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Impact of Location and Seasonal Variability on the Herbage and Essential Oil Yields of two Grown sweet Basil Varieties in Egypt.

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Abstract: Sweet basil, *Ocimum basilicum* L. is an important medicinal plant, has been used in various uses. Seeds of two Egyptian and French basil varieties were cultivated in two locations (clay soil at El-Jammal and sandy soil at El-Hammam) for two seasons in Egypt. The analysis of variance for six characters showed highly significant differences between varieties, locations and seasons. Oil percent was the most affected character by all the interaction sources of variation; therefore, it is the best trait can measure the environmental variation on the basil plant. The Egyptian variety gave the higher herbage yield, while the French variety gave the higher essential oil yield. On the other hand, correlation estimates showed that the selection must be for plant height and number of total branches per plant if the purpose was herbage production and for number of main branches per plant and essential oil percent if the purpose was oil production. According to the genetic parameters (GCV, PCV, H^2_b and GA), essential oil yield per plant and number of main branches per plant can be used as a base for simple selection to improve the basil plant. Essential oil composition differed between varieties and between locations. Estragol was the major compound in the oil of both varieties at El-Jammal location. Meanwhile, estragol and methyl cinnamate were the main components for both varieties at El-Hammam location.

Key words: Basil varieties, locations, genetic parameters, essential oil composition.

INTRODUCTION

The genus *Ocimum* that belongs to the family Labiatae comprises more than 150 species distributed from tropical to subtropical regions (De Paula *et al*. 2003 and Makonmen *et al*. 2003). Sweet basil *Ocimum basilicum* is one of the most popular aromatic plants and it has been used extensively in food industry (Simon *et al*. 1999). It is also used in the treatment of malaria, colic vomiting, common cold, cough, headaches, diarrhea, inflammation, pain, skin diseases and others (Pessoa *et al*. 2002, Adigüzel *et al*. 2005, Franca *et al*. 2008 and Venancio *et al*. 2011). The plant has shown to have biological activity as an insecticidal, nematicidal, fungicidal and antimicrobial (Ottai *et al*. 2012).

The importance of basil is increasing and has promising future in Egypt, especially when cultivated in new reclaimed soil under organic agriculture conditions (Aboud *et al*. 2006). The genetic improvement of any crop depends on the existence of initial genetic variability through its diverse genotypes (Ahmed and Khaliq 2002). Genetic studies in quantitative characters of aromatic plants are helpful to improve oil yield through selection of genotypes from population (Seidkr-Ozykowska and kazmterezak 2001). Genetic parameters estimating (GCV, PCV, H^2_b and GA) are important to determin genetic variability among selected genotypes of different species of basil (De Masi *et al*. 2006 and Nurzynska-Wierdak 2007).

The plant has an aniseed-like aroma and sweet taste due to the presence of estragol in its essential oil. Various basil species and cultivars provide essential oil with different compositions and aroma. The chemo taxonomical range of sweet basil is very wide (Kruger *et al*. 2002).

The objectives of this study were to determine the impact of location and seasonal variability on the herbage and essential oil yields of Egyptian and French sweet basils.

MATERIALS AND METHODS

Inbred seeds of two basil *Ocimum basilicum* varieties (Egyptian and French) were obtained from the group of Genetics and Breeding of Medicinal and Aromatic Plants in the Department of Genetics and Cytology, National Research Center (NRC), Egypt.

Cultivation Method:

Seeds of each basil variety were grown in two locations, clay soil at El-Jammal village, Tokh, Kalubia Governorate and sandy soil at El-Hammam city, Marsa Matrouh Governorate in Egypt for two summer successive seasons (2010 and 2011). The seeds were sown in bed on Marsh and 35 days later, planting seedlings

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were transplanted to the field. A complete random design with five replicates was established. Each replicate included ten lines of 5 m long and 70 cm wide. The plants were thinned to two plants per hill and the distance between hills was 35 cm. In the maturity phase six quantitative characters, plant height, cm (PH), number of main branches per plant (NMB), Number of total branches per plant (NTB), Leaves dry weight per plant, g (LDW), Essential oil content, % (EOC) and essential oil yield per plant, g (EOY) were recorded and the seeds were separately harvested for both seasons. Data of each character for each variety and location were statistically analyzed separately and combined using SPSS software 2001. Simple correlation coefficient for all possible pairs of essential oil yield/plant and its attributes was practiced for both seasons according to Gomez and Gomez 1984. The data of the second season were assigned for broad sense heritability (H^2_b) according to Robinson *et al.* 1951, the phenotypic coefficients of variance (P.C.V. %) and genotypic coefficients of variance (G.C.V %) according to Burton 1952 and the expected genetic advance ΔGA% according to Johnson *et al.* 1955.

