ORIGINAL RESEARCH ARTICLE

Effect of drying techniques on essential oil yield and their quality of lemongrass [Cymbopogon flexuosus (Nees ex Steud) Wats] cultivars Krishna and CKP-25

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ABSTRACT

An experiment was conducted on popularly cultivated two cultivars (cv. Krishna and cv. CKP-25) of Lemongrass (C. flexuous [Nees ex Steud) Wats] at FFDC, Kannauj in August 2015 and replicated in February 2016 to examine the effect on different drying techniques by adopting other parameters. The study recorded different parameters at intervals of 7 days during plantation. Cultivar Krishna was more favourable in herbage yield, oil percentage, and oil yield than cv. CKP-25. The semi-dried condition of CV. Krishna was found to be highest in oil percentage, i.e., $1.5 \pm 0.08\%$, followed by shade and sun-shade-dried oil percentage was found as $1.4 \pm 0.07\%$ each, and the most negligible value was by fresh leaves, i.e., $0.7 \pm 0.03\%$, whereas cv. CKP-25 oil percentages in semi-dried and shade-dried were found to be highest at 0.9 \pm 0.05% each; sun-shade-dried and fresh leaves were 0.8 \pm 0.04% and 0.6 ± 0.03%, respectively. In physical analysis, the value of cv. Krishna showed optical rotation in the ranges of -3.0 to -1.62, a refractive index of 1.4785 to 1.4791 at 27 °C, and a specific gravity of 0.8901- 0.8932 at 27°C. However, cv. CKP-25 showed optical rotation in the ranges of -2.4 to -1.31, a refractive index of 1.4724- 1.4829 at 27 °C, and a Specific gravity of 0.8819- 0.8854. All the values were under the range of BIS standards. In an instrumental analysis of the GLC report, citral content was recorded cv. CKP-25 showed a higher citral content of 83.95% in semi-dried conditions than cv. Krishna of 74.5%. The cv. (Krishna) in sun-shade dried, had 79.88%, followed by shade-dried at 76.83% and fresh leaves at 75.71%. Whereas, in cv. CKP-25, sun-shade-dried 83.24%, shade-dried 82.19%, and freshly harvested leaves showed 76.31% citral content, respectively.

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INTRODUCTION

Lemongrass is known as hardy perennial grass that belongs to one of the most important essential oils yielding monocot grass of family Poaceae, widely distributed worldwide and most predominantly found in warm temperate and

tropical regions of the world due to its interaction with the specific environments comprising of about 180 species, sub-species, varieties and sub-varieties out of which 45 are grown in India where most of these are aromatic essential oils yielding, used in cosmetics, pharmaceuticals, and perfumery

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applications (Khanuja et al., 2005; Hassan, 2016; Devi et al., 2019). The genus Cymbopogon can grow up to a height of 2 m with an infinite life span; in practice, it is uprooted and replanted after a growth period of 3 to 5 years to restore its vigour (Weiss, 1997; Elhag et al., 2018). In India, more than 60,000 hectares of area are under cultivation of aromatic grasses, which are distributed in different states like Madhya Pradesh, Gujarat, Karnataka, Assam, Kerala, Maharashtra, Uttar Pradesh and Andhra Pradesh (Padalia et al., 2011). The plant is native to Asia and Africa and cultivated in Guatemala (Srivastava et al., 2009). Three species of Cymbopogon yield lemongrass essential oil of commerce, with monoterpene citral as the principal constituent (75%). C. flexuosus (Steud) Wats is a species known to have several chemo-morphological cultivars. The "Lemongrass" plant yields one of the top 10 essential oils in the world (Lawrence, 1985), three species like Cymbopogon flexuous (Nee ex Steud) Wasts, East Indian, Cymbopogon citratus (DC). Stapf, West Indian, and other species Cymbopogon pendulus (Nee ex Steud), Jammu Lemongrass (Gupta, 1969; Chandra & Narayanan, 1971) contributed to oil production. East Indian Lemongrass (C. flexuosus), also known as Malabar or Cochin type, is preferred by the industry for its superior oil quality (Srivastava et al., 2009). The oil of West Indian lemongrass (*C. citratus*) has lower citral content and has mere trade in the country. Jammu lemongrass (C. pendulus) has high citral content, but its cultivation is mainly confined to North Indian states. It was reported that twentyseven species of Cymbopogon occurred in different parts of India and 406 accessions of C. flexuosus from Aromatic and Medicinal Plants Research Station, Odakkali (Kerala) based on their morphological and essential oils characters (Kuriakose, 1995). The commercially important *Cymbopogon* is *C*. flexuosus (Nees ex Steud) Wats (Poales: Poaceae), lemongrass, C. winterianus Jowitt ex Bor (Poales: Poaceae), citronella, C. martini Roxb. Wats (Poales: Poaceae), palmarosa, and C. pendulus (North Indian Lemongrass) are cultivated in tropical and subtropical regions of India (Devi et al., 2020). The term lemongrass was given because of its lemonlike solid odour due to the high citral content (75-90 %) of the essential oil present in the leaves. Citral derived from lemongrass oil takes a prominent place among the most widely consumed aromatic chemicals in the world, as it is the starting material for the preparation of essential ionones: α-ionone

