EFFECT OF PLANT ONTOGENY ON QUANTITY AND CHEMICAL CONSTITUENTS OF ESSENTIAL OIL IN SWEET BASIL (*OCIMUM BASILICUM* L.) GROWN IN SUDAN

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ABSTRACT: Sweet basil (*Ocimum basilicum* L.) is a very important food additive as well as for its therapeutic and cosmetic potential. The composition of essential oils in plants is affected by genetics and environmental conditions, which is determined by growth region and harvesting time in terms of ontogenetical variability. This study was carried out to assess the effect of plant ontogeny (pre-flowering, at flowering, fruiting) on essential oil content and chemical constituents for four sweet basil cultivated under irrigation conditions in the experimental farm of the National Oilseed Processing Research Institute (NOPRI), University of Gezira, Sudan. The essential oils were hydro-distilled from the leaves using Clevenger apparatus and the chemical constituents were determined by GC-MS. The results reveal that the essential oil yield content ranged from 0.1% to 0.2% at pre-flowering stage, whereas the oil content obtained at post-flowering stage was 0.1% for the investigated accessions. The highest essential oil content was recorded at flowering stage (0.2-0.5%), where the two Sudanese accessions had the maximum content (0.5%). The major chemical constituents, linalool, citral, methyleugenol, and eucalyptol reported at different developmental stages, punctuated between 5.73% and 32.93% in the four investigated accessions.

KEY WORDS: *Accessions, Chemical constituents, Essential oil, Plant ontogeny, Sweet basil.*

1. INTRODUCTION

Sweet basil (*Ocimum basilicum* L.), known as rihan in Sudan, is a perennial aromatic herb belonging to the Lamiaceae family and has been extensively utilized in food as a flavoring agent, in perfumery and in medical industries [1]. Also, the basil oil contains biologically-active constituents that possess insecticidal [2], nematicidal [3], and fungistatic properties [4]. Basil plants can be found in Sudan in wild populations.

The variation in yield and constituents of essential oils might be ascribed to genetic, developmental stages (ontogeny) and environmental factors [5, 6 & 7]. The plant ontogeny is considered as one of the most determining factors that influence amount and composition of essential oil [8]. The stage of full flowering is considered to be optimal for harvesting the herb due to the highest essential oil content [9, 10]. Environmental factors and harvest times have been observed to affect the chemical content and composition of essential oil [11]. The development of oil production by sweet basil accessions subjected to cultivated conditions could lead to optimization of oil yields [12, 13]. The essential oil of *O. basilicum*, harvested at flowering, was dominated by linalool (71.5%) and eugenol (10.16%) with an essential oil yield of 0.17% [14]. Harvesting the herb at the vegetative stage is the most popular method when basil is used as fresh, aromatic spice [15].

To meet the industrial growing demand for the essential oil of basil there is an urgent need for improving yield and identification of chemical constituents. The aim of this study is to investigate how both quality and quantity of sweet basil essential oil is influenced by plant ontogeny. This investigation of the distilled essential oils may give information about the optimum time to harvest for the highest yield and the most desirable aroma.

2. MATERIALS AND METHODS

2.1 Source of seeds

Four variants of sweet basil, two from Sudan (Abuharaz seeds were collected from the wild, and Lemon basil was obtained from Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, Sudan), and two from Germany and Thailand, were provided by the Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany.

2.2 Cultivation practices

Seeds were planted in the fields of the experimental farm of the National Oilseed Processing Research Institute (NOPRI), University of Gezira, Wad Medani, Sudan, June 2015. The Experiment was arranged in a randomized complete block design (RCBD) with three replicates using Fractional Factorial Design of Experiments (FFDOE) under Design of Experiments software. Leaves were collected in three different times of plant growth (before flowering, at flowering and at the fruiting stage).

