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Impact of shade net intensities on herb, essential oil yield and quality in holy basil, *Ocimum tenuiflorum* L. elite germplasm INGR18044

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Ocimum species reveal a huge variation in growth, biomass production, oil composition and yield based on different growing conditions including light intensities. Therefore, here, we analyzed the light requirement of tulsi in the non-traditional area to harvest the maximum quantity of leaf and essential oil yield. An elite germplasm 'INGR18044 (DOS-1)' was evaluated for its quantitative and qualitative traits under green coloured shade-net of different light intensities. The fresh leaf yield, stem weight, root weight, root length, root diameter, dry leaf yield and essential oil yield from fresh leaves were found highest under control conditions. Sole crop compared with intercrop in agroforestry module, intercrop in fruit crop module and crop under shade-net (50%) conditions were compared and significantly higher yield was observed in control. However, the sole crop resulted in small sized leaves with early maturity. The total chlorophyll and carotenoid content were maximum in 90% shade-net intensity and minimum in control conditions; while, Methyl eugenol was maximum in control and it was found minimum under 90% shade-net intensity. The fresh leaf yield, seed yield, essential oil yield, number of PGs per unit leaf area and methyl eugenol (ME) content showed a negative relationship and leaf area, total chlorophyll and carotenoid content showed a positive relationship with increased shade-net intensities. The poor leaf yield and oil yield were observed under different SNIs(Shade Net Intensities) but large-sized leaves stayed green and a continuous supply of fresh leaves was made possible under shade conditions and as an intercrop crop.

Keywords: Eugenol, Methyl eugenol, Peltate glands, Tulsi

Holy Basil (*Ocimumtenuiflorum* L.), an aromatic perennial plant commonly known as tulsi, is a member of Fam. *Lamiaceae* (Labiatae) which holds a wide variety of plants with biological and medical applications[1]. Though indigenous to India and Southeast Asia, it is largely distributed in Asia, Australia, West Africa and in some Arabian countries mainly in dry sandy areas[2]. Its wide-spread distribution covers the Himalayas in the north up to 1800 m height to the Andaman and Nicobar Islands in the south.

Out of 50 to 150 *Ocimum* species of herbs and shrubs, 'tulsi' is commonly described as *Ocimum sanctum* L., which includes two botanically and phytochemically distinct cultivars *i.e.,* Rama or Sri tulsi (green leaves) and Krishna or Shyama tulsi (purplish leaves) having almost the similar chemical constituents[3]. The main chemical constituents of Tulsi are: Oleanolic acid, Ursolic acid, Rosmarinicacid, Eugenol, Carvacrol, Linalool, and β-caryophyllene, have been used extensively for many years in food products, perfumery, and dental and oral products and plant extract continues the numerous searches for more effective drugs of plant origin which are less toxic and available for low socio-economic population in the treatment of diseases caused by pathogenic bacteria. Holy basil is a cheaper source of eugenol and methyl eugenolas compared to clove oil[2]. It is also used as a trap crop for male fruit fly management in mango orchards.

Medicinal plants and natural products are still considered promising alternatives to prevent or treat several diseases[4]. In Indian medicine, it is known to promote longevity and having healing power, and also used for applications in bronchial problems, liver disorders, hiccoughs, stomach disorders, genital and urinary disorders, dermatological diseases, ulcers and mouth infections, poisoning, mental stress disorders and sharpening the memory[5,6]. The daily addition of tulsi to the diet or as adjunct to drug therapy can potentially assist in prevention or reduction of different health conditions and warrants further clinical evaluation[7]. The green leaves and the tender parts of the shoots yield essential oils are the main economically important part of the plant. The

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essential oils of basils contain a heterogeneous group of aromatic compounds having immense value for flavour or fragrance in medicine. Aromatic compounds found in the essential oils are mainly the mono-terpenes, sesquiterpenes and phenols with their alcohols, esters, aldehydes, ketones and others. Recently, plant secondary metabolites like flavonoids, terpenes, alkaloids and α-tocopherol have gained major attention due to their antioxidant[8]. As a secondary metabolic product, the essential oil occurs in secondary tissues, glands, trichomes of the leaves and tender stemsare usually responsible for characteristic pleasant odours and flavours, which thus account in large parts for their value and determine their uses. These properties may be because leaves contain nearly 0.7% volatile oil that comprises about 71% eugenol and 20% methyl eugenol. It also contains carvacrol and sesquiterpine hydrocarbon caryophyllene, some amount of sesquiterpenes and monoterpenes *viz.,*bornyl acetate, α-elemene, neral, α- and β-pinenes, and camphene, the campesterol, cholesterol, stigmasterol and β -sitosterol are also found in the oil[9,10].

