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Influence of Different Methods and Time of Post Harvest Drying on The Essential Oil Content and Composition in Palmarosa (*Cymbopogon Martinii* (Roxb.) Wats. Var. *Motia* Burk.)

KEYWORDS: geraniol, geranyl acetate, linalool, method of drying

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ABSTRACT *Palmarosa (Cymbopogon martinii (Roxb.) Wats. var. motia Burk.) herb harvested at optimum time was dried both under shade and sunlight for a period of 23 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 and 23 days after harvesting. The essential oil was obtained by hydro-distillation of the leaves dried under both methods and 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 7 to 9 days after harvest. Sun drying resulted in significantly more geraniol content (77.02%) compared to shade drying (76.26%). The differences observed in the geraniol content in the essential oil for first five days of drying were not significant.The content of the geranyl acetate was more in shade dried herb (14.60%) and increasing post harvest drying period significantly increased geranyl acetate content of essential oil. Day temperature exhibited a significant negative influence on oil yield, geranyl acetate and linalool contents and a significant positive influence on geraniol content.*

NTRODUCTION

The leaves of palmarosa *(Cymbopogon martinii* (Roxb.) Wats. var. *motia* Burk. Family Poaceae) on steam distillation yield a pale yellow volatile oil possessing a pleasant odour with high content of compounds called Geraniol (75%) and Geraniol acetate (20%). Concentrations of linalool (2%), alpha-terpineol, geranyl isobutyrate and geraniol were relatively higher in the essential oils of mature to older leaves (Rao et al.,2005). Essential oil recovery and percentages of myrcene, beta-caryophyllene, geranyl acetate, (E.Z) farnesol and geranyl hexanoate were higher in the essential oils of young expanding leaves. Oil obtained from the palmarosa grass is widely used in many industrial applications like perfumes, cosmetics and bath products.

Palmarosa oil is valued highly in the perfumery industry as a source of high-grade geraniol (Mallavarapu et al.,1998) and it also is commercially important because of its antifungal action attributed to the presence of high concentration of geraniol (Bard et al.,1988). It is fungistatic against the filamentous fungi *Aspergillus niger*, *Chaetomium globosum* and *Penicillium funiculosum* (Delespaul et al.,2000) and is considered to provide protection against mosquitoes (*Anopheles culcifacies*) (Ansari and Razdan,1995). Essential oil also showed the highest activity against both gram positive and gram negative bacteria among the other tested essential oils (Lodhia et al.,2009).

Palmarosa oil has been shown to be an effective insect repellent when applied to stored grain and beans, an antihelmintic against nematodes, and an antifungal and mosquito repellent. It helps in clearing up minor infections and prevents ugly scarring in healing wounds.

The leaves of aromatic plants are often dried before extraction to reduce moisture content. During this process, many compounds which permeate to the leaf surface by the evaporating water are lost (Moyler,1994). The method of drying usually has a significant effect on the quality and quantity of the essential oils from such plants. A literature search revealed that drying method had a significant effect on oil content and composition of aromatic plants (Basver, 1993, Deans and Svoboda,1992, Karawya et al.,1980, Raghavan Rao et al.,1997). For example, the oil content of shade-dried Roman chamomil flowers was found to be higher (1.9% w/w) than that of sun-dried (0.4%) and oven-dried at 40^{0}C (0.9%) flowers. This type of information is not available for the aromatic grass palmarosa, hence this experiment is taken up to study the effect of different drying methods and post harvest storage on the quantity and chemical composition of the essential oil of palmarosa variety Trishna during February to April,2013.

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.

Experimental details

One experiment was conducted during the period February 2013 to April, 2013. Mature palmarosa herb ready for harvest was collected from 75 days old plants. The herb was made in to bundles of 250 g. In total 120 bundles were made. Sixty bundles were kept for shade drying and sixty bundles were kept in sun light for drying. The bundle 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

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

there were 15 lots in shade and 15 lots in sun light. Each lot served as a treatment. The samples were dried for 23 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 and 23 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 and 23 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 23 days after harvest (Table 2). It decreased from 250 g to 87.25 g in case of shade drying and to 83.76 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 to the tune of 109% in case of shade drying and 134% in case of sun drying occurred during the first five days after harvest. Thereafter there was loss in weight but the rate was slow.

