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Effects of different coloured LED light illumination on anthocyanin content of Nerium (*Nerium oleander* L.)

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Abstract
Nerium oleander L. is an ornamental flowering shrub suited for landscaping. It has gained worldwide reputation as an ornamental plant due to its prolific and perpetual flowering behaviour, along with its heat sustaining property, salinity, and drought adaptation. Flowers from the genus Nerium are generally used in home and temple for offerings to God. This research is carried out in local farmer's field in Salem district in order to understand the physiological and biochemical nature of Nerium plant by the introduction of different coloured LED lighting and fogging systems. The different coloured lights used were red, blue, white and yellow. The results revealed that the highest number of flower buds per inflorescence (10.0) was recorded by exposing the plant to red light for 6 hours + water spray (Fog) 15 min. for every one hour. The maximum weight was (23.1) observed in blue light for 6 hours + water spray (Fog) 15 min. for every one hour. The highest yield (129g/plant) was shown by giving Water spray (Fog) 15 min for every one hour. Red light for 6 hours + water spray (Fog) 15 min. for every one hourobtained the highest quantity of anthocyanin content of 0.86 µg/g in opened flowers and 0.81µg/g in unopened flower buds. The results reveal the fact that light plays a major role in influencing the physiology and biochemical nature of Nerium flowers.

Keywords: Nerium, anthocyanin, LED, Light, flower yield

Introduction
Nerium oleander L. is a flowering shrub with an attractive appearance. Its native species originated from Northern Africa and the Mediterranean region. The plant may reach upto a height of 20 feet with upright stems that extend laterally as plants flourish. The size of leaves vary from 10 to 22 cm in length. It is thin and sharp, with a notable mid rib and has a "leathery" feel. The plant terminates into 3 side branches from the central stem. Each flower is roughly 5 cm in diameter and has five petals. The fruit is made up of a thin follicle which is around 17 cm in length. It opens to disseminate fluffy seeds. The mode of reproduction is by seeds, but since it is allogamous and extremely heterozygous in nature, the seedling population is highly variable (Parashuram *et al.*, 2019) [16].

Light is the most significant environmental indicator that influences and governs a plant's growing pattern and behaviour. Plants have photoreceptors that sense Ultraviolet-A(UV-A), Ultraviolet-B(UV-B), blue light, red light, and far red light in diverse ways. Plants perceive the intensity, quality, direction and duration of light through these photoreceptors (Whitelam and Halliday, 2007) [19]. Chlorophylls are the primary photosynthetic pigments and various secondary plant pigments like carotenoids and anthocyanin are all capable of capturing light. These secondary pigments combine with the primary pigments to transmit light to the photosystems and emit regulated form of light (Morrow, 2008) [13]. The number of flower buds and yield were increased when chrysanthemum plants were exposed to artificial light, but the production time was reduced. (Nissim Levi *et al.*, 2019) [14]. In chrysanthemum, Red light treatment reduces the age of flowering compared to white and yellow light treatments (Kamelia *et al.*, 2018) [6].

Anthocyanins are water soluble pigments. It plays a major role in imparting colour to the flower. Depending on their pH it gives red, blue, purple or sometimes even black colour to the flower. Therefore it is necessary to understand the biosynthesis of anthocyanin in plant growth. Anthocyanin biosynthesis is also mediated by phytochrome in several plants such as tomato, cabbage and mustard (Mancinelli 1985) [11]. The present study was framed with the objective to study the effect of different LED light illumination on Anthocyanin content of Nerium flowers.

Material and Methods
Three-year-old Nerium plants of the commercially available variety 'local' with pink coloured flowers were studied at a Progressive Farmer's field in Salem district, Tamil Nadu, during period of 2020 and 2021. The experiment was designed in RBD, with three replications with the usage of various coloured LED lighting and fogging systems. Nerium flowers are harvested around 6.30 am early morning. The buds were collected in two stages: fully developed open buds and unopened buds.

