternaria alternata*, *Mucor hemilis*, *Helminthosporium solani*, *Humicola grisea*, and *Botrytis cinerea* were obtained from the Institute of Microbial Technology, Chandigarh, India. Recovery numbers for the above samples are given in Table 1. The culture of phyto-pathogenic fungi was grown on potatoes dextrose agar medium (200 g mashed potatoes in 1 L of distilled water, 15 g agar, 20 g dextrose, pH ± 5.6) and stored at –20°C with 20% glycerol water. The incubation period for different test materials was measured on the basis of the fungal growth pattern in selected growth sources. A 7-day-old fungal inoculum was used during the investigation in each case.

Minimum Inhibitory Concentration (MIC)

The micro-dilution method was used to determine MIC (NCCLS, 1999). All tests were performed on Czepak Dox Agar Media where dilutions were made with Tween-80 to obtain a final concentration of 0.5% (v/v). Media (20 mL) were poured into each plate and dried. Petri plates were traditionally inoculated and distributed evenly using a sterile glass dispenser. The final concentration of each type of mold was adjusted to 0.5 OD before use. Serial dilution of essential oils was prepared in a test tube to obtain 100, 200, 300, 400, 500, and 600 ppm concentrations. Plates were incubated at 27°C for 7 days. The minimum amount of oil that restricted or inhibited the growth of particular fungi was considered as MIC. However, other parameters were quantified at 500 ppm, the concentration at which the inhibition was recorded in all treatments.

Antifungal Activity Test

All plants' average concentration of essential oils was selected for antifungal properties based on the MIC results. The antifungal properties of essential oils of *A. conyzoides*, *L. camara*, *L. cubeba*, and *P. mullesua* were determined using the following techniques.

Antimycotic assay with disc diffusion technique

The oils were tested for their antifungal activity against *A. alternata*, *M. hemilis*, *H. solani*, *H. grisea*, and *B. cinerea*, by disk diffusion method (Boyer, 1976). The 7-day-old traditional mycelial mat was diluted, hung in a standard saline solution and filtered with aseptically dyed glass. Colon-forming units/mL for mold testing were determined, and the test inoculum was adjusted to 0.5 OD at 660 nm using distilled water. Inoculum (1 mL) was passed over a plate of Czapek's dox agar and dispensed using a sterile glass

Table 1. Various plants evaluated in the study and their ethnomedicinal uses by different tribes of North East India

Plant species	Tribe	Application	References
Ageratum conyzoides Linn.	Lepcha Tribe of Sikkim	Part use: Leaf	Pradhan and Badola (2008), Kala (2005)
	Arunachal Tribe	Disease: Cut, wounds, diarrhea, dysentery, intestinal colic with flatulence	Sajem and Gosai (2006)
	Jaintia Tribe	Part use: Leaf	
		Disease: Cuts, wounds	
		Part use: Leaf	
		Disease: Cuts, wounds	
Lantana camara Linn.	Lepcha Tribe of Sikkim	The juice of crushed leaves is applied to the fresh cut and wounds to heal. Crushed leaves are tied over the sprain to relieve pain.	Pradhan and Badola (2008)
Litsea cubeba (Lour) Pers	Arunachal tribe	Fresh ripe and unripe fruits are taken as a remedy for cold and cough and also for good sleep. Seeds are chewed in case of thread worm infection.	Srivastava and Adi (2009), Bhuyan (2007)
Piper mullesua Buch.-Ham.	Arunachal tribe	Leaves and inflorescence are used for cold and cough, stomach upset, case of thread worm infection.	Hussain and Hore (2008)

dispenser. As Connor and Beachat (1984) defined, sterile filters were immersed in essential oils. The concentration of essential oils was adjusted by dissolving the required amount in 0.5 mL of 0.1% Tween 80 and then mixed with 9.5 mL of sterile water. These containers were placed at 27°C, and the blocking area was recorded. The test was performed three times. The standard antibiotic Fluconazole (HiMedia) was used as an adequate control. Negative control included 0.5 mL water with 0.1% Tween 80. The growth barrier was measured using the HiAntibiotic zone scale-C (HiMedia).

