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Effects of removal of young leaves and kinetin on inflorescence development and bract enlargement of *Bougainvillea glabra* var. "Elizabeth Angus"

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Abstract

A study was carried out to investigate the effects of the removal of young leaves and different concentrations of kinetin on inflorescence and bract quality development in *Bougainvillea glabra* var. "Elizabeth Angus". Selected horticultural parameters were monitored at three day intervals during the experimental period, using different concentrations of kinetin and defoliation of young leaves as treatments. Results revealed that defoliation with or without kinetin reduced vegetative growth but promoted inflorescence development, probably as a result of the accumulation of photosynthetic assimilate at the reproductive shoot tip. It was observed that 50mg/L kinetin with defoliation produced the highest number of bracts, greatest blooming number, largest bract length and increased the bract longevity compared to other treatments and control. Furthermore, 50mg/L kinetin plus defoliation plants exhibited the highest chlorophyll fluorescence and quantum yield. On the other hand, 100mg/L kinetin treatment in defoliated plants increased the weight of individual bracts, including that of the flower. From this study, it can be concluded that defoliation of young leaves and 50mg/L kinetin treatment enhanced inflorescence development and improved the quality of *Bougainvillea* bracts.

Keywords: *Bougainvillea*, bract quality, inflorescence, defoliation and cytokinin

Introduction

The *Bougainvillea* is a flowering ornamental plant which belong to the family Nyctinaginacea (Four-o'clock), has 18 species. *Bougainvillea's* growth habit and beautiful showy bracts make it a popular plant for landscapes. *Bougainvillea* provides hedges, barriers, excellent ground and slope coverings. It can cover a whole hillside and can even choke out weed growth. *Bougainvillea* is also used as an accent plant, a specimen plant, in hanging baskets, in containers, and for bonsai. The true, perfect flower is small, tubular, commonly white or yellow, and surrounded by showy, vibrantly colorful petaloid bracts (Saifuddin et al., 2009).

Defoliation in photoperiodically sensitive plants can be used to promote flower formation. It has been reported that removal of the youngest, partially expanded leaves from short day flowering plants like *Bougainvillea* "Elizabeth Angus," promoted inflorescence development (Tse et al., 1973). One hypothesis accounting for this surprising result is that the young leaves act as 'sinks' and compete with the reproductive axes for assimilates coming from the leaves. Flower induction is the event that initiates the transition of a vegetative apex to a floral apex in response to an environmental development cue. In photoperiodically sensitive plants, the flowering signal is translocated from the perceiving organs (leaves) to the apex. Darnel et al., (2003) reported that flowering in many species can be induced by the application of a variety of environmental techniques and growth promoting chemicals. During inductive conditions for flowering, biochemical and physiological changes are recognized in the plants and one of the possible changes that might occur is in hormone content (Ito et al., 2001). Changes in endogenous plant growth regulators during flower induction are still unclear; although it has been pointed out that the regulators could be closely related to reproductive growth. Weidman and Stang, (1983) reported that exogenous application of cytokinin increased inflorescence number in both short-day and long-day strawberry cultivars. They also reported that it increased branch crown formation. During flowering in the pear plant the cytokinin content increases significantly in the leaves and in the leaf phloem sap (Ito et al., 2001; Lejeune et al., 1994) and in the shoot apical meristem, at the time of early mitotic activation (Jacqmard et al., 2002). Bemier et al., (2002) reported that an exogenous application of cytokinin in a short day plant can induce various cellular and molecular changes in the shoot apical meristem that are normally associated with floral transition. Dielen et al., (2001) investigated the positive effects of cytokinin on flowering in the late flowering *uniflora* mutant of tomato. In addition, cytokinin increases were well correlated with the early events of floral transition.

Although changes in endogenous cytokinins have an important role in flower induction in some plants, it has not been thoroughly investigated in *Bougainvillea.* Currently there is very little information available on the effects of removal of young leaves and cytokinin on inflorescence development and bract enlargement in *Bougainvillea.* In this study we attempt to improve the bract quality and enhance inflorescence development by defoliation and kinetin treatment.

Materials and Methods

Experimental site and plant material

The experiments were carried out at the Plant Physiology Garden, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia. Two years old *Bougainvillea* plants, 1.0 m of height and canopy length 1.5 m were selected for the study. Four field grown *Bougainvillea* plants were used in the experiment. Six selected branches per plant of the same length, same diameter and same number of leaves were selected for treatment application. The experiment consists of 4 treatments including control, with six replications and a single uniformed branch was taken as experimental unit. Treatments were set following randomized complete design. Each treatment was repeated by 6 replications.

