Influence of Foliar Application with Plant Aqueous Extracts on Growth, Yield and Chemical Constituents of Chamomile

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Authors' contributions

This work was carried out in collaboration between all authors. YMRA and HAI designed the study, wrote the protocol, cultured the experiment, performed the chemical and statistical analyses and prepared the manuscript. All authors read and approved the final manuscript.

Abstract

The main target of sustainable agriculture including organic farming is to use natural compounds such as plant aqueous extracts to elevate plant growth and productivity. The subject of the present study is to determine the plant growth and inflorescences production, some biochemical constituents of shoot and inflorescences and antioxidative activities of essential oil obtained from chamomile plants exogenously sprayed with aqueous extracts of dried roselle calyces, turmeric rhizomes, safflower flowers and red beet roots. A pot experiment was conducted during the two successive seasons of 2016/2017 and 2017/2018 in the open field of Experimental Farm of Agricultural Botany Department, Faculty of Agriculture, Ain Shams university, Qalyubia, Egypt. Transplants of chamomile, 45 days old, were separately sprayed after 15 days from transplanting by the four different aqueous extracts and distilled water was used as a control. Generally, spraying with tested plant aqueous extracts on chamomile plants caused high efficiency in growth promotion, inflorescences and essential oil production. Red beet and safflower extracts gave the highest number of branches and inflorescences per plant. Chlorophyll a, b, carotenoids, reducing sugars and amino acids were increased in chamomile shoots when red beet and safflower extracts were sprayed while flavonoids and phenolic compounds were significantly decreased in comparing with roselle and turmeric extracts treatments. Different concentrations of essential oil and inflorescences ethanolic extracts obtained from chamomile plants treated with safflower and red beet extracts showed the highest scavenging activities on DPPH radical and lowest IC_{50} values. Finally, it could be concluded that application of plant aqueous extracts considered as alternative method to chemical compounds which achieved sustainability of organic farming.

Key words: *Chamomile (Matricaria chamomilla. L), Roselle extract, Turmeric extract, Safflower extract, Red beet extract, Essential oil, DPPH radical, IC_{50}*

Introduction

Recently, public health and environmental safety encourage the use of plant extracts for improving growth, chemical composition and productivity of plants especially medicinal plants. Chamomile is one the important medicinal and aromatic plant belong to *Asteraceae* family, has a sweet, grassy and lovely fruity aroma. It has many medicinal uses due to its calming, carminative and spasmolytic properties, antimicrobial and anti-inflammatory effects **[1]**. The main bioactive constituents of chamomile essential oil are α-bisabolol, bisabolol oxide A, bisabolol oxide B, bisabolone oxide , α-pinene, β-pinene, chamazulene, camphene, myrcene, sabinene, 1,8-cineole y-terpinene, caryophyllene, propyl angelate and butyl angelate. Also, flavone glucosides (apigenin 7-O-glucoside and various acylated derivatives of

apigenin 7-O-glucoside) and flavonols (luteolin glucosides, quercetin and isohamnetin glucosides) were identified in chamomile [2].

However, plant extracts have recently become more common applications in modern agricultural production, among these substances are roselle, turmeric, safflower and red beet aqueous extracts.

Roselle (*Hibiscus sabdariffa* L.) is a tropical shrub with red, dark red or green inflated edible calyces belongs to family *Malvaceae* [3,4]. The calyces have been found to be rich in vitamin C and other antioxidants such as flavonoids [5] and minerals [6].

Turmeric, *Curcuma longa* L. (*Zingiberaceae*) rhizome commonly used as a spice [7]. The higher content of turmeric from amino acids, potassium, vitamins, antioxidants and plant pigments as curcumin and volatile oils encourage to undertake many attempts for using its aqueous extract as a stimulator for plant growth **[8].**

Safflower (*Carthamus tinctorius* L.) is world's oldest crop belonging to family *Compositae* which contains water soluble yellow dye (carthamidin), it has been used traditionally as an annual oil seed crop, medicinal herb and natural dye source for coloring food and textile [9].

Red beet (*Beta vulgaris* L.) is an herbaceous biennial crop belonging to family *Chenopodiaceae*, deep red-colored beet bulbs are the most common consumed for human [10]. Beet roots are rich in valuable bioactive compounds such as ß-cyanines [11], glycine betaine [12], saponins [13], carotenoids [14], betanin, polyphenols and flavonoids [15]. It considered as one of the most potent vegetables contains antioxidants due to the presence of ß-cyanins which are a group of compounds exhibiting antioxidant and radical-scavenging activities [16].

The purpose of this study is to investigate the effect of roselle, turmeric, safflower and red beet aqueous extracts as foliar sprayers on growth characters and yield production of chamomile plants and evaluate the stimulation of these extracts on the essential oil yield and the antioxidant potential of treated chamomile plants.

Material and Methods

Plant materials:

Seeds of chamomile (*Matricaria chamomilla*. L) were kindly produced from the Aromatic and Medicinal Plant Research Institute, ARC, Ministry of Agriculture, Egypt.

Preparation of plant aqueous extracts

Calyces of roselle (*Hibiscus sabdariffa* L.), rhizomes of turmeric (*Curcuma longa* L.), flowers of safflower (*Carthamus tinctorius* L.) were produced from Aromatic and Medicinal Plant Research Institute and roots of red beet (*Beta vulgaris* L.) were produced from Horticulture Research Institute, ARC, Ministry of Agriculture, Egypt, then dried, grinded and macerated 2g powder in 100 ml of distilled water and soaked for 24h then filtered and used freshly.

