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INFLUENCE OF COLOURED SHADE NETS ON GROWTH AND BIOCHEMICAL CONSTITUENTS IN FOUR SPECIES OF *OCIMUM*

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Abstract

Effects of different coloured shade nets (red, green, black, green, white net) and control (without net) on the biochemical and growth responses in four *Ocimum* spp. namely *O. tenuiflorum* L., *O. sanctum* L., *O. basilicum* L. and *O. gratissimum* L. were investigated. Results showed that plants kept under red net consistently outperformed other treatments recording significantly higher growth and biochemical characteristics for all selected *Ocimum* spp. after 120 days of transplantation. Parameters like shoot length (128.77 ± 4.00 cm), root length (81.48 ± 0.75 cm), leaf number (319.10 ± 3.38), total fresh biomass (158.17 ± 12.82 g/plant) and total dry biomass (63.88 ± 1.37 g/plant) witnessed a 3.38, 2.95, 2.76, 4.17 and 5.16% increase, respectively under red net over control. Plants under red net also recorded a significant increase of 9.68, 14.10 and 28.18% for total phenols (127.50 ± 2.95 mg/g DW), flavonoids (18.33 ± 2.71 mg/g DW) and alkaloids (32.31 ± 2.87 mg/g DW), respectively.

Introduction

Ocimum belonging to Lamiaceae has over 160 diverse pot-herbs species cultivated throughout the world (Kalita and Khan 2013). It is an upcoming potential crop with great relevance culturally and medicinally in the nutrition and pharmaceutical sector (Saran *et al.* 2017). Quality of light is known to alter plants' morpho-physiological characteristics altering the growth and biomass allocation (Brant *et al.* 2011), anatomical traits (Singla *et al.* 2021) pigments, biochemical traits and essential oil yield and quality (Saran *et al.* 2021). Long days and high temperatures have been found to be favourable for *Ocimum* plant growth, higher metabolite and oil production (Gingade *et al.* 2014).

Netting is a new contemporary agricultural practice being extensively implemented and evaluated for various crops, agricultural techniques and climatic conditions (Shahak *et al.* 2009). Coloured shade nets filter and modify a part of incident light; rest of the light remains unchanged, leading to a number of variations in the microclimatic traits and crop activity (Shahak *et al.* 2009). They are cheaper and less energy consuming alternative for improving plants' morpho-physiological traits and enhancing its photosynthetic efficiency. It also protects the crop from various pathogenic hazards and direct solar radiations that could have potentially caused chloroplast and pigment degradation, leaf and petal burn. The quality of light transmitted by these screens and shade nets depends upon their material properties as the coloured nets like green, red and blue nets increase the transmittance and scattering of light wavelength corresponding to its own colour while, absorbing light of all other wavelengths of the visible spectra (Shahak *et al.* 2009). White and black nets are neutral filters which do not alter the spectral quality of light but reduce its intensity and net temperature considerably differing only in the quality of deflecting back or absorbing sun's heat respectively (Costa *et al.* 2018).

Given the great medicinal and cultural importance of the crop, the information gained from the present study could be used to elucidate the biosynthetic pathway, potentially increasing the metabolites levels responsible for its medicinal and therapeutic properties. It was hypothesized

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that the photo selective nets can influence the growth and biochemical properties. Hence, the present experiments were conducted to study the influence of five different coloured shade nets on biochemical and growth responses on four prominent species of *Ocimum* in comparison to the control conditions.

Materials and Methods

The present investigation (2018-19) was conducted at the Research Farm, Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana. The four *Ocimum* spp. (*Ocimum tenuiflorum* L., *O. sanctum* L., *O. basilicum* L. and *O. gratissimum* L.) were grown in Herbal garden of the School of Organic Farming, Punjab Agricultural University. Seedlings at four leaf stage and uniform in age and vigour were selected and transplanted in polybags to the coloured shade nets at the experiment site and observed for the next 120 days after transplantation when the crops had attained a physiological maturity stage. The polybags were all of the same dimensions and filled with the same amounts of farmyard manure and soil in the ratio of 1 : 2. Six treatments - Control (without net) and five coloured shade nets (Green, Blue, Black, Red and White) were selected for the investigation. The experimental data for all the observations were recorded for two consecutive years. Plants from all species and treatments were randomly selected in a replication of three and their analysis was carried out in the plant physiology laboratory at Department of Botany, Punjab Agricultural University. Shoot and root length and stem diameter were measured in cm. The numbers of leaves were counted manually and leaf area was estimated using a portable leaf area meter. The total fresh biomass (TFB) and total dry biomass (TDB) of the 78 hrs oven dried whole plant were measured in g/plant.

