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Seaweed extract and brassinolide can enhance the dwarfing characteristics of potted chrysanthemum

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Abstract The search for environmental-friendly applications in agriculture is a priority and a necessity. The use of bio-stimulants such as seaweed extracts (SE) and brassinolide (BL) to improve plant growth, quality, and productivity is an effective and eco-friendly strategy. A pot experiment was carried out during the 2018 and 2019 seasons to investigate the effect of spraying plant growth retardants; cycocel (CCC) and paclobutrazol (PPP), in interaction with bio-stimulants; SE (Kelpak®) and BL (Milagrow®) on dwarfing characteristics of chrysanthemum [*Dendranthema grandiflorum* (Ramat.) Kitamura] cv. ‘Pink Zambla’ to achieve a desirable plant height with a high dwarfing value. Significant improvements of the studied traits; plant height, leaf number, branche number, plant biomass weight, flowering start, flower number, flower diameter, flowering period, and chlorophyll content was recorded for the combination treatments compared to the single application of the dwarfing factors. Concerning the key characteristics of chrysanthemum as potted plant, the best results were recorded in plants treated by combination of 3000 mg L^{-1} CCC or 50 mg L^{-1} PPP with different rates of Kelpak or Milagrow. It is possible to recommend the use of growth bio-stimulants along with growth inhibitors to produce a potted dwarf chrysanthemum plant with high flowering quality that increase its commercial value and meets consumer and market needs.

Keywords: *Dendranthema*, Cycocel, Paclobutrazol, Bio-stimulants, Brassinosteroids

Introduction

Floriculture is a vital activity in Egypt, and as such, it has made effective economic contributions. In particular, the cultivation of chrysanthemums is of great importance in Egyptian floriculture, and its cultivation has increased throughout the country. The genus *Dendranthema* (DC.) (*Chrysanthemum* L.) belongs to the family Asteraceae and includes about 40 species, widely distributed in China, Mongolia, Japan, and Eastern Europe (Mabberley, 2008;

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Youssef *et al*., 2020). *Dendranthema grandiflorum* (Ramat.) Kitamura (*Chrysanthemum indicum* L.) is one of the most important ornamental crops worldwide. Chrysanthemums are commonly used in bouquets and flower arrangements (Vijayakumar *et al*., 2021). In addition, recently, the importance of this flower as a pot plant has increased in Egypt.

The problem faced by chrysanthemums growers as potted plants is that their height is greater than the desired height beside the irregular growth habit. Controlling plant size is one of the most important aspects of potting plants that can be achieved chemically, genetically, or agronomically (Soliman *et al*., 2022; Li *et al*., 2023). Plant growth retardants (PGRs) can be an economical chemical option to control plant height and to improve the quality, balance, and overall appearance of many ornamental and flower plants (Asrar *et al*., 2014; Toaima *et al*., 2017; Abou Elhassan *et al*., 2021). Several reports have shown that there is a wide variation in the sensitivity of chrysanthemum species and cultivars to the application of PGRs (Pobudkiewicz, 2014; Ghatas, 2016; Vaghasia and Polara, 2016).

Among various PGRs, paclobutrazol and cycocel are well known for their use in the production of high-quality potted plants (Soliman *et al.*, 2022). Paclobutrazol (PPP), a triazole plant growth regulator, is effective in controlling vegetative growth and promoting compactness in several ornamental plants (Mishra and Yadava, 2011; Wickramasinghe *et al*., 2021; Abou Elhassan *et al*., 2021). Cycocel (CCC) is a synthetic plant growth inhibitor used in ornamental plants to induce dwarfing. In addition to the use of CCC to produce bedding and potted plants, it is used to enhance the green colour of the foliage, strengthen flower stems, and increase the resistance of plants to environmental stresses (Ghatas, 2016). However, damage to plant cells may occur because of cytotoxicity induced by these inhibitors, causing some problems such as delayed flowering, short flowering period, or small flower size. Thus, combining growth stimulants with dwarfing agents may be a good option to mitigate cytotoxicity and overcome this problem. In this context, Hu *et al*. (2013) found that a mixture application of abscisic acid and the bio-stimulants BL on *Leymus chinensis* enhanced the net photosynthesis rate, light saturation rate, water use efficiency, stomata conductivity, leaf respiration rate, maximum triphosphate utilization, maximum electron transfer rate, and carboxyl efficiency, thus increased density, plant height, and plant biomass.

Fertilization is one of the most important agricultural practices responsible for increasing the productivity of various crops. However, the improper use of chemical fertilizers by farmers to improve plant growth and productivity is harmful to the environment and human health (Nada *et al.*, 2022). Alternatively, the use of bio-stimulants such as seaweed extracts (SE) and brassinosteroids (BRs) in agriculture could be a feasible and environmentally friendly approach (Ghatas *et al.*, 2021). Bio-stimulants are a group of substances of natural origin that contribute to the absorption of nutrients, promote plant growth, and increase productivity while reducing dependence on chemical/inorganic fertilizers (Xu and Geelen, 2018). Kelpak, a liquid extract of the seaweed *Ecklonia maxima*, is a commercial product used as a spray or soil additive for improving plant health, anti-stress, and resistance to pathogenic fungi and nematodes (Oyoo *et al.*, 2010). On the other hand, brassinosteroids are a group of natural steroidal hormones (includes a class of more than 40 polyhydroxylated derivatives) present in plants and play an important role in many biological processes related to growth and development in plants (Kanwar *et al.*, 2022). Milagrow is a commercial natural growth stimulator extracted from the pollen of cabbage flowers (Seadh *et al.*, 2012). Milagrow promotes plant growth, increases yield, improves quality, promotes flower bud formation, and resists flower and fruit drops (Eid *et al.*, 2016). Its composition is 0.2% brassinolide (BL), 20% phosphorous, 10% potassium, and 3% boron (Seadh *et al.*, 2012).

The present study aimed to produce a potted chrysanthemum plant by applying PGRs such as cycocel (CCC) or paclobutrazol (PPP). To improve the dwarfing characteristics and quality of the product enough to meet market quality standards, the growth retardants were combined with growth bio-stimulants such as seaweed extracts (Kelpak®) and brassinolide (Milagrow®) as environmentally friendly natural stimulants instead of chemical fertilization. The vegetative growth, flowering aspects, and photosynthetic pigments of *D. grandiflorum* cv. 'Pink Zambla' were considered in the current study.

Materials and methods

The experiment was conducted in a private farm at El-Qurin (30°35'17.1"N 31°44'45.8"E), Sharkia, Egypt, during the two successive seasons 2018 and 2019. The experiment aimed to investigate the effect of the PGRs; CCC and PPP alone or in combination with the growth bio-stimulants; seaweed extracts (SE) (Kelpak®) and brassinolide (BL) (Milagrow®) on the dwarfing characteristics of *D. grandiflorum* (Ramat.) Kitamura cv. 'Pink Zambla'.

Plant materials

D. grandiflorum cv. 'Pink Zambla' cuttings were obtained from a private farm at El-Qanater El-Khayreya, Qalyubia, Egypt. Terminal cuttings (8-10 cm long) were treated with indole 3-butyric acid (IBA) at 2000 mg L^{-1}. The cuttings were then planted in a mixed medium of sand and peat moss in a ratio of 1:1 (v/v) and were incubated under long-day conditions and relatively high humidity (mist) for 10-15 days until fully rooting.

Mixture substrate (soil)

A commercial mixture substrate composed of 20% perlite and 80% peat moss (v/v) was prepared homogeneity before cultivation. The substrate pH was adjusted to 6.2 with calcium carbonate. The mixture substrate was chemically analyzed at National Research Centre, Dokki, Cairo, Egypt, and the results are presented in Table (1).

Table 1. Physical and chemical properties of the experimental mixture substrate (soil)

Items	**1st season**	**2nd season**
Moisture %	38	43
Ash %	51.61	63
Organic matter %	10.39	9.00
pH	7	6.3
E.C ds.m^{-1}	2.35	2.38
$CaCO_3$ %	2.85	2.60
Available macronutrients (mg kg^{-1} soil)		
N	50	51
P	11	13
K	78	80
Ca	1200	1142
Mg	162	170
Na	144	139
Available micronutrients (mg kg^{-1} soil)		
Fe	4.3	4.1
Mn	4.4	4
Zn	4.5	4.1
Cu	0.8	0.6

Cultivation and agricultural practices

Pots of 14 cm diameter were filled with the previously prepared mixture. The pots were arranged into groups of 15 pots for each treatment which contains 3 replicates of 5 pots each. The different groups of the prepared pots were placed in the spacing of 30 cm x 30 cm. Uniform rooted cuttings (15 days old) were planted on February 1st in both seasons. Containers containing plants (one plant per pot) were grown under a Multi Span greenhouse covered with plastic (120 microns thickness). Chrysanthemums are short-day plants as they only bloom in short daylight conditions. Therefore, flowering time can be regulated by controlling the photocycle to give marketable flowers during the year. Artificial light for 4 h (3 meters between lamps and 1.75 meters between plant and lamp) was employed to prolong the day for more than 16 h for all treatments for 28 days. After that, plants were covered with black plastic from 5 PM to 7 AM until color appeared in the flower bud. Pinching was carried out 2 weeks after transplanting. Plants were watered by drip irrigation to control irrigation management with a frequency depending on weather and plant conditions.