Tulsi growing in social forestry and backyard means require partial shade with high relative humidity and mild temperatures. Tolerance of plant under shade condition sustains growth due to increase in leaf area, photosynthetic capacity per unit of light and sugar content[10]. Shade leaves made efficient use of the less intense irradiation reaching up to them like Safed musli, rauvolfia, patchouli, turmeric, shatavari and brahmi[11]. Therefore, South Asian countries like India and Sri Lanka have been a tradition of practicing a mixed farming system depending upon the spacing and nature of trees[12]. It is a popular culinary herb used in many cuisines and medicines. It is used in both forms, fresh and dried; however, the predominant flavors diminish with drying, therefore, regular availability of fresh leaves is a major problem due to annual behavior. This crop can be harvested 65 days after transplanting (flowering stage) and start drying after 90 days. The leaf production taped up after initiation of reproductive phase due to diversion of food material from source to sink (spike or seed production). Therefore, a regular supply of leaves is possible by checking the reproductive stage. Shaded plants of tulsi had significantly greater plant biomass yield with higher leaf area index and leaf number than sun-exposed plants[13]. In this study, we have analyzed the light requirement in the non-traditional area to harvest the maximum quantity of farmers as well as industrial yield of Tulsi.

Materials and Methods

Experimental site

The experiment was carried out at the ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat (India) during the harvesting season of the year 2017-18. The experimental farm is located at 22°35' N and 72°55' E at an altitude of about 45.1 m above MSL.

Plant materials

For the first time, a selection INGR18044 (DOS-1) with having maximum dry leaf recovery (23.10 %), PGs in leaf (7/0.5 mm^2 area) and essential oil in green herbage (0.65%) was identified at the Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat and registered at ICAR-NBPGR, New Delhi. A nursery was established for germplasm INGR18044 (DOS-1) in mid-June, 2017 and transplanted after 45 DAS at a spacing of 45×45 cm. The germplasm was sown under different agro green shade-net intensities *i.e*., 0 (control), 50, 75 and 90%; where 0% intensity refers to control (open field) conditions. Agro green shade net intensity represents shade percent and measurement of the fabric weight, which is 84, 95 and 130 GSM (gram per meter square) in 50, 75 and 90 % SNI, respectively. The observations were recorded for plant height, plant spread, plant weight, number of branches per plant, fresh leaf yield, stem weight, root weight, root length, root diameter, dry leaf yield, seed yield, essential oil percent and essential oil yield from fresh leaves. Five replications with thirty-five plants in each replication were used for observation on yield and contributing parameters. These observations were averaged out to get the mean value of each replication. Plant height was measured as the length between the shoot tip of the main axis and the collar region. Sampled plants were separated into leaves, stem and root and the fresh weight of the leaves and stem were recorded. The dry leaf yield was measured after proper drying until constant moisture content.

Leaves were collected during October 2017 from the experimental field to measure unit leaf size, 10 leaves were used for measuring leaf size using a leaf area meter (LAM 3000). The number of Peltate Glands (PG) in 0.5 mm^2 area from the middle part of the abaxial surface of the leaf was counted under a light microscope at 10X visualization.

Analysis of pigments content

Mature fresh leaves from the main axis were collected during morning hours. Small discs of 100 mg weight was suspended in the test tubes filled with 10 mL of 80% acetone. The test tubes were made air-tight and kept in dark conditions for 24 h. After 24 h, the aliquot was taken in a cuvette for spectrophotometric data analysis at wavelengths of 470, 645 and 663 nm for carotenoids and chlorophyll content, respectively. The chlorophyll pigments (chlorophyll a and b) and total carotenoid content were estimated by the method described by literature[9].

