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# Effect of $GA_3$ and Growth Biostimulants on Growth and Chemical Composition of Calia *Secundiflora* Plants

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**Abstract:** This study was carried out at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University during two seasons of 2008 and 2009 to investigate the effect of some bio-stimulants and gibberellic acid ($GA_3$) on growth and chemical composition of *Calia secundiflora* Auqt. plants.The seedlings were treated with $GA_3$, yeast extract, humic acid, algae extract and mycorrhizae after a month from planting and repeated twice at two months intervals. The results revealed that $GA_3$ significantly increased plant height and stem diameter. The treatments of humic acid, algae and mycorrhizae significantly increased the plant height. Algae extract and mycorrhizae gave the thickest stems. The maximum number of leaves and branches per plant was obtained when seedlings were treated with mycorrhizae. Humic acid, algae and mycorrhizae treatments were the most effective on increasing fresh and dry weights of leaves. All treatments increased the fresh and dry weights of stems. Both humic acid and mycorrhizae significantly increased the root length; fresh and dry weights and the carbohydrates content in dried leaves to maximum values. Most of the treatments increased the pigments content of leaves. The treatments of humic acid, algae and mycorrhizae were the most effective treatments on increasing the contents of N, P and K in the leaves.

**Key words:** $GA_3$ · Biostimulants · Humic acid · Mycorrhizae · Algae · Yeast · Calia

## INTRODUCTION

*Calia secundiflora*, commonly known as mescal bean, is a flowering evergreen shrub or small tree native of South America and belongs to family Fabaceae. Leaves are compound, the leaflets are rather thick and waxy and the bark is smooth. It grows slowly to a height of 4-6 m and a crown diameter of 3 m. Fragrant purple flowers are produced in large clusters in late winter and early spring. Calia is a popular ornamental plant due to its showy flowers; it does well either in sun or light shade as well as in high pH soils and can tolerate other types of soils. The plants are extremely drought-tolerant and thus, has minimum water requirements once established. In addition to be used in gardens and streets, it can be used in reclaiming marginal lands, parking lots and highway medians. It's not invasive and adds dimension and beauty to unsightly areas.

Gibberellin is a naturally occurring plant hormone that regulates the growth of plants. It is a very potent hormone and regular applications of low concentrations can have a profound effect on flowering of plants, it maximizes the number and size of blossoms of the plant, making the garden a showplace. Nowadays, several biostimulants became the most important materials required to substitute chemical fertilizers for healthy and cheap production of many plants. Active dry yeast is a natural safe biofertilizer causes various promotive effect on plants, it is a natural source of cytokinins, simulates cell division and enlargement and it is used as an alternative source of growth substances in bio/organic fertilization system. Humic acid is a biostimulant which increases nutrient uptake, microbial activity, it is an excellent root stimulator and it increases the permeability of plant membranes and accelerates cell division. Humic acid affects physical and chemical properties of soils; it has been shown to increase the uptake of nitrogen,

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potassium, calcium, magnesium and phosphorus by plants [1]. Mycorrhizae fungi affect growth of most plant species through various ways,they increase phosphorus uptake, enhance uptake of nutrients by root system and are beneficial in the biological nitrogen fixation, bio-control of pathogens and drought resistance [ 2, 3]. The application of algae (seaweed extract) as an organic biostimulant is becoming an accepted practice in horticulture industry, it is available as an extract, powder or liquid form. It is believed to promote a healthy root system, helps plant becoming more disease resistant and helps provide rapid root development.

## MATERIALS AND METHODS

This study was carried out at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University during two seasons of 2008 and 2009 to investigate the effect of some bio-stimulants and $GA_3$ on growth and chemical composition of *Calia secundiflora* Auqt. Seedlings (4 month old, 10-12 cm height and 4-6 leaves were obtained from Orman Botanical garden). The seedlings were planted on 10 $^{th}$ March, 2008 and 25$^{th}$ March, 2009 in 30 cm clay pots filled with a mixture of clay and sand. The mechanical and chemical characteristics of the used mixture are shown in Table 1. The seedlings were sprayed with $GA_3$ at 100 ppm (Berlex tablets) and 1% of each of: yeast extract, humic acid,algae extract (Algifert ) and mycorrhizae (from Ain Shams University) applied as a soil drench. The water solutions of biostimulant substances were prepared using Bio-film at 1ml/L as a wetting agent for the different treatments. The treatments were applied a month from planting and repeated twice at 2 months intervals. The plants were fertilized with NPK (Krestalon at 20-20-20) at 5 g/ pot at monthly intervals. The plants were grown for 10 months for each season. The plants received a regular care of irrigation.

