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INFLUENCE OF YEAST NATURAL EXTRACT ON GROWTH, FLOWERING, YIELD AND SOME ANATOMICAL STRUCTURE OF MARIGOLD (*Calendula officinalis* L.) PLANT.

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ABSTRACT

The present investigation was conducted at the Faculty of Agric. Cairo Univ. Giza, Egypt during the two successive seasons of 2012 and 2013 to study the effect of foliar application of yeast natural extract at 2, 4, 8 and 12 g/L on the growth, flowering, yield and certain anatomical structure of marigold stem and leaf.

Results indicated that all studied traits of growth, flowering and yield well improved due to yeast extract application at the levels of 2, 4 and 8 g/L, whereas decreased at 12 g/L. The maximum increment was detected at 4 g/L due to its positive effect on the anatomical structure of stem and leaf.

Keywords: *Calendula officinalis* L., yeast extract, morphology, flowering, yield, anatomy

INTRODUCTION

Marigold (*Calendula officinalis* L.) belonging to family Asteraceae, is a herbaceous medicinal plant. The origins of this plant has been reported are Mediterranean, West Asia, Southern Europe and East Mediterranean (Omid Beygi, 2005).

It is an erect, annual herbaceous aromatic plant, growing to 60 cm high, with angular and glandular stems. The leaves are oblong-lanceolate, 5–17 cm, hairy on both sides, and occasionally waved or weakly toothed. Its yellow inflorescences comprise a flower head surrounded by two rows of hairy bracts. The fruit is usually a thorny curved achene (Branzil 2005, Re TA, *et al.* 2009 and Gharineh *et al.* 2013). Pot marigold inflorescences have many pharmacological activities including antioxidant, antibacterial, antifungal, anti-inflammatory and antiviral (Kasiram *et al*. 2000, Barbour *et al*. 2004, Preethi *et al*. 2006 and Danielski *et al*. 2007). Marigold plants used as cytotoxic and have tumor reducing potential (Chandran and Ramadasan 2008). It has a very good cutaneous wound healing and collagen efficiency skin antifungal and antiviral properties (Mohammad and Kashani, 2012). Calendula plant is widely cultivated for ornamental purposes and it has been used as a medicinal plant to treat various diseases for a long time (Torbaghan, 2012).

A great attention has been paid on the possibility of using natural and safety substances which are rich sources of phytohormones in order to improve plant growth. In this concern, yeast extract is a rich source of phytohormones especially cytokinins, that enhance cell division and enlargement (Khedr and Farid, 2000 and Mahmoud, 2001). It is also, rich with a mixture of amino acids, peptides, the best sources of the B-complex vitamins such as B1, B2, B6 and B12, carbohydrates, sugar, vitamins, enzymes, and minerals (Amer 2004, Dawood *et al.* 2013 and Marzauk *et al.* 2014).

Therefore, the present investigation was designed to detect the influence of different levels of yeast extract on vegetative growth, flowering, yield and anatomical observation of *Calendula officinalis* L. plant.

MATERIALS AND METHODS

The present study was carried out at the Agricultural Experiments and Researches Station, Faculty of Agriculture, Cairo University, Giza, Egypt through the two successive winter seasons of 2012 and 2013 to study the effect of foliar spray with different concentrations of yeast extract on morphological characters, flowering induction, yield and anatomical characters of Calendula plant.

Seeds of marigold (*Calendula officinalis* L.*)* were obtained from Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, Egypt. Seeds were sown in well-prepared seed beds at the nursery on 1st and 7th of October, 2012 and 2013 in the first and second seasons, respectively. After 30 days from sowing, with an average height 10 cm, marigold seedlings were transplanted to the open field in plots 2x2 m which contained 4 rows at distance of 40cm between plants, each plot contain 20 plants. The experiment was arranged in Randomized Complete Block Design with three replicates for each treatment. Common cultural practices were followed, including regular irrigation, fertilization and manual weed control. Yeast extract with tap water was sprayed at concentrations of 2, 4, 8 and 12 g/L. The control plants were sprayed with tap water.

