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Effect of harvesting time and height of harvest on the performance of tulsi (*Ocimum tenuiflorum* L.) under shade and open condition

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Abstract
Two separate field experiments, one under 50 % shade and another under open condition was conducted at Kerala Agricultural University, Thrissur during 2019-20 and 2020-21 to find out the effect of harvesting time and height of harvest on tulsi (*Ocimum tenuiflorum* L.). Present investigations were formulated in randomized block design (RBD) having four treatments and five replications. Treatments included, harvesting at 20 cm height above ground level at 75 and 135 DAT (T_1), harvesting at 30 cm height above ground level at 75 and 135 DAT (T_2), harvesting at 20 cm height above ground level at 90 and 150 DAT (T_3) and harvesting at 30 cm height above ground level at 90 and 150 DAT (T_4). Irrespective of growing conditions, harvesting at 20 cm height above ground at 75 and 135 DAT (T_1) and harvesting at 30 cm height above ground at 75 and 135 DAT (T_2) produced significantly higher biomass yield, essential oil yield and total chlorophyll. Among growing conditions, tulsi plants grown under 50 % shade was superior to open condition with respect to fresh biomass yield and oil yield, proving feasibility of growing tulsi as an intercrop.

Keywords: Biomass yield, essential oil yield, harvesting height, open, shade and tulsi

Introduction
Ocimum tenuiflorum L., popularly known as tulsi or holy basil, is an aromatic plant with multitude medicinal properties belonging to family Lamiaceae. Almost all parts such as leaves, stem, flower, roots and seeds of tulsi have been used in numerous formulations of Ayurvedha, Sidha, Unani and Homeopathy. These plants also have antifertility, antimicrobial, antifungal, anticancer, antidiabetic, analgesic, cardio protective and adaptogenic actions. *Ocimum tenuiflorum* L. is a rich source of biologically active compounds, the aromatic oil of this species possesses a characteristic, pleasant aroma and the main components are linalool, methyl chavicol, camphor and methyl euginol (Gill and Randhava, 1996) [1]. Tulsi have been described as the "Queen of plants" and the "mother medicine of nature" because of its perceived medicinal and therapeutic properties (Singh *et al*., 2010) [2].
These plants are distributed in the tropical, sub-tropical and warm temperate regions of the world and grown for the fresh market as well as for essential oil production (Zheljazkov *et al.*, 2008) [3]. According to National Medicinal Plants Board (NMPB), *Ocimum tenuiflorum* L. is a high demanded medicinal plant and it is prioritized for commercial cultivation. With rising global demand, expanding the cultivation of medicinal and aromatic plants appears to be an important strategy (Rao *et al.*, 2004) [4]. However, there exist numerous factors that influence growth, agronomic characteristics, biomass, essential oil yield and quality of aromatic and medicinal plants (Khazaie *et al*., 2007) [5]. Gupta (1996) [6] reported the differences in fresh biomass, oil yield and oil composition of *Ocimum* species with respect to ontogenetical stage during the time of harvest. As per Singh *et al*. (2010) [7], both harvesting stage as well as cutting height significantly influenced the growth and fresh herb yield of tulsi. The influence of date of seeding, transplanting and harvesting of *O. tenuiflorum* on content and composition of essential oil were reported by Sims *et al*. (2014) [8]. According to Suvera *et al*. (2015) [9], *Ocimum* spp. under silvi-medicinal systems registered significantly higher fresh above and below ground and total biomass and oil yield compared to sole cropping. Similarly Thakur *et al*. (2009) [10] also observed higher essential oil recovery from *O. sanctum* grown under agroforestry system. Shaded plants of tulsi had significantly greater plant biomass yield with higher leaf area index and leaf number than sun-exposed plants (Milenkoviic *et al*., 2019) [11].

In contrast Chang *et al*. (2008) [12] found that basil grows well in full sunlight. Harinkheda and Mishra (2009) [13] and Nagarajaiah *et al.* (2012) [14] also reported lower biomass production of medicinal and aromatic plants under different shaded conditions.

Ocimum species exhibit a lot of variation in growth, biomass production, oil yield, and oil composition based on the growing condition and ontogenetical stage during harvest. Therefore, it is necessary to identify optimum light intensity and method of harvesting to ensure high yield and quality. Hence, the present study was formulated to investigate the effect of harvesting time and height of harvest on the performance of tulsi (*Ocimum tenuiflorum* L.) under 50 % shade and open condition.