Chemical analysis of plant aqueous extracts

The pH values, titratable acidity, soluble phenolic compounds, total flavonoids, anthocyanin, reducing sugars, free amino acids and N, P, K percentages were determined in the previous aqueous extracts.

Total titratable acidity (mg citric acid 100 mg^{-1} d.wt.) of plant aqueous extracts was determined according to **A.O.A.C. [17]**.

Total soluble phenols, flavonoids, reducing sugars and free amino acids were extracted from chamomile shoots according to **Ackerson [18]** using 80% ethanol.

Soluble phenolic compounds were estimated as (g 100 g^{-1} d.wt.) by the method of Folin-Ciocalteu as described by **Shahidi and Naczk [19]** using gallic acid as a standard.

Total flavonoids concentration was determined as (g 100 g^{-1} d.wt.) by the aluminum chloride colorimetric assay according to **Marinova *et al.* [20]** using quercetin as a standard.

Anthocyanins concentration was colorimetrically proceeded as g $100g^{-1}$ d.wt. according to **Du and Francis [21]**.

Reducing sugars were determined colorimeterically (g $100g^{-1}$ d.wt.) by using 3,5dinitrosalsylic acid according to **Miller [22]** using glucose as a standard.

Free amino acids were estimated colorimetrically (g $100g^{-1}$ d.wt.) by using ninhydrin according to **Jayeraman [23]** using glycine as a standard.

Plant aqueous extracts were digested by using H_2SO_4 and H_2O_2 according to the method described by **Piper [24]** to determine N, P and K percentages according to the method described by **Black *et al.* [25] and Wilde *et al.* [26].**

Experimental set up

Pot experiment was carried out under open field condition in the Farm of Agricultural Botany Department, Faculty of Agriculture, Ain Shams University, Shoubra El Kheima, Qalyubia, Egypt, (30° 06′ 42″ N 31° 14′ 46″ E) during the two successive winter seasons of 2016/2017 and 2017/2018. Chamomile seeds were sown on 15th September in nursery beds. On 1st November of both seasons, when the grown seedlings reached about 10-15 cm in length, ten transplants were transferred in plastic pots (30 x 40 cm) filled with clay loamy/sand (3:1 v/v) soil (Table 1). The plants were thinned out into three uniform transplants per each pot after 10 days from transplanting. Three replicates for each treatment, three pots/replicate, were arranged in a randomized complete block design. The pots were regularly irrigated with tap water when plants needed. Each seedling was sprayed with 30 ml of the four previous prepared fresh aqueous extracts (2% w/v) and tap water was used as control. The volume of extracts was consequently increased with increasing plant growth. The foliar applications were applied four times, the first one was carried out 15 days after transplanting and others were applied with 2 weeks intervals. Tween 20 at 0.1 % was used as a wetting agent. All agricultural practices were done as the recommendations of Ministry of Agriculture.

Table 1. Mechanical and chemical analyses of the experimental soil

%						Soluble anions (meq $^{-1}$)			Soluble cations (meq $^{-1}$)			
Clay	Silt	Sand	Soil texture	EC dS m^{-1}	pH	HCO_3^-	SO_4^{--}	Cl^-	K^+	Na^+	Ca^{++}	Mg^{++}
48	24	28	Clay loamy	0.935	7.11	3.32	3.24	1.67	0.89	2.13	4.50	1.67

Soil and Water Research Centre, ARC, Giza, Egypt.

Chamomile plant samples

Three vegetative plant replicates were randomly taken from each treatment at 45 days after transplanting, after the third spray treatment, for the chemical analyses, *i.e.* chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids, flavonoids, total soluble phenolic compounds, reducing sugars and amino acids.

Another two samples (at 45 and 120 days after transplanting) were randomly collected to measure plant height (cm), number of branches plant^{-1}, shoot fresh and dry weights plant^{-1} (g). Each sample was contained three plants from each treatment.

Harvesting of inflorescences started at the last week of January until the middle of April for the both seasons. The picking of the inflorescences was done continuously when the ray flowers were in mood. Three plants were used to determine the total number of inflorescences plant^{-1}, total inflorescences fresh and dry weights plant^{-1} (g).

Total soluble phenolic compounds, flavonoids and carotenoids were determined in chamomile inflorescences. The essential oil yield plant^{-1}, its chemical components and scavenging activity on DPPH radical in both oil and inflorescences ethanolic extract were estimated.

Chemical analyses of chamomile samples

Chlorophylls a & b (Chl a, Chl b) and carotenoids concentrations were extracted and assayed as the procedure of **Costache *et al.* [27]**. Chl a, b and carotenoids were expressed in shoots as mg g^{-1} f.wt. whereas carotenoids were estimated in inflorescences as mg 100g^{-1} d.wt.

Soluble phenolic compounds, total flavonoids, reducing sugars and free amino acids concentrations were determined as mentioned before.

Inflorescences essential oil was distilled using a micro distilling apparatus and oil volume was measured as ml of oil 100g^{-1} d.wt. inflorescences according to **Guenther [28]**.

Chemical components of the chamomile essential oil were determined by Gas liquid chromatography-mass spectrometer (GLC-MS). Chromatographic analysis of essential oil using GC-MS was performed (Agilent Technologies 7890 GC system combined with 5977, A Mass Selective Detector) in National Research Center, El Dokki, Egypt.

