

Response of pot marigold plant (*Calendula officinalis* L.) to spraying with some growth biostimulants

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Abstract

Two field experiments were conducted in the farm of the Department of Agricultural Botany, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt. during the two winter seasons 2021/2022 and 2022/2023, in order to study the effect of some growth stimulants such as yeast extract (5, 10, 15 g/l), humic acid (0.5, 1, 1.5 g/l) and algae extract (4, 6, 8 cm³/l) on some morphological, physiological and floral characteristics of two cultivars of *calendula officinalis* plant double flower and single flower compared to control (tap water). The results showed that the double flower cultivar was significantly superior in morphological characteristics (plant height, number of branches / plants, stem diameter, number of leaves, leaf area (cm²) plant⁻¹, fresh weight and dry weight) except for the characteristics of plant height in the first season and the number of branches in the second season where the opposite happened. Single flower cultivar also caused significant superiority in the physiological characteristics, namely (chlorophyll a, chlorophyll b, total chlorophyll, total carotenoid in the leaves) except for total carotenoid in flowers for both seasons. As for the floral characteristics, which are (diameter of inflorescence, number of rays (flowers) / inflorescence, fresh and dry weight of the inflorescences / plant), the double flower cultivar recorded the highest results compared to the single flower cultivar except for number of inflorescences / plant where the opposite happened. The results also recorded that foliar spraying of yeast extract at (5, 10 and 15 g/l), humic acid at (0.5, 1, 1.5 g/l) and algae extract at (4 cm³/L) improved the morphological, physiological and floral characteristics of both cultivars of *calendula officinalis* during the two study seasons compared to control (tap water). On the contrary, spraying with algae extract at (6, 8 cm/l) caused a noticeable decrease in the morphological, physiological and floral characteristics mentioned above for both cultivars of *calendula officinalis* during the two seasons of the study compared to control (tap water). Finally, the study showed that the use of yeast extract at a concentration of 10 g/l followed by humic acid at a concentration of 1 g/l as growth biostimulants sprayed on the *calendula officinalis* plant has caused a significant improvement in morphological, physiological and floral characteristics, especially with the double flower cultivar, and this is confirmed by the results under the same study conditions.

Keywords: pot marigold, *Calendula officinalis*, yeast extract, algae extract, humic acid, morphology, physiology.

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1. Introduction

Calendula officinalis Linn. belongs to the Asteraceae (Compositae) family. It is an annual with bright or yellow orange daisy-like flowers which are used for medicinal or culinary purposes (Bcerentrup and Robbelen, 1987; Cromack and Smith, 1998), especially in phytotherapy (Isaac, 1992). The genus *Calendula* includes 15-20 species. Native to Egypt and the Mediterranean, *Calendula* is cultivated in temperate regions around the world. Easily naturalized, it grows readily in sunny locations throughout North America and Europe. It prefers previously cultivated positions (Mills, 1999; Chevallier, 1996). It grows to a height of 30-60 cm with multiple branching stems. Its leaves are spatulate or oblanceolate, sessile, with widely spaced tiny teeth on the borders, and the whole leaf is covered with very short fine hairs. *Calendula* has single flower heads situated on a green crown-shaped receptacle. The inner portion of the flower head consists of orange-yellow, tubular florets (often called petals). As the petals fall off, a circular corona of seeds remains in view. The plant is an annual, seldom biennial. It grows to between 30 and 50 cm high and has about 20 cm long tap root and numerous thin, secondary roots. The stem is erect, angular, downy and branched from the base up or higher. The alternate leaves are almost spatulate at the base, oblong to lanceolate above and are all tomentosae (Editorial Boards PDR

for Herbal Medicines 2003). Yeast (*Saccharomyces cerevisiae*) (dry bread yeast, DBY) is an enriched source of phytohormones especially cytokinins, vitamins, enzymes, amino acids and minerals as well as has a stimulatory effect on the cell division and enlargement, protein and nucleic acids synthesis, chlorophyll formation and protective role from different stresses (Shehata *et al.*, 2012). Yeast extracts contain trehalose-6-phosphate synthases which had a key enzyme for trehalose synthesis (Yeo *et al.*, 2000). Humic acid is a natural substance produced by the decay of organic materials can be utilized for increasing yield (Chen *et al.*, 2017; Primo *et al.*, 2011; Schiavon *et al.*, 2010; Selladurai and Purakayastha, 2016). Humic acid is a product contains many elements which improve the soil fertility and increase the availability of nutrient elements by holding them on mineral surfaces and consequently affect plant growth and yield (Akinici *et al.*, 2009; El-Desuki, 2004; Neri *et al.*, 2002). Seaweed extract is a new generation of natural organic fertilizer highly nutritious and encourage faster germination of seeds and enhance yield and resistant ability of several crops (Dhargalkar and Pereria, 2005). Seaweed is rich in macro and micronutrients (Chapman and Chapman, 1980). So, the main aim of this investigation was to study the influence of different levels of yeast and seaweed natural extracts as well as humic acid on morphological, physiological and flowering characteristics of two cultivars

of *Calendula officinalis* L. plant (Double and single flower).