Materials and methods

Two separate field experiments, one under 50 % shade and another under open condition, each for two years were conducted during *Kharif* 2019-20 and 2020-21 at the Agronomy Department, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala (13° 32'N latitude and 76° 26'E longitude, at an altitude of 40 m above mean sea level). The soil of the experimental site was sandy clay loam in texture, medium in organic carbon (1.02 %), available P_2O_5 (21.08 kg ha^{-1}) and K_2O (213.6 kg ha^{-1}) at 0-15 cm soil depth.

The experiment was arranged in randomized block design (RBD) with four treatments and five replications. The treatments included, harvesting at 20 cm height above ground level at 75 and 135 DAT (T_1), harvesting at 30 cm height above ground level at 75 and 135 DAT (T_2), harvesting at 20 cm height above ground level at 90 and 150 DAT (T_3) and harvesting at 30 cm height above ground level at 90 and 150 DAT (T_4). The same sets of treatments were repeated under open and 50 % shaded (artificial) condition. Shade was introduced artificially by providing green colour shade net with 50 % permeability of sunlight. For planting, seedlings were raised in small polythene bags in nursery and two month old healthy, uniform sized seedlings were transplanted in the main field (both 50 % shade and open) at 40 cm × 40 cm spacing. 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}. Harvesting was done manually with secateurs at different times and heights based on the treatments. The data on plant height and fresh biomass yield were recorded during each harvest. Oil concentration (%) in fresh herbage was estimated by hydro distillation method using Clevenger's apparatus (ASTA, 1968) [15]. Fresh sample of about 30 g was harvested and hydro distilled in a Clevenger's apparatus and the temperature was maintained at 90° C till boiling and then kept at 70° C for 3 hours for distillation. The essential oil yield was computed by multiplying the oil concentration (%) with respective biomass yield and expressed in kg ha^{-1} (Dhar *et al*., 1996) [16]. Chlorophyll and carotenoid content (mg g^{-1} of fresh weight) in leaves were estimated using Dimethyl sulphoxide (DMSO) extraction technique (Hiscox and Israelstam, 1979) [17]. The data were analysed statistically using analysis of variance (ANOVA) with statistical package 'WASP 2' (Statistical package, ICAR Goa).

Results and discussion

Plant height

Plant height of tulsi at first cut was not significantly affected by treatments in both the condition (50 % shade and open condition) during both years of study (Table 1). While harvesting height and harvesting date significantly influenced the plant heights at second cut. Both under 50 % shade and open condition, higher plant height was observed with harvesting at 30 cm height above ground level at 75 and 135 DAT (T_2) and it was statistically at par with harvesting at 30 cm height above ground level at 90 and 150 DAT (T_4). The higher harvesting height (*ie*., 30 cm above ground level) in these treatments compared to T_1 and T_3 (*ie*., 20 cm above ground level) helps to retain more food reserves in the plants and thus it enhances re growth after first cut. This might be the reason for higher plant height at second cut in T_2 and T_4. Among different growing conditions, plant height was higher under 50 % shade compared to open in both 2019-20 and 2020-21. Milenkovic *et al*. (2019) [11] reported that sweet basil plants under shade were taller than those grown under open due to increase in internode length. *Ocimum selloi* plants cultivated under coloured shade net were taller in comparison with those in full sunlight (Costa *et al*., 2010) [18]. Higher plant height due to shade in aromatic and medicinal plants has also been registered by Thakur *et al*. (2019) [19] and Huang *et al*. (2016) [20].

Biomass yield

The biomass yield at first cut and second cut were significantly influenced by the treatments (Table 2). In 2019-20 under 50 % shade, biomass yield at first cut was higher in T_1 (harvesting at 20 cm height above ground level at 75 and 135 DAT), while biomass yield at second cut and total biomass yield were higher in T_2 (harvesting at 30 cm height above ground level at 75 and 135 DAT) and these two treatments were statistically at par. The same trend was followed in 2020-21 also. The pooled data of total biomass yield under 50 % shade showed that harvesting at 30 cm height above ground level at 75 and 135 DAT (T_2) recorded the higher total biomass and which was statistically at par with harvesting at 20 cm height above ground level at 75 and 135 DAT (T_1).