Ethanolic solutions of chamomile essential oil (2, 4, 6, 8 and 10 µg ml^{-1}) and inflorescences ethanolic extract (0.66, 1.33, 2.00, 2.66 and 3.33 mg ml^{-1}) were prepared. The percent of scavenging activity of the different concentrations of both extracted essential oil and inflorescences ethanolic extracts on DPPH radical were evaluated by measuring from the bleaching of the violet colored ethanolic solution of DPPH according to **Gulluce *et al.* [29]**. This spectrophotometric assay uses the stable radical 2, 2′- diphenyl-1-picryl hydrazyl (DPPH) as a reagent. Inhibition of free radical DPPH calculated according to the following equation:

Scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}]x\ 100$

The concentration of the chamomile essential oil (µg ml^{-1}) and the inflorescences ethanolic extract (mg ml^{-1}) that needed to inhibit 50 % of the initial DPPH concentration (IC_{50}) was calculated for each treatment.

Statistical analysis

All experiment data was analyzed by analysis of variance (ANOVA) using the General Linear Model procedure of CoStat. Significance between means was tested by "F" test and the value of LSD (p=0.05) was calculated **[30]**. Significant differences of means at $P \leq 0.05$ were compared by different letters as described by Duncan test of **Gomez and Gomez [31].** Results expressed as mean ± standard deviation (SD)**.**

Results

Chemical analysis of plant aqueous extracts

Data in **table (2)** showed significant differences ($P \leq 0.05$) in the physical-biochemical analyses of all plant aqueous extracts. All extracts had acidity pH values.

Roselle aqueous extract had the lowest pH value (1.62) followed by red beet extract (4.42), safflower extract (4.77) and turmeric extract which had the highest pH value (5.84). In contrast, titratable acidity % showed the opposite trend where roselle extract contained the highest TA% (15.66%) while turmeric extract had the lowest percent (0.96%).

Safflower aqueous extract contained the highest concentrations of total soluble phenols (2.55 %), reducing sugars (15.47 %) and P (0.80 %) in addition to its high concentration of amino acids, whereas, the highest concentration of free amino acids (1.17 %), flavonoids (8.46 %) and K (7.85 %) were recorded in red beet extract. Rosella extract was found to contain the highest concentration of N compared to other extracts (Table 2). It seemed also that turmeric extract is low in most of biochemical estimates that were appreciated. As for anthocyanin, roselle and red beet extracts contained (0.29 % and 0.09 %), respectively while turmeric and safflower extracts recorded anthocyanin free aqueous extracts.

Table 2. Some physical properties and biochemical constituents of roselle, turmeric, safflower and red beet aqueous extracts

Physical properties and biochemical analyses	Plant water extracts			
	Roselle extract	**Turmeric extract**	**Safflower extract**	**Red beet extract**
pH	**1.62**^{d}±0.01	**5.84**^{a}±0.01	**4.77**^{b}±0.01	**4.42**^{c}±0.01
Titratable acidity (mg citric acid 100 g^{-1} d.wt.)	**15.66**^{a}±0.57	**0.96**^{d}±0.005	2.55^{c}±0.005	**4.76**^{b}±0.05
Soluble phenols %	**1.77**^{b}±0.15	**0.14**^{d}±0.002	**2.55**^{a}±0.02	**0.32**^{c}±0.015
Flavonoids %	**2.44**^{b}±0.08	**0.29**^{d}±0.001	**1.76**^{c}±0.01	**8.46**^{a}±0.007
Anthocyanin mg 100g^{-1} d.wt.	**0.29**^{a}±0.001	**0.00**	**0.00**	**0.09**^{b}±0.001
Reducing sugars%	**6.92**^{c}±0.01	**1.18**^{d}±0.01	**15.47**^{a}±0.02	**12.78**^{b}±0.01
Free amino acids %	**0.14**^{c}±0.006	**0.15**^{c}±0.002	**1.09**^{b}±0.02	**1.17**^{a}±0.04
N%	**40.92**^{a}±1.57	**11.52**^{c}±0.37	**20.55**^{b}±0.79	**21.39**^{b}±0.95
P%	**0.59**^{b}±0.07	**0.37**^{d}±0.001	**0.80**^{a}±0.001	**0.48**^{c}±0.001
K%	**7.57**^{b}±0.01	**5.65**^{d}±0.01	**6.18**^{c}±0.01	**7.85**^{a}±0.01

Data were presented as mean ± SD (n=3). Means in the same column with different letters are significantly different at P≤0.05.

Effects of foliar application with plant aqueous extracts on vegetative growth parameters and inflorescences yield of chamomile plants.

Significant differences in growth parameters of chamomile shoot was detected by foliar applications of plant aqueous extracts at 45 and 120 days after transplanting during the two seasons 2016/2017 and 2017/2018 (Tables 3 and 4).

Obtained results in Table (3) revealed that plant growth remarkably response to the different exogenous spray extracts compared to control. Chamomile plants sprayed by roselle or turmeric extracts increased plant height more than plants safflower and red beet extracts, on the other side, safflower or red beet extracts enhanced the number of branches/plant and shoot f.wt. and d.wt. at 45 days after transplanting (DAT) in the two seasons.

Table 3. Growth parameters "at 45 DAT" of chamomile plants sprayed with four different aqueous extracts during 2016/2017 and 2017/2018 successive seasons.

