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EFFECT OF B-SITOSTEROL AND GIBBERELLIC ACID ON LEAF ANGLE ,GROWTH, FLOWERING AS WELL AS BIOCHEMICAL CONSTITUENTS OF MARIGOLD (*Calendula officinalis*) PLANT

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ABSTRACT

Marigold plant growth and orientation were improved by adding gibberellic acid (25, 50 and 100 mgL^{-1}) and β-sitosterol (20, 40 and 80 mgL^{-1}) as well as their interaction treatments. GA_3 (25 mgL^{-1}) was more effective on number of branches, fresh and dry weight of shoots and roots/plant. While, GA_3 (100 mgL^{-1}) significantly increased plant height. Leaf angle was more decreased with GA_3 than β-sitosterol application. Both GA_3 (100 mgL^{-1}) or β-sitosterol (40 mgL^{-1}) significantly induced flowering earliness and number of marigold plant inflorescences. While low concentration of both substances improved the other characters of inflorescences. GA_3 (100 mgL^{-1}) and β-sitosterol (40 mgL^{-1}) interaction significantly increased earliness, number and stalk length of marigold plant inflorescences.

Pigments (Chl.a & b and carotenoids) and biochemical constituents (total sugars, indoles and free amino acids) were significantly enhanced with GA_3 (25 mgL^{-1}) and / or β-sitosterol (20 or 40 mgL^{-1}) as foliar application. However, both the highest concentration of two bioregulators gave the highest phenolic content of marigold plant leaves. Additionally, GA_3 (25 mgL^{-1}) and β-sitosterol (40 mgL^{-1}) interaction was more effective on leaves biochemical contents (Chl.a, carotenoids, total sugars, indols and free amino acids). Phenolic content was permanent increased with the highest concentration of both growth substances. GA_3 and β-sitosterol appeared positive correlation between fresh weight of inflorescences and Chl.(a+b). All the results were significantly negative attributed with phenolic content of marigold plant leaves.

Keywords: Marigold, Gibberellic acid, β-sitosterol, growth orientation, biochemicals.

INTRODUCTION

Marigold plant (*Calendula officinalis* L.) is a common herb, has some compounds that have a number of medicinal uses. Sterols are one of the present compounds in marigold plant.

Plant sterol composition varies within different species and physiological stage of development (Ebrahimzadeh *et al*., 2001). Despite the identification of plant steroids (Brs), dozens of other sterols are also found in plants. Major plant sterols such as sitosterol and stigmasterol are similar in structure of 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 Enricsson, 1999), carbohydrate distribution in maize (Abd El-Wahed, 2000), free amino acids, phenols and indoles in soybean (Abd El-Wahed, 2008). In addition, brassinosteroids could be generate erect leaf of rice plant (Morinaka *et al*., 2006).

GAs regulate 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). GAs changed the orientation of lateral branches of runners to that of erect ones. The amount of native gibberellic acid antagonists was higher in runner plants. Furthermore, runner plants contained a particular gibberellic acid inhibitor not found in erect plants. Addtionally, gibberellic acid 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 gibberellic acid (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).

Purpose of the present study:

While some studies claimed that GA_3 and steroids promote plant growth. However none of these studies have sought to examine β-sitosterol and GA_3 as single or combination on growth to obtain quantitative data on the actual characters of marigold plant. 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 gibberellic acid (Diterpene) and β-sitosterol (Triterpene) as well as their combinations on growth, flowering and some biochemical constituents of marigold plant. Carotenoids of *Calendula officinalisl* L. plant is Tetraterpene. Seeds were obtained from Medicinal and Aromatic plants Research Section, Ministry of Agriculture. Seeds were sown in bed on October 15th for both the two seasons. After one month, the uniform seedlings about 6 cm in height with 2 pairs of leaves were individually transplanted into pots of 30 cm diameter filled with 10kg 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 after 2 weeks later.

Plants were foliar sprayed with gibberellic acid (GA_3) at (0, 25, 50 or 100 mg/1), β-sitosterol at (0, 20, 40 or 80 mg/l) and control plants prayed with distilled water. Plants were twice sprayed with the growth regulators up the run-off occurred, the first sprayed was five weeks after transplanting and the second one was applied three weeks later. Treatments were arranged in

complete randomized design, replicated 3 times, each replicate contained 6 pots.

Biochemical constituents determination:

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

Statistical Analysis:

The data were statistically analyzed for each season and then combined analysis of the two seasons was carried out according to procedure outlined by Snedecor and Cochran (1990). For comparison between means, L.S.D. test at 5% was calculated.

