Industrial Crops & Products 108 (2017) 700–707

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Selection of superior *Ocimum sanctum* L. accessions for industrial application

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ARTICLE INFO

Keywords:
Ocimum sanctum
Eugenol
Chemical characterization
Leaf recovery
Harvesting stage

ABSTRACT

Holy basil (*Ocimum sanctum* L.), a sacred medicinal plant in India is widely used in Indian and other international traditional medicinal systems. Eleven accessions of the plant have been characterized for 21 quantitative and qualitative traits and six essential oil components. Three different harvesting stages of accessions grown in western India were collected for selecting superior accessions with desirable morphological and chemical characters affecting yield and contributing traits for industrial use. The essential oil was extracted by hydro distillation and the characterized by gas chromatograph-mass spectrometry. The colour of the leaves, new branches and inflorescence varied from green to purple-green. The basil accession, DOS-1, resulted in maximum dry leaf recovery (230 g kg^{-1}), total chlorophyll content (1.25 mg g^{-1}), carotenoid content (8.5 mg mL^{-1}) and number of peltate glands. The oil content in green herbage was maximum in DOS-1 (50 g kg^{-1}), followed by DOS-3 (44 g kg^{-1}) at crop maturity. Maximum oil and eugenol yield was also observed in DOS-1 (73 kg ha^{-1} and 67 kg ha^{-1}). Overall, DOS-1 accession was found superior for leaf recovery, oil yield and eugenol content and therefore, can be used further in crop improvement and commercial cultivation as a new selection.

1. Introduction

The basils are largely distributed in Asia, Australia, West Africa and also in some Arabian countries mainly in drier sandy areas (Pistrick, 2001). It is a group of 50–150 species comprising of herbs and shrubs from the tropical regions. The holy basil (*Ocimmum sanctum* L. syn. *Ocimum tenuiflorum*) (Family Lamiaceae), is the most sacred herb. It is a native of India and is cultivated largely as a house hold species in India. It is a diploid species having chromosome no. 2n = 32. Leaf types in basils are simple, ovate, elliptic-oblong, obtuse or acute and the leaf margins are usually slightly toothed with entire or sub-serrate or dentate. Both the adaxial and abaxial surfaces are pubescent and dotted with minute glands and slender hairy petioles (Malav et al., 2015).

Basil leaves find extensive use in Ayurvedic system of medicine for various ailments including the lowering of plasma glucose (Mukherjee et al., 2006). Essential oils and herbal extracts have attracted a great deal of scientific research interest due to potential as natural flavours. The most common uses of the holy basil include preparation of herbal tea, healing remedies, cosmetics and as preservative (Anbarasu and Vijayalakshmi, 2007). Essential oils of holy basil is valued due to active constituent eugenol that contributes to their therapeutic potential (Kothari et al., 2004). Other constituents in the essential oil include thymol, citrol, geraniol, camphor, linalool, and methyl cinnamate (Padalia and Verma, 2011; Singh et al., 2011; Verma et al., 2011). Methyl eugenol (ME), which is chemically known as 4-allylveratrole; 4-allyl-1,2- dimethoxybenzene; 1,2- dimethoxy-4-(2-propenyl) benzene; 3,4- dimethoxy-allylbenzene; 3-(3,4- dimethoxyphenyl) prop-ʟ-ene, is the chief aromatic compound in holy basils. The biosynthesis of ME is routed through formation of an essential amino acid, the phenylalanine through caffeic acid and ferulic acid (Herrmann and Weaver 1999). Synthetic ME is now in wide use in perfumery, aromatherapy as well as in processed food industry as flavoring agent (Tan and Nishida, 2012). Recently, antifungal activity of holy basils was documented (Sethi et al., 2013). Eugenol is usually extracted from clove buds (70–85%) as well as leaves and barks of Cinnamomum (20–50%). Although, these plants are rich in eugenol but their cost of commercial extraction is very high (Shasany, 2016).

Morphological and chemotypic characterization is the main criterion for selection of suitable *Ocimum sanctum* variety for herbal industry. The variants with different combinations of purple or green calyces and purple or white corolla are available in India and adjoining regions. These include light green-leaved (Rama tulsi) and purple/dark green-leaved (Shyama tulsi) (Kothari et al., 2004). Oil yield, leaf stage and no. of peltate gland (PG) also play important role in the content of essential oil obtained from plants.

Although various workers have reported the variation in the

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http://dx.doi.org/10.1016/j.indcrop.2017.07.028
Received 7 April 2017; Received in revised form 13 July 2017; Accepted 14 July 2017

chemical composition and essential oil content in the *Ocimum* sp., very few comprehensive studies have been conducted regarding the morphological and chemical characterization of the different accessions to select a superior accession, which can be commercially explored for industrial application. With this background in mind, the current study was undertaken to determine the important morphological parameters to select superior *O. sanctum* accessions from western regions of India that have better herbage, oil yield and chemical composition of leaf essential oils and the best stage of harvesting.

2. Materials and methods

2.1. Experimental site

The study was conducted at the experimental fields of the Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat (India) during two consecutive harvesting seasons in the years 2015 and 2016. The experimental farm is located at 22°35′N and 72°55′E at an altitude of about 45.1 m above mean sea level.