	Foliar applications

Seasons	Growth parameters	Control (distilled water)	Roselle extract	Turmeric extract	Safflower extract	Red beet extract
2016/2017	Plant height (cm)	21.17^{c}±2.31	42.23^{b}±3.46	56.77^{a}±0.64	35.67^{c}±3.05	27.30^{d}±2.99
	No. of branches	27.33^{d}±2.08	70.33^{c}±8.08	153.33^{b}±6.81	177.00^{a}±6.24	160.67^{b}±6.66
	Shoot f.wt.	12.73^{c}±1.48	36.80^{d}±4.29	40.57^{c}±1.17	68.73^{a}±3.69	55.17^{b}±2.77
	Shoot d.wt.	2.38^{d}±0.30	6.84^{c}±0.65	7.61^{c}±0.37	12.93^{a}±1.18	10.38^{b}±0.74
2017/2018	Plant height (cm)	21.63^{d}±3.45	41.85^{b}±1.13	53.80^{a}±3.93	37.90^{b}±1.44	26.77^{c}±1.75
	No. of branches	26.67^{d}±1.15	68.67^{c}±6.66	156.67^{b}±7.09	170.33^{a}±7.09	159.67^{b}±4.04
	Shoot f.wt.	13.38^{c}±0.32	36.57^{d}±4.30	42.60^{c}±2.08	67.52^{a}±5.93	54.65^{b}±3.75
	Shoot d.wt.	2.45^{c}±0.12	6.90^{d}±0.73	7.94^{c}±0.32	12.78^{a}±0.94	10.26^{b}±0.69

Data were presented as mean ± SD (n=3). Means in the same column with different letters are significantly different at P≤0.05.

Moreover, foliar spray with turmeric extract influenced the highest chamomile shoot height (70.50 and 73.50 cm) followed by roselle water extract (66.60 and 66.50 cm), at120 DAT of the two seasons, respectively. Both safflower and red beet extracts increased number of branches and shoot f.wt. and d.wt. comparing to control plants. Number of branches/plant increased about 3-4 folds more than the control plants with all different aqueous extracts at both seasons of experiment **(Table 4)**.

Table 4. Growth parameters "at 120 DAT" of chamomile plants sprayed with four different aqueous extracts during 2016/2017 and 2017/2018 successive seasons.

		Foliar applications				
Seasons	Growth parameters	Control	roselle extract	turmeric extract	safflower extract	red beet extract
2016/2017	Plant height (cm)	53.67^{c}±2.31	66.60^{ab}±1.68	70.50^{a}±5.0	60.50±2.18	59.17^{bc}±8.61
	No. of branches	53.67^{e}±2.52	141.00^{d}±2.65	157.33^{c}±6.66	236.00^{a}±7.81	166.00^{b}±7.0
	Shoot f.wt.	79.75^{e}±3.33	121.37^{d}±4.87	131.47^{c}±3.62	160.87^{a}±6.82	143.00^{b}±5.24
	Shoot d.wt.	18.61^{c}±1.01	27.70^{b}±2.26	29.65^{b}±1.71	34.73^{a}±0.48	32.67^{a}±0.57
2017/2018	Plant height (cm)	52.47^{c}±0.75	66.50^{b}±2.75	73.50^{a}±1.25	61.80^{c}±2.29	56.97^{d}±2.30
	No. of branches	53.00^{e}±2.0	141.67^{d}±9.5	159.67^{c}±1.53	219.00^{a}±6.0	177.00^{b}±3.46
	Shoot f.wt.	78.18^{d}±5.47	118.23^{c}±2.14	133.57^{b}±9.41	158.80^{a}±7.55	142.43^{b}±0.61
	Shoot d.wt.	17.89^{c}±1.02	27.19^{b}±1.01	28.33^{b}±2.22	35.88^{a}±1.83	34.35^{a}±1.14

Data were presented as mean ± SD (n=3). Means in the same column with different letters are significantly different at P≤0.05.

In the same trend foliar applied roselle, turmeric, red beet and safflower extracts gradually increased total no. of inflorescences, inflorescences fresh and dry weights per plant, these increases reached the significant level when compared to control **(Table 5)**.

Table 5. Inflorescences yield "at 150 DAT" of chamomile per plant sprayed with four different aqueous extracts during 2016/2017 and 2017/2018 successive seasons.

		Foliar applications				
Seasons	Yield	Control	Roselle extract	Turmeric extract	Safflower extract	Red beet extract
2016/2017	no. of inflorescences plant^{-1}	118.00^{e}±6.0	184.00^{d}±4.58	246.00^{c}±6.0	279.33^{a}±4.73	258.67^{b}±3.79
	Inflorescences f.wt. plant^{-1}	12.16^{d}±0.43	19.56^{c}±1.34	26.12^{b}±0.83	29.51^{a}±0.36	28.28^{a}±0.60
	Inflorescences d.wt. plant^{-1}	2.90^{c}±0.2	4.46^{b}±0.38	6.27^{a}±0.09	7.14^{a}±0.51	7.02^{a}±0.89
2017/2018	no. of inflorescences plant^{-1}	121.00e±2.65	181.67d±4.51	245.67c±5.03	281.00a±2.0	261.67b±3.06
	Inflorescences f.wt. plant^{-1}	11.85e±1.10	19.96d±0.15	26.48c±0.56	31.57a±0.43	28.68b±1.18

Inflorescences d.wt. plant^{-1}	2.39d±0.02	3.68c±0.17	4.79b±0.2	6.60a±0.78	6.20a±0.15

Data were presented as mean ± SD (n=3). Means in the same column with different letters are significantly different at P≤0.05.

Chemical constituents of chamomile shoot influenced with foliar applications of plant aqueous extracts.

Tables (6 and 7) showed significant increase (P≤0.05), in most cases, in the tested chemical constituents of chamomile shoots sprayed with the four plant aqueous extracts.