Total chlorophyll content (TCC) was measured using a SPAD-502 Chlorophyll Meter and was expressed in SPAD units. Care was taken that the midrib of the leaf did not fall under the sample area (sensor) of the instrument. Total soluble sugar content (TSS) was determined following the protocol of Dubois *et al.* (1956). Total phenolic content (TPC) and total flavonoid content (TFC) were determined following the protocol of Chang *et al.* (2002). Total alkaloid content (TAC) was determined following the protocol of Shamsa *et al.* (2008). Biochemical constituents were expressed in the units of mg/g dry weight (DW). The experimental design of the study was kept as a completely randomized design (CRD). The tabulated data for both the years (2018 and 2019) were obtained from all treatments in the form of triplicates and an average mean value is presented. The standard error of the mean average value was calculated using Microsoft Excel (2016). Data were subjected to Tukey's HSD post hoc test at $p \leq 0.05$ for the ANOVA using SAS 9.3 for windows and assessed as per cent variation with respect to the control.

Results and Discussion

In the current study, the use of coloured shade nets successfully altered the plant phenology and biochemical traits. *O. gratissimum* recorded the tallest plants among all species, with longest shoot and root length. A 3.38% higher shoot length (128.77 cm) was recorded in plants under red net than the control (124.57cm) while, a 14.38% decrease was recorded in the shoot length (106.65cm) in plants under black net (Fig. 1). The present findings are more of less similar to the results reported by Costa *et al.* (2018) in *Ocimum selloi*. Similarly in case of root length, Red nets recorded a 2.95% higher root length (81.48 cm) than the control (79.14cm) (Fig. 2). Roots could possibly develop better due to reduced light intensity and better micro-climate inside the coloured nets. Plants placed under black net recorded pronounced decrease of 15.85% in the root length (66.59cm) in plants (Fig. 2).

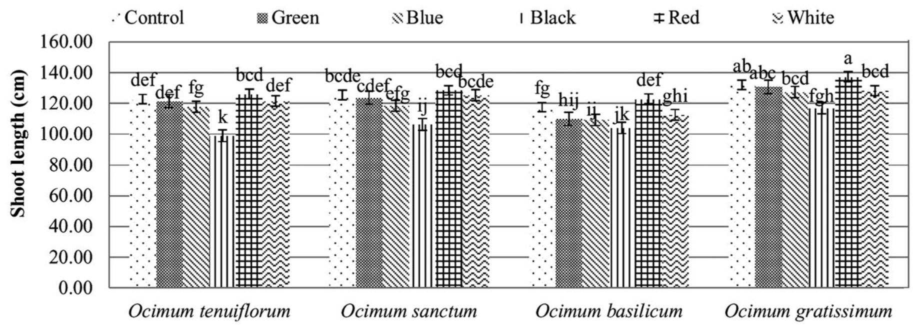

Fig. 1. Effect of coloured shade nets on shoot length in *Ocimum* spp. Least square means with different letters are significantly different.

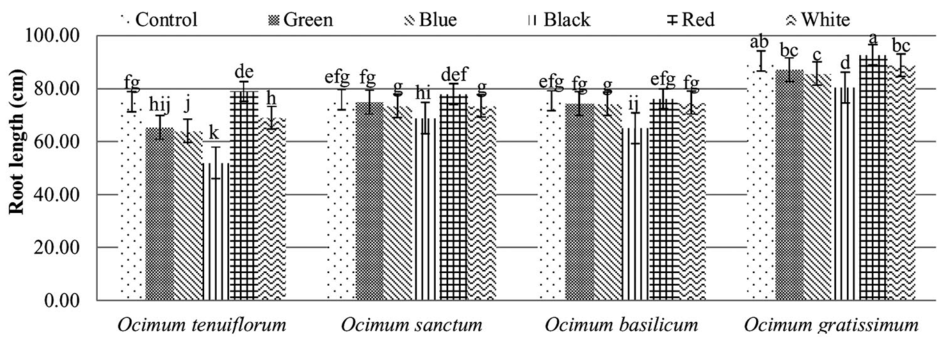

Fig. 2. Effect of coloured shade nets on root length in *Ocimum* spp. Least square means with different letters are significantly different.

