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STUDIES ON SOME CHEMICAL COMPONENTS OF NUTMEG *(MYRISTICA FRAGRANS* - HOUTT) LEAF DIRECTED AT DETERMINATION OF SEX OF SEEDLINGS

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Abstract : The essential oil content and the tlc phenolic profiles of the leaves of seedlings, 5 - year old and adult nutmeg, ***Myristica fragrans*** plants were studied. Significantly higher essential oil content and the absence of two phenolic spots were observed in female adult plants when compared with males. Differences in α - pinene, terpinolene, α - terpinine, myrcene and geranyl acetate content were also seen. Using other criteria described for the sexing of nutmeg seedlings to identify suspect male and female plants, it is shown that essential oil content and the phenolic profiles may also be used for this purpose.

1. Introduction

The nutmeg tree is typically dioecious with male and female flowers on different trees although hermaphrodite flowers and bisexual trees occur rarely.[3] After planting generally 50% of the trees turn out to be male, thereby decreasing productivity of the estate and being a burden to the planter until flowering takes place.

The literature describes methods of sexing nutmeg seedlings. For example Phadnis and Choudhari (1971)[6] advocated a colorimetric test that they claimed could distinguish sex in approximately two thirds of seedlings while Nayar et al. (1974)[4] examining the epidermal cells of nutmeg leaf described the different structures of calcium oxalate crystals in seedlings of male and female plants. We have in a preliminary study indicated that characteristics such as leaf shape, essential oil content and composition and profile of phenolics differ in male and female plants (both adults and seedlings).[5]

2. Experimental

2.1 Samples and Sampling

2.1.1 Adult trees

Male and female trees (over 50 years of age) were selected from different locations in Kandy and Matale (10 of each) and approximately 2kg samples of leaves were collected at random. Each sample was separately sun dried to 9.5 - 10%

moisture. The samples were then tested for the following : moisture content (Dean and Stark entrainment method),[2] essential oil content, essential oil composition, and tlc profile of phenolics. Analysis for each plant was carried out in duplicate.

2.1.2 Seedlings and young plants

Seedlings were maintained in a nursery at the CISIR. All leaves of 6 month seedlings were used for a sample (100-200g). Seedlings were differenciated into broad and narrow leaf types. The mean widths of the broad and narrow leaves were 6 and 4.2 cm respectively while both had roughly the same length (13-15 cm). Leaves (2kg) were taken from 5 year seedlings of the two types which have been grown since 1983 in the CISIR. The epidermal cells of the broad and narrow leaves were stained with safranin and viewed under the microscope (Carl-Zeiss D-7082) with in base halogen illuminator (6v, 10w). Samples were also treated as in 2.1.

2.2 Essential oil Extraction

In the case of 5 year plants, essential oils were analysed using a Clevenger arm[1] by water distillation of a 50g dried sample. In the case of the 6 month seedlings essential oils were trapped in (ether:hexane, 1:1) by the method of Senanayake,[7] due to the smaller sample size.

2.3 Essential oil Composition

This was determined using a Perkin-Elmer glc model sigma 2B. Column length, 2m; column diameter, 2mm; packing material, carbowax 20M (with chromosorb W). Injector temperature, 200 °C; detector temperature, 240 °C; detector FID; temperature programming, 60° to 200 °C (2 °C min^{-1}); carrier gas, N_2 (flow rate 30 ml min^{-1}); air flow rate, 60ml min^{-1}, H_2 flow rate, 25ml min^{-1}; recorder Perkin Elmer model 0560002, IMV chart speed 5mm min^{-1}. Identification was by peak enrichment and retention data only.

2.4 Tlc Profile of Phenolics

The dried leaf samples (30g for 5 year and adult plants and 5g for young plants) were extracted by percolation in acetone and separated on silica gel G-60 (300 μm) using n-butanol; ethyl acetate; acetic acid (4:2:4).

