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Influence of Leaf Blight Disease on Yield of Oil and its Constituents of Java Citronella and *In-vitro* Control of the Pathogen Using Essential Oils

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Abstact: Leaf blight of Java citronella (*Cymbopogon winterianus* Jowitt.), caused by *Cuvularia* spp., was found to cause a dramatic change in oil yield and its constituents. The major oil constituents decreased with increased disease incidence with the exception of geraniol, which increased 9.2% in diseased leaves. The percentage decrease of the major constituents of oil from diseased plants was found to be 52.8, 50.0, 47.1, 46.5, 30.7 and 19.1 for citronellol, linalool, citronellal, citranellyl acetate, geranyl acetate and limonene, respectively, compared to healthy plants. The causal organism, *Curvularia* sp., might have altered the monoterpenoid biosynthesis in the host. The *in-vitro* fungitoxicity of essential oils of *Citrus sinensis* (exocarp), *Curcuma longa* (rhizome) and *Elettaria cardamon* (seed) against the *Curvularia* spp. was studied and found to completely inhibit mycelial growth at different concentrations. The fungitoxicity of the oils was unaffected by variation in temperature and prolonged storage.

Key words: *Cymbopogon winterianus* · *Curvularia* sp. · *Citrus sinensis* · *Curcuma longa* · *Elettaria cardamon* · fungitoxicity

INTRODUCTION

Java Citronella (*Cymbopogon winterianus* Jowitt; family Poaceae) is cultivated under sub tropical and tropical climates. More than 400 tonnes of citronella oil are produced annually in North East India [1]. Oil obtained from the leaves is used by the perfumery, cosmetic and pharmaceutical industries for various purposes. Leaf blight is a serious disease of citronella, particularly in NE India, causing loss of herbage and oil yield. It initially appears as a brownish lesion in the leaf lamina and later spreads over the whole leaf. As a result, the entire leaf lamina dries up and herbage yield, as well as the quality and quantity of oil are seriously reduced. Several workers have reported previously about the disease and its effect on oil yield from the Northeastern region of India [2-4]. To reduce the loss of oil yield from the disease, chemical control with synthetic fungicides like benzimidazoles and heterocyclic nitrogenous compounds [2] and *in-vitro* biological control through biocontrol agent [5] were attempted. It has long been acknowledged that some plant essential oils exhibit antimicrobial properties [6, 7], but reports of biological control of leaf blight disease of citronella using essential oils are scarce. Therefore, a study was undertaken to observe the effect of leaf blight disease on citronella oil and its constituents and *in-vitro* control of the pathogen using essential oils.

MATERIALS AND METHODS

Experiments were carried out at Regional Research Laboratory, Jorhat, Assam during 2004 and 2005. The cultivation was done in an area of 2 hectares. The soil was sandy loam. The disease first appeared during April-May and continued till October-November. The climate was humid and having heavy rainfall 2266-2743 mm during the summer season.

The disease was first observed in April and diseased leaves were marked for the study. Observations of disease severity were recorded at 15, 30, 45 and 60 days. During these periods, the effects of leaf blight on the yield of oil and its constituents were recorded. Oil (w/v) was extracted from both diseased and healthy leaves using a Clevenger apparatus and stored at 0-4°C for later gas chromatographic analysis. The volatile constituents in the oil were determined by the GC method of analysis. A Chemito model 3865 NRGC with FID and a Hewlett Packard HP 3395 data integrator was used for the analysis. The constituents in the oil were separated in a packed glass column (2 mm id x 2 M length) of 15% SE 52

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Table 1: Effect of leaf blight disease on oil yield and major constituents at different incidence period

Parameter	Healthy leaf	Diseased leaf Incidence period (days) 15	30	45	60	Difference % at 60 days
A) Oil %	1.64	1.40	1.32	1.20	1.01	
Oil loss %	-	14.64	20.68	26.83	38.42	
B) Major constituents %						
1) Limonene	2.60	2.30	2.00	1.90	1.80	30.76
2) Linalool	0.80	0.70	0.60	0.50	0.40	50.00
3) Citronellal	34.40	34.00	32.00	30.00	27.80	19.18
4) Citronellol	16.70	16.10	14.20	9.10	7.90	52.69
5) Geraniol	22.70	23.20	24.00	24.90	25.00	9.20(+)
6) Citronellyl acetate	5.30	4.90	3.80	3.10	2.80	47.16
7) Geranyl acetate	8.60	7.90	6.80	5.10	4.60	46.51

+ Indicates gain %

Table 2: Fungitoxicity of essential oils against *Curvularia* sp.

