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ORIGINAL RESEARCH ARTICLE

Evaluation of essential oil yield and chemical compositions of Citronella (*Cymbopogon winterianus*) cultivars under Southern tropical regions of India

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ABSTRACT

A field study was conducted at CSIR-CIMAP, Research Centre, Bengaluru, during 2018-2019 to evaluate the performance and chemical compositions of six citronella cultivars (Medini, Jalpallavi, Bio-13, C-Line, CIM-Jeeva and Manjusha) under southern climatic regions of Karnataka, India. Results revealed that significant differences in growth, yield and chemical compositions among the cultivars studied. The plant height varies from 123.33-139 cm and number of tillers ranged from 37.56-45.67 cm. Significantly higher herb yield was recorded in CIM-Jeeva (217 kg/100m^2) >Bio-13 (207 kg/100m^2)>C-line (192.33 kg/100m^2). The essential oil recovery varied from 1.13-1.23% (v/w) and essential oil yield varies from 1.93-2.69L/100 m^2, respectively in different cultivars studied. Overall, twenty four constituents, comprising 92.05–98.07% of the total oil compositions, were identified using GC-FID.The varieties arranged according to the citronellal content as follows, CIM-Jeeva (44.60 ± 1.27%) >Jalpallavi (41.35 ± 0.92%) > Manjusha (39.15 ± 1.63%) > Bio-13 (37.65 ± 4.45%) > Medini (21.80 ± 3.11%) > C-line (8.10 ± 2.56%). C-line was found to be geraniol rich (59.35 ± 1.06%) among the citronella cultivars studied. Overall, cultivar CIM-Jeeva and Bio-13 were superior as compared to other cultivars in essential oil yield and market acceptable citronellal content.Hence, it can be concluded that CIM-Jeeva and Bio-13 are the ideal cultivars for commercial cultivation under South Indian climatic conditions.

INTRODUCTION

Citronella (*Cymbopogon winterianus* Jowitt) is an important perennial multi harvest aromatic grass belongs to the family Poaceae. Citronella essential oil is a natural source for insect repellent (Mahidol, 2004; Malaluan and Malaluan, 2015) antimicrobial activity (Ahmad et al., 2020) antiparasitic (Sabuna et al., 2018) antioxidant activities (Sharma et al., 2020, Ogbonna et al., 2020; Saha et al., 2020) acaricide activities (Blank et al., 2007) and has wide

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application in pharmaceutical (Sharma et al., 2018) and perfumery Industries. This essential oil contains citronellal, citronellol and geraniol which are extensively used in the preparation of soap, incense, candles, cosmetics, perfumes and flavouring industries. In addition, citronella oil has significant anti-bacterial (Sharma et al., 2020) and anti-fungal (Li et al., 2013) properties. Traditionally citronella oil is used for treatment of fever (Eden et al., 2020). Two *Cymbopogon* species are known according to their region Java citronella (*C. winterianus)* and Jamarosa (*C. nardus*). Both the types have similar essential oil odor and therapeutic uses, but exhibit variation in the citronellal content. Higher amount of citronellal and geraniol can be observed in Java citronella as compared to Jamarosa.

India is one of the leading essential oil producers in the world. It accounts production of many essential oils including citronella oil. The market demand up to the year 2025 in India is estimated to be 3200 ha and 66000 ha globally. However, in current scenario we are facing tough competition for price and quality of the essential oil from the developing countries. Majority of the area under citronella cultivation belongs to Northern India. Citronella requires high temperature, humid weather coupled with abundant sunlight and well distributed rainfall throughout the year. Natural habitat of *Cymbopogons* is found in elevation of 1500m. Sarma et al., (1977) reported the successful cultivation of citronella crop at 1200m altitude in the Yaongyemson valley of Nagaland. To meet the growing demand of this essential oil, the present study was proposed to select a promising variety for the Southern region with an objective of evaluation of essential oil yield and chemical compositions of superior genotypes for Southern tropical regions of India.

MATERIALS AND METHODS

Experimental location

The field experimentation was carried out in CSIR-Central Institute of Medicinal and Aromatic Plant Sciences (CIMAP), Research Center, Bengaluru. The location comes under Eastern dry zone (Zone-5) of Karnataka. The experimental location lies in the latitude of N: 13.085 and longitude of E: 77.592. The experimental soil was found to be neutral in soil reaction with sandy loam in texture. The location receives an average annual rainfall of 925 mm.

