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Effect of β-sitosterol and Gibberellic Acid on Leaf Angle, Growth, Flowering and Biochemical Constituents of Marigold (*Calendula officinalis* L.)

El-Sherbeny Mohamed Rashad* • **Mohamed Slama Ahmed Abd El-Wahed** • **Aboubaker Ahmed Amin**

Botany Department, National Research Centre, El-Tahrir Str., El-Dokki, Cairo, Egypt

Corresponding author: * **sherbeny_rashad56@yahoo.com**

ABSTRACT

Marigold (*Calendula officinalis* L.) plant growth and orientation were improved by foliar application of gibberellic acid (GA_3; 25, 50 and 100 mg L^{-1}) and β-sitosterol (20, 40 and 80 mg L^{-1}) singly, or in combination. GA_3 at 25 mg L^{-1} more effective increased the number of branches, fresh and dry weight of shoots and roots/plant while GA_3 at 100 mg L^{-1} significantly increased plant height. Leaf angle decreased more with GA_3 than with β-sitosterol application. Both GA_3 (100 mg L^{-1}) or β-sitosterol (40 mg L^{-1}) significantly induced flowering earliness and number of inflorescences while a low concentration of both substances improved other inflorescence characters. The GA_3 (100 mg L^{-1}) × β-sitosterol (40 mg L^{-1}) interaction significantly increased earliness, number and stalk length of marigold plant inflorescences. Pigments (Chlorophyll (Chl) *a* and *b*, and carotenoids) and biochemical constituents (total sugars, indoles and free amino acids) were significantly enhanced by GA_3 (25 mg L^{-1}) and/or β-sitosterol (20 or 40 mg L^{-1}) as a foliar application. However, the highest concentration of both bioregulators resulted in the highest phenolic content of marigold plant leaves. GA_3 and β-sitosterol were positively correlated to fresh weight of inflorescences and Chl *a* + *b* content. There was a significant negative correlation between all results and phenolic content of marigold plant leaves.

Keywords: bioregulators biochemical, earliness, growth orientation
Abbreviations: dw, dry weight; **EO**, essential oil; **fw**, fresh weight; **GA_3**, gibberellic acid; **Fd**, flowering date; **Rd**, ripe date; **La**, leaf angle

INTRODUCTION

Marigold (*Calendula officinalis* L.) is a common herb that has some compounds that have a number of medicinal uses; its orange petals are an excellent remedy for inflammation and skin diseases (Neto *et al*. 1996) and an antiseptic (Sakharkar *et al*. 2000). Sterols are one of the compounds present in marigold.

Plant sterol composition varies within different species and physiological stage of development (Ebrahimzadeh *et al*. 2001). Despite the identification of plant steroids, dozens of other sterols are also found in plants. Major plant sterols such as sitosterol and stigmasterol are similar in structure to the fungal ergosterol and cholesterol in animals. Sterols are known to regulate transcriptional and post transcriptional events, which in turn affect lipid synthesis, meiosis, apoptosis, developmental patterning, protein cleavage and protein degradation (Edwards and Ericsson 1999), carbohydrate distribution in maize, *Zea mays* (Abd El-Wahed 2000), and free amino acids, phenols and indoles in soybean, *Glycine max* (Abd El-Wahed 2008). In addition, brassinosteroids are able to generate erect leaves in rice, *Oryza sativa* (Morinaka *et al*. 2006).

Gibberellic acid (GA_3) regulates various developmental processes throughout the life cycle of the plant, from seed germination through leaf expansion, stem elongation, flower induction and development to seed development (Sun and Gubber 2004). According to these authors, GAs change the orientation of lateral branches of runners to that of erect ones, the amount of native GA_3 antagonists was higher in runner plants, furthermore, runner plants contained a particular GA_3 inhibitor not found in erect plants, additionally, GA_3 changes leaf orientation and prostate growth habit of short-day plant to that typical of the long-day and modifies leaf sharp in both types of plants. Geotropic responses of the stem appear to be modified by photoperiod, temperature and GA_3 (Wallenstein and Luke 1963). Robust target genes are affected specifically by a single hormone. However, in the cases of GA-induced genes, no specific robust targets were identified. This may suggest that interactions with other hormones play major roles in GA action which necessitates the existence of efficient and sensitive crosstalk mechanisms among the corresponding signaling pathways (Nemhauser *et al*. 2006). The stimulative effect of GA_3 on growth of medicinal and aromatic plants was studied by Farooqi *et al*. (1996) using GA_3 (25 and 50 mg/l) on *Artemisia annua* L. and Mohamed *et al*. (1996) on basil (*Ocimum basilicum* L.) plant using GA_3 at 50 ppm.

The purpose of the present study was to determine the effect of β-sitosterol and GA_3 on growth, leaf orientation, flowering as well as chemical contents of marigold plant.