Stem diameter was found to be significantly greater under the control than among all the treatments (Fig. 3). All coloured shade nets observed significant decrease in the stem diameter compared to plants under the control. The lowest stem diameter was recorded in plants under black net with a significant decrease of 41.82% in the stem diameter in comparison to the stem diameter (10.52 cm) of plants under the control (Fig. 3). Wu *et al* (2017) reported similar observations of shade negatively influencing the stem diameter due to decreased photosynthetic activity under shade nets and the utilization of assimilates present more for increasing shoot length,

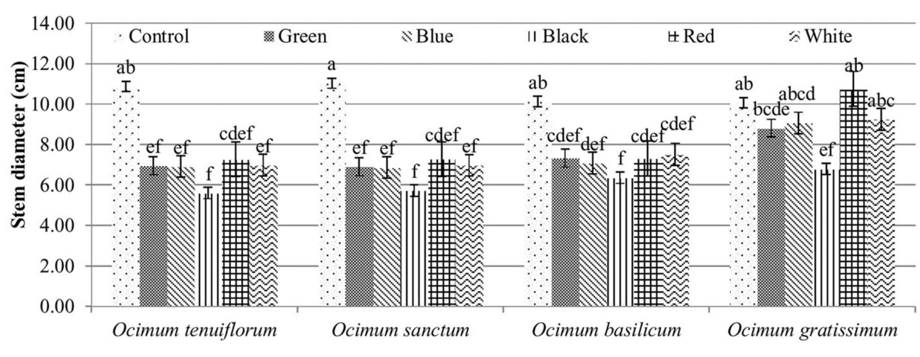

Fig. 3. Effect of coloured shade nets on stem diameter in *Ocimum* spp. Least square means with different letters are significantly different.

petiole length as a part of shade avoidance syndrome. All coloured shade nets recorded a decrease in mean leaf number with the exception of plants under red nets, which showed a 2.76% increase in the mean total number of leaves (319) compared to plants under control (310.53) (Table 1). There was a significant decrease in the leaf number of around 32.66% in plants under black net (209.12) (Table 1). The decrease in leaf number could be a direct result of higher branching in plants under control due to higher photosynthetic activity of plants without net. It also alludes to the high light intensity requirement of the crop.

Leaf area is another important growth parameter interpreting the utilization of intercepted radiation, crop's dry matter production and photosynthetic capacity. Significantly higher leaf area was recorded in plants under all coloured shade nets than under control (Fig. 4). The present results are in agreement with the reports of Dai *et al.* (2009) who reported lowest leaf area in plants grown under full sunlight in *Tetrastigma hemsleyanum*. Under low light intensities the leaf tissue cells expand more to optimize their effectiveness of light absorption by increasing the receptive area of a leaf for photosynthesis with lowest leaf area under full sunlight (Dai *et al.* 2009) as also evidenced in the present study. This can be attributed to the stress response of plant growing under nets (Saifuddin *et al.* 2010).

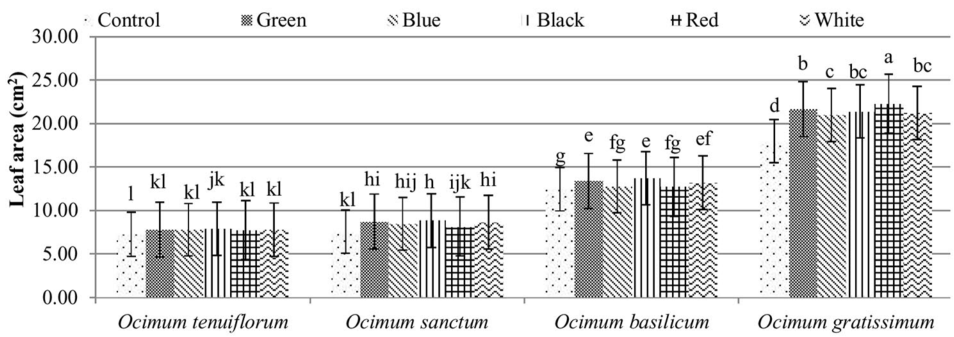

Fig. 4. Effect of coloured shade nets on leaf area in *Ocimum* spp. Least square means with different letters are significantly different.