At the end of the experiment, the plants were cut at 3 cm above soil surface,and the following data were recorded: Plant height,stem diameter,number of leaves and branches per plant, fresh and dry weights of leaves, stems and roots and root length. Chemical determinations including chlorophyll a, b and total carotenoids were determined according to Saric *et al.* [4], total carbohydrates in dried leaves were determined according to Herbert *et al.* [5]. N, P and K were determined using the wet digestion procedure,total nitrogen content was determined using Nesslar method [6], phosphorus content was determined according to Troug and Meyer [7]. The content of potassium was determined by using operation chart of Shimadzu Atomic Absorption Flame Spectrophotometer. The different treatments (9 pots / treatment) of the experiment were replicated 3 times and each replicate contained 3 plants (3 pots). The layout of the experiment was a complete randomized design. The differences between the means of the different treatments were compared using "Least Significant Difference (L.S.D)" test at 5% probability, according to Sendecor and Cochran [8].

## RESULTS AND DISCUSSION

**Plant Height:** Data in Table 1 show that in both seasons, treating the plants with $GA_3$ significantly increased plant height as compared with the control. This finding agrees with many researchers [9, 10]. Treating the plants with yeast extract had no clear trend on plant height,in both seasons. The treatments of humic acid, algae and mycorrhizae significantly increased the plant height as compared with the control. In the first season, the treatment of humic acid gave tallest plants (49.63 cm ) whereas in the second one,the treatment of mycorrhizae increased plant height to the maximum value. In this respect, Watfa [11] on carob found that mycorrhizae significantly increased plant height.Phanuphong and Gregory [12] found that HA increased plant height and relative growth rate and had a positive influence in promoting root system which increases the nutrients uptake. El-Attar [13] on *Ficus alii*, found that humic acid significantly increased plant height.

**Stem Diameter:** As shown in Table2, $GA_3$ treatment significantly increased the thickness of stem, in both seasons, compared with the control.Grzesik and Joustra [14] on Juniperus *communis* obtained similar findings. In both seasons, yeast extract had no significant effect on stem thickness. The treatments of algae extract and mycorrhizae significantly increased the stem thickness to the highest values (10.44, 9.88,12.76 and 12.33 mm) in both seasons respectively, against 8.14 and 9.55 mm, respectively,for the control. El-Attar [13] on *Ficus alii*, found that humic acid had no clear trend on stem thickness. Ordog [15] stated that algae excretes great number of substances that influences plant growth and development.

Table 1: The mechanical and chemical characteristics of the used mixture for growing *Calia secundiflora*

| Chemical characteristics | | | | Mechanical characteristics | | | |
|---|---|---|---|---|---|---|---|
| pH (1:2.5) | EC (dS/m). | $HCO_3$ (%) | Organic matter (%) | Clay (%) | Silt (%) | Sand (%) | Texture |
| 7.12 | 1.34 | 1.08 | 0.83 | 33.5 | 27.7 | 38.8 | Clay loam |

Table 2: Effect of $GA_3$ and biostimulants on plant height, stem diameter, number of leaves and number of branches of *Calia secundiflora* plants

| | Plant height cm | | Stem diameter mm | | Number of leaves | | Number of branches | |
|---|---|---|---|---|---|---|---|---|
| Treatments | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 |
| Control | 34.34 | 36.56 | 8.14 | 9.55 | 39.60 | 29.35 | 2.20 | 1.64 |
| $GA_3$ | 41.65 | 42.82 | 9.34 | 11.60 | 44.42 | 39.70 | 4.33 | 4.60 |
| Yeast extract | 32.32 | 37.44 | 8.23 | 9.56 | 37.61 | 34.66 | 2.15 | 2.52 |
| Humic acid | 49.63 | 39.33 | 8.87 | 11.44 | 39.00 | 48.20 | 4.10 | 5.28 |
| Algae extract | 37.66 | 39.89 | 10.44 | 12.76 | 45.22 | 42.00 | 4.67 | 4.54 |
| Mycorrhizae | 42.44 | 46.22 | 9.88 | 12.33 | 50.46 | 53.33 | 6.50 | 5.76 |
| LSD at 5% | 3.28 | 2.16 | 1.02 | 0.78 | 4.20 | 3.65 | 1.32 | 1.98 |