3. Results and Discussion

3.1 Studies Using Adult Male and Female Plants

3.1.1 Calcium oxalate crystals

It was not possible to correlate structure of calcium oxalate crystals in the epidermis to the male and the female plants. Further, the broad and the narrow leaves could not be correlated to the sex.

3.2 Essential oil Content Composition

Essential oil content in male and female plants, 1.41 ± 0.20% & 2.59 ± 0.26% respectively, showed significant variation. Ten plants of each sex were used. Essential oil composition of the above varied significantly with the sex especially in the case of -pinene, terpinoene, -terpinine, myrcene and geranyl acetate (Table 1).

Table 1: Some components of volatile oils adult male and female plants

Component	%	
	Adult Male	Adult Female
α - pinene	25.2 ± 1.0	15.4 ± 0.9
terpinolene	2.8 ± 0.7	1.3 ± 0.3
α - terpenine	1.9 ± 0.5	5.0 ± 0.3
myrcene	2.6 ± 0.5	11.0 ± 0.5
geranyl acetate	2.0 ± 0.4	5.5 ± 0.3

10 plants of each sex were used and duplicate determinations carried out.

3.1.3. Phenolics

The male plant showed the presence of two additional phenolics. Rate of flow is expressed relative to distance travelled by fastest moving common spot R_{cp} (Table 2).

Table 2 : Phenolics separated by tlc

Spot No.	R_{cp} Value	
	Male	Female
1.	0.13	0.13
2.	0.32	0.32
3.	absent	0.50
4.	0.64	0.64
5.	0.78	0.78
6.	1.00	1.00
7.	absent	1.15

Rcp value is distance of flow relative to fastest running common phenolic (No.6).

The above results suggest that the essential oil composition and tlc profile could be used to distinguish between adult male and female plants.

3.2 Studies Using Nutmeg Seedlings

3.2.1. Calcium oxalate crystals

As described by Nayar et al. ,[4] the narrower leaf had clusters of small calcium oxalate crystals while the broad leaf had large separate crystals.

3.2.2. Essential oil content and composition

Due to the method of essential oil collection[7] an accurate quantitative, estimation of essential oil content of 6 month seedlings was not possible. Essential oil composition varied considerably (Table 3). The narrower leaf has a significantly higher geranyl acetate content.

Table 3: Chemical composition of Nutmeg seedling leaf Oil

Component	Broad leaf		Narrow leaf		
α-pinene	15.2,	14.5	8.3	8.5	8.9
β - pinene	47.8,	28.9	27.6	26.4	21.2
Sabinene	1.8,	1.1	1.8	2.6	0.6
b-phellandrene	1.1,	2.5	0.7	1.8	1.6
α−terpenine	10.1,	8.8	6.5	7.2	7.0
1, 8-cineole	7.0,	4.8	2.9	3.2	4.5
α−terpinene	1.9,	1.35	0.6	1.8	1.6
p-cymene	3.3,	2.3	1.5	1.1	2.0
cis-p-meth-2en-1-ol	4.2,	5.9	4.2	4.0	4.6
geranyl acetate	4.2,	7.3	28.8	32.7	24.6
safrole	-	-	9.8	7.2	7.3
1-terpinene-4-ol	-	-	4.3	3.0	4.3

Results from two broad leaf and three narrow leaf plants are given above.

3.2.3. Phenolic compounds

The profile of phenolic components in the broad leaf was identical to that of the adult male plant leaf and the profile of the narrower leaf was identical to that of the adult female plant leaf. This strongly suggested that the tlc profile could be used to identify male and female seedlings.

3.3 Studies on 5 Year Plants

3.3.1. Calcium oxalate crystals

The plants arising from the broad leaf (male) seedlings had a few large crystals surrounded by smaller crystals, small independant crystals were also visible. Plants from the narrower leaf (female) seedlings maintained their calcium oxalate crystal structure. Although there is a difference, this difference appears to be less marked compared to the six month stage.

3.3.2. Essential oil composition and content

Leaf essential oil content of the suspected female plant was 1.02 ± 0.13 %. This was appreciably higher than the suspected male plant which was 0.6 ± 0.07%. Results have been calculated using data from 5 plants each.