2. Materials and methods

This research was conducted at the Experimental Farm and Laboratories of Faculty of Agriculture, Al-Azhar University, Assiut, Egypt, during two successive winter seasons of 2021/2022 and 2022/2023. The aim of this study is the effect of yeast extract at the levels of (5,10,15) g/l, humic acid at the levels of (0.5,1,1.5) g/l, and algae extract at the levels of (4,6,8) cm³/l on morphological, physiological and flowering characteristics of two cultivars of *Calendula officinalis* L. plant double flower and single flower. Seeds of marigold (*Calendula officinalis* L.) for single flower cultivar were obtained from Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. While seeds for double flower cultivar were obtained from Calendula Herbs Company, Fayoum, Egypt.

2.1 Experiment Design

The seeds were sown on September 1st of both seasons in pots of 40 cm diameter filled with loamy soil. Seedlings were transplanted on the 10th of October into the field in plots 2×2 m. which contained 4 rows at distance of 40 cm. between plants, each plot contained 20 plants. The experiment was arranged in a split split-plot design, with three replications, the cultivars occupied the main plots while biostimulants treatments represented the

sub plots and concentrations of those treatments represented sub-sub plots. All treatments were sprayed four times, after 4,6,8,10 weeks from transplanting date.

2.2 Studied Characters

Nine plants were chosen from each treatment to determine the studied characters.

2.2.1 Morphological characters

Three replicates were taken randomly from each treatment at 45 days after transplanting during two consecutive seasons of 2021/2022-2022/2023. Plants are dried in an oven at 70 °C for 48 h until a constant dry weight was reached and the following morphological characteristics were recorded *i.e.*, plant height (cm), branch number / plant, stem diameter (cm), number of leaves, fresh weight (g) and dry weight (g) of plant. Leaf area (cm²) plant⁻¹ was measured by the method described by Gao *et al.* (2011).

2.2.2 Physiological characters

Chlorophyll a, b and total chlorophyll and total carotenoids in fresh leaves as well as total carotenoid in flowers were determined according to Mornai (1982). One gram of the plant fresh weight sample of shoots was extracted with 5 ml of dimethyl-formamide for overnight at 5°C then determined chlorophyll a, chlorophyll b and total chlorophyll as well as total carotenoids using spectrophotometer at wavelength (663,

647 and 470 nm), respectively. The concentrations of this pigment were calculated by means of Nornai's formula: $Ch1.a = 12.76 A663 - 2.79 A647$ (mg/l) $Ch1.b = 20.76 A647 - 4.62 A663$ (mg/l). Total chlorophyll = $17.90 A647 - 8.08 A663$ (mg/l). Total carotenoids = $(1000 \times A470 - 3.72 \times Ch1.a - 104 \times Ch1.b) / 229$ (mg/l). All the above were calculated on fresh weight basis as mg/g F.W.

2.2.3 Floral Characters

Flowering data were recorded at 80,90 and 100 days from transplanting through three cuts. Floral data to flowering characters such as, number of inflorescence / plant, inflorescence diameter, number of rays (flowers) / inflorescence, fresh and dry weight of inflorescence / plant were recorded as an average of first three cuts. Whereas number of inflorescence / plant were recorded as total of first three cuts.

2.2.4 Statistical analysis

Collected data were subjected to a split split-plot analysis of variance (ANOVA) using Costat software package version 6.451. Significant differences among treatment means were executed using Fisher's least significant difference (LSD) test when the level of significance was 5% ($p \leq 0.05$).

3. Results and Discussion

3.1 The effect of foliar application with

yeast extract, humic acid, and algae extract on morphological characteristics flour and composite flours of raw and germinated quinoa

Data in Tables (1 and 2) clearly show the effect of spraying with yeast extract at (5,10 and 15 g/l), humic acid at (0.5,1 and 1.5 g/l) and algae extract at (4,6 and 8 g/l) on vegetative growth characteristics of calendula officinalis plant at 45 days after transplanting i.e., plant height (cm), stem diameter, No. of branches plant⁻¹, No. of leaves plant⁻¹, leaf area (cm²) plant⁻¹, fresh and dry weights (g) plant⁻¹ for the two cultivars (double flower and Single flower) during 2021/2022 and 2022/2023 seasons. The results showed that the double flower cultivar was significantly superior to all morphological characteristics except for the two characteristics of the height of the plant in the first season and the number of branches in the second season, where the single cultivar outperformed the double cultivar, this difference between the cultivars is due to the genetic variations among cultivars and their capability for utilizing the environmental sources especially light, CO₂, water, and nutrients. Regarding the effect of yeast extract, in both seasons, using yeast extract at 5,10 and 15 g/l concentrations increased all morphological characteristics such as (Plant height, branch number / plant, stem diameter, number of leaves, leaf area, fresh weight and dry weight of plant) of *Calendula officinailis* L. plant as compared with untreated

