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FLOWER INDUCTION, CHLOROPHYLL FLUORESCENCE AND CAROTINOID OF *ALLAMANDA* SP. AS AFFECTED BY GIBBERELLIC ACID AND ALUMINUM SULFATE

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Abstract

Allamanda sp. is an important ornamental plant for environmental and medicinal values. Plant growth hormone keeps a superlative role to improve the flower quality like early flower induction, color and size as well as hybrid innovative production in the floriculture industries. The study was carried out to evaluate the effects of gibberellic acid (GA_3) at 100 mg/l and aluminium salt (sulfate) on the flowers development and early initiation. Number of buds and flower, flower weight, chlorophyll fluorescence yield and total carotenoid contents were determined. The result showed that GA_3 at 100 mg/l application in bud initiated spot swabbing was most effective in getting early bud and flower initiation, delaying senescence and discoloration (fresh) followed by $Al_2(SO_4)_3$ and water control. Chlorophyll fluorescence yield and carotenoid contents were also highest in flowers treated with GA_3. The quantum yield (Fv/Fm) was maximal in GA_3 treated flower. These results indicate that hormone application (GA_3) was the best treatment to develop flower quality of *Allmanda sp.*

Keyword: *Gibberellic acid; flower initiation; weight; carotene, Allamanda flower.*

Introduction

Allamanda species are native to the Americas, where they are distributed from Mexico to Argentina. Allamanda species are familiar as ornamental plants cultivated for their large, yellow and pink colorful flowers and make attractive to the environmental beautification (De Souza-Silva and Rapini, 2009). The Allamanda vine and flower perfectly combine the beauty and make them garden favorite. *Allamanda cathartica* has almost year-round evening fragrance from the multitude of flowers. Flowers are mostly yellow and environmental friendly (Antosh, 2012). Allamanda is used throughout the tropics as ornamentals. The flowering is a complete developmental process consisting of the sequential phases namely flower induction, initiation, flower opening, pollination and flower senescence. Flower induction or the influence of flowering time depends on some known factors such as physiological stress, nutrient availability, light, day length and temperature. The timing of the transition from vegetative growth to flowering is of paramount importance in agriculture, horticulture and plant breeding because flowering is the first step of sexual reproduction (Koning, 1982, Georges *et al.*, 1993). The plant hormones may have a profound effect on the growth and the development of the plant organ. Plant hormones may control the plant growth and the development by affecting the cell division, elongation and differentiation (Hossain et al., 2008).

Environmental factors, such as natural stress and nutrient availability influence flowering time, but perhaps the most important are light intensity, day length and temperature (Ana *et al.*, 2004). Other factors also have been found to induce flower earlier or frequent flowering such as plant growth regulating hormones and chemicals (Hossain et al.2008). The most reliable method of flower promotion has been found to be the application of plant growth regulators such as gibberellins and cytokinins. In most experiments, the best results have been achieved by GA_3 and along with some additional chemicals such as ethanol, 8-hydroxyquinoline sulfate and aluminium sulfate (Hossain et al., 2008; Ichimura and Korenagea, 1998; Ichimura and Ueyama, 1998).

High concentration of GAs showed a positive role on flower formation in olive during induction and initiation period. In addition, the application of gibberellic acid (GA_3) has the potential to control growth and flowering and induce earliness of meristem (Khan and Chaudhry, 2006; Sharma and Room, 2009).

GA_3 promotes flowering in a range of plant species. Many species that flower early in response to GA_3 also flower early in response to long days or vernalization (Mariko *et al.*, 2001). Ogale *et al.* (2000) inferred that GA_3 spraying changed the mode of action, by increasing the flower size to varying degrees (20- 40%) in all *Portulaca grandiflora* cultivars.. Research is being carried out throughout the world on how to improve bloom cycle, flower size, flower color and flower longevity. Many techniques have been conducted but there is still a significant lack of knowledge on allamanda flower enlargement and early blooming. Therefore, the study was undertaken to

investigate the effects of swabbing and growth regulators (100ppm,GA_3 and aluminium sulphate) on the early bud and flower initiation and freshness (delay abscission) as well as phytochemical chemical content of *Allamanda* sp.