Chl a, Chl b and carotenoids

Foliar applications with red beet and safflower extracts induced markedly increase in Chl a (0.94 and 0.92), Chl b (0.35 and 0.34) and carotenoids (0.25 and 0.24) mg/g f.wt. in chamomile shoot in comparison with untreated plants which showed the lowest pigments concentration (0.79, 0.27 and 0.22 mg/g f.wt) respectively, at the first season. Also, the same behavior was detected at the second one (Table 7).

Table 6. Effect of foliar application of plant aqueous extract on some biochemical constituents in chamomile shoot during the season of 2016/ 2017.

First season 2016/2017					
Biochemical constituents	**Control**	**roselle extract**	**turmeric extract**	**safflower extract**	**red beet extract**
Chl a (mg g^{-1} f.wt.)	**0.79^{e}±0.001**	**0.89^{d}±0.001**	**0.85^{c}±0.001**	**0.92^{a}±0.009**	**0.94^{b}±0.001**
Chl b (mg g^{-1} f.wt.)	**0.27^{c}±0.01**	**0.30^{b}**±0.001	**0.30^{b}±0.001**	**0.34^{a}±0.001**	**0.35^{a}±0.001**
Carotenoids (mg g^{-1} f.wt.)	**0.22^{d}±0.001**	**0.23^{c}±0.002**	**0.23^{c}±0.003**	**0.24^{b}±0.001**	**0.25^{a}±0.001**
Flavonoids mg (100g^{-1} f.wt.)	**25.65^{d}±3.85**	**92.74^{b}±1.005**	**115.4^{a}±0.82**	**45.45^{c}±2.75**	**42.39^{c}±1.04**
Phenolic compounds mg (100g^{-1} f.wt.)	**88.03^{bc}±12.37**	**92.29^{b}±5.59**	**114.07^{a}±9.01**	**73.64^{d}±3.37**	**77.47^{cd}±1.03**
RS (mg 100g^{-1} f.wt.)	**220.23^{e}**±1.05	**295.28^{d}±1.009**	**305.14^{c}±0.95**	**429.22^{a}±1.105**	**339.45^{b}±1.05**
AA (mg 100g^{-1} f.wt.)	**76.44^{d}±0.09**	**77.44^{cd}±1.098**	**78.25^{c}±0.02**	**87.78^{a}±1.10**	**82.57^{b}±0.28**

Data were presented as mean ± SD (n=3). Means in the same column with different letters are significantly different at P≤0.05.

Flavonoids and Soluble phenolic compounds

Tables (6 and 7) showed also that exogenous applied turmeric extract elevated the total flavonoid concentrations (115.40 and 101.20 mg 100g^{-1} f.wt.) and soluble phenolic compounds concentrations (114.07 and 103.76 mg 100g^{-1} f.wt.) more than control palnts in chamomile shoots during the two successive seasons, respectively. Flavonoids concentration was also increased about 2- 3.5 times than control when plants treated with the other plant aqueous extracts. On the other hand, soluble phenols were decreased due to spraying with safflowers and red beet extracts at the both seasons when compared with control.

Reducing sugars and amino acids

Safflower extract treatment elevated both reducing sugars (429.22 and 295.24 mg 100g^{-1} f.wt.) and amino acids (87.78 and 99.74 mg 100g^{-1} f.wt.) in chamomile shoots during the two seasons, respectively (Tables 6 and 7). Application with other plant extracts clearly induced an increase in reducing sugars and amino acids in comparing to plants sprayed with water.

Table 7. Effect of foliar application of plant aqueous extract on some biochemical constituents in chamomile shoot during the season of 2017/ 2018.

Second season 2017 / 2018					
Biochemical	**Control**	**roselle**	**turmeric**	**safflower**	**red beet**

constituents		extract	extract	extract	extract
Chl a (mg g^{-1} f.wt.)	0.55^{e}±0.01	0.60^{d}±0.001	0.65^{c}±0.003	0.85^{a}±0.85	0.71^{b}±0.002
Chl b (mg g^{-1} f.wt.)	0.22^{e}±0.002	0.25^{d}±0.003	0.25^{c}±0.001	0.29^{a}±0.002	0.28^{b}±0.003
Carotenoids (mg g^{-1} f.wt.)	0.20^{e}±0.001	0.21^{d}±0.004	0.22^{c}±0.003	0.24^{a}±0.004	0.23^{b}±0.002
Flavonoids mg ($100g^{-1}$ f.wt.)	15.32^{e}±0.03	89.63^{b}±0.08	101.20^{a}±0.1	22.40^{d}±0.05	39.66^{c}±0.02
Phenolic compounds mg ($100g^{-1}$ f.wt.)	85.96^{c}±0.025	89.12^{b}±0.025	103.76^{a}±0.23	55.76^{e}±0.02	64.33^{d}±0.02
RS (mg $100g^{-1}$ f.wt.)	198.36^{e}±0.03	215.46^{d}±0.08	221.34^{c}±0.01	295.24^{a}±0.04	253.63^{b}±0.08
AA (mg $100g^{-1}$ f.wt.)	65.48^{e}±1.22	71.67^{d}±1.27	77.39^{c}±1.73	99.74^{a}±0.03	87.73^{b}±1.12
Data were presented as mean ± SD (n=3). Means in the same column with different letters are significantly different at P≤0.05.					

Effect of plant aqueous extracts on biochemical constituents of chamomile inflorescence

Exogenous spray with red beet extract improved the total soluble phenolic compounds (601 and 563 mg $100g^{-1}$ d.wt.) and flavonoids (1282 and 773 mg $100g^{-1}$ d.wt.) in chamomile inflorescences followed by roselle or safflower extracts when compared with control, while spraying with turmeric extract led to reduce both phenols (483 and 420 mg $100g^{-1}$ d.wt.) and flavonoids concentrations (485 and 296 mg $100g^{-1}$ d.wt.) than control at both seasons, respectively (Tables 8 and 9).