The tested concentrations of yeast extract were applied twice by means of an atomizer sprayer. The first application was four weeks from transplanting and the second was two weeks from the first one (at this age, opening of the flower buds started). Volume of spraying solution per plot was almost 1.5 and 3 liters for first and second applications; respectively. This volume was adequate to wet plants of the plot thoroughly with excess of dripping solution.

Recording of Data

A random sample of 15 plants for each tested treatment (5 plants from each replicate) was assigned for investigation. Vegetative characters were recorded after 60 days from transplanting; *i.e.,* two weeks after the second application of yeast extract. The following characters were studied in both growing seasons.

Morphological Characters

Plant height (cm), number of branches/plant, leaf number/plant, shoot fresh weight/plant (g/shoot), shoot

dry weight/plant (g/shoot) and leaf area (cm^2), measured by means of leaf area meter L1-3000A portable area meter.

Flowering parameters

Number of inflorescence/plant, inflorescence diameter (cm), number of ray flower/inflorescence, inflorescence fresh weight (g/inflorescence), inflorescence dry weight (g/inflorescence).

Yield and its components

Number of ahenes/capitulum, weight of ahenes /capitulum (g) and 100 achene weight (g)

Anatomical studies:

A comparative microscopical study was performed on plant materials for treatments which showed remarkable response to foliar spray with yeast extract in addition to control. Tested materials included the main stem at its median portion and its leaf. Specimens were taken throughout the second season of 2014 at the age of ten weeks. Specimens were killed and fixed for at least 48 hrs in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70 %). Specimens were washed in 50 % ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56 °C, sectioned to a thickness of 20 microns, double stained with crystal violet-erythrosin, cleared in xylene and mounted in canada balsam (Nassar and El-Sahhar, 1998). Slides were microscopically examined and the measurements were taken as an average of 10 readings from 3 slides calculated.

Statistical analysis:

The data were statistically analyzed using MSTAT – C software. The mean comparisons among treatments were determined by New Least Significance Differences (New L.S.D.) at 5% level of probability as reported by Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

Morphological characters

Data dealing with morphological characters includes mean values of; plant height, number of branches/plant, number of leaves/plant, leaf area and shoot fresh and dry weight of Calendula plants as affected by foliar spray with different concentrations of yeast at 0, 2, 4, 8 and 12 g/L, during the two growing seasons are presented in (Table 1). It is clear that, using yeast extract at 2 and 4 g/L concentrations increased significantly all characters of Calendula plant in both seasons. In addition, 8 g/L concentration caused significant enhancement in number of branches/plant in both seasons and total leaf area in the 2nd season only.

The maximum significant increment was detected when Calendula plants were treated with 4g/L, being 31.3 - 28.8, 31.6 - 29.5, 30.1 - 27.7, 28.0 - 26.6, 30.6 - 31.0 and 32.4 - 29.4 % more than the control for plant height, number of branches/plant, number of leaves/plant, leaf area and shoot fresh and dry weight in the 1st and 2nd seasons, respectively. By contrast, foliar spray with the relatively high concentration; 12 g/L caused significant decreases by 10.9 - 8.8, 13.0 - 11.1, 10.5 - 9.3, 8.5 - 8.0, 10.6 - 10.0 and 10.0 - 11.1% below the control in the 1st and 2nd seasons, respectively. In this concern, all the following reports are in agreement with the present findings; Ali (2001) on *Calendula officinalis* plants proved that foliar application by active dry yeast at 4.5 g/L concentration caused a significant increase in plant growth. The significant enhancement effect of yeast extract at 5 g/L on *Chrysanthemum grandiflora*, as aromatic and medicinal plants, was recorded by Hanafy *et al.* (2012) by using active dry yeast at 10 g/L was enhanced the plant growth of *Schefflera arboricola*. Moreover, Wahba (2002) on O*enothera biennis*, Abd El-Kafie *et al.* (2001) on *Chrysanthemum grandiflora*, El-Gamal (2005) on Sweet Basil and Abd El-Latif (2006) on *Salvia officinalis* and Azoz (2014) on Basil.

