CHAPTER 6

Effects of naphthaleneacetic acid (NAA) and gibberellic acid (GA_3) on bract longevity and senescence of *Bougainvillea spectabilis*

6.1 Introduction

It is well known and documented that plant hormones play an important role in controlling the growth, development, metabolism and morphogenesis of plants (Claus, 2008). One of these, the gibberellins are plant growth hormones that are well known for their ability to elicit different mode of actions (Hye and William, 2008). Ogale *et al.* (2000) reported that gibberellic acid (GA_3) induced changes in *Portulaca garndiflora* flower size by ~ 40% and color from crimson red to complete white. GA_3 has also been reported to reduce the time needed for inflorescence emergence, accelerated flowering and increased the number of flower buds and open flowers in most growing conditions (*Cucumis sativus*) (Khan and Chaudhry, 2006). In addition, the application of GA_3 has the potential to control growth, flowering and induce earliness meristem.

1-Naphthalene acetic acid, commonly abbreviated NAA, is an organic compound with the formula of $C_{10}H_7CH_2CO_2H$. NAA is a plant hormone in the auxin family and is an ingredient in many commercial postharvest horticultural products (http://www.sciencelab.com). Contrary to GA_3, the effect of the plant hormone NAA on a plant often depends on the stage of the plant's development (Chang and Chen, 2001). NAA have been reported to prevent flowers and fruits from dropping off trees before maturation. NAA has also been used to prevent the undesirable sprouting of stems from the base of ornamental trees. Although GA_3 is well-known in promoting flower growth and

development, its involvement in controlling the delay of senescence is less clear (Rosenvasser *et al*, 2006). There have been reports that GA_3 has little effect as an ethylene inhibitor, inhibiting both climacteric ethylene production and flower senescence (Kun *et al.*, 2008). In view of the above reports, it is possible that a combination of both GA_3 and NAA, when applied together, are able to produce larger flowers with good longevity (Luiz *et al.*, 2008).

Therefore, the purpose of this study was to investigate the flower blooming, discoloration and determine the influence of different concentrations of GA_3 and NAA on flower longevity when applied during different flower development stages of potted bougainvillea.

6.2 Materials and Methods

6.2.1 Plant Materials

Two-year-old bougainvillea plants were used in this study. The plants average height was 50 cm and canopy wide was 25 cm. The trees were chosen which consisted of 8 main branches and 5 minor branches. During the experiment, the plants were placed under exposed sunlight conditions (temperature, 21-32°C, maximum PAR 2100 μE m^{-2} s^{-1} and relative humidity of 60-90%).

6.2.2 Hormone Treatments

Treatments were set in a completely randomized design (CRD). Each treatment was carried out in three replications and sprayed on a two day interval. Nine plants bracts were sprayed with 50, 100 and 150 ppm NAA, respectively. Other nine plants bracts were sprayed with 50 ppm NAA + 100 ppm GA_3, 100 ppm NAA + 100 ppm GA_3, 150 ppm NAA + 100 ppm GA_3, respectively. Three plants bracts were sprayed with 100 ppm GA_3 and the controlled three plants bracts were sprayed with distilled water.

6.2.3 Data Collection

Bract development was divided into five stages (* 1st stage: initial budding, 2nd stage = bud opening, 3rd stage = partial blooming, 4th stage = full blooming, 5th stage = 50% discoloration or flower abscission) to measure and compare bract longevity and weight among the different treatments. Bract longevity, bract weight, bract discoloration were measured weekly. Each treatment had three plants, and each plant had all the four bract stages. Bracts of three flowers in the same stage were considered to be a unit of replication.

6.2.4 Bract Longevity

Bract longevity was counted as the number of days from treatment setting stages (1st, 2nd, 3rd and 4th) to 50% discoloration or abscission (5th stage).

6.2.5 Chlorophyll content

Chlorophyll content was measured as described in the previous chapter (5.2.8).

6.2.6 Bract Weight and Length Measurements

Bract weight and length measurements were measured as described in the previous chapter (5.2.5).

6.2.7. Evaluation of Abscission and Petal Discoloration

Abscission was counted in number of days from the treatment setting stage (1^{st}, 2^{nd}, 3^{rd} and 4th) to the flower abscission (5^{th}) stage. Bract (flower) status was observed everyday. Petal discoloration was also observed by observing petal symptoms. Color change (from pink to discolor) was determined according to the way of Hossain *et al* (2007).

6.2.8 Stomatal Conductance

Stomatal conductance was measured as described in the previous chapter (3.2.7).

6.2.9 Total Soluble Sugar

Total Soluble Sugar (Ts) was measured as described in the previous chapter (3.2.6).

6.2.10 Statistical Analysis

Statistical analysis was evaluated as described earlier (3.2.10).