that is used as a flavour, cosmetics and perfumes, whereas β -ionone is used for the synthesis of vitamin A (Ganjewala, 2013). Over the world, aromatic grass has been extensively used for cosmetics, flavouring, and clothing in the perfumery industry for many years (Katiyar, 2011). Besides being used in different industries, the infusion of lemongrass in traditional medicine for lowering blood sugar levels showed significant importance (Bharti et al., 2013). Many studies have also reported having some pharmacological properties in lemongrass essential oil, such as antifungal, antimicrobial, mosquito antimycotic, acaricide, repellent, insecticidal activity, and repellent properties against a variety of insects (Rajashekar et al., 2013; 2016; Devi et al., 2020; 2021).

During drying processes, various aromatic compounds are lost as they are volatile, and the changes occur in the volatile compounds in spices and other plants' composition (Figiel et al., 2010; Rocha et al., 2011; Orphanides et al., 2016). After drying, a specific compound increases (Yousif et al., 1999; Bartley and Jacobs, 2000;), or a new compound formation has been formed (Ozdemir et al., 2018; Zhang et al., 2018). In sweet basil, 36-45%, 23–33% in marjoram, and 6–17% in oregano during ambient temperature drying reduced the essential oils' total quantities (Nykanen and Nykanen, 1987). The shade-dried Roman chamomile had a markedly lower total content of essential oils compared with sun-dried and oven-dried (Omidbaigi et al., 2004). Consuelo Diaz-Maroto et al., 2002 stated that Laurus nobilis showed similar results in oven-drying and airdryingatambienttemperature, showing any hardloss in volatiles as compared to the fresh herb. Different drying methods significantly affected the oil content and composition of aromatic plants when dried at 60°C (Venskutonis, 1997). The essential oils extracted from fresh leaves (0.06%), dry leaves (0.03%), and fresh flowers (0.09%) of Calendula officinalis L. exhibit different oil yields, having a total of 30, 21 and 24 compounds (Okoh et al., 2008). The effect of the drying method (sun, shade and oven drying at 45 °C) on the essential oils content and composition of C. citratus showed significant differences, as oven drying gave the highest essential oils (2.45%) compared to shade-drying (2.12%) and sun-drying methods (2.10%) having geranial (citral-a), neral (citral-b) and myrcene as principal components (Hanna et al., 2012) – different drying methods in Lavandula angustifolia Mill. Like sun-drying (SD, 72 h under sunlight), closed shade-drying (CSD, 72 h under shade condition in a closed place), oven-drying (OD, 48 h heated at 40 °C) and microwave-drying (MD, 5 min irradiated at 55% energy of 900 W) had a significant effect on the essential oil content (w/w %) showing highest percentage by CSD of 3.4% with different constituents of 35, 36, 34 and 38 respectively (Mirjalili *et al.*, 2019). In *Origanum vulgare* L., the highest essential oil yield was achieved from microwave and shade drying methods with 39 components, with carvacrol being the main constituent ranging from 56.2 to 81.4% (Caputo *et al.*, 2022).