Yeast extract contains different nutrients (N, P, K, Fe, Zn and Mn), high values of vitamins, carbohydrates and valuable source of phytohormones (*i.e.* auxins, cytokinins) and protein that enhance cell division and enlargement, so it's important in improving plant growth (Abou EL-Yazied, and Mady 2012 and Marzauk *et al.* 2014).

Flowering parameters

The mean values of the studied characters included number of inflorescence/plant, inflorescence diameter (cm), number of ray flowers/ inflorescence, inflorescence fresh and inflorescence dry weight of Calendula plants as affected by foliar spray with different concentrations of yeast extract in the two successive seasons are given in Table (2).

It is obvious that the first two concentrations; 2 and 4 g/L increased significantly all the flowering characters of calendula plant, except the concentration 2 g/L for inflorescence fresh weight which caused insignificant effect. The maximum increase was achieved at the rate of 4 g/L being 29.4 - 27.9, 29.5 - 27.6, 27.9 - 26.4, 26.8 - 25.6 and 28.6 - 27.8 % more than the untreated plants (control) in the 1st and 2nd seasons for the previous characters, respectively.

Using the concentration of 8 g /L caused insignificant enhancement in all flowering parameters, except number of ray flowers/ inflorescence the 1st season which caused significant increment. Meanwhile, the highest concentration 12 g/L caused insignificant reduction in all characters reached to 7.1 - 7.6, 7.2 - 8.5, 8.6 - 8.8, 11.8 - 9.1 and 9.5 - 5.6 % below the control in the 1st and 2nd seasons, respectively.

These results were in agreement with Ahmed *et al.* (1998) stated that highest increment in yield of calyex of Roselle (*Hibiscus sabdariffa*) plants were obtained by spraying plants with 2 g/L, while Abd El-Kafie *et al.* (2001) showed that foliar application of active dry yeast at 5 g/L to *Chrysanthemum grandiflora* increased flower numbers/plant. Meanwhile, Ali (2001) stated that number of flowers of *Calendula officinalis* plants was significantly increased by foliar application of yeast extract at concentrations of 1.5, 3 and 4.5 g/L, while Azoz (2014) on Basil found that with 2, 4 and 8 g/L increased significantly number of inflorescence, meanwhile, 12 g/L caused insignificant reduction of Basil plant.

Table 1. Effect of yeast extracts on morphological characters of *Calendula officinalis* L. plants during two seasons; 2012 and 2013.

	Morphological characters					
	1st Season 2012					
	plant height (cm)	No. of branches / plant	No. of leaves / plant	Total leaf area (cm^2)	Fresh weight/plant (g)	Dry weight/plant (g)
Control	43.93	19.11	194.34	1575.81	217.12	40.23
Yeast extract 2g/L	49.92	22.13	228.96	1766.04	246.57	45.59
Yeast extract 4g/L	57.67	25.15	252.83	2017.15	283.47	53.27
Yeast extract 8g/L	48.51	21.56	214.36	1719.83	234.94	43.12
Yeast extract 12g/L	39.16	16.61	174.02	1441.26	194.00	36.21
New L.S.D. at 5%	4.60	2.33	21.85	133.25	21.87	3.82
	2nd Season 2013					
Control	40.86	17.86	177.57	1469.21	205.19	33.12
Yeast extract 2g/L	45.75	20.05	204.37	1614.64	231.98	36.88
Yeast extract 4g/L	52.63	23.12	226.67	1859.62	268.82	42.87
Yeast extract 8g/L	43.91	19.83	193.52	1581.24	223.91	34.71
Yeast extract 12g/L	37.25	15.87	161.07	1352.03	184.57	29.43
New L.S.D. at 5%	3.21	1.74	16.45	116.82	18.51	2.98

Table 2. Effect of yeast extract on flowering characters of *Calendula officinalis* plants during two seasons; 2012 and 2013.

