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Modification of growth, flowering and chemical composition of *Tagetes patula* plants

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ABSTRACT

A field experiment was conducted on French marigold plants to evaluate their behavior under spraying different concentrations of chlormequat chloride (CCC) in an open field of a private farm in kom-Hamada, EL-Behira governorate, Egypt during two successive seasons 2016 and 2017. Morphological characters yield and chemical constituents were studied. Spraying CCC with different concentrations showed differences on the studied parameters compared with the control treatment in both seasons. The application of 50 mg/l CCC recorded the maximum values of leaf area, length of inflorescence pedicel, minimum number of days to first visible flowering bud and minimum number of days to first inflorescence opening. However, the tallest plants, branch length, node length and maximum plant fresh weight, plant dry weight, numbers of inflorescence per plant, inflorescence fresh weight and inflorescence diameter were noticed with the application of 100 mg/l CCC. The treatment of 1000 mg/l gave maximum number of the primary branches, root fresh weight, root dry weight, vase life, chlorophyll A, total chlorophyll content, carotenoids content, carbohydrates percent, phosphorus content, potassium content, essential oil percent, essential oil content, polyphenols, flavonoids and antioxidant activity (IC_{50}). Moreover, the stem diameter, chlorophyll B content, nitrogen percent were found by the application of 2000 mg/l CCC in the first and second seasons, respectively. In view of the components of the volatile oil found that Piperitone, Caryophyllene, limonene, (-)-Caryophyllene oxide compounds were the main compounds. The percentages of these compounds were affected by factors under study.

Key words: Chlormequat chloride, *Tagetes patula*, vegetative growth, flowering; essential oil, polyphenols, antioxidant.

Introduction

The genus *Tagetes* consists of approximately 40-50 species and belongs to the Asteraceae (Lawrence, 1985). *Tagetes patula* L. is known as French marigold. It is native to Mexico and other warm parts of America. It is a well known worldwide as an ornamental annual plant with yellow or orange flower-head (Szarka *et al*., 2006). It is planted in mixed borders, the beds for mass display and pots. Marigold flowers are used in flower decorations and making garlands, during several religious and social functions and further the petals are used in dry flower making and the industry of pigment extraction. Recently, marigold is grown commercially for the extraction of carotenoid pigments mainly xanthophylls (Sanghamitra *et al*., 2015). Previous studies found the antioxidant properties of marigold extract were in correlation with its polyphenol content (Li *et al*., 2007). The isolation of polyphenols such as caffeic acid, gallic acid, acylated flavonoid-*O*-glycosides and methoxylated flavonoids from marigold was well literatured by many authors i.e. (Aquino *et al*., 2002 and Parejo *et al*., 2005). The flowers are used in drugs, and pharmaceutical products, processed food, confectionery and in the poultry industry. One of the most important effects of the plant is their use as very valuable intercrop for controlling plant parasitic nematodes and insecticidal activity (Darwish, 1992). Its essential oil is effective as antiparasitic, antispasmodic, antibiotic, antimicrobial and antiseptic (Chowdhury *et al*., 2009).

Plant growth regulators have been defined as one of the main factors influencing plant growth and their primary and secondary metabolites pool. Exogenous application of growth regulators has

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been reported for several horticultural and ornamental plants (Wareing and Phillips, 1985). Plant growth regulators have been reported to improve growth, fresh plant yield, and yield quality (Saimbhi, 1993).

Cycocel (CCC) (chlormequat; 2-chloroethyltrimethyl ammonium chloride) is a synthetic plant growth regulator. It is used as retardant of plant growth. Therefore, it is used to ornamental plants for inducing dwarfism in plants and shorter internodes, stronger stems and green leaves. It is also utilized to produce compact, sturdy potted and bedding plants, enhance the green color of the foliage, strengthen flower stems and promote resistance of foliage to environmental stresses. Although the growth reduction effect of cycocel is common, growth reduction percentage, flowering, leaf area and chlorophyll content, flower shape and colour responses of plants to this chemical can vary depending on the dose or concentration, method, site of application, species and cultivar and also growing season (Taiz and Zeiger, 2006). Generally, the exogenous applications of growth retardants play an important role in growth, flowering and yield and chemical constitute. For example, chloremquat enhanced the volatile oil accumulation in plants (Abou Zied and Sherbeany, 1971).

This work has bilateral purpose so deals with *Tagetes patula* plant as ornamental, aromatic and medicine plant.

Hence, the present investigation aimed to study the effect of different concentrations of cycocel on herb yield, inflorescences yield, quality of inflorescences, the ability of produce dwarfed plants, yield of essential oil from *Tagetes patula* plants and its composition, herb content of other chemical constituents such as polyphenols, flavonoids and antioxidant activity of herb.

Materials and methods

Plant material:

This study was conducted during two seasons of April-July of 2016 and 2017 in a completely randomized design (CRD) in an open field of a private farm in kom-Hamada, EL-Behira governorate, Egypt. Three seedlings with four true leaves of *Tagetes patula* were transplanted in 30 cm diameter pots. Each pot content about 10 kg of field soil. The physical and chemical properties of the soil samples were determined according to Jackson (1973) and Cottenie *et al*. (1982) as shown in table (1).

Table 1: The physical and chemical properties of the experimental soil.

O.M.	CaCO3%	Sand%	Silt %	Clay%	Texture class	pH	ECe (ds/m)
1.4	1.8	27	39	34	Clay loam	7.8	2.3
Soluble ions (meq/L)							
HCO^{3-}	Cl^{-}	SO^{2-}	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	
2.5	9.3	10.7	10.9	6.02	4.55	0.32	

Plant growth regulator:

There were ten pots (three seedlings each) for each treatment. The effect of plant growth regulators on *Tagetes patula* chemical composition, flowering and yield traits was evaluated by spraying plants foliage with solutions of chlormequat chloride (CCC) (50, 100, 500, 1000 and 2000 mg/l) one foliar sprays were given after two week from transplanting. Tween 80 (0.01%) was used as wetting agent. Plants of control treatment were sprayed with distilled water and Tween 80 (0.01%). The plants were harvested at maximum flowering stage.