Treatments

The growth stimulants

SE (Kelpak®) was sprayed at 0, 3, 4, and 5 ml L^{-1}, while BL (Milagrow®) was applied at 0, 60, 80, and 100 mg L^{-1}. Both stimulants were sprayed 4 times at 3, 5, 7, and 9 weeks from transplanting. The composition of Kelpak® is displayed in Table (2) according to Anton *et al*. (2010). Milagrow is a registered trademark product of Green India Co., India. It has been obtained from Jaara Company, Cairo, Egypt. The composition of Milagrow is 0.2% BL, 20% phosphorous, 10% potassium, and 3% boron (Seadh *et al*., 2012).

Table 2. Chemical composition of Kelpak®

Composition (mg L^{-1})							
Auxins	10.7	Cytokinins	0.03	Amino acids	1000	Carbohydrates	10000
Proteins	2000	P_2O_5	300	Mg	56.4	Ca	200
Tot N	400	K_2O	6100	Mn	0.8	B	3.2
Org N	4000	Fe	2.2	Cu	1.8	Zn	0.9

The growth retardants

CCC (cycocel, or 2-chloro ethyl trimethyl ammonium chloride) at rates of 1500, 3000, and 6000 mg L^{-1}, and PPP (paclobutrazol) at 25, 50, and 75 mg L^{-1} were applied 4 weeks after transplanting (2 weeks after pinching) and repeated 2 weeks later. The spray solution soaked both leaves and stems till running off point using a 2-liter hand pump sprayer. The treatments were applied in the afternoon (4.00 pm) for easier absorption.

Measurements

Plant height (cm) from the soil surface to stem apex, leaf number per plant, and branche number per plant were recorded at the beginning of flowering. Fresh weight (g $plant^{-1}$) of aerial parts including flowers was recorded at the stage of complete flowering opening. Dry weight (g $plant^{-1}$) was determined after drying in an oven at 65°C for 48 h.

Flowering start (days) was considered at the first bud sprouting. The flower number per plant was calculated for both opened and unopened flowers. Flower diameter (cm) was measured for fully opened flowers. The flowering period (days) was recorded from the first opened flower until the end of flowering.

The content of chlorophyll and carotenoids (mg g^{-1} FW) were determined according to Lichtenthaler (1987). About 0.2 g of the fresh leaf was mixed with 15 ml acetone (80%). After filtration, the volume was adjusted to 15 ml with acetone (80%), and the absorption was measured with a JENWAY 6800 UV/Vis. spectrophotometer at 663.2, 646.8, and 470 nm against acetone (80%) blank. The concentration of chlorophyll (Chl) and carotenoids (Car) was calculated using the following formula:

$$Chl.a = 12.25A_{663.2} - 2.79A_{646.8}$$
$$Chl.b = 21.50A_{646.8} - 5.1A_{663.2}$$
$$Total\ Chl = 7.15A_{663.2} + 18.71A_{646.8}$$
$$Car = (1000A_{470} - 1.8Chl.a - 85.02Chl.b) / 198$$

Statistical analysis

The experiment design was arranged in a randomized complete design during the two seasons. Each treatment contained three replicates and each replicate consisted of 5 potted plants. The statistical analysis of data was subjected to Analysis of Variance (ANOVA), and means were compared by L.S.D. at 5% using COSTAT package ver. 6.4 (CoHort software Monterey, USA) according to Snedecor and Cochran (1980).

Results

Plant height (cm)

Spraying the dwarfing agents, CCC and PPP, on chrysanthemum plants resulted in a significant reduction in plant height compared to control treatment in both seasons (Figure 1A). Plant height was decreased linearly with the increase in the concentration of CCC and PPP. Higher dosages of CCC (6000 mg L^{-1}) and PPP (75 mg L^{-1}) severely reduced the plant height to 12.00 and 14.67 cm in the 2018 season, and to 14.00 and 14.67 cm in the 2019 season, respectively. Moderate applications of dwarfing agents (CCC 3000 mg $L^{-1}$and PPP 50 mg L^{-1}) achieved stem lengths (20-25 cm) close to commercially desirable plant lengths (around 30 cm).

Within the combination treatments of plant retardants; CCC and PPP, and growth stimulants; Kelpak and Milagrow, the highest significant values of plant height (59.45, 60.53, 59.40, 59.43, and 61.60 cm) were recorded when 3, 4, and 5 ml L^{-1} Kelpak, and 80 and 100 mg L^{-1} Milagrow were sprayed to plants dwarfed with 25 mg L^{-1} PPP in the 1st season, respectively, without significant differences between them (Table 3). In the 2nd season, the same trend was observed. The suitable commercial length for chrysanthemum plants (25-35 cm) was gained when 3000 mg L^{-1} of CCC or 50 mg L^{-1} of PPP was applied in interaction with the growth stimulants Kelpak and Milagrow at any rate.

Table 3. Effect of cycocel (CCC) and paclobutrazol (PPP) in interaction with Kelpak and Milagrow on plant height of chrysanthemum

Treatments		Control	Kelpak (ml L^{-1})			Milagrow (mg L^{-1})			Mean A
		0	3	4	5	60	80	100	
Plant height (cm)									
First season (2018)									
CCC (mg L^{-1})	1500	49.33	49.97	51.23	52.91	48.83	53.13	55.53	51.56
	3000	20.00	27.13	29.13	30.23	28.13	29.27	32.18	28.01
	6000	12.00	12.67	14.13	14.63	13.50	15.72	16.38	14.15
PPP (mg L^{-1})	25	55.00	59.45	60.53	59.40	57.32	59.43	61.60	58.96
	50	23.01	30.85	32.00	32.48	30.20	31.23	34.23	30.57
	75	14.67	15.52	15.03	16.92	16.05	17.08	18.72	16.28
Mean B		29.00	32.60	33.68	34.43	32.34	34.31	36.44	
L.S.D. at 5%: A= 0.94, B=1.02, A*B= 2.49									
Second season (2019)									
CCC (mg L^{-1})	1500	50.33	50.43	53.33	53.40	49.87	52.83	57.80	52.57
	3000	22.00	27.60	30.50	29.67	30.87	30.07	34.00	29.24
	6000	14.00	16.59	17.33	16.88	14.96	16.44	17.96	16.31
PPP (mg L^{-1})	25	70.00	74.85	75.29	76.00	75.33	78.48	79.40	75.62
	50	25.00	30.11	33.59	34.18	28.67	33.11	37.22	31.70
	75	14.67	15.81	16.07	17.92	16.22	17.51	21.29	17.07
Mean B		32.67	35.90	37.69	38.01	35.99	38.07	41.28	
L.S.D. at 5%: A= 1.23, B=1.33, A*B= 3.26									

Leaf number per plant

As displayed in Figure (1B), treating chrysanthemum plants with CCC and PPP reduced leaf number compared with the control. The negative effect of CCC and PPP on leaves number was increased with the increase in dwarfing agent level. The lowest leaf number (9.33 and 11.00 leaves per plant) were counted for plants dwarfed by the higher dose of PPP (75 mg L^{-1}) in the first and second seasons, respectively, followed by a higher level of CCC (6000 mg L^{-1}).

A significant improvement was observed in the leaf number of dwarfed plants when they were sprayed with Kelpak or Milagrow (Table 4). The highest significant value of leaf number in 1st season was recorded for the treatment of CCC (1500 mg L^{-1}) with Milagrow (100 mg L^{-1}) (63.33 leaves per plant) and the treatment of PPP (25 mg L^{-1}) with Milagrow (100 mg L^{-1}) which recorded 58.00 leaves per plant.