Extraction Of Essential Oils:

One hundred grams of each Egyptian and French basil leaves for both El-Jammal and El-Hammam locations were subjected for hydrodistillation in microscale (v/w) return flow distillation apparatus (Guenther 1961). The extracted oil was over dried by anhydrous sodium sulphate and kept at refrigerator until used. The volume of essential oil was estimated as essential oil percent (EOP). Essential oil yield (g) per plant (EOY) was computed from multiplication of leaves dry weight (g) per plant with EOP.

Essential Oil Fractionation:

The essential oil of each variety was fractionated using GC model HP 6890 Series (Agilent) and Mass Spectrometer HP 5973 with TR-5MS capillary column with 5% phenyl polysil phenylene siloxane (30 mL x 0.25 mm ID x 0.25 film thickness). Oven temperature was programmed at 50 - 180 °C with rate of 5 °C per minute, then isothermal at 180 °C for 10 minute. Both the injector and detector temperatures were maintained at 250°C, and helium was the carrier gas at flow rate of 1 ml per minute. Identification of each compound was carried out by comparing mass spectra of each peak with those of Wile library and with mass spectrum of the standard materials.

Results:

Data presented in Table (1) showed the analysis of variance of six quantitative characters of two basil varieties (Egyptian and French) separately grown at two locations (El-Jammal and El-Hammam) with clay and sandy soil, respectively in two summer seasons. Highly significant differences were observed between locations and seasons in both varieties, except in plant height (PH) for locations as well as PH and number of total branches/plant (NTB) for seasons corresponded Egyptian variety. The interaction between locations and seasons appeared no significant differences in all traits for both varieties, except leaves dry weight/plant (LDW) and essential oil percentage (EOP) for the French variety which had significant differences (Table, 1).

The combined analysis for both varieties through the abovementioned traits, locations and seasons was illustrated in Table (2). The major sources of variability a) varieties, b) locations and c) seasons presented highly significant variation among all studied characters. Both duplicated interaction (axb, axc and bxc) and tri-interaction (axbxc) showed significant variance only corresponding to EOP. Significant interaction was found between varieties with locations in NTB and LDW. While, number of main branches/plant (NMB) showed significant interaction between varieties with seasons, essential oil yield (EOY) showed significant interaction between locations with seasons. Also, significant tri-interaction was found in LDW (Table, 2).

The mean value of the six traits for each variety, location and season were described as histograms in Fig. (1). Egyptian variety had the higher value for the plant morphological traits of PH, NMB, NTB and LDW in all locations and seasons. The highest mean values of Egyptian basil were PH= 99.6 cm, NMB= 26.4 branches/ plant, NTB= 166.4 branches/ plant and LDW= 129.44 g at El-Hammam location in the second season. Meanwhile, the highest values of French basil were PH= 77 cm, NMB= 23.4 branches/ plant and NTB= 74 branches/ plant at the same location and season. The highest French LDW was 72.92 at El-Hammam location in the first season. However, French basil had the maximum mean value for essential oil traits EOP and EOY (0.93 %and 72.75 g/ plant, respectively) at El-Hammam location in the second season, opposite to 0.44 % and 56.46 g/ plant which were found to be the highest Egyptian values for EOP and EOY, respectively in the same location and season. On the other hand, El-Hammam location presented the higher value than El-Jammal location for both varieties and seasons in all characters, except LDW of French variety which was higher at El-Jammal location in the second season. Contrary, all the traits were improved in the second season except LDW of French variety which was higher in the first one (Fig. 1).

Table 1: Analysis of variance (MS) of six traits for Egyptian and French basil varieties (separately) studied in two locations for two seasons.