Simple correlation coefficients between growth, flowering and biochemical contents under studies treatments of 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) showed that GA_3 significantly increased vegetative growth characters *i.e.* plant height, number of branches, fresh and dry weight of shoots and roots/plant. While, shoot/root ratio of fresh and dry weight were significantly decreased compared to control. GA_3 (100 mgL^{-1}) gave the highest value of plant height. GAs cause dramatic increases in plant height (King *et al*., 2008).

In the same trend, GA_3 (25 mgL^{-1}) was more effective on number of branches, fresh and dry weight of root and shoot/plant (Table 1). The reduction of leaf angle was attributed to increasing GA_3 concentration. These effects might be due to GA_3 role for improving vegetative growth characteristic, whereas GAs are plant hormones that participate in regulation of many growth developmental processes in plants (Hedden and Phillips, 2000 and Olszewski *et al*., 2002).

Effect of β-sitosterol on vegetative growth:

β-sitosterol application led to significant increase in vegetative growth characters (plant height, number of branches, fresh and dry weight of root and shoot) of marigold plant as shown in Table (2) and Fig.(2). Growth

characters were significantly differed in their response to β-sitosterol application. In addition, reducing leaf angle was related with increasing β-sitosterol concentration. Shoot dry weight and shoot/root rate of plant were more increased by 40 mgL^{-1} β-sitosterol, while shoot fresh weight and shoot/root ratio of fresh weight were improved by 20 mgL^{-1} β-sitosterol. The highest number of branches, root fresh weight and leaf angle was recorded with 80mgL^{-1} β-sitosterol. The increments of growth criteria might be due to the differentiation of morphogenesis mother cell during plant growth stages as a result to β-sitosterol application. The results were in agreement with Cao and Chen (1995) who reported that brassinosteroid induced inclination was accompanied by increasing lamina fresh weight. Also, Abd El Wahed *et al*.,(2001) showed stimulatory effect on vascular differentiation, thickness of upper epidermal mesophyll tissue layers growth and yield as well as chemical composition of wheat plant by β-sitosterol application . In addidion, brassinolide doubled the mean root length and the number of mitoses over that controls at low concentration (Howell *et al*., 2007).

Effect of GA_3 and β-sitosterol interaction on vegetative growth:

Data in Table (3) and Figures (3 to 5) showed that vegetative characters significantly increased as a result of GA_3 and β-sitosterol interaction application. Their improvements were related to increase both GA_3 and β-sitosterol concentrations. The highest values of vegetative growth characters (number of branches and fresh weight of shoots/plant) was obtained with GA_3 (25 mgL^{-1}) plus β-sitosterol (80 gL^{-1}), dry weight of shoots/plant with GA_3 (25 mgL^{-1}) + β-sitosterol (40mgL^{-1}) and shoot/root ratio with GA_3 (25 mgL^{-1}) plus β-sitosterol (20 mgL^{-1}) and β-sitosterol (80 mgL^{-1}). It appears from the results that both phytohormones play an important role in differentiation and morphogenesis of plant cell because their precoursor is isoprenoid. In relation, Hedden and Phillips (2000) reported that GAs are plant hormones that participate in regulation of many growth and developmental processes in plants and important in stem regulation.

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).

Characters / Gibberellic acid (mgL^{-1})	Plant height (cm)	Branches number / plant	Shoots weight (g/plant)		Root weight(g/plant)		Shoot/root ratio		Leaf angle
			F.W.	D.W.	F.W.	D.W.	F.W.	D.W.	
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
L.S.D. at 5%	0.85	0.82	1.81	0.66	0.55	0.15	0.06	0.03	2.04

Fig. (1): Leaf angle of marigold plant under gibberellic acid treatments.

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

Characters / B Sitosterol (mgL^{-1})	Plant height (cm)	Branches number / plant	Shoots weight (g/plant)		Root weight(g/plant)		Shoot/root ratio		Leaf angle
			F.W.	D.W.	F.W.	D.W.	F.W.	D.W.	
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
L.S.D. at 5%	0.85	0.82	1.81	0.66	0.55	N.S.	0.06	0.03	0.04

Fig. (2): Leaf angle of marigold plant under β-sitosterol treatment.Table

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

Characters / Interaction treatments		Plant height (cm)	Branches number / plant	Shoots weight (g/plant)		Root weight (g/plant)		Shoot/root ratio		Leaf angle
				F.W.	D.W.	F.W.	D.W.	F.W.	D.W.	
GA_3(mgL^{-1})	Sitosterol (mgL^{-1})									
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
L.S.D. at 5%		1.71	1.65	3.62	1.33	1.11	0.31	0.12	0.07	4.07

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 could be generate erect leaf of rice plant (Morinaka *et al*., 2006).

