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Effect of night-break on growth and flower production in *Kalanchoe*

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ABSTRACT

***Kalanchoe blossfeldiana* is a popular potted flowering plant with extended flowering periods and low demand for nutrients and water. Understanding photoperiodic response is a prerequisite for the management of the flowering period in photoperiodic crops like *Kalanchoe*. The night-break treatments were provided by an artificial light source (CFL bulbs) starting from 7th October 2019 for 20 (7th October - 26th October), 30 (7th October - 5th November) and 40 (7th October - 15th November) days as night interruption (NI) for 30 min. and 60 min. from 11:30 PM to 00:00 AM and 11:30 PM to 00:30 AM. Exposing the plants to a 60 min. night interruption for 20 days has proven to be crucial in achieving essential qualitative criteria for potted plants, specifically ensuring compact plant growth with minimal plant height (26.20 cm) and a controlled plant spread (19.17 cm). The maximum number of days to flower bud appearance (138.07 days), days to colour break stage (203.79 days) and days to 50% opening of flowers (232.23 days) was under 60 min. of night interruption for 40 days. Therefore, delayed flower production in *Kalanchoe* for up to 45 days can be achieved by 60 min. of night interruption for 40 days along with acceptable quality pot plants. A significant amount of anthocyanins and total phenols reported with photoperiodic treatment confirms that photoperiod influenced the biosynthesis of anthocyanin and phenolic compounds in *Kalanchoe*.**

Key words: *Kalanchoe blossfeldiana* Poelln., Night interruption, Photoperiod, Potted plant, Phytochrome, Short Day plant.

INTRODUCTION

In this new era of climate change, succulents like *Kalanchoe blossfeldiana* Poelln. will play a crucial role in fulfilling the needs of flowering pot plants for home decoration. Succulents are underutilized and underappreciated plants with the potential to be ornamental potted and landscape plants. Kalanchoes are one of the world's most economically valuable ornamental flowering plants due to their ease of growing and maintenance. The genus *Kalanchoe* comprises of 139 species belonging to the Crassulaceae family and are indigenous to Madagascar, an island on the South-East coast of Africa.

Short exposure to the suitable integral of light in the middle of the long night by breaking the long night into two short nights effectively suppresses the flowering in short-day plants and promotes flowering in long-day plants. This phenomenon is called night interruption (NI). It has been widely employed as a technique for investigating the photoperiodic regulation of light-responsive plants, and it is better understood when experimenting with short-day plants (Higuchi *et al.*, 7). In the genus *Kalanchoe*, the most important species, *K. blossfeldiana* Poelln., is classified as a qualitative Short-Day plant with a critical day length of 12 h (Currey and Erwin, 4). Under subtropical conditions like Punjab and other northern states of India, the flowering period is limited, *i.e.*, the end of February to mid-March. For commercial flower production, photoperiods are artificially extended (through night interruption) and shortened (through black cover) to maintain vegetative growth and induce flowering, depending upon the requirement. Understanding the point at which plants become responsive to inductive photoperiods, gauging market demand, and assessing the impacts of photoperiod regimes on crop quality characteristics will empower commercial growers to formulate production schedules to decrease the duration required for crop production, while ensuring the maintenance of satisfactory crop quality.

Further, one of the significant challenges in the pot plant industry is controlled plant growth with compact plants. However, potential potted plants like *K. blossfeldiana* Poelln. have diverse foliage and elongated natural growth habits, which chemical growth regulators control. Since these chemicals are hazardous to the environment and human health, finding alternative methods to achieve compact plants is necessary (Pearson *et al.*, 12). The objectives of the present investigation were to study the effect of the combination of days and time of night interruption on extension growth, flowering, and physiological and biochemical changes of *Kalanchoe* and to compare the plant responses with those under natural day length with an ultimate objective of flower regulation.

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MATERIALS AND METHODS

The study was conducted at the Department of Floriculture and Landscaping, Punjab Agricultural University, during 2019-21. Plants of *Kalanchoe* were raised through the terminal shoots. Rooted cuttings were transplanted in 15 cm (diameter) pots. Plants were grown under natural day length until transfer to the night interruption (NI) treatments. Long days (SD with 60 and 30 min. NI) were provided by night interruption through artificial light sources like compact fluorescent light bulbs of 23 Watts having light intensity 150 lux by hanging 50 cm above the tip of the plants. Plants were placed to photoperiodic set up of night interruption of 30 and 60 min. from 11:30 PM to 00:00 AM and 11:30 PM to 00:30 AM, respectively, starting from 7th October for 20 days (7th October- 26th October), 30 days (7th October - 5th November) and 40 days (7th October - 15th November). A digital timer programmable controller was used to control the ON and OFF of compact fluorescent lamp (CFL) bulbs for night-break. After providing the long days through night interruption, plants were shifted to natural day length for induction of flowering. The critical day length of 12 h was required to induce the *Kalanchoe* (SDP) flowering; hence, an uninterrupted long night longer than 12 h was sufficient to initiate flowering. Plants treated as control given natural day length confirm a clear photoperiodic flowering response.

