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Yield and Chemical Composition of the Essential Oils of Three *Cymbopogon* Species Suffering from Iron Chlorosis

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The yield and chemical composition of the essential oils of Java citronella (*Cymbopogon winterianus* Jowitt.), lemongrass (*Cymbopogon flexuosus* (Nees ex. Steud.) Wats. var. *flexuosus* Hack.) and palmarosa (*Cymbopogon martinii* (Roxb.) Wats. var. *motia* Burk.) plants suffering from iron chlorosis were examined. Iron chlorosis significantly reduced biomass and essential oil yields and total chlorophyll content of the leaves of all the three species. The citronellal, citronellol and geraniol contents of Java citronella, the (*E*)-citral percentage of lemongrass and the geraniol concentration of palmarosa were lower in plants affected by iron chlorosis as compared to healthy plants. The minor constituents of the essential oils of these crops were also affected by iron chlorosis, but the crops differed in their response to iron deficiency.

KEY WORDS: *Cymbopogon winterianus* Jowitt.; Java citronella; *Cymbopogon flexuosus* (Nees ex. Steud.) Wats. var. *flexuosus* Hack., lemongrass; *Cymbopogon martinii* (Roxb.) Wats. var. *motia* Burk., palmarosa; essential oil yield; chemical composition; iron chlorosis

INTRODUCTION

Three *Cymbopogon* grasses, namely, Java citronella (*Cymbopogon winterianus* Jowitt.), lemongrass (*Cymbopogon flexuosus* (Nees ex Steud.) Wats. var. *flexuosus* Hack.) and palmarosa (*Cymbopogon martinii* (Roxb.) Wats. var. *motia* Burk.) are widely cultivated for their essential oils used by the perfumery, flavouring and pharmaceutical industries. Java citronella and lemongrass were reported to suffer from iron chlorosis (deficiency of iron leading to loss of green colour of leaves) when irrigated with water containing bicarbonates[1] or when grown on calcareous soils;[2,3] however, iron chlorosis was not reported in palmarosa. Contradicting results were published on the effect of iron chlorosis on yield and terpenoid composition of Java citronella. A reduction in yield of oil and concentration of major compounds (citronellol, citronellal and geraniol) in chlorotic leaves of Java citronella was reported from Lucknow, North India,[2,4] while no such decreases were reported from Bangalore.[1] In lemongrass, the yield of oil and citral content declined in plants suffering from iron chlorosis.[3,5]

All three aromatic crops are grown on a commercial scale on our research farm. During the rainy season (June–September) of 1994, we observed chlorotic plants in patches in all the three species. The symptoms were typical of iron chlorosis[4,5] and the condition was resolved when the plants were sprayed with 3% ferrous sulphate ($FeSO_4.7\ H_2O$). This is the first time that iron chlorosis was observed in palmarosa. Analysis of the soil samples from affected areas showed higher concentrations of calcium carbonate ($CaCO_3$) than in adjoining areas, indicating that the iron chlorosis was lime-induced. The high soil moisture during rainy season can produce more bicarbonates in calcareous soil, which induces iron deficiency.[6] Earlier studies [2-5] reported the variations only in major components of the essential oils of Java citronella and lemongrass affected by iron chlorosis. In this study, we investigated in

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detail the changes in chemical composition of essential oils of Java citronella, lemongrass and palmarosa suffering from iron chlorosis.

EXPERIMENTAL

Sample Collection and Extraction of Essential Oil

The agroclimatic conditions of the experimental location were described previously.[7] Plants of the three aromatic grasses suffering from iron chlorosis were identified and marked in the field. They were harvested and their biomass yields were recorded. From these plants, severely chlorotic and partially chlorotic leaves were collected separately. Green leaves from healthy plants growing in the same field were collected simultaneously. Total chlorophyll content of healthy and diseased leaves was estimated by the Association of Official Analytical Chemists

Table 1. Biomass and essential oil yields and total chlorophyll content of three *Cymbopogon* species suffering from iron chlorosis

	Java citronella			Lemongrass			Palmarosa		
Factor	1	2	3	1	2	3	1	2	3
Biomass yield per plant (g)	526.3	–	261.9*	785.8	–	484.9*	621.8	–	387.9*
Essential oil yield (%)	1.32	0.80	0.72	0.71	0.51	0.47	0.67	0.56	0.43
Total chlorophyll (mg/g)	1.02	0.52	0.09	8.41	1.01	0.11	1.98	0.61	0.12

1 = Healthy plant; 2 = Partially chlorotic leaves from diseased plants; 3 = Severely chlorotic leaves from diseased plants; * total of partially chlorotic and severely chlorotic leaves from diseased plants.