Treatment Setting

For the treatment setting 24 branches were selected. Every branch was tagged below 15 cm from the apex of the shoot. In all treatment, the leaves below the 10^{th} leaf, counting basipetally from the first leaf unfolding from the terminal bud, were removed. Node 1 was termed n (Fig.1). Nodes above n were designated n + 1, n + 2, etc., whereas those below were referred to as n - 1, n - 2, etc. In the defoliation experiments leaves at nodes n through n – 5 were removed. During the course of the experiment new leaves on defoliated plants were continuously removed as they unfolded from the apical bud. The kinetin was applied twice in a 10-µl droplets to the terminal and partial bud.

Data Collection

The number of nodes to first inflorescence, number of buds/15cm branch, blooming rate, bract length and stem elongation were measured at three day intervals, whereas, Individual bract weight and weight of flower including bract were measured at (18 days) final days of observation.

Chlorophyll fluorescence yield measurements

Chlorophyll fluorescence yield was measured by Plant Efficiency Analyzer (Hansatech Instrument Ltd., England). A single leaf was attached to the leaf clip and kept in dark place for 30-45 minutes to maintain dark adaptation. The fluorescence signal was measured for 3 seconds and fluorescence yield observed where, Fo = Lower fluorescence, Fm = maximum fluorescence, Fv = relative variable fluorescence (Fm- Fo). Temperature = 27ºC, Time range = 10µs- 3 sec.

Bract length, weight, blooming and longevity of bougainvillea

Individual bract and cluster bract weight including flower were taken using a Mettler PJ3000 weighing machine and bract length was measured by Mitutoya Vernier Scale. From the beginning of the experiments, 15 buds were selected for full blooming and longevity measurements. Observations were made when all bracts are open and abscission has occurred.

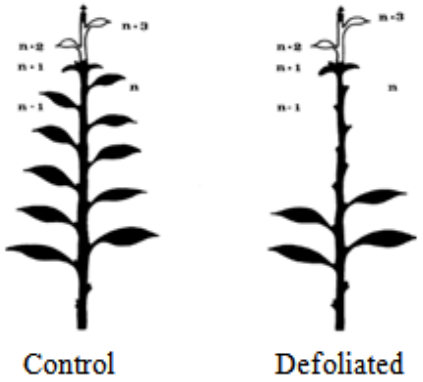

Fig.1 Diagrammatic sketch of intact and defoliated plants. Nodes are indicated by the letter n which is the first unfolding leaf from the terminal bud cluster.

Statistical analysis

The experimental design was a completely random design with six replications. All the data were analyzed using MSTAT statistical software. The one way ANOVA was applied to evaluate the significant difference of the parameter studied in the different treatments. Least significant difference (Fisher's protected LSD was calculated, Following significant F-test (p=0.05).

Results

Inflorescence development

From the results, it is clear that defoliated treatment enhanced the inflorescence development and anthesis earlier than the control treatment. Intact plants produced flower at the $n+6^{th}$ node, whereas, defoliated plant flowered at the n+4th node. As can be seen from Fig. 2, it was found to be statistically significant between the treatments and control in the case of inflorescence development of Bougainvillea. Inflorescence developed earlier in 50mg/L kinetin treated branches than in 100mg/L cytokinin treated branches. Control treatment produced the late flowering. Defoliation plus cytokinin treatment caused the greatest inflorescence development and was greater than the sum of the kinetin and defoliation only treatments.

Blooming number

Flower bud abscission before blooming is serious problem in *Bougainvillea* plants. As shown in Figure 3 cytokinin treatments had a significant effect on the blooming number of *Bougainvillea* bracts. Exogenous application of cytokinin stimulates the opening of the bud and reduces the bud abscission before blooming. It was observed that bract blooming increased with the advancement of time in the treated branches. From the results it can be seen that bract blooming increased up to 15 days of observation after which abscission started (Fig.5). Defoliation treatment with 100 mg/L cytokinin produced the highest number of blooming bracts (10) followed

Table1. Influence of defoliation and different concentration of kinetin on stem elongation (mm), bract blooming and longevity of Bougainvillea

