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Influence of Different Methods and Time of Post Harvest Drying on the Essential Oil Content and Composition In *Eucalyptus Citriodora*

KEYWORDS: citronellal, isopulegol, citronellol, method of drying.

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ABSTRACT *Eucalyptus citriodora herb harvested at optimum time was dried both under shade and sunlight for a period of 29 days after harvest. Observations on the loss of weight , essential oil content and composition were observed at 0,1,3,5,7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 and 29 days after harvesting. The essential oil was obtained by hydro-distillation of the leaves dried under both methods (sun and shade) and the oil samples were analyzed by GC. A significant decrease in the weight of herb was noticed due to drying. Significant differences were also noticed in the essential oil content and composition. Drying methods resulted in significant improvement in the oil content, which exhibited an increase up to 5 days after harvest. Sun drying resulted in significantly lesser citronellal (60.91%) and higher isopulegol content (10.73%) compared to shade drying (72.01% and 7.95%,respectively). Citronellol content of the essential oil increased up to five days after harvesting in case of shade drying. Isopulegol increased significantly with increase in time after harvest in both methods of drying.*

INTRODUCTION

Essential oils obtained by distillation of the leaves of *Eucalyptus* have essential oils characteristic of the particular species used. The oils may be roughly divided into three important classes viz., (1) the *medicinal* oils, which contain substantial amounts of cineole); (2) the *industrial* oils, containing terpenes, which are used for flotation purposes in mining operations; (3) the *aromatic* oils, such as *Eucalyptus citriodora (E.citriodora)*, which are characterized by their aroma. Some of the species commonly grown in India are red gum (*E. camaldulensis*), lemon-scented gum (*E. citriodora*), Tasmanian blue gum (*E. globulus*) and Cider gum (*E. tereticornis*). Among these, *E. citriodora* is a large, quick-growing tree with smooth and white bark and lemon-scented leaves. It is extensively planted and coppiced for the extraction of essential oil that is rich in citronellal and used in perfumery and as flavouring agent. The oil is known to possess a wide spectrum of biological activities including fungicidal (Ramezani et al., 2002), insecticidal (Isman, 2000), nematicidal (Pandey et al., 2000) and allelopathic properties (Kohli, 1990). . It was shown that the oil of *Eucalyptus citriodora* exerted it's antimicrobial activity through the synergistic action of citronellal and citronellol (Ramezani et al., 2002). The information available about post harvest drying of herb on the quality and content of essential oil in E. citriodora is meagre, hence this study was taken up to study the influence of two methods of drying over a period of one month on the essential oil content and composition.

MATERIAL AND METHODS

Experimental site

The experimental site is located at the altitude of 542 m above mean sea level with a geographical bearing of 78.38 longitudes and 17.32 latitude.

Weather condition

Semi-arid tropical climate zone of Hyderabad has the average rainfall of 800 mm per year. The average monthly weather conditions of the experimental site during the experimental period are presented in Table-1.

Table 1. Daily weather data at the experimental site during the period April to May, 2013

Days after harvest	Date	Max (°C)	Min (°C)	RH %	Sunshine (hrs)
0	10-Apr-13	32.00	16.60	56.50	7.70
1	12-Apr-13	29.40	19.40	82.50	9.80
3	14-Apr-13	29.40	13.00	64.50	10.20
5	16-Apr-13	32.50	17.70	82.00	7.90
7	18-Apr-13	31.00	17.20	68.50	8.50
9	20-Apr-13	32.60	15.50	59.50	9.70
11	22-Apr-13	33.00	16.40	58.00	9.50
13	24-Apr-13	33.20	16.50	56.00	9.80
15	26-Apr-13	32.80	14.50	52.00	9.90
17	28-Apr-13	33.00	14.60	70.50	8.20
19	30-Apr-13	33.00	17.00	75.00	9.50
21	02-May-13	34.00	18.00	71.00	9.30
23	04-May-13	35.60	21.00	62.00	4.40
25	06-May-13	39.40	23.90	83.00	38.00
27	08-May-13	38.80	27.00	69.00	33.00
29	10-May-13	41.00	29.30	54.00	23.00