Plant biomass was directly impacted by the variations in different spectral qualities and intensities of light and radiation. Among species, *O. gratissimum* recorded the highest total fresh biomass (164.83 g/plant) and total dry biomass (60.80 g/plant) (Table 2). Plants under red net recorded significantly highest total fresh biomass (158.17 g/plant) and total dry biomass (63.88 g/plant) among all treatments, with an increase of 4.17% and 5.16% respectively, in comparison to total fresh biomass (151.83 g/plant) and total dry biomass (60.75 g/plant) in plants under control (Table 2). The rapid increase in total fresh biomass under red nets could be attributed to the prolific growth factors such as increase in the shoot length, root length and leaf number in *Ocimum* spp (Fig. 1, Fig. 2, Table 1). Higher biomass accumulation was reported in many crops under red net as the red light wavelengths fall into chlorophyll absorption peak, potentially increasing the photosynthetic activity and accumulation (Shahak *et al.* 2009). Similarly, lowest total fresh biomass (109.86 g/plant) and total dry biomass (42.41 g/plant) were recorded in plants under black net with a decrement of around 27.65 and 30.18%, respectively (Table 2) alluding to the high light intensity requirement of the crop.

Table 1. Effect of coloured shade nets on leaf number and total chlorophyll content in *Ocimum* spp.

Treatments	Leaf number					Total chlorophyll content (SPAD units)				
	O. tenuiflorum	*O. sanctum*	*O. basilicum*	*O. gratissimum*	Mean	*O. tenuiflorum*	*O. sanctum*	*O. basilicum*	*O. gratissimum*	Mean
Control	321.33±6.87^{bcd}	317.33±7.41^{de}	284.11±7.34^{k}	319.33±4.73^{cde}	310.53	45.53±0.91^{a}	40.21±0.98^{cdefghi}	34.79±0.97^{jk}	32.79±0.62^{k}	38.33
Green net	295.67±5.40^{ij}	300.67±7.24^{ghi}	273.67±6.19^{lm}	298.17±4.08^{hij}	292.05	40.93±1.38^{bcdefgh}	45.44±1.85^{a}	39.38±1.59^{efghi}	44.05±1.04^{abc}	42.45
Blue net	267.33±8.41^{m}	292.33±8.13^{j}	256.78±10.02^{n}	279.83±3.74^{kl}	274.07	43.27±1.30^{abcde}	43.56±1.26^{abcd}	37.71±1.55^{hij}	39.92±0.58^{defghi}	41.12
Black net	199.67±3.26^{q}	221.67±3.54^{o}	204.45±3.22^{pq}	210.67±2.27^{p}	209.12	38.90±0.62^{ghi}	44.12±0.67^{abc}	36.91±0.61^{ij}	42.88±0.43^{abcdef}	40.70
Red net	329.33±3.48^{a}	326.33±3.30^{abc}	292.89±3.83^{ij}	327.83±2.91^{ab}	319.10	44.07±2.79^{abc}	43.01±2.64^{abcdef}	38.25±3.06^{ghij}	39.88±2.33^{defghi}	41.30
White net	307.67±12.28^{fg}	312.67±6.18^{ef}	281.89±5.48^{k}	305.17±2.87^{fgh}	301.85	41.80±2.76^{abcdefg}	44.27±1.39^{ab}	39.27±1.23^{fghi}	43.12±0.64^{abcdef}	42.12
Mean	286.83	295.17	265.63	290.17	284.45	42.42	43.44	37.72	40.44	41.00

Least square means with different letters are significantly different.

Table 2. Effect of coloured shade nets on the total fresh and dry biomass in *Ocimum* spp.