Table 4. Effect of cycocel (CCC) and paclobutrazol (PPP) in interaction with Kelpak and Milagrow on leaf number per plant of chrysanthemum

Treatments		Control	Kelpak (ml L^{-1})			Milagrow (mg L^{-1})			Mean A
		0	3	4	5	60	80	100	
Leaf number per plant									
First season (2018)									
CCC (mg L^{-1})	1500	42.00	46.67	53.00	56.67	56.00	57.67	63.33	53.62
	3000	35.33	37.00	38.00	45.00	49.33	52.33	53.33	44.33
	6000	12.00	17.67	19.00	25.00	21.00	22.00	22.67	19.90
PPP (mg L^{-1})	25	42.00	49.33	51.00	52.67	54.00	54.67	58.00	51.67
	50	28.33	35.33	37.67	39.67	37.67	41.33	42.33	37.48
	75	9.33	23.33	26.67	27.67	28.67	30.67	33.67	25.71
Mean B		28.17	34.89	37.56	41.11	41.11	43.11	45.56	
L.S.D. at 5%: A= 2.08, B= 2.24, A*B= 5.49									
Second season (2019)									
CCC (mg L^{-1})	1500	45.00	52.33	57.00	60.67	64.33	66.00	74.33	59.95
	3000	34.33	40.67	43.67	48.67	51.33	55.00	59.33	47.57
	6000	11.33	20.67	24.67	29.33	25.00	26.33	27.67	23.57
PPP (mg L^{-1})	25	44.00	48.67	54.67	58.33	59.33	63.00	66.00	56.29
	50	26.00	37.67	39.33	40.67	39.00	42.33	52.67	39.67
	75	11.00	22.33	29.67	32.33	31.67	34.67	39.67	28.76
Mean B		28.61	37.06	41.50	45.00	45.11	47.89	53.28	
L.S.D. at 5%: A= 2.43, B= 2.63, A*B= 6.44									

Branche number per plant

Application of CCC at low (1500 mg L^{-1}) and moderate (3000 mg L^{-1}) concentrations and PPP at a moderate level (50 mg L^{-1}) improved branching as compared to untreated plants (Figure 1C). The highest number of branches (4.00 branches per plant) was produced on plants treated with 1500 mg L^{-1} CCC in the 2018 season followed by 3000 mg L^{-1} CCC and 50 mg L^{-1} PPP (3.67 branches per plant). The same treatments recorded the highest branching in the 2019 season. Higher dosages of the dwarfing agents resulted in sever inhibition in branching.

An improvement in chrysanthemum branching was observed in the combination treatment (Table 5). Herein, in the second season, the highest values of branche number (4.40, 4.67, and 4.46 branches per plant) were noticed when 100 mg L^{-1} of Milagrow interacted with 1500, 3000 mg L^{-1} CCC, and 50 mg L^{-1} PPP, respectively.

Fresh and dry weight (g plant^{-1})

Fresh (FW) and dry weight (DW) were decreased in plants treated with high level of CCC or PPP separately (Figure 1D and 1E). The dwarfing caused by low and medium concentrations of CCC did not reduce the FW significantly when compared to control. In contrast, treating plants with 25 and 50 mg L^{-1} of PPP increased FW but insignificantly compared to control in both seasons. Low concentration of PPP (25 mg L^{-1}) recorded the highest significant DW in the first season (11.73 versus 11.17 g plant^{-1} of control).

Table 5. Effect of cycocel (CCC) and paclobutrazol (PPP) in interaction with Kelpak and Milagrow on branche number per plant of chrysanthemum

Treatments		Control	Kelpak (ml L^{-1})			Milagrow (mg L^{-1})			Mean A
		0	3	4	5	60	80	100	
Branche number per plant — First season (2018)									
CCC (mg L^{-1})	1500	4.00	4.00	4.03	4.00	4.33	3.87	4.47	4.10
	3000	3.67	3.70	3.93	4.33	3.83	4.33	4.33	4.02
	6000	1.33	1.70	1.77	1.87	1.40	1.67	1.50	1.60
PPP (mg L^{-1})	25	2.67	3.23	3.33	3.43	3.56	3.60	3.33	3.31
	50	3.67	4.30	4.33	4.57	3.50	3.90	4.40	4.10
	75	1.67	2.00	2.33	2.43	1.63	1.60	2.33	2.00
Mean B		2.83	3.16	3.29	3.44	3.04	3.16	3.39	
L.S.D. at 5%: A= 0.14, B= 0.15, A*B= 0.37									
Second season (2019)									
CCC (mg L^{-1})	1500	3.67	3.43	3.67	4.33	4.33	4.17	4.40	4.00
	3000	3.67	3.67	4.00	4.23	4.00	4.10	4.67	4.05
	6000	1.27	2.00	2.00	1.90	2.00	2.33	1.77	1.90
PPP (mg L^{-1})	25	2.33	3.07	3.23	3.63	3.47	3.43	3.00	3.17
	50	3.67	4.13	4.17	4.20	3.67	4.33	4.46	4.09
	75	1.77	1.83	2.07	1.93	1.80	1.90	2.20	1.93
Mean B		2.73	3.02	3.19	3.37	3.21	3.38	3.42	
L.S.D. at 5%: A= 0.10, B= 0.10, A*B= 0.25									

Regarding the interaction treatments, data tabulated in Table (6 and 7) revealed that the FW and DW of plants treated with Kelpak and Milagrow and dwarfed with CCC and PPP was enhanced in most treatments.

Table 6. Effect of cycocel (CCC) and paclobutrazol (PPP) in interaction with Kelpak and Milagrow on fresh weigh of chrysanthemum

Treatments		Control	Kelpak (ml L^{-1})			Milagrow (mg L^{-1})			Mean A
		0	3	4	5	60	80	100	
Fresh weight (g plant^{-1})									
First season (2018)									
CCC (mg L^{-1})	1500	69.50	71.89	77.03	85.00	78.49	84.78	87.93	79.23
	3000	67.00	68.30	68.73	73.59	71.82	74.85	81.87	72.31
	6000	56.33	58.28	66.05	70.79	60.12	61.77	64.92	62.61
PPP (mg L^{-1})	25	72.30	77.52	78.79	83.17	76.82	78.10	80.96	78.23
	50	72.72	75.02	76.91	79.37	72.89	75.48	83.74	76.59
	75	46.33	58.09	61.38	64.71	52.36	57.86	64.08	57.83
Mean B		64.03	68.19	71.48	76.11	68.75	72.14	77.25	
L.S.D. at 5%: A= 2.19, B= 2.37, A*B= 5.80									
Second season (2019)									
CCC (mg L^{-1})	1500	72.28	74.80	79.95	87.52	77.60	89.45	96.37	82.57
	3000	63.42	67.59	68.46	72.69	70.93	78.43	83.79	72.19
	6000	54.00	55.94	65.40	68.04	58.96	63.32	66.67	61.76
PPP (mg L^{-1})	25	74.30	79.16	81.82	92.26	78.83	81.99	83.89	81.75
	50	75.58	79.26	79.70	77.42	69.80	76.59	75.45	76.26
	75	48.55	63.45	66.59	65.41	59.23	62.98	65.08	61.61
Mean B		64.69	70.03	73.66	77.22	69.23	75.46	78.54	
L.S.D. at 5%: A= 2.42, B= 2.62, A*B= 6.42									

Table 7. Effect of cycocel (CCC) and paclobutrazol (PPP) in interaction with Kelpak and Milagrow on dry weight of chrysanthemum

Treatments		Control	Kelpak (ml L^{-1})			Milagrow (mg L^{-1})			Mean A
		0	3	4	5	60	80	100	
Dry weight (g plant^{-1})									
First season (2018)									
CCC (mg L^{-1})	1500	10.30	10.26	11.22	12.21	11.96	12.37	12.83	11.59
	3000	9.50	10.12	10.10	11.61	9.60	10.14	10.54	10.23
	6000	8.74	8.80	9.18	9.19	7.94	8.42	8.94	8.75
PPP (mg L^{-1})	25	11.73	12.15	12.21	12.95	11.99	12.19	12.62	12.26
	50	11.26	11.32	11.63	11.88	10.30	10.95	12.21	11.36
	75	9.61	11.18	11.83	12.61	10.53	10.75	10.93	11.06
Mean B		10.19	10.64	11.03	11.74	10.39	10.80	11.35	
L.S.D. at 5%: A= 0.35, B= 0.38, A*B= 0.93									
Second season (2019)									
CCC (mg L^{-1})	1500	11.03	10.67	11.49	11.85	11.67	12.41	13.31	11.77
	3000	10.06	10.54	10.75	12.22	9.72	10.06	12.01	10.77
	6000	9.14	8.83	9.47	10.03	7.78	9.00	9.35	9.09
PPP (mg L^{-1})	25	11.89	12.64	12.56	13.09	12.23	12.81	13.26	12.64
	50	10.99	11.37	12.09	12.10	9.71	10.53	11.14	11.13
	75	10.05	11.46	12.19	12.17	10.88	10.83	11.12	11.24
Mean B		10.53	10.92	11.43	11.91	10.33	10.94	11.70	
L.S.D. at 5%: A= 0.40, B= 0.43, A*B= 1.05									

Plant FW reached the highest value when the combination of 1500 mg L^{-1} CCC and 100 mg L^{-1} Milagrow was applied, which achieved 87.93 and 96.37 g plant^{-1} in the first and second seasons, respectively. The highest DWs were found for 1500 mg L^{-1} CCC with 100 mg L^{-1} Milagrow and 25 mg L^{-1} PPP with 5 ml L^{-1} Kelpak.

