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## Impacts of Shading on Flower Formation and Longevity, Leaf Chlorophyll and Growth of *Bougainvillea glabra*

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**Abstract:** Bougainvillea plants were exposed to artificially reduced light intensity to capture different Photosynthetic Photon Flux Density (PPFD), 0% (direct sunlight), 30, 50 and 75% shading and to observe the effects of shading on flower formation and longevity, leaf chlorophyll and sugar content and quality of plants. Plant attained the greatest leaf size with maximum branching when seedlings were grown in 30 and 50% shading, whereas 0% shading showed the lowest value. Growth parameters related to the plant characteristics such as branch initiation, branch growth and potassium content increased under high shading treatments. Plants exposed to 0% shade showed the highest sugar content and the sugar content decreased by increasing shading. The low light intensity that results in decreased sugar and chlorophyll contents may be attributed to the reduction of flower initiation and in turn to the acceleration of flower abscission. In addition, more than 30% shading led to stop up flower initiation completely. Hence, it is suggested that 0% shading can be applied to maintain frequent flower initiation and flower longevity.

**Key words:** Bougainvillea, light, shade, leaf, flower

### INTRODUCTION

Bougainvillea has about eighteen species and generally used in the arid landscapes for beautification, horticulture, pharmaceutical industries, agriculture and environmental industries on account of the large flexibility in different agro climatic regions of the world (Suxia *et al.*, 2009; Saifuddin *et al.*, 2009a; Simon *et al.*, 2006). Besides its ornamental value in the landscaping, recently it has been discovered that bougainvillea is a pollution tolerant plant and can help in the mitigation of air pollution (green house gases) (Kulshreshtha *et al.*, 2009; Sharma *et al.*, 2005). On the basis of leaves physical experiment of bougainvillea, it can be referred that this plant is a dust mitigator and it absorbs the pollutants from the environment in which it grows. Therefore, this plant was highly recommended for plantation in urban and industrial areas where particulate is a problem.

Light is an essential prerequisite factor for the plant growth and development. It has long been known that photoperiodic conditions bring around the transition from vegetative to productive development as distinguished from condition that influences the subsequent development of flower buds (Wurr *et al.*, 2000). In addition, physiologically, light has both direct and indirect effects. It affects metabolism directly through photosynthesis, growth and development indirectly (Dai *et al.*, 2009).

Moreover, formation of new structures in higher plants is controlled by light such as the regulation of flower bud formation and the accumulation of food reserves by cell in leaf bases (Irene *et al.*, 2007). In spite of the fact that light influences many phases of plant growth and development, relatively few photoreactions are probably involved (Ben and Bullockb, 2007). One of these is responsible for photosynthesis and the other for phototropism. In addition, Puech-Suanzes *et al.* (1989) reported that photosynthesis by canopy is proportional to its PAR interception, with a near constant light use efficiency for different growth stages prior to maturation and senescence.

Light provides the leaf with more photochemical energy that can be utilized by photosynthesis. Excessive light intensity results in lower quantum utilization and a lower assimilation yield (photoinhibition). Moreover, Goltsev *et al.* (2003) reported that extremely high irradiation destroys photosynthetic pigments. Osmond (1994) reported that high light intensity inhibits photosynthesis and may lead to photosynthetic apparatus (chloroplast) damage.

The quality of potted flowering plants (generally placed in shaded area) is often greatly affected by poor environmental conditions, such as low light intensity, high or low temperature, variation and water stress (Doi *et al.*, 1992). In Antirrhinum, low light intensity significantly increased the flowering time with higher leaf

number (Cremer *et al.*, 1998). Koji *et al.* (2005) reported that low light intensity increased ethylene production that resulted to the preceding reduction of CO<sub>2</sub> assimilation and sugar content, which in turn led to the acceleration of sepal abscission.

