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Evaluation of essential oil of *Cymbopogon distans* and *Cinnamomum tamala* against plant pathogenic fungi

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ABSTRACT

Resistance to conventional fungicides causes the poor disease control of agriculture plant essential oils have a great potential as novel fungicide sources for controlling pathogenic fungi. In this study antifungal activity of the essential oil of *Cymbopogon distans* and *Cinnamomum tamala* were evaluated *in vitro* against ten plants pathogenic fungal activity was evaluated with three replicates. The result showed *C. distans* and *C. tamala* essential oil with maximum zone of inhibition against *Fusarium* sp. (12.53 ± 0.97 mm) and *P. aurantiogriseum* (12.06 ± 0.52 mm) while minimum activity was seen against *R. solani* (6.83 ± 0.41, 6.16 ± 0.16 mm) zone of inhibition respectively. The highest efficacy was observed for *C.distans* essential oil where the MIC values 0.625 mg/ ml against *A. flavus* and *Pythium* sp. So, plant essential oils have the potential to replace the synthetic fungicides in the management of postharvest diseases of fruits and vegetables.

Key words: Antifungal activity, Essential oil, Post-harvest, MIC, *Fusarium* sp.

INTRODUCTION

Plant secondary metabolites are good source for the control of fungal pathogen because they consist of many bioactive constituents. Plant pathogens like fungi, nematodes, bacteria, viruses etc. damage plants and cause significant economic losses (Montesinos, 2003). Among these, fungi are one of the main agents that cause several diseases in plants. Essential oils from plants have great potential as novel fungicide sources for controlling pathogenic fungi. The fungicidal properties depended on various plant products including oil, alkaloids, resin, saponin, organic acid and gums (Sofoworo, 1984; Asthana *et al.,* 1989; Chaturvedi *et al.,* 1987; Daoud *et al.,* 1990; Cowan, 1999; Al-Mughrabi *et al.,* 2001). Chemically synthesized fungicides are widely used for the control of various plant diseases and their frequently uses have faced major obstacles like development of resistant population of the pathogen against various chemical fungicide groups (Lin, 1981) and also infections caused during post-harvest conditions which lowers the shelf life and adversely affect the market value of fruits (Tripathi, *et al.,* 2008). However, the development of new agents for plant disease control has attracted much attention of investigators. So, the uses of plant-derived products as disease control agents have been studied, since they have low toxicity, less environmental effects and wide public acceptance (Lee *et al*., 2007) and are safe natural substances and they have been considered at low risk for resistance development by pathogenic microorganisms (Angelini *et al.,* 2006). Plant essential oils are not only used as fragrance and flavouring agents (Lahlou, 2004) but also has a potential alternatives for use as plant fungal pathogenic control agents (Isman, 2000; Tripathi and Dubey, 2004) and a large number of plant essential oils have been reported to have such antifungal properties.

In present work, the aim of the study was to assess the antifungal activity of *Cymbopogon distans* (Gania Grass) and *Cinnamomum tamala* (tejpat) against 10 different plants fungal pathogen and to find out new and safe alternatives for controlling the growth of fungal diseases in plants.

MATERIALS AND METHODS

Plant material: The leaves of *C. distans* and *C. tamala* were collected from Bhawankhal district Almora and Centre for Aromatic Plants (CAP) farm, Dehradun (Uttarakhand) respectively. The plant specimens were dully identified and deposited in the herbarium of Botanical Survey of India (BSI) Dehradun, with BSI Id. No.115522 and 115183.

Oil isolation: The essential oil from shade dried leaves of *C. distans* and *C. tamala* was isolated by hydrodistilation for 4 hours using Clevenger apparatus. The oil obtained were dried over anhydrous sodium sulphate (Na_2SO_4) and kept in cool and dark place for further analysis.

Tested microorganisms: Antimicrobial activity from oil of *C. distans* and *C. tamala* against plant pathogenic fungi like *Aspergillus flavus, Fusarium oxysporum, Colletotrichum capsici, Ustilago maydis, Rhizoctonia solani, Aspergillus niger, Penicillium aurantiogriseum, Fusarium sp., Puccinia sp.* and *Pythium sp.* was carried out from Microbiology Division, Innovation Life Sciences, Lucknow.

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Antifungal activity of essential oil of *C. distans* and *C. tamala:* Durning the preparation of inoculumn the fungal culture was inoculated in Sabouaud's Dextrose Broth. The inoculum was standardized by adjusting the turbidity of culture to Mc Farland 0.5 standard (~ 10-6 cfu/ml) with sterile broth or by further incubation. (Malik *et al.,* 2008)

The antimicrobial activity of oil obtained from *C. distans* and *C. tamala* was determined by using disc diffusion method. 100 µl of each fungal suspension was spread over petri dish containing Potato Dextrose Agar medium (PDA) plate and sterile whatman filter paper discs 6 mm were soaked in 25 µl of different oils samples and the plates were incubated at 28°C for 5 days. (Sethi *et al.,* 2013). After incubation the antifungal activity was evaluated by measuring zone of inhibition. Each experiment was carried out in triplicate. The Minimum Inhibitory Concentrations (MIC) of essential oils against pathogenic fungi were determined using two fold serial dilution method (Joshi, 2013) and the minimal concentration at which there was no visible growth after 5 days incubation period at 28ºC was taken as the MIC of that essential oils.