MATERIALS AND METHODS

Greenhouse experiments were carried out at the National Research Centre, Cairo, Egypt, during two successive seasons (2005-2006, 2006-2007) to study the effect of foliar application of different concentrations of GA_3 (a diterpene) and β-sitosterol (a triterpene) as well as their combinations on growth, flowering and some biochemical constituents of marigold (*Calendula officinalis* L.). Seeds were obtained from the Medicinal and Aromatic Plants Research Section, Ministry of Agriculture. Seeds were sown in seed beds (using four beds, each bed 35 × 35 cm, 3.5 cm between seeds, 100 seeds per bed) on October 15th for both seasons. After one month, uniform seedlings about 6 cm in height with 2 pairs of

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leaves were individually transplanted into pots of 30 cm diameter filled with 10 kg of clay-loamy soil. The plants were fertilized with 2.5 (g) ammonium sulphate + 3.75 (g) calcium super phosphate + 1.25 (g) potassium sulphate per pot after 3 weeks from transplanting and repeated again 2 weeks later (fertilizers obtained from the Egyptian Ministry of Agriculture).

Plants were foliar sprayed with GA_3 (Berelex tablets, Imperial Chemical Industries Ltd., UK) at 0, 25, 50 or 100 mg/1 and/or β-sitosterol (Sigma-Aldrich, St. Louis, USA) at 0, 20, 40 or 80 mg/l while control plants were sprayed with distilled water. Plants were sprayed twice with the growth regulators (or distilled water in controls) until run-off occurred; the first spray was five weeks after transplanting and the second one was applied three weeks later. Treatments were arranged in a complete randomized design, replicated 3 times, each replicate contained 6 pots.

Determination of biochemical constituents

After two weeks from the second spray the fifth leaf from the top of the plant was collected for determination of photosynthetic pigments content (chlorophyll (Chl) *a*, *b*, total Chls and total carotenoids) spectrophotometrically as described by Saric *et al*. (1967). Flowering date was calculated as the number of days from sowing to the first flower open. The following data were recorded at the full blooming stage (April 15th): plant height, number of branches/plant, fresh and dry weights (fw and dw, respectively) of shoot and root/plant, shoot/root ratio, leaf angle, number of inflorescences/plant, stalk length and diameter of inflorescence as well as average fw and dw of one inflorescence. Samples of ray flowers were air-dried and their carotenoid contents were determined using the method described by the A.O.A.C. (1990). In addition, leaf samples were oven-dried at 70°C for 24 h, and total sugar (Dubois *et al*. 1956), total indoles (Bently 1961), phenols (Danial and George 1972) and free amino acids (Plummer 1978) contents were determined.

Statistical analysis

The data were statistically analyzed for each season and then a combined analysis of the two seasons was carried out according to the procedure outlined by Snedecor and Cochran (1990). Simple correlation coefficients between growth, flowering and biochemical contents of all treatments (GA_3, β-sitosterol and their interaction) were determined according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Effect of GA_3 on vegetative growth characters

Data in **Table 1** and **Fig. 1** show that GA_3 significantly increased vegetative growth characters, namely plant height, number of branches, fw and dw of shoots and roots/plant while the shoot/root ratio of fw and dw were significantly decreased compared to the control. GA_3 (100 mg L^{-1}) resulted in the tallest plants. GAs in general cause dramatic increases in plant height (King *et al*. 2008).

Fig. 1 Leaf angle of marigold plant under gibberellic acid (GA_3) treatments. Treatments: 1, Control; 2, 25 mg/l GA_3; 3, 50 mg/l GA_3; 4, 100 mg/l GA_3.

GA_3 (25 mg L^{-1}), following a similar trend, was more effective on number of branches, fw and dw of roots and shoots (**Table 1**). The reduction of leaf angle was attributed to an increase in GA_3 concentration. These effects might be due to the role of GA_3 in improving vegetative growth characteristic since GAs are plant hormones that participate in the regulation of many growth developmental processes in plants (Hedden and Phillips 2000; Olszewski *et al*. 2002).

Effect of β-sitosterol on vegetative growth

The application of β-sitosterol led to a significant increase in vegetative growth characters (plant height, number of branches, fw and dw of roots and shoots) of marigold plants as shown in **Table 2** and **Fig. 2**. Growth characters differed significantly in their response to β-sitosterol application. In addition, reduced leaf angle was related with increasing β-sitosterol concentration. Shoot dw and the shoot/root ratio of plant were increased more by 40 mg L^{-1} β-sitosterol while shoot fw and the shoot/root ratio of fw were improved by

Fig. 2 Leaf angle of marigold plant under β-sitosterol (β-ST) treatment. Treatments: 1, Control; 5, 20 mg/l β-ST; 6, 40 mg/l β-ST; 7, 80 mg/l β-ST.