Carotenoids concentration in chamomile inflorescences highly increased when safflower extract sprayed on plants which reached (40.64 and 37.48 mg $100g^{-1}$ d.wt.) followed by red beet extract (36.78 and 34.01 mg $100g^{-1}$ d.wt.) which also showed significant an increase in carotenoids concentration more than control (28.94 and 27.55 mg $100g^{-1}$ d.wt.) at both seasons, respectively.

Essential oil yield

Chamomile essential oil yield was increased when any of the tested plant aqueous extracts were applied in comparing to control (Tables 8 and 9). Essential oil yield increased to the maximum concentrations (2.43-2.25 and 2.07-2.08 ml $100g^{-1}$ d.wt.) in inflorescences of chamomile sprayed with safflower and roselle extracts at the two seasons, respectively, in comparing with other extracts and water control as shown in Tables (8 and 9).

Table 8. Effect of foliar application of plant aqueous extract on some biochemical constituents in chamomile inflorescences during the season of 2016/ 2017.

First season 2017					
Biochemical constituents	Control	roselle extract	turmeric extract	safflower extract	red beet extract
Phenolic compounds (mg/100g d.wt.)	562^{b}±10	569^{b}±10	483^{d}±9	538^{c}±1	601^{a}±5.29
Flavonoids (mg /100g d.wt.)	574^{d}±4.36	712^{c}±10	485^{e}±2	942^{b}±1	1282^{a}±9.5
Carotenoids (mg/100g d.wt.)	28.94^{e}±0.01	32.56^{d}±0.01	33.45^{c}±0.01	40.64^{a}±0.01	36.78^{b}±0.01
Oil yield (ml/100g d.wt.)	0.73^{e}±0.01	2.07^{b}±0.01	1.19^{d}±0.01	2.43^{a}±0.01	1.22^{c}±0.01
IC 50 of oil (µg ml^{-1})	14.36^{b}±0.03	35.82^{d}±0.02	37.87^{c}±0.026	20.74^{c}±0.025	0.638^{a}±0.002
IC 50 of inflorescences ethanolic extract (mg ml^{-1})	1.465^{d}±0.01	1.049^{c}±0.01	2.205^{e}±0.001	0.349^{a}±0.01	0.875^{b}±0.01
Data were presented as mean ± SD (n=3). Means in the same column with different letters are significantly different at P≤0.05.					

Table 9. Effect of foliar application of plant aqueous extract on some biochemical constituents in chamomile inflorescences during the season of 2017/ 2018.

	Second season 2018				
Biochemical constituents	**Control**	**roselle extract**	**turmeric extract**	**safflower extract**	**red beet extract**
Phenolic compounds (mg/100g d.wt.)	**501^{c}±2**	**515^{b}±10**	**420^{d}±4.58**	**525^{b}±10**	**563^{a}±1**
Flavonoids (mg /100g d.wt.)	**402^{c}±10**	**592^{b}±10**	**296^{d}±3.6**	**762^{a}±10**	**773^{a}±11**
Carotenoids (mg/100g d.wt.)	**27.55^{e}±0.01**	**31.32^{d}±0.01**	**31.98^{c}±0.01**	**37.48^{a}±0.01**	**34.01^{b}±0.01**
Oil yield (ml/100g d.wt.)	**0.58^{e}±0.01**	**2.08^{b}±0.01**	**1.12^{c}±0.01**	**2.25^{a}±0.01**	**0.801^{d}±0.001**
IC 50 of oil (µg ml^{-1})	**20.61^{b}±0.02**	**60.26^{d}±0.06**	**88.83^{e}±0.07**	**25.52^{c}±0.15**	**0.856^{a}±0.004**
IC 50 of inflorescences ethanolic extract (mg ml^{-1})	**1.345^{d}±0.016**	**0.939^{c}±0.01**	**1.512^{a}±0.01**	**0.235^{e}±0.01**	**0.778^{b}±0.01**

Data were presented as mean ± SD (n=3). Means in the same column with different letters are significantly different at P≤0.05.

Biochemical constituents of chamomile essential oil

GLC-MS analysis improved that chamomile essential oil contains 16 compouds *viz*, artimisia ketone, artimisia alcohol, iso-borneol, trans-β-farnesene germacrene D, germacrene B, cadinene, sapthulenol, farnesene epoxide, tau-cadinol, α bisabolol oxide B, caryphylene oxide, bisbolone oxide, α bisabolol, chamazulene and α bisabolol oxide A (Tables 10 and 11). The main components of chamomile volatile and essential oil as detected by GC-MS were bisabolol oxide A, α bisabolol oxide B, trans – β- farnesene, chamazulene, bisabolone oxide organized by concentration for the two growing seasons.

α Bisablol oxide A was the major compound that detected in chamomile volatile oil which increased with all exogenous spray treatments at the two growing seasons. The concentration of bisabolol oxide A reached about 90 % in the essential oil produced from plants sprayed with safflower and red beet extracts, where safflower extract recorded the highest value (90.87%) followed by red beet extract 90.16%, roselle extract (89.25%), turmeric extract (80.74%) while control treatment gave the lowest value (78.64%) during the first season (Table 10), on the other hand turmeric extract treatment gave the lowest value (78.88%) during the second season (Table 11).