Effect of GA_3 on flowering:

Data in Table (4) show that GA_3 application significantly decreased the number of days to appearance the first inflorescence. The contrast trend was obtained with numbers, stalk lengths, fresh weight and carotenoides content of inflorescences. Increasing or decreasing character values was related to GA_3 concentration. GA_3 at 100 mgL^{-1} was more effective on number of days the appearance of the opened first flower and stalk length, 50 mgL^{-1} on diameter, 25 mgL^{-1} on inflorescences fresh weight and carotenoides of flower petals. These effects show that GA_3 plays an important role in flowering of marigold plant. That might be due to its clear associations between applied and endogenous GAs and flowering in dicotyledonous species including *Arabidopsis* (Eriksson *et al*., 2006). Whereas, the level of bioactive GAs (Variously GA_1, GA_4, GAs and GA_6) increase rapidly in the leaf, petiole and shoot apex of both monocots and dicots (King *et al*., 2006). These results appeared an effect on flowering characters of marigold plant (Menesy *et al*., 1991).

Figs. (3 to 5): Leaf angle of marigold plant under interaction treatments

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 bedue 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 on chamomile plant (Abd El- Wahed and Gamal, 2004).

Effect of GA_3 and β-sitosterol interaction on the flowering:

Data in Table (6) show that interaction between GA_3 (100 mgL^{-1}) and β-sitosterol (40 mgL^{-1}) had significant effect on flowering earliness of marigold plant and increasing number of inflorescences While, GA_3 (50 mgL^{-1}) plus β-sitosterol gave the highest value of inflorescence diameter. In the same time, the highest fresh and dry weights of inflorescence were obtained with GA_3 (25 mgL^{-1}) and β-sitosterol (20 mgL^{-1}). Carotenoids content in ray flower was more significantly affected by GA_3 (25 mgL^{-1}) and β-sitosterol (40 mgL^{-1}) interaction treatment.

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

Flowering arameters / Gibberellic Acid (mgL^{-1})	Number of days to first flower open	Number of inflorescences plant	Stalk length of inflorescence	Diameter of inflorescence	Fresh weight of one inflorescence	Dry weight of one Inflorescence	Carotenoids content in ray flower (mg/g D.W.)
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
L.S.D. 5%	0.9	0.25	0.24	0.12	N.S.	0.01	0.038

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 the two seasons).

Flowering parameters / β-sitosterol (mgL^{-1})	Number of days to first flower open	Number of inflorescences plant	Stalk length of inflorescence	Diameter of inflorescence	Fresh weight of one inflorescence	Dry weight of one Inflorescence	Carotenoids content in ray flower (mg/g D.W.)
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
L.S.D. 5%	0.94	0.25	N.S.	0.12	0.12	0.01	0.038

This might be due to the both role of GA_3 and β-sitoserol in enhancing sexual hormone of meristemic tissues that led to earliness and increasing inflorescence characteristic. These results were in agreement with El-Shazly and El-Masri (2003) who found increasing numbers of total and open balls per cotton plant. Bandara and Tanino (1995) showed that GA_3 application on carrot plant advanced flowering at least 2 weeks but only increased flowering to 88%. In addition, flowering hormone may be a steroid or isoprenoid-like compound. Other steroid compound play an important role in flowering plant (Biswas *et al.*, 1967).

Effect of GA_3 on biochemical contents of marigold leaves:

Data presented in Table (7) indicated that there are significant increaments of biochemical contents (Chl.a,b Chl.(a+b), carotenoids, total sugar, indoles and free amino acid of marigold leaves related to GA_3 (25 mgL^{-1}) foliar application. While, total phenols was significantly enhanced with increasing GA_3 concentration. Maximum value of total phenols was obtained by the highest concentration of GA_3 (100 mgL^{-1}). It appears from our results, that increase GA_3 concentration led to increase phenolic compounds in marigold leaves. In the opposite trend, the other biochemical contents of marigold plant were improved with GA_3 at low concentration. It might be due to that GA_3 application resulted in activation of the different enzymes in marigold leave. These results were in accordance of Wu *et al* . (1993) who found that exogenous gibberellins led to an increase in acid invertase activitygiven the involvement of gibberellins in tulips and the dynamic changes in carbohydrates profiles. Whereas, high concentration of hexose sugar and increased activity of acid invertase were observed when internodes were rapidly elongating (Ranwala and Miller, 2008). In this trend, GA_3, treatments increased essential amino acid, total amino acid content of soybean 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 as shown in Table (8). The highest values of Chl.a, carotenoids, indoles and free amino acids were significantly advanced with β-sitosterol (40 mgL^{-1}) foliar application. While, 80 mgL^{-1} β-sitosterol gave the maximum value of phenolic compounds in the leaves.