Experimental details

The field experiment was conducted in Randomized complete block design (RCBD)with six citronella cultivars (Medini, Jalpallavi, Bio-13, C-Line, CIM-Jeeva and Manjusha) replicated four times. The experimental land was thoroughly ploughed and plots were made as per the experiment layout. Different cultivars were planted during the first week of August. The experimental plot size was 3.6mx3.6m. The spacing was followed row-to-row spacing of 45 cm and plant-to-plant spacing of 45 cm. A well-decomposed Farm yard manure (FYM) was applied before 15 days of planting. Calculated amount of fertilizers were added to the soil on the day of planting. Recommended dose of chemical fertilizers $N:P_2O_5:K_2O$ was applied at the rate of 100:40:40 kg/ha respectively. Nitrogen was applied in four splits. The crop was managed by standard package of practices. The field was irrigated immediately after planting. Subsequent irrigation was scheduled based on the soil moisture condition. The plots were kept weed free. The morphological characters like height (cm) of the plant, number of tillers per

Table 1: General information of different cultivars of citronella

Cultivars	Variety development	Citronellal (%)	Adaptability
CIM-Jeeva	Mutation breeding	42.97%	Suitable for both with 90% establishment capability
Jalpallavi	Extensive clonal selection	43.00%	Waterlogged conditions
Bio-13	Rigorous recurrent screening in soma clones	38.00%	Suitable for both the conditions
Manjusha	Clonal selection	38.61%	For genetic plains
Medini	Clonal selection	11.18%	Southern and northern sub Himalayan region of India
C-line	Clonal selection	Geraniol rich (60%)	For tropical and subtropical conditions

* Source: CIMAP, varietal portal

plant, leaf-width, leaf area (cm^2), and herbage yield, oil recovery were recorded before harvest of the crop.

Observations

At the time of harvest five plants were randomly selected for recording the observations. The plant height was recorded from base of the plant to the extreme growing tip using meter scale. Similarly, number of tillers per clump and number of leaves per tillers were also recorded. Yield parameters *viz.,* herb yield and essential oil yield was calculated based on the net plot yield and converted to ton per hectare.

Source of different lemongrass cultivars

Different *Cymbopogon* spp. (*viz.,*Bio-13, Medini, CIM-Jeeva, Jalpallavi, Manjusha and C-Line (selection line) evaluated in the present study were collected from CSIR-CIMAP, Research Centre gene bank. Whereas, C-line, geraniol rich variety was developed through selection method. The source and citronellal content of varieties and other relevant information are provided in Table 1.

Analytical methods

Extraction of the essential oil

The fresh leaves (100 g) of different citronella (*Cymbopogon spp*.) cultivars were harvested manually and were subjected to hydro-distillation for 3-3.30 hours in a Clevenger apparatus for the extraction of essential oil. The oils were dehydrated with anhydrous Na_2SO_4 and kept in a cool and dark place until further analysis.

Gas chromatography

Gas chromatography analysis was carried out on Thermo Fisher Trace GC-1300, equipped with TG-5 capillary column (30 m × 0.25 mm, 0.25 µm film thickness), with flame ionization detector (FID). The temperature of the oven column ranged from 70 to 230°C, programmed at 3°C min^{-1}, using N_2 as carrier gas at constant flow rate of 1.0 mL min^{-1}. The Injector and detector (FID) temperatures were maintained at 240°C and 260°C, respectively. Injection volumes of the oils were 0.02 ìL neat with a split ratio, 1:40. The relative content of constituents was quantified on the basis of percent peak area without using a correction factor.

Essential oil content (EOC)

The essential oil percentage in the herbage was calculated using the following formula:

$$EOC\ (\%) = \frac{\text{Volume of essential oil collected (ml) X 100}}{\text{Total weight of fresh leaves}}$$

Essential oil yield

Essential oil yield *(kg/litre per hectare)*was calculated based on the herb yield and oil content using the formula:

$$\text{Oil yield} = \frac{\text{Herb yield X EOC}}{100}$$

Statistical Analysis

The data collected from the experimental plot at the time of harvest were documented and subjected to analysis of variance (Gomez and Gomez, 1984). Fisher and Snedecor's 'f' test was used for testing the significance of different sources of variation at 0.05 level of probability. Duncan multiple range test (DMRT) was used for comparison of means at 0.05 probability.