At the first season, artimisia ketone, artimisia alcohol, trans-β-farnesene, bisabolone oxide and chamazulene were reduced when chamomile plants were sprayed with the tested plant extracts compared with control treatment, on the other hand, artimisia alcohol, artimisia ketone were increased in volatile oil when plants sprayed with turmeric or red beet extract on the second season. α Bisabolol was not detected with foliar spray of roselle, turmeric and safflower extracts at the first season, while it detected with roselle and turmeric extract treatments at the second season. Cadinene, which not detected in untreated plants was found in oil of chamomile treated with roselle (0.18 – 0.08%) and safflower extract (0.22 – 0.12%) at both seasons, respectively. Tau-cadinol showed the highest value in safflower extract treatment (1.33% and 1.40%) while it showed the lowest value in red beet extract treatment (0.47 % and 0.61%) during both seasons, respectively.

Table 10. Effect of foliar application with plant aqueous extracts on the biochemical composition of essential oil in chamomile inflorescences and their percentages during the season

of 2016 / 2017.

First season 2016 / 2017						
		Foliar application treatment				
biochemical composition	Retention time (R_t)	control	roselle extract	turmeric extract	safflower extract	red beet extract
Artimisia ketone	8.4	1.71	0.47	0.92	-	0.25
Artimisia alcohol	9.16	0.58	0.07	0.23	-	0.25
iso borneol	12.99	0.18	0.14	0.23	-	0.24
trans- β –farnesene	24.41	3.58	1.89	2.38	1.58	2.65
Germacrene D	25.48	0.14	0.25	0.22	0.26	0.26
Germacrene B	26.06	0.15	0.17	0.20	0.20	0.15
Cadinene	26.84	-	0.18	-	0.22	-
Sapthulenol	29.39	0.55	0.51	0.70	0.27	0.63
Farnesene epoxide	30.94	0.13	-	0.15	0.14	0.08
Tau-cadinol	31.92	0.67	0.85	0.52	1.33	0.47
α-Bisabolol oxide B	32.20	7.81	1.91	11.00	2.23	1.83
Caryphylene oxide	32.67	0.39	0.24	0.38	0.36	0.56
bisbolone oxide	33.38	2.46	2.65	2.00	1.32	1.82
α bisabolol	33.59	0.25	-	-	-	0.27
Chamazulene	35.24	2.71	1.44	0.25	1.09	0.38
α Bisabolol oxide A	35.86	78.64	89.25	80.74	90.87	90.16

Data is presented as percentage

Table 11. Effect of foliar application with plant aqueous extracts on the biochemical composition of essential oil in chamomile inflorescences and their percentages during the season of 2017/ 2018.

Second season 2017 / 2018						
		Foliar application treatment				
biochemical composition	Retention time (R_t)	control	roselle extract	turmeric extract	safflower extract	red beet extract
Artimisia ketone	8.40	0.72	0.20	1.55	-	0.40
Artimisia alcohol	9.16	0.32	-	0.39	-	0.50
iso borneol	12.99	0.18	0.06	0.24	0.29	0.49
trans- β –farnesene	24.41	2.9	3.32	1.15	1.01	1.71
Germacrene D	25.48	0.23	0.24	0.21	0.17	0.21
Germacrene B	26.06	0.23	0.16	0.18	0.18	0.16
Cadinene	26.84	-	0.08	-	0.12	-
Sapthulenol	29.39	0.49	0.34	0.76	0.39	0.68
Farnesene epoxide	30.94	0.12	0.06	0.13	0.06	-
Tau-cadinol	31.92	0.75	0.79	0.73	1.40	0.61
α-Bisabolol oxide B	32.20	4.84	1.25	11.39	3.48	1.98
Caryphylene oxide	32.67	0.32	0.29	0.33	0.17	0.25
bisbolone oxide	33.38	2.16	2.42	2.62	1.98	2.51
α bisabolol	33.59	0.12	0.08	0.09	-	0.19
Chamazulene	35.24	2.14	2.23	0.36	0.63	0.19
α Bisabolol oxide A	35.86	84.48	88.48	78.88	90.04	90.02

Data is presented as percentage

Scavenging activity of essential oil and ethanolic extract of chamomile inflorescences

Increasing the concentration of chamomile essential oil accompanied with increasing the scavenging activity on DPPH radical in all treatments (Figures 1 and 2). Essential oil from plants treated with red beet extract had the highest scavenging activity on DPPH radical and lowest value of IC_{50} (0.638 and 0.856 μg ml^{-1}) during 2017 and 2018, respectively, in comparison with other treatments (Figure 1 and Tables 8 & 9).