2.2. Plant materials

Plants of ten accessions of holy basil collected from different parts of India, were maintained and compared with check variety "Angana", for inclusion in the present study. The collection sites of accessions and their origin are listed in Table 1. Seeds were sown in the field at DMAPR, Boriavi, Anand (Gujarat) with a spacing of 45 cm × 45 cm. The crop was raised following the standard agronomic practices. Harvesting was done during September–October months of 2015 and 2016. Leaves were collected for analysis at full flowering stage from each accession. Field screening of different accessions was carried out for leaf yield and related traits. The plants selected for analysis were of uniform in age. In the field, three blocks of each germplasm were marked and ten plants in each block were randomly chosen for observations represented as a replication. The values of different observations obtained from these plants were averaged to get the mean value.

2.3. Extraction of essential oils

The fresh biomass of the tagged plants was harvested at maturity stage for extraction of essential oil. Freshly harvested leaves (1000 g each) were hydro-distilled for 3.0 h in a Clevenger-type apparatus in triplicate. The essential oil layer was separated. The distillate was extracted with diethyl ether and etheral layer was dried over anhydrous sodium sulphate. Ether was distilled off on gently heated water bath and oils were stored in amber colour vials at 4–8 °C for further analysis.

Table 1
Geographic information of collection site of different accession.

Accession	Type	State	Local area	Location	
				Lat ^{0}N	Long ^{0}E
TC-1	Local Selection	Gujarat	Boriavi	22° 61′	72° 93′
DOS-1	Local Selection	Gujarat	Mogar, Anand	22° 54′	73° 02′
DOS-3	Local Selection	Gujarat	Boriavi	22° 61′	72° 93′
DOS-4	Local Selection	Gujarat	Boriavi	22° 61′	72° 93′
DOS-5	Local Selection	Gujarat	Boriavi	22° 61′	72° 93′
DOS-7	Local Selection	Gujarat	Boriavi	22° 61′	72° 93′
CHES G-1	Local Selection	Gujarat	Vejalpur	22° 41′	73° 34′
DEDIYA G-1	Local Selection	Gujarat	Dediapada	21° 38′	73° 35′
DEDIYA S.P.	Local Selection	Gujarat	Dediapada	21° 38′	73° 35′
DEDIYA P-1	Local Selection	Gujarat	Dediapada	21° 38′	73° 35′
Angana	Variety	U.P.	CIMAP Lucknow	26° 87′	80° 98′

2.4. Leaf colour intensity

The intensity of the colour of the leaf, inflorescence and branches was determined based on a scale developed by a panel of five scientists for confirming the observations taken from experimental fields. The intensity of purple or green colour at the maturity stage was measured by the scientists. The aim was to morphologically characterize the accession for the extent to which colour was perceived as crucial factor. The scoring procedure was consummate with the help of check list of odd numbers from 0 to 13 put during the proper stage for intensity of purple colour of new branch, an inflorescence and the leaf including veins, margin and mid rib. For each trait, the score of individual scientists was added and each rank was confirmed into scores on majority basis. The maximum score for all the values were arranged in descending order (Table 2).

2.5. Relationship between peltate gland and age of leaf

Leaves were collected from experimental field at three different stages *viz*., young (fully expanded), recently mature (dark colored) and old (before yellowing). The leaves at tip of the axis to 3rd; 4th–6th and 7th–9th internode, respectively, were used for measuring leaf area using LAM (LAI 3000) and counting no. of PG in 0.5 mm^2 area under light microscope at 10 *X* visualization.

2.6. Analysis of the essential oil composition

The chemical characterization of the essential oils was performed on a Thermo Focus GC coupled with Thermo Polaris Q single quadruple mass spectrophotometer (MS) detector in Electron Ionization mode and Thermo triplus autosampler on a DB-5MS capillary column (30 m, 0.25 mm id, 0.25 μ film thickness) with the following operating conditions initial oven temperature 60 °C, held for 5 min, then a 5 °C/min tamp to 250 °C and held for 3 min; carrier gas he constant flow @ 1.0 mL min^{-1}, injection volume 0.5 μL (split flow-1:20), the temperature for Inlet, ion source and MS transfer line was 240 °C, 220 °C and 240 °C, respectively. The GC column was coupled directly to the spectrometer in EI mode at 70 eV with the mass range of 40–500 atomic mass unit at 1 scan/s. Individual compounds were identified by mass spectrometry and their identities were confirmed by comparing mass spectra with Mass Spectral Library and literature (Adams, 2007; NIST, 2005).

2.7. Economics

On an average, a yield of 1843 kg ha^{-1} dry leaf and 73 kg ha^{-1} oil was obtained from harvests. The farmer sold its dry leaf at US$ 2.32 kg^{-1} and oil at US$ 38.88 kg^{-1} in local market. The material was turned over on alternate days during drying under shed condition and oil was extracted from fresh herbage using Hydro-distillation. The primary data were collected through personal interview using a pre-tested questionnaire. To examine the economics, simple cost accounting method was followed and the financial feasibility was worked out by comparing costs and returns. The prices used in the analysis were averages for the period 2015–16.

2.8. Statistical analysis

The statistical analysis of the data was performed using standard statistical procedures. The analysis of variance was done in randomized block design for observations recorded during experiment by using statistical software SAS 9.2 (SAS, 2008). DMRT comparisons among the essential oil, compounds obtained from the accessions including check. The results were presented at 5% level of significance ($P = 0.05$). The critical difference (*CD*) values were calculated to compare the various treatment means.