Materials and Methods

Experimental site and plant materials

Methodology

Three trees were selected for the experiment. Plant to plant distance was 1.5 m. The tree was designed as triangle position. The treatments were employed on three selected branches. Bud initiated spot (base of flower bud) was chosen in each branch. For GA_3 100mg/l application, cotton was used to swab the solution to the base of flower bud. The swab was applied once a week and continued until 4 weeks after the treatment application. For $Al_2(SO_4)_3$ treatment the same procedure was followed. The following data was recorded.

Treatments setting

Treatments were applied maintaining completely randomized design (CRD). Each treatment was conducted in three replications and swabbed for 4 weeks. The selected branches flower bud (before initiation) were swabbed with GA_3 (100 ppm) and Aluminium S (100 ppm). The controlled branches were swabbed with the distilled water.

Quantum yield measurement

Chlorophyll fluorescence was measured by using Plant Efficiency Analyser (Hansatech Instrument Ltd., England). A leaf clip was attached to one of the leaves (selected closed to flower base) and kept in dark for 30-45 minutes to maintain dark adaptation. Then, the leaf clip was oriented with the shutter plate. When the light intensity was applied on the leaf, the fluorescence signal was counted for 3 seconds and observed the Quantum yield or Photosynthetic yield. The maximal fluorescence (Fm) and minimal fluorescence (Fo) value was taken from the display pad of Plant Efficiency Analyser machine. The yield of variable fluorescence (Fv) was calculated as Fm-Fo. Calculation of quantum yield was determined according to the equation Fv/Fm (Temperature = 28ºC, Time range = 10 µs- 3 sec).

Measurement of of bud and flower initiation and number

Bud and flower status was observed every week. Number of bud and flower, and longevity (freshness) was counted as the number of days from flower initiation to flower abscission and full blooming days was counted from flower initiation to full blooming stage.

Determination of Total Carotenoid Content

Total carotenoids were determined according to the methods of Lichtenthaler and Buschmann. The method consisted of repeated acetone extraction, until obtained colorless residue, with a pestle and mortar and filtered over filter paper. The extracts were made up to 50 ml with acetone. The concentration of carotenoids was measured at 470nm in a Shimadzu UV 160A spectrophotometer. The amount total carotenoid content was calculated were calculated according to the formula of Lichtenthaler and Wellburn(1985).

Results

The influence of all treatments on bud and flower initiation was observed throughout the experiments. In the present work, the number of bud initiation per branch was higher in GA_3 and aluminum sulfate than in water control **.** In fifth week bud number was observed to be two in water control, while it was nine and five in GA_3 and Aluminum sulfate respectively (Fig. 1). In the case of bud initiation, bud was initiated 3 days earlier in GA_3 and aluminum sulfate than in water control (Fig. 1)**.** The new bud initiation was particularly delayed and decreased by control (Fig. 2).

The influence of all treatments on flower initiation was observed throughout the experiments. The number of flower initiation per branch was higher in GA_3 and aluminum sulfate than in water control **.** In fourth week flower number was observed to be one in water control, while it was four and two in GA_3 and Aluminum sulfate respectively (Fig. 2).

Flower fresh weight was remained higher on 2nd day and from 3rd day it was decreasing for all treatments (Fig. 3). However, bract longevity, abscission and color was prolonged by applying GA_3 and aluminum sulfate compared to the water control.

It has been seen in Figure 3, flower treated in GA_3 showed the highest percentage of weight compared to other treatments. On the other hand, water exhibited lowest weight. The increasing effect of flower weight in GA_3 was significantly better than aluminum sulfate (Fig. 4a). Flower longevity was higher (8.7d) in aluminum sulfate followed by 5.5 and 4 d in GA_3 and water control.

The quantum yield of dark-adapted leaves determining the maximum efficiency of photosystem was showed (Table 1). The photosystem of dark-adapted leaves, measured by quantum yield showed lower values in control plants. Whereas, significantly highest value of photsynthetic yield, chlorophyll flourescence and quantum yield was observed in GA_3 treated plants (Table 1). In aluminum treated plants, the quantum value increased.

The carotinoid content showed a significant difference with respect to the applied chemical treatments compared to the water control (Table 1). The effects of the treatments on flower longevity were showed in Figure 4b. Very strong coorrelation was found between quantum photosynthetic yield and carotine content (Fig. 5).