Drying resulted in a loss of 130.4 g in case of shade drying and 143.52 g in case of sun drying at 5th day after harvest. Between 5th and 23rd day the loss in weight was 32.35 g in case of shade drying and 22.72 g in case of sun drying. The loss in moisture was very rapid in the first few days after harvest especially in the first three to five 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).

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 0.75% to 1.38 % at seven days after harvest and subsequently it decreases to 1.26% at 23 days after harvest. In case of sun drying also a significant increase in oil content was noticed from 0.76 % to 1.36 % at 9 days after harvest and later it decreased to 0.96 % at 23 days after harvest. In both the methods of drying the oil content at 23 days after harvest was significantly higher than that noticed in the fresh herb immediately after harvesting. On an average, among the methods of drying the differences in oil content were statistically not significant.

On an average, among the days the oil content exhibited an increase up to 7 to 9 days after harvest and thereafter it started decreasing and reached 1.11% by 23 days after harvesting. The differences observed in the oil content between the period 3rd to 17 days after harvesting were statistically not significant.

Generally, drying of the plant material before distillation results in both increased and reduced essential oil yield depending on time of drying and temperature 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 ◦C whereas it decreases at drying temperatures above 50 ◦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 29.4 to 36.6 ^{0} C during day and from 12.9 to 21.40 C during night. Lower day and night temperatures might have increased the oil content for a few days after harvest under controlled drying conditions. Similarly, highest essential oil content of *Cymbopogon winterianus* Jowitt was observed when dried at high temperature (Rocha et al., 2000). This may be specific to this particular aromatic grass palmarosa also.

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 1.88 to 1.10 ml / bundle in case of shade drying and it decreased from 1.90 to 0.80ml / 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 1.89 ml to 0.95 ml which is about 49.7 % loss in oil yield. There was significant decrease in the volume of herb to the tune of 30-35% 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

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

yield obtained will be more per unit volume. For example in case of shade drying by 5th day after harvest the loss in weight was 52 % and the loss in volume was 40 %. Hence when oil recovery is calculated on volume basis it works out to be about 3.00 ml. 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).

Oil composition

The major components of the essential oil were found to be geraniol, geranyl acetate and linalool (Table 4). This is in conformity with the essential oil composition of the palmarosa reported by many authors (the essential oil contains seventeen constituents accounting for 95.6–97.1% of the total and ◦-Ocimene (1.2–4.3%), linalool (0.8–2.0%), geraniol (70.1–85.3%) and geranyl acetate (4.3–14.8%) are the major components.

Geraniol

The content of geraniol in the essential oil as influenced by different methods of drying and post harvest drying period indicated that sun drying resulted in significantly more geraniol content (77.02%) compared to shade drying (76.26%). On an avergae , among the days the differences observed in the geraniol content in the first five days after harvest were not significant. Thereafter the geraniol content in the oil increased significantly (except on 9th and 15th day after harvest) up to 17 days after harvest. This indicates an improvement in the geraniol content of the oil due to drying at moderate temperatures under natural conditions. In practical terms drying the herb under natural conditions up to 5 to 15 days after harvest results in lesser cost of distillation and also provides a better quality oil.

Similarly in mentha 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).

Geranyl acetate and linalool

The content of the geranyl acetate was more in shade dried herb (14.60%) compared to sun dried herb (Table 4). Due to prolonged period of post harvest drying the content of geranyl acetate increased significantly. The content of geranyl acetate increased by 36.3% by drying the herb for twenty four hours. The increase ranged from 4.4 % to 36.3% due to drying. The content of linalool in the essential oil exhibited a decrease pattern in both the methods of drying up to 15 days after harvest (Table 4), thereafter it followed a increase-decrease pattern.

During drying of aromatic plants, the leaf moisture moves to the leaf surfaces through diffusion and essential oils components are therefore dragged following the moisture (Hamrouni-Sellami et al.,2011 b). 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 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.