	Flowering characters				
	1st Season 2012				
	Number of inflorescence / plant	Inflorescence diameter (cm)	Number of ray flower / inflorescence	Inflorescence fresh Weight (g)	Inflorescence dry weight (g)
Control	181.61	5.53	42.04	1.27	0.21
Yeast extract 2g/L	205.82	6.34	48.26	1.44	0.24
Yeast extract 4g/L	235.00	7.16	53.76	1.61	0.27
Yeast extract 8g/L	198.53	6.05	46.31	1.36	0.22
Yeast extract 12g/L	168.72	5.13	38.43	1.12	0.19
N.L.S.D. at 5%	22.73	0.59	3.85	0.21	0.03
	2nd Season 2013				
Control	177.74	4.93	39.88	1.21	0.18
Yeast extract 2g/L	200.11	5.57	45.31	1.36	0.20
Yeast extract 4g/L	227.34	6.29	50.41	1.52	0.23
Yeast extract 8g/L	191.88	5.26	43.02	1.19	0.19
Yeast extract 12g/L	164.32	4.51	36.39	1.10	0.17
N.L.S.D. at 5%	20.32	0.45	4.21	0.19	0.02

Yield and its component

Aspects of yield traits under consideration include number of ahenes/ capitulum, weight of ahenes /capitulum (g) and 100 achene weights (g) at harvest time as affected by foliar application with different concentrations of yeast extract in two successive seasons are columned in Table (3). It is clear that the first three concentrations 2, 4 and 8 g/L of yeast extract increased significantly number of ahenes/capitulum and weight of 100 achenes in both seasons, except the concentration of 8 g/L which induced insignificant enhancement in number of ahenes/capitulum in the 2nd season, and weight of 100 achene in both seasons. On the other hand, in both seasons, weight of ahenes /capitulum were significantly enhanced only by the concentration of yeast extract 4 g/L, and using the concentrations; 2 and 8 g/L increased it insignificantly. The maximum increase in number of ahenes/ capitulum, weight of ahenes /capitulum and weight of 100 achene was observed at the concentration of 4 g/L being 31.9 - 29.8, 31.8 - 30.0 and 31.0 - 28.2 % more than the control in the 1st and 2nd seasons, respectively.

On the other hand, the lowest values of these characters were obtained by the concentration of 12 g /L. It recorded 8.7 - 9.4, 13.6 – 10.0 and 7.1 - 5.5 % less than the control in the 1st and 2nd seasons, respectively. The same results were obtained by Eid (2001) on Coriander plant, Naguib and Khalil (2002) on *Nigella sativa* and Azoz (2014) on Basil plant.

Table 3. Effect of yeast extract on yield and its component of *Calendula officinalis* plants during two seasons; 2012 and 2013.

	Yield characters		
	1st Season 2012		
	Number of achenes / capitulum	Weight of achenes / capitulum (g)	Weight of 100 achene (g)
Control	20.19	0.22	1.13
Yeast extract 2g/L	23.13	0.25	1.27
Yeast extract 4g/ L	26.64	0.29	1.48
Yeast extract 8g/ L	22.13	0.24	1.19
Yeast extract 12g/ L	18.42	0.19	1.05
N.L.S.D. at 5 %	1.59	0.06	0.08
	2nd Season 2013		
Control	19.35	0.20	1.10
Yeast extract 2g/ L	21.98	0.23	1.23
Yeast extract 4g/ L	25.12	0.26	1.41
Yeast extract 8g/ L	20.28	0.22	1.16
Yeast extract 12g/ L	17.54	0.18	1.04
N.L.S.D. at 5%	1.37	0.05	0.07