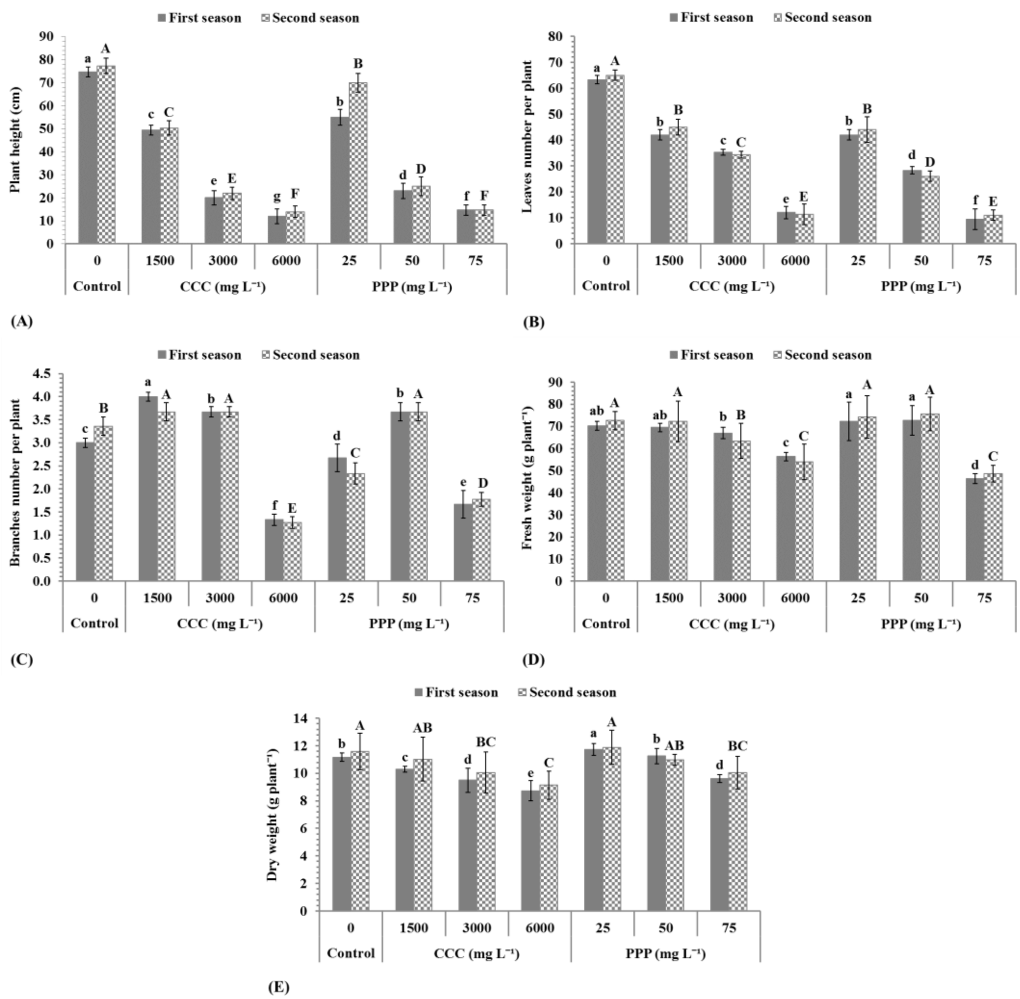

Figure 1. Effect of different concentrations of cycocel (CCC) and paclobutrazol (PPP) on vegetative characters: plant height (cm) (A), leaf number per plant (B), branche number per plant (C), fresh weight (g plant^{-1}) (D), and dry weight (g plant^{-1}) (E) of *D. grandiflorum* cv. 'Pink Zambla' during the seasons of 2018 and 2019. Bars represent ±Standard Deviation (n=3). Columns annotated with the same letters are not statistically different (*P*<0.05)

Flowering start (days)

CCC and PPP applied separately caused significantly earlier flowering compared to the control in both seasons except for treatment 75 mg L^{-1} PPP, which delayed flowering as compared with the control by 5 days and 1 day in the 1st and 2nd seasons, respectively (Figure 2A). In this context, the earlier flowering was achieved due to applying medium concentrations of CCC (3000 mg L^{-1}) and PPP (50 mg L^{-1}) (85.33 and 82.33 days in the 1st season, and 82.33 and 84.33 days in the 2nd season, respectively).

The interaction between PGRs; CCC and PPP, and the growth stimulants; Kelpak and Milagrow showed a positive effect on the time of the flowering start of the chrysanthemum plant (Table 8). The earliest flowering was achieved when 3000 mg L^{-1} of CCC was combined with 100 mg L^{-1} of Milagrow, as the plants flowered after 80.27 and 80.07 days of cultivation, in the 2018 and 2019 seasons, respectively. This means that these plants can be produced 2-5 days earlier than plants stunted with 3000 mg L^{-1} CCC only (85.33 and 82.33 days in the first and second seasons, respectively).

Table 8. Effect of cycocel (CCC) and paclobutrazol (PPP) in interaction with Kelpak and Milagrow on flowering start of chrysanthemum

Treatments		Control	Kelpak (ml L^{-1})			Milagrow (mg L^{-1})			Mean A
		0	3	4	5	60	80	100	
Flowering start (days) — First season (2018)									
CCC (mg L^{-1})	1500	98.33	87.13	86.00	84.80	87.26	87.67	85.87	88.15
	3000	85.33	84.73	84.33	83.87	83.47	82.27	80.27	83.47
	6000	95.33	89.93	88.00	85.13	92.13	87.33	85.67	89.08
PPP (mg L^{-1})	25	92.67	90.00	88.53	87.13	84.93	82.27	82.07	86.80
	50	82.33	82.33	81.80	82.27	83.33	83.83	81.13	82.43
	75	105.33	92.13	89.67	89.20	87.27	86.20	85.13	90.70
Mean B		93.22	87.71	86.39	85.40	86.40	84.93	83.36	
L.S.D. at 5%: A= 0.50, B= 0.54, A*B= 1.33									
Second season (2019)									
CCC (mg L^{-1})	1500	88.67	86.47	85.13	84.07	85.47	84.47	82.07	85.19
	3000	82.33	82.73	82.40	81.27	81.20	80.60	80.07	81.52
	6000	92.00	88.80	87.27	85.20	91.20	86.60	86.00	88.15
PPP (mg L^{-1})	25	90.00	88.13	86.80	86.07	86.27	84.13	82.13	86.22
	50	84.33	81.87	81.93	81.80	83.73	82.60	80.60	82.41
	75	102.33	91.73	89.80	88.53	88.67	87.07	85.20	90.48
Mean B		89.94	86.62	85.56	84.49	86.10	84.24	82.68	
L.S.D. at 5%: A= 0.55, B= 0.59, A*B= 1.45									

Flower number per plant

Application of CCC and PPP each alone at a moderate dose improved flower number as compared to non-dwarfed plants. The highest significant number of flowers was produced from plants treated with 3000 mg L^{-1} of CCC (12.10 and 12.90 flowers per plant) and 50 mg L^{-1} of PPP (10.93 and 11.93 flowers per plant) in 2018 and 2019 seasons, respectively, compared to control (9.47 and 10.03 flowers per plant) (Figure 2B).