The following data were recorded each season:

Vegetative and rooting parameters:

Plant height (cm), branch length (cm) (mean of all branches per plant), number of branches, stem diameter (mm) (at the base of plant), node length (cm): the average node length (cm), leaf area

(cm^2): as mentioned by Koller (1972), plant fresh and dry weight (g) and root fresh and dry weight (g).

Flowering parameters:

Numbers of days to first visible flowering bud (day), numbers of days to first inflorescence opening (day), numbers of inflorescence per plant, inflorescence fresh weight (g), inflorescence diameter (mm), length of inflorescence pedicel (cm), vase life (day): as described with Eason et al. (2001).

At harvest leaf greenness (chlorophyll content) was quantified using a SPAD-502 Chlorophyll Meter (Minolta Camera Co., Ramsey, NJ). Also, chlorophyll A, B and beta carotene were determined according to Wintermans and Mats (1965) and total carbohydrate (% of dry plants) was determined as reported by Dubios *et al.* (1956). Nitrogen content (%), phosphorus content (%) and potassium content (%) were determined according to the methods described by Kjeldahl (Nelson and Sommers, 1973), Murphy and Riley (1962) and Isaac and Kerber (1971), respectively.

Essential oil:

Essential oil extraction:

Essential oils were extracted from aerial parts of each treatment by water distillation using clevenger apparatus for 2 h according to Guenther 1961 and expressed as ml/100g, while essential oil yield was expressed as ml/plant. The extracted essential oil was dehydrated over anhydrous sodium sulphate and stored at freezer (-5 ^{o}C) till used for gas chromatography-mass spectrometry (GC-MS) analysis.

GC-MS analysis:

The GC-MS analysis of the essential oil of the different treatments was carried out in the second season using gas chromatography/mass spectrometry instrument stands at the Department of medicinal and aromatic plants research, National Research Center with the following specifications. Instrument: a traces GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadruple Mass Spectrometer). The GC-MS system was equipped with a Tr-5 MS column (30 m x 0.32 mm i. d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.3ml/min and a split ratio of 1:10 using the following temperature program: 60^{o}C for 1 min; rising at 4 ^{o}C /min to 160 ^{o}C and held for 6min; rising at 6 C/min to 210 ^{o}C and held for 1 min. The injector and detector were held at 210 ^{o}C. Diluted samples (1:10 hexane, v/v) of 0.1µL of the mixtures were injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Compounds were identified by matching of their mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Total phenolic content:

It was determined according to the method of Singleton and Rossi, (1965).

Total flavonoid content:

It was determined using the colorimetric method of Kim *et al*. (2003).

Antioxidant activity determinations:

The antiradical activity of different samples was determined by using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Brand-Williams *et al*.,1995).The extract concentration

(µg/ml) providing 50% of antioxidant activities (IC_{50}) were calculated by plotting in a graph inhibition percentage against extract concentration.

Statistical analysis:

This experiment was conducted in completely randomized design (CRD). The mean of three seedlings of each pot act as one repetition. Analysis of variance with SAS software (SAS Institute, 1988) was carried out on the test treatments data. Treatments' means were compared using the LSD test at 5% level of probability. The experiment was repeated in the second year at the same site using the same steps and techniques of the first year to compare the results of the two successive seasons**.**

Results

Plant height

Data presented in table 2 show that, spraying marigold plants with different concentrations of chlormequat chloride (CCC) (0, 50, 100, 500, 1000 and 2000 mg/l) solutions caused significant differences in plant height. The first and second concentration of CCC increased the plant height of marigold plants, but after that the height decreased with increasing CCC concentrations. Moreover, the tallest (60.5 and 62.5 cm) and shortest (35.3 and 37.1 cm) plants were observed by the application of 100 and 2000 mg/l CCC, respectively compared with the other treatments and control in both seasons.

Branch length

Different treatments resulted in significant differences in branch length (Table 2). The branch length firstly increased with increasing CCC until 100mg/l and then it decreased with increasing CCC concentration. It ranged from 6.1 to 27.0 cm in the first season and 6.4 to 27.4 cm in the second one for the treatments of 2000 mg/l to 100 mg/l CCC, respectively. Insignificant differences were found between 50 and 100 mg/l CCC in both seasons, respectively.

Branches number

The branches number was significantly different from each other among the studied treatments (Table 2). The maximum branches number was recorded with the application of 1000 mg/l CCC (11.4 and 11.2 for both seasons, respectively) which was closely followed by 2000 mg/l CCC. On the other hand, the minimum branches number (3.2 and 3.6) was observed with control.

Stem diameter

Significant differences in stem diameter were detected as a function of growth regulator dosage for most treatments of French marigold (Table 2). Comparing the impact of the CCC and control treatments on stem diameter it was noticed that, the stem diameter increases in parallel with increasing CCC dosage and the spraying with 2000 mg/l recorded the thickest plants (7.7 and 7.9 mm) compared with the other CCC concentrations in the first and second seasons, respectively with insignificant differences between 1000 and 2000 mg/l CCC dosage in both seasons, respectively.

Node length

The response of nodes to elongate decreased with increasing CCC concentration. In addition, the smallest nodes (1.96 and 2.04 cm) in the first and second seasons, respectively were observed after spraying plants with 2000 mg/l CCC comparing with all treatments and control plants. Insignificant differences observed between 1000 and 2000 mg/l CCC. On the contrary, the longest nodes (3.32 and 3.38 cm) were found with spraying 100 mg/l CCC in the first and second seasons compared with the control and the other treatments, respectively. The differences in nodes length between 50 and 100

mg/l CCC in the first season and between 0, 50 and 100 in the second season, respectively were insignificant.

