E3 Journal of Medical Research Vol. 1(3). pp. 034-037, April, 2012
Available online @ http://www.e3journals.org
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Full length research paper

Dynamics of essential oils of *Cymbopogon martinii* (Roxb.) Wats and *Cymbopogon citratus* (DC) Stapf. under rust disease indices

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Accepted 16 March, 2012

An investigation was carried out to study the difference in quality of essential oils obtained from healthy and rust infected *Cymbopogons* (*Cymbopogon martinii* (Roxb.) Wats and *Cymbopogon citratus* (DC) Stapf.) at various Per cent Disease Index (PDI) using GC and GC / MS analyses. Result showed a marked difference in the oils with regards to oil recovery. In case of *C. martinii* healthy leaves yielded 0.60% oil whereas leaves with 100 PDI yielded 0.40% oil. A trend of increase in percentage of geraniol was recorded with increase in PDI. The oil from healthy leaves yielded 69.48% geraniol while oil from 100 PDI yielded 93.46% geraniol. Another major component geranyl acetate was found higher (17.82%) in the oil from healthy leaves than the oils from 100 PDI (0.95%).

Key words : Essential oil, *C. martini*, *C. citrates*, rust, *Puccinia nakanishikii*

INTRODUCTION

Cymbopogon martinii (Roxb) Wats (Palmarosa) and *Cymbopogon citratus* (DC) Stapf. (Lemongrass) are essential oil bearing aromatic plants belonging to the genus *Cymbopogon*. The oil of *Cymbopogon martini* is used as base for fine perfumery and is valued because of its geranial content. Besides the perfumery value, the oil has a great wound healing effect. *Cymbopogon citratus* is one of the sources of citral, an important monoterpene aldehyde, large quantity of which is being utilized for production of ionones, vitamin A and geraniol besides the use in perfumery soaps and cosmetics .

Although *C. citratus* and *C. martinii* are two economically important essential oil yielding grasses, due to the infection by rust fungus these two species of *Cymbopogon* show serious losses in terms of herb yield, oil content and quality. Rust fungi are obligate parasites and are highly destructive. The pathogen associated with rust disease of these two cymbopogons is identified as *Puccinia nakanishikii* (Diet). Upadhaya & Bordoloi (1975), Upadhya & Dwivedi (1976) described about the reduction of herb and essential oil contents in Java citronella due to attack by *Curvularia eragrostidis* and in *Ocimum basilicum*. Tarabeih, *et al* (1980) reported about the loss of essential oil content on anise, carway and fennel due to attack by *Sclerotinia sclerotiorum*. Bharadwaj *et al* (1980) working on three mint species (*Mentha spicata*, *M.piperata* and *M.arvensis*) reported that oil and herb yields were reduced markedly by the attack of *Rhizoctonia solani*. Janardhanan *et al* (1980) reported about the loss of geraniol and oil content due to attack by *Curvularia andropogonis* in *Cymbopogon martini* (Palmarosa). It has been observed that detailed investigation on the disease and the dynamics of essential oils of these two valuable essential oil bearing crops under disease indices has so far not been done. Therefore, the present investigations have been done to study the dynamics of essential oils of these two valuable

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Table 1. Analysis (percentage) of *C. martinii* oils produced from plants with varying percent Disease indices.

PDI	Oil content w/w	Geraniol	Geranyl acetate
Healthy	0.60	69.48	17.82
11.10	0.58	71.32	10.23
22.30	0.55	74.43	8.10
34.25	0.52	76.86	7.02
46.52	0.48	78.66	5.32
62.15	0.46	82.45	3.02
75.66	0.44	84.96	2.10
87.20	0.43	86.74	1.30
100.00	0.40	93.46	0.953

essential oil bearing crops under disease indices.

Materials and Methods

Extraction of oils

Leaf samples were hydro distilled for about four hours by applying Clevenger type apparatus (Clevenger, 1928). After distillation oil collected in the glass container was analyzed by GLC method.

Analysis of oils (Gas chromatography)

A shimadju GC-IT A gas chromatograph equipped with a FID detector and a HP fused silica column (30 m x 0.32 nm, 0.25 µ m film thickness) was used. Samples were injected in the split mode, using pressure controlled helium as carrier gas at a linear velocity of 30 cm / s (at 60 ^{o} c). Injection and detector temperature were maintained, respectively, at 28^{0} and 30^{0}C. The column over temperature was programmed from 50^{0}C (After 3 min.) to 300^{0}C at 45^{0}C / min. The final temperature was hold for 20 min. Peak areas and retention times were measured by electronic integration. The relative amounts of individual components were based on the peak areas obtained without FID response factor correction. Temperature programmed (linear) retention indices of the compounds were determined relative to n- alkanes.

Gas chromatography / Mass spectroscopy (GC/MS)

Analysis were carried out on a Shimadju GC-17A / GCMS – QP 5000 system A 25 m / 0.20 mm fused silica HP – 1 column, with a film thickness of 0.33 mm, was employed. The column over temperature was programmed from 60^{o} C (After 30 min) to 300^{o} C at 5^{o} C/min. The injector and GL/MS interface temperatures were maintained at 280^{0} and 300^{o} C respectively. Helium carrier gas was pressure controlled to give a linear gas velocity of 44 cm/s (at 60^{o} C). Electron ionization mass spectra were acquired over the mass range 10 -400 Da at a rate of 2/s.