Experimental details

An experiment was conducted during the period April 2013

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to May, 2013. Mature eucalyptus citriodora herb ready for harvest was collected from 10 year old plants. The herb was made into bundles of 250 g. In total 128 bundles were made. Sixty four bundles were kept for shade drying and sixty four bundles were kept in sun light for drying. The bundles were loosely tied and care taken to see that excess heat does not develop inside.

The bundles were made in to lots of four each and each lot consisting of the four bundles were spread on papers. In total there were 16 lots in shade and 16 lots in sun light. Each lot served as a treatment. The samples were dried for 29 days. Essential oil was extracted from the entire herb in each bundle in corresponding lot at 0,1,3,5,7,9,11,13,15,17,19,21, 23,25,27 and 29 days after harvest (four bundles /lot served as four replicates). The days after harvest constituted the treatments and the four bundles in each lot served as replicates. Essential oil was extracted from all the four bundles in each lot. The data was fitted in a Randomized Block Design with factorial concept. Methods of drying (Sun and shade drying) constituted the two factors and different days of sampling after harvest constituted the thirteen levels in each method.

Weight of samples
At 0,1,3,5,7,9,11,13,15,17,19,21,23,25,27 and 29 days after harvest , four bundles in each lot were weighed and the loss in weight calculated in both shade and sunlight systems of drying. Similarly the loss in volume was also recorded.

Essential oil extraction
For the extraction of essential oils, all the herbage available in each bundle in each replicate after taking the weight as such was subjected to hydro-distillation using a Clevenger-type apparatus for 3.5 h. The essential oils obtained were collected, dried over anhydrous sodium sulphate and stored at 4 °C until the GC analysis was carried out. The data on the oil yield per bundle was recorded and data presented as oil yield /bundle. The oil content (%, v/w) was also calculated.

GC analysis
GC analysis was carried out using Varian CP-3800 with Galaxie chromatography data system fitted with flame ionization detector (FID) and an electronic integrator. Separation of the compounds was achieved employing a Varian CP-Sil 5CB capillary column (ID: 50 m X 0.25 mm; film thickness 0.25 µm) with 5% dimethyl polysiloxane. Nitrogen was the carrier gas at 0.5 ml/min constant flow rate. The column temperature program was: 120°C (2 min) to 240°C (6 min) at 8°C/min ramp rate. The injector and detector temperature were 250°C and 300°C respectively. Samples (0.2 µL) were injected with a 20:80:20 split ratio. Retention indices were generated with a standard solution of *n*-alkanes (C_6-C_{19}). Peak areas and retention times were measured by an electronic integrator. The relative amounts of individual compounds were computed from GC peak areas without FID response factor correction. The samples collected in all replicates were subjected to GC analysis for statistical analysis purpose.

Statistical analysis
Analysis of variance was performed to determine the effect of method of drying and days after harvest and their interaction [method x days after harvest) on herb weight, essential oil content and composition of individual constituents of the oil using statistical software IRRISTAT (IRRI, Manila, Philippines). Means were compared using least significant differences (LSDs) at 5% probability levels.

RESULTS AND DISCUSSION

Weight of herb
Methods and time of drying significantly influenced the weight of herb over a period of 29 days after harvest (Table 2). It decreased from 250 g to 104.33 g in case of shade drying and to 106.67 g in case of sun drying. On an average, between the methods of drying, sun drying resulted in a significantly more loss in weight compared to shade drying (Table 2). During the first five days after harvest there was rapid loss in weight. The reduction in weight was to the tune of 46.93% in case of shade drying and 48.40 % in case of sun drying. Thereafter the loss in weight was slow (9-12% during 7- 29 days after harvest). The loss in moisture was very rapid in the first few days after harvest especially in the first five to nine days after harvest. The rate of loss in moisture was low during the rest of the period. In case of *Mentha arvensis* also it was reported that there will be a loss of weight and volume to tune of one third or 66% in two days of shade drying (Hazra et al., 1990).