Extraction of essential oils

The fresh biomass of the tagged plants was harvested at maturity (105 DAS) for the extraction of essential oil. Freshly harvested leaves (three portions of 1000 g each) were hydro-distilled for 3 h in a Clevenger-type apparatus. The distillate was extracted with diethyl ether and the ether layer was dried over anhydrous sodium sulphate. Ether was distilled off on a gently heated water bath and oils were stored in amber colour vials at 4-8°C for further analysis[4].

Analysis of the essential oil composition

The chemical constituents of oil obtained from various samples were examined by the combination of Gas Chromatography-Mass Spectrometry (GC-MS). Analysis of the volatile oils was performed on a GC/MS benchtop ion trap mass spectrometer equipped with a ZB-5 capillary column (30 m×0.25 mm id, film thickness 0.25 m). In another set, an analysis of the essential oils was performed on a Thermo Focus GC coupled with Thermo Polaris Q single quadruple mass spectrophotometer detector in Electron Ionization mode and Thermo tri plus autosampler on a DB-5MS capillary column (30 m, 0.25 mm id, 0.25 µm film thickness) with the following operating conditions of initial oven temperature 60°C, held for 5 min, then a 5°C/min to 250°C temperature and held for 3 min; carrier gas constant flow @1.0 mL min^{-1}, injection volume 0.5 µL (split-flow-1:20), the temperatures for inlet, ion source and MS transfer line was 240, 220 and 240°C, respectively. The GC column was coupled directly to the spectrometer in EI mode at 70 eV with the mass range of 40-500 a.m.u at 1 scan/s. Individual compounds should be identified by mass spectra and their identities were confirmed by comparing their mass spectra with Mass Spectral Library and literature[15,16].

Statistical analysis

The analysis of variance was done in randomized block design for various observations made during the experiments by using statistical software SAS 9.2. DMRT comparisons were made among the different shade-net intensities. The results were presented at a 5% level of significance (P = 0.05). The critical difference (CD) values were calculated to compare the various growth conditions.

Results and Discussion

Morphological and carpological variation

Holy basil germplasm INGR18044 was observed under different shade-net intensities for yield and yield contributing parameters for sustainable production (Table 1). The result revealed that plant height was found maximum in holy basil grown under 50% shade-net intensity (116.60 cm), whereas a minimum in control conditions (75.40 cm). The maximum stem length was also reported under shade conditions compared to control in tomato[10]. Similarly, the plant spread was found to be maximum under 50% shade-net intensity (3340 cm^2), while the minimum plant spread was observed at 90% shade-net intensity (1724 cm^2). The fresh plant weight was maximum (487 g) under control conditions, whereas the minimum plant weight was found in 90% shade-net intensity (218 g). The highest number of branches was observed in 90% shade-net intensity (12), whereas the other three conditions gave the same number of branches (10). Degani *et al.*[17] also reported that the number of branches increased in eucalyptus with increased shade

Table 1 — Effect of shade net intensity on yield and yield contributing parameters in holy basil (DOS-1)

Shade net Intensity	Plant height	Plant spread	Plant weight	No. of branches /plant	Fresh leaf yield	Stem weight	Root weight	Root length	Root diameter	Dry leaf yield	Seed yield	Essential oil	Essential oil yield from fresh leaves
(%)	(cm)	(cm^2)	(g)		(kg ha^{-1})	(kg ha^{-1})	(kg ha^{-1})	(cm)	(mm)	(kg ha^{-1})	(kg ha^{-1})	(%)	(kg ha^{-1})
Control	75.40^{c}	2732.40^{b}	487.00^{a}	10.00^{b}	7973^{a}	10701^{a}	1610^{a}	10.30^{a}	15.48^{a}	1834^{a}	278^{a}	0.69^{a}	55^{a}
50	116.60^{a}	3339.84^{a}	363.20^{b}	10.00^{b}	6248^{b}	8659^{b}	1008^{b}	9.60^{b}	12.83^{b}	1012^{b}	135^{b}	0.65^{ab}	41^{b}
75	113.20^{ab}	2290.80^{c}	356.20^{c}	10.00^{b}	6002^{c}	7163^{c}	748^{c}	8.60^{c}	10.95^{c}	942^{c}	31^{c}	0.41^{b}	25^{c}
90	106.03^{b}	1724.27^{d}	218.80^{d}	12.00^{a}	4303^{d}	5016^{d}	466^{d}	7.90^{d}	10.94^{c}	502^{d}	0.60^{d}	0.41^{b}	18^{d}