Antimycotic assay by biomass measurement

The fungal activity was also evaluated by measuring dry weight biomass after normal growth intervals (i.e., 48–288 h). Peptone broth media (Peptone 0.5%, dextrose 1%, yeast extract 0.1%, $MgSO_4$ 0.05%, $CuSO_4$ 0.03%, and Tween-80 0.5%) was used for mildew. Broth media (10 mL) were added to various test tubes and sterilized for 30 min in an autoclave. After autoclave, test tubes were cooled, and essential oil was added at a maintained concentration of 500 ppm for each tube. In addition, as an inoculum, 1 mL of culture suspension (OD 0.5) was added and mixed well. Test tubes were mounted on an incubator shaker (Scigenics) at 27°C with continuous movement at 150 rpm. After the required incubation, the cultures were extracted and filtered using Whatman filter paper number 1, and the suspension dried at 50°C for 3–4 h. The dry weight of the mold was determined by removing the last dry weight of the filter paper from the first dry weight. Biomass was measured after 48 h of incubation to 288 h.

Antimycotic testing by spectrophotometric method

Although the spectrophotometric method is commonly used for bacterial growth analysis, few reports measure optical density to study the antifungal activities of essential oil (Wilson *et al*., 1997). In the present study, antifungal activity was evaluated by measuring conidia and mycelial growth at different incubation periods starting from 72 to 360 h. Peptone broth media (Peptone 0.5%, dextrose 1%, Yeast extract 0.1%, $MgSO_4$ 0.05%, $CuSO_4$ 0.03%, and Tween-80 0.5%) were used as growth media. Broth media (10 mL) were added to separate test tubes and sterilized for 30 min. After autoclaving, test tubes were cooled, and essential oil was added to each tube, maintaining 500 ppm concentration. After proper mixing, 1 mL of culture suspension (OD 0.5) was added and incubated in an incubator shaker at 27°C with continuous shaking at 150 rpm to avoid clumping of fungal mycelia. Growth was estimated by measuring optical density at 660 nm. Absorbance was calculated after 10 s of pouring the broth culture in a cuvette to avoid estimation error.

Determination of Strength of Essential Oils

The efficiency of essential oils was graded into five categories based on their durability compared to positive control. The strength of the selected essential oils was tested based on the durability of their action against phytopathogenic fungi. The grading was done as:

(1) Very strong: *Inhibition zone 3–4 times larger than the positive control*. (2) Strong: *Inhibition zone 2–3 times larger than the positive control*. (3) Moderate: *Inhibition zone 1–2 times larger than the positive control*. (4) Weak: *Inhibition zone less than the positive control* (5) No action: *No inhibition zone.*

RESULTS

Different MIC values were recorded for each phytopathogenic fungus (Table 2). *Litsea cubeba*,

Table 2. MIC (ppm) of essential oils against phytopathogenic fungi

Sample	Accession No.	*Ageratum conyzoides*	*Lantana camara*	*Litsea cubeba*	*Piper mullesua*
Alternaria alternata	MTCC 149	500	200	300	400
Mucor haemilis	MTCC 157	400	300	400	200
Helminthosporium solani	MTCC 1899	300	300	200	300
Humicola grisea	MTCC 352	400	400	300	400
Botrytis cinerea	MTCC 359	400	400	200	300

MIC: Minimum inhibitory concentration

Table 3. Inhibition in fungal growth (mm) by essential oil of plants at 500 ppm concentration. The zone is excluding the size of paper disc (±S.E.)