Discussion

It has been described well that the commercial value of flowers is very dependent on its early flower initiation and blooming including flower color and weight. Allamanda plant is well known for its beautiful flowers and in rising landscaping activities. Its commercial value could be improved through early blooming and increasing its quality in terms of size and weight (Tjosvold *et al.,* 1994). Flower initiation and blooming rate could be improved also by using growth regulating hormones, such as GA_3. 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 may increase the osmotic potential of the 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.

In this experiment, the GA exhibited short period for bud formation and flower number/branch, flower weight and longevity was increased by GA and Aluminium sulfate. Hossain et al (2008) has shown that GA_3 increased the supply of total sugar in petal and treated leaf. It might be responsible for stimulating cell division, new leaf formation and ultimately more flower and frequently flower bud initiation. However, in the case of control treatment, the number of flower/branch was low due to the prolonged vegetative stage for shoot initiation and lack of leaves to utilize in photosynthetic process or lack of contribution of cytokinins from root towards shoot (Hossain and Mizutani, 2009; Calatayud, 2004; Hossain et al, 2007a). In addition, plants produced fewer flowering buds in water control than in chemical treatment. This indicated that the switch to early flowering was maintained at subsequent flower formation under preferable condition. Hossain et al. (2008) reported a similar report that plants initiate more and larger leaves in order to capture more sunlight and decrease the flowering time. But in control treatment, the plant showed a greater tendency toward vegetative reproduction rather than reproductive growth**.** The quantum system of this sp declined in control and increased GA_3 chemical. The quantum efficiency in the dark adapted leaf (Fv, Fo and Fm) has been carried out extensively as an indicator of hormonal and chemical stress on leaves or plant morphology (Bibi *et al.,* 2008). However, measuring quantum yield of photosynthesis with the light-adapted test in our studies proved the positive effect of chemical on leaf photosynthesis process. The present value indicated that quantum system of PSII increased significantly in GA_3 treatment. The lowest values were obtained in water treated branch leaves. Maximum efficiency of photosystem of dark-adapted leaves, measured by fluorescence quantum showed lower values in control plant leaf. Fv and Fm reflected the potential quantum efficiency and were used as a sensitive indicator of plant performance, with optimal values of around 0.8 measured for most plant species (Calatayud *et al.,* 2002; Johnson *et al.,* 1993). The value obtained by fluorescence, indicate that GA treated branch promoted better photosynthetic light reaction than control branch. This fact can be attributed, among other causes, to a higher radiation intercepted by the leaves to the enhancement of photosynthetic rates in the remaining mature leaves or to changes in photosynthetic capacity of mature leaves (Mediene *et al.,* 2002).

The accumulation of chlorophyll was significantly higher in plants which underwent in GA. Enhanced synthesis of chlorophyll and carotinoid by GA treatment had previously been reported and it has been suggested that the enhanced synthesis was attributed to the increased hormone activity in rose and bougainvillea plants (Angeles *et al.,* 2008; Hossain et al., 2007a). Jidapha et al (2006) observed the carotine content was varied from 0.4 -4.5 g/kg at different species of *Allamanda* flower which agreed with our results.

Conclusion

It can be concluded that , 100ppm GA3 and aluminium sulphate showed the effective growth regulators to induce bud and flower production, increase flower weight and longevity, photosynthetic fluorescence, quantum yield and carotinoid content. In addition, the results suggested that these growth regulators[GA_3 and $Al_2(SO_4)_3$ 100ppm] using swabbing technique instead of spray were effective for the flower development and longer the flower longevity with less chemical cost and quantity of chemicals without hazarding the environment instead of spray.

References

Antosh, G., (2012). Allamanda plant care-A bush of beauty. http://www.plant-care.com/allamandas-epitome-of-a-shrub-beauty.html.

Ana, P.A.V., C.L. Rita, R. Figueiredo, and B.K. Gilberto, (2004). Photoperiod and temperature effects on in vitro growth and flowering of *P. pusilla*, an epiphytic orchid. Plant Physiology and Biochemistry, 42: 411–415.