RESULTS AND DISCUSSION

The antifungal efficacy of essential oils from C. distans and *C. tamala* against ten different pathogenic fungi was assessed by disc diffusion method. The result of which is presented in (Table 1). The oils showed antifungal activity and exhibited moderate to feeble inhibition against all the test pathogens. The essential oil of *C. distans* and *C. tamala* with maximum zone of inhibition was seen against *Fusarium* sp. (12.53 ± 0.97 mm) and *P. aurantiogriseum* (12.06 ± 0.52 mm) and minimum against *R. solani* (6.83 ± 0.41, 6.16 ± 0.16 mm) zone of inhibition respectively. Moreover, *C. tamala* and *C. distans* essential oils showed similar inhibitory effect against *A. Niger*, However, as compared to standard antifungal i.e. Amphotercine B, Gania grass essential oils showed a similar activity against *Aspergillus favus*, *Pythium sp.* and *Fusarium sp.* (Table 1)

Table 2: Minimum inhibitory concentration (MIC) in mg/ml

Pathogen	*C. tamala*	*C. distans*
Aspergillus favus	5	0.625
Fusarium oxysporum	5	1.25
Colletotrichum capsici	5	1.25
Ustilago maydis ,	10	2.5
Rhizoctonia solani	10	10
Aspergillus niger	1.25	2.5
Penicillium aurantiogriseum	1.25	2.5
Fusarium sp.	1.25	1.25
Puccinia sp.	2.5	1.25
Pythium sp.	2.5	0.625

MIC values were found in subsequent experiments determined for all essential oils based on the lowest determined MIC values of *C. distans and C. tamala* against target fungal species was clearly confirmed. The absolutely highest efficacy was observed for *C.distans* essential oil where the MIC values 0.625 mg/ ml against *A. flavus* and *Pythium* sp. and other fungi from 1.25 mg/ml to 10.0 mg/ml while the *C. tamala* ranged from 1.25mg/ ml to 10.0 mg/ml across the entire target fungal spectrum (Table 2)

Furthermore, the biological activity of these oils is probably due their prominent concentration of Piperitone in *C. distans* (Chauhan *et al.,* 2015) and cinnamaldehyde in *C. tamala*. (Simic *et. al.*, 2004) have reported that cinnamaldehyde *Cinnamomum* oil has antifungal activity upon many fungal species as major component against fungal growth. (Unlu *et al.,* 2010; Shahverdi *et al.,* 2007 and Ooi *e. al.,* 2006) and has also been confirmed the cinnamaldehyde activity against plant pathogen. Chauhan *et al.,* (2015) reported that *C.distans* has most prominent inhibitory activity against *F. oxysporum*, while Bhuyan *et al.,* (2010) reported the effect of essential oil from *Cymbopogon* sp. on *in vitro* growth of and sporulation of rice pathogens, *R. solani*.

Table 1: Antifungal activity of essential oils against plant pathogens

Pathogen	*C. tamala**	*C. distans**	Amphotercin B
Aspergillus favus	10.03 ± 0.40	12.06 ± 0.26	12.5
Fusarium oxysporum	8.46 ± 0.61	11.03 ± 0.20	12.0
Colletotrichum capsici	9.46 ± 0.79	11.97 ± 1.05	11.0
Ustilago maydis ,	6.96 ± 0.47	11.07 ± 0.42	11.5
Rhizoctonia solani	6.16 ± 0.16	6.83 ± 0.41	10.5
Aspergillus niger	11.0 ± 0.57	11.47 ± 0.79	12.5
Penicillium aurantiogriseum	12.06 ± 0.52	9.5 ± 1.06	12.5
Fusarium sp.	10.10 ± 0.64	12.53 ± 0.97	11.5
Puccinia sp.	8.46 ± 0.77	11.43 ± 0.74	13.0
Pythium sp.	9.03 ± 0.46	11.5 ± 0.88	11.5

*Mean± SD

In conclusion, the present results shows that among the tested phytopathogenic fungi, the antifungal activities of *C. distans* essential oils showed against all the tested fungi and *C. tamala* against *Fusarium sp.* and *P. aurantiogriseum* as compared to other fungi. So for the development of plant-derived secondary metabolites in post-harvest disease control is alternative for synthetic fungicides because of no animal toxicity, environment-friendly and wide public acceptance and they are quickly metabolized and excreted. However, further studies is necessary for determining the practical applicability and safety of gania grass essential oils and its constituents as a fungicides and developing formulations to improve its activity and stability as a noval fungicides for plants and need for further research in formulation of Gania grass oil based fungicides.

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