In 2019-20 under open condition, biomass yield at first cut and total biomass yield were higher in T_1 (harvesting at 20 cm height above ground level at 75 and 135 DAT), while biomass yield at second cut was higher in T_2 (harvesting at 30 cm height above ground level at 75 and 135 DAT) and these two treatments were statistically at par. In 2020-21, the same trend was followed for biomass yield at first cut and second cut, while total biomass yield was higher in T_1 and treatments T_1 and T_2 were statistically at par. The pooled data of total biomass yield under open condition revealed that harvesting at 20 cm height above ground level at 75 and 135 DAT (T_1) recorded higher total biomass and which was statistically at par with harvesting at 30 cm height above ground level at 75 and 135 DAT (T_2).

In both shade and open condition, early harvesting (75 & 135 DAT) at either 20 or 30 cm height above ground was significantly superior to late harvesting (90 &15O DAT) and biomass production was higher in first cut and it gradually decreased in the second cut. In *Ocimum* species biomass yield reached maximum when the plants are in between full flowering and initiation of seed formation stage (Gupta, 1996) [6]. According to Corrado *et al.* (2020) [21], basil can be harvested more than once and yield was significantly affected by the cut factor, the biomass yield was higher at the first cut because the average leaf size and total leaf area per plant was significantly higher at the first cut. Kothari *et al*. (2004) [22] reported that harvesting methods affected biomass yield of *Ocimum tenuiflorum* L. grown in south India and harvesting

of shoot biomass at 30 cm above ground gave maximum herbage yield and harvesting of secondary branches had minimum biomass yield. He also revealed that irrespective of methods of harvest, biomass production was higher in first cut and declined gradually in second, third and fourth cuts.

While comparing biomass yield between open and shade, it is clear that compared to open condition, plants grown under 50 % shade had higher biomass yield at all harvests. Suvera *et al*. (2015) [9] observed significantly higher fresh above and below ground and total biomass yield of *Ocimum* spp. under silvi-medicinal systems compared to sole cropping. Shaded plants of *Ocimum basilicum* L. recorded significantly greater plant biomass yield with higher leaf area index and leaf number than sun-exposed plants (Milenkoviic *et al*., 2019) [11].

Essential oil yield

Statistically significant differences in essential oil yield due to treatments were observed under 50 % shade and open condition during both years (Table 3). Under 50 % shade, at first cut T_1 recorded higher essential oil yield, in second cut T_2 recorded higher oil yield and these treatments were statistically at par. The same trend was observed in open condition also, *ie.*, early harvesting (75 & 135 DAT) either at 20 or 30 cm height above ground was significantly superior to late harvesting (90 &15O DAT) treatments.

The higher oil yield in T_1 and T_2 might be due to higher biomass production. While comparing oil yield between first cut and second cut, higher oil yield observed in first cut than second cut. Tansi and Nacar (2000) [23] stated that in lemon basil essential oil yield was maximum at the first harvest. Among growing conditions, plants grown under 50 % shade recorded higher oil yield than open grown plants and this might be due to higher biomass yield under shade. African basil produced comparatively high essential oil yields per plant when grown under natural shade compared to full sunlight (Ade-Ademilua *et al*., 2013) [24]. In *Ocimum* spp. oil yield was higher when plants raised in silvi-medicinal systems compared to sole cropping (Suvera *et al*., 2015) [9]. Similarly Milenkovic *et al*. (2019) [11] indicated that essential oil accumulation in sweet basil was higher in shade as compared to unshaded condition.

Total chlorophyll and total carotenoids

Total chlorophyll at second cut was not significantly influenced by treatments; while chlorophyll at first cut in shade grown as well as open grown plants were significantly influenced by treatments (Table 4). Harvesting at 20 cm height above ground level at 75 and 135 DAT (T_1) and harvesting at 30 cm height above ground level at 75 and 135 DAT (T_2) had significantly higher chlorophyll values compared to T_3 and T_4 in both open and shade. In this study, age of the leaf and plant might have affected the chlorophyll as in these two treatments plants were harvested early (75 DAT) compared to other treatments (90 DAT). Chlorophyll content in plants increases from youngest leaf to the leaf which can be called as "photosynthetically mature" and after attaining this maximum value the chlorophyll content decreases (Sestak, 1963) [25]. The significance of plant age on chlorophyll content was also reported by Mauromicale *et al*. (2006) [26]. There was no significant difference in total carotenoids at first cut as well as second cut in both first year and second year under shade as well as open (Table 5). Among the growing conditions, total chlorophyll was found higher for plants grown under 50 % shade, in contrast total carotenoids was higher in open conditions. Stagnari *et al*. (2015) [27] also reported higher chlorophyll content and lower carotenoids content under shade in lettuce.