Treatments	Total fresh biomass (g/plant)					Total dry biomass (g/plant)				
	O. tenuiflorum	*O. sanctum*	*O. basilicum*	*O. gratissimum*	Mean	*O. tenuiflorum*	*O. sanctum*	*O. basilicum*	*O. gratissimum*	Mean
Control	133.76±14.76^{hijk}	154.55±17.17^{def}	143.61±16.86^{fghi}	175.40±7.00^{ab}	151.83	53.38±2.91^{fg}	59.97±3.14^{cde}	62.44±3.11^{bcd}	67.20±1.99^{ab}	60.75
Green net	122.86±13.67^{klm}	145.95±24.57^{fgh}	128.35±17.92^{jkl}	162.96±7.80^{cd}	140.03	46.77±3.10^{ij}	54.32±4.15^{fg}	55.80±3.55^{ef}	63.46±2.33^{bc}	55.09
Blue net	114.37±9.57^{mno}	125.56±8.95^{klm}	119.71±13.57^{lmn}	159.21±1.89^{cde}	129.71	44.19±3.11^{j}	53.07±3.01^{fg}	52.05±3.70^{fgh}	54.59±1.38^{fg}	50.98
Black net	89.32±7.25^{p}	102.55±8.54^{o}	108.60±7.06^{no}	138.95±3.51^{ghij}	109.86	31.52±1.94^{k}	46.32±2.10^{ij}	47.22±1.91^{hij}	44.59±1.35^{j}	42.41
Red net	138.19±13.47^{ghij}	160.75±12.11^{cde}	149.42±16.30^{efg}	184.31±9.42^{a}	158.17	57.22±1.99^{def}	63.44±1.14^{bc}	64.97±1.32^{abc}	69.89±1.00^{a}	63.88
White net	126.80±56.12^{jkl}	149.65±14.23^{efg}	131.46±11.18^{ijkl}	168.13±2.95^{bc}	144.01	49.98±4.70^{ghi}	56.04±2.36^{ef}	57.16±2.10^{ef}	65.07±1.08^{abc}	57.06
Mean	120.88	139.84	130.19	164.83	138.94	47.18	55.53	56.61	60.80	55.03

Least square means with different letters are significantly different.

Table 3. Effect of coloured shade nets on total flavanoid content and total alkaloid content in *Ocimum* spp.

Treatments	Total flavonoid content (mg/g DW)					Total alkaloid content (mg/g DW)				
	O. tenuiflorum	*O. sanctum*	*O. basilicum*	*O. gratissimum*	Mean	*O. tenuiflorum*	*O. sanctum*	*O. basilicum*	*O. gratissimum*	Mean
Control	15.36±0.91^{bcdefgh}	15.79±0.99^{bcdefg}	19.11±0.97^{ab}	13.98±0.62^{defghi}	16.06	25.87±0.85^{ghijk}	24.88±0.91^{hijkl}	21.54±1.07^{l}	28.54±1.25^{efgh}	25.21
Green net	13.67±1.38^{efghi}	13.30±1.85^{fghi}	15.38±1.57^{bcdefgh}	11.51±1.04^{hi}	13.47	26.24±1.28^{fghijk}	27.10±1.38^{fghij}	22.25±1.64^{kl}	32.25±1.88^{bcde}	26.96
Blue net	14.50±1.30^{cdefghi}	14.59±1.26^{cdefghi}	17.54±1.55^{bcde}	12.16±0.58^{ghi}	14.70	28.63±1.17^{efgh}	28.74±1.26^{defgh}	22.91±1.49^{kl}	35.11±1.73^{b}	28.85
Black net	12.29±0.62^{ghi}	15.14±0.67^{cdefgh}	17.72±0.61^{bcd}	11.19±0.43^{i}	14.09	26.02±0.56^{fghijk}	27.44±0.60^{fghi}	22.82±0.72^{kl}	32.82±0.83^{bcd}	27.28
Red net	16.54±2.79^{bcdef}	18.00±2.64^{bc}	22.76±3.06^{a}	16.00±2.33^{bcdefg}	18.33	32.46±2.37^{bcde}	31.60±2.55^{bcde}	24.09±3.03^{ijkl}	41.09±3.51^{a}	32.31
White net	13.11±2.76^{fghi}	13.31±1.39^{fghi}	15.92±1.23^{bcdefg}	13.24±0.64^{fghi}	13.90	30.04±1.29^{cdef}	29.83±1.39^{cdefg}	23.14±1.65^{jkl}	33.14±1.92^{bc}	29.04
Mean	14.25	15.02	18.07	13.01	15.09	28.21	28.27	22.79	33.83	28.27

Least square means with different letters are significantly different.

Light is a key factor affecting leaf pigment levels and composition. Lowest TCC levels in plants were recorded (Table 1) as the high degree of incident light leads to degradation of the chlorophyll pigment. The highest TCC levels were recorded in plants under green net (42.45 SPAD units) with the increment of 10.75%, followed by plants under white net (42.12 SPAD units) with a 9.87% increase in comparison to the TCC levels recorded in plants under control (38.33 SPAD units). Shading also significantly increased leaf SPAD value in other crops (Mu *et al.* 2010). Restrepo-Diaz and Garces (2013) supported the present results with similar findings reporting higher leaf chlorophyll content in rice leaves under shaded conditions than under full sunlight.