Table 2. Weight of herb (g) and loss in weight(g and %) as influenced by different methods of drying and post harvest period of drying in *E.citriodora* .

Days after harvest	Weight of herb, g			Loss in weight , g			Loss in weight, %		
	Method of drying			Method of drying			Method of drying		
	Shade	Sun	Average	Shade	Sun	Average	Shade	Sun	Average
0	250.00	250.00	250.00	0.00	0.00	0.00	0.00	0.00	0.00
1	180.00	152.00	166.00	70.00	98.00	84.00	28.00	39.20	33.60
3	147.00	134.33	140.67	103.00	115.67	109.34	41.20	46.27	43.73
5	132.67	129.00	130.84	117.33	121.00	119.17	46.93	48.40	47.67
7	130.33	126.67	128.50	119.67	123.33	121.50	47.87	49.33	48.60
9	130.33	124.67	127.50	119.67	125.33	122.50	47.87	50.13	49.00
11	130.00	122.00	126.00	120.00	128.00	124.00	48.00	51.20	49.60
13	128.67	120.67	124.67	121.33	129.33	125.33	48.53	51.73	50.13
15	125.00	119.67	122.34	125.00	130.33	127.67	50.00	52.13	51.07
17	125.00	117.33	121.17	125.00	132.67	128.84	50.00	53.07	51.53
19	121.67	115.33	118.50	128.33	134.67	131.50	51.33	53.87	52.60
21	120.67	115.00	117.84	129.33	135.00	132.17	51.73	54.00	52.87
23	117.67	114.00	115.84	132.33	136.00	134.17	52.93	54.40	53.67
25	116.67	113.33	115.00	133.33	136.67	135.00	53.33	54.67	54.00

27	108.66	111.16	109.91	141.34	138.84	140.09	56.54	55.54	56.04
29	104.33	106.67	105.50	145.67	143.33	144.50	58.27	57.33	57.80
Average	135.54	129.49		114.46	120.51		45.78	48.20	
	'F' test	C.D(P=.05)	C.V.%	…	…	…	…	…	…
Methods	*	4.53	9.01	…	…	…	…	…	…
Days	*	12.82		…	…	…	…	…	…
Interaction	*	18.13		…	…	…	…	…	…

Oil content

Both the methods of drying resulted in significant improvement in the oil content (Table 3). In case of shade drying, the oil content (%) increased from 1.57 % to 2.54 % at five days after harvest and subsequently it decreased to 1.05% at 29 days after harvest. In case of sun drying also a significant increase in oil content was noticed from 1.45 % to 3.16 % at 3 days after harvest and later it decreased to 0.64 % at 29 days after harvest. In both the methods of drying the oil content at 29 days after harvest was significantly less than that noticed in the fresh herb immediately after harvesting. On an average, among the days the oil content exhibited an increase up to 3 days after harvest and thereafter it started decreasing and reached 0.87% by 29 days after harvesting.

Generally, drying of the plant material before distillation results in both increased and reduced essential oil yield depending on time and temperature of drying which are two crucial parameters for determining the essential oil yield (Hamrouni-Sellami et al.,2011 a). Low temperature and relatively short drying time improve the essential oil yields and increasing drying temperature results in a significant decrease in the essential oil content and the rate of decrease can be different in various plants (Hamrouni-Sellami et al.,2011 a). Previous reports have also shown that drying process results in a higher essential oil yield at temperatures up to 50 ^{0}C whereas it decreases at drying temperatures above 50 ^{0}C (Hamrouni-Sellami et al.,2011 a). The variations might be due to differences in species or sub-species, secretory tissue, their localization and essential oil constituents of plant (Khangholi and Rezaeinodehi, 2008, Hamrouni-Sellami et al.,2011 b).