Table 1 Effect of gibberellic acid and β-sitosterol on vegetative growth characters of marigold plant during full blooming stage (average of the two seasons).

Gibberellic acid (mgL^{-1})	Ph	Bn	Sh w		R w		Sh/rr		La
			fw	dw	fw	dw	fw	dw	
Control	27.92	9.75	63.36	11.48	27.55	7.23	2.29	1.57	66.25
25	30.58	12.33	71.73	13.08	33.97	9.46	2.12	1.39	60.25
50	34.75	11.50	63.08	11.97	33.19	8.55	1.93	1.35	48.75
100	37.92	10.00	54.62	10.32	30.45	8.25	1.80	1.25	33.17
LSD 5%	0.85	0.82	1.81	0.66	0.55	0.15	0.06	0.03	2.04

Ph = plant height, Bn = No. of branches/plant , Sh w = Shoots weight (g/plant) , R w = Root weight (g/plant), Sh/rr = Shoot/root ratio, La = leaf angle, fw = Fresh weight, dw = Dry weight

Table 2 Effect of β-sitosterol on vegetative growth characters of marigold plant during full blooming stage (average of the two seasons)

B Sitosterol (mgL^{-1})	Ph	Bn	Sh w		R w		Sh/rr		La
			fw	dw	fw	dw	fw	dw	
Control	32.25	9.00	57.96	10.68	31.32	8.42	1.87	1.27	52.83
20	33.25	11.08	66.93	12.59	30.42	8.35	2.20	1.51	52.17
40	34.25	11.67	66.22	13.42	31.41	8.37	2.12	1.56	55.00
80	31.42	11.83	61.68	10.17	32.45	8.35	1.95	1.23	48.42
LSD 5%	0.85	0.82	1.81	0.66	0.55	N.S.	0.06	0.03	0.04

Ph = plant height, Bn = No. of branches/plant , Sh w = Shoots weight (g/plant) , R w = Root weight (g/plant), Sh/rr = Shoot/root ratio, La = leaf angle, fw = Fresh weight, dw = Dry weight

Table 3 Effect of the interaction between gibberellic acid and β-stosterol treatments on vegetative growth characters of marigold plant during full blooming stage (average of both seasons).

Interaction treatments		Vegetative growth characters								
GA_3	Sitosterol	Ph	Bn	Sh w		R w		Sh/rr		La
(mgL^{-1})	(mgL^{-1})			fw	dw	fw	dw	fw	dw.	
0	0	22.33	7.00	47.64	8.11	27.27	7.28	1.75	1.78	77.33
	20	30.00	9.00	74.89	13.38	31.30	7.51	2.39	1.78	66.67
	40	31.00	13.00	70.36	14.20	27.00	7.40	2.57	1.92	61.67
	80	28.33	10.00	60.55	10.25	24.65	6.71	2.46	1.50	59.33
25	0	27.00	12.00	68.38	12.15	34.51	10.33	1.98	1.18	61.67
	20	32.00	11.33	71.46	13.18	31.34	8.53	2.28	1.51	57.67
	40	33.00	12.33	72.34	14.39	34.89	9.31	2.07	1.56	63.33
	80	30.33	13.67	74.73	12.61	35.13	9.68	2.13	1.32	58.33
50	0	36.67	9.00	60.94	12.18	32.46	8.27	1.98	1.47	46.67
	20	34.00	13.00	66.64	12.82	32.56	9.30	2.05	1.39	47.67
	40	35.67	11.33	63.29	13.32	31.12	8.02	2.03	1.41	57.33
	80	32.67	12.67	61.42	9.57	36.60	8.63	1.65	1.11	43.33
100	0	43.00	8.00	54.86	10.27	31.06	7.80	1.77	1.32	25.67
	20	37.00	11.00	54.72	10.97	26.47	8.08	2.07	1.36	36.67
	40	37.33	10.00	58.87	11.77	32.63	8.76	1.80	1.34	37.67
	80	34.33	11.00	50.01	8.26	31.64	8.35	1.58	0.99	32.69
LSD 5%		1.71	1.65	3.62	1.33	1.11	0.31	0.12	0.07	4.07

Ph = plant height, Bn = No. of branches/plant , Sh w = Shoots weight (g/plant) , R w = Root weight (g/plant), Sh/rr = Shoot/root ratio, La = leaf angle, fw = Fresh weight, dw = Dry weight

20 mg L^{-1} β-sitosterol. The highest number of branches, root fw and leaf angle were recorded with 80 mg L^{-1} β-sitosterol. The results were in agreement with Cao and Chen (1995) who reported that brassinosteroid-induced rice (*Oryza sativa* L.) lamina joint inclination was accompanied by increasing lamina fresh weight. Also, Abd El Wahed *et al*. (2001) showed a stimulatory effect on vascular differentiation, thickness of upper epidermal mesophyll tissue layers, growth and yield, as well as chemical composition of wheat (*Triticum aestivum* L.) by β-sitosterol application (10^{-4} M). In addition, brassinolide doubled the mean root length and the number of mitoses in onion (*Allium cepa*) over controls at a low concentration at 0.005 ppm (Howell *et al*. 2007).