Angeles, C., R. Dolors, G. Elisa, and F.M. Pedro. (2008). Light acclimation in rose (Rosa hybrida cv. Grand Gala) leaves after pruning: Effects on chlorophyll a fluorescence, nitrate reductase, ammonium and carbohydrates. Scientia Horticulturae, 111: 152–159.

Bibi, A.C., D. M. Oosterhuis and E. D. Gonias. (2008). Photosynthesis, quantum yield of photosystem ii and membrane leakage as affected by high temperatures in cotton genotypes. The Journal of Cotton Science, 12: 150–159.

Calatayud, A., D.J. Iglesias, M. Talon and E. Barreno. (2004). Response of spinach leaves (Spinacea oleracea L.) to ozone measured by gas exchange, chlorophyll a fluorescence, antioxidant system, and lipid. Peroxidation. Photosynthetica, 42: 23–29.

Calatayud, A., J.W. Ramirez, D.J. Iglesias and E. Barreno. 2002. Effects of ozone on photosynthetic CO2 exchange, chlorophyll a fluorescence and antioxidant systems in lettuce leaves. Physiol. Plant, 116: 308–316.

De Souza-Silva, R.F. and A. Rapini, (2009). *Allamanda calcicola* (Apocynaceae), an overlooked new species from limestone outcrops in the States of Minas Gerais and Bahia, Brazil. Kew Bull., 64: 171-174

Emongor, V.E. (2004). Effect of gibberellic acid on postharvest quality and vase life of gerbera cut flowers (Gerbera *jamesonii*). *Journal of Agronomy*, 3: 191-195.

Georges, B., H. Andree, H. Claude, P. Anne, and L. Pierre. 1993. Physiological signal that induce flowering. *The Plant Cell*, 5: 1147-1155.

Hossain, A.B.M.S., N.B. Amru and O. Normaniza. 2007a. Postharvest quality, vase life and photosynthetic yield (Chlorophyll Fluorescence) of bougainvillea flower by applying ethanol. Australian Journal of Basic and Applied Sciences, 1: 733-740.

Hossain, A. B. M. S., F. Mizutani, J. M. Onguso, Ali. R. Shereif and H. Yamada. (2007b). Inhibiting peach-trees growth with Abscisic acid, hinokitiol, and tropolone applied to partially ringed bark strips. Journal of the Horticultural Science and Biotechnology 82 (2): 175-178.

Hossain, A. B. M. S. and F. Mizutani. (2009). Growth regulatory effect of cytokinin in peach trees as affected by ringed bark strip by using bioassay in vitro. Canadian Journal of Pure and Applied Sciences. 3 (1): 655-659.

Hossain, A.B.M.S., A.N. Boyce and H. M. A. Majid. (2008). Vase life extension and chlorophyll fluorescence yield of bougainvillea flower as influenced by ethanol to attain maxmum environmental beautification as ornamental components. American Journal of Environmental Sciences 4 (6): 625-630.

Ichimura, K. and M. Korenagea. (1998). Improvement of vase life and petal color expression inseveral cultivars of cut Eustoma flowers using sucrose with 8-hydroxyquinoline sulfate. Bull, Nati.Res. Veg. Ornam. Plants&Tea. Japan 13:31-39.

Ichimura, K. and S. Ueyama. (1998). Effects of temperature and application of aluminum sulfate on the postharvest life of cut rose flower. Bull. Natl. Res. Inst. Veg., Ornam. Plants& Tea,Japan 13: 51-60.

Jidapha T, N. Rakariyatham and R. L. Deming. (2006). Determination of Major Carotenoid Constituents in Petal Extracts of Eight Selected Flowering Plants in the North of Thailand. Chiang Mai J. Sci. 2006; 33(2) : 327 - 334. www.science.cmu.ac.th/journal-science/josci.html.

Johnson, G.N., A.J. Young, J.D. Scholes, and P. Horton. (1993). The dissipation of excess excitation energy in British plant species. Plant Cell Environ, 16: 673–679.

Khan, A.S., and N.Y. Chaudhry. (2006). GA_3 improves flower yield in some cucurbits treated with lead and mercury. African Journal of Biotechnology, 5: 149-153.