Variety	S.O.VA	df	PH	NMB	NTB	LDW	EOP	EOY
Egyptian	Location(a)	1	396.05 ns	583.2**	17052.8**	4393.62**	0.079**	2524.10**
	Season (b)	1	174.05 ns	231.2**	1479.2 ns	1443.31*	0.030**	1022.88**
	a x b	1	8.45 ns	9.8 ns	88.2 ns	562.85 ns	0.001 ns	259.30 ns
	Replicates	4	6.68 ns	0.45 ns	67.93 ns	251.89 ns	0.001 ns	28.44 ns
	Residual	12	102.48	2.65	665.53	192.42	0.001	39.09
French	Location(a)	1	819.2**	638.45 **	2714.45*	938.45**	0.305**	3485.86**
	Season (b)	1	180**	110.45**	1940.45*	390.73**	0.133**	1510.67**
	a x b	1	0.0 ns	0.05 ns	1566.45 ns	77.61*	0.040*	159.50 ns
	Replicates	4	3.83 ns	2.80 ns	98.95 ns	6.09 ns	0.002 ns	26.13 ns
	Residual	12	5.19	0.73	394.45	9.26	0.005	32.65

** &* = significant at p <0.001 and 0.005, respectively. ns = nonsignificant value, PH = Plant height (cm), NMB = Number of main branches/ plant, NTB = Number of total branches / plant, LDW = Leaves dry weight (gm / plant), EOP = Essential oil percent, and EOY = Essential oil yield (gm / plant).

Table 2: Combined analysis of variance (MS) of six traits of two basil varieties grown in two locations for two seasons.

S.O.VA	df	PH	NMB	NTB	LDW	EOP	EOY
Varieties (a)	1	5736.02**	81.23**	48094.22**	10429.67**	1.257**	1839.41**
Locations (b)	1	1177.22**	1221.03**	16687.22**	4698.06**	0.340**	5968.98**
Seasons (c)	1	354.02**	330.63**	3404.02*	1667.97**	0.145**	2506.68**
Axb	1	38.03 ns	0.635 ns	3080.03*	636.04*	0.039**	38.91 ns
Axc	1	0.03 ns	11.03*	15.63 ns	166.06 ns	0.018*	24.01 ns
Bxc	1	4.23 ns	5.63 ns	1199.03 ns	111.22 ns	0.028**	415.32**
Axbxc	1	4.22 ns	4.23 ns	455.62 ns	529.22*	0.018*	6.34 ns
Replicates	4	7.48 ns	0.50 ns	9.65 ns	130.33 ns	0.001 ns	15.27 ns
Residual	28	46.57	1.86	476.74	104.67	0.003	36.36

** &* = significant at p <0.001 and 0.005, respectively. ns = nonsignificant value, PH = Plant height (cm), NMB = Number of main branches/ plant, NTB = Number of total branches / plant, LDW = Leaves dry weight (gm / plant), EOP = Essential oil percent, and EOY = Essential oil yield (gm / plant).

Table 3: Correlation coefficients among six studied characters of basil in the first and second season.

characters	PH	NMB	NTB	LDW	EOP
First season					
NMB	0.5192				
NTB	0.9492**	0.7048*			
LDW	0.9730**	0.6550*	0.9961**		
EOP	-0.6736*	0.2451	-0.5132	-0.5695	
EOY	-0.1707	0.7278**	0.0281	-0.0348	0.8388**
Second season					
NMB	0.7253**				
NTB	0.9320**	0.6356*			
LDW	0.6670*	0.0201	0.8540**		
EOP	-0.5307	0.1871	-0.6059	-0.8505**	
EOY	-0.0051	0.6844*	-0.0518	-0.4631	0.8269**

** &* = significant at p <0.001 and 0.005, respectively. PH = Plant height (cm), NMB = Number of main branches/ plant, NTB = Number of total branches / plant, LDW = Leaves dry weight (gm / plant), EOP = Essential oil percent, and EOY = Essential oil yield (gm / plant).

Table 4: Four Genetic parameters estimated for six traits of Egyptian and French basil varieties in two locations.