Effect of GA_3 × β-sitosterol interaction on vegetative growth

Data in **Table 3** and **Figs. 3-5** show that vegetative characters significantly increased proportionately as a result of the GA_3 × β-sitosterol application. The highest values of vegetative growth characters (number of branches and fw of shoots/plant) were obtained with GA_3 (25 mg L^{-1}) + β-sitosterol (80 g L^{-1}), dw of shoots/plant with GA_3 (25 mgL^{-1}) + β-sitosterol (40 mgL^{-1}) and shoot/root ratio with GA_3 (25 mg L^{-1}) + β-sitosterol (20 or 80 mg L^{-1}). It appears from the results that both phytohormones play an important role in differentiation and morphogenesis of plant cells because their precursor is isoprenoid. Hedden and Phillips (2000) reported that GAs participate in many growth and developmental processes in plants, particularly in stem growth regulation. Additionally, brassinosteroids are involved in many morphological and physiological processes in rice, including the elongation and unrolling of leaves and skotomorphogensis (Mori *et al*. 2002). Therefore, brassinosteroids are able to generate erect leaves in rice (Morinaka *et al*. 2006).

Effect of GA_3 on flowering

Data in **Table 4** shows that GA_3 application significantly decreased the number of days to appearance of the first inflorescence. A contrasting result was obtained with numbers, stalk lengths, fw and carotenoid content of inflorescences, dependent on GA_3 concentration. GA_3 at 100 mg L^{-1} affected most the number of days until the appearance of the first opened flower and stalk length, 50 mgL^{-1} the diameter, 25 mg L^{-1} the inflorescence fw and flower petal carotenoid content. These effects show that GA_3 plays an important role in flowering of marigold. A clear association between applied and endogenous GAs and flowering in dicotyledonous species, including *Arabidopsis*, is well known (Eriksson *et al*. 2006). The level of bioactive GAs (GA_1, GA_4 and GA_6) increase rapidly in the leaf, petiole and shoot apex of both monocots and dicots (King *et al*. 2006).

Fig. 3 leaf angle of marigold plant as affected by GA_3 concentrations under 20 mg/l β-ST. Treatments: 1, Control; 8, 25 GA_3 + 20 β-ST; 9, 50 GA_3 + 20 β-ST; 10, 100 GA_3 + 20 β-ST.

Fig. 4 leaf angle of marigold plant as affected by GA_3 concentrations under 40 mg/l β-ST. Treatments: 1, Control; 14, 25 GA_3 + 40 β-ST; 15, 50 GA_3 + 40 β-ST; 16, 100 GA_3 + 40 β-ST.

Fig. 5 leaf angle of marigold plant as affected by GA_3 concentrations under 80 mg/l β-ST. Treatments: 1, Control; 11, 25 GA_3 + 80 β-ST; 12, 50 GA_3 + 80 β-ST; 13, 100 GA_3 + 80 β-ST.

Table 4 Effect of gibberellic acid on flowering parameters and carotenoids content in ray flowers of marigold plant during full blooming stage (average of both seasons).

Gibberellic Acid (mgL^{-1})	Nd	Ni	Si	Di	Fwi	Dwi	Cc
Control	104.6	11.50	13.57	6.86	5.46	0.78	1.374
25	97.8	13.93	14.81	6.54	5.54	0.77	1.461
50	93.3	13.42	17.19	6.93	5.41	0.73	1.254
100	85.7	14.82	21.52	6.59	5.42	0.75	1.263
LSD 5%	0.9	0.25	0.24	0.12	N.S.	0.01	0.038

Nd = Number of days to first flower open, Ni = Number of inflorescences, Si = Stalk length of inflorescence, Di = Diameter of inflorescence, Fwi = Fresh weight of one inflorescence, Cc = Carotenoids content in ray flower (mg/g D.W.)

Table 5 Effect of β-sitosterol on flowering parameters and carotenoids content in ray flowers of marigold is L. plant during full blooming stage (average of both seasons).

β-sitosterol (mgL^{-1})	Nd	Ni	Si	Di	Fwi	Dwi	Cc
Control	99.50	13.73	16.78	6.09	4.95	0.67	1.293
20	97.25	13.73	16.85	7.25	5.98	0.83	1.449
40	91.75	14.74	16.77	6.75	5.40	0.73	1.358
80	92.83	11.47	16.69	6.83	5.50	0.80	1.252
LSD 5%	0.94	0.25	N.S.	0.12	0.12	0.01	0.038

Nd = Number of days to first flower open, Ni = Number of inflorescences, Si = Stalk length of inflorescence, Di = Diameter of inflorescence, Fwi = Fresh weight of one inflorescence, Cc = Carotenoids content in ray flower (mg/g D.W.)