Growth parameters, such as plant height, plant spread, the thickness of the matured leaf, days after transplanting to visible flower bud appearance or days to flower bud appearance, colour break stage and days to 50% opening of flowers were measured at the time of peak flowering. The freshly matured 50 mg leaves were immersed in 10 mL of dimethyl sulfoxide. They were placed in a hot air oven at 60°C to extract pigment for 2 h. After a requisite period, absorbance was recorded on a spectrophotometer at 645 and 665 nm. The total chlorophyll content was estimated using Hiscox and Israelstam (8). To estimate carotenoid content, the fresh leaves of 50 mg were homogenized with 3 mL of 80% acetone and centrifuged at 3000 rpm for 10 min. Then, the pellet was extracted again with 2 mL of 80% acetone and re-centrifuged. The absorbance was recorded at 480, 645 and 663 nm (Kirk and Allen, 9). Leaf tissue of 5 g was homogenized in 10 mL of 0.1% HCl in methanol for total anthocyanins content. The extract prepared was put for 20 h at room temperature, and the absorbance was noted at 530 nm (Ranganna, 14). Folin-Ciocalteu reagent was used to determine total phenols (Bray and Thorpe, 3). Total phenols were calculated against a standard curve of gallic acid. This experiment was laid out in Factorial Completely Randomized Design with 5 replications comprising of 6 pots per replication, and the data were analyzed using SPSS software (Version 25; I.B.M. Developer), and treatment means were compared using Tukey's honestly significant difference (HSD) test at 5% level of significance.

RESULTS AND DISCUSSION

In this study, plant height, spread, and leaf thickness were the greatest for plants under an uninterrupted short-day condition (natural day length). Additionally, the plant height decreased, corresponding with the exposure to NI treatment of 30 (26.47 cm) and 60 min. (26.62 cm) as compared with control (28.34 cm) plants (Fig.1A). Both plant height and plant spread revealed significant differences with the interaction among different times and duration of NI plants exposed to 60 min. for 20 days had the shortest (Fig. 1A) plant height (26.20 cm) and minimum (Fig. 1B) plant spread (19.17 cm). However, the maximum plant height (28.56 cm) and spread (22.41 cm) were recorded in plants without any NI *i.e.*, in control for 30 and 40 days duration, respectively. The leaf thickness decreased correspondingly with increasing the time interval of NI treatment ranging from 2.47 to 1.83 mm, and among interaction, the maximum (2.52 mm) value was observed under control for 40 day plant, which were at par with that of 20- and 30-days duration and minimum (1.76 mm) was noticed under 30 min. of NI for 30 days (Fig. 1C). The value of leaf thickness was found to be statistically at par with 60 min for 30 days and 60 min for 40 days. In *K. blossfeldiana*, all NI treatments promoted overall vegetative growth. During the critical phase, plants exposed to long-day treatment shifted the hormone balance, leading to an increase in gibberellins that resulted in increased vegetative growth (Brian, 2).

The manipulation in light conditions with NI 60 min for 20 days can effectively produce compact plants with minimal plant height and spread. Recent research findings have indicated that exposure to a far-red light or low R: F.R. light tends to favour the presence of phytochromes in the Pr form, thereby stimulating plant stem elongation. Conversely, red light or high R: F.R. light tends to shift the phytochromes towards the Pfr form, which hinders plant stem growth (Smith, 16), which might be due to C.F.L. bulbs used in our experiment emitting higher R: F.R. light and establish higher Photosynthesis Photon Efficacy (PPE), which result in shorter plant height. In the present experiment, leaf thickness was decreased with increasing exposure to night interruption, resulting in extended Long-Day conditions. The results are in agreement with the findings of Friend and Lydon (6), who found that leaf thickness was