Koning, R.E. 1982. Control of flower opening by plant hormones in Gaillardia garndiflora. The university of Michigan, Phd thesis, pp1-189

Mariko, O., T. Yasushi, I. Masaki, and M. Masanobu. (2001). Elevated sensitivity to gibberellin by vernalization in the vegetative rosette plants of Eustoma grandiflorum and Arabidopsis thaliana. Plant Science, 160: 1237-1245.

Mediene, S., M.O. Jordan, L. Pages, J. Lebot, and S. Adamowicz. (2002). The influence of severe shoot pruning on growth, carbon and nitrogen status in young peach trees (*Prunus persica*). Tree Physiol., 22: 1289–1296.

Ogale, V.K., P.V. Babu, and S.D. Mishra. (2000). GA-induced stage specific changes in flower color and size of portulaca grandiflora cv NL-CRyP. Current Science, 79: 889-894.

Sharma, R.R. and S. Room. (2009). Gibberellic acid influences the production of malformed and button berries, and fruit yield and quality in strawberry (*Fragaria* × *ananassa* Duch.). Scientia Horticulturae, 119: 430-433.

Tjosvold, S.A., M.J. Wu and M.S. Reid. (1994). Reduction of postproduction quality loss in potted miniature roses. Hort. Science, 29: 293-294.

Annexure

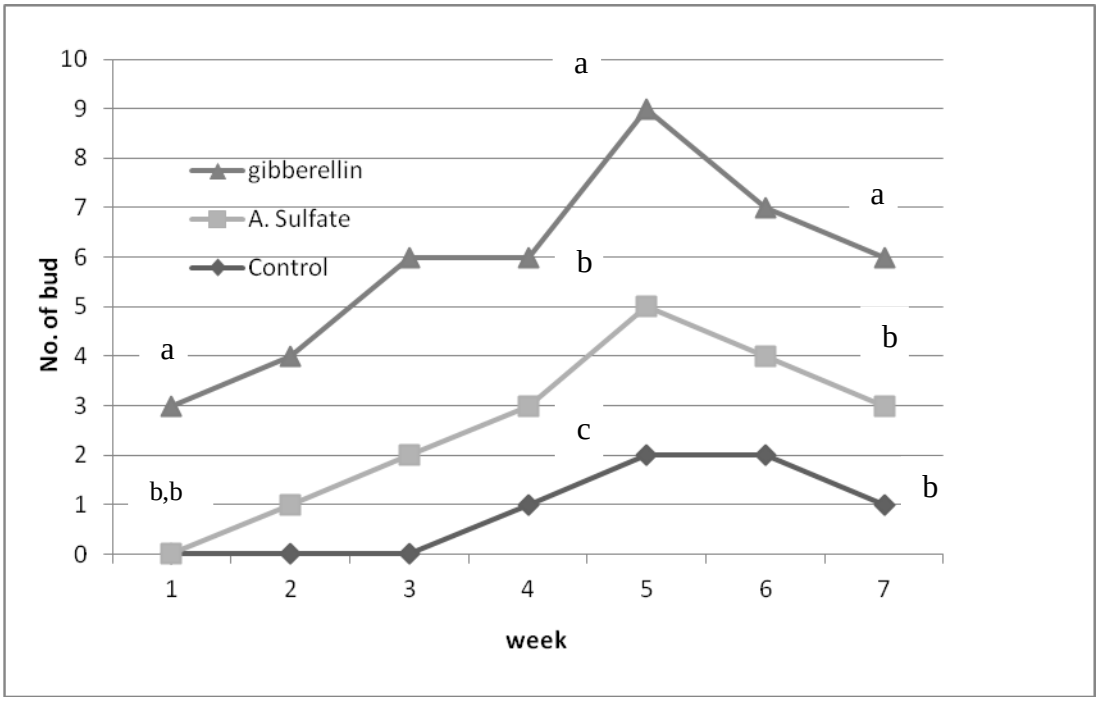

Fig. 1. Number of flower bud at different treatments. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test.