A decrease in light intensity can be naturally caused by clouds or artificially by shading nets. However, shading nets are commonly used in countries like Pakistan and the U.K., during summer months to decrease the temperature inside the glass-houses and to protect the plants from the harsh effects of severe sunshine. As described in the general photo-thermal model, the major influencing factors were considered as photoperiod and temperature on flowering time (Munir *et al.*, 2004; Ellis *et al.*, 1990). The effect of light intensity was successfully incorporated in the same model in *Petunia*, *Viola* and *Antirrhinum* (Adams *et al.*, 1997; Munir, 2003). Potassium acts as catalysts for many of the enzymatic processes in the plant that are necessary for plant growth to take place. On the other hand, key role of potassium is the regulation of water use in the plant (osmoregulation). This osmoregulation process affects water transport in the xylem, maintains high daily cell turgor pressure which affects cell elongation for growth and most importantly it regulates the opening and closing of the stomates which affect transpirational cooling and carbon dioxide uptake for photosynthesis (Yang and Zhang, 2006).

However, it is precisely that these relationships which drive plant growth and determine competitive success when light limits growth. Limited attention was paid previously on *Bougainvillea* to explore how these environmental factors are related to plant life, structure and flowering process. Therefore, the objective of the present study was to determine the effects of different shading on the flowering time, flower longevity, leaf chlorophyll and soluble sugar content of *Bougainvillea* sp.

## MATERIALS AND METHODS

**Experimental site and plant materials:** The experiment site was the Plant Physiology Garden, Institute of Biological Sciences, University of Malaya. Two-year-old (total of plants 12) *bougainvillea* plants were grown in a small polythene bag. The plants were irrigated twice a day to avoid water stress condition and placed under prevailing conditions (relative humidity 60-90% and temperature 21-32°C).

**Treatment setting:** For the different types of shade plot, light intensity was artificially reduced using a black plastic, cloudy white plastic and transparent plastic to allow 25, 50 and 70% Photosynthetic Photon Flux Density

(PPFD), respectively, covering (300 cm long × 300 cm wide) three plants per treatment. For the control treatment, three plants were placed under direct sun light (0% shade). The light intensity under the each shaded net was measured and each value was converted into  $\mu\text{E}/\text{m}^2 \text{ sec}$ . Then, the value was calculated into a shading percentage comparing with the non-shaded or control light intensity which resulted as follows:

- The Photosynthetically Active Radiation (PAR) under direct sunlight (L1) is 2100  $\mu\text{E}/\text{m}^2 \text{ sec}$  (0% shade or control)
- The PAR under transparent plastic (L2) is 1470  $\mu\text{E}/\text{m}^2 \text{ sec}$  (30% shade)
- The PAR under cloudy Plastic (L3) is 1050  $\mu\text{E}/\text{m}^2 \text{ sec}$  (50% shade)
- The PAR under black Plastic (L4) is 525  $\mu\text{E}/\text{m}^2 \text{ sec}$  (75% shade)

The shading net was set up 250 cm above from the ground at the beginning of experiment. The plants were irrigated twice a day to avoid water stress condition. Each plant was 50 cm of height and plant consisted of eight primary branches and five secondary branches. Each replicates were equally fertilized with 5 g of NPK (12:12:17) at fifteen days interval. Plants in each treatment were observed daily until the first flowering of the controlled plant. Flowering and others vegetative parameters were recorded during six month experimental periods.

**Branch length measurement:** Branch length was measured by measuring tape.

**Flower longevity:** Flower longevity was counted as the number of days from flower initiation to flower abscission.

**Stomatal conductance:** Stomatal conductance was measured in different seasons stomatal using a portable porometer (Leaf Porometer, Model SC-1, USA). A leaf clamber was attached to one of the leaf and kept in ambient temperature for 10-15 min to maintain sunlight adaptation. A stomatal conductance was measured in 3 replicates from different spots of a single leaf.

