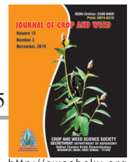
Journal of Crop and Weed, 18(2): 30-35 (2022)

ISSN- O : 2349 9400 ; P : 0974 6315



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Effect of growing condition and harvesting method on essential oil and eugenol contents of tulsi (*Ocimum tenuiflorum* L.)

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Received : 14.12.2021 ; Revised : 10.04.2022 ; Accepted : 01.05.2022

DOI: https://doi.org/10.22271/09746315.2022.v18.i2.1569

ABSTRACT

A study was conducted during two consecutive kharif seasons of 2019 and 2020 to standardize the shade requirement and method of harvesting for obtaining high essential oil and eugenol contents in tulsi. Experiments were laid out in split plot design with two main plots and four sub plot treatments. Growing of tulsi under 50 per cent shade and harvesting at 20 - 30 cm height from ground level at 75 and 135 days after transplanting (DAT) can be recommended for higher essential oil production. For open growing condition, harvesting at 20 - 30 cm height at 90 and 150 DAT can be recommended for higher eugenol content.

Keywords: Essential oil, eugenol, harvesting method, shade, tulsi

Tulsi (*Ocimum tenuiflorum* L.) is an aromatic plant having multitude medicinal properties. It is a species prioritized by National and State Medicinal Plant Boards for commercial cultivation and the estimated annual trade is 2000-3000 MT (NMPB, 2020). With steadily rising interest in herbs, attention should be given to the sustainable production and utilization of aromatic and medicinal plants.

Essential oils extracted from *Ocimum* plants are complex mixtures of natural organic compounds and possess several biological properties. Eugenol, the active constituent present in tulsi essential oil is mainly responsible for its therapeutic potentials. The essential oil yield of tulsi usually ranges from 0.2 to 1.0 per cent but even reaches 1.7 per cent based on the source and phenological stage. Essential oil content and composition in *Ocimum* spp depended on edaphic and climatic conditions of the location, growing season and maturity stages of the plant (Laskar and Majumdar, 1988)**.** Among the environmental factors that have an impact on plant growth, development and yield, both quality and quantity of light transmitted to canopy play a vital function (Shahak *et al*., 2004). As per Milenkovic *et al*. (2019) accumulation of essential oil was more in shaded plants compared to unshaded condition. In *Ocimum basilicum* L., higher irradiance (no shade) significantly improved the linalool and eugenol contents, whereas content of methyleugenol was increased by lower irradiance. Ontogeny or growth stage of plant had close association with secondary metabolite buildup in plants and thus had major influence on oil yield and its composition (Dhar *et al*., 2006; Verma *et al*., 2010). In Indian basil, main crop harvested after 40 days at 15 or 7.5 cm height from the ground level and ratoon crop at 50 days after the first harvest produced maximum oil yield (Singh *et al*., 2010).

The growth, development, yield and quality of aromatic and medicinal plants are drastically influenced by many extrinsic and intrinsic factors. Hence the present study has been conducted to standardize the shade requirement and method of harvesting for obtaining high essential oil and eugenol content in tulsi.

MATERIALS AND METHODS

The present study was conducted in *kharif* 2019 and repeated in *kharif* 2020 at Agronomy Department Farm, College of Agriculture, Vellanikkara, Thrissur, Kerala 13^0-32'N latitude and 76^0-26'E longitude, 40 m above mean sea level). Field soil was sandy clay loam, acidic in reaction (4.68), low in available nitrogen (142 kg ha^{-1}), high in available phosphorus (27 kg ha^{-1}) and medium in available potassium (214 kg ha^{-1}). The average annual rainfall was 3128.3 mm during 2019 and 2697.3 mm during 2020. The maximum, minimum and average temperature prevailed during 2019 was 32.9 ^{0}C, 21.7 ^{0}C and 26.87^{0}C, respectively. During 2020, maximum, minimum and average temperatures were 33.0 ^{0}C, 21.5^{0}C and 27.02^{0}C, respectively.

Experiments were laid out in split plot design with two main plots and four sub plot treatments and five replications.