[Means with the same letter (superscript) in the columns do not showing significantly different (P = 0.05) – (Duncan Multiple Range Test]

intensity. Cut greens of Dracaena grown under shade-nets gave a better performance in terms of plant height, number of leaves, biomass yield and harvest index compared to control[18].

The parameters *viz.,* fresh leaf yield (kg ha^{-1}), stem weight (kg ha^{-1}), root weight (kg ha^{-1}), root length (cm), root diameter (mm), seed yield (kg ha^{-1}) and dry leaf yield (kg ha^{-1}) were found highest (7973, 10701, 1610, 10.30, 15.48, 278 and 1834, respectively) in control condition, whereas the lowest was observed (4303.20, 5016, 466.40, 7.90, 10.94,0.60 and 502, respectively) in 90% shade-net intensity. The oil percent and essential oil yield from fresh leaves were found highest (0.68% and 55 kg ha^{-1}) in a control condition, whereas the lowest was found (0.41% and 18 kg ha^{-1}) in 90% shade-net intensity. Similarly, the maximum number of peltate glands (PGs) were observed under control condition while minimum in the case of 90% shade-net intensity (Fig. 1). The maximum essential oil yield values were reported under control conditions as compared to shade conditions in *Myrtuscommunis* L.[20]. Spike length, number of seeds per spike, number of nodes per spike and weight of seeds per plant were observed maximum in control followed by 50% shade-net intensity as compared to 75 and 90% shade-net intensities (Fig. 2). The better performance of flower parameters in sunlight was also reported in *Salvia sclarea* L. as compared to shade conditions[14]. Growing of tulsi plants at 50% shade is superior to open condition with respect to fresh biomass yield and oil yield[20]. There was a non-significant difference between control conditions and 50% shade-net intensity in terms of spike parameters (Fig. 3). This led us to investigate the effect of 50% shade-net intensity, sole crop (control) and two farmer-friendly crop regimens (intercrops with forestry plants and fruit orchards). The performance of different regimens was compared using leaf yield per plant, seed yield per plant and essential oil content (%) in leaves. All three crop regimens gave significantly higher yield as compared to 50% shade-net crop regimens (Fig. 4).

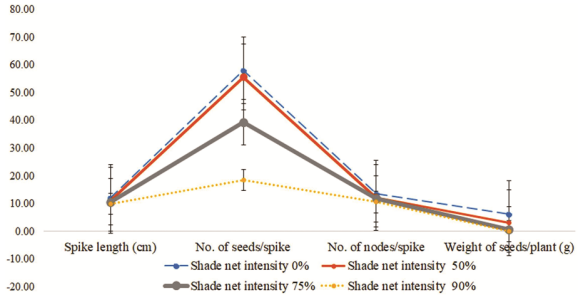

Fig. 2 — Performance of spike and relevant parameters under different shade-net intensities

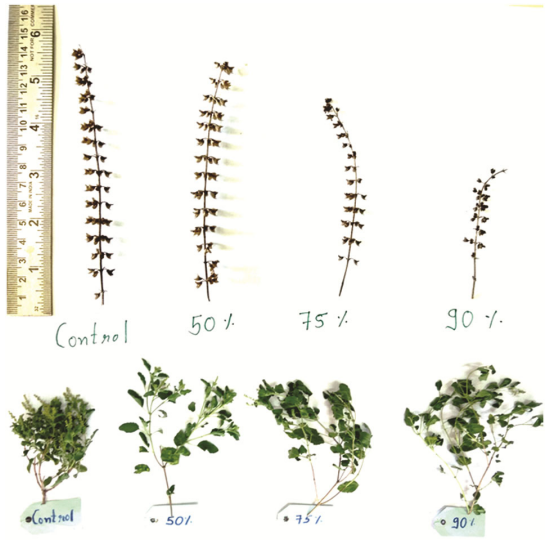

Fig. 3 — Morphology of INGR18044 branch, leaf and spike under different shade-net intensities