Treatment Details

Treatments	Particulars
T_1	Blue light (450-495 nm) for 6 hours
T_2	Red light (620-750 nm) for 6 hours
T_3	White light (450-500 nm) for 6 hours
T_4	Yellow light (560-580 nm) for 6 hours
T_5	Blue light for 6 hours + water spray (Fog) 15 min. for every one hour
T_6	Red light for 6 hours + water spray (Fog) 15 min. for every one hour
T_7	White light for 6 hours + water spray (Fog) 15 min. for every one hour
T_8	Yellow light for 6 hours + water spray (Fog) 15 min. for every one hour
T_9	Water spray (Fog) 15 min. for every one hour
T_{10}	Control (Farmers practice)

Lighting time and water spray (fog) will be carried out from 10.00 P.M to 4.00 A.M (6 hours)

Estimation of Anthocyanin
One gram of Nerium flower petals was ground with 10 ml of 80% ethyl alcohol and centrifuged for eight minutes at 2000 rpm. One ml of the supernatant liquid was mixed with 3 ml of hydrochloride in aqueous methanol. One ml of peroxide reagent was added to the supernatant liquid, whereas one ml of methanolic hydrochloride was added to the blank tubes. For 15 minutes, the compositions were maintained in the darkness. On 525nm, the intensity was measured in OD values (i.e., µg/g). (Swain and hills, 1959) [17]

Flower colour
The Royal Horticultural Society's Colour Chart (RHSCC), Sixth edition (2015) was used to determine the colour of the flowers.

Shelf life (hours)
50 flower buds which were about to open were harvested from each treatment and stored in polythene cover of 100 gauge thickness having ventilation of 1% and used to study the shelf life of the flowers.

Data collection and statistical analysis
The observations were taken from five plants in each replication and the mean data was statistically analyzed and the critical differences were calculated with a 5% (0.05) probability based on Panse and Sukhatme method (2000) [15].

Result
Flower yield parameters
The impact of different coloured LED light and fogging system on flower yield characteristics of Nerium revealed statistically significant variations among the individual and also the combination of treatments.

Number of inflorescence per plant
Maximum number of inflorescence per plant (25.4) has been recorded in treatment T_6 (Red light for 6 hours + water spray (Fog) 15 min. for every one hour) which was (24.3) on par with treatmentT_9 (Water spray (Fog) 15 min for every one hour). The treatment T_2 (Red light for 6 hours) has recorded the least number of inflorescence per plant (13.6).

Number of flower buds per inflorescence
The highest number of flower buds per inflorescence (10.0) was observed in treatment T_6 (Red light for 6 hours + water spray (Fog) 15 min. for every one hour) which was significantly higher compared to treatment T_9 (Water spray (Fog) 15 min for every one hour) (9.0). The lowest number of flower buds per inflorescence (6.0) was recorded in treatment T_1 (Blue light for 6 hours) (Fig 1).

Hundred flower buds weight (g/Plant)
The maximum weight of hundred flower buds was (23.1) observed in treatment T_5 (Blue light for 6 hours + water spray (Fog) 15 min. for every one hour). The lowest weight of hundred flower buds (18.4) was observed in treatment T_{10} (Control) (Fig 2).

Flower yield (g/plant)
Among the treatments, the highest flower yield (129g/plant) was observed in treatment T_9 (Water spray (Fog) 15 min for every one hour). Followed by, the plants treated with White light for 6 hours (T_3) which was also on par with the plants treated with Yellow light for 6 hours (T_3). The lowest flower yield (106.1g/plant) was recorded in plants treated with White light for 6 hours (T_3) (Fig 2).

Shelf life (hours)
Among the treatments, the maximum shelf life (79 hours) was observed in the treatment T_9 (Water spray (Fog) 15 min for every one hour). The lowest shelf life (45 hours) was observed in treatment T_4 (Yellow light for 6 hours).

Biochemical parameters
Anthocyanin content of Opened flower buds (µg/g)
The illumination of Nerium plants with Red light for 6 hours + water spray (Fog) 15 min. for every one hour) recorded the highest amount of anthocyanin content of 0.86 µg/g. The lowest amount of anthocyanin content (0.71µg/g) was recorded in the plant treated with White light for 6 hours (T_3). (Fig 3)

Anthocyanin Content of Unopened flower buds (µg/g)
The highest amount of anthocyanin content of 0.81 µg/g was recorded in plants treated with red light for 6 hours + water spray (Fog) 15 min. for every one hour. Among the treatments, the plants treated with White light for 6 hours was recorded the least amount of Anthocyanin content (0.66 µg/g). (Fig 3)

Flower colour (RHSCC)
The illumination to different LED lights did not showed any significant difference on flower colour of Nerium. Flower colour obtained was Moderate Purplish Pink (B) under the group Red Purple Group 62.