A few years back, from being one of the importers of lemongrass essential oil, India became one of the largest exporters in the world (CSIR Aroma Mission). High-yielding varieties developed by CSIR-CIMAP, such as Krishna, produce more essential oil and increase the crop yield twofold. The recently developed CIM-Shikhar also showed great potential for commercial cultivation. Under the CSIR Aroma Mission, more than 3500 hectares have been cultivated, and about 350 tons of essential oil worth Rs 50 crores have been contributed to the Indian Aroma industries. This has not only reduced the import burden of the country but also helped enhance exports through the initiatives taken up by our government through Atma Nirbhar in Lemongrass essential oil productions. During the year 2021-22, India exported more than 610 tons of lemongrass essential oil worth Rs.102 crores, whereas about 350 tonnes of essential oil worth Rs. 49 crores in 2017-18. This oil is being exported to countries like the USA, the UK, France, Germany, Spain, Australia, Canada, etc. Therefore, the process of choosing the best methods for essential oil extraction for quality and quantity analysis is much needed to enhance the growth of the Indian economy.

MATERIALS AND METHODS

Propagation

The experimental field was harrowed twice to bring the soil to fine tilth, and the field was marked into different plots. Plantation of different cultivars (Krishna and CKP-25) of lemongrass was done by using disease-free healthy slips 20cm long after removing the top leafy portion from the fields of FFDC Kannauj, UP in August 2015. The collected cultivars were planted in a complete randomized

block design at 4×3 sq. meter plot size, and line-to-line and plant-to-plant spacing was 60×45 cm with six replicas for experimental purposes. Irrigation was done immediately after the plantations, and it continued for two weeks for the plants to grow correctly. During plantation, 5kg each of neem cake was applied in both plots. The plots were maintained to keep the weed-free by manual weeding, and the cultivars were replicated in February 2016.

Parameters Recorded

Different parameters were recorded like the plants' height, the tillers' height, the number of leaves, and the number of tillers for both cvs. Krishna and cv. CKP-25. Irrigation was carried out five times for both varieties to the plant specimens' specific conditions and physiological needs. Different parameters like temperature (27-34 °C) and humidity (36-82%) were also recorded during the lemongrass plantation. No diseases were recorded during the analysis. Later, by sickle, the leaves were harvested 10 cm above the ground and chopped into 2-3 cm before distillation.

Drying Methods

For the extraction of essential oil, drying of the leaves was performed under four different conditions as follows:

a. Fresh/wet leaves, b. Semi-dried (5 h in sunlight and 19 h in shade), c. Shade-dried (24 h in shade condition), and d. Sun-shade-dried (5 h sunlight and 43 h shade).

The essential oils were isolated from the leaves of two cultivars of lemongrass by a hydrodistillation method using a Clevenger-type apparatus (Guenther, 1950) for four hours. Isolations were carried out in triplicates for both the cultivars studied. The Hydrodistillation flask was charged with 500 g each. The oil content in percentage (v/w) was estimated on a fresh weight basis. The following formula calculation of the essential oil concentration (%) was estimated,

Essential oil (%) = amount of essential oil extracted (g) / amount of crop biomass distilled (g) $\times 100$

The oil percentage was calculated by the oil yield of each isolate over a w/w basis (Schaneberg and Khan, 2002). The essential oils were dried using anhydrous sodium sulfate (M. wt = 142.04)

and stored at 4 °C in sealed amber vials for further evaluation.

Physicochemical parameters

The parameters like oil yield after removing the moisture content through anhydrous sodium sulfate addition were recorded. The oil percentage can be calculated by knowing the weight of the oil and material taken. Specific Gravity is measured using a Pyknometer of 5ml at 27 °C of a given volume of oil to an equal amount of distilled water. The optical rotation of the oils was measured by putting a polarimeter (M/S Perkin Elmer 243B Model) containing essential oil between the polarizer and analyzer, which indicated either dextra or levo rotation. The refractive index was measured by putting the oil on the prism of the Abbe refractometer adjusted a standard temperature. The compounds were identified using the GLC (Hewlett Packard Model) and GC-MS (Agilent) report, which were automated for 62.20 minutes. From the graph, the major and minor components can be visualized, i.e., neral and geranial as principal components and minor components like linalool, geraniol, etc., and the recorded data were also compared with the standard data. All the oils received during the experiment were analyzed using the laboratory's standard operating manual at FFDC, Kannauj, UP, India.