**Photosynthetic pigments estimation:** The photosynthetic pigments chlorophyll a, chlorophyll b and total chlorophyll were estimated after homogenizing 0.5 g of the fresh leaf sample in 80% ethanol. The absorbance was measured at 663 and 645 nm and the chlorophyll content was measured using the following formula (Asare-Boamah *et al.*, 1986).

$$\text{Chl a (mg g}^{-1}\text{)} = [(12.7 \times A_{663}) - (2.6 \times A_{645})] \times V \text{ mL mg}^{-1} \text{ leaf tissue}$$

$$\text{Chl b (mg g}^{-1}\text{)} = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times V \text{ mL mg}^{-1} \text{ leaf tissue}$$

**Estimation of total soluble sugars:** For total soluble sugars, one gm of leaf was homogenized in 4 ml of 0.5 N of sodium hydroxide and ground in a mortar with a pestle and then centrifuged at 3,500 rpm for 20 min at 4°C. The pellet was discarded and the supernatant was neutralized with 0.5 N acetic acid. The resulting solution was made up to 40 mL and was stored at 4°C until use. These extracts were then used for the determination of total soluble sugars according to the phenol-sulphuric method by Dubois *et al.* (1956). One milliliter of the leaf extract was placed in a test tube and 1 mL of Phenol [5% w/v] was added followed by 5 mL of concentrate Sulphuric acid and the contents were mixed and left at room temperature for 10 min. Spectrophotometers reading were taken at 490 nm absorbance. The sugar concentration was obtained by referring to the standard glucose graph. The assay for this standard glucose graph was carried out by adding phenol and sulphuric acid to a standard glucose solution with concentrations varying between 0-100  $\mu\text{g mL}^{-1}$ . Total soluble sugars was expressed in mg/100 g leaf fresh weight.

**Estimation of potassium content:** The most recent fully expanded same age and relative position on the plant leaves were taken from each treatment. For potassium estimation, one gram leaf was homogenized in 5 mL distilled water in a motor with a pestle and then centrifuged at 3,500 rpm for 20 min. The 3 to 5 drops of the supernatant liquid were transpired onto the calibrated sensor pad (Cardy Potassium Meter, Model-2400, USA). A sampling paper was also be placed on the sensor and saturated with the liquid. After the value has stabilized (30 to 45 sec), reading (ppm) was taken from the display pad.

**Leaf size measurement:** Leaf size was measured by Vernier scale.

**Statistical analysis:** Statistical analysis was performed using SPSS software. The one way ANOVA was applied to evaluate the significant difference of the parameters studied in the different treatments. The LSD ( $p = 0.05$ ) was calculated using the error mean squares of the analysis of variance.

## RESULTS

There was a hyperbolic relationship between new branch number/plant and shade percent (Fig. 1). The

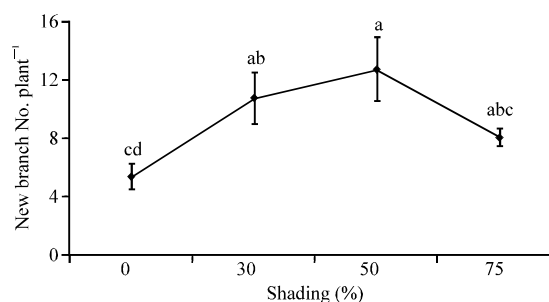


Fig. 1: New branch initiation under different shading. Means followed by different alphabets above bar chart are statistically different at 5% level of significance, using DMR test

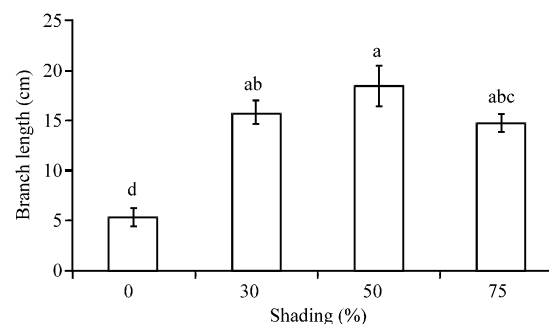


Fig. 2: Branch growth affected under different shading condition. Means followed by different alphabets above bar chart are statistically different at 5% level of significance, using DMR test

number of branch/plant increased when the shade percentages were increasing up to 50%. Further increase in shading percentage had reduced the number of branch/plant. The new branch initiation under direct sunlight (0% shade) was observed to be the lowest. However, in 50% shade, the branch initiation was increased by 116%. Whilst the plants regenerated ten branches in 30% shade and seven branches in 75% shade.