**Number of Leaves per Plant:** Data in Table 2 show that in the first season, the treatment of $GA_3$, algae extract and mycorrhizae significantly increased the formation of leaves/plant, compared to the other treatments and control. In the second season, all treatments of biostimulants significantly increased the number of leaves/plant compared to the control. The maximum increment in leaf formation (53.33 leaves /plant) was obtained when the seedlings were treated with mycorrhizae, compared with 29.35 leaves/ plant for the control. Ajiboya [10] stated that $GA_3$ increased the number of leaves of *Prosopis africana* and *Dialium gueenensis;* Watfa [11] found that gibberellic acid and Mycorrhizae fungi significantly increased the number of leaves of *Ceratonia siliqua* and *Pinus halepensis* seedlings. Whereas, El-Attar [13] on *Ficus alii*, found that the formation of leaves was greatly responded to the application of humic acid.

**Number of Branches per Plant:** Regarding the effect of the $GA_3$ and biostimulants on number of branches, the obtained data (Table2) indicate that the treatments of $GA_3$, humic acid, algae extract and mycorrhizae significantly increased the number of branches /plant compared to the yeast treatment and control In both seasons. treating the plants with mycorrhizae significantly increased the number of branches /plant to the maximum values of 6.50 and 5.76,respectively compared to 2.2 and 1.64 branches /plant, for the control. El-Attar [13] on *Ficus alii*, found that the formation and growth of side shoots were greatly responded to the application of humic acid. Kung [16] on *Senna spectabilis* reported that inoculation with mycorrhizae significantly improved the growth performance, which was attributed to improvement in nutrient uptake.

**Fresh and Dry Weights of Leaves:** As shown in Table3, in the first season,all treatments significantly increased the fresh weight of leaves. $GA_3$, algae and mycorrhizae treatments were the best as they gave marked increases in the fresh weight of leaves giving 51.81, 54.44 and 60.66 g, respectively compared with 40.08 g. for control. Humic acid, algae and mycorrhizae treatments showed also the great influence in this concern in the second season. In both seasons, the effect of $GA_3$ as well as biostimulants treatments on dry weight of leaves were in parallel with their effects on fresh weight of leaves. Humic acid as well as mycorrhizae treatments were the most effective. The increase in leaves fresh and dry weights can be attributed to the increase in both plant height and number of leaves / plant. The increment in growth of leaves by spraying plants with humic acid may be due to that humic acid contains many elements which improve the plant growth. These results are in harmony with those reported by some researchers who reported that soil inoculation with VA mycorrhizae lead to best seedling growth and nutrient uptake [12, 17, 18 ].

**Fresh and Dry Weights of Stems:** Data shown in Table 3 reveal that in the first season, all treatments increased the fresh weight of stems. $GA_3$, algae and mycorrhizae treatments significantly increased it giving 10.45, 9.36 and

Table 3: Effect of $GA_3$ and biostimulants on fresh and dry weights of leaves and stems of *Calia secundiflora* plants

| | F.W. of leaves | | D.W. of leaves | | F.W. of stems | | D.W. of stems | |
|---|---|---|---|---|---|---|---|---|
| Treatments | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 |
| Control | 40.08 | 36.71 | 5.00 | 4.30 | 7.37 | 6.12 | 2.98 | 2.64 |
| $GA_3$ | 51.81 | 44.36 | 8.16 | 6.24 | 10.45 | 7.25 | 4.62 | 3.10 |
| Yeast extract | 43.47 | 41.73 | 6.39 | 6.25 | 7.56 | 6.83 | 2.34 | 2.00 |
| Humic acid | 47.42 | 54.42 | 7.48 | 7.80 | 8.80 | 8.50 | 4.11 | 3.60 |
| Algae extract | 54.44 | 55.70 | 8.58 | 7.35 | 9.36 | 8.44 | 4.18 | 3.18 |
| Mycorrhizae | 60.66 | 65.19 | 9.13 | 8.77 | 11.00 | 10.25 | 4.04 | 4.22 |
| LSD at 5% | 3.25 | 4.00 | 2.88 | 1.65 | 1.74 | 1.90 | 0.86 | 0.70 |

