



Evaluation of Morphological Characteristics, Yield Potential, and Essential Oil Composition of *Ocimum sanctum* Genotypes for Crop Improvement

Mirudhula S. ^a, Nalina L. ^{b*}, Kokila Devi E. ^c,
Bini Sundar S. T. ^a and Meenakshi P. ^d

^a Department of Medicinal and Aromatic Crops, HC&RI, TNAU, Coimbatore, India.

^b Controller of Examinations, TNAU, Coimbatore, India.

^c Department of Plant Biotechnology, CPMB&B TNAU, Coimbatore, India.

^d Department of Biochemistry, TNAU, Coimbatore, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i193606

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/105437>

Original Research Article

Received: 17/06/2023

Accepted: 21/08/2023

Published: 25/08/2023

ABSTRACT

Ocimum sanctum, also known as Holy Basil, is an aromatic herb with a long history of use in traditional medicine. It is native to tropical and subtropical regions of Asia and is now cultivated worldwide. Plants have erect, much-branched sub-shrub with simple opposite green or purple leaves that are strongly scented and hairy stems and leaves has a strong, characteristic aroma that occurs mainly because of the volatile oils present.

*Corresponding author: E-mail: nalina@tnau.ac.in;

In this experiment, 30 genotypes of *Ocimum sanctum* were evaluated for morphological, yield and quality traits. At nursery stage, germination percentage, seedling shoot length and seedling root lengths were recorded. Among the accessions, OS-22 exhibited the highest germination percentages (82%), Seedling shoot and root length were highest in Os-18 and OS 21 (35 cm and 11 cm) respectively. In the main field, the accessions were characterized for qualitative traits viz growth habit, leaf colour and stem colour. The accessions were grouped into two, based on growth habit. Based on stem and leaf colour the accessions were grouped into five and four groups respectively. In case of quantitative traits, plant height achieved maximum in the accession OS 22 (88cm) followed by OS 17(75cm). The accessions OS-15 and OS-22 exhibited more extensive plants spread, while OS-21 and OS-13 had higher number of primary branches. Accessions OS-20 and OS-23 recorded more number of leaves (409 and 422) and the accessions OS-22 and OS-11 recorded highest fresh leaf weight (370.2 g & 301.67 g). The essential oil recovery and oil yield was highest in the accession OS 20 (green type) followed by OS 22 (purple type). Characterization of oil in GCMS revealed that the key component was eugenol, which showed highest content in accession OS-20 (46 %). This study can be used for further crop improvement studies and breeding programs.

Keywords: *Ocimum sanctum*; eugenol; essential oil; GC-MS.

1. INTRODUCTION

Ocimum sanctum, commonly known as holy basil, comes under the genus *Ocimum* belongs to the family Lamiaceae and has rich historical use in traditional medicinal systems, particularly in Ayurveda [1]. This aromatic plant holds profound significance in various cultures. The therapeutic potential of *Ocimum sanctum* has been recognized for its extensive health benefits [2]. The herb's multifaceted pharmacological actions encompass the ability to counteract physical, chemical, metabolic, and psychological stress [3]. It offers protection against chemical stress like pollutants and heavy metals, as well as physical stressors such as exertion, ischemia, and exposure to extreme conditions [4]. Moreover, it has been shown to positively impact metabolic parameters, including blood glucose, blood pressure, and lipid levels. Its anxiolytic, anti-depressant, and cognitive-enhancing properties contribute to its reputation as a medicinal herb [5]. It exhibits a broad-spectrum antimicrobial activity. It is rich in diverse metabolites, including essential oils, flavonoids, phenolic compounds, and terpenes. [6] The key compound, eugenol, is responsible for its distinct aroma and many of its medicinal effects, including antioxidant, anti-inflammatory, and antimicrobial activities [7]. Other significant metabolites such as caryophyllene, linalool, and ursolic acid further contribute to tulasi's adaptogenic and stress-relieving properties. Collectively, these metabolites interact to provide a range of health benefits, encompassing immune system modulation, cardiovascular support, and cognitive enhancement [8].