Table 2: The mean values of plant height (cm), branch length (cm), branches number, stem diameter (mm), node length (cm), and leaf area (cm^2) as affected by CCC treatments.

CCC Conc.	Plant height (cm)		Branch length (cm)		Branches number		Stem diameter (mm)		Node length (cm)		Leaf area (cm^2)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	43.6c	44.6c	16.5b	16.8b	3.2 d	3.6d	5.0d	5.2 d	3.09 b	3.30a	5.80a	5.86ab
50mg/l	59.4b	60.5b	25.9 a	26.3a	7.4c	7.8c	6.4c	6.9c	3.20ab	3.32a	5.94a	6.32a
100mg/l	60.5a	62.5a	27.0 a	27.4a	8.4bc	8.0c	6.9bc	7.1bc	3.32a	3.38a	5.82a	6.18a
500mg/l	41.9d	43.8c	11.6 c	11.7c	9.0b	9.4b	7.0b	7.3bc	2.38c	2.42b	4.18b	4.02b
1000mg/l	36.6e	37.9d	9.0d	9.2d	11.4a	11.2a	7.3ab	7.5ab	2.16cd	2.24bc	4.0bc	3.58b
2000mg/l	35.3f	37.1d	6.1e	6.4e	11.0a	10.0b	7.7a	7.9a	1.96d	2.04c	3.12c	3.40b

Means in columns followed by the same letter are not statistically different at the 0.05 probability level. 1st and 2nd means first and second seasons, respectively.

Leaf area

Comparing the effect of all studied treatments on plant leaf area revealed significant differences between treatments. Plant leaf area decreased with increasing CCC concentrations. So, the minimum leaf area of plants (3.12 and 3.40 cm2) in both seasons, respectively were found with spraying plants with 2000 mg/l CCC comparing with all CCC concentrations and control treatments. Moreover, the largest leaves of French marigold (5.94 and 6.32 cm^2) were measured with the application of 50 mg/l CCC in the first and second season respectively. The differences between the 0, 50 and 100 mg/l CCC in the first and second seasons, respectively were insignificant (Table 2).

Plant fresh and dry weight

Fresh and dry weight of French marigold plants were significantly increased by all treatments of CCC compared to the control in both seasons. Moreover, the heaviest fresh weights (85.40 and 88.16 g /plant) and dry weights (14.51 and 14.96 g/plant) of plants for the first and second seasons, respectively were found by using 100 mg/l CCC. The plant dry weight had insignificant differences with using the concentrations of 50, 100, 500 and 1000 mg/l CCC in the first and second seasons, respectively. Whereas, the significant lightest fresh weights (44.06 and 44.95 g) and dry weights (9.33 and 9.60 g/plant) of the plants were recorded with conrol in both seasons, respectively (Table 3).

Table 3: The mean values of plant fresh weight (g/plant), plant dry weight (g/plant), root fresh weight (g/plant) and root dry weight (g/plant) as affected by CCC treatments.

CCC Conc.	Plant fresh weight(g/plant)		Plant dry weight(g/plant)		Root fresh weight(g/plant)		Root dry weight(g/plant)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	44.06 e	44.95 f	9.33 c	9.60 c	8.10 c	8.42 c	3.02 c	3.21 c
50mg/l	66.40 d	68.41 e	11.94 a	12.31 a	8.52 c	8.86 c	3.06 c	3.17 c
100mg/l	85.40 a	88.16 a	14.51 a	14.96 a	11.69 b	12.14 b	3.71 bc	3.89 bc
500mg/l	80.58 b	81.86 c	14.09 a	14.91 a	11.80 b	12.28 b	4.16 ab	4.36 ab
1000mg/l	80.42 b	84.00 b	14.19 a	14.84 a	13.93 a	14.48 a	4.52 a	4.75 a
2000mg/l	75.20 c	78.01 d	13.79 b	14.32 b	13.33 a	13.86 a	4.23 ab	4.44 ab

Means in columns followed by the same letter are not statistically different at the 0.05 probability level. 1st and 2nd means first and second seasons.

Root fresh and dry weight

Data presented in table 3 shows that on both seasons root fresh and dry weights were increased significantly as a result of increasing CCC concentration and the maximum root fresh weight (13.93

and 14.48 g/plant) and dry weight (4.52 and 4.75 g/plant) of plants were found by spraying plants with 1000 mg/l CCC. On the contrary, the control treatment had the lightest root fresh weight (8.10 and 8.42 g/plant) and minimum root dry weight (3.02 and 3.21 g/plant). Insignificant differences were detected between the application of 1000 and 2000 mg/l for root fresh weight and between 50, 1000 and 2000 mg/l for root dry weight CCC and also between 0 and 50 mg/l CCC for root fresh and dry weight in the first and second seasons, respectively.

Days to first visible flowering bud

The treatment with CCC of French marigold caused significant differences between all treatments in number of days to first visible flowering bud in both seasons, respectively. The fasted visible flowering bud was found in the treatment of 50 mg/l CCC (45.8 and 44.0 day for both seasons, respectively). From the other hand, spraying 2000 mg/l CCC had the maximum period to produce the first visible flowering bud (85.6 and 83.6 day) in both seasons, respectively.

Days to first inflorescence opening

The effects of CCC on the number of days to the opening of first inflorescence are shown in table 4; it revealed generally that all treatments effected significantly on the number of days to the opening of the first inflorescence per plant (Table 4). Moreover, the earliest opening (69.8 and 66.8 day) was appeared by spraying 50 mg/l CCC in the first and second seasons, respectively compared with the other concentrations of CCC and control treatments. On the contrary, 2000 mg/l CCC achieved the maximum number of days to the opening of the first inflorescence per plant (118.6 and 114.6 day) compared with its other concentration treatments in both seasons, respectively.