Phyto pathogens Accession No.	Incubation period	Inhibition zone size (mm)				
		+ve control*	*Ageratum conyzoides*	*Lantana camara*	*Litsea cubeba*	*Piper mullesua*
Alternaria alternata	6 days	10±1.00	10±1.00	22±2.00	40±0.60	28±2.00
MTCC 149	12 days	10±1.00	10±1.00	22±2.00	40±0.60	26±1.50
	18 days	10±1.00	8±1.00	18±1.80	40±0.62	20±0.60
	24 days	10±1.20	0±0.00	18±1.80	40±0.62	18±2.52
	32 days	10±1.20	0±0.00	15±1.00	40±0.62	18±1.53
Mucor heamalis	6 days	12±1.20	18±2.00	22±1.75	40±0.62	18±1.58
MTCC 157	12 days	10±1.20	18±1.60	22±1.80	40±0.62	18±1.52
	18 days	10±1.20	18±1.60	18±1.80	24±0.15	18±1.50
	24 days	9±0.50	16±1.50	16±1.65	16±0.15	18±1.50
	32 days	9±0.50	16±1.50	16±1.65	10±0.50	15±1.10
Helminthosporium solani	6 days	12±1.00	12±1.00	15±1.30	40±0.60	19±1.60
	12 days	12±1.00	12±1.00	15±1.30	40±0.60	19±1.60
MTCC 1899	18 days	12±1.00	12±1.00	15±1.30	40±0.60	19±1.60
	24 days	12±1.00	12±1.00	15±1.30	40±0.60	15±1.50
	32 days	10±1.00	12±1.00	15±1.30	36±0.55	15±1.50
Humicola grisea	6 days	14±1.20	16±1.10	22±2.10	40±0.66	26±2.00
MTCC 352	12 days	14±1.00	16±1.10	22±2.10	40±0.66	26±2.10
	18 days	14±1.20	16±1.10	22±2.10	38±0.68	26±2.10
	24 days	14±1.00	14±1.00	22±2.20	36±0.55	26±2.10
	32 days	14±1.30	12±1.00	22±2.10	34±0.40	19±1.75
Botrytis cinerea	6 days	14±1.20	16±1.00	24±2.40	40±1.66	20±1.75
MTCC 359	12 days	14±1.00	16±1.20	19±2.00	40±1.66	20±1.80
	18 days	14±1.20	16±1.20	19±2.00	40±1.55	20±1.80
	24 days	14±1.20	16±1.20	19±2.00	40±1.65	19±1.75
	32 days	14±1.20	16±1.20	16±1.50	30±1.50	0±0.00

*Fluconazole is used as Positive control

P. mullesua, and *L. camara* essential oils showed remarkable effects on selected phytopathogenic fungi selected up to 500 ppm. *L. cubeba* was inhibitory at 200–400 ppm and was pathogen-specific. However, *A. conyzoides* oil was active at 300–500 ppm for various fungal pathogens. Overall, 500 ppm was considered the best for the antifungal activity of essential oils. *L. cubeba* oil showed a blocking effect 3–4 times compared to controls. The oil completely inhibited the growth of *A. alternata. M. haemalis*, *H. solani*, and *Humicola grisea* restricted the fungal growth up to 92 h at 500 ppm concentrations. However, *B. cinerea* showed signs of development after 216 h of incubation. *A. conyzoides* oil

showed a positive effect against *A. alternata* and *M. hemilis*. *L. camara* essential oil was effective against *A. alternata*, *M. hemilis*, and *H. grisea*. *P. mullesua* oil showed a high inhibition in the case of *H. grisea*, *A. alternata*, and *M. hemilis* (Table 3).

Biomass analysis showed complete inhibition of the growth of fungi by *L. cubeba* essential oil (Table 4). *L. cubeba* oil was effective against all selected phytopathogenic fungi. Other essential oils were comparatively less effective against selected phytopathogens. *A. conyzoides* performed best in *A. alternata* while the *Lantana camera* limited the biomass of *H. grisea* and *A. alternata*. Similarly, *P. mullesua* oil was very effective in controlling the biomass of *A. alternata*, *H. solani*, and *M. hemilis* (Table 4).