Branch length was significantly increased by high shade application on plants (Fig. 2). The increase in branch length could be due to the enhanced cell division activity by high shade. Branch length was enlarged by 260% in 50% shade and the lowest branch length was recorded in 0% shade. Branch length increased gradually with decreasing light intensity ( $p < 0.05$ ) and then in 75% shade, the branch length decreased. The present results are supported by Lindstrom *et al.* (2006), who described how tissue activity and gene metabolism work in early plant and fruit developing under high shade.

High light intensity (0% shade) allowed more flower than low light intensity (30% shade). Flower number was

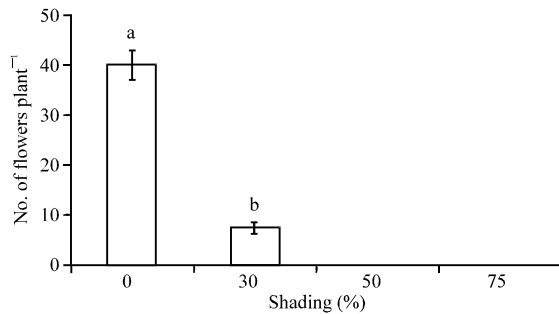


Fig. 3: Flower initiation was affected by different shading condition. Means followed by different alphabets above bar chart are statistically different at 5% level of significance, using DMR test

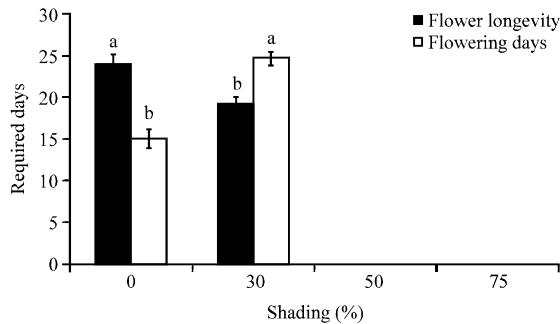


Fig. 4: Effect of shading on flower longevity and flower production in potted bougainvillea. No flowers were initiated in 50 and 75% shade

40 in 0% shade, while it was only seven in 30% shade (Fig. 3). Most significant results were expressed when the plant was placed in both 50 and 75% shading conditions. In these two shading conditions, plants were not capable to initiate a single flower. These two light transmission shade nets referred the inclusive inability to flower initiation of bougainvillea.

The flower longevity and required days to initiate flower were twenty four and fifteen days, respectively, in 0% shade. Whereas, it was nineteen and twenty four days, respectively, in 30% shade (Fig. 4). However, in both 50 and 75% shade treatments, flower initiation was not observed at all. The 50 and 75% shade indicated that light intensity is an important factor for the flower production of bougainvillea plants. Both 0 and 30% shade treatments refer that the longevity was also affected by light intensity (shade). Low shade or high light intensity was preferable for keeping flower fresh for long days. Comparatively higher shading was not suitable for flower production and longer vase life of *Bougainvillea glabra*.