RESULTS AND DISCUSSION

The significant result showed the effect of drying techniques on the essential oil using different drying processes for both CVs Krishna and CV. CKP-25 has differences in their percentage of oil and significant chemical components. Cultivar Krishna got the highest mean values of 119.78 ± 5.99 cm and 72.92 ± 3.65 cm of plants' height and tillers' heights, respectively, compared to cv. CKP-25 of 54.71 ± 2.73 cm and 50.94 ± 2.55 cm, respectively.

Meanwhile, in the case of tillers and leaves present, cv. CKP-25 got a higher number, i.e., 11 ± 0.55 and 83.84 ± 4.19 , respectively, compared to cv. Krishna of 8 ± 0.4 and 60.98 ± 3.05 , respectively (Figure 1). The present work showed similarity with the findings of Lal *et al.*, 2018 as *C. khasianus* reached 98 cm in vegetative plant height, having 93 and 870 tillers and several leaves. The plant height of Jor Lab L-15 was 109 cm, and the check variety, i.e., IIMJ (CK) -10, reached an average height of 79 cm of C.

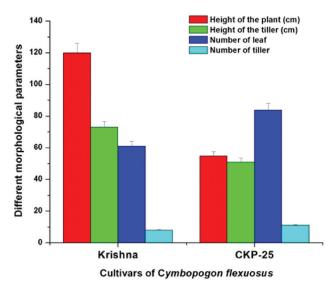


Figure 1: Comparison of different morphological parameters of selected cultivars of Cymbopogon flexuosus

khasianus (Lal et al., 2019). Among the cultivars of C. flexuosus, CIM-Shikar showed a significantly higher plant height of 136.75cm, followed by CIM-Suwarna (131) cm. The presence of a higher number of tillers per clump was noticed in CIM-Shikar (47.60), which is followed by Krishna (47.53) and CKP-25 (42.13), where C. flexuosus cultivar, Nima obtained a lesser number of tillers per clump of 38.13 respectively (Yogendra et al., 2022). Different morphological studies had also been done by Shamsheer et al., 2020 in 50 accessions of C. citratus collected from different regions showing a range of 67cm to 168cm plant height having 2 to 56 tiller numbers, and the highest number of tillers produced was from the accession of Khairpur having 21 and 56 tillers in the first and second year of growth. It showed that the environment affects the morphological traits, and the plant hormones are essential in regulating the number of tillers. Different grass species showed fluctuation in the number of tillers, which might be due to the presence of zeatin, which is responsible for increasing the tiller number in plants like maize and C. citratus (Shamsheer et al., 2020). The height of the plant influenced the productivity of plant biomass was also reported by Iqbal et al., 2015. Cymbopogon species showed variation in plant height, leaf length and width, which may be due to the intensity of light as well as the presence of auxin (plant growth hormone) in different concentrations that might affect the plant growth (Barišić et al., 2006). The morphological characters also showed variation in different characters; however, the plant belongs to the same genus (Poehlman and Sleper, 1995), and this might be due to hereditary differences in the plant and various environmental conditions or both. Environmental factors, such as rainfall, relative humidity, temperature, etc., were responsible for various morphological characteristics that could cause differential performance (Frankel *et al.*, 1994).