6.3 Results

Bract longevity was prolonged by applying NAA (50, 100 and 150 ppm) at all stages of flower development (Table 6.1). Application of 100 ppm GA_3 showed the shortest duration of (20 days) flower life compared to the other treatments. However, bract longevity was prolonged by applying NAA (50, 100, 150 ppm) + GA_3 at the stage of one, two, three and four by 157%, 146%, 155% and 216%, respectively, compared to the control. When single treatments of NAA or GA_3 were compared with combined treatments (NAA + GA_3), the prolonging effect of NAA + GA_3 was significantly better. Among the combined treatments of NAA plus GA_3, the best result was exhibited by GA_3 (100 ppm) plus NAA (100 ppm). These results showed that harvest quality of bougainvillea bracts were vastly improved as a result of the combined activity of NAA and GA_3 throughout the different developmental stages (Table 6.1).

There was a considerable increase in petal chlorophyll content from 13% at stage one to over 170% at stage four by the 100 ppm NAA treatments. The values were lower in the case of the combined hormonal treatments (NAA and GA_3). At stages three and four, the chlorophyll content was very low in the NAA treatments whilst in the GA_3 and GA_3 plus NAA treatments no chlorophyll content was recordable. The results imply that there was a little effect of NAA on the bract natural color. Consequently, the chlorophyll content of the bracts at the subsequent stages were affected. Furthermore, it was observed that the bract chlorophyll content in the GA_3 and GA_3 plus NAA treatments during all the stages were lower by 71% than the control. In addition, as for the leaf chlorophyll content, it was lower (average 11% in NAA and 20% in NAA plus GA3) in all the treatments when compared to the control. The observation could possibly be attributed to the effect of NAA and GA_3 on chlorophyll synthesis.

Table 6.1: The effects of NAA and GA_3 solutions on bract longevity at different stages* of bract development in *Bougainvillea* sp. Means followed by different alphabets within column are statistically different at 5% level of significance, using DMR test.

Treatment	**Bract Longevity (days)**			
	Stage1	**Stage2**	**Stage3**	**Stage4**
Control	19.33±1.65 gh	13.33±0.88 g	9±1.15 gh	6.33±0.33 g
50ppm NAA	28.33±1.76 def	23.33±1.2 fe	20.66±1.76 cdef	14.33±1.20 f
100ppm NAA	30±2.08 de	27.66±0.88 cd	22.66±1.2 abcde	19.33±0.88 bc
150ppm NAA	30.33±1.2 d	25.66±0.33 de	21.33±1.76 bcde	18.0±1.15 cde
100ppmGA	20±1.15 g	12.33±1.2 gh	11.33±0.88 g	5.33±0.33 gh
100ppmGA+ 50ppm NAA	52±1.52 ab	35.66±1.2 a	23.33±4.7 abc	21.66±1.45 ab
100ppmGA+100ppm NAA	54.66±1.76 a	34.66±1.45 ab	26.66±0.88 a	23.0±1.15 a
100ppmGA+150ppm NAA	50.66±1.33 abc	32±1.15 abc	24.66±0.88 ab	19±0.57 bcd

* Stage 1: initial budding, Stage 2: bud opening, Stage 3: partial blooming, Stage 4: full blooming.

Table 6.2: Leaf and bract Chlorophyll content (chlorophyll) at the different stages of bract development in different NAA and GA_3 treatments, Means followed by different alphabets within columns are statistically different at 5% level of significance, using DMRT test.

Treatment	**Chlorophyll Content**				
	Stage1	Stage2	Stage3	Stage4	Leaf*
Control	7.8±1bc	5.86±0.23 bd	1.63±0.24 cd	0	54.2±0.98 a
50ppm NAA	8.83±0.71 abc	6.26±0.3 abc	2.13±0.20 ab	1.26±0.12 c	50.46±1.31 b
100ppm NAA	8.86±1.09 ab	6.66±0.20 a	2.7±0.17 a	1.7±0.28 ab	48.36±0.63 bc
150ppm NAA	9.63±1.21 a	6.4±0.49 ab	2.26±0.24 abc	2.1±0.26 a	45.1±1.05 cde
100ppm GA_3	2.23±0.23 defg	0.6±0.05 fg	0	0	46.5±0.25 cd
100ppm GA_3+ 50ppm NAA	2.6±0.35 def	0.86±0.08 f	0	0	44.23±0.31def
100ppm GA_3+100ppm NAA	3.2±0.32 d	1.26±0.17 e	0	0	43.3±1.59defg
100ppm GA_3+150ppm NAA	2.93±0.34 de	0.5±0.08 gh	0	0	42.46±1.12efgh

*Leaf SPAD value were measured at the end of experiment

Table 6.3: The effect of NAA and GA_3 treatments on bract discoloration at the different stages of bract development in *Bougainvillea* sp. Mean separation within columns by analysis of Duncan's multiple ranges.