plants (control). The use of concentration of 10 g/l of yeast extract led to a significant increase in all the characteristics mentioned followed by the concentration of 5 g/l and then 15 g/l except for the characteristics of plant height and stem diameter, where the concentration exceeded 15 g/l on the concentration 5 g/l and these results for both cultivars in both seasons. These results are in line with those obtained by El-Shawa *et al.* (2020) found that foliar application of yeast extract led to improve and increase plant growth of calendula plants significantly more than those obtained by control, such as plant height, number of branches plant, plant fresh and dry weights. Atteya (2018) found that using yeast extract on zinnia plants improved vegetative characteristics such as plant height, branch length, branches number, stem diameter, leaf area, plant fresh and dry weight. Yeast extract contain different nutrients (N, P, K, Fe, Zn and Mn), higher values of vitamins, carbohydrates and valuable source of phytohormones, like Auxins, cytokinins and protein that enhance cell division and enlargement, so it's important in improving plant growth (Abou El-Yazied and Mady, 2012; Marzauk, 2014). With regard to humic acid, the results obtained showed that the use of humic acid as a foliar spray on the calendula plant in its two cultivars of double flower and single flower led to an increase in all the aforementioned morphological characteristics compared to the untreated plants (control), where the

best concentration was 1g / liter, followed by the concentration of 1.5 g/l and finally the concentration of 0.5 g / liter for both cultivars in both seasons. These findings corroborated those of El-Nashar (2021) who indicated that foliar application of humic acid on Calendula (*Calendula officinalis* L.) plant enhanced vegetative traits as plant height, the number of leaves, leaf area and shoot dry mass by using the two dose 1.5 and 2.0 g/L at three times. Shalaby *et al.* (2022) who showed that foliar application of potassium humite treatment on Marigold (*Tagetes Erecta* L.) significantly increased all studied vegetative growth parameters except plant height, which remained non significant. The reason for this may be due to Zhang and Ervin (2004) where they indicated that the attendance of iron element in the HAs or their colloidal nature have a real effect on the growth of diverse micro-organisms which may excrete a variety of antibiotics, vitamins, and growth substances and these can actively plant growth. As for algae extract, its use at a concentration of 4 cm³/l led to an increase in all morphological characteristics. The present results were in line with those found by Tirki and Gantait (2021) showed that *Ascophyllum nodosum* (seaweed) extract 0.7% was found to be significantly superior for all growth parameters of chrysanthemum cv. Poornima White. The maximum plant height (48.55 cm), number of leaves (179.9) and leaves length (7.5cm) were noticed in *Ascophyllum nodosum* 0.7%. and Thirumaran (2009) who found

seaweed extract enhanced all growth parameters of *Abelmoschus esculentus* plant. The increased growth of seaweed extract was reported by Zamani et al. (2013) who mentioned that the presence of some growth promoting substances as auxins, gibberellins, cytokinins, precursors of ethylene and betaine and cytokinins which are present and potentially involved in enhancing plant growth responses. while the concentration of 8 cm³/l led to a decrease in all morphological characteristics, followed by a concentration of 6 cm³/l, except for the leaf area, where the concentration of 6 cm³/l led to a greater decrease of concentration of 8 cm³/l compared to untreated plants (control). These results are in harmony with Seleem (2016) found that spraying of *calendula officinalis* plant with seaweed

extract at the concentration of 4cm³/l reduced all vegetative traits. Whereas lower concentration (0.5, 1, 2 cm³/l) increased all the previous traits. Ferreni and Nicese (2003) found no statistical differences in shoot growth of *Quercus robur* L. plant treated with algae compared to control. These results were for both cultivars in both seasons. Regarding the interaction between cultivars and treatments at different levels. The results indicated that the highest values for all vegetative growth characteristics of *Calendula officinalis* plant were obtained with double flower cultivar and yeast extract at a concentration of 10 g/l compared to untreated plant (control) and other applied treatments. These results were true during the two experimental seasons.

Table (1): Morphological characteristics of calendula cultivars as affected by spraying with yeast extract, humic acid and algae extract at 45 days after transplanting during 2021/2022 and 2022/2023 seasons.