Varieties	Characters	El-Jammal				El-Hammam			
		G.C.V	P.C.V	H^2b	G.A	G.C.V	P.C.V	H^2b	G.A
Egyptian	PH	8.04	8.35	0.9272	13.89	10.77	11.86	0.8245	19.33
	NMB	99.95	100.0	0.9890	26.41	35.80	36.03	0.9877	17.37
	NTB	22.80	33.34	0.4679	32.61	2.11	13.03	0.0262	1.12
	LDW	11.34	11.73	0.9340	19.41	31.65	37.74	0.7032	63.22
	EOP	36.04	36.42	0.9792	0.20	36.89	38.02	0.9412	0.29
	EOY	47.21	47.91	0.9711	22.27	74.25	74.47	0.9939	69.68
French	PH	15.42	15.50	0.9889	19.33	12.14	12.82	0.8972	17.53
	NMB	76.50	77.44	0.9757	15.25	33.95	34.47	0.9698	14.53
	NTB	55.95	59.49	0.8845	10.76	4.14	4.33	0.8846	5.97
	LDW	27.78	28.47	0.9521	34.44	10.08	10.27	0.9623	15.36
	EOP	16.63	20.86	0.6350	0.15	48.14	49.32	0.9528	0.78
	EOY	52.75	53.27	0.9805	37.47	58.19	59.47	0.9572	71.82

GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, H^2b = Broad sense heritability and GA= Genetic advance. PH = Plant height (cm), NMB = Number of main branches/ plant, NTB = Number of total branches / plant, LDW = Leaves dry weight (gm / plant), EOP = Essential oil percent, and EOY = Essential oil yield (gm / plant).

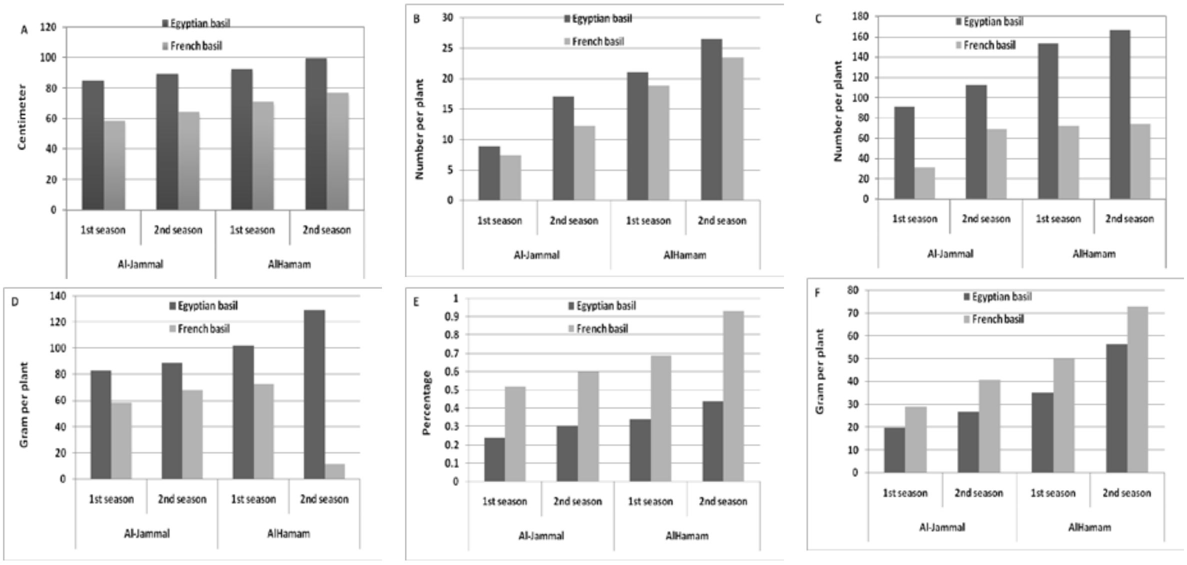

Fig. 1: Histogram of mean performance of six traits for the Egyptian and the French basil studied in two locations and two seasons. A- Plant height (PH), B- Number of main branches/plant (NMB), C- Number of total branches/plant (NTB), D- leaves dry weight/plant (LDW), E- Essential oil percent (EOP) and F- Essential oil yield/plant (EOY).