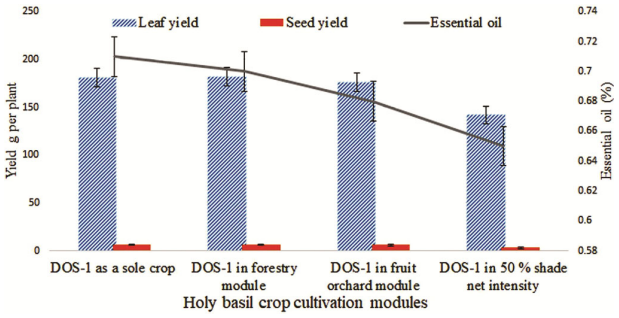

Fig. 4 — Performance of INGR18044 (DOS-1) under different farmer friendly crop modules

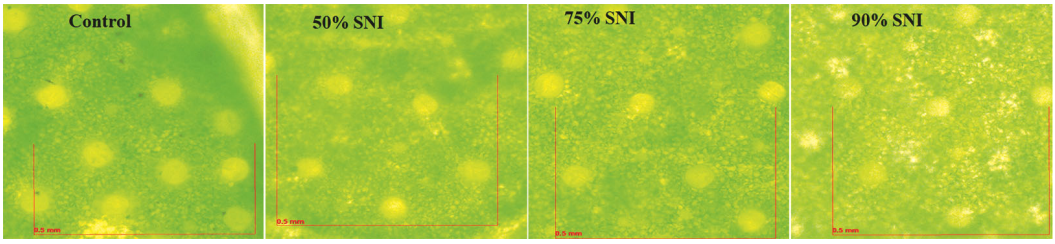

Fig. 1 — Variation for density (0.5 mm at 10X zoom) of peltate glands (PG) in INGR18044 under different shade-net intensities

Table 2 — Effect of shade net intensity on leaf area, chlorophyll and carotenoids content in holy basil (DOS-1)

Shade net Intensity (%)	Leaf area (cm^2)	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Chlorophyll a:b ratio	Total Chlorophyll (mg g^{-1})	Carotenoids (mg mL^{-1})
Control	1.35^{d}	1.01^{d}	0.23^{c}	4.39^{a}	1.24^{d}	3.01^{d}
50	2.16^{c}	1.18^{c}	0.27^{c}	4.35^{a}	1.46^{c}	4.68^{c}
75	2.72^{b}	1.70^{b}	0.41^{b}	4.18^{ab}	2.11^{b}	6.87^{b}
90	3.81^{a}	1.96^{a}	0.50^{a}	3.96^{b}	2.45^{a}	7.86^{a}

[Means with the same letter (superscript) in the columns do not showing significantly different (P = 0.05) – (Duncan Multiple Range Test)]

Table 3 — Effect of shade net intensity on chemotypic composition of essential oils (g kg^{-1}) in holy basil (DOS-1)

Shade net Intensity (%)	Methyl eugenol	Eugenol	Caryophyllene oxide	cis-alpha-bisabolene	Borneol	Bicyclo[3.1.1] heptane	Azulene
Apex RT	21.09	19.30	24.62	21.53	13.76	20.93	19.82
Control	920^{a}	28^{d}	11^{b}	00^{d}	00^{c}	00^{c}	00^{c}
50	840^{b}	67^{b}	7^{d}	11^{c}	00^{c}	00^{c}	00^{c}
75	780^{c}	77^{a}	8^{c}	24^{b}	7^{b}	13^{b}	9^{a}
90	710^{d}	50^{c}	14^{a}	28^{a}	10^{a}	17^{a}	6^{b}

[Means with the same letter (superscript) in the columns do not showing significantly different (P = 0.05) – (Duncan Multiple Range Test]

Early leaf maturity and smaller leaf size were observed in the sole crop.