Anatomical characters

I- Anatomy of the Main Stem

Microscopical measurements of certain histological characters in transverse sections through the median portion of the main stem of Calendula plant sprayed with 4 g/L yeast extract and those of control are presented in Table (4). Also, microphotographs depict these treatments are shown in Figure (1). It is clear that yeast extract treatment resulted in larger stem diameter by 31.9 % more than the control. Different tissues which comprise the stem shared by various extents in this increment *i.e.* epidermis, cortex, fiber group, phloem, xylem and pith diameter by 10.5, 30.8, 25.9, 54.5, 48.5 and 29.8 % over than the control, respectively. The increment in cortex thickness could be attributed to the increment in cell diameter as well as another increase in number of parenchyma cell layers; the average elevated percentages of parenchyma cell layers were 16.3 % over the control. It is apparent that, the increase in vascular bundle dimension was mainly due to the increase in vessel diameter and average number of vessel rows by 58.0 and 31.8 % compared with control, respectively. It is interesting to mention that, relative to the control, pith diameter enlargement was mainly due to the increase in average pith parenchyma cell number and diameter. Effect of yeast extract on anatomical characteristics of different plants reported previously by Azoz (2014) on Basil and Nassar *et al.* (2011) on Bean plants.

Table 4. Measurements in microns (µ) of anatomical characters in transverse sections through the median portion of the main stem of *Calendula officinalis* plant grown in the 2nd season 2013 and sprayed with yeast extract.

	Treatments	
Characters	**Control**	**Yeast extract 4g/L**
Stem diameter	4850	6400.7
Epidermis thick.	20	22.1
Cortex thick.	390	510.4
No. of cortical layer	11	12.8
Fiber cap thick.	97.5	122.8
Phloem thick.	82.5	127.5
Xylem thick.	212.5	315.6
Vessel diameter	19.8	31.3
No. of xylem rows / bundle	11	14.5
Pith diameter	3250	4218.6
Parenchymatous pith thick.	110	135.7

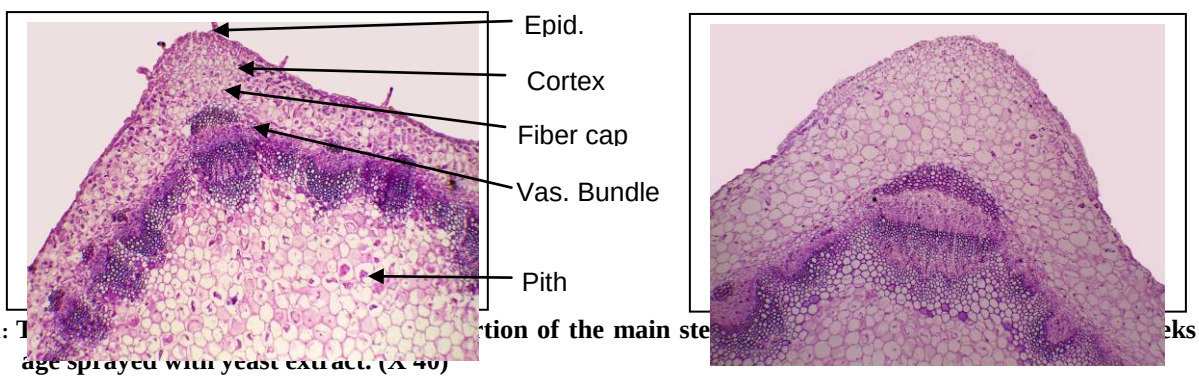

Fig. 1: T…rtion of the main ste…eks age sprayed with yeast extract. (X 40)

A- Control. B- Plant sprayed with 4 g/L.

II- Anatomy of the leaf

Microscopical counts and measurements of certain histological characters in transverse sections through the blade of the leaf developed on the median portion of the main stem as treated with yeast at 4 g/L and those of control are presented in Table (5) and Figure (2). It is noted that spraying yeast extract at concentration of 4 g/L increased thickness of both midvein and lamina of leaf blades of Calendula plant by 33.5 and 23.4 % more than the control, respectively. It is clear that, the increase in lamina thickness was accompanied with 30.0 and 20.4 % increments in thickness of palisade and spongy tissues compared with the control, respectively. Likewise, the main vascular bundle of the midvein was increased in size as a result of spraying yeast extract. The increment was mainly due to the increase in length by 45.4 % and in width by 54.3 % more than the control. Also average number of xylem rows per midvein bundle was increased by 40.0 % over the control. Which amounted to more total active conducting area to cope with vigorous growth resulting from treatment with 4 g/L. The above mentioned results are in accordance with the findings reported by Azoz (2014) on Basil, reported that foliar spray with yeast extract at 4 g/L increased the anatomical structure of the plant. Nassar *et al.* (2011) on Bean, stated that yeast extract at 100 ml/L had positive effect on plant anatomical character.