RESULTS AND DISCUSSION

The growth and yield parameters, *viz.,* plant height (cm), number of tillers, herb yield (kg/ $100m^2$), essential oil content and oil yield (L/$100m^2$) of the six different citronella cultivars are presented in Table 2. Growth parameters of citronella cultivars differed significantly due to differential performance by the cultivars. The maximum plant height was noticed in CIM-Jeeva (139 cm), followed by Bio-13 (134.67 cm) and the lowest plant height was noticed in Jalpallavi (123.33 cm). The higher numbers of tillers per clump were recorded in CIM-Jeeva (45.67) followed by Bio-13 (42.33) while the minimum was recorded in Jalpallavi (37.56). The difference in number of tillers is accredited to variation in the genotypes and interaction with environmental variations which are in line with earlier reports in *Cymbopogan pendulus* (Sharma

Table 2: Growth and yield parameters of citronella cultivars under Southern region of Karnataka, Bengaluru

Cultivar	Plant height (cm)	Number tillers	Number of leaves/tiller	Biomass (kg/100m^2)	Oil recovery (%)	Oil yield (L/100m^2)
Medini	135.55 ^{ab}	43.22 ^{ab}	5.83 ^{a}	192.67 ^{abc}	1.13 ^{a}	2.16 ^{bc}
Jalpallavi	123.33 ^{de}	37.56^{cd}	5.90 ^{a}	167.83 ^{cd}	1.15 ^{a}	1.93 ^{cd}
Bio-13	134.67 ^{abc}	41.67 ^{abc}	5.43 ^{a}	207.32 ^{abc}	1.20 ^{a}	2.49 ^{ab}
C-Line	125.00 ^{bcd}	42.33 ^{abc}	5.74 ^{a}	192.33 ^{abc}	1.17 ^{a}	2.24 ^{abc}
CIM-Jeeva	139.00 ^{a}	45.67^{a}	5.45 ^{a}	217.50 ^{a}	1.23 ^{a}	2.69 ^{a}
Manjusha	127.00 ^{bcd}	40.17 ^{abc}	5.77 ^{a}	185.00 ^{bc}	1.15 ^{a}	2.13 ^{bc}

et al., 2005) and in citronella (Ibrahim and Khalid 2013). Poehlman and Sleper (1995) reported that the occurrence of variation in a plant can be due to hereditary differences in the plant and environment conditions in which plants are being grown or sometimes the combination of both.

The yield characteristics of different cultivars of citronella varied significantly due to the difference in growth and yield potential. The maximum herb yield was found in cultivar CIM-Jeeva (217.50 kg/100m^2), followed by Bio-13 (207.32 kg/100m^2) and C-line (192.33 kg/100 m^2); while the cultivar Jalpallavi showed the lowest herb yield (167.83 kg/100 m^2) among the cultivars evaluated in the present study. The essential oil content ranges from 1.13% to 1.23%. The highest recovery was recorded in CIM-Jeeva (1.23%) followed by Bio-13(1.20%) and lowest was noticed in Jalpallavi (1.13%). This variation in the oil content is attributed to the variation in inherent capacity of genotypes, season of harvest, harvesting time and herbage drying period. Similarly, the highest essential oil yield was recorded in the cultivar CIM-Jeeva (2.69L/100m^2), followed by Bio-13 (2.49L/100m^2). The increase in the herb yield and essential oil yield maybe due to the production of more tillers per plant and, number of leaves/tillers. Plant height had a positive and strong correlation/association with yield parameters (Table 2). Sarma et al., (2001) reported that variation in essential oil content and yield is mainly due to seasonal changes and harvesting time of the crop. Further he concluded that higher yield of java citronella can be obtained during September to October.