Treatment	**100 % Discoloration (days)**			
	Stage1	**Stage2**	**Stage3**	**Stage4**
Control	19.57±2.54 *ef	13.60±0.75 *f	8±1.29*fg	7.63±0.29 *e
50ppm NAA	35.33±1.62 cd	29.33±2.17 de	26.66±1.79cd	20.33±1.50 cd
100ppm NAA	37±1.0 c	32.54±0.48 cd	29.21±1.24b	23.45±1.78 ab
150ppm NAA	39.63±1.3 c	29.66±0.93 d	24.33±1.65de	20±1.18 cd
100ppmGA	21±2.26 *e	12.33±0.7 *fg	11.33±0.74*f	5.33±0.31 *ef
100ppmGA+ 50ppm NAA	59±2.15 ab	40.66±0.24 a	31.33±4.7a	23.66±2.3 ab
100ppmGA+100ppm NAA	62.66±2.76 ab	38.66±2.55 ab	30.66±0.45ab	25±1.65 a
100ppmGA+150ppm NAA	60.66±2.52 a	36±2.5 bc	28.66±0.45bc	21±0.87 bc

*Abscission stage

It has been reported that NAA not only inhibit ethylene biosynthesis and contribute to longevity but also slow down natural shoot growth (Zhu and Davies, 1997). The influence of all hormonal treatments on bract discoloration was observed throughout the experiment by the method of Hossain *et al.* (2007). Research results showed that there was a significant delaying in discoloration in the NAA and GA_3 plus NAA treatments compared to the control and singly GA_3 treatment. The control and GA_3 treatments showed abscission whereas the other treatments exhibited a gradual discoloration without abscission. Bract discoloration was delayed when NAA was applied in all the stages. However, bract discoloration process was remarkably reduced from 216% at stage one to over 200% at stage four in the combined NAA and GA_3 treatments. The results suggest that there was a positive effect of the combined treatments of NAA and GA_3 on the bract discoloration process (Table 6.3).

Total soluble sugar (Ts) at the end of the experiment was significantly higher in GA_3 and GA_3+NAA than NAA and control. TSS level was enhanced by 45% in 100 ppm GA_3 treated plant leaf. The minimum total soluble sugar was obtained in all NAA treated leaf compared with all treatments. TSS level was 40% lower in 150 ppm NAA than in control (Fig. 6.1).

Bract weight was almost the same in all the treatments at the 1^{st} stage (Fig. 6.2). From the 2^{nd} stage, onwards change was observed and the maximum bract weight was recorded in 100 ppm GA_3 treatment. When the individual treatments of NAA and the combined treatments of NAA and GA_3 were compared, the bract weight was significantly higher in all the NAA plus GA_3 treatments and bract weight was decreased as the NAA concentration increased. At the 4^{th} stage, the highest bract weight was observed in the 100 ppm GA_3 treatment. It is clear that 100 ppm GA_3 had a positive effect on bract weight rising compared to all treated concentration of NAA.

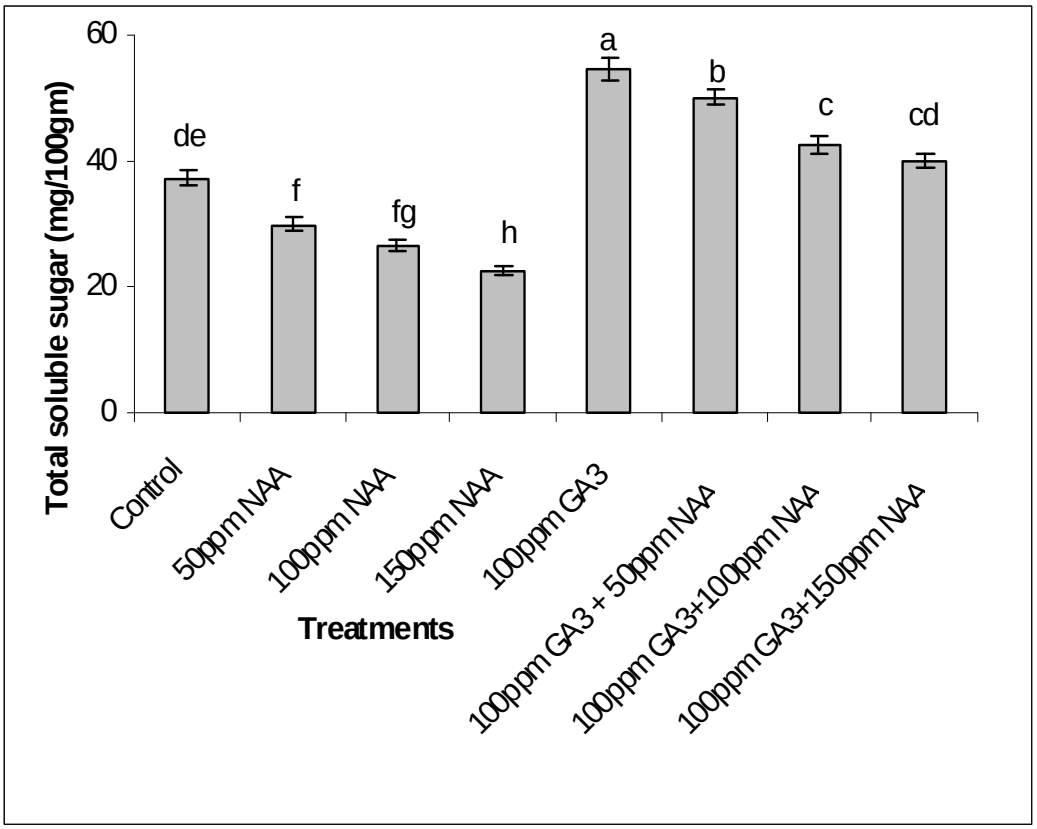

Figure 6.1: The effect of NAA and GA_3 treatments on Ts of leaves. Means followed by different alphabets above bars are statistically different at 5% level of significance, using DMR test.