Cultivar	Treatments	Characters	Plant height		Stem diameter		Number of main branches		Number of leaves	
			1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Double flower	Control	0.0	27.85	31.33	0.67	0.70	9.00	8.67	142.67	177.67
	Yeast extract	5 g/l	33.60	33.53	0.73	0.70	11.33	9.33	219.33	213.33
		10 g/l	36.17	35.17	0.83	0.80	14.67	10.00	251.00	258.67
		15 g/l	33.73	34.40	0.73	0.73	10.33	9.00	211.00	197.00
	Humic acid	0.5 g/l	31.63	31.93	0.73	0.73	9.67	9.00	205.67	194.67
		1 g/l	37.03	35.27	0.83	0.77	14.33	10.00	221.33	252.33
		1.5 g/l	35.07	34.93	0.77	0.77	13.00	9.67	220.33	244.00
	Algae extract	4 cm/l	30.77	31.37	0.70	0.77	9.00	9.00	173.67	183.67
		6 cm/l	27.23	29.03	0.60	0.65	7.67	7.00	138.33	141.67
		8 cm/l	27.77	30.10	0.67	0.70	7.67	8.33	139.00	156.33
	Mean		31.38	32.48	0.72	0.73	10.39	8.94	183.97	197.89
	Single flower	Control		30.20	31.80	0.52	0.50	7.67	8.67	112.67
Yeast extract		5 g/l	31.70	32.17	0.55	0.55	10.33	11.00	164.33	150.67
		10 g/l	37.63	35.27	0.67	0.73	15.00	13.00	220.00	272.00
		15 g/l	33.10	32.97	0.63	0.57	9.67	10.33	132.00	135.00
Humic acid		0.5 g/l	31.57	32.07	0.60	0.57	9.67	10.00	122.33	125.33
		1 g/l	33.83	34.37	0.63	0.73	12.67	12.67	210.67	238.00
		1.5 g/l	33.73	33.00	0.63	0.60	11.00	11.67	183.00	151.33
Algae extract		4 cm/l	30.60	31.80	0.53	0.53	7.67	9.00	116.67	121.00
		6 cm/l	28.23	28.67	0.42	0.40	7.33	8.00	90.33	95.33
		8 cm/l	30.15	28.97	0.48	0.50	7.33	8.67	99.00	105.00
Mean			31.76	32.06	0.56	0.56	9.47	10.03	139.69	143.89
L.S.D at 5%		Cultivars		ns	ns	0.06	0.07	0.55	0.55	4.20
	Treatment		2.18	0.95	0.05	ns	0.85	1.34	1.63	4.51
	Concentration		ns	ns	ns	ns	0.47	ns	1.55	3.06
	Interaction		ns	ns	ns	ns	ns	ns	4.37	8.65

1st season = 2021/2022 season, 2nd season = 2022/2023 season.

Table (2): Morphological characteristics of calendula cultivars as affected by spraying with yeast extract, humic acid and algae extract at 45 days after transplanting during 2021/2022 and 2022/2023 seasons.

Cultivar	Treatments	Characters		Leaf area		Plant fresh weight		Plant dry weight	
		1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season		
Double flower	Control	0.0	30.86	31.13	214.57	227.19	16.93	17.25	
	Yeast extract	5 g/l	39.09	35.36	375.39	331.34	23.97	21.87	
		10 g/l	47.59	51.59	457.59	391.33	29.06	24.83	
		15 g/l	36.66	33.54	351.41	320.49	22.25	21.75	
	Humic acid	0.5 g/l	36.37	32.95	281.26	263.88	19.37	19.57	
		1 g/l	41.16	38.76	391.02	369.95	25.46	23.47	
		1.5 g/l	39.83	38.08	389.92	363.61	25.11	22.26	
	Algae extract	4 cm/l	34.68	32.46	348.95	297.85	21.87	21.42	
		6 cm/l	26.78	27.60	169.56	145.06	13.11	13.73	
		8 cm/l	27.61	29.07	213.94	224.25	16.61	17.02	
	Mean		35.20	34.40	301.90	282.44	20.63	19.81	
	Single flower	Control		27.44	24.57	193.87	214.73	16.48	15.50
		Yeast extract	5 g/l	30.11	31.59	297.30	311.21	20.78	22.09
			10 g/l	46.14	41.19	436.45	461.39	26.62	26.42
15 g/l			29.25	30.86	278.85	235.81	19.56	16.93	
Humic acid		0.5 g/l	29.04	29.44	235.66	225.38	18.15	16.66	
		1 g/l	41.11	39.57	328.82	344.74	23.93	24.83	
		1.5 g/l	30.66	34.54	311.29	336.79	23.88	24.27	
Algae extract		4 cm/l	28.44	25.62	250.60	225.52	18.65	16.85	
		6 cm/l	25.53	20.06	137.78	165.90	9.42	11.31	
		8 cm/l	18.97	19.44	169.72	214.06	14.88	14.45	
Mean			30.13	28.83	252.34	263.75	18.78	18.36	
L.S.D at 5%		Cultivars		0.68	1.96	8.95	13.57	1.81	ns
		Treatment		1.68	1.47	4.62	7.30	0.57	0.99
		Concentration		1.37	0.90	2.69	4.05	ns	0.47
	Interaction		Ns	2.54	7.61	11.46	ns	1.34	

1st season = 2021/2022 season, 2nd season = 2022/2023 season.