Table 2. Percentage composition of essential oils of healthy and diseased (affected by iron chlorosis) plants of *Cymbopogon winterianus* Jowitt. (Java citronella)

Compound	RRI*	Healthy plant	Diseased plant	
			Partially chlorotic leaves	Severely chlorotic leaves
6-Methyl hept-5-en-2-one	967	0.2	–	tr.
Myrcene	985	1.3	9.1	4.9
α-Phellandrene	997	0.1	0.2	0.1
p-Cymene	1019	0.3	–	tr.
Limonene	1025	4.2	2.6	2.2
(*Z*)-β-Ocimene	1030	0.3	1.9	1.1
(*E*)-β-Ocimene	1042	0.4	3.2	1.7
Terpinolene	1082	0.1	0.2	0.1
Linalol	1087	1.5	1.5	1.1
Citronellal	1138	40.2	26.6	29.4
Isopulegol	1148	0.9	1.4	2.2
Borneol	1156	0.2	0.6	0.4
Terpinen-4-ol	1166	0.1	0.2	0.2
α-Terpineol	1176	0.1	0.1	0.1
n-Decanal	1187	0.1	0.1	0.1
Citronellol	1213	9.1	6.0	6.2
Geraniol	1241	20.4	10.5	14.4
Citronellyl acetate	1336	3.2	6.2	5.7
Neryl acetate	1344	tr.	0.5	0.4
Geranyl acetate	1362	4.7	8.1	9.3
β-Bourbonene	1382	0.1	0.6	0.1
β-Elemene	1390	2.3	1.6	1.2
β-Caryophyllene	1423	0.2	0.1	tr.
α-Humulene	1456	0.2	0.1	tr.
γ-Cadinene	1517	1.7	0.5	0.6
Elemol	1539	2.3	7.1	7.6
α-Cadinol	1644	0.5	3.1	2.6

*On BP-1 (dimethyl siloxane); tr = <0.1%.

(AOAC) method.[8] The leaf samples from healthy and diseased plants of all the three species were hydrodistilled in Clevenger-type glass apparatus for 3 h. The oils were collected and their volumes measured before drying over anhydrous sodium sulphate. The oil samples were stored at 0°C for GC analysis.

Component Identification and GC Analyses

GC analyses of the oil samples from healthy and diseased plants were performed on a Perkin-Elmer gas chromatograph 8500 equipped with flame ionization detector using a glass BP-1 column (25 m × 0.5 mm i.d.) coated with dimethyl siloxane. Nitrogen at 10 p.s.i. inlet pressure was used as a carrier gas. The temperature was programmed from 60°C to 220°C at a rate of 5°C/min. Samples were injected with a split ratio of 1:80. Essential oil components were identified by comparing the relative retention indices (RRI) of the peaks with literature data[9,10] and by peak enrichment on co-injection of standard compounds.

RESULTS AND DISCUSSION

Iron chlorosis decreased the biomass and essential oil yields as well as total chlorophyll content of the leaves of all the three *Cymbopogon* species (Table 1). Iron plays an important role in the formation of amino-levulinic acid, which is a precursor of chlorophyll.[11] A strong correlation between leaf chlorophyll and iron content was also observed.[12] Thus, iron deficiency, in the present study, resulted in the decrease of total chlorophyll of partially chlorotic and severely chlorotic leaves of diseased plants in comparison to healthy plants. Essential oil is a secondary plant metabolite synthesized from the products of photosynthesis. As chlorophyll and iron (a constituent of cytochrome and ferrodoxin) are essential for photosynthesis, reduced chlorophyll

Table 3. Percentage composition of essential oils of *Cymbopogon flexuosus* (Nees ex Steud.) Wats. var. *flexuosus* Hack. (lemongrass) from healthy plants and plants affected by iron chlorosis

Compound	RRI*	Healthy plant	Diseased plant	
			Partially chlorotic leaves	Severely chlorotic leaves
6-Methyl hept-5-en-2-one	967	0.5	1.9	1.4
Myrcene	986	tr	0.4	0.4
α-Phellandrene	1000	tr	0.2	0.2
p-Cymene	1018	0.1	0.2	0.2
Limonene	1022	0.3	0.1	0.1
(*Z*)-β-Ocimene	1035	tr	0.2	0.2
(*E*)-β-Ocimene	1046	tr	0.2	0.1
Terpinolene	1083	0.1	0.1	0.1
Linalol	1088	0.5	1.0	0.9
Camphor	1132	0.2	0.5	0.5
Citronellal	1137	0.1	0.2	0.2
Isopulegol	1147	0.5	1.1	1.1
Borneol	1164	tr	0.1	0.1
Terpinen-4-ol	1167	tr	0.2	0.2
α-Terpineol	1172	0.1	–	–
Citronellol	1209	tr	–	–
(*Z*)-Citral	1222	36.1	38.2	38.1
Geraniol	1242	0.2	1.3	0.8
(*E*)-Citral	1253	53.6	46.4	47.9
Citronellyl acetate	1340	0.9	0.1	–
Geranyl acetate	1361	0.1	1.5	1.6
β-Elemene	1388	0.3	0.1	0.1
β-Caryophyllene	1420	0.7	0.3	0.2
α-Humulene	1453	0.1	0.1	0.1
β-Bisabolene	1496	0.1	0.4	0.5
β-Caryophyllene oxide	1574	1.2	0.1	0.1