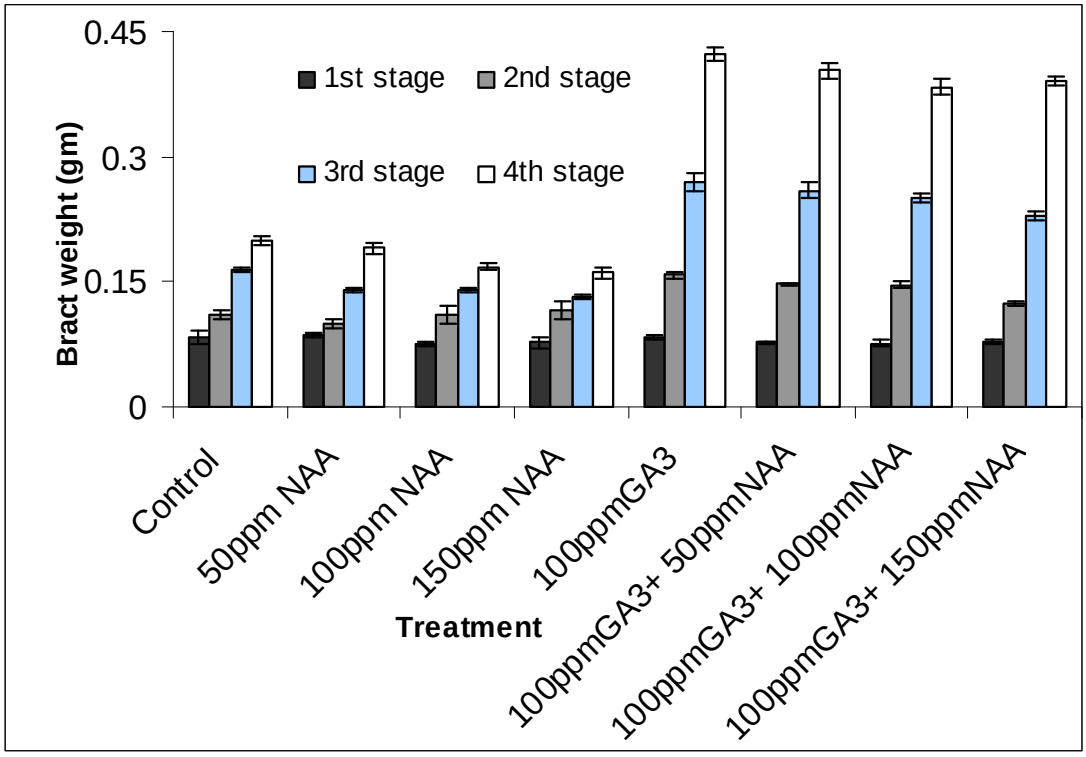

Figure 6.2: The effects of NAA and GA_3 treatments on bract weight at different developmental stages (5% level of significance, using DMR test).

With regard to stomatal conductance (40.3 m^2s/mol), it was higher in the control treatment compared to all the hormonal treatments (Fig. 6.3). Amongst the hormonal treatments, stomata frequency was significantly higher in the GA_3 treated branches leaves than in the NAA and NAA plus GA_3 treatments. The difference in stomatal conductance between NAA and NAA plus GA_3 leaf surfaces were probably related to the fact that NAA caused a reduction in stomata number. The negative effects of NAA on growth are again exhibited here. All the leaves treated with NAA showed a little wrinkle on its surface area. In the case of bract diameter, the mixed treatments of GA_3 and NAA and the single treatment of GA_3 clearly showed a positive effect by enlarging bract size. However, the single NAA treatments showed a slightly negative effect with regard to bract size compared to the control. The diameter of fully open bracts was about 45 mm (Fig. 6.4 and Fig. 6.7) in GA_3. Spraying with low concentrations of NAA plus GA_3 stimulated flower size with diameters ranging from 44 to 39 mm.

Shoot length was extremely affected by all the NAA treatments when applied singly, compared to the control (Fig. 6.5). Shoot length was significantly increased by 71% by the influence of GA_3, probably due to its well known effect on early cell development. However, it decreased remarkably up to 14% in the presence of NAA in all the GA_3 plus NAA treatments.