Table 6 Effect of the interaction between gibberellic acid and β-sitosterol treatments on flowering parameters and carotenoids content in ray flowers of plant during full blooming stage (average of both seasons).

Interaction treatments		Flowering parameters						
GA_3 (mgL^{-1})	Sitosterol (mgL^{-1})	Nd	Ni	Si	Di	Fwi	Dwi	Cc
0	0	115.00	9.00	10.92	5.25	3.87	0.62	1.069
	20	102.00	12.50	15.75	7.84	7.03	0.94	1.484
	40	96.33	15.92	14.38	7.52	5.97	0.79	1.511
	80	105.00	8.58	13.25	6.82	4.99	0.76	1.431
25	0	98.00	16.83	15.69	6.12	5.92	0.78	1.572
	20	104.00	12.79	13.52	7.36	6.79	0.95	1.438
	40	99.00	14.03	13.75	6.33	4.22	0.61	1.617
	80	90.33	12.08	16.28	6.34	5.21	0.75	1.218
50	0	94.00	15.83	17.50	6.35	5.16	0.67	1.342
	20	98.00	13.64	16.92	7.14	5.31	0.73	1.467
	40	92.00	11.28	16.80	6.69	5.67	0.70	1.047
	80	89.00	12.92	17.53	7.53	5.48	0.81	1.161
100	0	91.00	13.25	23.00	6.63	4.84	0.61	1.188
	20	85.00	16.00	21.19	6.67	4.80	0.70	1.406
	40	79.67	17.75	22.17	6.47	5.73	0.82	1.259
	80	87.00	12.28	19.72	6.61	6.32	0.89	1.199
LSD 5%		1.87	0.50	0.48	0.24	0.24	0.03	0.076

Nd = Number of days to first flower open, Ni = Number of inflorescences, Si = Stalk length of inflorescence, Di = Diameter of inflorescence, Fwi = Fresh weight of one inflorescence, Cc = Carotenoids content in ray flower (mg/g D.W.)

Menesy *et al*. (1991) first studied the effect of GA_3 (at 50, 100 and 200 ppm) on flowering characters of marigold; all concentrations significantly accelerated flowering date (107.30, 108.30 and 112.30 days, respectively) compared with the control (122 days). In addition, GA_3 at 100 ppm resulted in the heaviest weight of a single flower.

Effect of β-sitosterol on flowering

β-sitosterol application appeared significant decrease on number of days to appearance the first flower and enhancement of inflorescence characteristic (number, diameter, fresh and dry weight) and carotenoids content of flower petioles as shown in **Table 5**. The lowest value of days number to appearance the first flower and number of inflorescences/plant was more affected by increasing β-sitosterol concentration. These results show that β-sitosterol had stimulatory effect on marigold flowering, that meight be due to flowering hormone role as a steroid or an isoprenoid-like compound. In this trend unsaponifiable liquid fractions from flowering plants promote flowering in the vegetative ones. The active substance might include vitamin E and certain unidentified sterols (Biswas *et al*. 1967). This effect appeared at increasing number, fresh and dry weigh of flowers/plant as a result of stigmasterol application (25, 50, 75 and 100 mg/l) especially at 100 mg/l on chamomile (*Chamomilla recutita* L. Rausch) (Abd El-Wahed and Gamal 2004).

Effect of GA_3 and β-sitosterol interaction on the flowering

Data in **Table 6** shows that interaction between GA_3 (100 mg L^{-1}) and β-sitosterol (40 mg L^{-1}) had a significant effect on flowering earliness of marigold plant and increased the number of inflorescences. While GA_3 (50 mgL^{-1}) + β-sitosterol (40 mgl^{-1}) gave the highest value of inflorescence diameter, the highest inflorescence fw and dw were obtained with GA_3 (25 mg L^{-1}) + β-sitosterol (20 mgL^{-1}). Carotenoid content in ray florets was more significantly affected by GA_3 (25 mgL^{-1}) + β-sitosterol (40 mg L^{-1}) interaction treatment. El-Shazly and El-Masri (2003) also found that the number of total and open balls per cotton plant increased with the application of GA_3. Bandara and Tanino (1995) showed that the application of GA_3 to carrot plants advanced flowering by at least 2 weeks but only increased flowering to 88%.