With regard to stomatal conductance, it was found that at the first season, stomatal conductance was higher

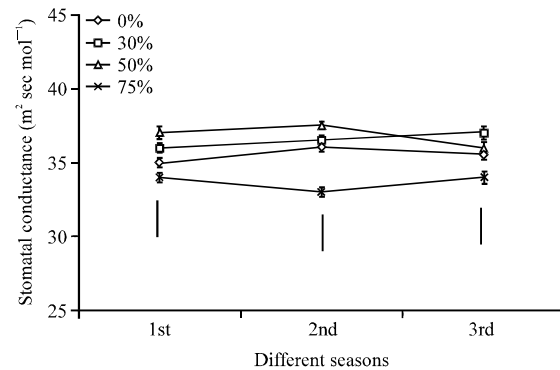


Fig. 5: Stomatal conductance was affected by different shading treatments at different seasons. Means followed by different lines are statistically different at 5% level of significance, using DMR test

Table 1: Comparison of photosynthetic pigments or chlorophyll content of bougainvillea leaves grown under full sunlight (0% shade), 30% shade, 50% shade and 75% shade. Values are Means $\pm$ SE (Different alphabets mark significant differences,  $p < 0.05$ )

Different shading (%)	Chlorophyll-a (mg/100 g)	Chlorophyll-b (mg/100 g)	Ratio(Chl-a/Chl-b)*
0	11.63 $\pm$ 0.15a	24.50 $\pm$ 0.35a	0.47 $\pm$ 0.002*
30	11.25 $\pm$ 0.09ab	23.87 $\pm$ 0.36ab	0.47 $\pm$ 0.003*
50	10.45 $\pm$ 0.09c	22.40 $\pm$ 1.11abc	0.46 $\pm$ 0.028*
75	9.65 $\pm$ 0.27d	19.15 $\pm$ 0.33d	0.50 $\pm$ 0.012*

\*Means not significant (4th column)

in 50% shade compared to all shading treatments (Fig. 5). Among all shading treatments, stomatal conductance was significantly lower in 75% shade plants leaf. At the second season, the stomatal conductance was gradually increased except in 75% shade. The difference of stomatal conductance among the shading treatments was probably related to the fact that 75% shade caused a reduction of stomatal conductance to maintain the physiological coherence or due to the extreme growth rate. That means leaf contains low stomatal conductance at growing or vegetative stage. This might be referred to a negative effect by high shading (75%) on plant morphology. Nabi *et al.* (2000) proved that the stomatal conductance was closely related to leaf age and leaf position. In the case of 0 and 50% shading, stomatal conductance decreased slightly after the 2nd season compared to the previous season.

The content of photosynthetic pigments in bougainvillea leaves was exposed at different shading. The photosynthetic pigment chlorophyll a and b showed a significant difference in presence of different shading treatments (Table 1). The accumulation of chlorophyll-a and b was significantly low in plants leaves in 75% shade. The highest amount of chlorophyll a and chlorophyll b was observed in 0% shading or control plant. Thus, the

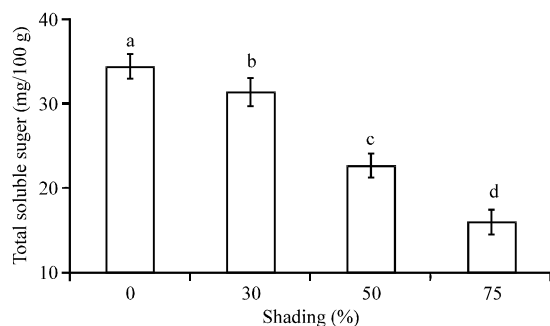


Fig. 6: Sugar content in bougainvillea leaf under various light levels. Means followed by different alphabets above bar chart are statistically different at 5% level of significance, using DMR test

enhancement of the photosynthetic pigments was because of low shading and reduction in chlorophyll a and b or high shading (50 and 75%) or the damage of photosynthetic component in leaves. Synthesis of chlorophyll pigment was enhanced by low shading treatment has previously been reported by Dai *et al.* (2009) and it has been suggested that the synthesis of chlorophyll enhancement was attributed to the utilization of light activity by leaves.