Chlorophyll and carotenoids content

The observations were recorded for leaf area, chlorophyll and carotenoid content under different shade-net intensities exhibited that the total chlorophyll and carotenoids content found maximum in 90% shade-net intensity (3.81 cm^2, 2.45 mg $g^{-1}$and 7.86 mg mL^{-1}, respectively) followed by 75% shade-net intensity (2.72 cm^2, 2.11 mg g^{-1} and 6.87 mg mL^{-1}, respectively), whereas the minimum estimates were found in control conditions (1.35 cm^2, 1.24 mg g^{-1} and 3.01 mg mL^{-1}, respectively) (Table 2). Similarly, shaded leaves recorded higher content of total chlorophyll (chlorophyll a and chlorophyll b) as compared to control conditions. The leaf area, the content of carotenoids and chlorophyll were significantly higher under increasing shade-net intensities in cucumber[20]. An increase in biomass (vegetative and reproductive) exhibits coincides with increases in chlorophyll content. Shade-grown leaves get less opportunity to harvest light, and thus to compensate for this, contain more chlorophyll than leaves exposed to direct Sun light[21]. The photosynthetic rate of shade growing leaves associated with higher chlorophyll contents is likely due to a higher number of photosystems in the membranes of the thylakoid of these leaves[20], indicating that shade leaves made efficient use of the less intense irradiation reaching up to them. Besides the light effect on the photosynthetic process itself, synthesis and degradation of chlorophylls were also directly associated to light intensity[6].

Compositions of essential oil (%)

The essential oil of INGR18044 was subjected to detailed chemical characterization to determine its impact on the composition of volatile constituents under different shade-net intensities (Table 3 and Fig. 5). The highest methyl eugenol (920 g kg^{-1}) was found in the control condition followed by 50% shade-net intensity (840 g kg^{-1}), whereas the minimum was found in 90% shade-net intensity (710 g kg^{-1}). Eugenol (77 g kg^{-1}) was highest in 75% shade-net intensity followed by 50% shade-net intensity (67 g kg^{-1}), whereas it was minimum in control (28 g kg^{-1}). The caryophyllene oxide was maximum in 90% intensity (14 g kg^{-1}) followed by control (11 g kg^{-1}) and it was minimum (7 g kg^{-1}) in 50% shade-net. The cis-alpha-bisabolene, borneol and bicyclo (3.1.1) heptane were found highest (28, 10 and 17 g kg^{-1}, respectively) under 90% shade-net intensity. Maximum azulene (9 g kg^{-1}) was found in 75% and minimum (6 g kg^{-1}) was observed in 90% shade-net intensity. Light reflection from different colored mulches influences aroma or chemical content in sweet basil. The variation for quality parameters or essential oil compositions in *Ocimum* species was also reported[22,23]. Significant differences were observed in volatiles and biochemical classes as compared to protected cultivation[24]. At the same time, the specific light treatment also increases volatile content and mass in basils.

Correlation among shade-net intensities and yield parameters

At the crop harvesting stage, there was a significant positive relationship observed between shade-net intensities with leaf area, total chlorophyll and carotenoid content. Leaf area, total chlorophyll and