Chemical composition of essential oil

About 24 main chemical compounds were identified in the present study by gas chromatography which constitute about 92.05-97.30% citronella essential oil (Table 3). Citronellal, neral, geraniol, elemol and geranial and geranyl acetate are the important chemical constituents. Citronella essential oil containing about 35% citronellal is considered the best quality commercial oil. The highest citronellal was recorded in CIM-Jeeva (44.60±1.27%) followed by Jalpallavi (41.35 ± 0.92%), Manjusha (39.15±1.63%), Bio-13 (37.65 ± 4.45%) and Medini (21.80±3.11%) and the lowest amount was found in C-line (8.60 ± 0.57%). According to citronellol content, the varieties can be arranged as follows: CIM-Jeeva (11.30 ± 0.28%) >Jalpallavi (9.25 ± 1.91%) >Bio-13 (9.15 ± 1.48%) > Medini (8.20 ± 0.14%) > Manjusha (8.15 ± 1.20%) > C-line (2.0±0.00%). The C-line was superior in geraniol content compared to citronellal content followed by Medini (50.25 ± 1.77%). Fatima et al., (2002) reported that the decrease in the geraniol and citronellal content was due to drought condition which implies increased amounts of citronellol. Elemol content of different citronella cultivars varied from 0.15-3.96%. The highest percentage of elemol was recoded in Jalpallavi (3.96%) and the lowest (0.15%) was noticed in C-line. Higher amount of geranial (8.28%) was noticed in C-line followed by Medini (5.65%), Bio-13 (1.35%) and the lowest was recorded in CIM-Jeeva (0.60%) cultivar. Overall, cultivars CIM-Jeeva and Bio-13 were superior compared to other cultivars in terms of essential oil yield and market acceptability of citronellal and citronellol content.

Table 3: Chemical composition of essential oil of citronella cultivars

Compounds	RI_{cal}	RI_{exp}	Percentage composition (%)					
			Bio-13	Manjusha	Medini	Jal Pallavi	Jeeva	C-line
6-methyl hept-5-en-2-one	978	981	0.15±0.07	0.15±0.07	0.20±0.14	0.10±.000	0.10±.00	0.30±0.14
Limonene	1018	1024	2±1.27	2.5±0.57	0.1±0.00	2.6±0.71	2.45±0.07	2.7±0.42
(*Z*)-beta-Ocimene	1030	1032	t	t	t	t	0.10±0.00	t
(*E*)-beta-Ocimene	1042	1044	t	t	0.10±0.00	t	t	t
Linalool	1100	1095	0.50±0.01	0.60±0.00	1.00±0.00	0.70±0.02	0.60±0.01	0.95±0.21
Citronellal	1146	1148	37.65±4.45	39.15±1.63	21.80±3.11	41.35±0.92	44.60±1.27	8.60±0.57
Terpinen-4-ol	1172	1174	0.20±0.14	0.30±0.28	0.20±0.00	0.40±0.42	0.10±0.00	0.25±0.07
α-Terpineol	1184	1186	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00	0.15±0.07	0.10±0.01
Citronellol	1226	1223	9.15±1.48	8.15±1.20	8.20±0.14	9.25±1.91	11.30±0.28	2.00±0.00
Neral	1235	1235	1.10±0.71	1.05±0.64	4.15±0.49	0.60±0.00	0.55±0.07	5.95±0.21
Geraniol	1245	1249	23.40±6.22	24.70±3.25	50.25±1.77	19.55±0.49	22.75±0.49	59.35±1.06
Geranial	1262	1264	1.35±0.78	1.30±0.71	5.65±1.06	0.80±0.00	0.60±0.14	8.20±0.28
Citronellyl Acetate	1352	1630	1.60±0.42	1.55±0.64	0.80±0.85	2.10±1.41	1.43±0.81	0.50±0.00
Geranyl Acetate	1381	1379	4.05±1.77	4.05±0.64	3.30±2.12	5.95±0.78	4.20±1.98	7.15±0.07
β-Elemene	1390	1389	0.45±0.07	0.55±0.07	0.25±0.07	0.55±0.07	0.70±0.00	0.15±0.07
β-Caryophyllene	1418	1417	_t	_t	0.10±0.00	_t	0.10±0.00	0.80±0.00
Germacrene-D	1480	1484	0.50±0.14	0.50±0.14	0.10±0.00	0.45±0.07	0.30±0.28	0.10±0.00
Citronellyl butyrate	1532	1530	6.40±0.00	6.10±0.00	1.40±0.00	6.20±0.00	0.30±0.00	0.50±0.02
Elemol	1548	1548	3.50±3.39	3.65±3.61	1.40±0.00	3.80±3.96	3.20±3.68	0.15±0.07
Germacrene-D-4-ol	1576	1574	t	1.05±0.07	t	0.65±0.07	0.75±0.07	0.15±0.07
Caryophyllene oxide	1585	1582	0.15±0.07	0.15±0.07	t	0.20±0.00	0.15±0.07	t
10-epi-γ-Eudesmol	1618	1622	t	t	t	0.15±0.07	0.10±0.00	t
epi-α-Cadinol	1636	1638	0.45±0.07	0.50±0.14	0.20±0.14	0.55±0.07	0.45±0.07	t
β-Eudesmol	1644	1649	0.10±0.00	t	t	0.80±0.00	0.70±0.00	0.10±0.00
Total			**92.05±4.88**	**92.75±2.90**	**97.50±0.57**	**93.40±2.55**	**95.13±0.67**	**97.30±0.57**