Sugar content was affected significantly by the different shading treatments (Fig. 6). The 50 and 75% shading treatments resulted significant reduction of sugar content in leaf tissue. Whereas, the highest sugar content was observed in 0% shade, through affecting the metabolism of high photosynthetic process which led to increase sugar content in leaf. Hence, it was observed that 0% (direct sunlight) shade was the optimum for bougainvillea to maintain the highest sugar content using photochemical energy. These results are in agreement with those obtained by Ralph (1999). In high shade, the TSS content was low, due to reserved TSS was consumed as an energy source in high shade or low photosynthetic capacity by plant leaves (Iwona *et al.*, 2005).

Potassium content was improved in 30% shading compared to others shading. The most effective treatment was 30% shade for increasing potassium content in leaf by 6%. In case of 0% shade or direct sun light, potassium content was reported lower compared to 50 and 75% shading. But the most effective treatment for reducing plant potassium content was 75% shade.

Leaf size was the smallest when plants were grown under full sun light or in 0% shade (Fig. 7). Dai *et al.* (2009) also reported similar results which showed that as light intensity decreased, the leaf number and size increasing in *Tetrastigma hemsleyanum* plants.



Fig. 7: Randomly chosen *Bougainvillea glabra* leaves from the various shading treatments for leaf size and color assessment. (L1) 0% shade, (L2) 30% shade, (L3) 50% shade, (L4) 75% shade

Experimental results showed that bougainvillea attained largest leaf size when cultivated in 50% shade. Leaf color of plants grown in 75 and 50% shade was yellowish-green and light green, respectively. This adjustment reduced the respiratory demand of shoot to help compensate the greatly decreased the photosynthetic capacity of the leaves (Campbell and Miller, 2002). This suggests that although this shade-tolerant plant can adapt under a light of 75% shade, such low light intensity will still decrease its growth. The lower chlorophyll values of 75% shade plantlets suggests that they adapted to high shade and used to close stomata to decrease water loss (Dai *et al.*, 2009).

## DISCUSSION

**Plant branching, flower initiation, shading and number of flower:** The time from treatment setting to flowering varied with different shades. Plant that received high light intensity (0% shade) was dense and produced maximum flower per plant and allowed the plants to initiate flower by fifteen days. Thirty percent shading net allowed more photosynthesis to take place at a higher rate from the early stage and produced more branches and leaves and allowed the plants to flower by twenty four days (Fig. 4). In a *vice versa* effect, high shading (50 and 75%) completely blocked up flower initiation but did not reduce the branching and leaf number. Cremer *et al.* (1998) reported similar results which showed that flower initiation decreased with the increase of shading. The possible reason was that in high shading or low light intensity, plants were unable to perceive the developmental signal in the leaves that induced

incompetence in flowering. Therefore, in this present study a linear decrease in the number of flowering buds was observed when the light transmission (30, 50 and 75% shading) was gradually reduced.

Plants produced fewer flowering buds in low light or 30% shading than in 0% shading. This indicated that the switch to flowering was only maintained at subsequent flower formation under direct sunlight (0% shade) or higher light intensity. Ballare (1999) referred a similar report that in high shading plants produced larger leaves and taller stem, in order to capture more light, probably because of a shade avoidance mechanism which resulted in decreasing the flower buds and delaying flowering time. Plant fresh weight was increased in a similar logical pattern from lower to higher shade levels showing that plants under low light conditions tended more towards to vegetative rather than reproductive growth (Munir *et al.*, 2004).

**Sugar content and flower longevity:** The flower longevity on plants held under direct sunlight or in 0% shade was twenty four days. Whereas, the longevity was nineteen days in 30% shade (Fig. 4). But the 50 and 75% shade were absolutely unable to induce flower formation. On the other hand, the similar results have been obtained regarding sugar content (Fig. 5) that as the shading increased, the sugar synthesis were decreased. Ichimura *et al.* (2000) reported that flower longevity was related to light intensity and sugar supply to the sepals after flower opening. Similar report was referred by Serek *et al.* (2006) that the increase of shading promoted ethylene production. This ethylene gas is involved in petal and/ or sepal abscission and wilting of flowers in many plants including bougainvillea (Saifuddin *et al.*, 2009b). In this content it might be assumed that high shading accelerated flower abscission due to low sugar supply and early ethylene production.