Originating from the Indian subcontinent, *Ocimum sanctum* is identifiable by its potent aroma, petite leaves and vibrant green or sometimes purple coloration. Typically, it assumes the form of a compact, bushy annual herb, reaching heights of up to 18 inches. Leaves are in vibrant green, regal purple, or captivating red shades, exhibiting ovate, lanceolate, or oblong shapes and varying in size from small (1-2 cm long) to large (5-10 cm long) [9]. Stem is green with hues of green, purple, or red, while flowers showcase the elegance of white, the softness of pink, or the majesty of purple, with forms ranging from tubular to bell-shaped. Among the different variants of *Ocimum sanctum*, "sri tulasi" and "krishna tulasi" stand out, distinguished by the colour of their leaves [10]. Morphological variation in holy basil is thought to be due to a combination of genetic and environmental factors. Hence, the research was conducted to identify a genotype with high yield, oil recovery along with unique morphological traits [6].

2. MATERIALS AND METHODS

Seeds of *Ocimum sanctum* accessions were collected from different parts of Tamil Nadu and the experiment was conducted in RBD with three replication and 30 treatments in the year 2022-23. Seeds were raised in nursery. Seeds germinated after 10 to 14 days of sowing. In the nursery, observation on germination percentage, seedling shoot length and root length were recorded. Seedlings were transplanted to main field at a spacing of 45x 45cm. Regular cultural operations were followed [3]. In the main field, both qualitative (plant growth habit, leaf colour,

stem colour). Royal Botanical society colour chart was used for screening the germplasm. Quantitative parameters viz. plant height, plant spread, number of primary branches, number of secondary branches, number of leaves per plant, fresh weight of leaves were recorded.

2.1 Essential Oil % Estimation

To extract essential oil fresh leaves were harvested and weighed, leaves were cut into small pieces and water was added with ratio of 3:1 after adding water sample was boiled by maintaining temperature ranging from 70 to 80° C it takes around 3 hours for distillation by condensation process oil get collected in upper layer of water and they are separated and collected in tube [11].

Essential oil was distilled and oil recovery and oil yield were calculated.

2.2 Gas Chromatography and Mass Spectroscopy

Essential oil of high yielding accessions were analyzed using a Perkin-Elmer (Italy) gas chromatograph (Model 8500) equipped with flame ionization detector (FID), GP-100 printer-plotter and an electronic integrator using BP-1 (SGE, USA) (25 m × 0.5 mm i.e. × 0.25 mm film thickness) capillary column coated with polydimethylsiloxane. Nitrogen was used as carrier gas at 10 psi inlet pressure with a flow rate of 0.4 ml/min (linear velocity 14 cm/s). Temperature was programmed from 60 to 220 °C at a ramp rate of 5 °C/min with a final hold time of 10 min, Identification of individual compounds was achieved by comparing their mass spectra with a relevant database, and quantification was done based on peak areas [12].

2.3 Statistical Analysis

The experiment was carried out in randomized block design with 3 replications and 30 treatments, each genotype is considered as one treatment. The mean performance and ANOVA was calculated using SPSS software.

3. RESULTS AND DISCUSSION

In this study, thirty germplasm accessions of Tulsi (*Ocimum sanctum*) were collected and evaluated for morphological, yield and quality traits. In the nursery, germination percentage (ranged from 5% to 95%). The accession OS12 exhibited the highest germination percentage 95%, followed by OS20 (75%). However, the accessions OS10 and OS-3 had much lower

germination rates, at 5% and 10%, respectively (Fig. 4). Significant variations were observed for seedling shoot and root length. Shoot length ranged from (8cm to 35 cm). The accession OS18 and OS16 recorded highest lengths 35cm and 30cm respectively, the accession OS10 recorded the lowest shoot length (8cm). Root length ranged from 3cm to 11cm) It was highest in OS21 and OS22 (11 cm and 10 cm) respectively and lowest in OS7 and OS19. Variation was observed for growth habit, stem and leaf colour. Owing to the ease of crosspollination, morph types, chemo types and genotypes are available in this genus [13]. The accessions were grouped into two based (Fig. 1) on growth habit viz. [14], erect and spreading. Erect growth habit was observed in 26 accessions and spreading habit was observed in 4 accessions. Based on stem colour the accessions were grouped into 5 (Fig. 2) (Table 2), Brilliant purple colour was observed in 4 accessions. Light purple stem colour was observed in 8 accessions were under, Yellowish green stem colour was observed in 6 accessions. Deep purple colour of stem was recorded in 7 accessions. Viz., Dark green was recorded in 3 number of accessions. Based on leaf colour the accessions were grouped into 4 in which accessions are grouped under vivid yellowish green colour leaf (Fig. 3). Accessions were grouped under dark green leaves. Light olive green was observed in 5 accessions [6]. Observations in the main field after 45 days (Table 3) of transplanting revealed significant variations for growth and yield parameters. Plant height (ranged from 15cm to 88cm), the accession OS22 recorded the maximum height (88cm, while OS8 and OS4 had lower height 22.1cm and 23cm, respectively. Plant spread (ranged from 10cm to 62cm) the accessions OS15 and OS18 recorded highest spreads at 58cm and 59cm, respectively. Number of primary branches ranged from 3 to 22 with OS21 having more primary branches [4], followed by OS22 with 15 branches. OS6 had the fewest primary branches [15]. The number of secondary branches ranged from 9 to 76 the accession OS20 having the more number of secondary branches 72, and OS6 having the fewest 9 [16]. Further the accessions were grouped based on statistical analysis and are arranged in a group based on least significant difference. totally 18 groups were classified and those which have similar characters are arranged in same group and its said to be on par with each other (Table 4) Yield observation like number of leaves per plant also varied significantly, with OS20 and