Treatment	Stem elongation		Full blooming	Bract longevity	Quantum yield
	1 (d)	9 day	(Days)	(Days)	(Fv/Fm)
Control	3.0±1	30±1a	21.23±0.45	22.66±1.20c	0.68±0.040d
Leaf removal	3.0±1	27±1a	20.55±0.77	24.78±1.55b	0.94±0.023a
LR+ 50mg/L	2.5 ±1	21±1b	19.39±0.33	27.00±1.00a	0.75±0.011c
LR+ 100mg/L	2.5±1	19±1c	19.43±0.88	26.00±1.65a	0.81± 0.017b
	ns	*	ns	*	**

Means (±S.E) within the same column followed by the same letter, do not differ significantly according to the LSD test at α=0.01, ns=non significant* significant at 0.05%.

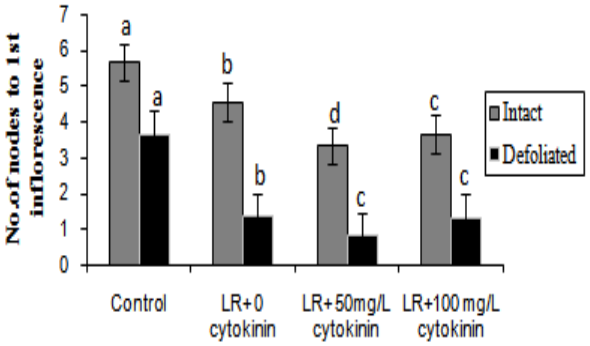

Fig.2 Influence of defoliation and different concentration of kinetin on inflorescence development.

Fig.3 Photograph showing the effects of defoliation and cytokinin on number of buds and blooming rate of Bougainvillea

by 50 mg/L cytokinin plus defoliation treatment and defoliation only treatment with a value of 8 and 7 respectively. Whereas control produced the lowest number (2) of blooming bracts (Fig. 5). The blooming number of *Bougainvillea* bracts increased with cytokinin concentration in defoliated branches.

Number of buds

It is well documented that in the literature, that cytokinin treatment can increase the number of flowering buds (Dielen et al., 2001). Results showed that defoliated branches with cytokinin treatment had a significant effect on the number of buds per branch (Fig.4). Cytokinin (100 mg/L) plus defoliated treatment produced the highest number of buds (23) followed by 50mg/L cytokinin with defoliation and defoliated treatment only, with 19 and 15 number of buds respectively. The control branches produced the lowest number of buds (12) at the 15th day of observations (Fig.4). Among the treatments, 100mg/L kinetin with defoliation showed the highest increasing trend in bud number every three days of observation.

Bract growth

As can be seen in Figure 6, kinetin with leaf defoliation treatment exhibited higher bract growth from the 1st day till the 15th day of observations. At the 3rd day of observations, the highest bract length was 4.2 cm, observed in the 100 mg/L kinetin with leaf removal treatment followed by 50mg/L kinetin with leaf removal and leaf removal treatment with bract lengths of 3.8 cm and 3.5 cm respectively, whereas the lowest bract length of 1.4 cm was observed in the control branches (Fig. 6). Bract growth was rapid initially until the 9th day of observation, after which it was relatively slower between the 9 to 15th day. Bract length was found to be statistically significantly higher than the control in all the treatments with defoliation plus kinetin treatments exhibiting the faster growth rate.

Bract weight

As shown in Figure 7, all the treated branches in this study yielded higher bract weight than the control treatment. Kinetin 50 mg/L plus defoliation treatment increased the bract weight by 127%, followed 100mg/L kinetin and defoliation treatment and defoliation only treatment with values of 70% and 60% increases on the 18th day of observations. The data between the treatments and control was found to be statistically significant different.

Bract length and width

Kinetin plus defoliation treatment had significant effects on bougainvillea bract length and width. As can be seen in Figure 8 all the different treatments produced larger bract size compared to the control treatment. Kinetin 50 mg/L plus defoli-

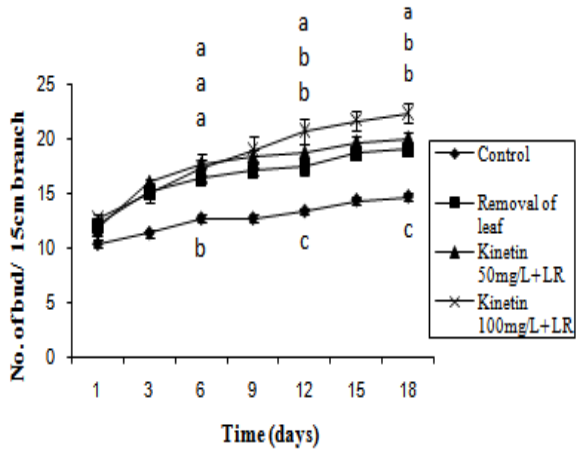

Fig.4 Number of bud/15 cm treatment branch for Bougainvillea as affected by different treaments