Numbers of inflorescence per plant

All plant retardant treatments affected significantly on the number of inflorescences per plant. It was increased by increasing CCC concentrations and the application of 100mg/l CCC gave the highest number of inflorescences per plant (16.1 and 16.9) in both seasons, respectively. However, this situation was reversed markedly as a result of using the highest concentrations of CCC in both seasons, respectively. The control treatment recorded the lowest numbers of inflorescence (3.6 and 3.8) compared with all CCC treatments in the first and second seasons, respectively (Table 4).

Table (4): The mean of days to first visible flowering bud (day), days to first inflorescence opening (day), numbers of inflorescence per plant, inflorescence fresh weight (g/ inflorescence), inflorescence diameter (mm), length of inflorescence pedicel (cm) and vase life (day) for as affected by CCC treatments.

CCC Conc.	Days to first visible flowering bud (day)		Days to first inflorescence opening (day)		Numbers of inflorescence per plant		Inflorescence fresh weight (g)		Inflorescence diameter (mm)		length of inflorescence pedicel (cm)		Vase life (day)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	57.2 d	55.2 d	88.2 d	84.2 d	3.6 e	3.8 e	3.0 e	3.1 d	2.0 e	2.1 d	6.4 a	6.7 a	6.8 d	7.8 e
50mg/l	45.8 f	44.0 f	69.8 f	66.8 f	13.0 b	12.8 b	3.3 d	3.6 c	3.2 d	3.3 c	6.8 a	7.2 a	9.4 c	10.8d
100mg/l	49.0 e	47.0 e	74.0 e	71.0 e	16.1 a	16.9 a	4.4 a	4.5 a	4.0 a	4.1 a	6.1 a	6.5 a	10.2 bc	11.6 cd
500mg/l	60.2 c	58.2 c	97.0 c	93.0 c	10.9 c	11.6 c	4.1 b	4.2 b	3.9 ab	3.9 ab	4.2 b	4.6 b	11.0 ab	12.6 bc
1000mg/l	79.6 b	77.6 b	109.6 b	105.6 b	9.9 c	10.8 c	3.8 b	4.0 b	3.7 bc	3.7 b	3.1 bc	3.9 bc	11.8 a	13.8 a
2000mg/l	85.6 a	83.6 a	118.6 a	114.6 a	8.4 d	8.8 d	3.5 c	3.7 c	3.6 c	3.7 b	2.5 c	2.8 c	11.2 ab	13.2 ab

Means in columns followed by the same letter are not statistically different at the 0.05 probability level. 1st and 2nd means first and second seasons, respectively.

Inflorescence fresh weight

The obtained results (Table 4) have revealed that, inflorescence fresh weight increased significantly with the application of chlormequat chloride in this experiment. So, the control treatment had the lightest inflorescences (3.0 and 3.1g), while spraying with 100 mg/l gave the heaviest inflorescences (4.4 and 4.5 g) in both seasons, respectively.

Inflorescence diameter

Inflorescence diameter was significantly affected with treatments (Table 4).With increasing the concentration of CCC from 100 to 2000 mg/l, inflorescence diameter decreased in both seasons. The largest inflorescence diameter (4.0 and 4.1 cm) was recorded with the application of 100 mg/l CCC. On the contrary, the smallest inflorescence (2.0 and 2.1 cm) were observed with the control treatment in the first and second seasons compared with other treatments

Length of inflorescence pedicel

It is clear from the results that the studied treatments significantly affected the length of inflorescence pedicel of plants (Table 4). Moreover, the longest inflorescence pedicel (6.8 and 7.2 cm) was noticed with the application of 50 mg/l CCC and the shortest one (2.5 and 2.8cm) was observed with the application of 2000 mg/l CCC in the first and second seasons, respectively compared with the other treatments and control.

Vase life

Generally, all chlormequat chloride treatments significantly improved *Tagetes* vase life compared to control treatment in two studied seasons, respectively. Therefore, the longest vase life of *Tagetes* inflorescences (11.8 and 13.2 day) was observed with the application of 1000 mg/l CCC while, the shortest vase life for the inflorescences in this study was found in control in the first and second seasons, respectively compared with the other treatments (Table 4).

Chlorophyll A and B and total chlorophyll

All the concentrations of chlormequat chloride used in this study significantly had the ability to raise chlorophyll A and B and total chlorophyll content in the leaves of *Tagetes* plants compared to the control plants in both seasons, respectively. Moreover, the application of 1000 mg/l CCC recorded the maximum values of chlorophyll A (0.754 and 0.792 mg/g) and total chlorophyll (33.3 and 35.6 Spad unit). While, the highest values of chlorophyll B (0.268 and 0.288 mg/g) were observed with the application of 2000 mg/l CCC. Insignificant differences were detected between 500, 1000 and 2000mg/l CCC in the chlorophyll A, B and total chlorophyll content in both studied seasons, respectively. From the other hand, the minimum values of chlorophyll A (0.452 and 0.476 mg/g) and B (0.204 and 0.218 mg/g) and total chlorophyll (23.0 and 24.5 Spad unit) were found with control treatment in the first and second seasons, respectively (Table 5).

Carotenoids

Table (5) shows that all chlormequat chloride treatments effected significantly on leaves content of carotenoids in both seasons, respectively. The values of carotenoids ranged from 301.1 to 334.1 mg/g in the first season and from 304.1 to 337.5 mg/g in the second one, respectively for the control and 1000 mg/l CCC treatments, respectively.

Total carbohydrate content

The percentage of total carbohydrate in dry plant leaves showed that treatments with different concentrations of CCC had different significant effects on treated plants. Spraying 50, 100, 500 and

1000 and 2000 mg/l CCC resulted in an increment in the percentage of total carbohydrate content in both seasons, respectively, compared with the control. Moreover, the treatment of 1000 mg/l had the maximum carbohydrate content (29.2 and 29.5 %) compared with the other studied treatments and control in the first and second seasons, respectively. Insignificant differences were detected between 1000 and 2000 mg/l CCC in the first season and between 500, 1000 and 2000 mg/l CCC in the second one, respectively (Table 5).