Among the different parameters recorded, fresh leaves had the highest herbage yield in both the cultivars of lemongrass of 34.9 ± 1.74 tons and 30.5 ± 1.52 tons of cv. Krishna and cv. CKP-25, followed by semi-dried, shade-dried, and sun-shade-dried leaves for each cultivar. Semi-dried leaves of cv. Krishna has the highest oil percentage of $1.5 \pm 0.08\%$, 1.4 ± 0.07% each of shade-dried and sun-shadedried leaves, whereas fresh leaves had a minor oil percentage of $0.7 \pm 0.03\%$ of cv. Krishna. Among cv. CKP-25, semi-dried, and sun-shade-dried leaves recorded higher percentages of 0.9± 0.05% each and $0.8 \pm 0.04\%$ and $0.6 \pm 0.03\%$ by shade-dried and fresh leaves. The present findings were similar to the study of Edwin et al., 2012 - semi-dried condition of cv. Krishna got the highest weight of 418.5 ± 20.92 oil yield/herbage yields compared to cv. CKP-25 of 233.1 ± 11.65 oil/herbage yield (Table 1). In comparison to the present findings, the reported average herbage yield was 27.6-30 ton/herbage yield /year for Jor Lab L-10, whereas 21.45-22.33 in the check variety for the first and second year of analysis, 30.32, 36.5, 29.57ton/herbage yield /year for Jor Lab L-8, Jor Lab C-5 of Java Citronella and Jor Lab L-14 (Lal et al., 2019; Lal et al., 2016a; 2016b; Lal et al., 2018). Comparable herbage yield of Cymbopogon species from other findings like Yogendra et al., 2022; Kumar et al., 2023 also reported that the herbage yield of CIM-Shikar (24.25 ton/herbage yield / year), followed by Krishna (22.50 ton/herbage yield /year) and CKP-25 (20.72 ton/herbage yield /year) and 373.61 to 434.36 quintal/herbage yield /year of C. spring. Different studies were also reported by Lal et al., 2018; Lal et al., 2020; Shamsheer et al., 2020; Munda et al., 2022; and Mehrotra et al., 2023. C. flexuosus produced 1% essential oil content, and Jor Lab C-5 of Java Citronella (C. winterianus) had a 1.20% essential oil yield (Lal et al., 2016a; 2016b). Shahi and Sharma, 1997 reported an essential oil yield of 0.305%. Kassahun et al., 2011 reported that fresh essential oil content and essential oil yield/ herbage yield varied from 0.34-0.53 and 0.45-0.55% and 42.29-108.16 and 155.09-342.69 kg for the first and second year of analysis. The overall mean performance of lemongrass in terms of citral content over the tested locations varied from 70.81-82.68% (Kassahun et al., 2011). Chauhan et al., 2017 reported that the essential oil of the palmarosa (C. martini) crop yields comparatively less oil recovery of about (0.56%) with an average oil yield of 139.7 kg herbage yield-1. Kumar et al., 2023 stated that C. spring produced an annual essential oil yield ranging from 0.51 to 0.86 % and an essential yield of 210.03 to 375.13 kg/herbage yield/year. Yogendra et al., 2022 reported that the essential oil content was in the range of 0.70-1.35% (v/w), and the essential yield ranged from 133.56-302.40 kg herbage yield⁻¹ year-1 in different cultivars of lemongrass.

In cv. Krishna, the result showed the highest citral content found in sun and shade-dried leaves at 79.88% and that of cv. CKP-25, the highest citral content in semi-dried leaves of 83.95%, respectively (Table 2). The high citral content in the oil by adopting different drying methods makes it useful in the pharmaceutical, perfumery, and flavour industries. All the values of both varieties' specific gravity, optical rotation, and refractive indices were recorded under the normal range of the BIS report (Table 3). A comparable mean range of the main composition of lemongrass varieties viz. citral, neral, geraniol, citronellal, citronellol, geranyl acetate,

Table 1: Comparative studies on herbage and essential oil yield of selected cultivars of *Cymbopogon flexuosus* (Mean ± SE)

S1.		Cv. CKP-25					
no.	Parameters	Herbage Yield/ha (tons)	Oil (%)	Oil Yield/ha (kg)	Herbage Yield/ha	Oil (%)	Oil Yield/ha (kg)
1.	Fresh (wet)	34.9±1.74	0.7±0.03	244.3±12.21	30.5±1.52	0.6±0.03	183±9.15
2.	Semi-dried	27.9±1.39	1.5±0.08	418.5±20.92	25.9±1.29	0.9±0.05	233.1±11.65
3.	Shade-dried	25.7±1.28	1.4±0.7	359.8±17.99	24.9±1.24	0.8±0.04	199.2±9.96
4.	Sun-Shade-dried	24.2±1.21	1.4±0.7	338.8±16.94	22.7±1.13	0.9±0.05	204.3±10.21