2.3 Hydro distillation of essentials oils

The leaves of basil were harvested, weighed and dried at room temperature for 72 hours. For distillation of essential oil, 200 gram of each sample was ground and subjected to hydro distillation for 4 hours at 65°C using Clevenger apparatus. The oil was collected and dried over anhydrous sodium sulphate and stored in a brown bottle under refrigeration for further analysis. The essential oil was calculated as a percentage based on dry weight.

2.4 Gas Chromatography – Mass Spectrometry Analysis

To identify the constituents of essential oil extracted from the leaves of the 4 accessions, GC analysis was performed using Agilent Technologies 7890 A, gas chromatograph, 5975c inert MS with a capillary column (30 m x 250µm with film thickness 0.25µm) was used. Helium was used as a carrier gas, at a flow rate of 3 ml/min. The oven temperature was increased between 25°C/min to 200°C for 35 min, and then 5°C/min to 250°C for 18min. Essential oil solution (5µl) in hexane was injected with split less mode. The oil constituents were identified by comparison of their retention time (RT) with the mass spectral library of the GC-MS data software system (NIST11 library). The total running time for a sample was 35 min.

3. RESULTS AND DISCUSSION

3.1 Essential oil content

The essential oil content in basil ranged from 0.1% to 0.5% in the accessions from Sudan, from 0.1% to 0.27% in accessions from Thailand and from 0.1% to 0.2% in accessions from Germany (Table 1). Stanescu, [16], reported the level of essential oil in *Ocimum basilicum* between 0.2% and 1%. This corroborates the findings of Verma and his group, [10], who mentioned that, the highest content of essential oil was at the flowering stage. Abuharaz accession gave the highest content followed by the Sudanese lemon accession, while the Germany accession was the lowest in essential oil content, which might be due to weather and the hour/day sunlight in the subtropical area (Abuharaz, Sudan), and Germany [17].

Table 1: Essential oil content and yield of four basil accessions at different developmental stages

Accessions	Pre- flowering	At Flowering	Fruiting
Abuharaz*	0.1%	0.50%	0.1%
Lemon*	0.1%	0.49%	0.1%
Thailand	0.2%	0.27%	0.1%
Germany	0.1%	0.20%	0.1%

* Wild Sudanese accessions

3.2 Oil constituents

Twenty one (21) compounds were identified in the leaf essential oil of basil accessions from Sudan (Abuharaz) in the pre-flowering stage. The major component was citral (29.58%). Other components present in ratable content were 1-octanol (3.31%) and photocitral B (0.47%). As for the leaf, essential oil of basil accessions from Sudan (Abuharaz) is in the flowering stage. The major component was linalool (12.51%). The other component present in ratable content was 1-octanol (3.88%). In the leaf essential oil of basil accessions from Sudan (Abuharaz), in the fruiting stage, there were forty three (43) identifiable compounds. The major components were eucalyptol (21.38%) and linalool (18.39%). The other component present in ratable content was methyl eugenol (8.33%) (Table 2). The variations in these compounds content is mainly due to the plant ontogenetic behavior that built on species history while cultivated in specific climatic region. In the leaf essential oil of basil accessions from Sudan (Lemon) in the pre-flowering stage, the major component was citral (23.56%). Compounds presented in the essential oil extracted from the leaf of basil accessions from Sudan (Lemon) in the flowering stage were identified. The major component was citral (12.51%). The other component present in ratable content was 1-octanol (3.88%). In the leaf essential oil of basil accessions from Sudan (Lemon) in the fruiting stage, tenty three (23) compounds were identified. The major component was linalool (20.23%).The other component present in ratable content was eucalyptol (18.15%) (Table 2). In basil accessions from Thailand in pre-flowering stage, citral (5.73%) was abundant. In basil accessions from Thailand in flowering stage, forty three (43) compounds were identified. Methyl eugenol (10.86%) and linalool (9.98%) were the main components. Other components present in appreciable content were 2,6-dimethyl-6-(4-methyl-3-pentenyl) (7.72%) and estragol (6.55%). The variation in essential oil composition is mainly due to seasonal or regional variations as well as the method of extraction [18].