Similar results were obtained in present study. The ambient temperature in both systems ranged from 32.00 to 35.00 ^{0} C during day and from 16.60 to 21.00 C during night. Lower day and night temperatures might have increased the oil content for a few days after harvest under controlled drying conditions. Highest essential oil content of *Cymbopogon winterianus* Jowitt was observed when dried at high temperature (Rocha et al., 2000).

Oil yield

The oil yield per bundle as such (original weight 250 g/bundle at zero days) was estimated using Clevenger apparatus replication wise and the data presented in Table 3. The oil yield decreased from 3.93 to 1.10 ml / bundle in case of shade drying and it decreased from 3.63 to 0.68ml / bundle in case of sun drying. Among the methods of drying sun drying resulted in significantly higher loss in oil yield compared to shade drying.

On an average, over methods the oil yield decreased from 3.78 ml to 0.89 ml which is about 76.6 % loss in oil yield. There was significant decrease in the volume of herb to the tune of 30-45% by the fifth day in both the methods of drying. The volume reduced to 50% by 15 days after harvest and thereafter the reduction in volume is slow. The reduction is volume of the herb was more in sun drying. This facilitates loading of more herb for extraction of oil and the oil yield obtained will be more per unit volume. More oil can be obtained because of loss in volume and more herb can be loaded. Controlled drying at moderate ambient temperatures can be taken advantage for recovering more oil and reducing the cost of distillation.

At the beginning of drying aromatic plants, the leaf moisture moves to the leaf surfaces through diffusion and essential oils are therefore dragged following the loss of moisture (Hamrouni-Sellami et al.,2011 b) and in oregano an essential oil of approximately 3.7% on dry weight was obtained corresponding to approximately 1.52% on a fresh weight (Azizi et al.,2009). In *O.micranthum*, it was observed that the of oil content obtained from dry tissue was higher than from fresh tissue due to the lower water content but no significant

Table 3. Oil yield (ml/bundle) and Oil content (%) as influenced by different methods of drying and post harvest period of drying in *E.citriodora*

Days after harvest	Oil yield /bundle, ml			Oil content , %		
	Method of drying			Method of drying		
	Shade	Sun	Average	Shade	Sun	Average
0	3.93	3.63	3.78	1.57	1.45	
1	3.83	4.80	4.32	2.13	3.16	
3	3.77	3.60	3.69	2.56	2.68	
5	3.37	3.10	3.24	2.54	2.40	
7	3.07	2.77	2.92	2.36	2.19	
9	2.80	2.30	2.55	2.15	1.84	
11	2.40	2.30	2.35	1.85	1.89	
13	2.33	1.73	2.03	1.81	1.43	
15	2.03	1.67	1.85	1.62	1.40	
17	1.90	1.57	1.74	1.52	1.34	
19	1.73	1.20	1.47	1.42	1.04	
21	1.60	1.07	1.34	1.33	0.93	
23	1.47	1.07	1.27	1.25	0.94	
25	1.26	0.93	1.10	1.08	0.82	
27	1.23	0.76	1.00	1.13	0.68	
29	1.10	0.68	0.89	1.05	0.64	
Average	2.36	2.07		1.71	1.55	
	'F' test	C.D (P=.05)	C.V.%	'F' test	C.D (P=.05)	C.V.%
Methods	*	0.084	9.969	*	0.083	13.354
Days	*	0.237		*	0.234	
Interaction	*	0.336		*	0.331	

differences were observed in the concentration of the constituents. Similarly in case of basil dried at low temperatures (25 to 30 ^{0}C), stored properly, and freshly ground before oil extraction, the oil composition was found to be similar to extraction from fresh plant material. Essential oil crops, such as peppermint and spearmint, are partially field-dried or cured after harvest prior to distillation for improving the oil yield and quality.