Table 4: Effect of $GA_3$ and biostimulants on root length; fresh and dry weights of *Calia secundiflora* plants

| | Root length (cm) | | Root fresh weight (g) | | Root dry weight (g) | |
|---|---|---|---|---|---|---|
| Treatments | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 |
| Control | 16.8 | 17.6 | 2.67 | 2.55 | 0.87 | 0.68 |
| $GA_3$ | 21.7 | 22.3 | 3.03 | 2.69 | 1.02 | 0.96 |
| Yeast extract | 17.8 | 20.6 | 2.40 | 3.32 | 0.72 | 1.13 |
| Humic acid | 24.3 | 22.0 | 3.60 | 3.87 | 1.29 | 1.22 |
| Algae extract | 23.0 | 21.3 | 3.28 | 2.82 | 1.07 | 0.90 |
| Mycorrhizae | 26.2 | 23.0 | 3.82 | 4.24 | 1.22 | 1.32 |
| LSD at 5% | 4.80 | 3.05 | 0.66 | 0.52 | 0.34 | 0.25 |

11 g, respectively, compared with 7.37 g for the control. In the second season, humic acid, algae and mycorrhizae treatments significantly increased the fresh weight of stems. Yeast treatment was the least effective treatment in this respect. In the first season, $GA_3$ followed by humic acid and algae treatments showed the greatest effect on increasing the dry weight of stems. In the second season, humic acid and mycorrhizae treatments gave the heaviest dry weight of stems. These findings are in agreement with some researches on some trees seedlings which concluded that VA- mycorrhizael fungi and humic acid significantly enhanced the weight of stems [12, 19, 20 ].

**Root Length; Fresh and Dry Weights:** Data in Table 4 show the effect of $GA_3$ and biostimulants on root length; fresh and dry weights. In both seasons, treating the plants with $GA_3$ significantly increased root length compared with the control. The treatments of humic acid and mycorrhizae were the most effective treatments, giving the maximum length. Watfa [11] on *Ceratonia siliqua* and *Pinus halepensis*, found that $GA_3$ increased the root length. Venkatech *et al.* [21] on *Pongamia pinnata* seedlings reported that VAM enhanced root length.

All treatments except $GA_3$ and yeast treatments, in the first season, significantly increased the fresh weight of roots. Humic acid and mycorrhizae treatments were the best in this respect giving 3.60 and 3.82 g,respectively compared with 2.67 g for control. In the second season, yeast extract,humic acid and mycorrhizae treatments resulted in the heaviest fresh weight of roots giving 3.32, 3.87 and 4.24 g, respectively. Table3 shows that in the first season,mycorrhizae and humic acid treatments showed the greatest effect on increasing dry weight of roots. In the second season,yeast extract; humic acid and mycorrhizae treatments were the most effective. Mukerji *et al.* [19] on some tree seedlings, obtained significant increase in root weight by using VAM.

**Total Carbohydrates Content:** Data in Table 5 show the effect of $GA_3$ and different biostimulants treatments on the carbohydrates content of the leaves of *Calia secundiflora* seedling. It is clear from data that mycorrhizae, humic acid and algae treatments greatly increased it in the first season, comparing with control,whereas yeast extract decreased it. In the second season, the highest value of the carbohydrates in the leaves (25.44%D.W.) was obtained with mycorrhizae,

Table 5: Effect of $GA_3$ and biostimulants on total carbohydrates and pigments contents in leaves of *Calia secundiflora* plants

| | Total carbohydrate % D.W. | | Chl.A mg/g F.W. | | Chl.B mg /g F.W. | | Caroteniods mg /g F.W. | |
|---|---|---|---|---|---|---|---|---|
| Treatments | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 |
| Control | 16.33 | 18.52 | 0.88 | 0.65 | 0.35 | 0.37 | 0.52 | 0.43 |
| $GA_3$ | 19.89 | 21.88 | 1.06 | 0.86 | 0.55 | 0.44 | 0.66 | 0.57 |
| Yeast extract | 15.67 | 16.89 | 0.77 | 0.74 | 0.30 | 0.41 | 0.38 | 0.54 |
| Humic acid | 24.50 | 23.60 | 1.16 | 1.45 | 0.51 | 0.40 | 0.76 | 0.67 |
| Algae extract | 22.80 | 21.65 | 1.08 | 1.26 | 0.52 | 0.44 | 0.79 | 0.70 |
| Mycorrhizae | 23.66 | 25.44 | 0.95 | 1.28 | 0.38 | 0.49 | 0.55 | 0.74 |
| LSD at 5% | 2.65 | 2.44 | 0.30 | 0.24 | 0.16 | 0.20 | 0.21 | 0.18 |