Under spectrophotometric measurements, phytopathogenic fungi have shown a slight growth in the *L. cubeba* oil treated culture, followed by *P. mullesua* and *A. conyzoides* oils. *A. alternata* was more sensitive to *L. cubeba* and *A. conyzoides* oils. *H. grisea* and *B. cinerea* were more susceptible to *P. mullesua* oil. However, *H. solani* was more sensitive to the essential oils of *A. conyzoides* and *M. hemilis* against *L. camara* than other species tested (Table 5).

Most importantly, *L. cubeba* was an effective antifungal agent followed by *P. mullesua* and *L. camara*. *A. conyzoides* did not work well among the selected essential oils. The intensity of *L. cubeba* oil action was highest in all phytopathogens tested except for *M. haemilis*, when its activity decreased after 18 days of incubation (Fig. 1). The *L. camera* was effective against *M. hemilis* and *Humicola grisea* up to 32 days of incubation. Similarly, *P. mullesua* effectively controlled *A. alternata*, *M. hemilis*, and *H. grisea*. However, *A. conyzoides* had intense action against *M. hemilis* for up to 18 days of incubation.

Table 4. Effects of essential oils (500 ppm concentration) on the biomass (mg) of fungi at different intervals of time (h) in broth culture (±S.E.)

Sample	Incubation period (h)	*Ageratum conyzoides*	*Lantana camara*	*Litsea cubeba*	*Piper mullesua*	Negative control
Alterneria alternata	48	22±1.50	15±1.50	0±0.00	10±1.50	50±2.00
	92	31±1.70	16±1.50	0±0.00	20±0.50	81±2.00
	144	80±2.00	33±1.90	0±0.00	22±1.50	142±1.50
	216	83±2.00	31±1.90	0±0.00	25±1.50	153±2.50
	288	81±2.00	30±1.70	0±0.00	26±0.50	180±2.50
Mucor haemilis	48	33±1.50	17±1.50	0±0.00	11±1.50	57±1.00
	92	31±1.50	19±1.00	0±0.00	22±1.00	63±2.00
	144	52±1.50	31±1.50	11±1.00	31±1.00	124±2.50
	216	54±2.00	33±1.50	13±1.00	34±1.50	151±3.00
	288	50±2.00	31±1.50	23±1.80	30±1.50	166±2.50
Helminthosporium solani	48	30±1.50	19±1.00	0±0.00	11±1.50	71±2.00
	92	32±1.50	23±1.00	0±0.00	12±1.50	109±1.50
	144	51±2.30	30±1.50	10±0.50	36±2.00	122±3.00
	216	51±2.30	32±1.50	12±0.50	35±2.00	143±3.00
	288	50±2.30	61±2.00	61±2.00	37±1.50	162±2.50
Humicola grisea	48	42±1.50	13±0.50	0±0.00	19±1.50	86±1.40
	92	53±2.30	17±1.50	0±0.00	20±1.50	94±1.50
	144	67±2.00	18±1.50	12±1.00	22±1.50	133±2.00
	216	94±2.00	32±0.50	25±1.50	26±1.50	162±2.00
	288	60±2.00	46±0.50	43±2.00	26±0.50	186±2.00
Botrytis cinerea	48	44±1.50	20±1.00	0±0.00	17±1.50	72±1.00
	92	41±1.50	22±1.50	0±0.00	18±0.50	88±1.00
	144	47±2.70	28±1.00	0±0.00	31±0.50	123±1.50
	216	40±2.60	28±1.00	0±0.00	33±0.50	141±1.50
	288	40±2.60	40±1.50	35±1.50	66±1.00	157±1.50