It might be concluded that foliar application of β-sitosterol concentration was sufficient to improve the biochemical contents of marigold leaves. It seemed that a shift between the previous compounds has been done within and between the plant organs at different stages. This metabolic shift may involved translocation and reconstructed of amino acids (Osaki *et al*., 1991), activation of dehydrogenase activity (Wang & Wang, 1997) or stimulation of DNA and RNA replication (Szekers & Konez, 1998). This might explain the obtained changes in the biochemical contents in the marigold leaves.

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 the seasons).

Interaction treatments / Flowering parameters		No. of days to first flower open	No. of inflorescence/ Plant	Stalk length of inflorescence	Diameter of inflorescence	Fresh weight of one inflorescence (g)	Dry weight of one inflorescence (g)	Carotenoids content in rays flowers (mg/g D.W.)
GA_3(mgL^{-1})	Sitosterol (mgL^{-1})							
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
L.S.D. at 5%		1.87	0.50	0.48	0.24	0.24	0.03	0.076

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

Data presented in Table (9) emphasized that biochemical contents in marigold leaves significantly decreased with increasing both GA_3 and β-sitosterol concentration in the interaction treatment as foliar application except the phenolic compounds that significantly enhanced with increasing both two applied substances. Maximum values of the biochemical (Chl.a, carotenoids, total sugars, indoles and free amino acids) were obtained with both GA_3 (25 mgL^{-1}) plus β-sitosterol (20 mgL^{-1}). It noted that this effect is parallel to β-sitosterol (40 mgL^{-1}) result on leaf pigments. It might be due to antagonistic relation between these substances in their interaction. It appears that both gibberellic acid and β-sitosterol have a role in leave biochemical contents.

This metabolic effect might be due to their precoursor. That is attributing with the balance of both substances in the leave. These results were in line with Abd El-Wahed (2000) on maize, Abd El-Wahed (2008) on soybean who found that sterols compound application improved the biochemical reconstruction in the plants.

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

Correlation coefficient between plant growth, leaf angle, flowering and biochemical contents of marigold leaves related to GA_3 effect are presented in in Table (10). The results show highly and positively correlation

coefficient between leaf angle, plant growth (Fresh weight/plant, and shoot-root ratio; flowering (No. of days to first flower) and biochemical contents Chl.(a+b), carotenoids in ray flower, indoles and free amino acids). These effects reflected on the correlation between diameter of inflorescine. From the present results, it could be concluded that GA_3 foliar application pronounced increases in growth characters, flowering and biochemical content of marigold leaves.

Concerning of β-sitosterol foliar application correlation coefficient, the results appeared highly and positively correlation 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 compound. The interaction between leaf angle and number of days to first flower was significantly attributed with both used substances.

From the results, it be concluded that both substances play an important role in improving marigold plant growth but there is a antagonistic effect between them. This might be due to the same precoursor isoprene.

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

Characters / Gibberellic acid (mgL^{-1})	Chl. a (mgg^{-1} F.W)	Chl. b (mgg^{-1} F.W)	Total Chl (a+b) (mgg^{-1} F.W)	Total Carotenoids (mgg^{-1} F.W)	Total sugars (%)	Total indoles (mgg^{-1})	Total free phenols (mgg^{-1})	Total free amino acids (mgg^{-1})
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
L.S.D. 5%	0.01	0.01	0.02	0.02	0.52	0.137	0.27	0.19

Table (8): Effect of β-sitosterol on leaf pigments content and some biochemical composition of marigold plant (Average of the two seasons).