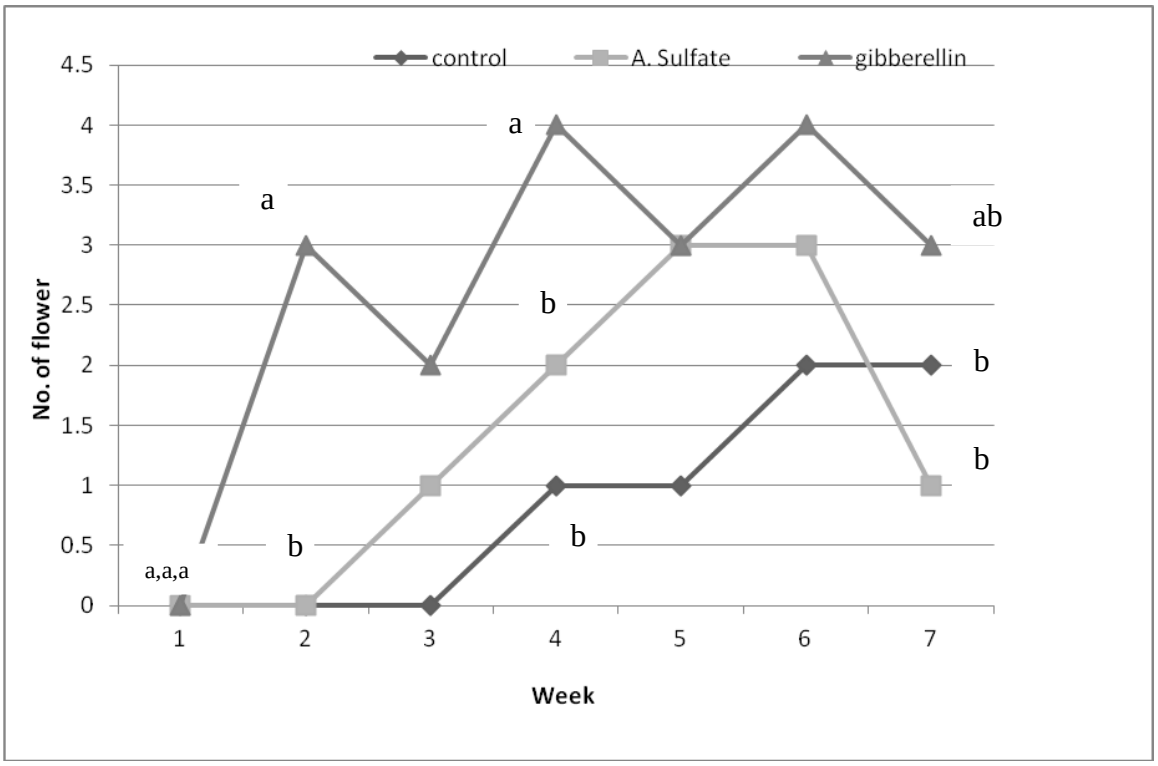

Fig. 2. Number of flower initiation at different treatments. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test.