t- trace amount

Citronella essential oil obtained in the present study was compared with those reported previously from other Indian states in terms of major components (Table 4).The higher amount of citronellal (50.93%) was noticed from Andhra Pradesh region (Kaul et al.,1997) whereas relatively very less amount was noticed in Medini cultivar from Karnataka. Gereniol and elemol content was comparatively higher in Eastern dry zone of Karnataka region as compared to other parts of

Table 4: Comparisation of major terpenoids in the essential oil of Java citronella (*Cymbopogon winterianus*) grown in different parts of India

Major Compounds	Concentrations (%)												
	Karnataka						Uttarakhand (Tarai region)	Uttar Pradesh	Andhra Pradesh	Jammu &Kashmir	Maharashtra	West Bengal	Assam
	Bio-13	Manjusha	Medini	Jal Pallavi	CIM-Jeeva	C-line	-	-	-	-	-	-	-
Citronellal	34.5	38.0	19.6	40.7	43.7	8.2	43.6	26.2-42.9	50.93	41.6	37.2-45*	36.0-44.6*	40.7-45.5
Citronellol	10.2	9.0	8.1	10.6	11.1	10.2	14.4	12.3-20.1	6.06	9.0	-	-	8.5-18.4
Geraniol	27.8	27.0	51.5	19.9	22.4	60.1	19.7	17.9-28.6	16.47	20.7	-	-	22.7-23.5
Elemol	5.9	6.2	1.4	6.6	5.8	0.1	3.50	- -	1.15	--	--	--	--
References	Present study						Singh etal., 2002	Sharma et al., 1988	Kaul et al., 1997	Kaul et al., 1997	Malwatkar et al., 1984	Nandi and Chatterjee 1991	Sarma 1977

* Total aldehyde content

the country. Variations in the chemical composition of natural essential oils are common occurrence mainly due to the source of cultivars, ecological conditions, chemotype/genotype, and crop growth and developmental stage (Verma et al.,2010, Verma et al.,2012).

The results of the present study showed a considerable variation in the quantitative composition of the essential oils obtained among six different cultivars. This may be due to influence of factors such as environmental and genetic character of a cultivar/genotype/chemotype (Kulkarni, 1999) and geographical differences in cultivation, (Kaul et al.,1997, Singh et al., 2002), cultivation season (Malwatkar et al., 1984; Singh et al, 1994), season of harvest (Verma et al., 2009; Kakaraparthi et al., 2014) and pedogenetic factors. The study demonstrated that potential cultivation of Java citronella for the production of quality essential oil in Southern zone of Karnataka.The ISO specification for the major essential oil constituents is as follows: citronellal (31.0-40.0%), geraniol (20.0-25.0%), citronellol (8.5-14.0%), geranyl acetate (2.5-5.5%), limonene (2.0-5.0%) etc. The results summarised in Table 3 for essential oil composition of different cultivars studied, showed that in term of major constituents (citronellal, geraniol, citronellol), the cultivars (Bio-13, Manjusha, Jalpallavi, Jeeva) have specification parallel to ISO specification, except Medini and C-line. The latter two cultivars show a dominance of geraniol in the essential oil, which is specific for both these cultivars. Moreover, the content of essential oil constituents is also dependent on various environmental and agronomic factors.