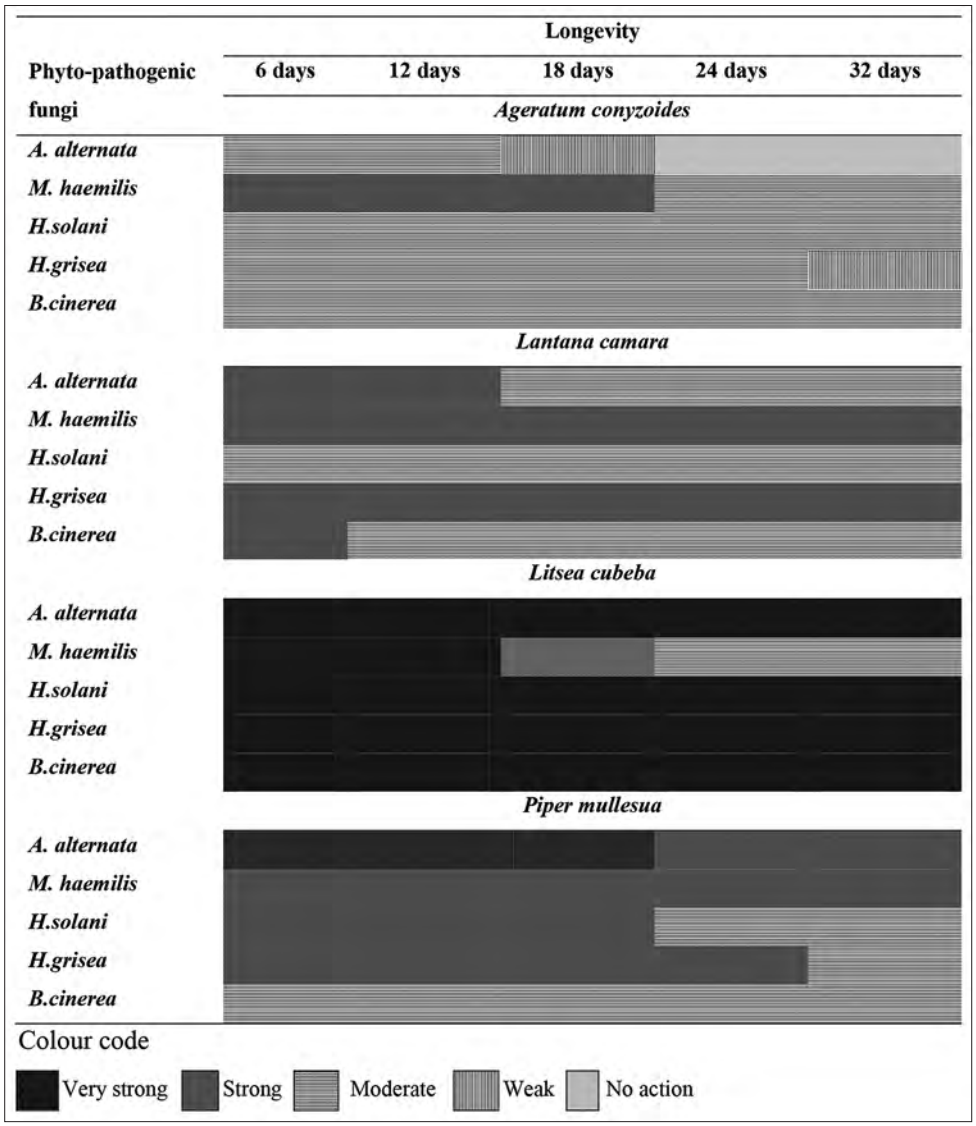

Fig. 1. Grading of the strength of four essential oil against phytopathogenic fungi. ***A. alternate: Alternaria alternata, M. hemilis: Mucor hemilis, H. solani: Helminthosporium solani, H. grisea: Humicola grisea, B. cinerea: Botrytis cinerea.***