Essential oil content of the two types of plants are given in Table 4 showing that the plants still shown considerable difference in the essential oil profiles. Special attention is drawn to α and B pinene, geranyl acetate, safrole, 1-terpinene-4-ol, myrcene and myristicin.

Table 4 : Essential oil composition of 5 year plants

Component	Suspected male	Suspected female
α - pinene	19.82 ± 0.29	12.56 ± 1.05
β - pinene	34.34 ± 2.31	23.46 ± 1.86
sabinene	1.08 ± 0.27	1.06 ± 0.24
α - terpinene	12.26 ± 1.56	9.64 ± 1.04
1, 8-cineole	6.42 ± 1.77	4.42 ± 1.44
2, terpinene	0.70 ± 0.24	1.10 ± 0.07
geranyl acetate	6.26 ± 1.75	1.0 ± 2.24
safrole	1.48 ± 0.41	7.46 ± 1.09
1-terpinene-4-o1	0.86 ± 0.14	4.98 ± 0.64
mycene	0.18 ± 0.14	8.70 ± 1.13
myristicin	4.88 ± 1.20	0.30 ± 0.29

Five plants were used and a duplicate reading taken for each.

3.3.3. Tlc Profile of Phenolics

The profile remained virtually the same as in the 6 month seedlings although additional spots at R_{cp} 1.30 and 1.48 appeared in the suspected male plants and at R_{cp} 0.55 in the suspected female plants. (These probably reflect advances in our techniques of tlc separation over the five years rather than the presence of additional compounds).

3.4 General Discussion

It appears that several indicators are available for sexing of nutmeg seedlings.

(1) the method of Nayar (structure of epidermal calcium oxalate crystals)
(11) leaf type
(111) essential oil composition
(1V) profile of phenolics

(111) and (1V) above could be used in cases of doubt when morphological and histological observations give ambiguous results.

Although essential oil content is a good indicator this can only be used at a later stage as sufficient leaves are not available at the seedling stage.

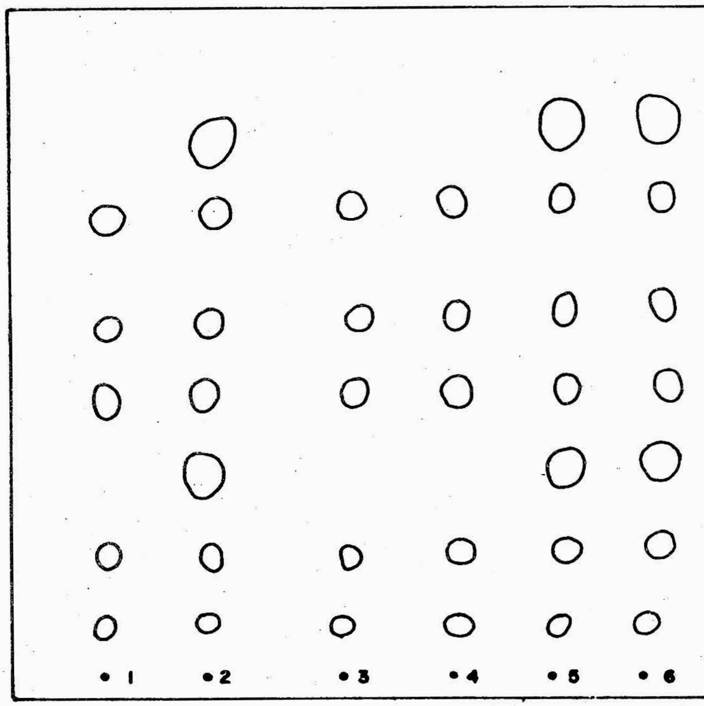

Figure 1 : Diagrammatic tlc separation of phenolics
Phenolics for adult male and female plant and seedlings of broad and narrow leaves are shown.

1. Adult female
2. Adult male

3.,4. Narrow leaf seedling
5,6. Broad leaf seedling

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