Plant Archives Vol. 25, Supplement 1, 2025 pp. 2096-2101 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-1.287

BIOCHEMICAL CHARACTERIZATION STUDIES IN NUTMEG (*MYRISTICA FRAGRANS* HOUTT.) ECOTYPES

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(Date of Receiving : 26-09-2024; Date of Acceptance : 02-12-2024)

ABSTRACT

Nutmeg (*Myristica fragrans* Houtt.) is an introduced crop to India. Assessment of the existing variability is a prerequisite for taking up successful crop improvement programmes, which is very much limited in this tree spice. In this context, the present study entitled "Biochemical characterization studies in nutmeg (Myristica fragrans Houtt.) ecotypes" was taken up at College of Horticulture, Mudigere during the year 2023-2024. A field survey was conducted in the nutmeg growing tracts of Chikkamagaluru district of Karnataka with the objective of studying the biochemical parameters of selected nutmeg ecotypes. The study revealed that Two ecotypes (Acc. 05 and Acc. 10) were found to be promising in terms of volatile oil and oleoresin content. Acc. 05 found to be promising in terms of bio-chemical characters like volatile oil in both mace and kernel and oleoresin content in kernel. Therefore, considering all the characteristics the ecotypes Acc. 05 and Acc. 10 were found to be elite ecotype for mace and nut with quality compound. GC-MS analysis of two distinct accessions *viz.,* Acc. 05 and Acc. 10 for mace and kernel volatiles revealed that Cyclohexene, 4-methylene-1-(1-methyl ethyl), 1,3-Benzodioxole, 4-Methoxy-6-(2-Propenyl), 3- Benzene, 1,2,3-trimethoxy-5-(2-propenyl), were seen in the kernel and mace volatile oil of both the accessions and it was highest in kernel volatile oil.
***Keywords* :** Mace, nut, volatile oil, oleoresin, GC-MS analysis.

Introduction

Nutmeg (*Myristica fragrans* Houtt.), a tropical tree species belonging to the family Myristicaceae, is a highly valued spice and medicinal plant cultivated primarily for its aromatic seeds and mace. Native to the Banda Islands of Indonesia, nutmeg has gained global importance due to its diverse culinary, pharmaceutical, and industrial applications. The seeds and arils of nutmeg are rich in secondary metabolites, including essential oils, phenolic compounds, alkaloids, and terpenoids, which contribute to its distinct aroma, flavor, and bioactivity.

Nutmeg holds significant promise in the spice industry for its flavour in food and its role as an ingredient in various value-added products. Its medicinal properties also make it a valuable component in the pharmaceutical industry. From dried nutmeg and mace, oils, oleoresin, and fixed oil are extracted. These extracts contain a mix of compounds such as myristicin, elemicin, sabinene, and safrole, which collectively constitute 80 percent of the oils. There is notable variability in the composition and proportions of these components across extracts from nutmeg and mace (Rema *et al.,* 2015).

Despite its widespread usage and economic significance, limited research has focused on the biochemical variation among different nutmeg accessions, which could provide critical insights for genetic improvement and crop development. Exploring the phytochemical diversity and antioxidant potential of nutmeg could not only aid in selecting elite genotypes for breeding programs but also promote its utilization in nutraceutical and pharmaceutical industries. This study aims to comprehensively analyse the biochemical properties of nutmeg.

Material and Methods

The present investigation "Biochemical characterization studies in nutmeg ecotypes" was carried out at the Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Horticulture, Mudigere during 2023-2024. Details of the materials used and methods followed in the study are described in this chapter.

Study area

Accessions of nutmeg were collected from diverse locations of Chikkamagaluru district of Karnataka. The study area comprised of Five taluks *viz*., Mudigere, Shringeri, Koppa, N R Pura and Tarikere. The geographical location of each study site was recorded. The accessions identified were in the age group of twelve to fifteen years. Thirty nutmeg accessions were selected from these plantations after thorough visual evaluation. The standard procedure of recording the qualitative and quantitative characters was followed by taking samples from all the four sides of the tree.

The selected 30 nutmeg accessions were evaluated for essential oil content of kernel and mace and oleoresin content of nut in the laboratory of College of Horticulture, Mudigere. The procedures followed for estimation of contents of volatile oil and oleoresin are furnished below.