DISCUSSION

Among the essential oils tested, *L. cubeba* was the best antifungal product. The importance of *L. cubeba* as an antifungal agent has been reported against common human fungal species such as *Aspergillus fumigatus*, *Trichophyton mentagrophytes* var. *interdigitale*, and *Candida albicans*. The citral compound produced by *Litsea* has antifungal and antitumor properties and helps prevent experimental atherosclerosis (Chen *et al*., 1994; Wang *et al*., 1999). However, its importance as an anti-phytopathogenic agent has not yet been reported. MIC of *L. cubeba* has been reported against *Aspergillus niger, Penicillium citrinum,* and *Trichoderma viride*; however, there is no record of its action against *A. alternata, M. hemilis, H. solani, H. grisea,* and *Boticola grisea.* Variable MICs for *L. cubeba* depended on metabolites and the types of molds tested (Chiang *et al.*, 2007). Si *et al*. (2012) reported 59 compounds from *L. cubeba* oil, out of which dominant components were monoterpenes (94.4–98.4%), represented mainly by neral and geranial (78.7–87.4%), and D-Limonene as a minor constituent (0.7–5.3%). Several components were only detected in specific regions, and compounds such as o-cymene and eremophilene have never been reported. Su and Ho (2012) identified main components in oil as β-caryophyllene (13.0%), τ-cadinol (11.1%), α-cadinol (8.6%), α-humulene

Table 5. Effect of essential oils (500 ppm) on the growth of fungi under the broth culture at different periods of incubation measured spectrophotometrically

Essential oils	Fungi	72 h	144 h	216 h	288 h	360 h
Negative control	*A. alternata*	0.34	0.74	0.79	0.86	1.07
	M. haemilis	0.64	0.7	0.77	0.83	1.08
	H. solani	0.64	0.69	0.75	0.82	1.19
	H. grisea	0.78	0.81	0.86	1.04	1.34
	B. cinerea	0.729	0.8	0.88	0.95	1.31
Ageratum conyzoides	*A. alternata*	0.176	0.232	0.461	0.49	0.613
	M. hemilis	0.125	0.622	0.697	0.877	0.905
	H. solani	0.005	0.087	0.278	0.295	0.401
	H. grisea	0.208	0.297	0.26	0.344	0.506
	B. cinerea	0.377	0.354	0.447	0.604	0.78
Lantana camara	*A. alternata*	0.14	0.141	0.155	0.193	0.214
	M. hemilis	0.024	0.046	0.065	0.091	0.204
	H. solani	0.102	0.102	0.124	0.212	0.272
	H. grisea	0.044	0.16	0.101	0.250	0.295
	B. cinerea	0.024	0.041	0.087	0.152	0.306
Litsea cubeba	*A. alternata*	0.008	0.013	0.026	0.059	0.108
	M. hemilis	0.012	0.016	0.027	0.125	0.315
	H. solani	0.031	0.033	0.056	0.059	0.209
	H. grisea	0.022	0.053	0.089	0.102	0.246
	B. cinerea	0.021	0.124	0.156	0.357	0.588
Piper mullesua	*A. alternata*	0.020	0.023	0.121	0.222	0.377
	M. hemilis	0.016	0.019	0.167	0.237	0.311
	H. solani	0.030	00.37	0.153	0.265	0.319
	H. grisea	0.011	0.016	0.053	0.069	0.492
	B. cinerea	0.004	0.014	0.118	0.137	0.249

A. alternate: Alternaria alternata, M. hemilis: Mucor hemilis, H. solani: Helminthosporium solani, H. grisea: Humicola grisea, B. cinerea: Botrytis cinerea

(7.5%), α-pinene (7.0%), globulol (6.6%), and β-eudesmol (6.1%). Yang (2010), through a preliminary bioassay study, showed *L. cubeba* oil had suitable fungicidal activities against *Sclerotinia sclerotiorum*, *Thanatephorus cucumeris*, *Pseudocercospora musae,* and *Colletotrichum gloeosporioides* at the concentration of 588 and 272 μM. The essential oil had good fungicidal activities against *T. cucumeris* and *S. sclerotiorum*, with IC_{50} values of 115.58 and 151.25 μg/mL, respectively. *B. cinerea* is a parasite that attacks the most common fruits such as grapes, oranges and kiwi, etc. The fungus attacks the fruit with a picking wound and grows on the fruit even if stored at 0°C. The ripe fruit's effects could be seen, especially during post-harvest operations.