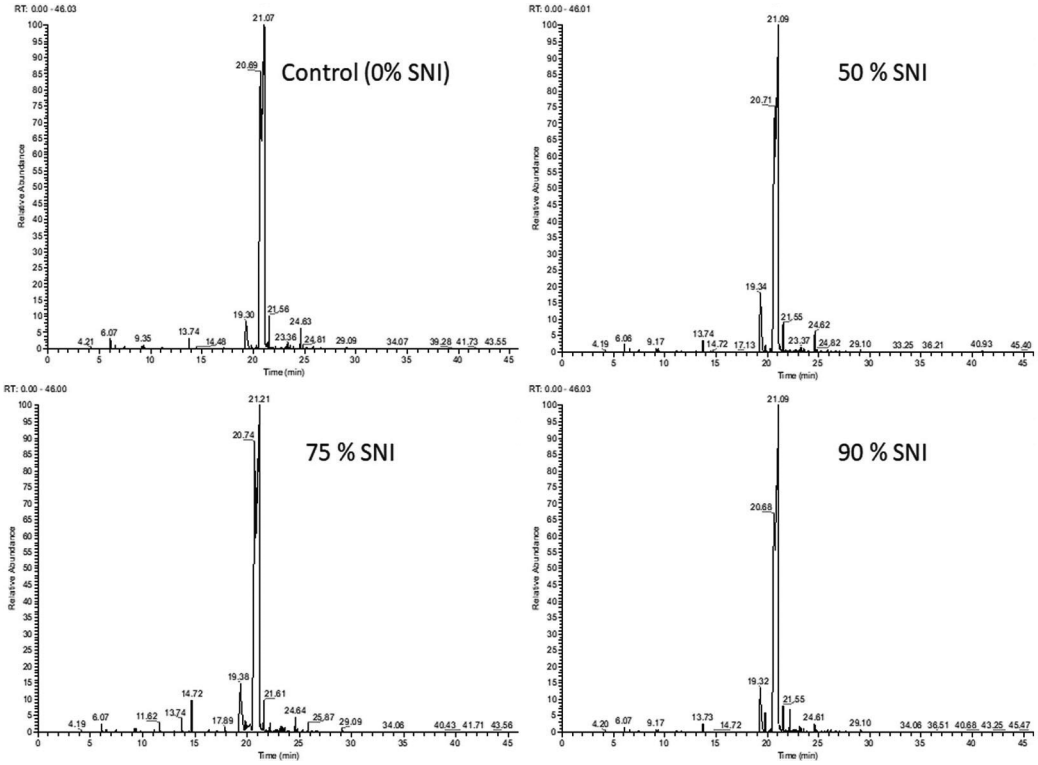

Fig. 5 — Chromatogram of volatile compounds identified under different shade-net intensities

Table 4 — Correlation coefficient for the association among shade net intensities, yield and contributing parameters of tulsi

Parameters	Shade net intensity	Fresh leaf yield	Essential oil yield	Seed yield	Leaf area	Number of PGs	Total Chlorophyll	Carotenoids
Fresh leaf yield	–0.948**							
Essential oil yield	–0.982**	0.936**						
Seed yield	–0.996**	0.926**	0.989**					
Leaf area	0.943**	–0.984**	–0.963**	–0.934**				
Number of PGs	–0.988**	0.918**	0.996**	0.997**	–0.939**			
Total Chlorophyll	0.925**	–0.909**	–0.980**	–0.939**	0.964**	–0.960**		
Carotenoids	0.974**	–0.934**	–0.999**	–0.983**	0.966**	–0.993**	0.986**	
Methyl eugenol	–0.974**	0.977**	0.988**	0.971**	–0.992**	0.974**	–0.973**	–0.988**

** significant at $P < 0.01$

carotenoid contents were increased with progressive shade-net intensities due to light stresses[10]. At the same time, significant negative relationships were observed between shade-net intensities with fresh leaf yield, essential oil yield, seed yield, number of PGs and methyl eugenol content (Table 4). As shade-net intensity increases, the essential oil yield, number of PGs per unit leaf area and methyl eugenol (ME) content decrease due to a positive relationship among essential oil yield, ME and number of PGs. Similarly, because of the position of young leaves (remain at top of the plant) and old leaves (remain within canopy/partial shade), the highest number of peltate glands (PG) were reported in young leaves as compared to old leaves[3,4].

Conclusion

Overall, yield parameters were observed higher in control, whereas estimates of total chlorophyll and carotenoid content were found higher under shade-net conditions. Under shade-net conditions, the leaf stayed green and larger in size and thus made possible a continuous supply of fresh leaf through cultivation under partial shade. Carpological and related parameters like spike length, number of seeds per spike, number of nodes per spike, and weight of seeds per plant were observed maximum in control condition followed by 50% shade-net intensity. Holy basil as a sole crop observed a significantly higher yield as compared to rest.

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Conflict of interest

Authors declare no competing interests.

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