Table 5: The mean values of chlorophyll A (mg/g), chlorophyll B (mg/g), total chlorophyll (spad unit) and carotenoids (mg/g) and carbohydrates (%) as affected by CCC treatments.

CCC Conc.	Chlorophyll A (mg/g)		Chlorophyll B (mg/g)		Total chlorophyll (Spad unit)		Carotenoids (mg/g)		Carbohydrates (%)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	0.452 c	0.476 c	0.204 b	0.218 c	23.0 c	24.5 c	301.1 e	304.1 e	22.0 e	22.7 d
50mg/l	0.542f b	0.572 b	0.222 b	0.238 bc	26.5 b	28.3 b	306.9 d	310.0 d	25.1 d	25.9 c
100mg/l	0.570 b	0.596 b	0.226 b	0.244 b	27.2 b	29.0 b	311.8 c	314.9c	26.3 cd	27.1 bc
500mg/l	0.680 a	0.716 a	0.252 a	0.272 a	31.0 a	33.2 a	323.6 b	326.8 b	27.4 bc	28.3 ab
1000mg/l	0.754 a	0.792a	0.254 a	0.274 a	33.3 a	35.6 a	334.1a	337.5 a	29.2 a	29.5 a
2000mg/l	0.694 a	0.730 a	0.268 a	0.288 a	31.5 a	33.6 a	324.2 b	327.4 b	28.1 ab	28.8 a

Means in columns followed by the same letter are not statistically different at the 0.05 probability level**.** 1st and 2nd means first and second seasons, respectively.

Nitrogen, phosphorus and potassium content

Comparison of the chlormequat chloride treatments and control treatment indicates that, CCC induced a significant increase in the percentages of macro-element in both seasons, respectively (Table 6). This stimulation of increase reached the maximum level with the application of 2000 mg/l CCC which gave the highest content of nitrogen (3.508 and 3.608 %). However, the maximum content of phosphorus (0.430 and 0.454 %) and potassium (2.534 and 2.604 %) were found with the application of 1000 mg/l CCC compared with all other treatments including the control. On the contrary, the control treatment recorded the lowest content of nitrogen (2.186 and 2.246 %), phosphorus (0.268 and 0.270 %) and potassium (1.698 and 1.748 %) compared with all treatments in the first and second seasons, respectively.

Table 6: The mean value of Nitrogen content (%), phosphorus content (%) and potassium content (%)as affected by CCC treatments.

CCC Conc.	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	1st	2nd	1st	2nd	1st	2nd
Control	2.186 d	2.246 d	0.268 f	0.270 f	1.698 f	1.748 f
50mg/l	2.898 c	2.982 c	0.322 e	0.332 e	1.894 e	1.944e
100mg/l	3.304 b	3.398 b	0.340 d	0.352 d	2.294 d	2.354d
500mg/l	3.438 ab	3.536 ab	0.370 c	0.402 b	2.378 c	2.432c
1000mg/l	3.488 a	3.588 a	0.430 a	0.454 a	2.534 a	2.604 a
2000mg/l	3.508 a	3.608 a	0.390 b	0.380 c	2.492 b	2.562 b

Means in columns followed by the same letter are not statistically different at the 0.05 probability level. 1st and 2nd means first and second seasons, respectively.

Essential oil percentage and essential oil content

The data tabulated in table 7 shows that there were significant differences between treatments in the essential oil percentage and content as a result of the application of CCC compared to the control treatment in both seasons, respectively. While the control treatment recorded the minimum essential oil percent (0.0342 and 0.0366 %) and essential oil content (0.0032 and 0.0035 ml/plant), the treatment of 1000 mg/l CCC achieved the maximum essential oil percent (0.0858 and 0.0822 %). While, the maximum essential oil content (0.0107 and 0.0108 ml/plant) was measured with spraying

200 mg/l CCC in the first and second seasons, respectively. There weren't significant differences between 500, 1000 and 2000 mg/l CCC treatments in the content of essential oil per plant in the first and second seasons respectively.

Table 7: The mean value of essential oil %, essential oil (ml/plant), polyphenols, flavonoids and antioxidant activity (IC_{50}) as affected by CCC treatments.

CCC Conc.	Essential oil %		Essential oil (ml/plant)		Polyphenols Mg Gallic/1 g herb		Flavonoids mg Rutin/1 g herb		Antioxidant activity IC_{50}[b]	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	0.034 f	0.0366 e	0.0032 d	0.0035 d	0.26 e	0.28 f	0.17 c	0.15 f	5.45 a	5.32 a
50mg/l	0.0438 e	0.0414 e	0.0062 c	0.0062 c	1.47 de	1.54 e	0.91 c	0.87 e	2.45 b	2.33 b
100mg/l	0.0528 d	0.0510 d	0.0074 b	0.0076 b	3.27 cd	2.89 d	2.87 b	2.64 d	1.87 c	1.67 c
500mg/l	0.0702 c	0.0684 c	0.0102 a	0.0101 a	4.46 bc	4.21 c	3.48 b	3.22 c	1.65 d	1.55 cd
1000mg/l	0.0858 a	0.0822 a	0.0103 a	0.0102 a	6.56 a	6.46 a	4.89 a	4.88 a	1.28 e	1.24 e
2000mg/l	0.0780 b	0.0756 b	0.0107 a	0.0108 a	5.32 ab	5.12 b	3.76 b	3.87 b	1.42 e	1.44 de

Means in columns followed by the same letter are not statistically different at the 0.05 probability level, [b]Concentration (µg/ml) for 50% inhibition. 1st and 2nd means first and second seasons, respectively.