Treatments

Main plot: Growing condition

M_1:- Open

M_2:- 50 per cent shade

Sub plot: Harvesting method

S_1:- Harvesting at 20 cm height from ground level at 75 and 135days after transplanting (DAT)

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S_2:- Harvesting at 30 cm height from ground level at 75 and 135 DAT

S_3:- Harvesting at 20 cm height from ground level at 90 and 150 DAT

S_4:- Harvesting at 30 cm height from ground level at 90 and 150 DAT

Experimental area was thoroughly ploughed with a disc plough and made into fine tilth by working with cultivator. The individual plots were laid out in open and 50 per cent shade as per the layout plan. The plot size adopted was 4.8 x 2.0 m. Artificial shading was provided by using green colour shade net with 50 per cent permeability to sunlight. Two months old healthy, uniform sized seedlings were transplanted in the main field at a spacing of 40 $\times$ 40 cm. The crop was uniformly fertilized with farm yard manure (FYM) @ 10 t ha^{-1} and N: P_2O_5: K_2O @ 120: 60: 60 kg ha^{-1}. After leveling FYM @ 10 t ha^{-1}, half dose of N and K and full dose of P were applied as basal. Remaining N and K were given in two equal split doses at 45 DAT and after first harvest. Experimental area was kept weed-free by hand weeding at 30, 60 DAT and at first harvest. Harvesting was done manually using secateurs at different times and heights as per the treatments.

Essential oil content (%) in fresh herbage was determined by hydro distillation method using Clevenger's apparatus (ASTA, 1968). For this, 30 g fresh sample of tulsi was collected and hydro distilled in a Clevenger's apparatus, the temperature was kept at 90°C till boiling and then maintained at 70 °C for 3 hours for distillation. The distillate was cooled to room temperature and oil was allowed to settle to obtain a clear layer. The volume was measured and oil concentration (%) was calculated as

$$\text{Essential oil (\%)} = \frac{\text{Volume of oil (ml)}}{\text{Weight of sample (g)}} \times 100$$

Essential oil yield was calculated by multiplying the oil concentration (%) with respective biomass yield and expressed in kg ha^{-1} (Dhar *et al*., 1996). Eugenol content of tulsi at first and second harvests was estimated by Gas Chromatograph-Mass Spectrometry (GC-MS) analysis (Thyagaraj *et al*., 2013). GC-MS analysis of the essential oil samples was done in an instrument model QP2010S manufactured by Shimadzu, Tokyo, Japan. The column used was ELITE-5MS of 30 $\times$ 0.25 mm $\times$ 0.25 ìm dimensions, column temperature was started at 50 °C, held for 2 min, stepped up to 250 °C for 6 min and finally boosted to 280 °C and held for 22 min. Helium gas (99.999 %) was used as carrier gas with a constant flow rate of 16.3 ml min^{-1}. Column flow rate was maintained 1.2 ml min^{-1}. Total volatile oil was calculated as the sum of all GC peak areas of individual compounds in the chromatogram, and the spectra was compared using two spectral libraries NIST 11 and Willey 8 (Willey Registry TM, 8^{th} Edition Mass Spectral Library, and NIST 11 Mass Spectral Library (NIST/EPA/NIH)). Eugenol content was expressed as relative percent area (%).

The data were analysed statistically using analysis of variance (ANOVA) with statistically package 'WASP 2.0' (Statistical package, ICAR - Central Coastal Agricultural Research Institute, Goa).

RESULTS AND DISCUSSION

Essential oil content and essential oil yield of tulsi

The influence of growing condition, harvesting method and their interaction on essential oil concentration and essential oil yield of tulsi is given in Table 1. During first year, plants raised under 50 per cent shade registered significantly higher essential oil at first harvest and second harvest (0.785 % and 0.739 %). During second year at second harvest also plants under 50 per cent shade had significantly higher essential oil content (0.702 %). Similarly, plants grown under 50 per cent shade recorded greater essential oil yield at first harvest (61.34 kg ha^{-1} and 49.87 kg ha^{-1}) and second harvest (20.78 kg ha^{-1} and 17.07 kg ha^{-1}) during both years. According to Ade-Ademilua *et al.* (2013), African basil recorded fairly more essential oil yield under natural shade compared to full sunlight. *Ocimum* spp. cultivated under silvi-medicinal systems produced higher oil yield than those raised under sole cropping (Suvera *et al*., 2015).

Harvesting method also influenced essential oil yield of tulsi. Harvesting at 20 cm or 30 cm height from ground level at 75 and 135 DAT recorded significantly higher essential oil yield. It was found that compared to harvesting at 90 and 150 DAT, early harvesting of tulsi (75 and 135 DAT) resulted in higher essential oil yield. Padalia *et al.* (2013) reported that the essential oils of genus *Ocimum* were found to vary significantly during different phenophases. In *Ocimum basilicum*, full flowering stage of the crop was the most profitable time of harvest with respect to oil yield and quality (Bahl *et al.,* 2000). Maturity of the plant at the time of harvesting significantly affected the essential oil content in *O. tenuiflorum* (Sims *et al*., 2013). Experimental results also revealed that, essential oil yield of tulsi were found to be higher at first harvest as compared to second harvest. Similarly, Tansi and Nacar (2000) and Moghimipour *et al.* (2017) reported that in tulsi, essential oil yield was maximum at the first harvest compared to second harvest.