Table 6: Effect of $GA_3$ and biostimulants on N; P and K contents in leaves of *Calia secundiflora* plants

| | N% D.W. | | P% D.W. | | K% D.W. | |
|---|---|---|---|---|---|---|
| Treatments | 2008 | 2009 | 2008 | 2009 | 2008 | 2009 |
| Control | 2.11 | 1.86 | 0.16 | 0.13 | 1.45 | 1.38 |
| $GA_3$ | 2.62 | 2.33 | 0.13 | 0.15 | 1.62 | 1.54 |
| Yeast extract | 2.21 | 1.92 | 0.20 | 0.18 | 1.05 | 1.45 |
| Humic acid | 2.36 | 2.08 | 0.09 | 0.11 | 2.53 | 1.93 |
| Algae extract | 2.29 | 2.45 | 0.26 | 0.22 | 2.38 | 2.18 |
| Mycorrhizae | 2.58 | 2.32 | 0.25 | 0.27 | 2.26 | 2.06 |
| LSD at 5% | 0.32 | 0.27 | 0.54 | 0.33 | 0.58 | 0.62 |

compared with control) 18.52%D.W.). In general, mycorrhizae and humic acid were the most effective treatments in this respect. El-Attar [ 13] on *Ficus alii*, found that humic acid increased the accumulation of total carbohydrates in the different plant parts.

**Pigments Content:** Concerning the effect of $GA_3$ and different biostimulants treatments on the contents of chlorophyll a;b and carotenoids in the leaves of *Calia secundiflora* seedlings, data in Table 5 reveal that in the first season,most of the treatments increased the chlorophyll a,as compared with the control except yeast extract, the highest content was recorded with humic acid. In the second one, there were pronounced increases in chlorophyll a with the treatments of humic acid, algae and mycorrhizae, compared to the control.

In the first season, the highest value of chlorophyll b was obtained with $GA_3$, algae extract and humic acid treatments. Mycorrhizae treatment was the most effective treatment in the second season. Regarding the effect on the content of carotenoids, the results reveal that the highest carotenoids content was obtained with algae and humic acid in the first season and algae and mycorrhizae in the second one.Watfa [11] found that gibberellic acid and mycorrhizae increased chlorophylls content of carob and Aleppo Pine. El-Attar [13] on *Ficus alii*, Ferrara *et al.* [22] on grapes and El-Bassiony *et al.* [23] on snap bean stated that humic acid significantly increased chlorophylls content.

**N; P and K Contents:** Table 6 shows that treating the seedlings with mycorrhizae as well as $GA_3$ showed the greatest effect on increasing the content of nitrogen in the first season. Whereas in the second one, the treatments of algae, mycorrhizae and $GA_3$ were the most effective in this respect. It is clear that mycorrhizae, algae and yeast extract, in both seasons, markedly increased P% in the leaves, compared with control. Concerning K content, Table 6 indicates that treating the plants with humic acid, algae and mycorrhizae increased the potassium content in the leaves, compared with control and other treatments in both seasons. It can be concluded that treatments of humic acid, algae and mycorrhizae were the most effective treatments on increasing the contents of N; P and K in the leaves of the *Calia secundiflora* seedlings. In this respect, Dubey and Ginwal [24] reported that mycorrhizae increased the absorption area of the roots and provide host plants with nutrients. Mycorrhiza improves the uptake of nutrients. Humic acids has been shown to increase the uptake of nitrogen by plants and to increase soil nitrogen utilization efficiency, it also enhances the uptake of potassium, calcium, magnesium and

phosphorus [11, 16, 21, 25]. This goes in harmony with researches on *Ficus alii* stating that humic acid had a great effect in increasing the N; P and K contents of the plants [12, 13].

## CONCLUSION

$GA_3$ significantly increased plant height and stem diameter. The treatments of humic acid, algae and mycorrhizae significantly increased the plant growth. Algae extract and mycorrhizae were the best treatments.

**Recommendation:** The seedling growth and quality of *Calia secundiflora* plants were significantly responded to treatments of $GA_3$, humic acid, algae and mycorrhizae.

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