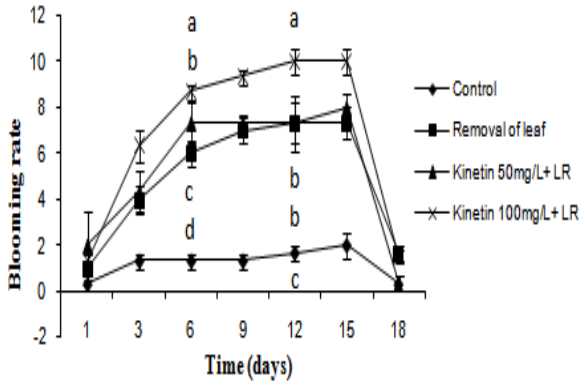

Fig.5 Effect of defoliation and different concentrations of cytokinin on bract blooming rate for Bougainvillea

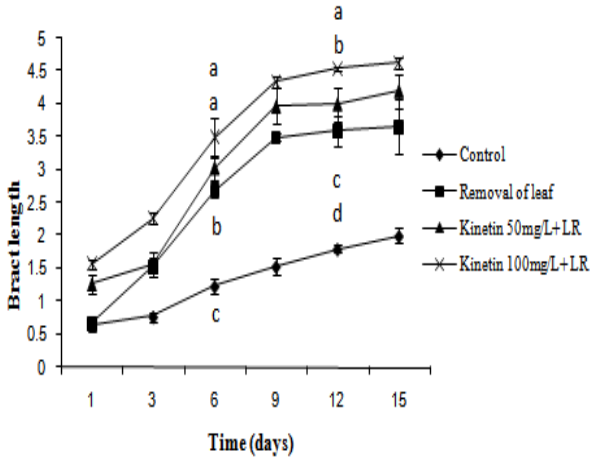

Fig. 6 Effect of defoliation and different concentrations of cytokinin on bract growth (length) of Bougainvillea

ation treatment produced the largest bract size (length and width) followed by 100 mg/L kinetin with defoliation and defoliation only treatment.

Weight of flower

From the results it was observed that defoliation of young leaves plus kinetin treatment produced a bigger sized flower (Fig. 9). Kinetin treatment with defoliation also had a significant effect on the weight of the flowers, including bracts. Kinetin (50 mg/L) plus defoliation treatment produced the highest bract weight (0.69g) including flower followed by 100 mg/L kinetin plus defoliation treatment and defoliation only treatment, with a weight of 68 and 67 g respectively. The control branches exhibited the lowest bract and flower weight.

Chlorophyll fluorescence

Chlorophyll fluorescence has become one of the most powerful and widely used techniques available to plant physiologist and ecophysiologist. Chlorophyll fluorescence gives information about the state of photosystem II in the chloroplasts thylakoid membranes. Chlorophyll fluorescence was different in the different cytokinin treated leaves and the control (Fig.10). Results showed that kinetin plus defoliation treatment had a significant effect on chlorophyll fluorescence. The highest maximum fluorescence (Fm) was observed in 50 mg/L kinetin plus defoliation treatment followed by defoliation treatment alone and treatment with 100 mg/L kinetin plus defoliation. Similarly variable fluorescence (Fv) was highest in 50 mg/L cytokinin plus defoliated branches followed by defoliation treatment alone and 100 mg/L kinetin plus defoliation (Fig.10). From the results shown in Table 1, it can be seen that optimum quantum yield (Fv/Fm) was highest in defoliation treatment alone, followed by 50 mg/L and 100 mg/L cytokinin plus defoliation treatments. These results were found to be in consonance with that of Zhang et al. (2003) who reported that plant growth regulators increased the photochemical efficiency (Fv/Fm) in Bluegrass Sod.