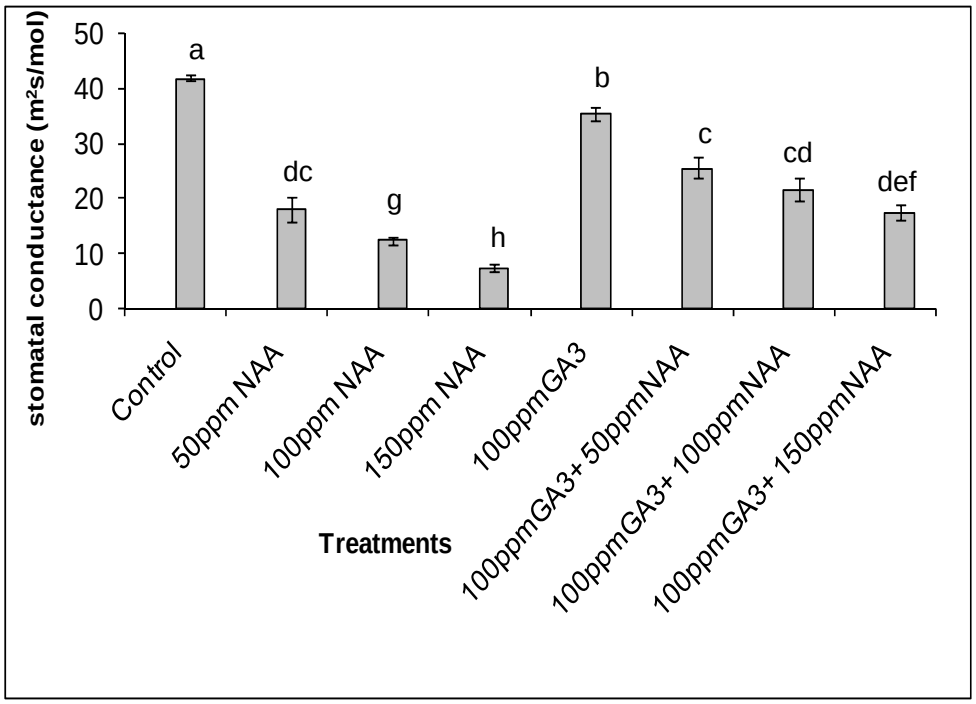

Figure 6.3: Stomatal conductance in leaves of branches treated with NAA and GA_3. Means followed by different alphabets are statistically different at 5% level of significance, (using DMR test)

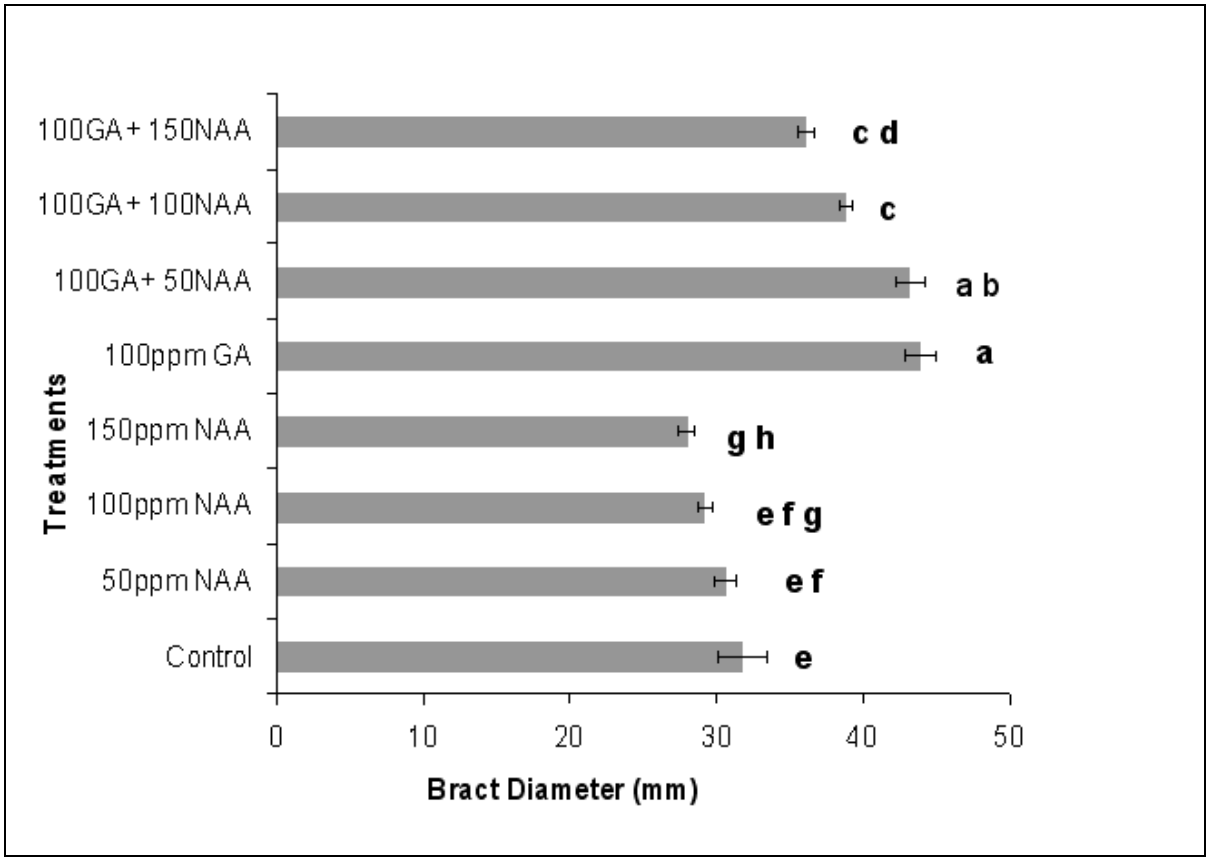

Figure 6.4: The effects of the different hormonal treatments on bract diameter and size (mm) at the 4th developmental stage in *Bougainvillea* sp. Means followed by different alphabets are statistically different at 5% level of significance, using DMR test.