3.2 The effect of foliar application with yeast extract, humic acid, and algae extract on physiological characteristics

Data presented in Table (3) show the effect of spraying with yeast extract at (5,10 and 15 g/l), humic acid at (0.5,1 and 1.5 g/l) and algae extract at (4,6 and 8 cm³/l) on chlorophylls content mgg⁻¹ F.W (chlorophyll a, chlorophyll b, total chlorophyll, total carotenoid in leaves and total carotenoids in flowers) of *Calendula officinalis* plant cultivars for the two cultivars (double flower and single flower) at 45 days after transplanting during 2021/2022 and 2022/2023 seasons. The results showed the superiority of single flower cultivar on the content of the determined

photosynthetic pigments compared to double flower cultivar except for the characteristic of total carotenoids in flowers and plant height in the first season, where the double cultivar outperformed the single cultivar, the differences between the cultivars is due to the genetic differences between the cultivars and their ability to benefit from environmental resources, especially light, carbon dioxide, water and nutrients. Regarding the effect of yeast extract, in both seasons, using yeast extract at 5,10 and 15 g/l concentrations increased all chlorophylls content (chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids) of *Calendula officinailis* L. plant as compared with untreated plants (control). The use of concentration of 10

g/l of yeast extract led to a significant increase in all the characteristics mentioned followed by the concentration of 5 g/l and then 15 g/l except for the characteristics of chlorophyll b in Single flower cultivar in second season, where the concentration exceeded 15 g/l on the concentration 5 g/l and these results for both cultivars in both seasons. These results are in harmony with El-Shawa *et al.* (2020) were showed that foliar application of yeast extract led to improve and increase physiological character such as carotenoid and total chlorophyll on calendula plant leaves. Atteya (2018) found that using yeast extract on zinnia plants increased leaves content of total chlorophyll compared with the control treatment. The obvious increase in chlorophyll contents of the yeast treated leaves (Table 3) can be interpreted by two functions. At the first, yeast as a source of cytokinins (Ferguson *et al.*, 1987) delays the degradation of chlorophyll via obstructing activity of both chlorophyllase and magnesium-dechelataze enzymes (Idso *et al.*, 1995). Secondly, this treatment provides the plant with certain macro and micro elements which play a direct or indirect role in the biosynthesis of chlorophylls. Yeast can activate magnesium chelatase through providing Mg and P (Pis needed for ATP production). With regard to humic acid, the results obtained showed that the use of humic acid as a foliar spray on the calendula plant in its two cultivars of double flower and single flower led to an increase in all the

aforementioned photosynthetic pigments content compared to the untreated plants (control), where the best concentration was 1 g/l, followed by the concentration of 1.5 g/l and finally the concentration of 0.5 g /l except for the characteristic of total carotenoid of flowers in single flower cultivar, were the best concentration was 1.5 g/l followed by 1 g/l then 0.5 g/l for both cultivars (Double flower and Single flower), the results were recorded in both seasons. These findings corroborated those of Tina *et al.* (2015) found that effect of humic acid on the chemical parameters such as carotenoid content was significant and 500 mg had highest significant effect, therefore the use of humic acid is recommended. Also, Samadimatin and Hani (2017) showed that humic acid as foliar spray on plant of *Dracocephalum moldavica* increased chlorophyll a, chlorophyll b and total chlorophyll, carotenoid. The highest positive effect was observed in 400 mg/L humic treatment. These results are due to humic acid increases Zn, Cu, and Mn absorption in plants. Zn is a catalyst in many plant enzyme systems which is involved in protein synthesis and analysis. One of the important roles of Zn is synthesizing the amino acid, tryptophan, which is a precursor for the auxin, indoleacetic acid promoting branch length growth; Cu plays a role in the activation of plant oxidases and Mn has an essential role in chlorophyll production in plants. Furthermore, humic acid increases Mn absorption in plants which inturn plays

an essential role in chlorophyll production in plants. Humic acid can increase the production of chlorophyll by placing more water and nutrients on the plant (Delfine *et al.*, 2005). As for algae extract, its use at a concentration of 4 cm³/l led to an increase in all photosynthetic pigments. These results are consistent with Sayed *et al.* (2018) showed that globe artichoke plants which sprayed with algae extract (4 ml/L) recorded significant increase chlorophyll a, b and total chlorophyll followed by plants sprayed with algae extract (2 ml/L) in the two successive seasons when compared with untreated plants. Also, Arafa *et al.* (2011) indicated that spraying potato plants with 500 mg/l seaweed extract

significantly increased photosynthetic pigment concentration as compared with control plant in the first and second growing seasons. Earlier studies by Whapham *et al.* (1993) had shown that the betaines present in extracts of *A. nodosum*, when used in the cucumber cotyledon bioassay devised for cytokinins (Fletcher, 1982), resulted in enhanced chlorophyll levels in comparison to the controls. These data strongly indicated that the effects on leaf chlorophyll contents produced using seaweed extracts are due to the betaines contained in them. while the concentration of 8 cm³/l led to a decrease in all aforementioned characteristics, followed by a concentration of 6 cm³/l compared to untreated plants (control).

Table (3): Photosynthetic pigments content of calendula cultivars as affected by spraying with yeast extract, humic acid and algae extract at 45 days after transplanting during 2021/2022 and 2022/2023 seasons.