Discussion

Effects of different coloured LED light and fogging system on flower yield parameters of Nerium

The impact of various coloured LED lights and fogging systems on flower yield was highest in treatment (T_9) as water spray (Fog) given for 15 minutes at one hour intervals, with the highest shelf life. Flowers show higher yield and increased shelf life due to the higher content of moisture present in the plant cell which enables the plant cells to be in turgid condition and enhances the keeping quality. Therefore, the outcome resulted in more production of buds on the plant. This is mainly due to increased carbohydrate transportation in the plant stem. Moreover, the air and plant temperature is reduced, which resulted in obtaining higher flower yield. This is in accordance with the findings of Khayat *et al*., 1988 [8], Dayan *et al.*, 2005 [2], Jiao and Grodzinski, 1998 [5].

Effects of different coloured LED light and fogging system on Biochemical parameters of Nerium

Anthocyanin accumulation in plants is influenced by many abiotic factors, among which light is the most important one (Grisebach 1982) [4]. Light-dependent anthocyanin biosynthesis significantly depends on plant species and experimental conditions (Mancinelli *et al.*, 1991) [10]. Plant physiological responses to red and far-red light are mediated by phytochrome. The photocontrol of Chalcone synthase (CHS) expression in petunia plant have been studied in detail (Katz and Weiss 1998) [7].

Anthocyanin biosynthesis in flowers has been studied in apple, petunia and rose where light is the key role in regulating the biosynthesis pathway (Biran and Halevy 1974; Weiss and Halevy 1991; Dong *et al.*, 1998) [1, 18]. Red light influences the enzyme Dihydroflavonol-4-reductase (DFR) in Arabidopsis (Kubasek *et al.*, 1998) [9]. *In vivo* and *in vitro* experiments conducted in Gerbera substantiates the fact that light is one of the most important factors for inducing flower pigmentation by reducing the gene expression of CHS and DFR when kept in dark condition. This confirms the role of CHS and DFR as two key enzymes responsible for light-induced anthocyanin biosynthesis (Meng *et al.*, 2004) [12].

Our above experimental results show, T_6 (Red light for 6 hours + water spray (Fog) 15 min. for every one hour) showed the highest anthocyanin content in both opened and unopened flower buds. Therefore, it is evident that red light selectively promotes anthocyanin biosynthesis. This is in accordance with the findings in cranberry fruits whereby the effects of red light are thought to be mediated-through phytochrome (Zhou and Singh 2004) [20].

Table 1: Effects of different coloured LED light and fogging system on flower yield parameters of Nerium.

Treatments	Number of inflorescence per plant	Number of flower buds per inflorescence	Hundred flower buds weight (g/Plant)	Flower yield (g/plant)	Shelf life
T_1- Blue light (450-495 nm for 6 hours	17.0	6.0	19.4	115.0	64 hours
T_2 - Red light (620-750 nm) for 6 hours	13.6	8.1	20.7	122.0	55 hours
T_3- White light (450-500 nm) for 6 hours	16.0	7.3	20.2	106.1	48 hours
T_4 - Yellow light (560-580 nm) for 6 hours	15.5	6.5	18.6	106.7	45 hours
T_5 - Blue light for 6 hours + water spray (Fog) 15 min. for every one hour	20.5	8.3	23.1	126.5	72 hours
T_6 - Red light for 6 hours + water spray (Fog) 15 min. for every one hour	25.4	10.0	20.6	118.6	69 hours
T_7 - White light for 6 hours + water spray (Fog) 15 min. for every one hour	19.0	7.5	21.2	121.9	72 hours
T_8 - Yellow light for 6 hours + water spray (Fog) 15 min. for every one hour	18.50	8.6	20.2	115.1	50 hours
T_9 - Water spray (Fog) 15 min. for every one hour	24.3	9.0	21.9	129.0	79 hours
T_{10} - Control (Farmers practice)	15.1	6.5	18.4	109.7	60 hours
SEd±	1.01	0.61	1.08	0.31	NS
CD (p=0.05)	2.03	1.26	2.20	0.65	

Table 2: Effects of different coloured LED light and fogging system on Biochemical parameters of Nerium