OS22 having the more number of leaves 422 and 409, respectively, while OS9 had the fewest leaves 65. Leaf yield ranged from 115g to 371.5g, with OS20 producing the highest yield at 371.5g and OS10 producing the lowest yield at 115g. among the accessions OS20 recorded more plant height, number of primary branches, secondary branches which in turn resulted in high leaf yield. The genetic improvement of the crop depends mostly on the nature and relative magnitude of genetic variance components concerned with yield and yield related attributes yield [17] of essential oil recovery % ranged from (0.01 to 1%) were accession Os20, OS22 observed 0.9 and 0.85% of essential oil (Fig. 5) [18,19].

Since the yield of OS20 (Fig. 6) and OS22 (Fig. 7) is more due to various factors. This essential oil were subjected to screening of major secondary metabolites like eugenol percentage in OS20 is (46%) OS22 (20%), caryophyllene in

OS20 (10%) and OS22 (30%), eucalyptol in OS20(2%) and OS22 (0.17%), Methyl eugenol in OS20 (51.78%) and OS22 (34%), elemene in OS20 (6.8%) and OS22 (11.6%), and germacrene D in OS20 (5.3%) and OS22 (7.2%) [20,21]. The compound alpha-terpinol in OS20 (5%) and it was absent in OS22. chemical composition of essential oil mainly depends on soil and climatic condition on the location, growing season and maturity stage however plants subjected to present study were cultivated at the same location under same soil and climatic conditions and harvested at the same age [3]. Therefore variability of essential oil composition could be considered as variation between two morphotypes [6]. Considering all these observations, it is evident that Os20 performed exceptionally well in terms of morphological characteristics, yield, and containing a higher percentage of secondary metabolites, indicating valuable medicinal and pharmacological properties.

Table 1. List of germplasm accessions in Ocimum sanctum

S.NO	Accession number	Place collected	Latitude (° N)	Longitude (° E)
1	OS-1	Tiruchirappali	10.7905	78.7047
2	OS-2	Dindigul	10.3624	77.9695
3	OS-3	Pudukkottai	10.3833	78.8001
4	OS-4	Madurai	9.9252	78.1198
5	OS-5	Salem	11.6643	78.1460
6	OS-6	Erode	11.3410	77.7172
7	OS-7	Dharmapuri	12.1211	78.1582
8	OS-8	Thanjavur	10.7870	79.1378
9	OS-9	Ariyalur	11.1401	79.0786
10	OS-10	Namakkal	11.2194	78.1678
11	OS-11	Karur	10.9601	78.0766
12	OS-12	Vellore	12.9165	79.1325
13	OS-13	Thiruvallur	13.1231	79.9120
14	OS-14	Perambalur	11.2342	78.8807
15	OS-15	Viluppuram	11.9401	79.4861
16	OS-16	Theni	10.0079	77.4735
17	OS-17	Musiri	10.9549	78.4439
18	OS-18	kulithalai	10.9426	78.4172
19	OS-19	Neyveli	11.5390	79.4794
20	OS-20	Coimbatore	11.0168	76.9558
21	OS-21	Kanyakumari	8.0844	77.5495
22	OS-22	Tirunelveli	8.7150	77.7656
23	OS-23	Krishnagiri	12.5266	78.2150
24	OS-24	Sivagangai	9.8433	78.4809
25	OS-25	Virudhunagar	9.4218	77.8367
26	OS-26	Ramanathapuram	9.3639	78.8395
27	OS-27	Thoothukudi	8.7642	78.1348
28	OS-28	Thiruvarur	10.7661	79.6344
29	OS-29	Chennai	13.0827	80.2707
30	OS-30	Thuraiyur	11.1519	78.5951