Essential oils	Inhibition (%) at different oil concentrations (ppm) 500	1000	1500	2000	2500
Citrus sinensis (exocarp)	77	100	100	100	100
Curcuma longa (rhizome)	19	37	62	88	100
Elettaria cardamon (seed)	40	86	100	100	100

on gas chrom Q, 80/100 mesh. The predominating major compounds and their percentages in the oil of healthy and diseased leaves are presented in Table 1.

To isolate the fungus for *in-vitro* study, newly developed disease spots were collected from the leaf lamina and surface sterilized in a 1% solution of sodium hypochlorite for four minutes. Single disease spots were excised from leaves and plated on 10 pairs of petri dishes containing Potato Dextrose Agar medium under aseptic conditions. A pure culture was isolated and the mycelia were observed under microscope and the fungus identified.

Essential oils of *Citrus sinensis* (exocarp), *Curcuma longa* (rhizome) and *Elettaria cardamon* (seed) were used for *in-vitro* study to control the mycelia growth of the *Curvularia* sp. isolated above. Five concentrations of oils viz. 500, 1000, 1500, 2000 and 2500 ppm were prepared in acetone. Conical flasks (250 ml) containing 20 ml of Potato Dextrose Broth were inoculated with the fungal isolate, treated with different concentrations of these oils and replicated for 10 times. After an incubation period of 1 week, the broths were filtered and filtrates were oven dried at 65°C and fungitoxicities (% reduction in fungal mass) were calculated against the oil concentrations (Table 2).

RESULTS AND DISCUSSION

Citronella leaf oil was found to be colourless, mobile and liquid, possessing a sweet odour. GLC analysis of both healthy and diseased leaf oils indicated the presence of seven major constituents. Most of the components were found to decrease in diseased leaf oil except geraniol. With the increased disease severity, the recovery of oil as well as its major components, except geraniol, gradually declined. The oil yield was decreased from 1.64 to 1.01% after 60 days of disease development. Gupta *et al.* [4] also reported similar results in case of essential oil of Palmarosa. Of the major constituents of the leaf oil, citronellol was reduced the most (52.8%), followed by linalool (50%), citronellyl acetate (47.16%), geranyl acetate (46.5%), limonene (30.7%) and citronellal (19.1%). Interestingly, geraniol concentration increased with the increase of the disease, reaching a maximum at 60 days to be 9.2% in the oil of diseased leaf as compared to healthy one. Similar results were also reported by Sarma *et al.* [5]. Misra *et al.* [3] reported the increase of geraniol as well as geranyl acetate in infected leaf oil of citronella.

The fungal colonies were blackish green, cottony with dark black bottom [8]. The essential oils of *C.*

sinensis, C. longa and *E. cardamon* exhibited antifungal activity against *Curvularia* species at different concentrations. *C. sinensis* was found to be most affective with complete mycelia inhibition at 1000 ppm followed by *E. cardamon* and *C. longa* at 1500 ppm and 2500 ppm, respectively. It was observed that the growth and spore germination of the pathogen was found to decrease with the increase in concentration of the oil. Babu *et al.* [9] reported that essential oils have inhibitory affect on the growth of pathogen. Burt and Reinders [6] reported the significant *in-vitro* colicidal and colistatic properties of essential oils on *E. coli* under broad temperature. Juliano *et al.* [7], Cosentino *et al.* [10] and Raina [11] also reported similar *in-vitro* antimicrobial effect of essential oils.

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