Interaction effect of growing condition and harvesting method on essential oil yield was significant at first harvest and second harvest. At first harvest, 50 per cent

Table 1: Direct and interaction effect of growing condition and harvesting method on essential oil content (%) and essential oil yield of tulsi

Treatments	2019 -2020				2020-2021			
	1st harvest		2nd harvest		1st harvest		2nd harvest	
	Essential oil (%)	Essential oil yield (kg ha^{-1})	Essential oil (%)	Essential oil yield (kg ha^{-1})	Essential oil (%)	Essential oil yield (kg ha^{-1})	Essential oil (%)	Essential oil yield (kg ha^{-1})
Growing condition								
M1-Open	0.698	49.93	0.676	16.41	0.666	36.45	0.623	11.74
M2- 50 % shade	0.785	61.34	0.739	20.78	0.733	49.87	0.702	17.07
LSD (0.05)	**0.046**	**5.64**	**0.038**	**3.01**	**NS**	**7.12**	**0.036**	**1.77**
Harvesting method								
S1- Harvesting at 20 cm height from ground at 75 and 135 DAT	0.760	63.36	0.725	21.92	0.732	51.36	0.677	14.21
S2-Harvesting at 30 cm height from ground at 75 and 135 DAT	0.752	59.29	0.712	23.13	0.707	46.29	0.673	19.31
S3-Harvesting at 20 cm height from ground at 90 and 150 DAT	0.728	46.74	0.697	14.72	0.677	38.27	0.651	11.37
S4- Harvesting at 30 cm height from ground at 90 and 150 DAT	0.727	45.17	0.696	14.62	0.694	36.72	0.650	12.74
LSD (0.05)	**NS**	**4.62**	**NS**	**2.33**	**NS**	**5.02**	**NS**	**1.88**
Treatment combination								
M1 X S1	0.704	53.60	0.690	19.58	0.692	44.02	0.642	11.66
M1 X S2	0.710	50.00	0.682	20.48	0.674	38.05	0.624	14.62
M1 X S3	0.686	41.01	0.670	13.14	0.640	32.21	0.618	10.12
M1 X S4	0.692	39.11	0.662	12.43	0.664	31.49	0.610	10.56
M2 X S1	0.816	73.11	0.760	24.26	0.772	58.70	0.712	16.75
M2 X S2	0.794	68.54	0.742	25.77	0.740	54.52	0.722	24.00
M2 X S3	0.770	52.47	0.724	16.29	0.714	44.33	0.684	12.62
M2 X S4	0.762	51.23	0.730	16.81	0.724	41.94	0.690	14.91
LSD (0.05)	**NS**	**6.54**	**NS**	**3.30**	**NS**	**7.01**	**NS**	**2.65**

Table 2: Major components identified in tulsi essential oil under open and 50 per cent shade

Open	50 % shade
Eugenol	Eugenol
Germacrene-D	β-caryophyllene
β-elemene	Germacrene-D
β-caryophyllene	β- elemene
Germacrene -A	β- ocimene
Copaene	4-allyl-1,2-dimethoxy benzene
β- ocimene	Endo-borneol
4-allyl-1,2-dimethoxy benzene	β-linalool
Elemol	β-cubebene
Delta - cadinene	β-cadinene
Endo-borneol	Copaene

Table 3: Direct and interaction effect of growing condition and harvesting method on eugenol content (%) of tulsi

Treatments	2019 -2020		2020-2021		Pooled data (2019-20 and 2020-21)	
	1st harvest	2nd harvest	1st harvest	2nd harvest	1st harvest	2nd harvest
Growing condition						
M1-Open	65.95	57.05	56.97	46.97	61.46	52.01
M2- 50 % shade	47.79	36.45	45.06	31.90	46.42	34.18
LSD (0.05)	**3.77**	**1.68**	**3.73**	**2.84**	**1.96**	**1.31**
Harvesting method						
S1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	52.63	46.73	47.99	39.61	50.31	43.17
S2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	51.93	46.56	46.97	39.70	49.45	43.13
S3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	62.17	46.30	55.19	40.01	58.68	43.15
S4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	60.75	47.41	53.92	38.42	57.33	42.91
LSD (0.05)	**3.43**	**NS**	**3.32**	**NS**	**2.53**	**1.94**
Treatment combination						
M1 X S1	62.12	56.75	54.94	45.90	58.53	51.33
M1 X S2	61.21	58.11	53.55	47.19	57.37	52.65
M1 X S3	70.77	56.19	60.47	48.25	65.61	52.22
M1 X S4	69.71	57.15	58.94	46.53	64.32	51.84
M2 X S1	43.13	36.70	41.03	33.32	42.08	35.01
M2 X S2	42.65	35.02	40.39	32.22	41.52	33.62
M2 X S3	53.56	36.41	49.94	31.76	51.75	34.08
M2 X S4	51.80	37.66	48.89	30.31	50.34	33.98
LSD (0.05)	**4.85**	**4.64**	**4.70**	**3.99**	**3.58**	**2.74**