Oil composition

The major components of the essential oil were found to be citronellal, isopulegol, and citronellol (Table 4). This is in conformity with the essential oil composition of the eucalyptus

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citriodora reported by many authors (the essential oil contains three major constituents accounting for 90-95 % of the total and they are citronellal (75-82%), isopulegol (4-16.00%) and citronellol (4.00-8.00%) .

Citronellal

The content of citronellal in the essential oil as influenced by different methods of drying and post harvest drying period indicated that shade drying resulted in significantly more citronellal content (71.75%) compared to sun drying (59.82%). Shade drying resulted in a significant increase in the citronella content up to five days after harvest and thereafter prolonging the period of storage resulted in significant decrease of the citronellal content. Under sun drying system the differences in the citronellal content were not significant in the first five days after harvest and storing the herb after five days resulted in a significant decrease in the citronellal content of the essential oil. In practical terms drying the herb under natural conditions up to five days after harvest results in lesser cost of distillation and also provides better quality oil.

Table 4. Citronellal (%), Isopulegol (%) and citronellol (%) content in the essential oil of *E.citriodora* as influenced by different methods of drying and post harvest storage

Days after harvest	Citronellal, %			Isopulegol , %			Citronellol , %		
	Method of drying			Method of drying			Method of drying		
	Shade	Sun	Average	Shade	Sun	Average	Shade	Sun	Average
0	75.93	77.24	76.58	4.37	5.18	4.77	4.90	7.88	6.39
1	81.76	78.35	80.05	5.10	4.81	4.96	4.92	7.27	6.10
3	81.37	76.03	78.70	5.59	5.87	5.73	5.35	6.48	5.91
5	80.44	72.75	76.60	4.95	7.95	6.45	6.94	6.71	6.83
7	69.60	63.55	66.57	6.83	10.88	8.86	8.23	7.85	8.04
9	77.05	64.03	70.54	6.67	10.10	8.39	6.78	7.71	7.25
11	76.08	62.29	69.18	7.00	9.54	8.27	7.06	7.34	7.20
13	72.87	65.86	69.36	8.04	9.08	8.56	7.75	7.13	7.44
15	72.09	65.39	68.74	8.65	10.07	9.36	7.25	7.59	7.42
17	69.90	61.70	65.80	8.50	10.44	9.47	7.73	8.35	8.04
19	68.25	52.12	60.19	9.80	13.81	11.80	7.33	7.85	7.59
21	67.15	52.42	59.78	8.35	13.84	11.10	6.41	6.40	6.40
23	66.33	53.41	59.87	10.77	13.16	11.96	7.51	7.12	7.31
25	67.33	43.82	55.58	9.51	14.99	12.25	8.23	7.79	8.01
27	63.98	44.58	54.28	11.46	16.00	13.73	8.69	7.46	8.07
29	62.00	41.00	51.50	11.60	16.00	13.80	8.00	7.50	7.75
Average									
	'F' test	C.D(P=.05)	C.V.%	'F' test	C.D(P=.05)	C.V.%	'F' test	C.D(P=.05)	C.V.%
Methods	NS	1.67	6.62	*	0.26	6.87	*	0.19	6.87
Days	NS	4.72		*	0.74		*	0.53	
Interaction	*	6.68		*	1.05		*	0.75	

Similarly in *Mentha arvensi* also it was observed that the highest essential oil content was found in plant material dried at low temperature, then dried naturally, and the lowest content was found in the plant material dried in laboratory oven. The content of the major constituent, piperitone, is reduced in the same order (71.7%, 50.8%. 43.1%). Pharmacologically active menthol, cineole, limonene and pinene are the most represented in the oil from the naturally dried herb. So, drying of plant material for isolation of essential oils at low temperatures was reported as the best method. Simultaneously, the herb dried in the natural way is quite acceptable (Asekun et al.,1995). In this experiment also the herb dried under shade or sunlight for a period of five days resulted in better quality oil and reduces the cost of production also.