Cultivar	Treatments	Chlorophyll a		Chlorophyll b		Total Chlorophyll		Total carotenoid					
		1 st season		2 nd season		1 st season		2 nd season		In leaves		In flowers	
		1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Double flower	Control	0.0	0.66	0.57	0.34	0.33	1.00	0.90	0.30	0.27	1.34	1.36	
	Yeast extract	5 g/l	0.70	0.60	0.39	0.35	1.09	0.95	0.33	0.29	1.39	1.41	
		10 g/l	0.78	0.83	0.47	0.53	1.24	1.36	0.36	0.38	1.94	1.46	
		15 g/l	0.70	0.60	0.39	0.35	1.09	0.95	0.32	0.29	1.41	1.45	
	Humic acid	0.5 g/l	0.67	0.59	0.35	0.34	1.02	0.93	0.31	0.28	1.39	1.42	
		1 g/l	0.75	0.73	0.46	0.39	1.20	1.11	0.34	0.34	1.43	1.46	
		1.5 g/l	0.70	0.62	0.39	0.36	1.09	0.97	0.33	0.29	1.43	1.45	
	Algae extract	4 cm/l	0.69	0.59	0.35	0.35	1.04	0.93	0.32	0.29	1.39	1.35	
		6 cm/l	0.53	0.45	0.33	0.32	0.86	0.77	0.26	0.24	1.24	1.30	
		8 cm/l	0.64	0.55	0.33	0.32	0.96	0.86	0.26	0.27	1.26	1.30	
Mean		0.68	0.60	0.37	0.36	1.05	0.96	0.31	0.29	1.41	1.39		
Single flower	Control		0.55	0.64	0.36	0.36	0.91	1.00	0.29	0.30	0.84	0.87	
	Yeast extract	5 g/l		0.75	0.73	0.46	0.45	1.21	1.18	0.36	0.35	0.95	0.89
		10 g/l		0.82	0.86	0.60	0.49	1.41	1.35	0.38	0.43	1.03	1.04
		15 g/l		0.70	0.70	0.44	0.46	1.14	1.16	0.35	0.35	0.98	0.92
	Humic acid	0.5 g/l		0.61	0.65	0.38	0.38	0.98	1.02	0.29	0.30	0.95	0.91
		1 g/l		0.78	0.76	0.57	0.47	1.35	1.24	0.37	0.37	0.99	0.99
		1.5 g/l		0.76	0.74	0.47	0.45	1.23	1.19	0.37	0.37	1.00	1.00
	Algae extract	4 cm/l		0.69	0.68	0.42	0.42	1.11	1.10	0.33	0.34	0.87	0.88
		6 cm/l		0.50	0.56	0.34	0.33	0.84	0.89	0.27	0.28	0.81	0.81
		8 cm/l		0.55	0.60	0.35	0.33	0.90	0.93	0.28	0.29	0.84	0.86
Mean		0.65	0.69	0.43	0.40	1.08	1.09	0.32	0.33	0.91	0.91		
L.S.D at 5%	Cultivars		ns	0.05	0.03	ns	ns	0.12	ns	0.03	0.14	0.15	
	Treatments		0.09	ns	0.06	0.05	0.15	0.14	0.04	0.04	0.72	0.08	
	Concentrations		ns	ns	0.04	ns	ns	0.07	ns	0.02	ns	ns	
	Interaction		ns	ns	ns	ns	ns	ns	ns	ns	0.17	ns	

1st season = 2021/2022 season, 2nd season = 2022/2023 season.

These results are in harmony with Seleem (2016) found that spraying of *calendula officinalis* plant with seaweed extract at the concentration of 4cm³/l reduced all vegetative, flowering and yield traits. Whereas lower concentrations (0.5, 1, 2 cm³/l) increased all the previous traits. These results were for both cultivars in both seasons. Regarding the interaction between the two cultivars of *calendula officinalis* plant (double flower and Single flower) with the different levels of treatments (yeast extract, humic acid and algae extract) with them concentrations resulted in a clear superiority of (single flower) cultivar over double flower cultivar and yeast extract at a concentration of 10 g/l compared to untreated plant (control) in the content of the photosynthetic pigments (chlorophyll a, b, total chlorophyll, total carotenoids in leaves and total carotenoid in flowers) in the calendula leaf and flower tissues. These results were similar during the two growing seasons.