Volatile oil content of kernel and mace

The volatile oil content of both kernel and mace was estimated by extracting the oil using Clevenger's apparatus through hydro distillation method (Clevengers, 1982). Fifteen grams of dried powdered sample (mace and kernel respectively) was mixed with 150 ml distilled water and then fed into a round bottom flask attached to the Clevenger's apparatus with condenser. Then the flask was heated gently up to a temperature of 70°C - 80°C and continued for three hours. The oil was collected in the receiver end of the Clevenger apparatus, cooled to room temperature. The volume of oil collected was expressed as per cent volume per unit mass of the sample

$$\text{Volatile oil}\,(\%) = \frac{\text{Volume of oil collected (ml)}}{\text{Total weight of the sample (g)}} \times 100$$

Oleoresin content of kernel

Oleoresin extraction from kernel was done by using Soxhlet apparatus through solvent extraction method with petroleum benzene. Five grammes of dried powdered sample was packed in thimble and then placed in extraction tube. Seventy-five milliliters of petroleum benzene were then added to the extraction tube, and then kept in water bath along with a condenser. The extraction continued for three to four hours till no colour was observed for the solvent in extraction tube. At the final stage of extraction, removed the thimble and distilled further to remove all the solvents. Results were expressed in percentage (ASTA,1968).

$$\text{Oleoresin}\,(\%) = \frac{\text{Weight of extraced oleoresin}}{\text{Initial weight of the sample (g)}} \times 100$$

GC-MS analysis of essential oil

The GC-MS analysis was performed using a Shimadzu QP2020 instrument with an MS detector and an SH-RTX WAX capillary column (0.50 µm diameter). Helium served as the carrier gas, with a constant flow rate of 1.5 mL/min, and an injection volume of 1 µL was used (split ratio 1:100). The inlet and ion-source temperatures were both maintained at 200°C. The total run time was 43 minutes, with the oven temperature initially set at 50°C, then increased at a rate of 5°C/min to 240°C, followed by a 5-minute hold. Methanol was used as the blank solvent, and the solvent cut time was set to 3.5 minutes. Data acquisition was conducted in scan mode.

Result

Mace oil

Perusal of the data presented (Table 1), clearly showed that the nutmeg accessions exhibited significant differences for volatile oil content of mace. The highest volatile oil content in mace was significantly observed in Acc. 05 (6.25 %), followed by Acc. 10 (5.79 %) and the least volatile oil content in mace was recorded in Acc. 28 (2.46 %).

Kernel oil

As evident from the data presented in Table 1, the nutmeg accessions exhibited significant differences in volatile oil content of the kernel. Highest volatile oil content was observed in Acc. 05 (6.46 %), with Acc. 08 following at 4.95 % and the least volatile oil content in the kernel was recorded in Acc. 22 (1.90 %).

Oleoresin content

The data in Table 1 revealed a significant difference among the nutmeg accessions for oleoresin content in the kernel. Significantly highest oleoresin content in the kernel was recorded in Acc. 05 (33.89 %), followed by Acc. 10 (32.15 %) and the least was observed in Acc. 26 (19.81 %).

Chemoprofiling based on kernel and mace volatiles

GC-MS profile of volatile oil of both mace and kernel of Acc. 05 and Acc. 10 revealed wide variation. Data pertaining to the content of various constituents of volatile oil are furnished in Table 2 and Table 3.

Chemoprofiling based on mace volatiles

A total of Ten constituents were identified from the mace volatile oils of both Acc. 05 and Acc.10 nutmeg accessions. Wide range of variation was observed among the components of mace oil in both the accessions.

A total of Ten six constituents were identified from the mace volatile oil of Acc. 05 and Five constituents were identified in Acc. 10.

Wide variations were observed in the components of mace oils among the accessions. Cyclohexene, 4-methylene-1-(1-methyl ethyl) (27.0 %), 1,3-Benzodioxole, 4-Methoxy-6-(2-Propenyl) (21.0 %), 3-Cyclohexene-1-ol, 4-Methyt-1-(1-methylethyl) (15.0 %), Beta-pinene (6.7 %), Benzene, 1,2,3-trimethoxy-5-(2-propenyl) (5.7 %), Gamma-Terpinene (4.5 %), Bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methylethyl) (3.4 %), Cyclopentene, 3-isopropenyl-5, 5-dimethyl (3.1 %), Cyclohexene, 1-methylene-4-(1-methylethenyl) (2.7 %), and Cyclohexene, 1-methyl-4-(1-methyl ethylidene) (1.2 %) were identified in Acc. 05.

Cyclohexene, 4-methylene-1-(1-methyl ethyl) (30.6 %), 1,3-Benzodioxole, 4-Methoxy-6-(2-Propenyl) (28.0 %), Benzene, 1,2,3-trimethoxy-5-(2-propenyl) (23.7 %),3-Cyclohexene-1-ol, 4-Methyt-1-(1-methylethyl) (12.7 %), Bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methylethyl) (0.6 %) were identified in Acc. 10.

Chemoprofiling based on kernel volatiles

A total of Seven constituents were identified from the kernel volatile oils of both Acc. 05 and Acc.10. Wide range of variation was observed among the components of kernel oil in both the accessions.