Effect of GA_3 on biochemical contents of marigold leaves

There were significant increments in the biochemical contents of Chl *a* and *b*, total Chls and total carotenoids, total sugar, indoles and free amino acid of marigold leaves related to GA_3 (25 mgL^{-1}) foliar application (**Table 7**). Total phenols were significantly enhanced by increasing GA_3 concentration (maximum at 100 mgL^{-1} GA_3). In contrast,

Table 7 Effect of gibberellic acid on leaf pigments content and some biochemical composition of marigold plant (average of both seasons).

Gibberellic acid (mgL^{-1})	Chl. *a*	Chl. *b*	Tchl (*a*+*b*)	Tc	Ts	Ti	Tph	Ta
Control	0.38	0.25	0.63	0.52	34.02	6.055	18.92	18.70
25	0.36	0.27	0.64	0.55	37.72	6.313	16.34	21.00
50	0.31	0.24	0.55	0.46	35.21	6.091	18.40	18.45
100	0.30	0.24	0.54	0.48	31.80	5.781	20.02	16.33
LSD 5%	0.01	0.01	0.02	0.02	0.52	0.137	0.27	0.19

Chl. *a* = Chlorophyll *a* (mmg^{-1} F.w), Chl. *b* = Chlorophyll *b* (mmg^{-1} F.w), Tchl (*a*+*b*) = Total Chlorophylls (*a*+*b*) (mgg^{-1} F.w), Tc = Total carotenoids (mgg^{-1} F.w), Ts = Total sugars (mgg^{-1} D.w), Ti = Total indoles, Tph = Total free phenols (mgg^{-1}), Ta = Total free amino acids (mgg^{-1})

Table 8 Effect of β-sitosterol on leaf pigments content and some biochemical composition of marigold plant (average of both seasons).

B-sitosterol (mgL^{-1})	Chl. *a*	Chl. *b*	Tchl (*a*+*b*)	Tc	Ts	Ti	Tph	Ta
Control	0.31	0.26	0.56	0.49	32.63	5.892	20.04	17.34
20	0.37	0.26	0.63	0.55	36.32	6.289	16.40	20.07
40	0.34	0.26	0.60	0.51	37.21	6.169	17.14	19.81
80	0.33	0.23	0.57	0.47	32.58	5.891	20.10	17.26
LSD 5%	0.01	0.01	0.02	0.02	0.52	0.137	0.27	0.19

Chl. *a* = Chlorophyll *a* (mmg^{-1} F.w), Chl. *b* = Chlorophyll *b* (mmg^{-1} F.w), Tchl (*a*+*b*) = Total Chlorophylls (*a*+*b*) (mgg^{-1} F.w), Tc = Total carotenoids (mgg^{-1} F.w), Ts = Total sugars (mgg^{-1} D.w), Ti = Total indoles, Tph = Total free phenols (mgg^{-1}), Ta = Total free amino acids (mgg^{-1})

Table 9 Effect of interaction between gibberellic acid and β-sitosterol treatments on leaf angle, leaf pigments content and some biochemical composition of marigold plant (average of both seasons).

Interaction treatments		Leaf pigments					Biochemical composition		
GA_3 (mgL^{-1})	Sitosterol (mgL^{-1})	Chl. *a*	Chl. *b*	Tchl (*a*+*b*)	Tc	Ts	Ti	Tph	Ta
0	0	0.29	0.21	0.50	0.40	27.47	5.512	15.03	23.31
	20	0.42	0.27	0.68	0.56	38.71	6.818	15.19	22.91
	40	0.40	0.28	0.68	0.57	36.76	6.026	17.68	20.05
	80	0.40	0.26	0.66	0.54	33.12	5.866	19.50	16.80
25	0	0.36	0.32	0.68	0.59	36.61	6.179	16.70	19.40
	20	0.39	0.25	0.65	0.54	37.98	6.339	15.54	21.67
	40	0.40	0.28	0.68	0.61	41.94	6.647	14.59	23.78
	80	0.31	0.24	0.55	0.46	34.75	6.085	18.55	19.16
50	0	0.31	0.26	0.57	0.51	34.75	0.065	19.09	18.51
	20	0.36	0.27	0.63	0.55	35.91	6.245	16.57	18.95
	40	0.25	0.21	0.46	0.37	36.93	6.148	17.38	18.57
	80	0.31	0.22	0.52	0.43	33.22	5.907	20.55	17.77
100	0	0.26	0.23	0.49	0.45	31.68	5.811	21.05	16.40
	20	0.31	0.25	0.57	0.53	32.69	5.752	18.33	16.75
	40	0.31	0.26	0.57	0.48	33.22	5.856	18.91	16.84
	80	0.30	0.22	0.52	0.45	29.60	5.705	21.79	15.32
LSD 5%		0.01	0.01	0.02	0.02	1.03	0.275	0.54	0.39