Polyphenols, flavonoids content and antioxidant activity

Data presented in table 7 show that, spraying marigold plants with different concentrations of chlormequat chloride (CCC) (0, 50, 100, 500, 1000 and 2000 mg/l) solutions made statistically significant differences in plant polyphenols, flavonoids content and antioxidant activity. It was noticed from the results that, the trend of the *Tagetes* herb content of polyphenols, flavonoids content increased with increasing CCC concentration. Moreover, the concentration of 1000 mg/l CCC made a pronounced increase in content of polyphenols (6.56 and 6.46 mg Gallic/1 g), flavonoids (4.89 and 4.88 mg Rutin/1 g) and as a result of that, the application of this treatment recorded the lowest concentration (1.28 and 1.24 µg/ml) of marigold herb extract needed for the inhibition of 50% DPPH.

Essential oil constituents

The effect of foliar spraying with CCC on chemical composition of the essential oil of *Tagetes patula* plants are given in Table 8. Sixty two components were identified in the essential oil of *Tagetes patula*. at different treatments that represented 98.15–99.98 % of the oils. The major components were Piperitone (4.85–53.32%), Caryophyllene (8.63–21.47%), α- terpinolene(5.73–15.04%), D-Limonene (4.98–11.10%) and (-)-Caryophyllene oxide (3.12–22.3%). Other components were present in amounts less than 2 % in most treatments**.**

Table 8: Chemical analysis of *Tagetes patula*Lessential oil as affected by CCC treatments.

	Name	RT	KI	Control	CCC 50 mg/l	CCC 100 mg/l	CCC 500 mg/l	CCC 1000 mg/l	CCC 2000 mg/l	Molecular Formula
1	α-Pinene	4.71	906.6	0.17	0.17	0.07	0.28	0.41	0.17	$C_{10}H_{16}$
2	Sabinene	5.73	943.0	0.30	0.34	0.19	0.40	0.65	0.36	$C_{10}H_{16}$
3	Linalyl acetate	6.17	959.2		0.34	0.16	0.30	0.53		$C_{12}H_{20}O_2$
4	D-Limonene	7.46	1004.0	4.98	10.35	5.99	7.72	11.10	5.50	$C_{10}H_{16}$
5	Trans-Ocimene	7.65	1008.9		0.59	0.14	0.15	0.35		$C_{10}H_{16}$
6	cis-Ocimene	8.04	1019.2	1.03	2.15	1.91	1.77	4.01	0.69	$C_{10}H_{16}$
7	δ 3-carene	8.47	1030.3	0.19	0.17	0.16	0.23	0.28	0.16	$C_{10}H_{16}$
8	α-terpinolene	9.41	1055.7	6.68	15.04	12.75	9.38	14.15	5.73	$C_{10}H_{16}$
9	p-Cymenene	9.88	1067.9	0.26	0.15	0.46	0.25	0.59		$C_{10}H_{12}$
10	Linalool	10.09	1073.7	0.63	0.19	0.37	0.34	0.46	1.11	$C_{10}H_{18}O$
11	(-)-Carvyl Acetate	10.64	1088.2	0.26	0.20	0.45	0.30	0.67	0.25	$C_{12}H_{18}O_2$
12	Perilla alcohol	11.09	1100.0			0.07			0.30	$C_{10}H_{16}O$
13	1,3,8-p-Menthatriene	11.45	1108.4	0.36	0.19	0.52	0.37	0.61	0.23	$C_{10}H_{14}$
14	cis-p-Mentha-2,8-dien-1-ol	11.74	1115.1	0.28	0.26	0.34		0.34	1.65	$C_{10}H_{16}O$
15	Camphor	12.08	1122.8			0.06			0.16	$C_{10}H_{16}O$
16	Borneol	13.08	1145.7			0.06			0.22	$C_{10}H_{18}O$
17	Terpinene-4-ol	13.38	1152.6	0.51	0.15	0.28	0.33	0.36	0.82	$C_{10}H_{18}O$
18	p-Cymen-8-ol	13.90	1164.6	0.28		0.29		0.25	0.37	$C_{10}H_{14}O$
19	trans-p-Mentha-2,8-dienol	14.17	1170.8			0.19		0.10	0.62	$C_{10}H_{16}O$
20	Acetic acid, octyl ester	14.63	1183.1			0.12		0.17	4.40	$C_{10}H_{20}O_2$
21	Thymyl Methyl Ether	15.36	1198.1	0.15	0.16	0.14		0.13	0.20	$C_{11}H_{16}O$
22	Piperitone Oxide	16.40	1221.1		0.09	0.12		0.21	0.52	$C_{10}H_{16}O_2$
23	Piperitone	16.58	1225.1	35.02	4.85	19.50	17.36	21.34	53.32	$C_{10}H_{16}O$
24	.l-Bornyl acetate	17.58	1247.1	0.73					1.02	$C_{12}H_{20}O_2$
25	dihydroedulan II	17.64	1248.2	0.88	1.59	0.77	1.01	0.64		$C_{13}H_{22}O$
26	Ocimenyl acetate	17.72	1250.3	1.70	0.63	0.14	0.35	0.16	0.82	$C_{12}H_{18}O_2$
27	dihydroedulan I	17.88	1253.9	0.40	0.30	0.25	0.30	0.19		$C_{13}H_{22}O$
28	α-Terpinyl propionate	19.53	1290.2	0.47	0.27	0.12	0.16		0.38	$C_{13}H_{22}O_2$
29	(+)-3-Carene, 10-(acetylmethyl)-	22.13	1348.2	0.29	0.32	0.44	0.37	0.34		$C_{13}H_{20}O$
30	α-Gurjunene	22.33	1352.7	0.40		0.42	0.45	0.31	0.27	$C_{15}H_{24}$
31	Caryophyllene	23.00	1367.6	21.47	21.07	14.58	16.35	11.15	8.63	$C_{15}H_{24}$
32	trans-Sesquisabinene hydrate	23.56	1380.1	0.22		0.24	0.21	0.16		$C_{15}H_{26}O$
33	(E)-β-Famesene	24.49	1401.0	6.25	3.45	5.06	4.19	4.07	1.21	$C_{15}H_{24}$
34	Tetradecane, 2,6,10-trimethyl-	25.52	1424.9	0.26	0.30	0.08			0.30	$C_{17}H_{36}$
35	(Z)-β-Farnesene	25.69	1428.7	0.91	0.23	1.12	0.91	0.77	0.48	$C_{15}H_{24}$
36	Farnesol	26.12	1438.8		0.26	0.21	0.19	0.18		$C_{15}H_{26}O$
37	α-Farnesene	26.70	1452.2	0.36		0.30		0.22		$C_{15}H_{24}$
38	7-epi-cis-sesquisabinene hydrate	27.38	1467.8	0.31	0.21	0.11				$C_{15}H_{26}O$
39	humuladienone	28.38	1491.1	1.18	1.49	1.27	1.08	0.76	0.77	$C_{15}H_{24}O$
40	Isoaromadendrene epoxide	28.63	1497.0	0.41	0.34	0.67	0.59	0.33	0.37	$C_{15}H_{24}O$
41	(±)-trans-Nerolidol	28.94	1504.2	1.16	1.32	1.48	0.96	0.84		$C_{15}H_{26}O$
42	Longipinocarvone	29.33	1513.7	0.11	0.29	0.14	0.15			$C_{15}H_{22}O$
43	(-)-Spathulenol	29.46	1517.0	0.19	1.42	0.86	0.90	1.08		$C_{15}H_{24}O$
44	(-)-Caryophyllene oxide	29.61	1520.6	5.23	22.30	4.71	4.12	3.12	3.25	$C_{15}H_{24}O$
45	trans-Z-α-Bisaboleneepoxide	29.72	1523.2	0.67		0.68	0.41	0.32		$C_{15}H_{24}O$
46	β-Guaiene	30.14	1533.5		0.24	0.37	0.44			$C_{15}H_{24}$
47	Humulene epoxide 2	30.70	1547.0		0.31	0.38	0.18			$C_{15}H_{24}O$
48	Calarenepoxide	31.89	1575.7	0.54	1.03	0.89	1.03	0.67		$C_{15}H_{24}O$
49	cis-Z-α-Bisabolene epoxide	32.51	1590.7		0.36	0.38				$C_{15}H_{24}O$
50	Alloaromadendrene oxide-(2)	32.62	1593.3			0.40	0.47	0.29		$C_{15}H_{24}O$
51	Aromadendrene oxide-(1)	33.09	1605.0		1.01					$C_{15}H_{24}O$
52	Calarene epoxide	33.49	1615.1	0.23		0.09	0.19		0.28	$C_{15}H_{24}O$
53	2,6,10-Trimethylundecan-(5E)-2,5,9-trien-4-one	35.87	1675.7			0.34				$C_{14}H_{22}O$
54	Neophytadiene	38.67	1749.0	3.32	1.97	4.30	4.89	2.95	0.30	$C_{20}H_{38}$
55	Hexahydrofarnesyl acetone	39.00	1758.0		1.77	0.61	0.48	0.31		$C_{18}H_{36}O$
56	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, cis-	39.86	1780.7		0.85	0.46	0.31	0.21		$C_{12}H_{20}$
57	Cembrene	41.39	1822.5			0.18	0.28	0.22		C20H32
58	β-elemene	42.42	1851.4			0.47	0.74	0.61	0.65	$C_{15}H_{24}$
59	Verticellol	44.31	1904.5	0.22		0.86	1.62	1.27	1.21	$C_{20}H_{34}O$
60	Thunbergol	48.21	2026.8			0.38	0.74	0.39		$C_{20}H_{34}O$
61	Squalene-2,3-Epoxide	48.62	2043.9			3.13	5.27	3.38	0.39	$C_{30}H_{50}O$
62	Nerolidol-Epoxyacetate	48.95	2057.9	0.66		6.92	11.08	7.39	2.87	$C_{17}H_{28}O_4$
	Total			99.67	98.72	98.15	99.6	99.04	99.98	