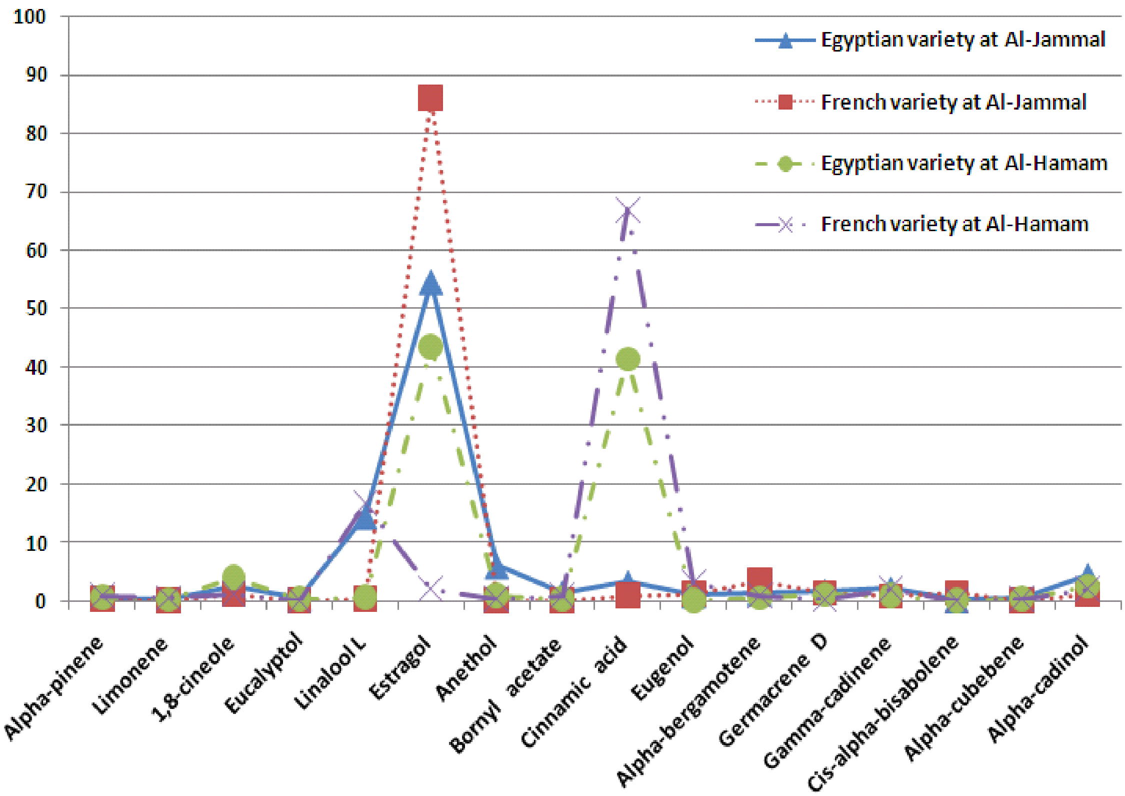

Fig. 2: GC/MS analysis of essential oil composition for Egyptian and French basil in two locations.

Phenotypic correlation between all possible pairs of all the studied characters were estimated for the basil varieties together in both seasons separately and shown in Table (3). Highly significant positive correlations were found for PH with both NTB and LDW, for EOY with both NMB and EOP as well as for NTB with LDW in the first season. Also significant positive correlations were found between NMB with both NTB and LDW. While negative significant association was observed between PH with EOP in the same season. However, PH associated positively and highly significant with both NMB and NTB in the second season. Also highly significant positive correlations were estimated for NTB with LDW and for EOP with EOY, but negative association was found between LDW with EOP in the same season. Additions to significant positive correlations were found between PH with LDW and between NMB with both NTB and EOY (Table, 3).

On the other hand, four genetic parameters; genotypic and phenotypic coefficients of variation (GCV and PCV), broad sense heritability **(**H^2_b) and genetic advance (GA) were studied for each character of both basil varieties and locations shown in Table (4). NMB of the Egyptian variety had the higher values of all genetic parameters than those in the French variety for both locations. French PH was higher than that in Egyptian for all parameters except GA at El-Hammam location. Meanwhile, LDW and EOY had the higher values for all parameters in French basil only at El-Jammal locality. All parameters of EOP were higher in Egyptian at El-Jammal, but higher in French at El-Hammam location. NTB genetic parameters had the higher values in French except for GA at El-Jammal and PCV at El-Hammam which were higher in Egyptian variety. Furthermore, the maximum value of GCV, PCV and H^2_b were 99.95, 100.0 and 0.9890, respectively for NMB of Egyptian variety at El-Jammal as well as 74.25, 74.47 and 0.9939, respectively for EOY of Egyptian variety at El-Hammam location. The maximum GA 37.47 and 71.82 were related to EOY of French variety at both El-Jammal and El-Hammam, respectively (Table, 4).