Chl. *a* = Chlorophyll *a* (mmg^{-1} F.w), Chl. *b* = Chlorophyll *b* (mmg^{-1} F.w), Tchl (*a*+*b*) = Total Chlorophylls (*a*+*b*) (mgg^{-1} F.w), Tc = Total carotenoids (mgg^{-1} F.w), Ts = Total sugars (mgg^{-1} D.w), Ti = Total indoles, Tph = Total free phenols (mgg^{-1}), Ta = Total free amino acids (mgg^{-1})

the other biochemical contents of marigold leaves were improved by GA_3 at low concentration (25 mg L^{-1}). Wu *et al*. (1993) who found that exogenously-applied gibberellins led to an increase in acid invertase activity in tulip (*Tulipa gesneriana* L.) whereas a high concentration of hexose sugar and increased activity of acid invertase were observed when internodes rapidly elongated (Ranwala and Miller 2008). GA_3 treatments (50 mg L^{-1}) similarly increased essential amino acid and total amino acid content of soybean (*Glycine max* L.) seed (Kushubakova 2008).

Effect of β-sitosterol on biochemical contents of marigold leaves

Biochemical contents of marigold leaves were significantly increased by β-sitosterol application (**Table 8**). The highest values of Chl *a*, carotenoids, indoles and free amino acids were obtained with the application of 40 mg L^{-1} β-sitosterol while 80 mg L^{-1} resulted in the maximum value of phenolic compounds in the leaves.

Metabolic shifts may involve translocation and reconstructed of amino acids (Osaki *et al*. 1991), activation of dehydrogenase activity (Wang and Wang 1997) or stimulation of DNA and RNA replication (Szekers and Konez 1998). This might explain the obtained changes in the biochemical contents in the marigold leaves.

Effect of GA_3 and β-sitosterol interaction on biochemical contents of marigold leaves

Increasing both GA_3 and β-sitosterol concentration simultaneously decreased biochemical contents in marigold leaves, except for phenolic compound content (**Table 9**). GA_3 (25 mg L^{-1}) + β-sitosterol (20 mg L^{-1}) resulted in the same effect as 40 mg L^{-1} β-sitosterol with respect to all leaf biochemical parameters.

Abd El-Wahed (2000) in maize (*Zea mays* L.) using 150 mg/l β-sitosterol and Abd El-Wahed (2008) in soybean using β-sitosterol (40 mg/l) found that application of sterol compounds improved the biochemical constituents of the plants.

Correlation coefficient of growth, flowering and biochemical content of marigold plant

The correlation coefficients between plant growth, leaf angle, flowering and biochemical contents of marigold leaves related to GA_3 effect are presented in **Table 10**. There is a highly positive correlation between leaf angle, plant growth (fw/plant, and shoot/root ratio; number of days to first flower and all biochemical contents. These effects consequently reflected on inflorescence diameter. Concerning the β-sitosterol foliar application correlation coefficient, the results appeared highly and positively correlated between leaf angle and plant height while there are a positive correlation between fresh weight of shoot and diameter of inflorescence and biochemical content, except phenolic com-

Table 10 Correlation between growth, flowering and biochemical contents under gibberellic acid concentration.

	La	Ph	Bn	Sh w	Shrr	Nd	Di	Fi	Tchl	Cc	Tc	Ti	Tph	Ta
La	1.000													
Ph	-0.986**	1.000												
Bn	0.194	-0.060	1.000											
Sh w	0.769*	-0.688*	0.763*	1.000										
Shrr	0.965**	-0.994**	-0.44	0.609	1.000									
Nd	0.982**	-0.988**	0.013	0.636	0.985**	1.000								
Di	0.259	-0.202	-0.255	-0.116	0.221	0.352	1.000							
Fi	0.616	-0.628	0.493	0.803**	0.582	0.505	-0.589	1.000						
Tchl	0.899**	-0.926**	0.214	0.774*	0.907**	0.856	-0.178	.870**	1.000					
Cc	0.747*	-0.761*	0.419	0.828**	0.722*	0.655	-0.446	0.983**	0.946**	1.000				
Tc	0.632	-0.525	0.881**	0.976**	0.432	0.479	-0.122	0.717*	0.620	0.717*	1.000			
Ti	0.753*	-0657	0.791*	0.994**	0.575*	0.619	-0.038	0.735*	0.717*	0.764	0.986**	1.000		
Tph	-0.596	0.504	-0.879**	-0.971**	-0.413	-0.436	0.269	-0.800**	-0.653	-0.782*	-0.988**	-0.966**	1.000	
Ta	0.772*	-0.698*	0.748*	0.998**	0.621	0.640	-0.152	0.830**	0.796*	0.854**	0.969**	0.988**	-0.970**	1.000