Isopulegol and citronellol

The content of the isopulegol was more in sun dried herb (11.10%) compared to sun dried herb (Table 4). Due to prolonged period of post harvest drying the content of isopulegol increased significantly. The content of isopulegol increased by 200% by drying the herb for twenty nine days. The increase ranged from 4.77 to 13.80 % due to drying. The content of citronellol in the essential oil increased with prolonged storage in case of shade drying and remained almost same in sun drying method. (Table 4).

Extensive decrease of many essential oil components dried at higher temperatures were reported in sage and thyme (Venskutonis, 1997), in basil (Yousif, 1999), in mint (Diaz et al., 2003), in sage (Hamrouni-Sellami et al.,2011 b) and in bay laurel (Hamrouni-Sellami et al.,2011 a). At high temperatures, the biological structure of the oil glands of medicinal and aromatic plants can be affected and the epithelial cells in the dried samples of some sensible plants can be observed to have been collapsed (Hamrouni-Sellami et al.,2011 a). Furthermore, more destruction might occur in structure of the plasma membrane at higher temperatures and it may in turn influence the permeability of plasma membrane (Hamrouni-Sellami et al., 2011 a).

But in this study slight improvement in the quality of essential oil was noticed in case of shade drying up to five days after harvest only.

Correlations

The components of the essential oil in different methods of drying were correlated with the average weather parameters /day (Table 5). Under shade drying system, dry weight of herb, oil yield / bundle, oil content, and Citronellal content exhibited significant negative correlation with minimum temperature. Isopulegol and citronellol showed significant positive correlation with minimum temperature. Similar type of relations was observed under sun drying system also. Under sun drying the correlations between dry weight and citronellol content with minimum temperature were not significant. Citronellal content exhibited a significant negative correlation with maximum temperature in both systems of drying. Isopulegol exhibited a significant positive relation with maximum temperature in sun drying only. A progressive decrease in citronellal and increase in isopulegol was noticed with increase in day and night temperatures.

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CONCLUSIONS

E citriodora herb harvested at optimum time was dried both under shade and sunlight for a period of 29 days after harvest. Observations on the loss of weight , essential oil content and composition were observed for sixteen times on alternate days. A significant decrease in the weight of herb was noticed due to drying. Significant differences were also noticed in the essential oil content and composition. Drying methods resulted in significant improvement in the oil content, which exhibited an increase up to 5 days after harvest. Sun drying resulted in significantly lesser citronellal (60.91%) and higher isopulegol content (10.73%) compared to shade drying (72.01% and 7.95%). Citronellol content of the essential oil increased up to five days after harvesting in case of shade drying. Isopulegol increased significantly with increase in time after harvest in both methods of drying.

Table 5. Correlation coefficient values (r) between observed parameters and some weather parameters during the experimental period.

Method of drying	Character	Correlation values (r values)			
		Max (°C)	Min (°C)	RH %	Sunshine (hrs)
Shade drying	Dry weight	-0.303	-0.545	0.393	-0.110
	Oil Yield / bundle	-0.408	-0.665	0.343	-0.010
	Oil content, %	-0.394	-0.512	0.204	0.016
	Citronellal, %	-0.515	-0.817	0.450	-0.188
	Isopulegol, %	0.463	0.719	-0.401	0.097
	Citronellol , %	0.173	0.639	-0.220	0.078
Sun drying	Dry weight	-0.285	-0.432	0.401	-0.085
	Oil Yield / bundle	-0.317	-0.712	0.266	-0.010
	Oil content, %	-0.303	-0.671	0.167	0.009
	Citronellal, %	-0.593	-0.760	0.404	-0.194
	Isopulegol, %	0.579	0.788	-0.456	0.218
	Citronellol, %	-0.039	0.129	0.060	0.056

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