Treatments	Anthocyanin content (µg/g)		Flower colour (RHSCC)	
	Opened flowers	Unopened flowers buds		
T_1	0.76	0.71	Red Purple Group 62	Moderate Purplish Pink (B)
T_2	0.78	0.73	Red Purple Group 62	Moderate Purplish Pink (B)
T_3	0.71	0.66	Red Purple Group 62	Moderate Purplish Pink (B)
T_4	0.72	0.65	Red Purple Group 62	Moderate Purplish Pink (B)
T_5	0.83	0.78	Red Purple Group 62	Moderate Purplish Pink (B)
T_6	0.86	0.81	Red Purple Group 62	Moderate Purplish Pink (B)
T_7	0.74	0.69	Red Purple Group 62	Moderate Purplish Pink (B)
T_8	0.73	0.68	Red Purple Group 62	Moderate Purplish Pink (B)
T_9	0.76	0.71	Red Purple Group 62	Moderate Purplish Pink (B)
T_{10}	0.73	0.68	Red Purple Group 62	Moderate Purplish Pink (B)
SEd±	0.04	0.037	NS	
CD (p=0.05)	0.0849	0.078		

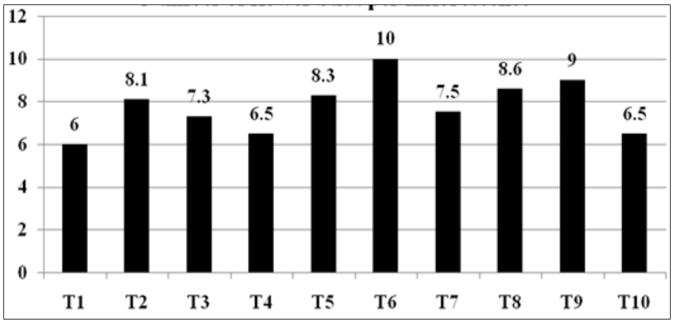

Fig 1: Number of flower buds per inflorescence

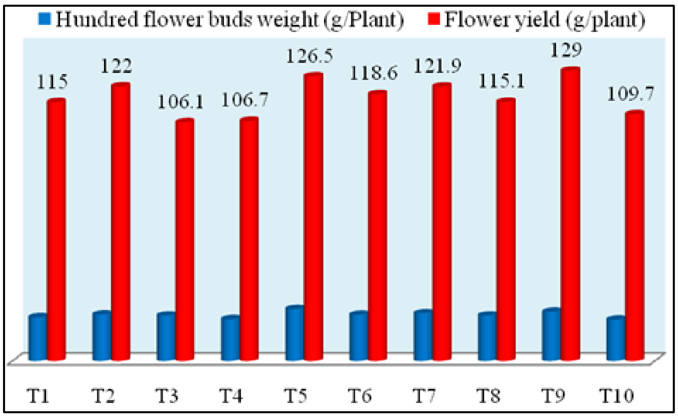

Fig 2: Hundred flower bud weight (g/plant)

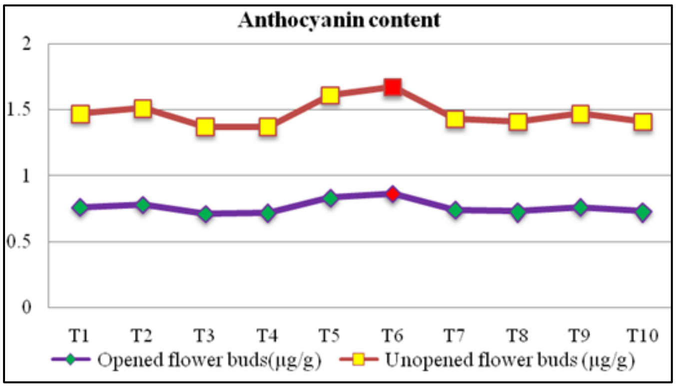

Fig 3: Anthocyanin content of opened and unopened flower buds

Conclusion

From the research findings, it has been concluded that the use of different LED lighting systems had influenced the inflorescence, flower bud weight, flower yield and shelf life. The highest amount of anthocyanin content was found in both opened flowers and unopened flower buds in the treatment of red light for 6 hours with water spray (Fog) 15 minutes for every one hour (T_6).

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