Regarding the interaction treatment, the highest significant number of flowers (14.29 and 16.07 flowers per plant) was counted for the treatment of 3000 mg L^{-1} of CCC in interaction with Milagrow at 100 mg L^{-1} compared to the single spraying with 3000 mg L^{-1} CCC (12.10 and 12.90 flowers per plant) in the first and second seasons, respectively (Table 9). The following combinations also gained the highest production of flowers without significant differences between them: 1500 mg L^{-1} CCC with 100 mg L^{-1} Milagrow, 3000 mg L^{-1} CCC with 4 and 5 ml L^{-1} Kelpak, 50 mg L^{-1} PPP with 5 ml L^{-1} Kelpak and 100 mg L^{-1} Milagrow, in the first season. Within different combinations, flower number was increased by the raising rate of Kelpak and Milagrow.

Table 9. Effect of cycocel (CCC) and paclobutrazol (PPP) in interaction with Kelpak and Milagrow on flower number of chrysanthemum

Treatments		Control	Kelpak (ml L^{-1})			Milagrow (mg L^{-1})			Mean A
		0	3	4	5	60	80	100	
Flower number per plant									
First season (2018)									
CCC (mg L^{-1})	1500	10.10	12.52	12.92	12.96	13.17	13.30	13.95	12.70
	3000	12.10	12.78	13.71	14.13	13.32	13.64	14.29	13.42
	6000	2.97	3.28	3.35	3.62	3.13	3.58	4.00	3.42
PPP (mg L^{-1})	25	8.00	9.68	9.66	10.27	10.77	10.98	11.00	10.05
	50	10.93	12.08	12.71	14.17	12.65	13.35	14.16	12.87
	75	2.57	3.68	3.98	4.32	3.78	3.85	4.15	3.76
Mean B		7.78	9.00	9.39	9.91	9.47	9.78	10.26	
L.S.D. at 5%: A= 0.24, B= 0.26, A*B= 0.63									
Second season (2019)									
CCC (mg L^{-1})	1500	9.10	12.88	13.58	13.80	13.32	13.73	14.25	12.95
	3000	12.90	13.88	14.10	15.12	14.02	14.78	16.07	14.41
	6000	2.10	3.55	3.70	3.29	3.23	3.62	3.75	3.32
PPP (mg L^{-1})	25	8.90	10.00	9.98	11.02	10.95	11.18	11.65	10.53
	50	11.93	12.95	13.37	14.30	13.00	14.03	15.10	13.52
	75	2.10	3.49	3.78	4.37	3.95	4.27	4.50	3.78
Mean B		7.84	9.46	9.75	10.32	9.74	10.27	10.89	
L.S.D. at 5%: A= 0.26, B= 0.28, A*B= 0.69									

Flower diameter (cm)

The data presented in Figure (2C) revealed that the chrysanthemum plants dwarfed by CCC and PPP each alone produced flowers smaller than those of non-dwarfed plants in both seasons. A slight increase in flower diameter (4.47 and 4.57 cm) was noticed for CCC application at 3000 mg L^{-1} in the 1st and 2nd seasons, respectively, without significant differences with the control (4.40 and 4.43 cm).

The flower size was increased upon spraying the bio-stimulants (Table 10). In the first season, the largest sizes of flower (5.47, 5.87, 5.77, and 6.03 cm) were recorded when 4 ml L^{-1} Kelpak, 80 or 100 mg L^{-1} of Milagrow were sprayed on plants dwarfed with 3000 mg L^{-1} of CCC, as well as when 100 mg L^{-1} of Milagrow applied on plants stunted with 50 mg L^{-1} of PPP, respectively, without significant differences between them. In the second season, the following combinations also resulted in the highest value for flower diameter without statistical differences: 3000 mg L^{-1} CCC with 4 or 5 ml L^{-1} Kelpak, 80 or 100 mg L^{-1} Milagrow; 25 mg L^{-1} PPP with 5 ml L^{-1} Kelpak or 100 mg L^{-1} Milagrow; 50 mg L^{-1} PPP with 80 or 100 mg L^{-1} Milagrow.

Flowering period (days)

There was no significant increase in the flowering period of dwarfed chrysanthemum plants in the first season (Figure 2D). In the second season, only 3000 mg L^{-1} CCC recorded a significant increase in the flowering period when compared with non-dwarfed plants (42.23 versus 35.93 days). The longest periods of flowering (44.20 and 42.23 days) were observed for 3000 mg L^{-1} of CCC in the first and second seasons, respectively.

The treatment of moderate concentrations of CCC (3000 mg L^{-1}) and PPP (50 mg L^{-1}) combined with different rates of Kelpak and Milagrow achieved a significant prolongation of the flowering period (Table 11). Herein, the flowering period was increased up to 11 days than plants received the dwarfing agents only. In the 1st season, the longest significant periods of flowering were recorded when 100 mg L^{-1} of Milagrow was applied to plants treated with 1500 mg L^{-1} CCC (49.67 days), 3000 mg L^{-1} CCC (52.20 days), 25 mg L^{-1} PPP (48.83 days), or 50 mg L^{-1} PPP (50.00 days), beside combination of 5 ml L^{-1} Kelpak with 3000 mg L^{-1} CCC (50.50 days) or with 50 mg L^{-1} PPP (48.63 days), without significant differences between them. The same trend of combinations was observed in the second season.

Table 10. Effect of cycocel (CCC) and paclobutrazol (PPP) in interaction with Kelpak and Milagrow on flower diameter of chrysanthemum

Treatments		Control	Kelpak (ml L^{-1})			Milagrow (mg L^{-1})			Mean A
		0	3	4	5	60	80	100	
Flower diameter (cm)									
First season (2018)									
CCC (mg L^{-1})	1500	3.70	3.90	4.07	4.27	4.37	4.43	5.00	4.25
	3000	4.47	5.23	5.47	5.30	5.10	5.87	5.77	5.31
	6000	1.60	2.50	2.97	3.03	2.30	2.37	3.00	2.54
PPP (mg L^{-1})	25	4.10	4.67	5.10	5.27	4.77	4.73	5.00	4.80
	50	4.17	4.60	4.63	4.80	4.87	5.30	6.03	4.91
	75	3.00	3.10	3.33	3.30	3.10	3.17	3.23	3.18
Mean B		3.51	4.00	4.26	4.33	4.08	4.31	4.67	
L.S.D. at 5%: A= 0.27, B= 0.29, A*B= 0.71									
Second season (2019)									
CCC (mg L^{-1})	1500	3.73	4.40	4.73	4.40	4.47	5.07	5.30	4.59
	3000	4.57	5.50	5.73	6.10	5.53	5.77	6.13	5.62
	6000	1.63	2.60	3.23	3.10	2.73	2.60	2.87	2.68
PPP (mg L^{-1})	25	4.13	5.07	5.43	5.73	4.67	4.97	5.70	5.10
	50	4.27	4.83	5.17	5.63	4.93	6.47	6.13	5.35
	75	2.93	3.17	3.27	3.70	3.07	3.50	3.90	3.36
Mean B		3.54	4.26	4.59	5.78	4.23	4.73	5.01	
L.S.D. at 5%: A= 0.30, B= 0.32, A*B= 0.79									

Table 11. Effect of cycocel (CCC) and paclobutrazol (PPP) in interaction with Kelpak and Milagrow on flowering period of chrysanthemum

Treatments		Control	Kelpak (ml L^{-1})			Milagrow (mg L^{-1})			Mean A
		0	3	4	5	60	80	100	
Flowering period (days)									
First season (2018)									
CCC (mg L^{-1})	1500	43.20	45.23	47.10	47.20	43.07	45.27	49.67	45.82
	3000	44.20	45.23	46.93	50.50	44.43	47.90	52.20	47.34
	6000	30.47	37.17	37.10	40.43	32.23	35.93	38.30	35.95
PPP (mg L^{-1})	25	41.13	44.53	47.00	48.43	41.90	46.43	48.83	45.47
	50	42.00	45.10	47.10	48.63	42.47	47.10	50.00	46.06
	75	24.60	30.30	33.00	36.87	34.97	38.23	40.60	34.08
Mean B		37.60	41.26	43.04	45.34	39.84	43.48	46.60	
L.S.D. at 5%: A= 1.38, B= 1.49, A*B= 3.64									
Second season (2019)									
CCC (mg L^{-1})	1500	40.03	41.63	44.27	46.20	40.83	42.93	48.10	43.43
	3000	42.23	41.07	41.67	51.93	43.27	44.83	51.27	45.18
	6000	26.66	32.83	35.20	37.67	30.23	32.50	35.33	32.92
PPP (mg L^{-1})	25	39.90	40.60	42.90	45.20	40.00	43.70	46.27	42.65
	50	40.37	43.03	44.60	50.16	45.40	48.30	51.26	46.16
	75	26.67	29.10	35.33	38.66	37.00	39.93	40.17	35.27
Mean B		35.98	38.04	40.66	44.97	39.46	42.03	45.40	
L.S.D. at 5%: A= 1.52, B= 1.64, A*B= 4.01									