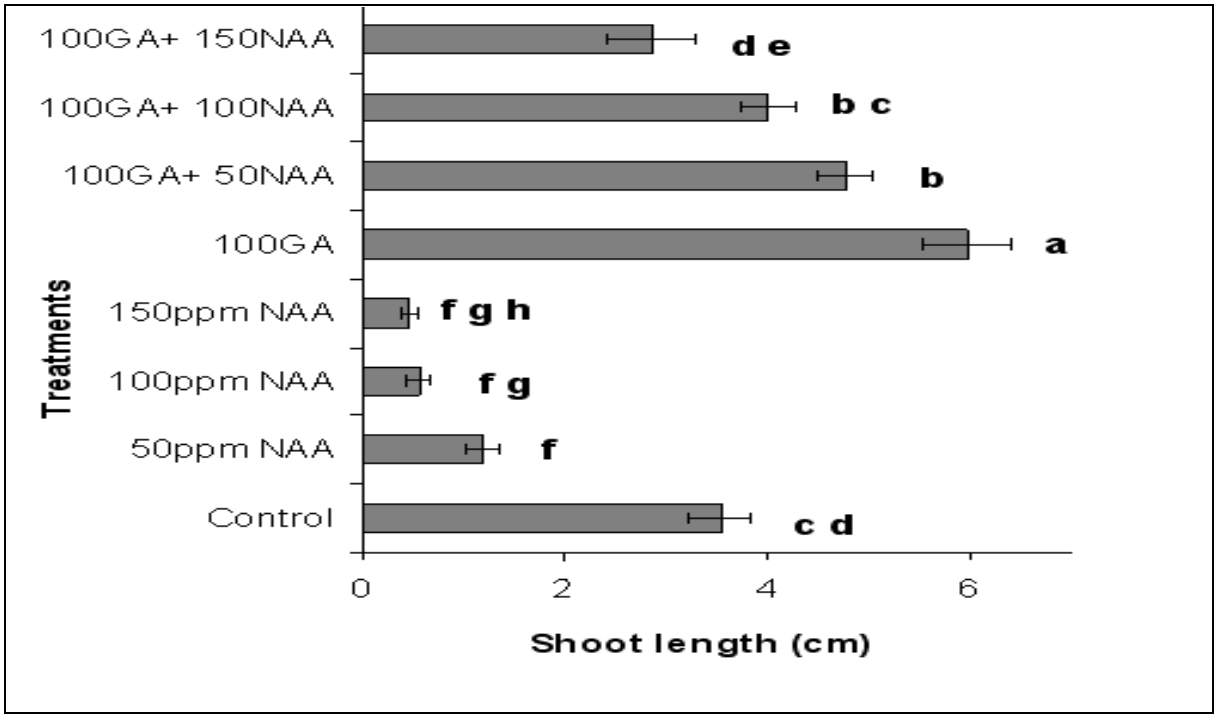

Figure 6.5: The effect of NAA and GA_3 treatments on shoot length. Means followed by different alphabets are statistically different at 5% level of significance, using DMR test.