Table 2. Qualitative characterization of *Ocimum sanctum* genotypes

Plant habit	Genotypes
Spreading	Os-1, Os-2, Os-3, Os-4, Os-5, Os-7, Os-8, Os-9, Os-11, Os-12, Os-13, Os-14, Os-15, Os-16, Os-18, Os-19, Os-20, Os-21, Os-22, Os-23, Os-25, Os-26, Os-27, Os-28, Os-29, Os-30
Erect	Os-6, Os-10, Os-17, Os-24
Stem colour	
Yellowish green	Os-1, Os-4, Os-13, Os-16, Os-26, Os-28, Os-30, Os-26, Os-21
Brilliant purple	Os-2, Os-8, Os-15, Os-22
Light purple	Os-3, Os-5, Os-7, Os-10, Os-12, Os-19, Os-21, Os-24
Dark Green	Os-9, Os-20, Os-29
Deep Purple colour	Os-6, Os-11, Os-14, Os-17, Os-18, Os-23, Os-27
Leaf colour	
Vivid yellowish green	Os-1, Os-4, Os-5, Os-7, Os-8, Os-10, Os-12, Os-15, Os-16, Os-21, Os-23, Os-24, Os-26
Light olive green	Os-2, Os-3, Os-6, Os-18, Os-20, Os-25, Os-30
Dark green	Os-9, Os-11, Os-14, Os-19, Os-28
Brilliant yellow green	Os-27, Os-29
Deep purple	Os-13, Os-17, Os-22