In fruiting stage basil accessions from Thailand, eleven (11) compounds were identified of which citral (25.61%) was the main constituent. Other components present in appreciable content were chavibetol (8.08%) and methyl eugenol (3.68%) (Table 2). As for the basil accessions from Germany in pre-flowering stage, citral (17.37%) was the main constituent. Other components present were *cis*-2,6-dimethyl-2,6-octadiene (5.14%), eugenol (4.67%) and methyl eugenol (0.77%). As compared to the flowering stage from Germany, citral (32.93%) was also abundant, which was related to the genetic materials that were used to classify basil into eight clusters according to the chemical composition of the essential oils [19]. Other components present were methyl eugenol (9.37%), 1-octanol (3.00%) and geranyl acetate (0.83%). In the basil accessions from Germany in the fruiting stage, compounds 3,6,9,12-Tetraoxahexadecan-1-ol (8.16%), crown-5 (4.15%) and hexaethylene glycol monododecyl ether (4.79%) were the main constituents. Other component present was 1,4,7,10,13,16 hexaoxacyclooctadecane (3.64%).

Table 2: Major chemical constituents of the essential oils at three developmental stages (pre-flower, at flower, and fruiting) for four basils obtained from Sudan*, Thailand and Germany.

Accession	R.T.[¥]	Compound	Peak Area		
			Pre-flower	**At- flower**	**Fruiting**
Abuharaz*	6.94	Citral	29.58±2.2	-	-
	5.89	Linalool	-	12.51±0.5	-
	4.27	Eucalyptol	-	-	21.38±1.1
Lemon*	5.32	Citral	23.56±0.8	-	-
	6.87	Citral	-	12.51±0.4	-
	4.34	Linalool	-	-	20.23±1.4
Thai	6.88	Citral	5.73±0.1	-	-
	8.18	Methyl eugenol	-	10.86±0.6	-
	6.89	Citral	-	-	25.61±1.8
German	6.88	Citral	17.37±0.6	-	-
	6.91	Citral	-	32.93±1.7	-
	16.07	3,6,9,12-Tetraoxahexadecan-1-ol	-	-	8.16±0.6

[¥]Retention Time

*Results are the mean of three replicates ± Standard Deviation (SD)

The amount and composition of essential oil is strongly dependent on the developmental stage of the plant (ontogeny), and therefore harvesting time is one of the most important factors influencing basil oil quality. In a study carried out in Poland [15], the oil extracted at the flower bud stage was characterized by the highest proportion of linalool (in both cultivars) and of 1,8-cineole (only in the cultivar 'Kasia'). The concentration of methyl chavicol and methyl eugenol in the oil decreased together with the development of the basil plants studied, similarly to the concentration of limonene, α-humulene, cis-muurola-4(14),5-diene, and transcalamene. Monoterpenes in their natural environment give different properties to the plant in which they are present. Citral, for example, is an important constituent of the smell of lemon. Thymol is involved in the flavor of mandarin oranges. Other monoterpenes, such as limonene and geranyl are constituents of flower scents and attract plant pollinators. High concentrations of monoterpenes in plants will repel many potential predators, but may attract other animals. Animals use citronellal, citral, and a- and ~-pinenes as feeding deterrents and geraniol, geranyl esters, myrcene, and terpinolene as pheromones. Some others, such as 1,8-cineole and camphor, are involved in plant-plant interactions. Some commercially important monoterpenes are menthol, camphor, carvone, thymol, fenchone and a-pinene [20]. Recently, application of monoterpenes as anticancer drugs based on the studies showing this effect in animal models, has been demonstrated [21]. Linalool is also used as a chemical intermediate and has found its usage as a scent in 60–80% of perfumed hygiene products and cleaning agents including detergents, soaps, shampoos, and lotions. Linalool is used by pest professionals as an insecticide against fleas, fruit flies and cockroaches.

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