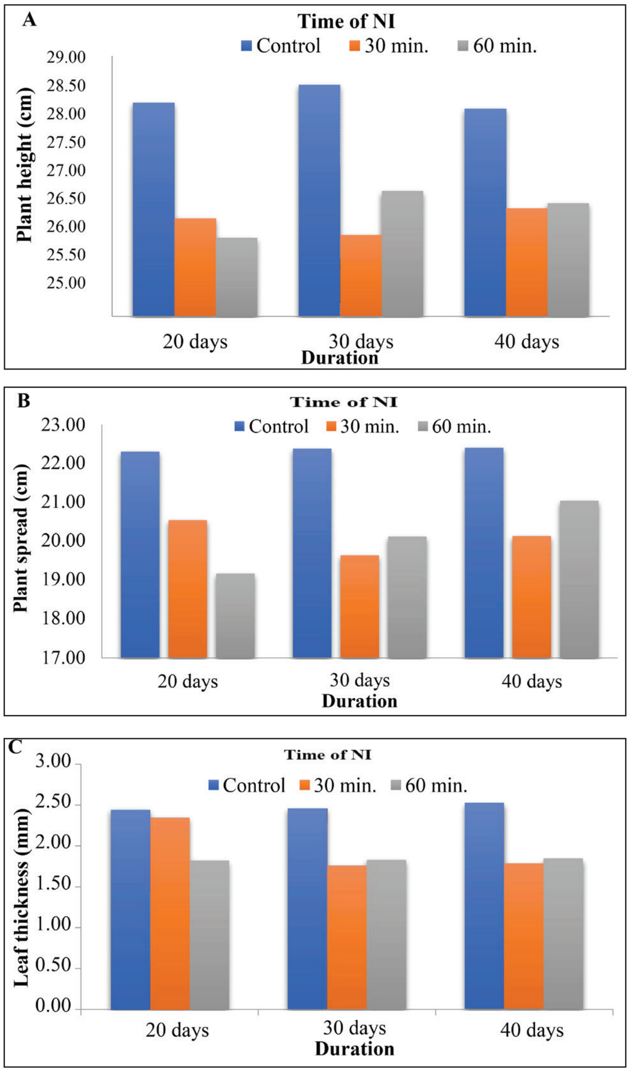

Fig. 1. Effect of different durations of time and days of night interruption (NI) on plant height (A), plant spread (B), and leaf thickness (C) of *K. blossfeldiana* Poelln.

inversely related to day length while working on pineapple, a similar short day and crassulacean acid metabolism (CAM) plant like *K. blossfeldiana*. The relationship between flowering and leaf thickness is believed to be linked, with thicker leaves being connected to additional cell layers or elongated palisade cells, thereby enhancing the photosynthetic capacity on an area basis (Pons and Pearcy, 13).

In *K. blossfeldiana,* the effect of different durations of time and days of NI was significant ($P< 0.05$) on the days to flower bud appearance, colour break stage and 50% opening of flowers. Flower bud development was slower in the photoperiodic NI treatments than in control (S.D., natural day length). Days taken to flower bud appearance were found significantly more in NI of 60 min. for 40 days (138.07 days, beginning of Jan.) than the control (93.40 days, end of Nov.), and this treatment significantly differed from all other treatments, where flower bud appearance was delayed by 1½ months as compared to control. NI significantly delayed the colour break stage and 50% of the flower opening. However, under the NI, the colour break stage and 50% flower opening recorded the longest durations (203.79 and 232.23 days, respectively) 60 min. for 40 days.

In contrast, the shortest durations (162.53 days for colour break and 187.24 days for 50% flower opening) were observed under control, *i.e.*, devoid of any NI (Table 1). Under natural short-day conditions from Oct. to Mar. in the Northern Hemisphere, night interruption exposure breaks up a long night, simulating extended long-day conditions for plants and its effect in Kalanchoe (SDP) was reported highest in the middle of the night (Thomas and Vince-Prue, 17). For all NI treatments, NI 60 min for 40 days significantly delayed flower bud appearance, colour break stage and 50% opening of flowers by 1½ months. Similar results were reported in photoperiodic crops (Singh and Bala, 15). Phytochromes are the primary photoreceptors responsible for controlling the flower induction process in plants. These are blue protein pigments with a molecular mass of about 125 kDa (kilodaltons), which are primarily present in leaves of plants and photo periodically adequate light is determined by the circadian clock, and plants use these pigments to sense the photoperiod. In photoperiodic crops, Pfr is the active form of phytochrome, which translocates to the nucleus when light signals are received and

Table 1. Effect of durations and days of night interruption (NI) on flower bud appearance, colour break stage and days to 50% opening of flowers.