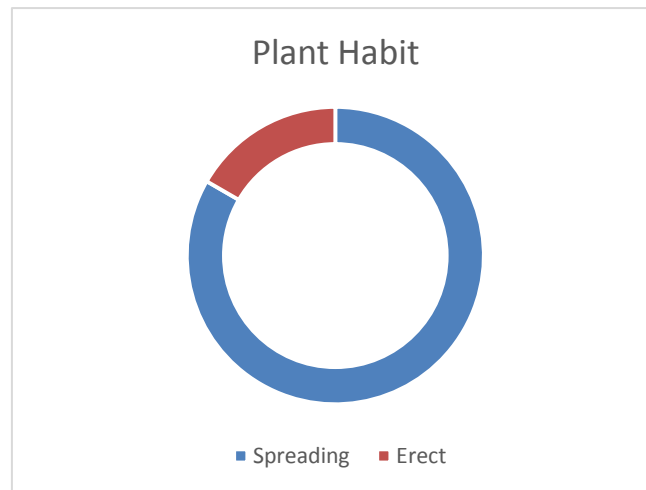


Fig. 1. Plant habit of *O. sanctum*

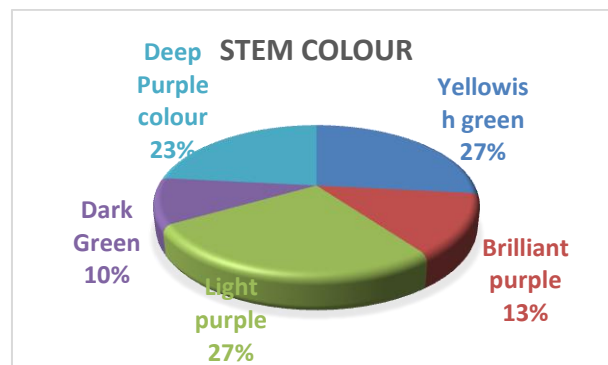


Fig. 2. Variations among stem colour *O. sanctum*

Table 3. morphological parameters of *Ocimum sanctum* genotypes

Accession name	Germination %	Shoot length (cm)	root length(cm)	Plant height (cm)	Plant spread (cm)	No of primary branches	No of secondary branches	No of leaves	Leaf fresh wt. (g)	Oil recovery percentage
OS-1	50.41	18.12	5.00	29.40	24.75	9.00	18.00	75	302.67	0.25
OS-2	29.93	23.30	6.12	39.40	31.23	14.00	31.00	98	210.30	0.20
OS-3	10.10	22.22	4.21	27.36	22.13	9.00	27.00	69	333.00	0.01
OS-4	40.72	19.12	5.52	23.21	20.51	8.00	16.00	88	227.31	0.10
OS-5	19.88	29.52	9.00	56.53	44.55	10.00	25.00	150	215.33	0.50
OS-6	29.88	25.30	4.41	36.42	25.88	3.00	9.00	121	290.71	0.05
OS-7	9.79	15.12	3.33	33.63	22.19	7.00	28.00	89	183.92	0.03
OS-8	19.55	20.30	6.00	22.10	15.43	5.00	10.00	97	222.13	0.10
OS-9*	20.17	17.20	10.12	47.66	41.24	11.00	33.00	160	235.67	0.40
OS-10	4.92	8.15	4.01	15.62	10.22	9.00	18.00	65	115.78	0.02
OS-11	49.69	32.22	7.20	50.55	46.15	10.00	20.00	189	354.67	0.54
OS-12	95.73	27.15	9.33	61.24	51.97	15.00	35.00	210	283.67	0.23
OS-13**	77.60	34.33	5.20	55.12	57.79	9.00	27.00	250	211.25	0.36
OS-14	59.52	29.01	6.12	46.42	50.79	13.00	26.00	291	297.62	0.59
OS-15	64.74	30.25	7.55	57.80	64.27	19.00	29.00	198	369.32	0.45
OS-16	70.01	32.23	8.52	62.43	58.65	20.00	48.00	269	274.67	0.39
OS-17	49.78	25.10	6.30	73.44	33.29	10.00	22.00	150	335.95	0.16
OS-18	83.52	35.31	9.20	78.33	61.30	21.00	33.00	215	367.33	0.62
OS-19	45.16	18.21	3.12	34.24	20.80	8.00	16.00	101	337.15	0.09
OS-20**	72.58	33.11	8.33	57.52	45.58	19.00	45.00	325	433.70	0.85
OS-21	78.45	24.22	11.01	67.41	51.98	22.00	35.00	269	280.91	0.59
OS-22**	82.92	27.32	9.20	84.30	46.18	15.00	21.00	356	371.52	0.90
OS-23*	90.51	22.10	5.30	73.13	23.26	21.00	36.00	158	282.57	0.31
OS-24	40.18	20.22	6.41	28.62	25.71	11.00	15.00	198	216.01	0.11
OS-25	45.30	16.20	8.20	36.41	20.42	12.00	12.00	120	253.02	0.23
OS-26	71.96	23.30	5.15	38.45	45.81	9.00	15.00	164	314.00	0.25
OS-27	41.58	25.15	6.23	36.34	25.25	10.00	20.00	99	233.66	0.30
OS-28	53.31	22.12	9.00	33.11	14.73	13.0	19.00	222	301.67	0.50
OS-29	28.71	28.30	6.10	46.71	18.13	7.00	31.00	133	235.71	0.22
OS-30	19.28	15.12	4.15	37.30	19.46	11.00	22.00	167	217.50	0.03

Accession name	Germination %	Shoot length (cm)	root length(cm)	Plant height (cm)	Plant spread (cm)	No of primary branches	No of secondary branches	No of leaves	Leaf fresh wt. (g)	Oil recovery percentage
mean	48.6000	23.76	6.43	45.99	34.26	11.65	31.23	199.3	294.6	0.32
SEd	1.1412	0.40	0.14	2.34	0.85	0.26	0.60	4.99	6.12	0.0063
CD(.05)	2.2847	0.81	0.29	4.70	1.71	0.52	1.21	9.99	12.26	0.012