Table 2: Physical and chemical analysis of selected cultivars of *Cymbopogon flexuosus*

Sl. No.	Cv. Krishna					Cv. CKP-25			
	Parameters	Specific Gravity at 27 °C	Optical Rotation	Refractive index at 27 °C	Citral content	Specific Gravity at 27 °C	Optical Rotation	Refractive index at 27 °C	Citral content
1.	Fresh (Wet) Leaves	0.890	-1.62	1.478	75.71%	0.882	-1.31	1.478	76.31%
2.	Semi-dried Leaves	0.893	-3.0	1.479	74.45%	0.885	-2.4	1.483	83.95%
3.	Shade-dried Leaves	0.891	-1.75	1.479	76.83%	0.883	-1.45	1.472	82.19%
4.	Sun-Shade- dried Leaves	0.891	-1.65	1.479	79.88%	0.884	-1.34	1.485	83.24%
	Mean ± SE	0.891±0.04	-2.00±0.1	1.479±0.07	76.71±3.83%	0.883±0.04	1.62±0.08	1.479±0.07	81.42±4.07%

Table 3: Physico-chemical properties of selected cultivars of *Cymbopogn flexuosus*

S1.	Characteristic	Range				
No.		Cv. Krishna	Cv. CKP-25			
1.	Odour	Lemony	Lemony			
2.	Colour and appearance	Light yellow to dark yellow	Light yellow to dark yellow			
3.	Oil yield	0.70-1.50%	0.6-0.90%			
4.	Specific Gravity (27 °C)	0.890-0.893	0.882-0.885			
5.	Optical Rotation	-3.0 to -1.75	-2.4 to -1.31			
6.	Refractive Index (27 °C)	1.478-1.479	1.472-1.485			
7.	Citral content	74.5%-79.88%	76.31%-83.95%			

elemicin, methyl iso-eugenol etc., from different studies had been reported of 54.1%, 30-93.74%, 82-88%, 72-75%, 30-94%, 78%, 13.05-82.26%, 2.87-69.99%, 2.20-88.14%, 65.09-85.33% from Sidibe *et al.*, 2001; Negrelle *et al.*, 2007; Ganjewala, 2008; Kumari *et al.*, 2009; Avoseh *et al.*, 2015; Lal *et al.*, 2016a; Chauhan *et al.*, 2017; Devi *et al.*, 2020; Yogendra *et al.*, 2022; Mehrotra *et al.*, 2023, respectively. Essential oils exhibit variations in the chemical composition that can be possibly caused by origin, genetic structure, climatic factors, chemotype/genotype, developmental stage of the collected plant materials, season of harvest, and the agronomical practices (Verma *et al.*, 2010).

CONCLUSION

It has been concluded that the selected cultivars were adapted to the study site, the cv. Krishna showed more adaptation in terms of plant height, whereas cv. CKP-25 showed a higher tiller number and several leaves, the cv. Krishna contains a higher oil percentage in the semi-dried conditions of 1.50 \pm 0.08 % compared to CKP-25 of 0.9 \pm 0.05 %. In the case of citral content, a higher value was found in CKP-25 of 83.95% in the Semi-dried condition compared to the cultivar Krishna of 74.5%. Among Krishna cultivars, the sun-shade condition showed the highest citral content of 79.88%, respectively. Thus, by adopting different drying processes, the oil percentage in different parameters of shade-dried and sun-shade-dried also showed a high percentage of oil contained (1.4% each). For both varieties, semi-dried conditions showed more favourable than others. Though both the cultivars belong to the same genus, they exhibit significant differences in various morphological parameters, possibly due to hereditary and the influence of various environmental factors such as rainfall, relative humidity, temperature, different soil parameters, etc. The cultivation of different suitable varieties of lemongrass should be encouraged in order to improve the ecological and economic values of bare land that can be offered as an alternate cash crop to the local farmers, which is not harmed by animals and needs less maintenance against different diseases as the plant works against various pest and pathogens.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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