Component identification

The components were identified by matching their retention times on various columns with those of authentic samples scanned under identical conditions. Identities of many compounds were further verified by GL/MS where peaks were compared with reference compounds and by matching their 70_{C} VEI mass spectra with those of library search data (Davies 1990, Sandra & Bicchi 1987; Masada 1967; Libbey 1991, Ramaswami *et al* 1988, Adams 1995, Henneberg *et al* 1998).

Dynamics of essential oil under rust disease indices

Essential oils of *C. martinii* and *C. citratus* were obtained on hydrodistillation of diseased leaves with different disease indices. Disease index was measured with a 4 points rating scale, where 0 – no disease (healthy); 1 = 1 – 25 %, 2 = 26 – 50 %; 3 = 51- 75%; 4 = 76 – 100 % leaf area infected, using the following formula:

Sum of all numerical rating x 100 Percent Disease Index (PDI)=Total number of leaves rated x maximum disease grade.

The neral, geranial and geranyl acetate contents of the oil samples were measured by GC using Varian 2440 (FID) equipped with a 5 mm X 2 mm, 10 % OV – 101 packed column temperature programmed from 90^{o} c to 160^{o} at 2^{o} c / min. The injector and detector temperatures were maintained, .respectively, at 210 ^{o} C and 220 ^{o}C. The carrier gas flow was N_2 (30 ml/min) (Boruah *et al,* 1995).

Results and Discussion

In case of *C. martinii* there was no marked difference ofoil recovery in between healthy leaves and leaves upto 22.30 PDI (Table 1). A sharp fall of oil recovery from the infected leaves with PDI of 46.52 and above was observed. Healthy leaves yielded 0.60 % oil and leaves with 100 PDI yielded 0.40% of oil. A trend of increase in

Table 2. Analysis of (percentage) *C. citratus* oils produced from plants with varying percent Disease indices

PDI	Geranial %	Neral %
Healthy	49.44	36.25
10.40	49.28	36.02
22.20	49.12	35.80
36.45	48.66	34.28
50.35	47.50	33.82
64.40	47.10	32.75
75.60	46.62	32.05
86.25	45.26	31.62
100.00	43.78	30.73

percentage of geraniol was recorded with increase in PDI. The oil from healthy leaves yielded 69.48 % geraniol while oil from 100 PDI contained 93.46% geraniol. Another component geranyl acetate was found to decrease. From healthy leaves the geranyl acetate content was 17.82% while at 100 PDI it was only 0.953%.

Table 2 indicated that the healthy leaves in case of *C. citratus* yielded 0.80% oil content while the leaves with 75.60 PDI gave a recovery of 0.50% oil content exhibiting reduction of 37.50 % in oil yield. There was no marked difference recovery of oil between healthy leaves and leaves with 64.40 PDI. A sharp fall of oil recovery from the infected leaves with PDI of 75.60 and above was observed. A trend of decrease in percentage of major component geraniol was recorded with increase in PDI. The oil from healthy leaves yielded 51.30 % geraniol, while oil from leaves with 100 PDI contained 47.69 % geraniol. Another major component neral was found to increase though not markedly with increase in disease index, in healthy leaves the neral content was 34.67% while at 100 PDI neral increased to 36.34%.

Relationships of essential oils and metabolic constituents of palmarosa and lemongrass are linked with sugars, peroxidase enzyme and protein. Burbott and Loomis (1967) while studying with peppermint observed that carbohydrate might serve as a ubstrate for essential oil metabolism. Essential oils are produced in special types of cells or in some glands. The oxidation-reduction state of these cells or glands depends upon the carbohydrate level. Ghosh and Chatterjee (1976) have recorded a reduction in the protein content during maximum oil formation in palmarosa and lemongrass. Loomis *et al*, (1979) are of the opinion that the oil formation is closely associated with protein content of the glands or tissues. According to Loomis and Croteau (1980) sugars and proteins are the primary metabolites linked with monoterpene (oil) metabolism. The oil and citral and oil and geraniol contents of *C. citratus* and *C. martini,* respectively, depend upon environmental and seasonal factors and on the enzymes system. Enzyme system depends upon the maturity of plants (Croteau & Hooper, 1978). It has been noticed that in lemongrass when the amount of oil is high, the amount of citral is low. While working with peppermint Croteau and Hooper (1978) noticed that accumulation of menthol is accompanied by depletion of oil content. In *Mentha arvensis*, Tyagi *et al* (1983) have observed an increase in oil content accompanied by a decrease in menthol.

In diseased leaves, the levels of each of sugar, chlorophyll, protein, ascorbic acid are reduced and because of this the amount of essential oils is also reduced. Due to infection, the oil producing cells may perhaps be destroyed or supply of oil producing substrates to the secretory cells may also be disturbed. It may be the cause for lesser amount of oil synthesis in diseased tissues. Carbohydrate content are the probable factors which accelerate disease infection and result in the yield of oil. The loss of essential oil and its chief constituent due to disease is in conformity with the reports of Goto (1986) in *Mentha citrate*, in Java citronella, palmarosa and Ocimum (Upadhaya *et al*, 1975) in *Mentha piperata* (Felklova, 1978), in *Mentha arvensis*, *Mentha spicata* and *Mentha piperata* by Bharadwaj *et al*, (1980), in palmarosa by Janardhanan *et al*, (1980) and in Anise, Caraway and Fennel (Tarabeih *et al*, 1980).

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