La = Leaf angle, Ph = Plant height, Bn = No. of branches/plant, Sh w = Fresh weight of shoots, Shrr = Shoot-root ratio (F.Wt.), Nd = No. of days to first flower, Di = Diameter of inflorescence, Fi = F.Wt. of one inflorescence, Ta = Total free amino acid in leaves, Tp = Total free phenols in leaves, Tc = Total carbohydrates in leaves, Ti = Total indoles in leaves, Cc = Carotenoids content in ray flower, Tchl = Total Chlorophylls (*a*+*b*).
*: Significant at 0.05 level
**: Significant at 0.01 level

Table 11 Correlation between growth, flowering and biochemical contents under β-sitosterol concentration

	La	Ph	Bn	Sh w	Shrr	Nd	Di	Fi	Tchl	Cc	Tc	Ti	Tph	Ta
La	1.000													
Ph	0.885**	1.000												
Bn	-0.242	0.197	1.000											
Sh w	0.311	0.715*	0.702*	1.000										
Shrr	0.351	0.723*	0.571	0.981**	1.000									
Nd	0.066	-0.235	-0.884*	-0.472	-0.296	1.000								
Di	-0.184	0.274	0.756*	0.864**	0.855*	-0.379	1.000							
Fi	-0.193	0.236	0.638	0.820*	0.842*	-0.218	0.986**	1.000						
Tchl	0.333	0.641	0.343	0.880**	0.953**	0.001	0.806*	0.844*	1.000					
Cc	0.494	0.705*	0.126	0.780*	0.883**	0.175	0.638*	0.697	0.970**	1.000				
Tc	0.685	0.943**	0.439	0.906**	0.906**	-0.345	0.577	0.537	0.815*	0.809*	1.000			
Ti	0.504	0.795*	0.365	0.918**	0.972**	-0.105	0.735*	0.749*	0.974**	0.961**	0.923**	1.000		
Tph	-0.558	-0.842*	-0.380	-0.924**	0.968**	0.155	-0.704*	-0.706*	-0.950**	-0.941**	-0.954**	-0.996**	1.000	
Ta	0.605	0.882**	0.394	0.925**	0.957**	-0.206	0.668	0.660	0.920**	0.913**	0.976**	0.984**	-0.996**	1.000

La = Leaf angle, Ph = Plant height, Bn = No. of branches/plant, Sh w = Fresh weight of shoots, Shrr = Shoot-root ratio (F.Wt.), Nd = No. of days to first flower, Di = Diameter of inflorescence, Fi = F.Wt. of one inflorescence, Ta = Total free amino acid in leaves, Tp = Total free phenols in leaves, Tc = Total carbohydrates in leaves, Ti = Total indoles in leaves, Cc = Carotenoids content in ray flower, Tchl = Total Chlorophylls (*a*+*b*).
*: Significant at 0.05 level
**: Significant at 0.01 level

Table 12 Correlation between growth flowering, and biochemical contents under interaction treatments

	La	Ph	Bn	Sh w	Shrr	Nd	Di	Fi	Tchl	Cc	Tc	Ti	Tph
Ph	-0.85**												
Bn	0.02	-0.027											
Sh w	0.43	-0.159	0.583										
Shrr	0.50	-0.262	0.236	0.66									
N d	0.79*	-0.727*	-0.307	0.124	0.367								
Di	-0.14	0.227	0.359	0.487	0.462	-0.152							
Fi	-0.09	0.046	0.215	0.416	0.279	-0.163	0.68						
Tchl	0.42	-0.358	0.305	0.659	0.706*	0.342	0.37	0.301					
Cc	0.24	-0.145	0.339	0.597	0.609	0.199	0.31	0.198	0.94**				
Tc	0.33	0.042	0.478	0.870**	0.576	0.080	0.45	0.320	0.64	0.68			
Ti	0.38	-0.045	0.300	0.853**	0.505	0.195	0.47	0.416	0.62	0.61	0.92**		
Tph	-0.31	-0.019	-0.471	-0.849*	-0.615	-0.069	-0.45	-0.393	-0.69*	-0.74*	-0.95**	-0.90**	
Ta	0.45	-0.108	0.388	0.894**	0.577	0.233	0.45	0.344	0.68	0.67	0.95**	0.95**	-0.90**

La = Leaf angle, Ph = Plant height, Bn = No. of branches/plant, Sh w = Fresh weight of shoots, Shrr = Shoot-root ratio (F.Wt.), Nd = No. of days to first flower, Di = Diameter of inflorescence, Fi = F.Wt. of one inflorescence, Ta = Total free amino acid in leaves, Tp = Total free phenols in leaves, Tc = Total carbohydrates in leaves, Ti = Total indoles in leaves, Cc = Carotenoids content in ray flower, Tchl = Total Chlorophylls (*a*+*b*).
* Significant at 0.05 level
** Significant at 0.01 level

pounds (**Table 11**). The interaction between leaf angle and number of days to first flower was significantly attributed to both substances used (**Table 12**).

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