Conclusion

Based on the data of two year study, it is clear that harvesting method as well as growing condition had influence on the performance of tulsi. The two treatments, T_1 (harvesting at 20 cm height above ground level at 75 and 135 DAT) and T_2 (harvesting at 30 cm height above ground level at 75 and 135 DAT) produced higher biomass yield, essential oil yield and total chlorophyll under 50 % shade and open. Hence irrespective of growing conditions, harvesting tulsi plants at 20 or 30 cm height above ground at 75 & 135 DAT can be practiced to obtain maximum biomass and oil yield. Further, growing of tulsi plants at 50 % shade is superior to open condition with respect to fresh biomass yield and oil yield and hence it can be successfully grown as an intercrop.

Table 1: Effect of harvesting time and height of harvest on plant height (cm) of tulsi during 2019-20 and 2020-21

50 % Shade						
Treatments	**Height from ground**	**Harvesting (DAT)**	**2019 -2020**		**2020-2021**	
			1st Cut	**2nd Cut**	**1st Cut**	**2nd Cut**
T1	20 cm	75 & 135 DAT	64.80	39.80 b	53.80	35.00 b
T2	30 cm		60.20	50.60 a	56.40	46.40 a
T3	20 cm	90 &15O DAT	64.00	38.00 b	61.00	34.20 b
T4	30 cm		67.60	47.20 a	58.60	44.80 a
SEm (±)			1.526	2.99	1.54	3.19
LSD (0.05)			NS	6.728	NS	6.633
Open						
Treatments	Height from ground	Harvesting (DAT)	2019 -2020		2020-2021	
			1st Cut	2nd Cut	1st Cut	2nd Cut
T1	20 cm	75 & 135 DAT	48.80	35.40 bc	42.60	32.20 b
T2	30 cm		45.60	42.40 a	43.80	43.20 a
T3	20 cm	90 &15O DAT	52.60	34.00 c	45.60	33.00 b
T4	30 cm		50.40	40.80 a b	48.20	40.00 a
SEm (±)			1.473	2.04	1.217	2.683
LSD (0.05)			NS	6.226	NS	4.950

Table 2: Effect of harvesting time and height of harvest on fresh biomass yield (t ha^{-1}) of tulsi during 2019-20 and 2020-21

50 % Shade									
Treatments	**Height from ground**	**Harvesting time (DAT)**	**2019-20**			**2020-21**			**Pooled data of total yield**
			1st cut	**2nd cut**	**Total**	**1st cut**	**2nd cut**	**Total**	
T1	20 cm	75 & 135 DAT	8.99 a	3.19 a	12.17 a	7.64 a	2.35 ab	9.99 a	11.08 a
T2	30 cm		8.59 a	3.75 a	12.34 a	7.40 ab	3.32 a	10.72 a	11.53 a
T3	20 cm	90 &15O DAT	6.85 b	1.99 b	8.84 b	6.24 bc	1.85 b	8.09 b	8.46 b
T4	30 cm		6.73 b	2.30 b	9.02 b	5.80 c	2.17 b	7.97 b	8.50 b
SEm (±)			0.584	0.405	0.961	0.445	0.317	0.688	0.821
LSD (0.05)			1.678	0.656	1.858	1.187	0.547	1.372	1.208
Open									
Treat ments	Height from ground	Harvesting time (DAT)	2019-20			2020-21			Pooled data of total yield
			1st cut	2nd cut	Total	1st cut	2nd cut	Total	
T1	20 cm	75 & 135 DAT	7.564 a	2.83 a	10.39 a	6.40 a	1.82 b	8.22 a	9.31 a
T2	30 cm		6.988 ab	2.98 a	9.96 a	5.59 ab	2.35 a	7.94 a	8.95 a
T3	20 cm	90 &15O DAT	5.952 b	1.96 b	7.91 b	5.01 b	1.64 b	6.65 b	7.28 b
T4	30 cm		5.652 b	1.89 b	7.54 b	4.77 b	1.75 b	6.51 b	7.02 b
SEm (±)			0.446	0.284	0.717	0.363	0.158	0.438	0.577
LSD (0.05)			1.462	0.531	1.648	1.066	0.477	1.152	1.079