Higher content of 1,3-Benzodioxole, 4-Methoxy-6-(2-Propenyl) was found in Acc. 05 (55.7 %), followed by 3-Cyclohexene-1-ol, 4-Methyt-1-(1-methylethyl) (18.2 %). The minimum constituents in mace oil of Acc. 05 were Bicyclo[3.1.0] hex-2-ene, 4-methyl-1-(1-methyl ethyl) (8.7 %), Benzene, 1,2,3-trimethoxy-5-(2-propenyl) (6.8 %) and Bicyclo[3.1.0] hexane, 4-methylene-1-(1-methyl ethyl) (1.9 %).

Higher content of Benzene, 1,2,3-trimethoxy-5-(2-propenyl) was also found in Acc. 10 (37.6 %), followed by 1,3-Benzodioxole, 4-Methoxy-6-(2-Propenyl) (26.1 %). The trace amount of constituents were Beta-pinene (8.3 %), Terpinen-4-ol (7.1 %) and Bicyclo[3.1.0] hexane, 4-methylene-1-(1-methyl ethyl) (3.4 %).

Discussion

Volatile oil of mace

The nutmeg accessions showed significant differences in volatile oil content in both mace and kernel. Significantly, maximum volatile oil in mace was observed in Acc. 05 (6.25 %) and minimum in Acc. 02 (2.46 %). Nutmeg accessions exhibited significant differences in volatile oil content of the kernel. The highest volatile oil content was observed in Acc. 05 (6.46 %) and the lowest volatile oil content in the kernel was recorded in Acc. 22 (1.90 %). This variation in volatile oil content in mace and kernel of different nutmeg accessions is due to genetic constitution and environmental conditions favouring biosynthesis and accumulations. The present results agree with the findings of earlier studies conducted by Maya *et al.* (2004), Kumar (2012) and Naveen (2003).

Volatile oil of kernel

As evident from the data presented in, the nutmeg accessions exhibited significant differences in volatile oil content of the kernel. The highest volatile oil content was significantly observed in Acc. 05 (6.46 %), followed by Acc. 08 following at 4.95 %. The lowest volatile oil content in the kernel was recorded in Acc. 22 (1.90 %). This variation in volatile oil content in mace and kernel of different nutmeg accessions is due to genetic constitution and environmental conditions favouring biosynthesis and accumulations. The present results agree with the findings of earlier studies conducted by Maya *et al.* (2004), Kumar (2012) and Naveen (2013).

Oleoresin of kernel

The oleoresin content showed significant variation among the different nutmeg accessions in the present study. Significantly, maximum oleoresin content was recorded in Acc. 05 (33.89 %) and was minimum in Acc. 26 (19.81 %). This may be attributed to genetic constitution of accessions and environmental conditions. These results confirm the findings of Naveen (2013), Teena (2015) and Vikram (2016) in nutmeg.

Chemo-profiling of kernel and mace volatiles

GC-MS analysis (Plate 3) of two distinct accessions *viz.*, Acc. 05 and Acc. 10 for mace and kernel volatile oil resulted identification of Ten and Seven constituents respectively. Volatile oil composition exhibited wide variability and also recorded presence of some unique compounds in the accessions. Major compounds *viz.*, Cyclohexene, 4-methylene-1-(1-methyl ethyl), 1,3-Benzodioxole, 4-Methoxy-6-(2-Propenyl), 3- Benzene, 1,2,3-trimethoxy-5-(2-propenyl), were seen in the kernel and mace volatile oil of both the accessions and it was highest in kernel volatile oil. Similar findings were also reported by Bakki *et al.* (2008) and Teena (2015).

Conclusion

From the present study, we can conclude that considerable variation found in nutmeg ecotypes under evaluation. All ecotypes had a huge variation in biochemical characters. Two ecotypes (Acc. 05 and Acc. 10) were found to be promising in terms of volatile oil and oleoresin content.

Acc. 05 found to be promising in terms of bio-chemical characters like volatile oil in both mace and kernel and oleoresin content in kernel. Therefore, considering all the characteristics the ecotypes Acc. 05 and Acc. 10 were found to be elite ecotype for mace and nut with quality compound.

GC-MS analysis of two distinct accessions *viz.,* Acc. 05 and Acc. 10 for mace and kernel volatiles revealed that Cyclohexene, 4-methylene-1-(1-methyl ethyl), 1,3-Benzodioxole, 4-Methoxy-6-(2-Propenyl), 3- Benzene, 1,2,3-trimethoxy-5-(2-propenyl), were seen in the kernel and mace volatile oil of both the accessions and it was highest in kernel volatile oil.

Table 1 : Variability for biochemical characteristics in nutmeg (*Myristica fragrans* Houtt.) accessions.