Table 5. Measurements in microns of certain histological features in transverse sections through the leaf blade of *Calendula officinalis* plant sprayed with yeast extract.

	Treatments	
Characters	**Control**	**Yeast extract 4g/L**
Midvein thickness	1610	2150
Lamina thickness	640	790
Palisade tissue thickness	200	260
Spongy tissue thickness	410	494
Dimension of the main vascular bundle:		
Length	460	669
Width	410	633
No. of xylem rows/ mid-vein bundle	15	21

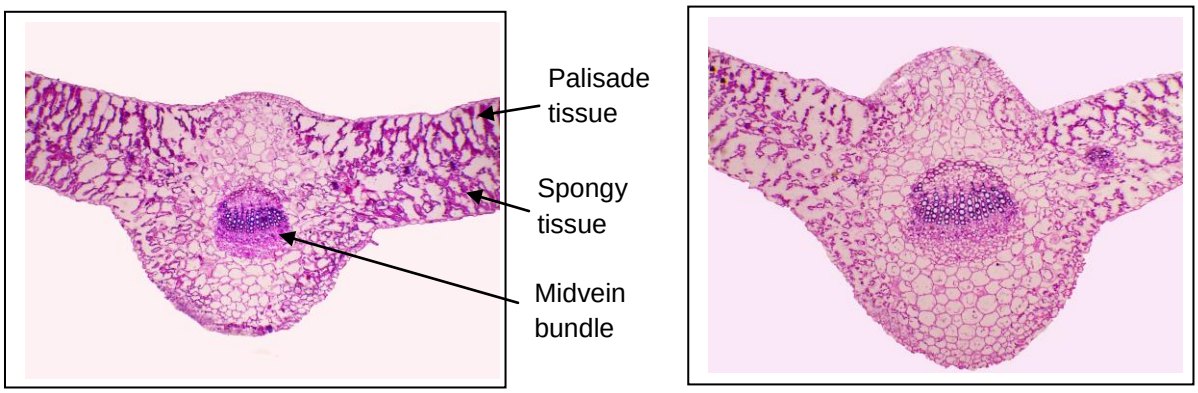

Fig. 2: Transverse sections through the blade of the leaf developed on the median portion of the main stem of *Calendula officinalis* plant ten weeks age sprayed with yeast extract. (X40)
A- Control. Pants sprayed with yeast 4 g/L.

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استخدام المستخلص الطبيعي للخميرة لتحسين النمو الخضري و التزهير و المحصول و الصفات التشريحية لنبات الأقحوان

فايزة حسين نوفل ، محمد اسامة السجاعي وانجي علاء سليم
قسم النبات الزراعي- كلية الزراعة – جامعة القاهرة – الجيزة – مصر.

اجري هذا البحث بمحطة التجارب الزراعية, كلية الزراعة, جامعة القاهرة في موسمين متتاليين ٢٠١٢ و ٢٠١٣ لدراسة تاثير مستخلص الخميرة بتركيزات ٢, ٤, ٨ و ١٢ جم / لتر على النمو الخضري، التزهير و المحصول اضافة الى الصفات التشريحية لنبات الأقحوان.

وكانت اهم النتائج المتحصل عليها: أن المعاملة بالتركيزات ٢, ٤ و٨ جم / لتر قد سببت زيادة في جميع الصفات المورفولوجية و صفات التزهير و الصفات المحصولية و التشريحية بينما ادت المعاملة بالتركيز ١٢ جم / لتر الى انخفاض في جميع الصفات السابقة. و اكدت النتائج ان المعامله بتركيز ٤ جم / لتر هي المعاملة التي يمكن التوصية بها لتحقيق اقصى زيادة في كل الصفات المدروسة و التي قد ترجع الى زيادة صفات التركيب التشريحي المدروسة في الساق و الورقة.