Table 3: Effect of harvesting time and height of harvest on oil yield (kg ha^{-1}) of tulsi during 2019-20 and 2020-21

50 % shade						
Treatments	**Height from ground**	**Harvesting (DAT)**	**2019 -2020**		**2020-2021**	
			1st Cut	**2nd Cut**	**1st Cut**	**2nd Cut**
T1	20 cm	75 & 135 DAT	73.11 a	24.26 a	58.70 a	16.75 a
T2	30 cm		68.54 a	25.77 a	54.52 a	24.00 a
T3	20 cm	90 &15O DAT	52.47 b	16.29 b	44.33 b	12.62 c
T4	30 cm		51.23 b	16.81 b	41.94 b	14.91 bc
SEm (±)			5.562	2.465	4.012	2.46
LSD (0.05)			7.989	3.614	9.364	3.424
Open						
Treatments	Height from ground	Harvesting (DAT)	2019 -2020		2020-2021	
			1st Cut	2nd Cut	1st Cut	2nd Cut
T1	20 cm	75 & 135 DAT	53.60 a	19.576 a	44.02 a	11.66 a
T2	30 cm		50.00 a	20.48 a	38.05 b	14.62 b
T3	20 cm	90 &15O DAT	41.01 b	13.14 b	32.21 c	10.12 b
T4	30 cm		39.11 b	12.43 b	31.49 c	10.56 b
SEm (±)			3.489	2.104	2.922	1.013
LSD (0.05)			5.604	3.345	4.955	1.993

Table 4: Effect of harvesting time and height of harvest on total chlorophyll (mg g^{-1}) of tulsi during 2019-20 and 2020-21

50 % shade						
Treatments	**Height from ground**	**Harvesting (DAT)**	**2019 -2020**		**2020-2021**	
			1st Cut	**2nd Cut**	**1st Cut**	**2nd Cut**
T1	20 cm	75 & 135 DAT	1.632 a	1.312	1.482 a	1.156
T2	30 cm		1.739 a	1.334	1.373 b	1.153
T3	20 cm	90 &15O DAT	1.119 b	1.292	1.089 c	1.152
T4	30 cm		1.203 b	1.284	1.003 c	1.150
SEm (±)			0.154	0.011	0.114	0.001
LSD (0.05)			0.222	NS	0.183	NS
Open						
Treatments	Height from ground	Harvesting (DAT)	2019 -2020		2020-2021	
			1st Cut	2nd Cut	1st Cut	2nd Cut
T1	20 cm	75 & 135 DAT	1.176 a	1.157	1.169 ab	1.098
T2	30 cm		1.157 a	1.080	1.208 a	1.060
T3	20 cm	90 &15O DAT	0.910 b	1.054	1.024 bc	1.099
T4	30 cm		0.946 b	0.997	0.909 c	1.057
SEm (±)			0.070	0.033	0.069	0.012
LSD (0.05)			0.211	NS	0.146	NS

Table 5: Effect of harvesting time and height of harvest on total carotenoids (mg g^{-1}) of tulsi during 2019-20 and 2020-21

50 % shade						
Treatments	**Height from ground**	**Harvesting (DAT)**	**2019 -2020**		**2020-2021**	
			1st Cut	**2nd Cut**	**1st Cut**	**2nd Cut**
T1	20 cm	75 & 135 DAT	0.662	0.958	0.764	1.005
T2	30 cm		0.699	1.022	0.711	0.973
T3	20 cm	90 &15O DAT	0.696	1.086	0.740	1.017

T4	30 cm		0.684	0.998	0.804	0.946
SEm (±)			0.008	0.027	0.012	0.016
LSD (0.05)			NS	NS	NS	NS
Open						
Treatments	Height from ground	Harvesting (DAT)	2019 -2020		2020-2021	
			1st Cut	2nd Cut	1st Cut	2nd Cut
T1	20 cm	75 & 135 DAT	0.883	1.100	0.850	1.017
T2	30 cm		0.873	1.150	0.891	1.091
T3	20 cm	90 &15O DAT	0.897	1.161	0.858	1.085
T4	30 cm		0.866	1.109	0.882	1.112
SEm (±)			0.007	0.015	0.001	0.02
LSD (0.05)			NS	NS	NS	NS

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