Correlation between the constituents of essential oil

The majority of growth parameters of citronella were positively correlated with yield of citronella (Table 5). Plant height (0.594*) and number of tillers/

Table 5: Correlation of growth, yield and chemical parameters of different citronella cultivars

Parameters	Plant height	Number tillers	Number of leaves	Biomass	Oil recovery	oil yield	Linalool	Citronellal	Citronellol	Neral	Geraniol	Geranial	Citronellyl Acetate	Geranyl Acetate	Elemene	Elemol
Plant height	1															
Number tillers	0.818**	1														
Number of leaves	-0.696	-0.618	1													
Biomass	0.594*	0.931***	-0.636	1												
Oil recovery	0.295ns	0.605*	-0.658	0.799**	1											
oil yield	0.522ns	0.867***	-0.663	0.979***	0.903***	1										
Linalool	-0.267	0.134 ns	0.542 ns	0.184	-0.100	0.096ns	1									
Citronellal	0.215ns	-0.168	-0.297	-0.260	0.078ns	-0.148	-0.897	1								
Citronellol	0.424ns	-0.084	-0.288	-0.299	-0.120	-0.245	-0.824	0.921***	1							
Neral	-0.225	0.207 ns	0.264ns	0.328	0.003ns	0.225ns	0.887***	-0.993	-0.954	1						
Geraniol	-0.102	0.286 ns	0.279ns	0.341	-0.064	0.214ns	0.912***	-0.986	-0.887	0.982***	1					
Geranial	-0.238	0.199 ns	0.278ns	0.322	0.007ns	0.222ns	0.897***	-0.992	-0.956	0.999***	0.981***	1				
Citronellyl Acetate	-0.102	-0.540	0.111ns	-0.651	-0.260	-0.543	-0.681	0.863***	0.846***	-0.900	-0.892	-0.892	1			
Geranyl Acetate	-0.757	-0.441	0.732**	-0.279	-0.160	-0.255	0.755**	-0.603	-0.666	0.587*	0.527ns	0.607*	-0.215	1		
Elemene	0.178 ns	-0.138	-0.340 ns	-0.181	0.189ns	-0.054	-0.898	0.988***	0.856***	-0.963	-0.977	-0.963	0.807**	-0.594	1	
Elemol	0.051 ns	-0.350	-0.270 ns	-0.396	-0.008	-0.278	-0.940	0.965***	0.860***	-0.961	-0.988	-0.963	0.870***	-0.548	0.958***	1

***significance at *P* <0.001, **significance at *P* <0.01, *significance at *P* <0.5; ns=non-significant; *P* <0.05 probability level

clump (0.931***) was significantly correlated with biomass. Citronellol has positive correlation with citronellyl acetate (0.846***), β-elemene (0.856***) and elemol (0.860***) and it has negative and non-significant correlation with rest of the constituents. Similarly, geraniol has positive correlation with linalool (0.912***) and neral (0.982***) and it has negative and non-significant correlation with rest of the constituents. Whereas, β-elemeneand elemolhas found to have a similar type of correlation with chemical constituents of citronella. Both β-elemene and elemol have positive correlation with citronellal (0.988*** and 0.965***), citronellol (0.856***and 0.860***) and citronellyl acetate (0.807*** and 0.870***) respectively. Linalool is positively and highly significantly correlated with neral (0.887***), geraniol (0.897***) geranial (0.912***) and geranyl acetate (0.755**) only and exhibit negative correlation with rest of oil constituents. A positive correlation with citronellol and citronellal content was observed with geraniol (Luthra et al., 1991) in citronella, whereas in the present study it was negatively correlated. Similarly, Rao et al., (1995) reported that negative correlation of geraniol with citronellol. Similar kind of results were noticed in the present study. Minor constituents like elemol and β-elemene showed positive correlation with each other and citronellal, citronellol and citronellyl acetate (Kakaraparthi et al., 2014).

CONCLUSIONS

In conclusion, the results of the present study clearly demonstrated the cultivar CIM-Jeeva recorded significantly higher amounts of herb and essential oil 217.50 kg/100m^2 and 2.69 L/100m^2, respectively. CIM-Jeeva and Bio-13 were superior compared to other cultivars in terms of essential oil yield and market acceptable citronellal content. Hence, we can recommend CIM-Jeeva and Bio-13 as the best cultivars for commercial cultivation under Southern tropical regions of India.

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Conflict of interest

The authors declare that they have no conflict of interest.

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