Table 4. Grouping of genotypes based on parameters by statistical analysis

Parameter	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Germination %	OS12	OS23	OS13 OS18 OS22	OS22 OS18 OS21	OS20	OS16 OS26	OS15	OS14	OS28	OS1 OS11 OS17	OS19 OS25	OS19 OS27 OS24	OS4 OS24	OS2 OS6 OS29 OS9 OS30	OS5 OS8	OS3 OS7	OS10	
Shoot length	OS18	OS13	OS20 OS22	OS11 OS16	OS15	OS5 OS14	OS29	OS12	OS6 OS17 OS27	OS21	OS2 OS26	OS3 OS23 OS24 OS28	OS8 OS4	OS4 OS1 OS19 OS25 OS30	OS9 OS7 OS25 OS30	OS7 OS10		
Root length	OS21	OS9 OS20	OS5 OS12 OS18 OS22 OS28	OS16 OS25	OS11 OS15	OS2 OS8 OS14 OS17 OS24 OS27 OS29	OS1 OS4 OS13 OS10 OS30	OS3 OS6 OS19	OS7	OS19								
Plant height	OS22	OS17 OS18 OS20	OS17 OS23 OS20	OS21	OS5 OS12 OS15 OS16	OS5 OS13 OS15 OS16	OS9 OS11 OS14	OS9 OS14 OS29	OS6 OS26 OS27 OS30	OS6 OS19 OS25 OS27 OS30	OS6 OS7 OS19 OS19 OS7 OS27 OS28 OS28	OS2 OS7 OS19 OS19 OS7 OS24 OS28 OS28	OS1 OS2 OS3	OS3 OS4 OS8	OS10			
Plant spread	OS15 OS22	OS16 OS18 OS20	OSs13	OS12 OS14 OS21	OS5 OS11 OS26	OS5 OS11	OS9	OS17	OS2	OS1 OS6 OS24 OS27	OS3 OS7 OS23 OS25 OS30	OS4 OS19 OS25 OS27 OS28	OS29	OS8 OS28	OS10			
No of primary branches	OS21 OS22	OS18 OS23	OS16 OS20	OS15	OS12	OS2	OS14 OS28	OS25	OS9 OS24 OS30	OS5 OS11 OS17	OS1 OS3 OS10	OS4 OS19	OS7 OS29	OS8	OS6			

Parameter	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
No of secondary branches	OS20	OS22	OS12 OS21	OS2 OS18 OS23	OS16 OS28	OS15 OS28	OS25	OS9 OS26	OS5 OS17	OS3 OS7 OS13 OS14 OS29	OS3 OS13	OS24 OS30	OS11 OS27	OS1 OS10 OS19	OS4 OS19	OS6 OS8		
	OS11	OS13 OS16 OS18 OS20 OS22	OS19	OS13 OS15 OS17 OS19	OS1 OS26	OS1 OS5 OS12 OS28	OS5 OS10 OS21 OS23	OS10 OS14 OS21 OS23	OS25	OS4 OS8 OS 27 OS29	OS4 OS7 OS27	OS4 OS7 OS24 OS30	OS2 OS7 OS24 OS30	OS16				
	OS20 OS22	OS19	OS14 OS21	OS11	OS5	OS28	OS15	OS9	OS16 OS17	OS13	OS23 OS27	OS1 OS26	OS12 OS25 OS29	OS2	OS18	OS4 OS8 OS24	OS6	OS7 OS10 OS30 OS3