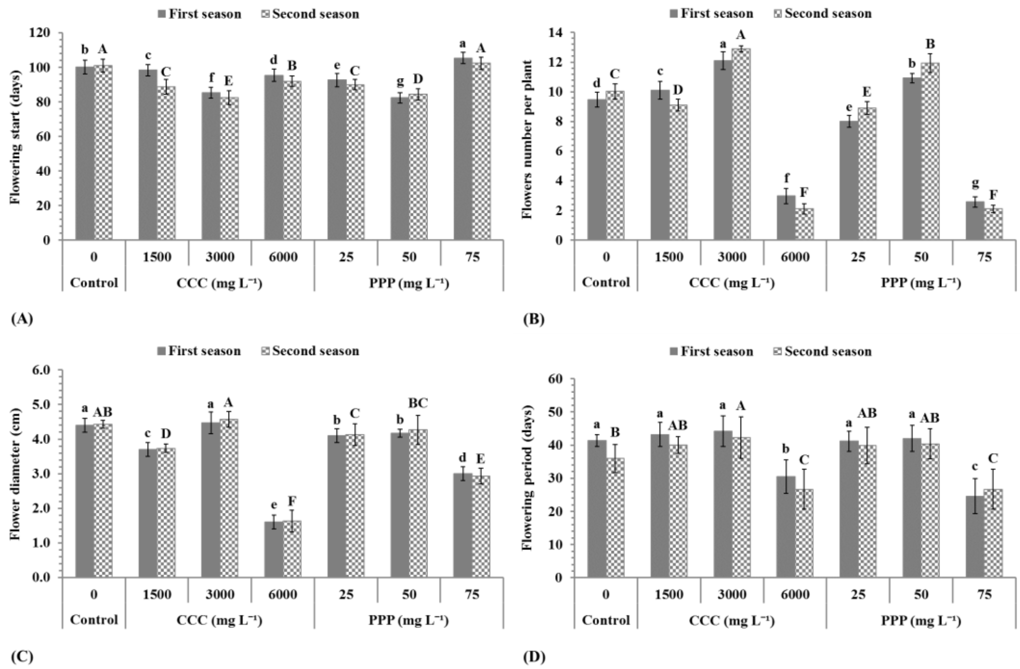

Figure 2. Effect of different concentrations of cycocel (CCC) and paclobutrazol (PPP) on flowering aspects: flowering start (days) (A), flower number per plant (B), flower diameter (cm) (C), and flowering period (days) (D) of *D. grandiflorum* cv. 'Pink Zambla' during the seasons of 2018 and 2019. Bars represent ±Standard Deviation (n=3). Columns annotated with the same letters are not statistically different (P<0.05)

Chlorophyll content (mg g^{-1} FW)

The content of chlorophyll was increased in CCC and PPP single sprays compared to untreated plants (Figure 3A). The total chlorophyll content gradually increased with increasing concentrations of both dwarfing agents. The highest significant value of total chlorophyll (1.39 and 1.50 mg g^{-1} FW) was recorded for the treatment of 75 mg L^{-1} PPP while the lowest value was recorded for control plants (0.70 and 0.76 mg g^{-1} FW) in 1st and 2nd seasons, respectively.

Respecting the interaction treatments, the total chlorophyll accumulation was enhanced due to the combined impact of growth retardants and bio-stimulants (Table 12). Combination of 75 mg L^{-1} PPP with 4 or 5 ml L^{-1} Kelpak and 80 or 100 mg L^{-1} Milagrow recorded the highest significant value of total chlorophyll (1.52, 1.62, 1.65, and 1.49 mg g^{-1} FW, respectively) in the first season. The medium dose of PPP (50 mg L^{-1}) came in the second order. The same findings were recorded in the second season. Raising the doses of Kelpak and Milagrow caused a significant increase in the accumulation of total chlorophyll.

Table 12. Effect of cycocel (CCC) and paclobutrazol (PPP) in interaction with Kelpak and Milagrow on total chlorophyll content in chrysanthemum

Treatments		Control	Kelpak (ml L^{-1})			Milagrow (mg L^{-1})			Mean A
		0	3	4	5	60	80	100	
Total chlorophyll content (mg g^{-1} FW)									
First season (2018)									
CCC (mg L^{-1})	1500	0.94	1.28	1.30	1.43	1.03	1.23	1.35	1.22
	3000	1.09	1.27	1.34	1.42	1.13	1.26	1.30	1.26
	6000	1.18	1.17	1.28	1.35	1.21	1.28	1.35	1.26
PPP (mg L^{-1})	25	0.91	1.27	1.39	1.43	1.08	1.23	1.29	1.23
	50	1.11	1.30	1.45	1.43	1.26	1.39	1.45	1.34
	75	1.39	1.44	1.52	1.62	1.42	1.65	1.49	1.51
Mean B		1.10	1.29	1.38	1.45	1.19	1.34	1.37	
L.S.D. at 5%: A= 0.077, B= 0.084, A*B= 0.205									
Second season (2019)									
CCC (mg L^{-1})	1500	1.06	1.27	1.40	1.53	1.11	1.19	1.51	1.30
	3000	1.21	1.45	1.45	1.57	1.29	1.30	1.34	1.37
	6000	1.13	1.22	1.26	1.38	1.28	1.32	1.35	1.28
PPP (mg L^{-1})	25	1.02	1.33	1.44	1.51	1.13	1.31	1.30	1.29
	50	1.23	1.35	1.46	1.41	1.24	1.42	1.40	1.36
	75	1.50	1.56	1.66	1.80	1.42	1.67	1.57	1.60
Mean B		1.19	1.36	1.44	1.53	1.25	1.37	1.41	
L.S.D. at 5%: A= 0.067, B= 0.072, A*B= 0.177									

Carotenoids content (mg g^{-1} FW)

As shown in Figure (3B), low and moderate rates of CCC significantly increased the content of carotenoids. The carotenoids content reached the highest value in plants dwarfed with CCC at concentrations of 1500 and 3000 mg L^{-1} (0.58 and 0.57 mg g^{-1} FW, respectively) in the first season as compared to the control (0.36 mg g^{-1} FW). In the 2nd season, the highest content of carotenoids was measured in plants treated with 1500 and 3000 mg L^{-1} CCC, 50 and 75 mg L^{-1} PPP (0.59, 0.60, 0.62, and 0.58 mg g^{-1} FW, respectively) compared to control (0.40 mg g^{-1} FW).

Data displayed in Table (13) show that combination treatments did not significantly enhance the accumulation of carotenoids in chrysanthemum leaves when compared with the single application of the dwarfing agents except for 25 mg L^{-1} PPP. A significant increase in the carotenoids content was observed when Kelpak was sprayed on plants treated with 25 mg L^{-1} PPP. The results revealed that Kelpak was more effective than Milagrow in increasing the carotenoids content in the leaves of plants treated with CCC or PPP. The highest content of carotenoids (0.66 and 0.70 mg g^{-1} FW) was measured for the combination of 1500 mg L^{-1} CCC with 5 ml L^{-1} Kelpak in the 1st and 2nd seasons, respectively.