*On BP-1 (dimethyl siloxane); tr = <0.1%.

levels and deficiency of iron adversely affected biomass and essential oil yields of the affected plants. The biomass yield reductions were 49.8% in Java citronella, 61.7% in lemongrass and 62.4% in palmarosa. The essential oil yield decreases were greater in severely chlorotic leaves in all the three species. Similar results were reported earlier in Java citronella[2,4] and lemongrass.[3,5] Among the three species, lemongrass recorded highest biomass yield and total chlorophyll content and Java citronella produced maximum essential oil yield.

Both major and minor components of the volatile oils of Java citronella, lemongrass and palmarosa were affected by iron chlorosis (Tables 2,3,4). In Java citronella, the concentrations of limonene, citronellal, citronellol, geraniol, β-elemene and γ-cadinol were lower, while the contents of myrcene, (*Z*)-β-ocimene, isopulegol, citronellyl acetate, geranyl acetate, elemol and α-cadinol were higher in plants affected with iron chlorosis as compared to healthy plants (Table 2). The severely chlorotic leaves had higher concentrations of citronellal, isopulegol, geraniol and geranyl acetate when compared with partially chlorotic leaves. Although the reasons for such variations in the volatile oil constituents are not known, decreases in the percentages of citronellal, citronellol and geraniol in Java citronella due to iron chlorosis were reported by other workers also.[2,4]

In lemongrass, the percentages of (*E*)-citral and β-caryophyllene oxide declined, whereas those of 6-methyl hept-5-en-2-one, linalol, camphor, isopulegol, (*Z*)-citral, geraniol, geranyl acetate and some other minor constituents increased due to iron chlorosis (Table 3). The severely chlorotic leaves had slightly higher percentage of (*E*)-citral than partially chlorotic leaves. Our results slightly differ from those of Subrahmanyam and Chattopadhyay,[3] who reported reductions both in citral a and citral b due to iron chlorosis under North Indian conditions. Decreased total citral content due to iron deficiency was reported by Farooqi *et al.*[5] also.

In palmarosa, linalol, geraniol and β-caryophyllene contents decreased, while geranyl acetate and geranyl butyrate contents increased due to iron chlorosis (Table 4). The severely chlorotic leaves contained higher percentages of geranyl acetate and geranyl butyrate than the partially chlorotic leaves.

A striking similarity in all the three species was

Table 4. Percentage composition (%) of essential oils of *Cymbopogon martinii* (Roxb.) Wats. var. *motia* Burk. (palmarosa) from healthy plants and plants affected by iron chlorosis

Compound	RRI*	Healthy plant	Diseased plant	
			Partially chlorotic leaves	Severely chlorotic leaves
Sabinene	968	0.1	–	–
Myrcene	984	3.3	3.6	2.9
α-Phellandrene	997	0.1	0.1	0.1
α-Terpinene	1010	0.1	0.1	0.1
Limonene	1024	0.3	0.3	0.2
(*Z*)-β-Ocimene	1030	0.9	1.0	0.8
(*E*)-β-Ocimene	1044	2.0	1.9	1.6
γ-Terpinene	1053	0.1	–	0.1
Terpinolene	1081	0.1	0.1	tr
Linalol	1086	2.2	1.9	1.8
Citronellol	1212	0.1	0.1	0.1
Geraniol	1241	75.8	63.2	59.3
Citronellyl acetate	1335	0.1	0.1	0.1
Geranyl acetate	1363	11.4	25.3	28.6
β-Caryophyllene	1422	1.3	0.2	0.5
Geranyl *n*-propionate	1447	0.1	0.1	0.1
Geranyl isobutyrate	1491	–	0.1	0.1
Geranyl butyrate	1529	0.1	0.1	1.2
Geranyl hexanoate	1718	0.7	0.2	0.5
Geranyl heptanoate	1810	0.1	0.1	0.3

*On BP-1 (dimethyl siloxane); tr = <0.1%.

the reduction of major constituents due to iron chlorosis; however, the crops responded differently to iron deficiency in respect of changes in minor components.

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