3.3 The effect of foliar application with yeast extract, humic acid, and algae extract on floral characteristics

Floral characteristics i.e., number of inflorescence / plant, Inflorescence diameter, number of rays (flowers) / inflorescence, fresh and dry weight of inflorescence / plant were affected by spraying with yeast extract at 5, 10 and 15 g/l, humic acid at 0.5,1 and 1.5 g/l and algae extract at 4,6 and 8 g/l during the

two growing seasons of 2021/2022 and 2022/2023. Regarding for cultivars there was significant superiority of double flower over single flower in the studied floral traits except for the characteristic of number of inflorescence /plant, where the single flower cultivar outperformed the double flower cultivar. At the same time, the results indicated that the floral traits of marigold plant increased by using yeast extract at concentrations of 5, 10 and 15 g/l compared to control plant as shown in Table (4) during the two growing seasons. The use of concentration of 10 g/l of yeast extract led to a significant increase in all the characteristics mentioned followed by the concentration of 5 g/l and then 15 g/l. This result was in agreement with that obtained by Khudair and Hajam (2021) on Chinese carnation (*Dianthus chinensis* L.) found that the treatment with a concentration of 2 ml l⁻¹ yeast extract increased the fresh and dry weight of the flowers, while the fresh weight of the shoot increased at a concentration of 1 ml l⁻¹. Also, El-Shawa *et al.* (2020) showed that Application of yeast extract increased floral characters such as number of flowers plant⁻¹, flowers fresh and dry weight on calendula plants. The positive effects of dry yeast on root, vegetative and flowering growth due to the favorable influence on metabolism and biological activity and its stimulating effect on photosynthetic pigments and enzyme activity which in turn encourage vegetative growth, improving flower formation and their set in some plants

and enhancement chlorophyll formation and carbohydrates accumulation (Barnett *et al.*, 1990; El-Sherbeny *et al.*, 2007). Also, data shown in Table (4) indicated that treated marigold plants with humic acid as foliar application led to a significant increase in the aforementioned floral characteristics. where the best concentration was 1 g/l, followed by the concentration of 1.5 g/l and finally the concentration of 0.5 g/l for both cultivars (double flower and Single flower), the results were recorded in both seasons. These findings are in agreement with Ahmad *et al.* (2019) showed that Significant differences were found in treated flowers with humic acid on marigold plant. The higher values were noted in flower length, number of flowers per plant and flower width. The higher values of number of flowers per plant (2.9), flowers width (9.92 cm) and flowers length (20.18 cm) were found by the application of 750 ml/l of HA. However, the maximum number of flowers per plant was (5), flower width (1.48 cm) and flower length (3.28 cm) were found with the application of 1000 ml/L Humic acid. The similar results were reported by Hasan (2019) reported that treatment of pot marigold (*Calendula officinalis* L.) plants with humic acid as foliar application at 0.15 g l⁻¹ led to positive effect in flowering traits as days required to inflorescence bud initiation, number of days remain inflorescence on the plant, number of ray flowers / inflorescence, dry weight of inflorescence and vas life. this effect may

be attributed as mentioned by Azooz (2009) and Abd El-Aal *et al.* (2005) that humic acid increase photosynthesis, chlorophyll density and plant root respiration which resulted in major plant growth also the beneficial effect of humic acid on plant growth may be because to its playing as source of plant growth hormones yield. As for algae extract, its foliar spraying at a concentration of 4 cm³/l led to an increase in all floral characteristics. These effects are agreement with Alkarawi and Abd Alkadim (2019) showed that the spraying of Dahlia plant cv. Albion with seaweed at a level of 100 mg/l⁻¹ had significant differences in number of flowers per plant, flower diameter, fresh weight of flower, fresh weight of the stalk compared with the control treatment. The increase was 12.0, 15.53, 25.66 and 46.56 for the above qualities respectively. Also, Emam *et al.* (2016) indicated that foliar spray with 1500 ppm of seaweed extract on pot marigold (*Calendula officinalis* L.) significantly increased number of flowers per plant and vase life in the two tested seasons. However, foliar spray with the lowest concentration of seaweed extract (500 ppm) resulted in significant increments in flower stalk length, flower diameter. Seaweed extracts have stimulating effects on growth characteristics which may be due to its important action on improving cell division since it contains higher amounts of nutrients, namely N, Mg, S, P, Cu, K, Mn, Ca, Mo, Fe, and B, natural hormones such as cytokinins, IAA and

GA3, amino acids, vitamins and antioxidants (James, 1994; Soliman *et al.*, 2000). While the concentration of 8 cm³/l led to a decrease in all aforementioned floral characteristics, followed by a concentration of 6 cm³/l compared to untreated plants (control). These results are in harmony with Seleem (2016) found that spraying of *calendula officinalis* plant with seaweed extract at the concentration of 4cm³/l reduced all flowering traits. Whereas lower concentration (0.5, 1, 2 cm³/l) increased all the previous traits. These results were for both cultivars in both seasons. In relation to the interaction

between the two cultivars of calendula officinalis plant (double flower and Single flower) with the different levels of treatments (yeast extract, humic acid and algae extract) with them concentrations resulted in a clear superiority of double flower) cultivar over single flower) cultivar and yeast extract at a concentration of 10 gl⁻¹ compared to untreated plant (control) in the floral characteristics i.e., number of inflorescence / plant, inflorescence diameter, number of rays (flowers) / inflorescence, fresh and dry weight of inflorescence / plant in calendula plant. These results were similar during the two growing seasons.