Characters / B-sitosterol (mgL^{-1})	Chl.a (mgg^{-1} F.W)	Chl.b (mgg^{-1} F.W)	Total Chl (a+b) (mgg^{-1} F.W)	Total carotenoids (mgg^{-1} F.W)	Total sugars (mgg^{-1})	Total indoles (mgg^{-1})	Total free phenols (mgg^{-1})	Total free amino acids (mgg^{-1})
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
L.S.D. 5%	0.01	0.01	0.02	0.02	0.52	0.137	0.27	0.19

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

Character / Interaction treatments		Chl.a (mgg^{-1} F.W)	Chl.b (mgg^{-1} F.W)	Total Chl. (a+b) (mgg^{-1} F.W)	Total carotenoids (mgg^{-1} F.W)	Total sugars (mgg^{-1} D.W	Total indoles (mgg^{-1} D.W)	Total phenols (mgg^{-1} D.W)	Total free amino acid (mgg^{-1} D.W)
GA_3 (mgL^{-1})	Sitosterol (mgL^{-1})								
0	0	0.29	0.21	0.50	0.40	27.47	5.512	23.31	15.03
	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
L.S.D. 5%		0.01	0.01	0.02	0.02	1.03	0.275	0.54	0.39

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تأثير البيتاسيتوسيترول وحمض الجبريلليك على زاوية الورقة و النمو والتزهير والمكونات الكيماوية لنبات الأقحوان

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تحسن نمو نبات الأقحوان بإضافة حمض الجبريلليك (٢٥، ٥٠، ١٠٠مجم/لتر) والبيتاسيتوستيرول (٢٠، ٤٠، ٨٠ مجم/لتر) وكذلك التفاعلى بينهما: وكان الرش بحمض الجبريلليك ٢٥ مجم/لتر هو الأكثر فعالية فى زيادة كل من عدد الأفرع، الأوزان الغضة والجافة للمجموع الخضرى والجذرى بينما الرش بتركيز ١٠٠ مجم/لترجاء فى المرتبة الثالثه فى زيادة طول النبات . أدى الرش بحمض الجبريلليك (١٠٠ مجم/لتر) والبيتاستيرستيرول ٤٠مجم/لتر متفردين إلى تبكير التزهير وزيادة عدد النورات الزهرية بينما حسنت التركيزات المنخفضة منها باقى الصفات الزهرية، كذلك أدى التفاعل بين حمض الجبريلليك(١٠٠ مجم/لتر) مع البيتاسيتوسيترول (٤٠ مجم/لتر) إلى تبكير تزهير النورات الزهرية وزيادة عددها وطول الساق الزهرية.

أدى الرش بحمض الجبريلليك ٢٥ مجم/لتر، البيتاسيتوستيرول ٢٠، ٤٠مجم/ لتر إلى زيادة معنويه فى محتوى كلورفيل (أ) كلورفيل (ب) والكاروتينات والسكريات الكلية والأندولات والأحماض الأمينية الحره , بينما أدى الرش بالتركيز الأعلى لكلا منهما إلى زيادة محتوى الأوراق من الفينولات . كان التفاعل بين حمض الجبريلليك ٢٥مجم/لتر والبيتاسيتوستيرول ٤٠ مجم/لتر هو الأكثر فاعلية فى زيادة محتوى الأوراق من كلورفيل (أ) والكاروتينات والسكريات الكلية والأندولات والأحماض الأمينية الحره , فى حين زادت الفينولات مع التركيزات العالية لهما.

ظهر ارتباط موجب بين إضافة حمض الجبريلليك والبيتاسيتوستيرول فى زيادة وزن النورات الزهرية وكلورفيل (أ ,_ب)، وارتباط سالب مع محتوى الفينولات فى الأوراق.

Table (10): Correlation between growth, flowering and biochemical contents under gibberellic acid concentration

Characters	Leaf angle	Plant height	No. of branches/plant	Fresh wt. of shoots	Shoot-root ratio (F.W.)	No. of days to first flower	Diameter of inflorescence	F. wt. of one inflorescence	Chla (a+b)	Carot. Content in ray flower	Total carbohydrates in leaves	Total indoles in leaves	Total free phenols in leaves	Total free amino acid in leaves
Leaf angle	1.000													
Plant height	-0.986**	1.000												
No. of branches/plant	0.194	-0.060	1.000											
Fresh weight of shoots	0.769*	-0.688*	0.763*	1.000										
Shoot-root ratio (F.W.)	0.965**	-0.994**	-0.44	0.609	1.000									
No. of days to first flower	0.982**	-0.988**	0.013	0.636	0.985**	1.000								
Diameter of inflorescence	0.259	-0.202	-0.255	-0.116	0.221	0.352	1.000							
F.W. of one inflorescence	0.616	-0.628	0.493	0.803**	0.582	0.505	-0.589	1.000						
Chla (a+b)	0.899**	-0.926**	0.214	0.774*	0.907**	0.856	-0.178	.870**	1.000					
Carot. content in ray flower	0.747*	-0.761*	0.419	0.828**	0.722*	0.655	-0.446	0.983**	0.946**	1.000				
Total carbohydrates in leaves	0.632	-0.525	0.881**	0.976**	0.432	0.479	-0.122	0.717*	0.620	0.717*	1.000			
Total indoles in leaves	0.753*	-0657	0.791*	0.994**	0.575*	0.619	-0.038	0.735*	0.717*	0.764	0.986**	1.000		
Total free phenols in leaves	-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	
Total free amino acid in leaves	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