shade condition with harvesting at 20 cm height from ground level at 75 and 135 DAT recorded greater essential oil yield, at second harvest 50 per cent shade condition with harvesting at 30 cm height from ground level at 75 and 135 DAT recorded greater essential oil yield and these two treatments were statistically on par. However harvesting method and their interaction did not showed any significant on essential content of tulsi.

GCMS analysis and eugenol content of tulsi essential oil

GCMS analysis of tulsi essential oil showed that the components varied slightly under open and 50 per cent shade (Table 2). The principal constituent of tulsi essential oil under open as well as 50 per cent shade was eugenol. The second and third major component under open was germacrene-D and β-elemene respectively. However under 50 per cent shade, second major constituent was β- caryophyllene, germacrene-D was third major component. The compositions of essential oils of *Ocimum selloi* plants varied according to the quality of light (Costa *et al*., 2010). According to Padalia and Verma (2011), the major compound in the essential oil of *O. sanctum* was eugenol (67.4 % - 72.8 %). Awasthi and Dixit (2007) conducted GC and GC-MS analysis of *O. sanctum* oils and found eugenol as the major constituent.

Data pertaining to the effect of growing condition, harvesting method and interaction effect of growing condition and harvesting method on eugenol content at harvests is presented in Table 3. In this study, plants grown under open condition had significantly greater eugenol content at first harvest (65.95 % and 56.97 %) and second harvest (57.05 % and 46.97 %) during both the years of study. According to Chang *et al*. (2008), essential oils composition of basil was strongly affected by light intensity and eugenol concentration was higher under higher light integrals. The concentration and composition of volatile compounds in basil oil was affected by solar irradiance and the content of eugenol was relatively high under shade (50 % shading) (Milenkovic *et al*., 2019). Similarly, in *O. gratissimum* L., eugenol content was maximum under open conditions compared to shade (Pillai, 1990).

Harvesting method influenced the eugenol content of tulsi. At first harvest, harvesting of tulsi at 20 cm height from ground at 90 and 150 DAT recorded greater eugenol content during both years (62.17 % and 55.19 %) and it was on par with harvesting at 30 cm height from ground level at 90 and150 DAT (60.75 % and 53.92 %). At second harvest, treatments found non significant with respect to eugenol content. Harvesting of tulsi at 90 DAT (S3 and S4) produced significantly higher eugenol content than harvesting at 75 DAT (S1 and S2). This might be due to increase in the maturity of leaves in above treatments. *Ocimum* spp. exhibited large variation in the essential oil composition due to variation in the ontogenetical stage of the plant at the time of harvest (Gupta, 1996). According to Sims *et al*. (2013), eugenol content of tulsi increased with a delay in harvest time. Increase in eugenol concentration of *O. sanctum* with the advancement of harvesting stage was reported by Saran *et al*. (2017). It was also evident from the study that eugenol was higher at first harvest than second harvest. Similarly, Tsasi *et al*. (2017) reported higher eugenol concentration in tulsi in the first harvest under field conditions. Interaction effect of growing condition and harvesting method on eugenol content was significant at first harvest and second harvest. Plants grown under open condition with harvesting either at 20 or 30 cm height from ground level at 90 and 150 DAT recorded higher eugenol content.

This study revealed that growing condition and harvesting method had profound influence on tulsi essential oil and eugenol. Essential oil production in tulsi was higher in shaded plants, while eugenol content was more when grown under open condition. Early harvesting at 75 and 135 DAT was superior with respect to essential oil production; however delayed harvest at 90 and 150 DAT recorded higher eugenol content.

ACKNOWLEDGEMENT

Authors express their sincere thanks to Kerala Agricultural University, Thrissur, Kerala (India) for providing all essential advice and facilities for the present study.

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