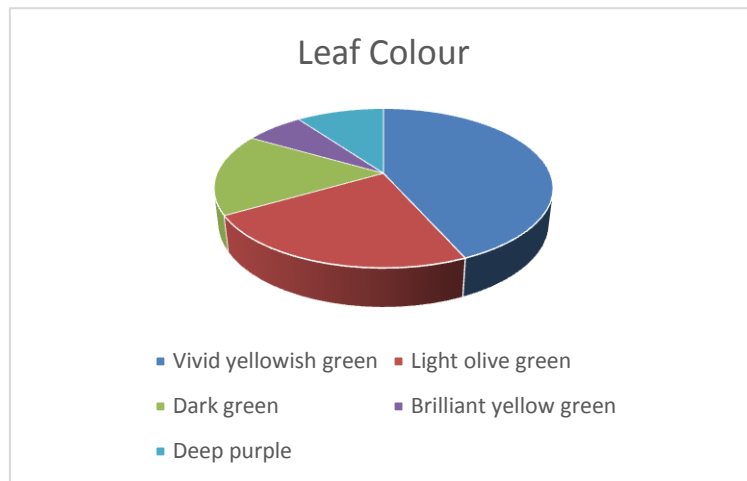


Fig. 3. Variation of leaf colour in *O. sanctum*

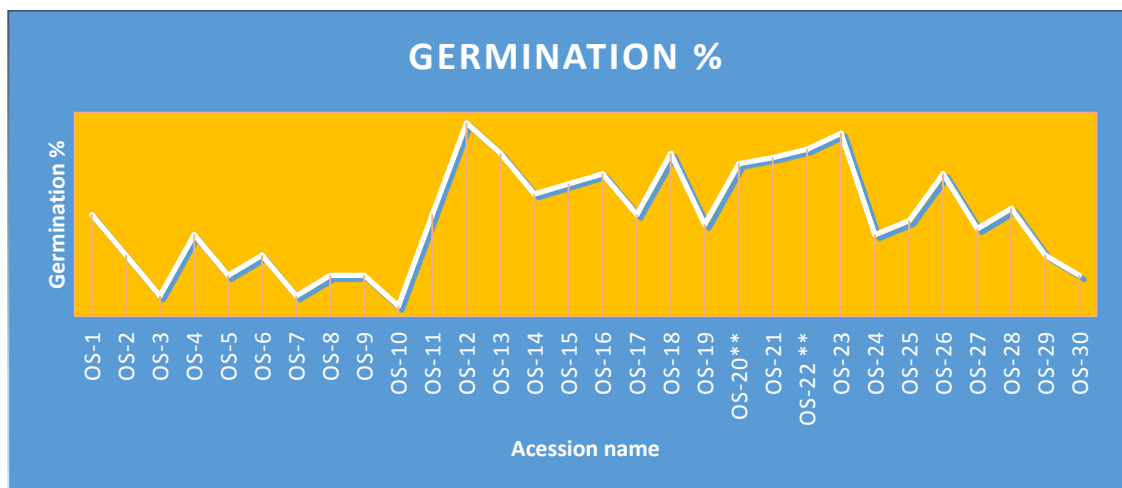


Fig. 4. Germination percentage of *O. sanctum* genotypes

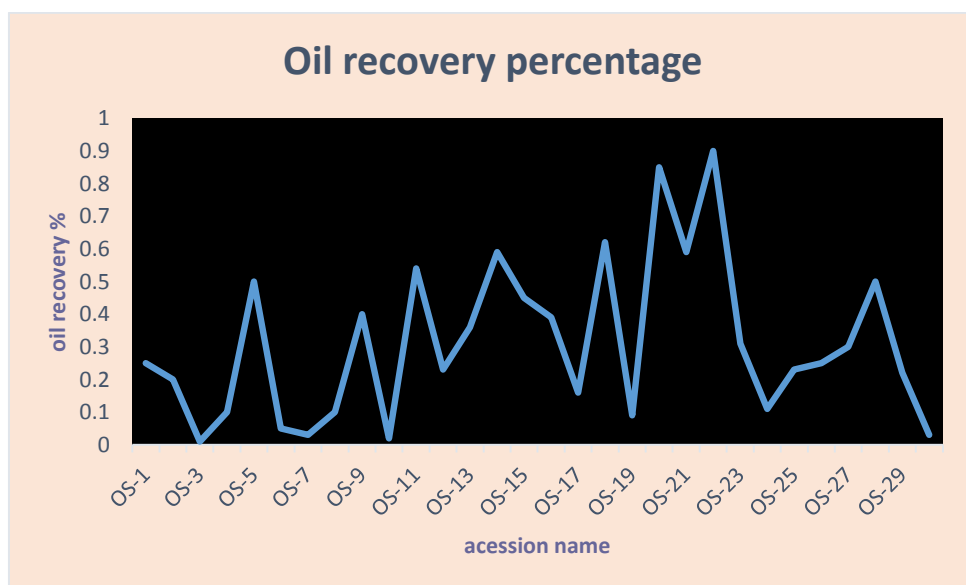


Fig. 5. Essential oil recovery percentage of *O. sanctum* genotypes

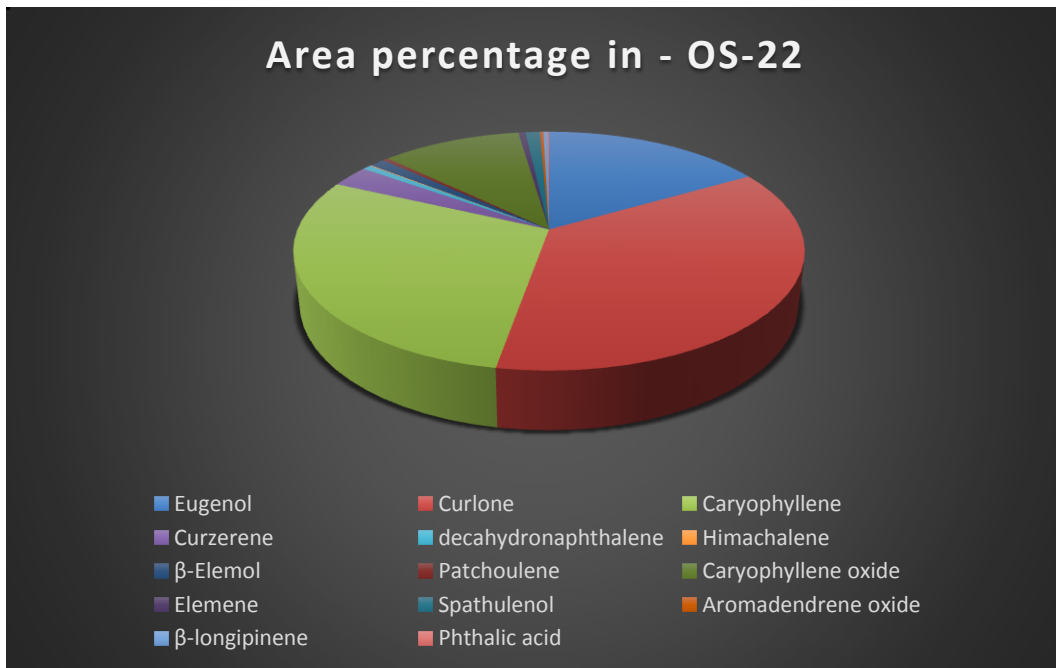


Fig. 6. Secondary metabolites and its area percentage through GC-MS in Os-20

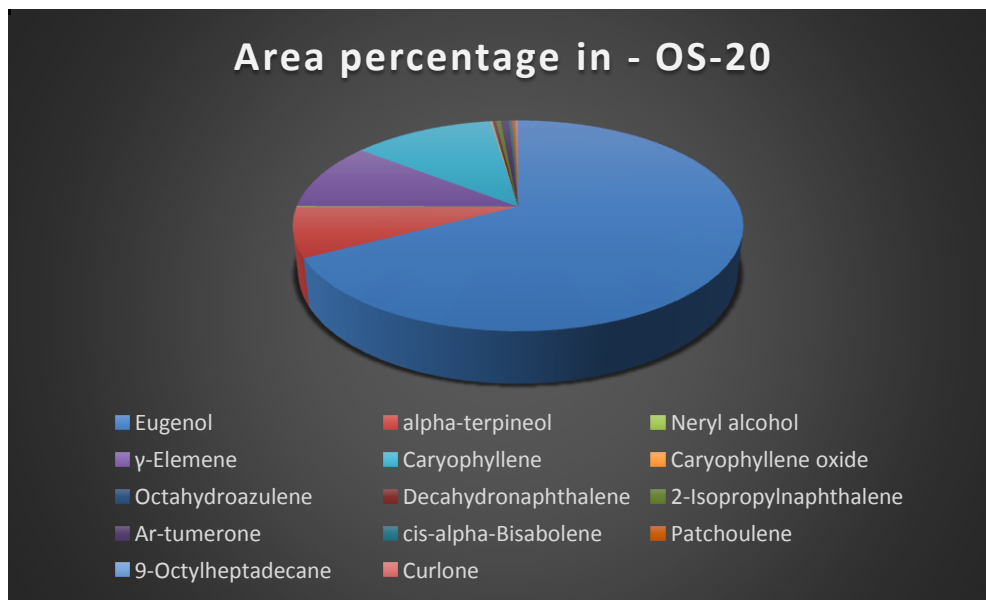


Fig. 7. Secondary metabolites and its area percentage through GC-MS in Os-22

4. CONCLUSION

Evaluated 30 *ocimum sanctum* genotypes for their morphological, yield, and quality traits. The findings highlighted significant genetic diversity, ranging from germination percentages to growth habits, stem colors, and essential oil composition. Notably, OS12 exhibited the highest germination rate at 95%, while OS20 and OS22 excelled in growth parameters and

essential oil content. OS20 stood out for its exceptional morphological traits, yield, and higher secondary metabolite percentages, suggesting promising medicinal potential. The study underscores the importance of preserving this genetic diversity for sustainable agriculture and pharmaceutical applications, emphasizing the need for further exploration of the genetic mechanisms and utilization of *ocimum sanctum* diverse traits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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