Accessions	Volatile oil content of mace (%)	Volatile oil content of kernel (%)	Oleoresin content of kernel (%)
01	4.55	4.23	27.26
02	4.19	4.04	30.09
03	5.43	4.27	25.13
04	4.25	4.26	29.00
05	6.25	6.46	33.89
06	4.40	4.64	26.18
07	4.64	4.13	29.04
08	3.35	4.95	26.94
09	3.53	3.44	26.11
10	5.79	4.76	32.15
11	3.26	4.10	24.04
12	4.55	4.43	23.20
13	3.38	4.50	23.35
14	3.23	3.41	26.06
15	3.36	3.56	27.40
16	3.75	3.61	21.37
17	4.17	4.62	25.19
18	4.47	2.66	29.20
19	3.54	4.79	31.03
20	4.56	3.39	22.33
21	5.13	2.94	25.27
22	4.32	1.90	29.20
23	3.37	4.64	22.96
24	4.18	4.73	26.17
25	4.53	3.11	27.94
26	3.56	3.53	19.81
27	3.49	4.33	27.11
28	2.46	4.91	26.47
29	4.33	3.63	26.47
30	4.18	3.56	23.56
S.Em±	**0.19**	**0.19**	**1.30**
CD (5%)	**0.55**	**0.53**	**3.68**

Table 2 : Variability for biochemical constituents of volatile oil of mace in select nutmeg accessions

Sl. No	Compound /accessions	Acc. 05	Acc. 10
1	1,3-Benzodioxole, 4-Methoxy-6-(2-Propenyl)	21.0	28.0
2	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)	5.7	23.7
3	Beta-pinene	6.7	
4	Cyclohexene, 4-methylene-1-(1-methyl ethyl)	27.0	30.6
5	Cyclohexene, 1-methylene-4-(1-methylethenyl)	2.7	*
6	Cyclopentene, 3-isopropenyl-5, 5-dimethyl	3.1	*
7	Cyclohexene, 1-methyl-4-(1-methyl ethylidene)	1.2	*
8	Gamma-Terpinene	4.5	*
9	Bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methylethyl)	3.4	0.6
10	3-Cyclohexene-1-ol, 4-Methyt-1-(1-methylethyl)	15.0	12.7

*Not detected

Table 3 : Variability for biochemical constituents of volatile oil of kernel in select nutmeg accessions

Sl. no	Compound /accessions	Acc. 05	Acc. 10
1	1,3-Benzodioxole, 4-Methoxy-6-(2-Propenyl)	55.7	26.1
2	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)	6.8	37.6
3	Bicyclo[3.1.0] hexane, 4-methylene-1-(1-methyl ethyl)	1.9	3.4
4	Bicyclo[3.1.0] hex-2-ene, 4-methyl-1-(1-methyl ethyl)	8.7	*
5	Beta-pinene	*	8.3
6	3-Cyclohexene-1-ol, 4-Methyt-1-(1-methylethyl)	18.2	*
7	Terpinen-4-ol	*	7.1

***Not detected**

Plate 1 : GC analysis of Mace and Kernel oil in laboratory

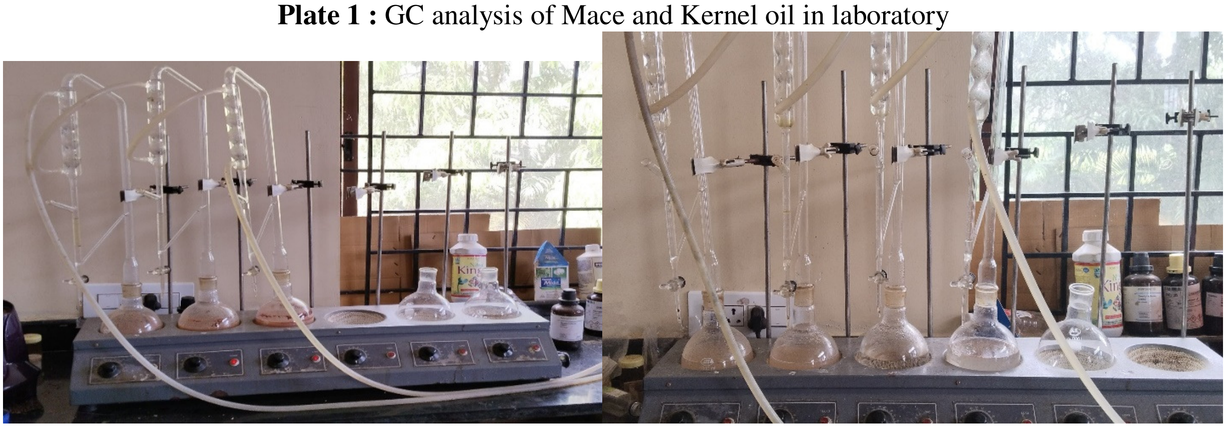

Plate 2 : Volatile oil extraction of Mace and Kernel in laboratory

Plate 3 : Oleoresin extraction of Kernel in laboratory

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