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 due to drying under moderate conditions.

Correlations

The components of the essential oil in different methods of drying were correlated with the average weather parameters /day (Table 5). Under shade system of drying, day and night temperatures, average relative humidity and sunshine hours per day did not show any significant relationship with dry weight, oil yield/bundle, oil content (%), geraniol (%), geranyl acetate (%) and linalool (%) in the essential oil except geranyl acetate which showed a significant negative correlation with maximum temperature.

Under sun drying maximum or day temperature exerted a significant negative influence on oil yield /bundle, geranyl acetate and linalool contents and a significant positive influence on geraniol content. This explains the increase in geraniol content in the sundried samples compared to shade dried samples. A progressive increase in day temperature was recorded during the period under study.In case of citronella cultivar Medini the concentration of geraniol was higher during summer (45.74%) followed by winter (22**).**

CONCLUSIONS

Palmarosa herb harvested normally was dried both under shade and sunlight for a period of 23 days after harvest. 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 7 to 9 days after harvest. Sun drying resulted in significantly more geraniol content (77.02%) compared to shade drying (76.26%). The content of the geranyl acetate was more in shade dried herb (14.60%) and increasing post harvest drying period significantly increased geranyl acetate content of essential oil. Day temperature exhibited a significant negative influence on oil yield, geranyl acetate and linalool contents and a significant positive influence on geraniol content.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

Table 1. Daily weather data at the experimental site during the period February to March, 2013

Days after harvest	Date	Max (°C)	Min (°C)	RH %	Sunshine (hrs)
0	15-Feb-13	32.0	16.6	56.5	7.7
1	17-Feb-13	29.4	19.4	82.5	9.8
3	19-Feb-13	29.4	13.0	64.5	10.2
5	21-Feb-13	32.5	17.7	82.0	7.9
7	23-Feb-13	31.0	17.2	68.5	8.5
9	25-Feb-13	32.6	15.5	59.5	9.7
11	27-Feb-13	33.0	16.4	58.0	9.5
13	01-Mar-13	33.2	16.5	56.0	9.8
15	03-Mar-13	32.8	14.5	52.0	9.9
17	05-Mar-13	33.0	14.6	70.5	8.2
19	07-Mar-13	33.0	17.0	75.0	9.5
21	09-Mar-13	34.0	18.0	71.0	9.3
23	11-Mar-13	35.6	21.0	62.0	4.4

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 palmarosa.

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	158.46	149.75	154.11	91.54	100.25	95.90	36.62	40.10	38.36
3	121.30	106.50	113.90	128.70	143.50	136.10	51.48	57.40	54.44
5	119.60	106.48	113.04	130.40	143.52	136.96	52.16	57.41	54.78
7	105.30	96.50	100.90	144.70	153.50	149.10	57.88	61.40	59.64
9	104.60	95.75	100.18	145.40	154.25	149.83	58.16	61.70	59.93
11	102.15	93.25	97.70	147.85	156.75	152.30	59.14	62.70	60.92
13	101.06	93.03	97.05	148.94	156.97	152.96	59.58	62.79	61.18
15	96.52	90.25	93.39	153.48	159.75	156.62	61.39	63.90	62.65
17	91.80	88.00	89.90	158.20	162.00	160.10	63.28	64.80	64.04
19	91.31	87.75	89.53	158.69	162.25	160.47	63.48	64.90	64.19
21	88.51	87.75	88.13	161.49	162.25	161.87	64.60	64.90	64.75
23	87.25	83.76	85.51	162.75	166.24	164.50	65.10	66.50	65.80
Average	105.66	98.23		133.24	140.09		53.30	56.04	
	'F' test	C.D(P=.05)	C.V.%	…	…	…	…	…	…
Methods	*	2.52		…	…	…	…	…	…
Days	*	6.17		…	…	…	…	…	…
Interaction	*	8.73	5.82	…	…	…	…	…	…