Stem elongation

Stem elongation in the defoliated plants were found to be much less compared to the intact plants. It was found to be statistically significant on the 9th day of observations (Table I). As can be seen, that highest stem length of 30cm was observed in the control branches followed by the defoliated branches and defoliation with 50mg/L cytokinin treatments which showed a stem length of 27 and 21 cm respectively. Defoliated branches with 100 mg/L kinetin treatment exhibited the lowest stem length of 19 cm. Our results suggest that kinetin showed an inhibitory function with regard to stem elongation and enhanced flowering.

Bract blooming and longevity

As shown in Table 1, kinetin had an effect on early flower blooming in *Bougainvillea.* It was observed that 21.23 and 20.55 days were required for the flowers to bloom in intact (control) and defoliated branches, whereas, 19 days was required for blooming in defoliated plants plus 50 and 100 mg/L kinetin treatment. Defoliation with kinetin treatment had a significant effect on bract longevity in *Bougainvillea.* It was

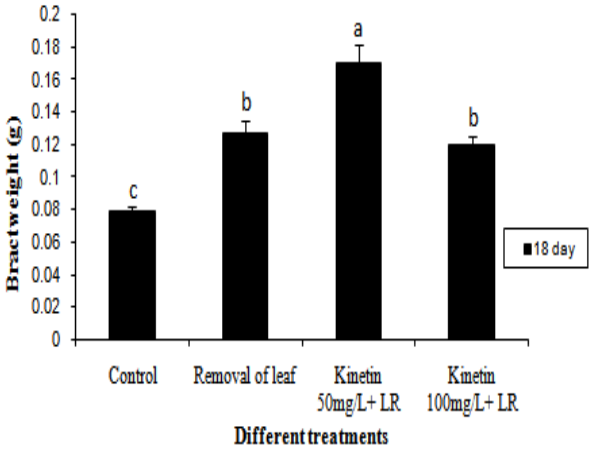

Fig.7 Effect of defoliation and different concentrations of cytokinin on bract weight of Bougainvillea at 18 day of observation

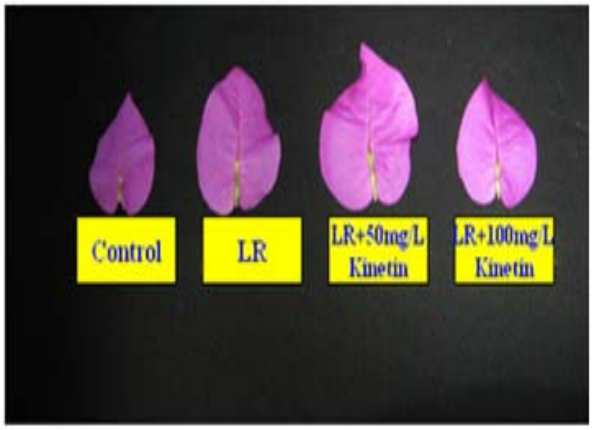

Fig.8 Photograph showing the effects of defoliation and cytokinin treatments on bract length and wide of Bougainvillea (LR= Leaf removal)

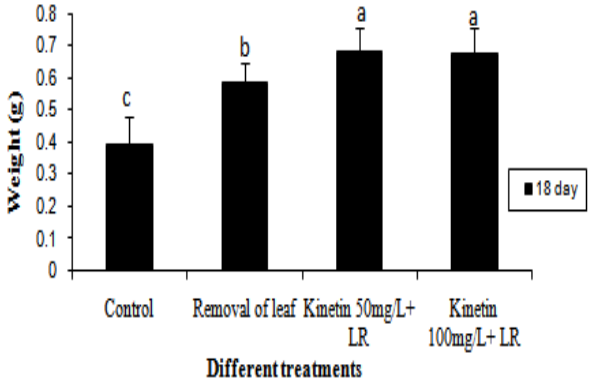

Fig.9 Effect of defoliation and cytokinin treatments on bracts weight including flower at 18 th day of observation

found that defoliation plus 50mg/L kinetin increased the bract longevity by about 5 days followed by defoliation plus 100mg/L kinetin treatment and defoliation only treatment which increased bract longevity by four days and two days more respectively compared to the control treatment.