Table (4): Photosynthetic pigments content of calendula cultivars as affected by spraying with yeast extract, humic acid and algae extract at 45 days after transplanting during 2021/2022 and 2022/2023 seasons.

Cultivar	Characters	Number of inflorescence/plant		Inflorescence fresh weight		Inflorescence dry Weight		Diameter of Inflorescence		Number of rays/inflorescence				
		1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season			
Double flower	Control	0.0	20.54	19.54	7.57	7.83	0.97	1.03	9.31	9.47	353.00	361.75		
	Yeast extract	5 g/l	25.50	25.42	8.69	9.46	1.20	1.38	9.73	9.85	390.50	456.25		
		10 g/l	31.13	31.42	9.36	10.39	1.33	1.42	10.28	10.48	443.50	545.00		
		15 g/l	24.58	21.50	8.65	9.44	1.12	1.30	9.65	9.84	373.75	420.50		
	Humic acid	0.5 g/l	24.46	21.38	7.85	8.53	1.07	1.12	9.36	9.49	354.00	397.25		
		1 g/l	26.42	31.42	10.26	10.92	1.46	1.87	10.20	10.44	426.25	502.75		
		1.5 g/l	26.13	30.25	7.98	8.74	1.12	1.16	9.89	10.38	409.50	465.25		
	Algae extract	4 cm/l	21.29	21.38	7.79	7.92	1.03	1.09	9.40	9.75	373.00	419.50		
		6 cm/l	10.63	10.54	5.30	6.07	0.93	0.75	9.20	9.37	274.50	297.75		
		8 cm/l	19.25	18.50	6.97	7.07	0.94	0.90	9.30	9.42	340.50	348.25		
	Mean		22.58	22.54	7.96	8.50	1.09	1.17	9.58	9.79	370.38	411.48		
	Single flower	Control		52.13	52.54	1.66	1.50	0.25	0.25	7.19	7.24	50.20	51.50	
		Yeast extract	5 g/l		56.17	66.50	1.89	1.94	0.29	0.32	7.71	7.45	60.50	57.60
			10 g/l		86.54	91.17	2.09	2.04	0.34	0.41	8.07	7.92	64.90	68.40
15 g/l				55.50	63.38	1.87	1.89	0.29	0.28	7.57	7.43	57.00	57.50	
Humic acid		0.5 g/l		52.63	57.50	1.77	1.76	0.28	0.26	7.25	7.27	54.70	56.40	
		1 g/l		83.42	75.25	1.96	1.97	0.32	0.32	7.76	7.53	63.30	65.20	
		1.5 g/l		60.33	70.29	1.85	1.83	0.28	0.27	7.73	7.46	62.50	59.70	
Algae extract		4 cm/l		52.38	52.54	1.72	1.74	0.27	0.26	7.27	7.38	55.40	56.70	
		6 cm/l		36.46	48.13	1.60	1.34	0.17	0.19	6.94	6.86	47.50	45.90	
		8 cm/l		50.54	48.50	1.65	1.36	0.23	0.24	7.09	6.95	49.60	49.30	
Mean			57.53	60.91	1.78	1.70	0.27	0.28	7.41	7.33	55.50	55.93		
L.S.D at 5%	Cultivars		2.26	0.46	0.03	0.93	0.01	0.04	0.64	0.54	69.37	4.75		
	Treatments		0.68	0.55	0.36	0.37	0.01	0.02	0.27	0.31	25.98	9.82		
	Concentrations		0.42	0.37	ns	0.21	0.01	0.02	0.08	0.15	ns	ns		
	Interaction		1.19	1.03	0.67	0.58	0.02	0.04	ns	ns	ns	64.62		

1st season = 2021/2022 season, 2nd season = 2022/2023 season.

4. Conclusion

In conclusion, it could be observed that the studied morphological characteristics i.e., plant height, branch number / plant, stem diameter, number of leaves, leaf area (cm²) plant⁻¹, fresh weight and dry weight of plant. Also, physiological characteristics i.e., chlorophyll a, b and total chlorophyll and total carotenoids in fresh leaves as well as total carotenoid in flowers. As well as floral characteristics i.e., number of inflorescence / plant, inflorescence diameter, number of rays (flowers) / inflorescence, fresh and dry weight of inflorescence / plant of pot marigold were increased, in both seasons, due to the use of the three rates of yeast extract (5, 10 and 15 g/l) and humic acid (0.5, 1 and 1.5 g/l). Also, algae extract at rate of 4 cm³/l in comparison with the untreated plants (control). Whereas, spraying with algae extract at concentration of (6 and 8 cm³/l) led to decrease in all the aforementioned characteristics. However, yeast extract at (10 g/l) and humic acid at (1 g/l) were much more effective than the other rates. Thus, results obtained in the present study recommended using of yeast extract up to a concentration of 10 g/l and humic acid up to a concentration of 1 g/l for increasing morphological, physiological and floral characteristics of marigold plant, especially double flower cultivar under Assiut governorate, Egypt growing conditions.

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