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

Days after harvest	Oil yield /bundle, ml			Oil content , %		
	Method of drying			Method of drying		
	Shade	Sun	Average	Shade	Sun	Average
0	1.88	1.90	1.89	0.75	0.76	0.76
1	1.38	1.26	1.32	0.74	0.80	0.77
3	1.51	1.50	1.51	1.24	1.41	1.33
5	1.54	1.30	1.42	1.29	1.22	1.25
7	1.58	1.30	1.44	1.50	1.35	1.42
9	1.40	1.30	1.35	1.34	1.36	1.35
11	1.45	1.29	1.37	1.42	1.38	1.40
13	1.35	1.20	1.28	1.34	1.29	1.31
15	1.21	1.13	1.17	1.25	1.25	1.25
17	1.10	1.13	1.12	1.20	1.28	1.24
19	1.10	1.03	1.07	1.20	1.17	1.19
21	1.18	1.07	1.13	1.33	1.22	1.28
23	1.13	0.80	0.97	1.30	0.96	1.13
Average	1.31	1.19		1.27	1.23	
	'F' test	C.D (P=.05)	C.V.%	'F' test	C.D (P=.05)	C.V.%
Methods	*	0.052		*	0.05	
Days	*	0.128		*	0.11	
Interaction	*	0.181	9.83	*	0.16	8.71

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

Table 4. Geraniol (%), Geraniol Acetate (%) and Linalool (%) content in the essential oil of palmarosa as influenced by different methods of drying and post harvest period of drying in palmarosa.

Days after harvest	Geraniol, %			Geranyl acetate, %			Linalool , %		
	Method of drying			Method of drying			Method of drying		
	Shade	Sun	Aver-age	Shade	Sun	Average	Shade	Sun	Average
0	75.60	75.40	75.50	12.00	12.06	12.03	2.36	2.24	2.30
1	76.41	73.76	75.08	16.35	16.48	16.41	1.83	2.33	2.08
3	73.71	76.44	75.07	17.45	14.29	15.87	2.58	2.00	2.29
5	76.57	75.12	75.85	14.46	15.74	15.10	2.06	2.41	2.23
7	77.96	76.87	77.42	14.60	14.25	14.42	1.78	2.52	2.15
9	78.58	73.31	75.95	14.24	16.46	15.35	1.29	2.39	1.84
11	75.87	78.72	77.29	13.92	12.54	13.23	1.49	1.37	1.43
13	76.03	79.92	77.97	15.08	13.03	14.05	1.63	1.43	1.53
15	77.65	75.34	76.50	12.81	15.46	14.14	1.96	1.51	1.73
17	77.83	79.03	78.43	12.57	12.56	12.56	2.37	1.98	2.18
19	75.48	76.53	76.00	14.47	14.37	14.42	1.88	1.80	1.84
21	72.15	80.47	76.31	15.44	10.74	13.09	2.37	1.71	2.04
23	76.94	78.74	77.84	13.78	12.30	13.04	1.96	1.68	1.82
Average	76.26	77.02		14.6	14.02		1.93	1.93	
	'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	*	0.71		*	0.17		*	0.14	
Days	*	1.74		*	0.42		*	0.35	
Interaction	*	2.46	2.18	*	0.60	2.85	*	0.49	17.41

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	-0.416	0.024	-0.056	-0.053
	Oil Yield /bundle	-0.365	-0.219	-0.251	0.252
	Oil content, %	0.388	-0.158	-0.208	0.014
	Geraniol, %	0.050	-0.012	-0.145	-0.208
	Geraniol acetate, %	-0.525*	0.017	0.407	0.414
	Linalool , %	-0.154	-0.210	0.197	-0.155
Sun drying	Dry weight	0.352	0.055	-0.073	-0.086
	Oil Yield /bundle	0.547*	-0.430	-0.212	0.252
	Oil content, %	0.014	-0.606*	-0.193	0.436
	Geraniol, %	0.552*	0.105	-0.176	-0.209
	Geraniol acetate, %	0.525*	-0.135	0.276	0.358
	Linalool , %	0.530*	0.069	0.507	-0.070

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