Discussion

Kinet et al. (1985) reported that cytokinin application in short day flowering plants can induce flowering earlier compared to the control plant. From this study, it was observed that both the removal of young leaves and exogenous application of cytokinin promoted flower development. It has been suggested that defoliation of young leaves removes competing sinks for assimilates (Tse et al., 1973). Response to the combined effect of defoliation plus kinetin treatment was apparent in this study by the promotion of well formed inflorescences at node n. Cytokinin applications further increased the accumulated in defoliated plants. Removal of young leaves plus cytokinin 50mg/L treatment showed a positive effect on the number of nodes in the 1st inflorescence, individual bract weight, weight of bracts including flower and fluorescence intensity and bract longevity. This is possibly be due to an increase in the cytokinin level in shoot apex, although this was not determined. These findings are in agreement with those obtained by Chang et al. (1999). They reported that cytokinin levels in tuberose corms during floral induction increased and suggested that cytokinins have a role in tuberose apex evocation. In addition, kinetin have been shown to occur in higher concentration in short-day flowering plant (Henson and Wareing, 1974). An additional factor that can promote flower bud development is the endogenous cytokinin level in the shoot apex. Wim et al. (1990) reported that exogenous application of cytokinin in *in vitro* cultures of tobacco stimulated flower bud formation. From our results, it is also clear that cytokinin treatment with defoliation increased the production of floral buds. Similar findings were also reported by Even-Chen et al., (1979). They reported that in Bougainvillea, cytokinin treatments promoted flower bud formation and decreased endogenous gibberellin content was necessary for flower bud formation. Treatment with kinetin plus defoliation enhanced the blooming numbers and increased the bract length of *Bougainvillea.* Chang et al. *(*1999) made similar observations and reported that during floral induction, cytokinin levels in tuberose corms increased and suggested that cytokinins have a role in tuberose apex evocation . Ulger et al. (2004) reported similar results and suggested that an increase in cytokinin during the flower induction period in olive may have a positive role on floral formation. Corbesier et al. (2003) reported that cytokinin increases were well correlated with the early events of floral transition. In short day condition, defoliation plus kinetin treatment performed the best result incase of bract length and weight of bracts including flower in Bougainvillea. These results are partially supported by the findings of Bemier et al. (2002). They reported that an exogenous cytokinin application to vegetative *Sinapis* plants grown in short days can induce various cellular and molecular changes in the SAM that are normally associated with floral transition. Our results show that the intact (control) plants exhibited the lowest numbers in all the parameters studied compared to the defoliated plants and the cytokinin treated plants. It is well documented that removal of young leaves may remove the source of inhibitor(s) of inflorescence development. There is no direct evidence to support this idea although it has

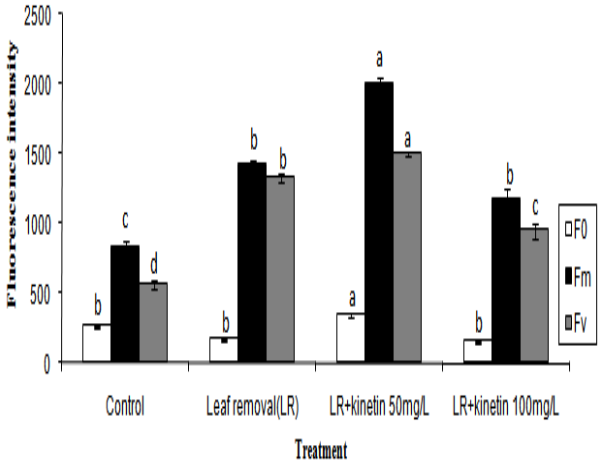

Fig.10 Effect of leaf removal and cytokinin treatment on florescence intensity (yield) of leaves of treated branch. F0= Lower fluoresenece, Fm= Higher fluorescence and Fv= Relative variable florescence

been shown that defoliation strongly inhibits stem elongation (Ito et al., 2001) and the young leaves are undoubtedly a source of naturally occurring growth regulators. This was suggested by Hackett and Sachs in 1968 who reported that in *Bougainvillea,* gibberellic acid strongly inhibits inflorescence development and the young leaves are a source of gibberellins. Jacqmard et al. (2002) reported similar results in sunflowers and suggested that one would expect removal of young leaves to lower gibberellin levels and thereby promote flowering.

Conclusion

From the above results, it can be concluded that to enhance the inflorescence development and improve the bract quality in Bougainvillea, defoliation plus cytokinin treatment was effective compared to only defoliation treatment. It can be summarized that cytokinin plus removal of young leaves are promising in enhancing inflorescence development, improving bract quality and increasing the bract longevity in *Bougainvillea* flowers under field or home garden conditions. These techniques can be applied in *Bougainvillea* or other ornamental flowering plants to make our environment esthetically beautiful.

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