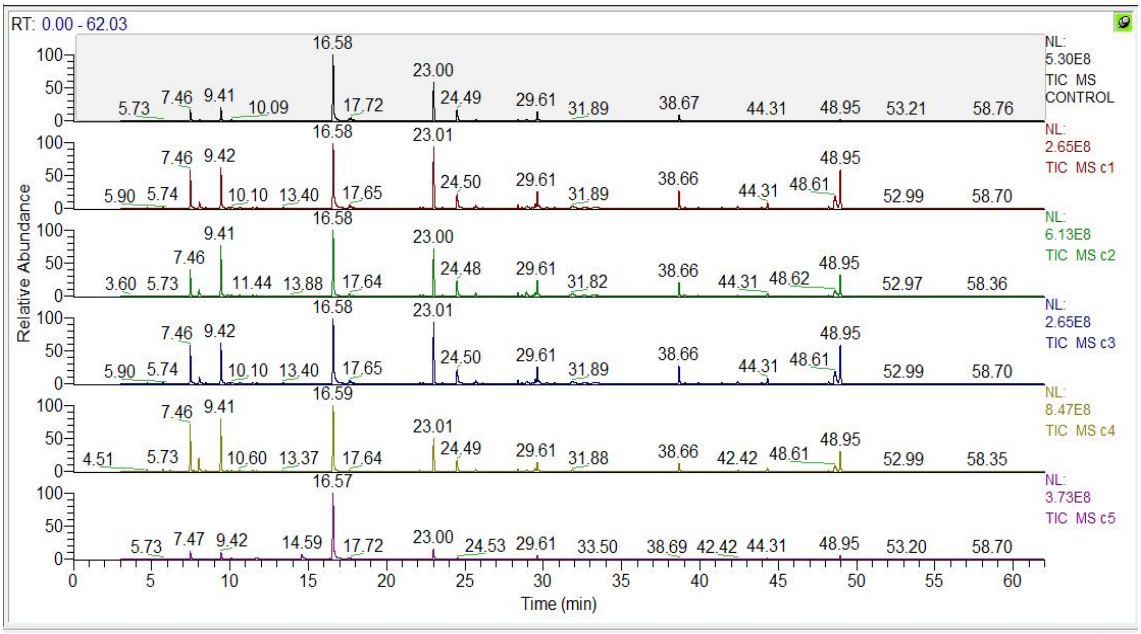

Fig. 1: The GC-MS profile of chemical analysis of *Tagetes patula* L. essential oil as affected with different concentrations of CCC.