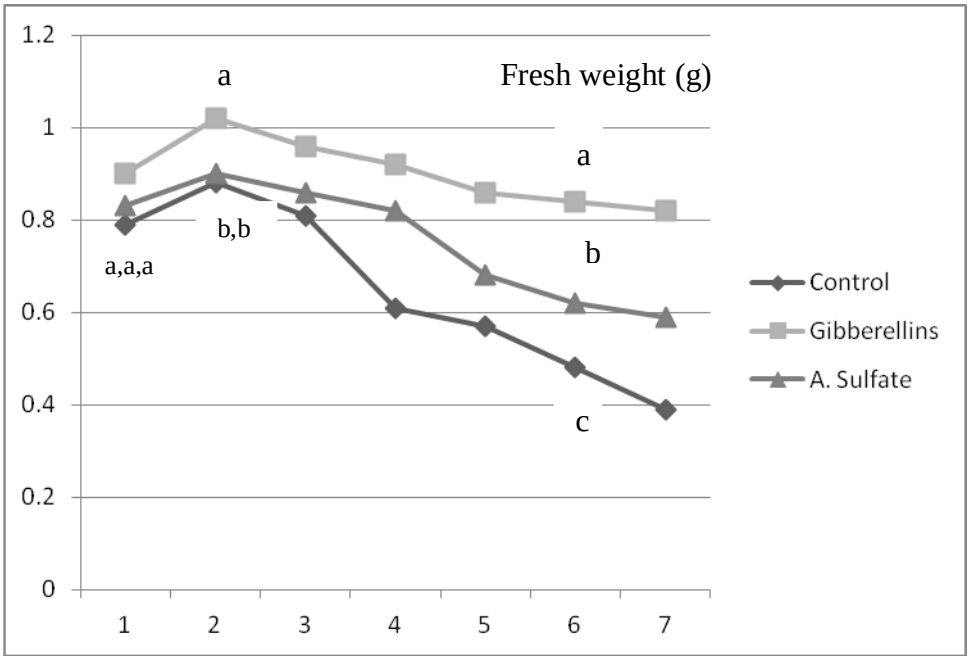

Fig. 3. Fresh weight at different days. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test.