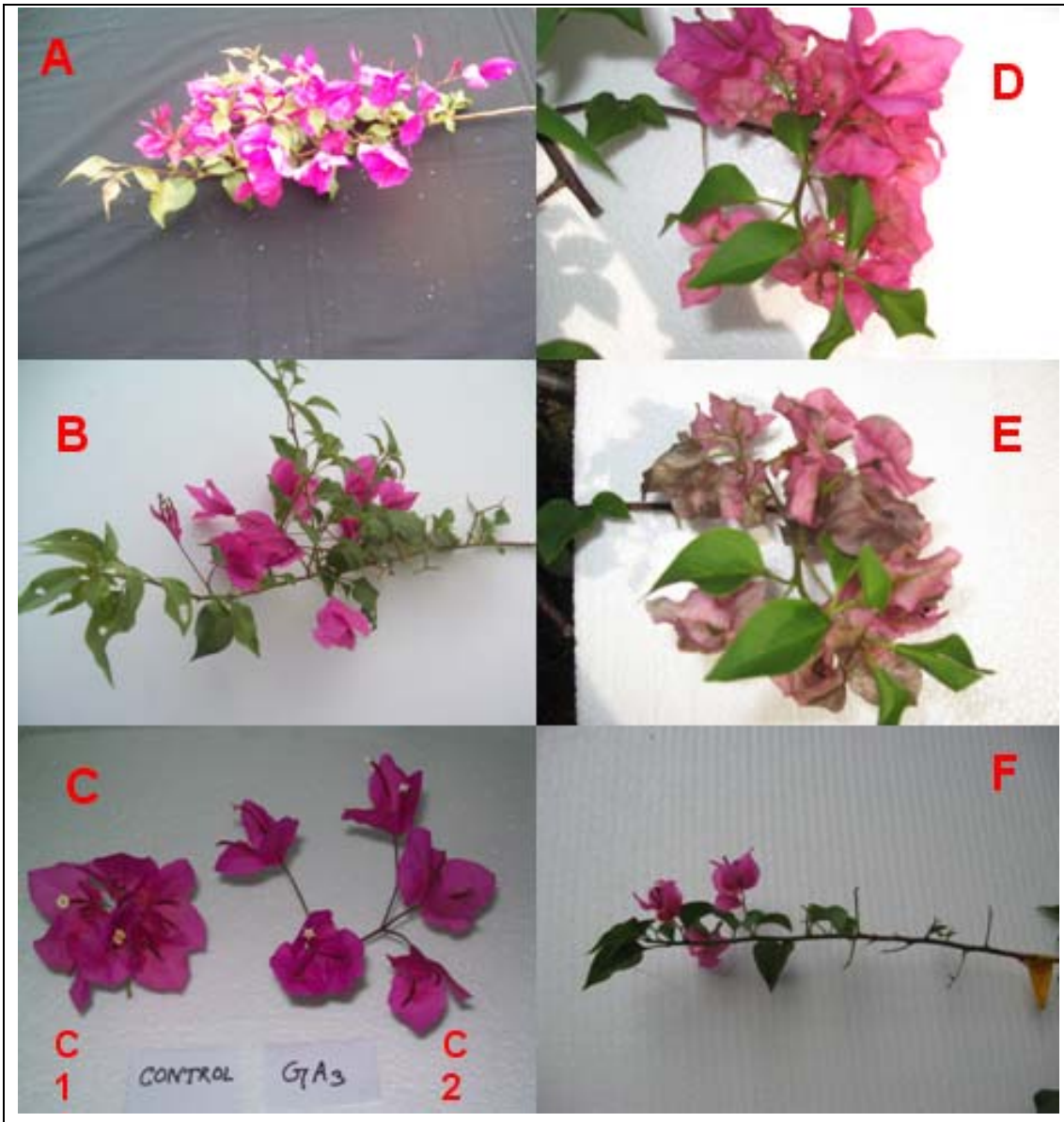

Figure 6.6: Photographs show the effects of mixed treatments (100 ppm GA_3 plus 100 ppm NAA) and 100 ppm NAA on the different developmental flowering stages in *Bougainvillea* sp. A shows the petal architecture and size after 25 days; B, flowers 40 days after applying mixed treatment (100 ppm GA_3 + 100 ppm NAA); C represents the control (C1) and 100 ppm GA_3 (C2) treatments; D and E represents 100 ppm NAA treatment, 25 and 40 days after application respectively; F shows control treatment after 25 days.

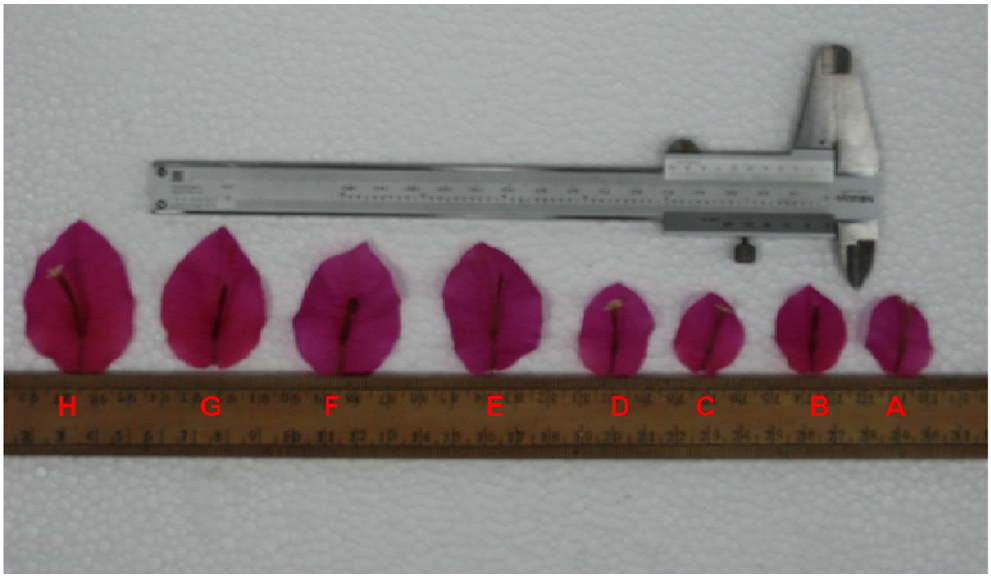

Figure 6.7: The effects of NAA and GA_3 treatments on 4th stage bract. Where, A is control, B is 50 ppm NAA, C is 100 ppm NAA, D is 150 ppm NAA, E is 100 ppm GA_3+50 ppm NAA, F is 100 ppm GA_3+100 ppm NAA, G is 100 ppm GA_3+150 ppm NAA. H is 100 ppm GA_3.