The present results show that the essential oil of *L. camera* inhibited the growth of phytopathogenic fungi. The MIC for *L. camera* oil ranged from 200 to 400 ppm. The antifungal properties of *L. camara* have been previously reported against fungal species such as *A. alternata*, *Curvularia lunata*, *Fusarium equiseti*, *Botryodiplodia theobromae*, *C. albicans*, and *Trichophyton mutagraphytes*, using filter paper disk diffusion method (Begum *et al.*, 2007; Sharma and Kumar, 2009). However, the phytopathogenic fungus selected in the current study is different from the previously reported species except for *A. alternata*. The antimicrobial properties of *L. camara* are due to its extracted leaf containing alkaloids, phenolics, terpenoids, phytosterols, tannins, and saponins (Ganjewala *et al*., 2009). However, these activities were attributed to the extracts from leaves and roots (Mdee *et al.*, 2009). Supporting current research, Saksena and Tripathi, (1985) reported the leaf extract of *L. camara* as an effective biopesticide to suppress spore germination of *A. alternata*. However, essential oils from *L. camara* are reported to be effective against a variety of pathogenic bacteria at higher concentrations of 1600 to 4000 ppm (Dharmagadda *et al.*, 2005). In contrast, current studies have shown that *L. camara* essential oil is effective at concentrations of 500 ppm.

Essential oil of *Ageratum conyzoides* also inhibited the growth of phytopathogenic fungi. In broth and solid culture growth of phytopathogenic fungi was always lesser than the controls. A literature survey reveals that antifungal activity of *A. conyzoides* against fungi *M. hemilis, H. solani,* and *H. grisea* has not been reported. The essential oils of *A. conyzoides* have been reported to inhibit the growth of *A. alternata* and *Aspergillus flavus* (Singh and Gupta, 1992; Kumar *et al*., 2010). Kamboj and Saluja (2008) found a wide range of chemical compounds, including alkaloids, coumarins, flavonoids, chromenes, benzofurans, sterols, and terpenoids from *A. conyzoides* essential oil. Inhibitory impact attributed to flavonoids, chromenes, benzofurans, sterols, and terpenoids in the oil. *A. conyzoides* leaf extracts are also effective against specific pathogens (Wiart *et al*., 2004; Mahato and Chaudhary, 2005). *A. conyzoides* causes inhibition of 60% growth of *B. cinerea* at 500 ppm concentration (Tripathi *et al*., 2008). *A. conyzoides* effectively prevented mycelial growth and germination of *D. bryoniae* spores (Fiori *et al*., 2000). *A. alternata* can also be controlled using *Cuminum cyminum* essential oil, *Luvunga scandens* and *Caryophyllene* fruit oil and *Ocimum sanctum* leaf oil at 1:100 to 1:200 dilutions (Garg and Siddique, 1992). Hadizadeh *et al*. (2009) also reported a few essential oils from medicinal plants with antifungal properties against *A. alternata. Alternaria* brown spot of tangerines and *Alternaria* leaf spot of rough lemon is a significant disease in India. Using the essential oils suggested above can reduce its effects on the plant.

Essential oil of *P. mullesua* restricted the growth of tested phytopathogenic fungi, and MIC ranged between 200 and 400 ppm. Studies on *P. mullesua* as an antifungal agent have not been reported; however, other piper species such as *Piper betel* and *Piper nigrum* do control several species of bacteria and fungi (Cowan, 1999; Erturk, 2006). Its insecticide properties of extracts and essential oils as antimicrobial compounds in the medical and agriculture industry are known (Srivastava *et al*., 2001).

In conclusion, essential oils from *Ageratum, Lantana,* and *P. mullesua* are effective against selected fungal pathogens, while *L. cubeba* was effective against all fungal pathogens with exceptional action levels. The essential oils of *Ageratum, Lantana, L. cubeba,* and *P. mullesua* thus can be used as a biocontrol agent in integrated pest management strategies, expressly when essential oils are widely accepted as biodegradable and eco-friendly fungi toxicants worldwide (Tripathi and Dubey 2004).

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