**Shade chlorophyll, stomatal conductance and leaf color:** Leaf chlorophyll content is well established as a common reference system when physiological reactions are quantified. Decreases in chlorophyll b content have been suggested to be an indication of chlorophyll destruction by excess irradiance (Jason *et al.*, 2004). Our results showed significant ( $p < 0.05$ ) decreases of chlorophyll content in 75 and 50% shaded by turning leaf color dark green into yellowing and light green, respectively (Fig. 7). Therefore, it is suggested that a shading higher than 50% conditions may seriously impair or partially active for chlorophyll synthesis. In this present study, plants grown under shaded conditions have been able to optimize their effectiveness of light absorption by increasing branches,

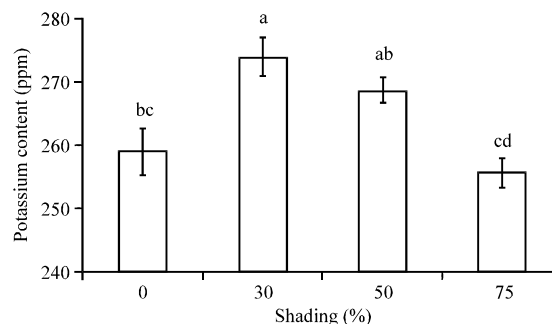


Fig. 8: Potassium content of different shading treated plants. Values are means of 3 measurements  $\pm$  SE. Means followed by different alphabets above bar chart are statistically different at 5% level of significance, using DMR test. Data were obtained at the end of experiment

leaf number and leaf size (Fig. 1, 7). Therefore, pigment density per unit leaf area was low under high shaded plant (Wittmann *et al.*, 2001). Among the shaded (30, 50 and 75%) plants, the smallest leaf size (Fig. 7) was exhibited in 75% shade. This result confirms the report by Gordon *et al.* (1994) in which leaf size decreased under low-light or extreme shading conditions in *Posidonia sinuosa* plants. The overall results also imply that *Bougainvillea glabra* is a shade tolerant plant. In the shaded treatments, plants can absorb sufficient light to maintain photosynthesis (Galmes *et al.*, 2007; Rena *et al.*, 1994).

In addition, Potassium availability was observed to be higher in 50 and 75% shade (Fig. 8) which may be in charge to increase plant growth as well as stomatal conductance. The lower stomatal conductance values of 0 shade and 75% shade plantlets suggests that they adapted to high light and low light irradiance and used stomatal closure to decrease water loss and reduced transpiration compared to photosynthesis.

Despite the smallest leaf size exhibited in full sunlight (0% shade), the number of flower and flower longevity was the highest. Therefore, it is suggested that full sunlight is to be applied on the bougainvillea plant in maintaining flower production with maximum longevity.

## CONCLUSION

In a conclusion, based on the current research findings, non-shaded condition (0% shade) showed the best methods to induce frequent flower and prolong flower vase life. In addition, it can be recommended that *Bougainvillea glabra* can be grown as a shade-tolerant plant. It has been showed that in 75% shade, plant can



still maintain growth by changing some physiological characters. In spite of low light intensity, plants maintain the growth due to the availability of potassium nutrient and high stomatal conductance. From the experimental observation, it can be inferred that flower initiation occurred due to sufficient light, sugar and chlorophyll content. None of the flower was initiated in both 50 and 70%. Apart from that, the early flower senescence was found to be due to 30% shading. This prominent effect would really recommend the species studied as landscaping and environmental beautification purposes. Finally, it is suggested that a molecular approach can be applied to genetically regulate flowering process and vase-life of *Bougainvillea glabra* in the future research.

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