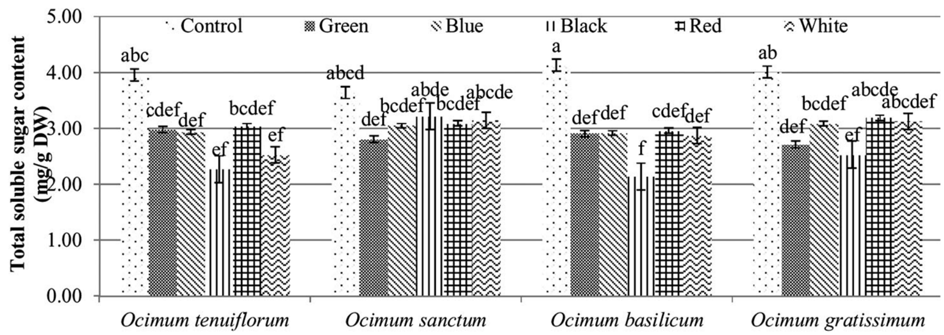

Fig. 5. Effect of coloured shade nets on total soluble sugar content in *Ocimum* spp. Least square means with different letters are significantly different.

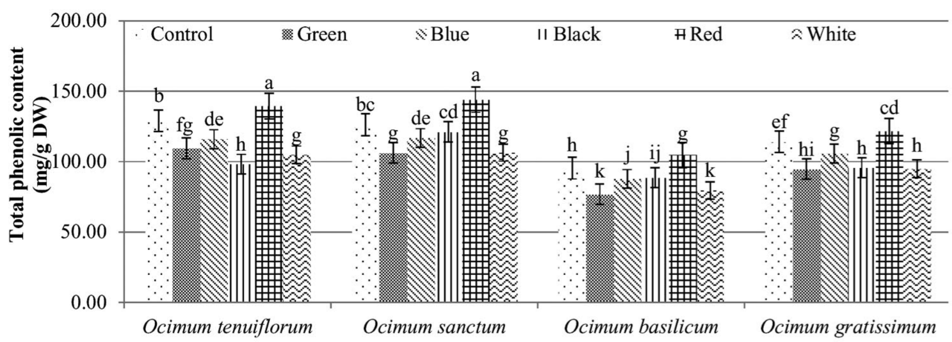

Fig. 6. Effect of coloured shade nets on the total phenolic content in *Ocimum* spp. Least square means with different letters are significantly different.

Figure 5 showed that control recorded significantly higher total soluble sugars (TSS) content was recorded in leaves than under coloured shade nets. All coloured nets in the present study showed a significant decline in TSS content ranging between 21.98 and 35.45% decrease than TSS content in plants under control (3.94 mg/g DW) (Fig. 5). The present results are supported by the similar findings of Koji *et al.* (2005) and Saifuddin *et al.* (2010), reporting that the sunlight is used to harvest photochemical energy and increase the photosynthetic process to increase the sugar content in leaves. Lowering of light intensity or high shade leads to a reduction of CO_2 assimilation and net sugar content in plant as a result of higher consumption of reserved total soluble sugars than the amount assimilated owing to the low photosynthetic capacity of plant leaves (Iwona *et al.* 2005).

The application of red coloured nets recorded a 9.68% increase in the mean total phenolic content (127.50 mg/g DW) and a 14.10% increase in the mean total flavonoids content (18.33 mg/g DW) than in plants under control (116.25 mg/g DW; 16.06 mg/g DW) (Fig. 6; Table 3). A 16.16% decline was recorded in the TFC levels (13.47 mg/g DW) in plants under green net (Table 3). In the present study, the lowest TPC levels were recorded in plants under white net (96.81 mg/g DW) followed by green net (96.50 mg/g DW) with an estimated decrease of 16.99 and 16.72%, respectively (Fig. 6). Manivannan *et al.* (2015) reported similar findings in Chinese foxglove (*Rehmannia glutinosa*) plants of red light wavelength increasing the phenolic and flavonoids compounds in the plants than white light. *O. gratissimum* recorded the significantly highest mean total alkaloid content (TAC) (33.83 mg/g DW) among all species (Table 3). Plants under colour nets recorded a significant percentage increase in TAC levels, ranging between 6.95-28.18% in comparison to the lowest mean TAC levels (25.21 mg/g DW) in plants under control. The highest mean TAC levels were obtained in plants under red net (32.31 mg/g DW) with a 28.19% increase. The reports of Liu *et al.* (2011) corroborated the present findings; alkaloid production is highest in white light and followed closely by red light than control.

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