Discussion

The response of plants to any plant growth regulator differ according to different species or varieties, concentration of growth regulators, number of application... ext. In this study six concentrations of chlormequat chloride (0, 50, 100, 500, 1000 and 2000 mg/l) on *Tagetes patula* L. were tested on *Tagetes patula* L. In this respect, 50 mg/l CCC hurried flowering compared with other treatments and control because it increased rooting compared to control, so it increased the elements reaching the plant and from the other side, gave light retardant effect compared to other high concentrations of CCC. It appears in the largest leaf area and tallest pedicel of inflorescence of it compared to the other CCC concentrations. 100 mg/l CCC was more effective in increasing plant height, branch length, plant fresh and dry weight. It is besides, achieving the maximum number, heaviest fresh weight and biggest diameter of inflorescences. These are due to the moderate effect of 100 mg/l CCC on increasing branching and hurrying flowering comparing with high concentrations of CCC. So, the possibility of harvesting inflorescences from this treatment early was available. Cutting one inflorescence from the branch (apical inflorescence) encourages secondary branching by deleting apical dominance. For that, despite the maximum number of primary branches with the high concentration of CCC, the concentration of 100 mg/l CCC had the heaviest fresh and dry weight of French marigold plants because of enhancing the formation of secondary branches by early inflorescences' cutting. In addition, the late flowering of high concentrations of CCC treatment despite the high number of primary branches, these high concentrations gave smaller number of inflorescence compared with medium concentrations. This is due to the end of growth season of French marigold as annual plant. 1000 mg/l CCC recorded the maximum number of primary branches, longest vase life of French marigold inflorescence and the highest essential oil percentage, polyphenols, flavonoids content and antioxidant activity compared with the control and the other treatments. It is due to the ability of this concentration to achieve the maximum fresh and dry weight of roots. Meanwhile, increasing the uptake of macro-elements, increase chlorophyll content and finally gave the maximum carbohydrate content of these treated plants. The high concentrations of CCC dwarfed the treated plants. So, the plants chemical contents had concentrated in the tissues of treated plants. As above mentioned, CCC is growth retardants, it is able to modify the endogenous levels of phytohormones i.e. auxin, gibberellins, ABA and cytokinins level. So the application of CCC

according to Youssef and Abd El-Aal (2013) increased the endogenous levels of cytokinins and decreased gibberellins and auxins level. Cytokinins retard chlorophylls degradation, preserve it and increase its synthesis (Devlin and Witham, 1983). Also, the increase in chlorophyll content due to growth retardant treatments might be attributed to the character of some growth retardants on depressing leaf area which lead to intensification of pigments in leaves. Cytokinins stimulate lateral shoots and roots initiation. Thus, increasing the fresh and dry weights of different plant parts (Devlin and Witham, 1983). Such results showed similar trends to those obtained by many investigators working on CCC on other plants. In this concern, Abou Zied and Sherbeany (1971) on *Matricaria chamomilla,* Saker (2004) on *Hibiscus rosasinensis and Tabernae montanacoronaria*, Youssef (2004) on *Strelitzia reginae*, Abd El-Kader (2009) on *Cestrum elegans* and *Tecomastans*, Sibel *et al*. (2009) on *Consolida orientalis,* Gosh *et al*. (2010) on *Jatropha curcas,* Ribeiro *et al*. (2011) on sunflower, Jungklang and Saengnil (2012) on patumma cv. Chiang Mai Pink. gibberellins are known as a stimulating and hormone for longitudinal growth in different plants (Devlin and Witham, 1983). Hence, reduction of endogenous gibberellins level due to the use of growth retardants treatments (CCC) with high concentration 1000 and 2000 mg/l led to reduction in the length of different cell types and consequently reduction in the plant height and leaf area. These treatments gave shortest plants and smallest leaves compared with the control plants. These results are in harmony with Youssef and Abd El-Aal (2013) who found that the *Tabernaemontana coronaria* plants received the different 1000, 1500 and 2000 mg/l CCC treatments were shorter than the untreated control plants. Joshi and Reddy (2006) reported a reduction in plant height with increase in concentration (500 to 2000 mg/l) of cycocel and alar application over control in China aster.

The essential oil percents in this experiment were less than obtained by Negi *et al*. (2013) (0.18%) it may be due to they determined this percent in the inflorescences only while we determined them in the total plant (inflorescences, leaves, stem and roots). Another reason may be due to planting in pots where the space available to roots is limited.

There are four main components for the essential oil of *Tagetes patula* L. were found in the GC/MS analysis in this investigation. These results are in agreement with Hethelyi *et al.,* (1998) who noticed that, the oils of *Tagetes patula* were found to contain limonene, α-terpinolene, piperitone and caryophyllene as major components. The variability in essential oil content and constituent due to CCC application may be due to the role of CCC as one of the plant growth regulators which defined as one of the main factors influence plants growth and their primary and secondary metabolites pool. This is in harmony with Abou Zied and Sherbeany (1971) and Eid and Rofaeel (1980).

Conclusion

The application of 100 mg/l CCC is recommended to obtain the maximum yield of *Tagetes patula* herb and flowers in the earliest date. The application of 1000 mg/l CCC is the best to have the maximum essential oil percentage, polyphenols, flavonoids content and antioxidant activity beside the longest vase life of *Tagetes patula* inflorescences. The application of 2000 mg/l CCC is beneficial to have the most dwarfed *Tagetes patula* plants with the maximum essential oil content per plant.

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