***: Significant at 0.05 level**
****: Significant at 0.01 level**

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

Characters	Leaf angle	Plant height	No. of branches/plant	Fresh wt. of shoots	Shoot-root ratio (F.W.)	No. of days to first flower	Diameter of inflorescence	F. wt. of one inflorescence	Chla (a+b)	Carot. Content in ray flower	Total carbohydrates in leaves	Total indoles in leaves	Total free phenols in leaves	Total free amino acid in leaves
Leaf angle	1.000													
Plant height	0.885**	1.000												
No. of branches/plant	-0.242	0.197	1.000											
Fresh weight of shoots	0.311	0.715*	0.702*	1.000										
Shoot-root ratio (F.W.)	0.351	0.723*	0.571	0.981**	1.000									
No. of days to first flower	0.066	-0.235	-0.884*	-0.472	-0.296	1.000								
Diameter of inflorescence	-0.184	0.274	0.756*	0.864**	0.855*	-0.379	1.000							
F.W. of one inflorescence	-0.193	0.236	0.638	0.820*	0.842*	-0.218	0.986**	1.000						
Chla (a+b)	0.333	0.641	0.343	0.880**	0.953**	0.001	0.806*	0.844*	1.000					
Carot. content in ray flower	0.494	0.705*	0.126	0.780*	0.883**	0.175	0.638*	0.697	0.970**	1.000				
Total carbohydrates in leaves	0.685	0.943**	0.439	0.906**	0.906**	-0.345	0.577	0.537	0.815*	0.809*	1.000			
Total indoles in leaves	0.504	0.795*	0.365	0.918**	0.972**	-0.105	0.735*	0.749*	0.974**	0.961**	0.923**	1.000		
Total free phenols in leaves	-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	
Total free amino acid in leaves	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

***: Significant at 0.05 level**
****: Significant at 0.01 level**

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

Characters	Leaf angle	Plant height	No. of branches/plant	Fresh wt. of shoots	Shoot-root ratio (F.Wt.)	No. of days to first flower	Diameter of inflorescence	F. wt. of one inflorescence	Chla (a+b)	Carot. Content in ray flower	Total carbohydrates in leaves	Total indoles in leaves	Total free phenols in leaves	Total free amino acid in leaves
Leaf angle	1.000													
Plant height	-0.852**	1.000												
No. of branches/plant	0.019	-0.027	1.000											
Fresh weight of shoots	0.432	-0.159	0.583	1.000										
Shoot-root ratio (F.Wt.)	0.503	-0.262	0.236	0.660	1.000									
No. of days to first flower	0.788*	-0.727*	-0.307	0.124	0.367	1.000								
Diameter of inflorescence	-0.143	0.227	0.359	0.487	0.462	-0.152	1.000							
F.Wt. of one inflorescence	-0.058	0.046	0.215	0.416	0.279	-0.163	0.684	1.000						
Chla (a+b)	0.423	-0.358	0.305	0.659	0.706*	0.342	0.366	0.301	1.000					
Carot. content in ray flower	0.242	-0.145	0.339	0.597	0.609	0.199	0.309	0.198	0.944**	1.000				
Total carbohydrates in leaves	0.332	0.042	0.478	0.870**	0.576	0.080	0.446	0.320	0.639	0.679	1.000			
Total indoles in leaves	0.377	-0.045	0.300	0.853**	0.505	0.195	0.473	0.416	0.619	0.614	0.917**	1.000		
Total free phenols in leaves	-0.306	-0.019	-0.471	-0.849*	-0.615	-0.069	-0.450	-0.393	-0.691*	-0.735*	-0.954**	-0.898**	1.000	
Total free amino acid in leaves	0.453	-0.108	0.388	0.894**	0.577	0.233	0.445	0.344	0.675	0.670	0.947**	0.952**	-0.903**	1.000

***: Significant at 0.05 level**
****: Significant at 0.01 level**