Table 13. Effect of cycocel (CCC) and paclobutrazol (PPP) in interaction with Kelpak and Milagrow on carotenoids content in chrysanthemum

Carotenoids content (mg g^{-1} FW)									
First season (2018)									
Treatments		Control	Kelpak (ml L^{-1})			Milagrow (mg L^{-1})			Mean A
		0	3	4	5	60	80	100	
CCC (mg L^{-1})	1500	0.58	0.63	0.62	0.66	0.44	0.43	0.45	0.54
	3000	0.57	0.58	0.63	0.59	0.45	0.46	0.48	0.53
	6000	0.51	0.52	0.45	0.43	0.39	0.40	0.40	0.44
PPP (mg L^{-1})	25	0.48	0.52	0.53	0.55	0.42	0.47	0.49	0.49
	50	0.54	0.53	0.57	0.56	0.43	0.49	0.50	0.51
	75	0.52	0.53	0.53	0.56	0.47	0.51	0.50	0.52
Mean B		0.53	0.55	0.55	0.56	0.43	0.46	0.47	
L.S.D. at 5%: A= 0.053, B= 0.057, A*B= 0.139									
Second season (2019)									
CCC (mg L^{-1})	1500	0.59	0.61	0.65	0.70	0.45	0.46	0.44	0.56
	3000	0.60	0.60	0.65	0.63	0.53	0.51	0.55	0.58
	6000	0.55	0.57	0.54	0.48	0.44	0.47	0.45	0.50
PPP (mg L^{-1})	25	0.45	0.54	0.57	0.61	0.45	0.50	0.53	0.52
	50	0.62	0.52	0.55	0.58	0.50	0.54	0.56	0.55
	75	0.58	0.57	0.56	0.58	0.45	0.53	0.49	0.54
Mean B		0.57	0.57	0.59	0.60	0.47	0.50	0.50	
L.S.D. at 5%: A= 0.046, B= 0.050, A*B= 0.122									

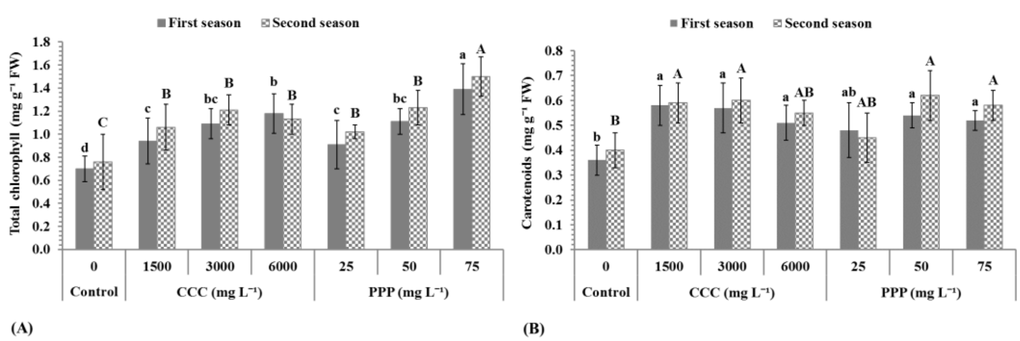

Figure 3. Effect of different concentrations of cycocel (CCC) and paclobutrazol (PPP) on total chlorophyll (A), and carotenoids (B) content (mg g^{-1} FW) of *D. grandiflorum* cv. 'Pink Zambla' during the seasons of 2018 and 2019. Bars represent ±Standard Deviation (n=3). Columns annotated with the same letters are not statistically different (P<0.05)

Discussion

To produce chrysanthemum plants as potted plant, improving the vegetative growth characteristics such as dwarfing height and increasing branching is one of the most desirable traits to obtain a dwarfed plant full of flowering heads. Application of PGRs (CCC and PPP) on *D. grandiflorum* cv. 'Pink Zambla' caused a significant inhibition in the stem height compared to untreated plants. The commercially desirable stem length (around 30 cm) can be achieved by using moderate rates of CCC (3000 mg L^{-1}) and PPP (50 mg L^{-1}). The mechanism of the reduction in plant height here appears to be related to the effect of PGRs in slowing cell division and restricting cell elongation (Magnitskiy *et al*., 2006; Karunananda and Peiris, 2010). CCC is known to completely inhibit the biosynthesis of gibberellins (GA) (Li *et al*., 2023); the main plant hormone responsible for cell elongation. To achieve a desirable plant height of high quality suitable for commercial production of potted chrysanthemum, combinations of PGRs and growth stimulants; SE (Kelpak) and BL (Milagrow), were applied. The interaction of CCC or PPP with Kelpak or Milagrow has enhanced these traits. In this connection, Ghoneim (2016), found that *Pelargonium zonale* plants treated with foliar spray of Milagrow at 80 mg L^{-1} and Kelpak at 4 ml L^{-1} recorded the highest plants. Moreover, Zong *et al*. (2019) reported that application of BRs increased plant height of *Leymus chinensis* due to improvements in physiochemical. The most appropriate result here (25-35 cm for stem length) is obtained due to applying moderate doses of the dwarfing agents in combination with any rate of both stimulants.

Decreasing leaves number in the dwarfed plants may be attributed to the resulting inhibition of stem height under the influence of PGRs. However, leaf number was increased when the stunted plants were sprayed with bio-stimulants. This increment may be related to the positive impact of bio-stimulants on increasing stem length and branching, as well as providing the nutritional growth necessities which were contained in these extracts. Moreover, it has been proven that these bio-stimulants act as anti-stress (Samira *et al*., 2012; Behnamnia, 2015). Promotion of shoot growth and leaf number is an indicator that BL (Milagrow) mitigated the negative impact of the dwarfing agents on the potted chrysanthemum.

PGRs are known to activate lateral buds to grow and fill in with a greater number of branches (Benjawan *et al*., 2007). In the current experiment, low and moderate levels of PGRs improved branching. Abbas *et al.* (2007) found that CCC levels (500, 1000 and 1500 mg L^{-1}) promoted shoot development in *Rosa damascene* and the highest branching was recorded for 1500 mg L^{-1}CCC. Also, the maximum branching in *Tagetes erecta* was reported at 2000 mg L^{-1} of CCC

(Khan *et al*., 2012). The enhancement of branching due to the application of low and medium levels of CCC and PPP may be attributed to the inhibitory action of these growth regulators on cell division in the apical bud, which may arrest the growth of the main axis and enhance lateral production and may also be due to the inhibition of auxin activity in the apical bud as it acts as an auxin antagonist (Prashanth *et al*., 2006; Di Benedetto and Molinari, 2007). Hence, special care is needed to establish bushy and dwarfed chrysanthemum plants to suit market specifications mainly in terms of plant height and branche number. The lowest significant number of branches was recorded with the single application of high levels of CCC (6000 mg L^{-1}) and PPP (75 mg L^{-1}), due to the toxic effect on the plant cell. However, the application of the growth bio-stimulants mitigated this toxicity and increased branching. Mohamed (2020) showed that BL at 50 mg L^{-1} and its combination with mycorrhizal fungi increased the number of branches in fennel, however, SE (2 ml L^{-1}), as well as its combination with mycorrhizal fungi resulted in the highest values for branch count. The effect of BL (Milagrow®) on vegetative parameters may be due to the improvement of cell growth, differentiation, division and enlargement, alteration of membrane potential, and metabolism of nucleic acids and proteins (Müssig, 2005; Dehghan *et al*., 2020).

As expected, the biomass FW and DW of chrysanthemum plants treated with a high rate of PGRs alone were decreased because of the severe reduction in plant height, branche number, and leaf number compared to the non-sprayed plants. Al-Shaer (2004) reported a similar observation that CCC increased the number of branches per plant but reduced both FW and DW in *Grindelia camporum*. Enhancing the FW with 25 and 50 mg L^{-1} PPP implies that the plants may have absorbed more water and nutrients. The decrease in DW was linearly related to the increase in the concentration of CCC and PPP. North *et al*. (2010) on *Dombeya burgessiae* reported the same observation where the FW and DW of plants were severely reduced with the increase of CCC concentration. Spraying plants with the bio-stimulants increased the FW and DW of the stunted plants. In general, the FW and DW were increased by increasing the concentrations of both bio-stimulants. This observation is in harmony with that recorded by Ferreira dos Santos *et al*. (2019) on ornamental sunflower. This could be related to the accumulation of phytonutrients and water in plant tissues. Similarly, Zong *et al*. (2019) found that the enhancement in physiochemical attributes due to BR application increased FW and DW of *Leymus chinensis*. BL is well known to protect plants exposed to abiotic stress and enhance plant growth and biomass FW and DW (Fariduddin *et al*., 2014).

The stimulating impact of SE on vegetative growth characteristics may be attributed to its primary action in promoting cell division and elongation as it contains high amounts of natural nutrients such as N, P, K, Ca, Mg, S, Fe, Cu, Mn, B and Mo, natural hormones (cytokinins, auxins, and gibberellins), amino acids, and vitamins (Begum *et al*., 2018). Moreover, studies with *Arabidopsis thaliana* cells revealed the ability of BRs to stimulate cell expansion which was accompanied by the amplitude of the hyperpolarization of the cell membrane (Zhang *et al*., 2005).