The essential oil for both basil varieties grown at both locations was analyzed by GC/MS and shown in Fig (2). Sixteen components were detected for the basil essential oil. Estragol was the major compound in the oil of both Egyptian and French varieties at El-Jammal location with 54.70 and 86.09 %, respectively. Meanwhile, estragol with 43.72 % and methyl cinnamate with 66.96 % were the main components for Egyptian and French varieties, respectively at El-Hammam locality. In addition to high percentage value (41.59 %) which was detected for methyl cinnamate corresponding to Egyptian basil at El-Hammam location. Five components (limonene, eucalyptol, anethol, bornyl acetate and alpha-cubebene) had trace amounts for French oil at El-Jammal as well as two compounds (eucalptol and cis-alpha-bisabolene) had the same amounts at El-Hammam location. While, two compounds (eugenol and cis-alpha-bisabolene) presented trace amounts for Egyptian oil only at El-Hammam location (Fig 2).

Discussion:

The analysis of variance showed highly significant differences between varieties, locations and seasons for all studied traits, suggesting that:

a) Wide variation between Egyptian and French basil varieties and the studied traits were governed by additive gene effect. These results are in accordance with those of Aboud *et al*. 2006 and Ottai *et al*. 2012. In this respect, basil types according to the basis of geographical origin were classified into four groups; European, French, Egyptian and Reunion or Comoros basils (Vieira *et al*. 2003, Vieira and Simon 2006 and Gautam *et al*. 2010).

b) The location and season variation affect significantly on the plant growth traits confirming the finding of Pamplona-Roger 2002, Kothari *et al*. 2004 and Raseetha-Vani *et al*. 2009 who report that basil plants were affected by various factors such as seasonal variation, method of harvest, climate and soil type.

c) Oil percent (OP) was the most affected character by all the interaction sources of variation therefore; it is the best trait to measure the effect of environmental variation on the basil plant.

d) The Egyptian variety gave the higher herbage yield, while the French variety gave the higher essential oil yield therefore determining that cultivating either Egyptian or French basil should be based on the goal of agriculture (herbage production or oil production).

On the other hand LDW correlated significantly with PH and NTB, while EOY correlated with NMB and EOP guiding to the selection for PH and NTB if the purpose was herbage production and/or for NMB and EOP if the purpose was oil production. Amoli 2012 found that the highest correlation value was between LDW with the total stem weight, while Aboud *et al*. 2006 reported that the associations between essential oil yield and other characters were different in each generation suggesting performance of cultivars changes from generation to another.

Moreover, the trait of EOY characterized with high values of GCV, PCV, H^2_b and GA for both varieties in both locations except for Egyptian basil at El-Jammal location where NMB had the high values. Therefore both EOY and NMB can be used as a base for simple selection to improve the basil plant which is in agreement with Blank *et al*. 2004 and Aboud *et al*. 2006. The proportion of variation, which is heritable, was not sufficient to determine the GCV alone. This could be done with the help of heritability estimates and genetic advance (Shehbaz Khan *et al*. 2012).

GC/MS analysis of essential oil composition differed between varieties and between locations confirming the results of Grayer *et al*. 1996 and Marotti *et al*. 1996 who reported that basil herb shows significant differences in essential oil content and composition, which depends on genotype, developmental stage or environmental conditions. Meanwhile, estragol and methyl cinnamate were the main components in the basil essential oil. Abdulrahman *et al*. 2009 studied 19 accessions of basil and classified them into 7 groups namely, high methyl chavicol, high linalool, high geraniol, linalool-methyl cinnamate, linalool-geraniol, methyl cinnamate-linalool and eugenol-linalool. However, Kruger *et al*. 2002, Trevisan *et al*. 2006 and Gautam *et al*.

2010 found that linalool was the major compound in the basil essential oil. According to Marotti *et al*. 1996, the European basil type has linalool and methyl chavicol, the Reunion basils have methyl chavicol, whereas tropical basil types have methyl cinnamate as the major constituents.

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