Treatment	Days to flower bud appearance	Days to colour break stage	Days to 50% opening of flowers
Control for 20 days	93.53^{a}	162.53^{a}	187.30^{a}
Control for 30 days	94.02^{b}	163.83^{a}	187.60^{a}
Control for 40 days	93.40^{a}	163.08^{a}	187.24^{a}
30 min. NI for 20 days	118.33^{b}	182.13^{b}	212.23^{b}
30 min. NI for 30 days	126.47^{bc}	190.63^{bc}	220.33^{bc}
30 min. NI for 40 days	127.40^{bc}	194.46^{cd}	221.73^{bc}
60 min. NI for 20 days	120.10^{b}	186.53^{bc}	214.17^{b}
60 min. NI for 30 days	127.03^{bc}	192.50^{bd}	219.37^{bc}
60 min. NI for 40 days	138.07^{c}	203.79^{d}	232.23^{c}

Mean values in each column with the same letters are not significantly different according to Tukey's HSD test at $P < 0.05$

activates downstream pathways (Franklin and Quail, 5). Under an uninterrupted long night, the Pfr form of phytochrome is slowly converted to the Pr form, leaving insufficient Pfr to inhibit flowering. Before exposure to darkness, phytochromes predominantly exist in the Pfr form. Upon entering dark conditions, the Pfr undergoes a process of dark recovery, gradually transforming into the inactive Pr form.

In our study, we also observed significant variation in production of leaf chlorophyll content (mg/g FW), total carotenoids (mg/g FW), anthocyanin content (mg/100g FW) and total phenols (mg/100 g) to the extended day length by NI treatments. The plants exposed to the NI condition had significantly lower leaf chlorophyll content than those under the uninterrupted Short-Day (control) condition. Under NI 60 min. for 40 days. The carotenoids and anthocyanin content of leaves displayed a similar tendency to chlorophyll content. However, the maximum carotenoid content (1.008 mg/g FW) was found in control plants, whereas the minimum (0.198 mg/g FW) value was observed in NI 60 min for 40 days, and the maximum anthocyanin content (0.271 mg/100 g FW) was reported in plants kept devoid of any NI for 40 days and minimum (0.199 mg/100 g FW) in NI 60 min. for 40 days. It is reported that natural short days try to induce anthocyanins, while night interruption results in a decreased accumulation of anthocyanin in leaves. Among different treatments, the maximum phenolic content (404.22 mg/100 g leaf) was obtained under night interruption of 30 min. for 40 days, which was statistically at par with NI 60 min. for 40 days (384.79 mg/100 g leaf), and the minimum content was found under control, *i.e.*, 144.95 mg/100 g leaf.

The findings of this study revealed that the biosynthesis of many compounds in *K. blossfeldiana* is often dependent on NI treatments, and it would be interesting to know how closely photoperiod is related to several pigments. The chlorophyll and carotenoids were lower for plants exposed to NI than for plants in the SD control. Similar results were reported that a "dilution" effect by substantially increased biomass results in a decline in chlorophylls and carotenoids (Li and Kubota, 10). In our study with *K. blossfeldiana*, the leaves showed red colouration at the apical end and on the underside along the leaf margins. Since the formation of bright red flowers is induced by exposing plants to short days, the formation of anthocyanin in several parts of *Kalanchoe* may be a photoperiodic stimulus. It is suggested that developing a significant amount of anthocyanins pigment in the herbaceous leaves of *Kalanchoe* is a photoperiodical response. It takes place only on short days, but short days with interrupted long nights behave as long days, producing a similar physiological effect, *i.e.*, preventing anthocyanins formation as in the case of flower induction (Neyland *et al*., 11). Tannins are the main phenolic fraction, comprising 70 - 80% of total phenolics content irrespective of age. Light plays a vital role in changing the content of phenolic compounds. Over a wide temperature range, Short Days will cause the synthesis of phenols in smaller amounts, whereas a significantly higher level of phenolic compound is induced by Long Day length (Balsa *et al*., 1). It is concluded that 60 min. of night interruption for 40 days can delay the flowering up to 45 days compared to control. Flowering in *Kalanchoe* can be extended up to mid-April. It also exhibits specific beneficial effects on plant growth in terms of plant height and spread, which are desirable characteristics for commercial potted plants for sale.

AUTHORS' CONTRIBUTION

Conceptualization of research (RS, PS); Designing of the experiments (RS, PS); Contribution of experimental materials (RS, PS); Execution of field/lab experiments and data collection (SRK); Analysis of data and interpretation (SRK, RS); Preparation of the manuscript (RS, SRK).

DECLARATION

The authors declare that they do not have any conflict of interest.

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