Flowering characteristics, such as the beginning of flowering, flower number per plant, flower diameter, and flowering period are important characteristics that producers of potted plants strive to enhance and increase. PGRs applied separately to chrysanthemum caused significantly earlier flowering compared to the control. Close to our results, Vaghasia and Polara (2016) found that CCC at 2500 mg L^{-1} gave the earliest flowering date in *C. morifolium* cv. IIHR-6. In contrast, Ghatas (2016) found that treatments of CCC (1000, 2000, and 3000 mg L^{-1}) and PPP (20, 40, and 60 mg L^{-1}) delayed the flowering of *C. frutescence* plants as compared with untreated plants, which indicates that the effect of these PGRs depends on many factors, including the plant species. Early flowering is a desirable trait for the producer to reduce production costs. The number of days required for flowering has gradually decreased with increasing concentration of the bio-stimulants sprayed on the dwarfed plants. In parallel with our results, Kandil *et al*. (2007) found that foliar application of 50 mg L^{-1} GA3 + 15 mg L^{-1} BL + 40 mg L^{-1} Kin promoted flowering start in *Rosa hybrida*. The presence of very high levels of BRs in pollen and seeds indicates the involvement of BRs in the regulation of reproductive growth and reproduction (Ali, 2017). Müssig (2005) suggested that BRs can determine branching and flower formation by modulating metabolic pathways and nutrient allocation or interacting with other signaling pathways. The early flowering of chrysanthemum plants sprayed with Kelpak may be caused by the early development of the plant due to the availability of phytonutrients.

As for the commercial production of potted plants, producing a plant full of flowers as much as possible is one of the most important goals that pot plant producers seek. Plants treated with the PGRs at moderate rates produced a higher number of flowers. Also, Vaghasia and Polara (2016) found that 2500 mg L^{-1} of CCC enhanced flower number in *C. morifolium* cv. IIHR-6. Moreover, Ghatas (2016) found that the highest flower number was recorded for *C. frutescence* plants subjected to 60 mg L^{-1} of PPP. A high concentration of dwarfing factors led to a sharp decrease in flower number, which may be due to the decrease in the number of shoots for these treatments, as previously mentioned. The number of flowers of chrysanthemum received both bio-stimulants and dwarfing compounds was better than that of plants treated with

PGRs separately. This increase in the number of flowers is attributed to the increase in branche number. Kandil *et al*. (2007) noticed an increase in the flower number in *Rosa hybrida* when a conjunction of 50 mg L^{-1} GA3 + 15 mg L^{-1} BL + 40 mg L^{-1} Kin was applied. Moreover, a stimulating effect of seaweed liquid fertilizer on flower number in *Tagetes erecta* was also reported by Sridhar and Rengasamy (2010).

Application of CCC and PPP each alone reduced the flower diameter. In conformity with this observation, Mishra and Yadava (2011) recorded a decrease in flower size and flower stalk length by increasing PPP concentration in China aster cv. Poornima. This may be related to the decrease in leaf number and therefore fewer overall nutrients needed to increase the flower size. However, a slight increment in flower size was recorded for moderate application of CCC, which may be due to the increased content of chlorophyll and the rate of photosynthesis, and thus the ultimately higher manufacture of photosynthetic increased flower size as reported by Singh *et al*. (2018). Similar results were recorded on *Primula forbesii* plants dwarfed with CCC (Zhang *et al*., 2020). The flower size was significantly improved when the growth stimulants and dwarfing agents were crossed. The positive effect of BL (Milagrow) on flower diameter could be due to the promoting of petal growth through cell expansion. In this concept area, Huang *et al*. (2017) found that BL promoted petal growth in *Gerbera hybrida* by elongating cells in the central and basal regions of the petals. The authors demonstrated that such effect on petal growth was greater than that of GA. Moreover, increasing flower diameter with Kelpak and Milagrow application could be correlated with the increase in photosynthesis rate (Fariduddin *et al*., 2014).

Only 3000 mg L^{-1} of CCC significantly enhanced the flowering period in the second season. In line with these results, Kumar *et al*. (2019) found that CCC at 2500 mg L^{-1} recorded the early flowering and maximum duration of flowering in *Nerium odorum* L. On chrysanthemum cv. Birbal Sahni, Singh *et al*. (2018) found that the maximum flowers yield, and shelf life of flowers were achieved with 5000 mg L^{-1} of CCC. However, in our study, high concentration of CCC (6000 mg L^{-1}) decreased the flowering period. This may be due to the difference in the cultivar and agricultural conditions. The flowering period was positively prolonged for the combination application. Bosila *et al*. (2016) on *Pelargonium zonale* ‘Serena’ reported that application of Kelpak gave the highest value of flowering period compared with chemical fertilization and control treatments.

The enhancement of flowering traits as a result of spraying SE products could be due to promoting plant growth (Mohamed, 2020). Moreover, the use of SE led to the activity of enzymes and thus increased biological processes within the plant cell, resulting in increased plant growth and productivity (EL Boukhari *et al*., 2020). Recently, Sheng *et al*. (2022) reported that BL signaling acts as a growth enhancer during vegetative and reproductive development in lotus (*Nelumbo nucifera*). The authors observed growth-promoting phenotypes and long-lasting flowering because of the exogenous application of 28-epihomobrassinolide.

It is known that biosynthesis and accumulation of pigments in plant tissues are affected by many factors, including nutrients and various stress factors, such as chemical compounds used in dwarfing potted plants (Abdel-Gawad, 2016; Ghoneim, 2016; Abbas, 2017). The content of chlorophyll was increased in chrysanthemum plants treated with PGRs which has also been confirmed in *C. frutescence* (Ghatas, 2016) and *Tabernaemontana coronaria* (Youssef and Abd El-Aal, 2013). This may be due to the inhibitory effect of PGRs which produced smaller cells and subsequently increased the concentration of chlorophyll within the reduced cell volume (Thakur *et al*., 2006). In addition, Tsegaw *et al*. (2005) suggested that the increased accumulation of chlorophyll in potato leaves upon application of PPP was due to enhanced chlorophyll biosynthesis as well as to more densely spaced chloroplasts per unit leaf area. It was reported that treatment with CCC and PPP resulted in an increase in chlorophyll content and photosynthesis rate, and consequently, increased the accumulation of carbohydrates in plant leaves (Zheng *et al*., 2012). Thus, this may be the reason for the increase in plant biomass, flower number, and the prolongation of the flowering period in some treatments in the current investigation compared to the control. Kelpak acted as a stronger growth stimulant than Milagrow in increasing the chlorophyll and carotenoids content in the stunted plants. A similar observation was recorded by Mohamed (2020) on the Dutch fennel as SE (2 ml L^{-1}) and its combination with mycorrhizal fungi recorded the highest values of chlorophylls compared to other stimulants i.e., amino acids, BL, humic acid, and salicylic acid. Carotenoids are a class of terpenoids with multiple functions such as photosynthetic pigments and plant protection under different stresses (Salam *et al*., 2023). Hu *et al.* (2013) observed that the photosynthetic capacity, net photosynthetic rate, light saturation rate, and quantitative efficiency of PSII were improved in *Leymus chinensis* treated with a mixture of abscisic acid and BL. Furthermore, under abiotic stress, BL can stimulate chlorophyll synthesis (Yadava *et al*., 2016). Increased level of bio-stimulants was accompanied by an increase in chlorophyll content. In this regard, Al-Dulaimy *et al.* (2021) found that *Gazania splendens* treated with SE at 4 ml L^{-1} recorded the highest leaf content of chlorophyll and total carbohydrates compared to control and low level. The stimulatory effects of the PGRs on enhancing the biosynthesis of carotenoids have been confirmed by Lodeta *et al*. (2010) and Abbas (2017) on poinsettia plants, and Abdel-Moniem (2016) on sunflower plant.

In conclusion, when producing potted plants, controlling stem height for proper length, good branching and a consistent vegetative shape with the flowers is often necessary to achieve the desired size and shape for marketing ornamental crops. In the current study, moderate and low concentrations of growth retardants (CCC and PPP) had the desired effects in manipulating the growth parameters of *D. grandiflorum* cv. 'Pink Zambla', and thus shaped as a potted plant. Concenrning the key characteristics of potted chrysanthemum, the best results were recorded in plants treated by a combination of 3000 mg L^{-1} CCC or 50 mg L^{-1} PPP with different rates of Kelpak or Milagrow. This makes us recommend the use of growth stimulants along with growth inhibitors when the purpose is to produce potted dwarfed chrysanthemum plants with high flowering quality that increase their commercial value and meet the needs of the consumer and market of potted flowering plants. Thus, combining growth stimulants with dwarfing agents may be a good option to mitigate cytotoxicity caused by growth retardants. On the other hand, the farmers' improper use of inorganic fertilizers to improve plant growth and productivity is harmful to the environment and human health. The use of bio-stimulants such as SE (Kelpak) and brassinosteroid (Milagrow) in agriculture could be an efficient and eco-friendly alternative to chemical fertilizers.

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