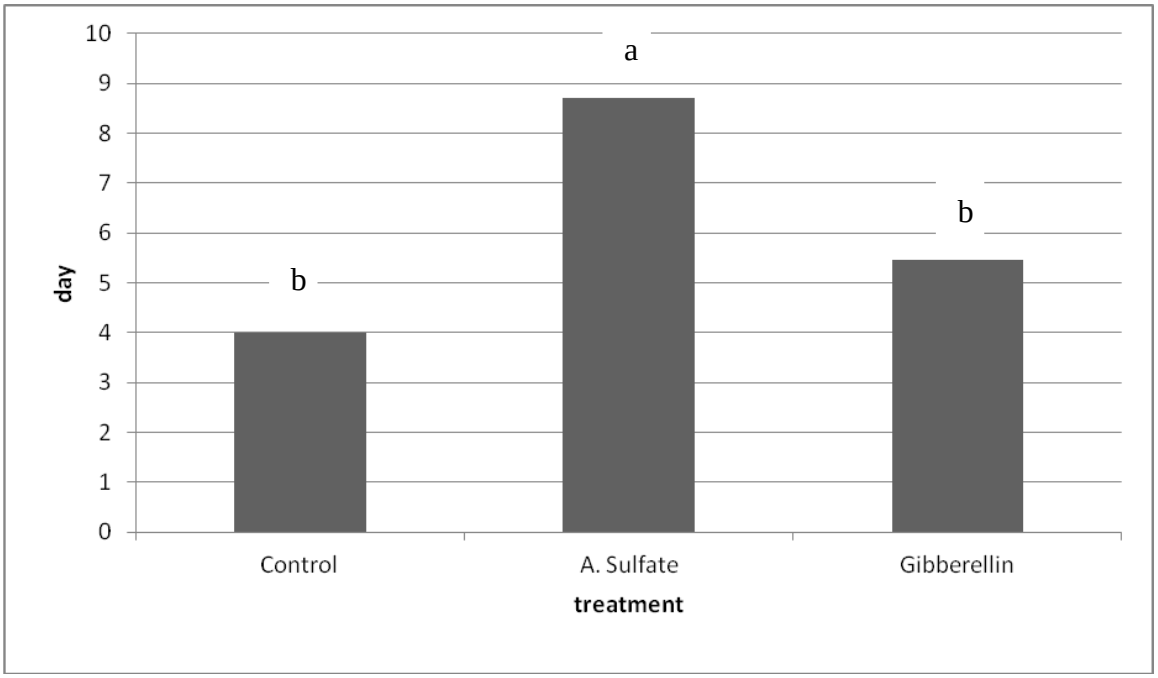

Fig. 4a. Flower longevity at different treatments

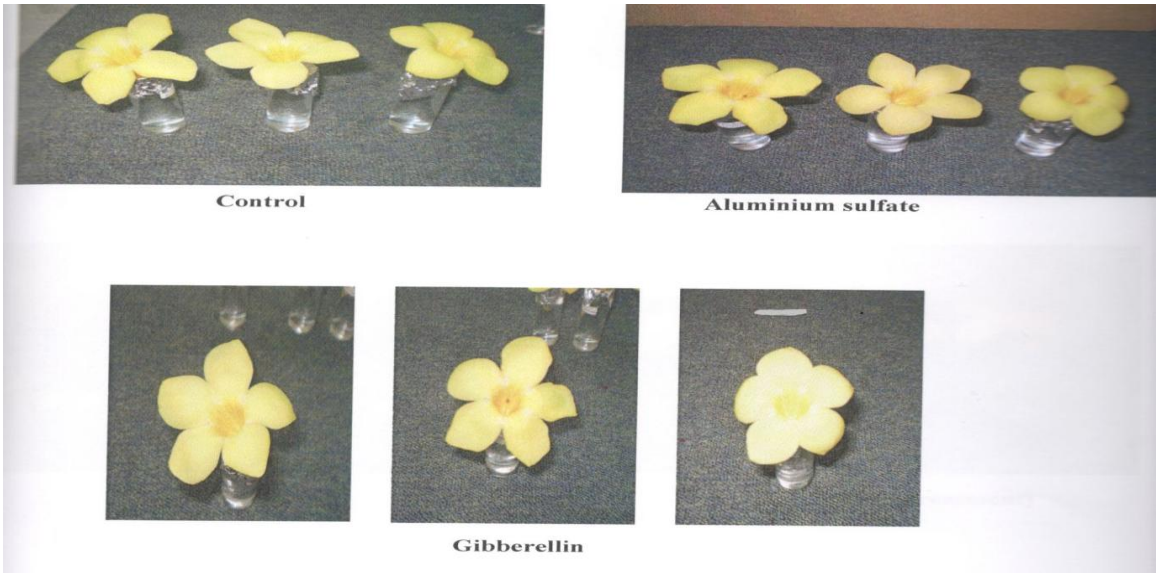

Fig 4b. Flower longevity observation at different treatments on 2nd day

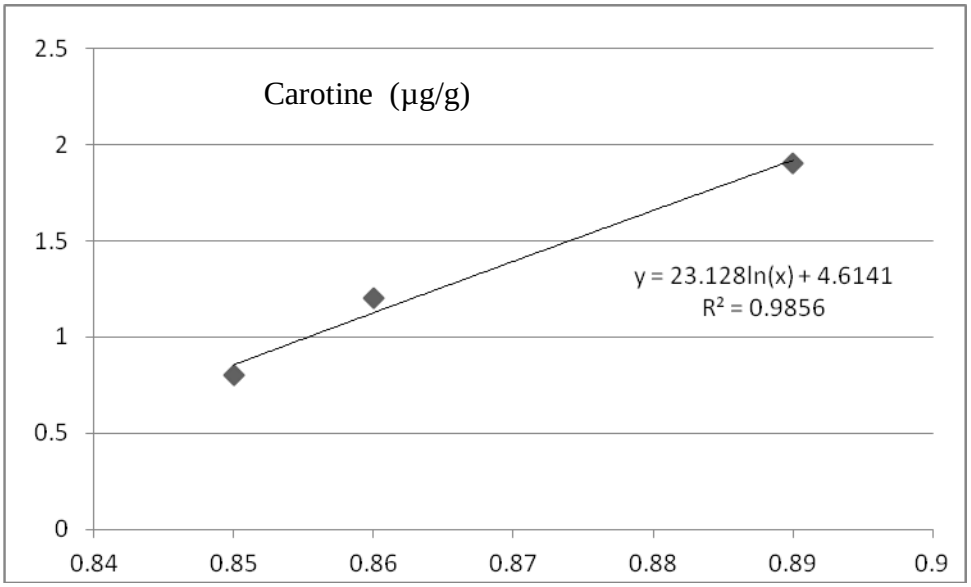

Fig. 5. Coorrelation between quantum photosynthetic yield and carotine content.

Table 1. Determination of Chlorophyll fluorescence (photosynthetic yield, quantum yield) in all treatments. Values are means of 3 measurements ± SE. Values followed by different alphabets indicate the existence of significant differences according to LSD $_{0.05}$ test.

Treatments	Fo	Fm	Fv	Fv/Fm	Carotinoid (µg/gfw)
Control	230a	1620b	1418b	0.85b	0.8b
GA	201a	1978a	1777a	0.89a	1.9a
Aluminium S.	222a	1683b	1461b	0.86b	1.2b

Fo: Lower photosynthetic yield, Fm: Higher photosynthetic yield, Fv: Variable of photosynthetic yield, Fv/Fm: Quantum yield