6.4 General Discussion

In the present study, it was observed that the treatments of NAA with GA_3 significantly improved flower size, weight, and longevity of bougainvillea. The improvement of flower longevity and delay in discoloration could possibly be the result of inhibiting ethylene action of NAA (Chang and Chen, 2001). The variable effects of GA_3 on bract weight and delay in discoloration was probably due to cultivar differences in soluble carbohydrate level of leaves. The action of GA_3 with NAA might have been to increase soluble carbohydrates in the abscission zones. As a result, the soluble sugar content of flower petals are increased at the time of flower opening and reaches a maximum before full opening stage. Consequently, premature abscission was prevented at the different flowering stages (Van Doorn, 2004 and Van Doorn, 2002).

Some studies have suggested that bougainvillea, like *Christmas cactus*, might be sensitive to ethylene in the early flower bud stages. Therefore, a lot of buds were abscised at the budding stage before full bloom (Han and Boyle, 1996). It was also suggested that the bracts were less sensitive to ethylene in the early stages of development (Elgar *et. al.*, 2003), thereby allowing NAA to inhibit abscission more effectively, as ethylene production was neutralized by NAA in the early stages of bract development. In addition, the variable effects of mixed GA_3 and NAA treatments at different stages of bract development could be due to differences in ethylene production and or sensitivity at different bract maturities. Chang and Chen (2001) reported that NAA delayed bract abscission in bougainvillea. In this study, bract longevity was significantly prolonged by 100 ppm NAA plus 100 ppm GA_3 and in treatments with 50 ppm NAA. However it was observed that the prolonging effect of NAA in this cultivar was more effective in the early stages of bract development

and the effects decreased as the bracts matures (Table 6.1 and Table 6.3). These results are in agreement with studies conducted on the *Eustoma* flower (Ichimura and Goto, 2002; Ichimura *et al.*, 2000) and *Easter* cactus (Han and Boyle, 1996).

6.4.1 Stomatal Conductance and Chlorophyll Content

The leaf tends to have its stomata closed during severe chemical (NAA) stress. The stomata adjustment towards progressively severe stress indicates their chemical resistance mechanisms as reported by Yoko *et al.* (2006). Amongst the numerous theories, this response of the stomata may be regarded as a feedback response where a signal from the NAA is transmitted to the leaves and bracts so that natural growth and cell activity is postponed by NAA to protect ethylene production through ACC path. The effect of NAA was more pronounced when its concentration was increased. Low stomata conductance reduced leaf chlorophyll content when compared to the control leaves. There was a positive correlation between NAA plus GA_3 treatment and stomatal conductance even though it was lower than the control leaf. Therefore, in the presence of GA_3, stomatal conductance was higher than in the NAA treatments alone. Consequently, chlorophyll content was higher in NAA than in NAA plus GA_3 treated plants leaf and petal. This implies that GA_3 might be effective in blocking NAA activity and stomatal conductance might be increased by the availability of concentrated external nutrients (GA_3).

In the water control, bracts were abscised rapidly, within twenty days (Table 6.1). In the presence of GA_3, bract discoloration was delayed in all GA_3 plus NAA treatments (Table 6.3 and Fig. 6.6). This hormonal effect was due to exhaustive and rapid nutrient utilization by plant. On the other hand when NAA was applied singly the bracts showed rapid discoloration (in the 50, 100 and 150 ppm) than NAA plus GA_3. In addition, the GA_3

treatment increased flower size and weight (Fig. 6.7). On the other hand, flower size and weight were not increased by NAA. Andrea *et al.* (2004) also observed a similar effect of NAA on flower size and weight. The reason behind this NAA effect was, it blocked the ethylene action and delayed growth process or less active in cell elongation processing. Therefore, stomatal conductance (Fig. 6.3) and chlorophyll content were also lower in NAA treated plants.

6.4.2 Total Soluble Sugar, Bract Weight and Discoloration

Gibberellins are well known to increase hydrolysis of starch and sucrose into glucose and fructose, which were utilized by the flowers for floret opening (Emongor, 2004). The increased sugars in the flower heads and stems of bougainvillea flowers may increase the osmotic potential of the flower bracts and petals, thus improving their ability to absorb nutrients and maintain their turgidity, which may explain the increase of flower weight in different developmental stages and observed in this study too (Fig. 6.2). NAA in presence of GA_3 delayed bract abscission and color fading because of GA_3 increased the hydrolysis of TSS which delayed petal abscission and color fading (senescence) (Fig. 6.6). There is a possibility of NAA to interact with GA_3 and hence delaying the senescence of flower by either altering the sensitivity of the tissue to ethylene or by delaying the natural rise in ethylene production or both (Zhu and Davies, 1997).