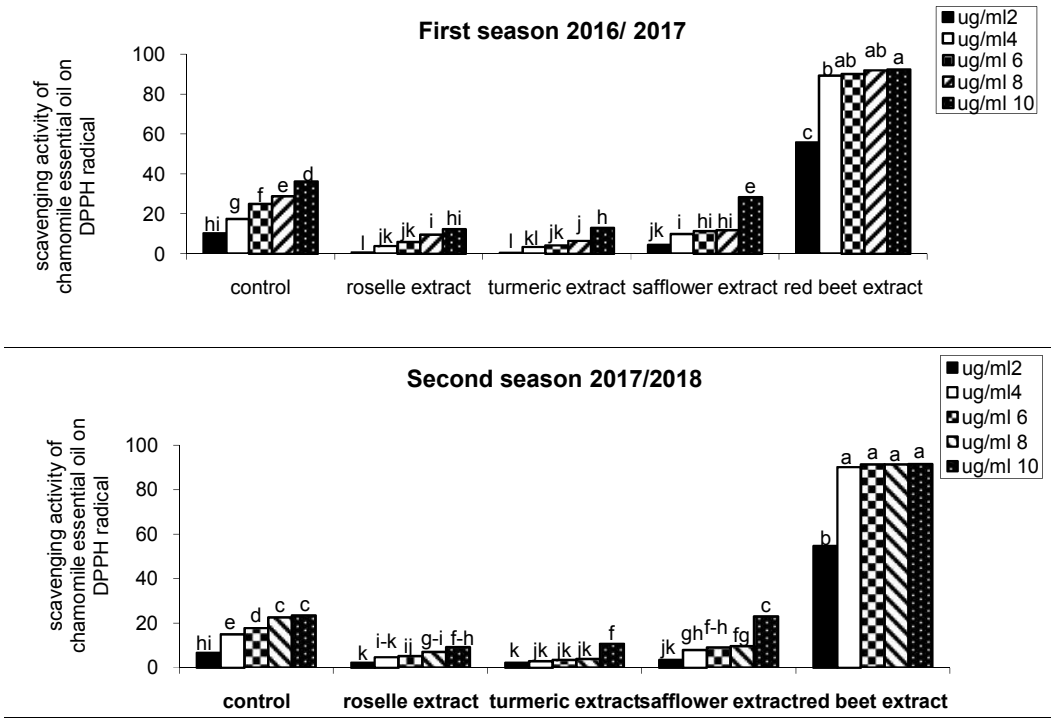
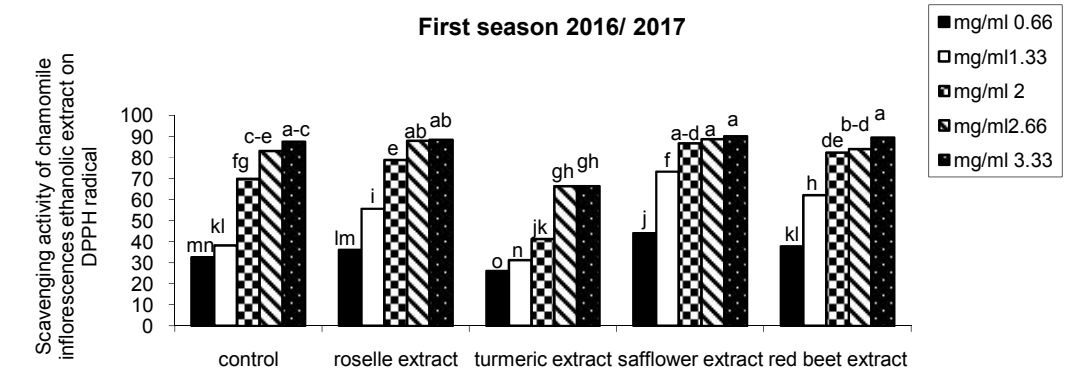


Figure 1. Effect of foliar application of plant aqueous extracts on scavenging activity % of chamomile essential oil on DPPH radical during the season of 2016/2017.

While ethanolic extract of chamomile inflorescences treated with safflower extract showed the highest scavenging activity and lowest IC_{50} (0.349 and 0.235mg ml^{-1}) during 2016/2017 and 2017/2018, respectively, followed by red beet, roselle while turmeric aqueous extract treatment showed the lowest scavenging activity on DPPH radical and the highest IC_{50} value (2.205 and 1.512 mg ml^{-1}), respectively, as showed in Figure 2 and Tables 8 & 9.



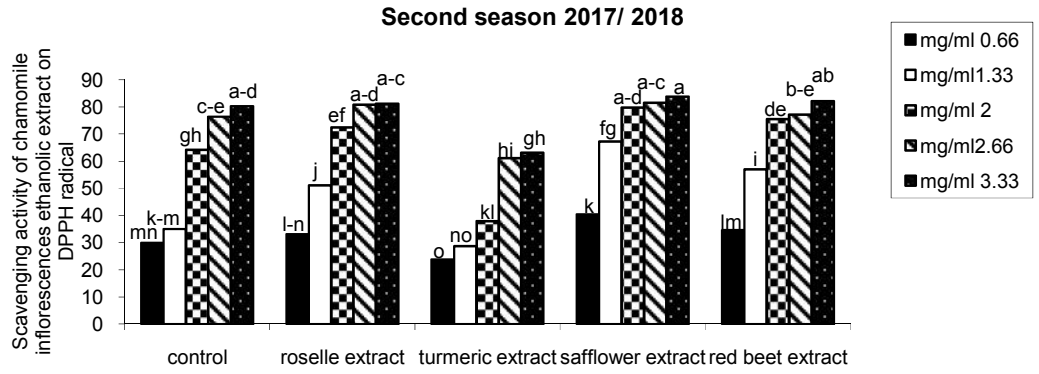


Figure 2. Effect of foliar application of plant aqueous extracts on scavenging activity % of chamomile inflorescences ethanolic extract on DPPH radical during the season of 2017/2018.

Discussion

In this study, spraying plant aqueous extracts of roselle, turmeric, safflower and red beet on chamomile plants showed high efficiency in growth promotion and essential oil production. This growth stimulation may be due to the high content of sugars, amino acids and various secondary metabolites in these plant aqueous extracts. Red beet and safflower extracts contained the highest concentrations of soluble phenolic compounds, flavonoids, reducing sugars, free amino acids in addition to N, P, K % in the present study. These results were in agreement with **Jasna *et al.* [32]** who stated that red beet contained high concentrations of phenols, flavonoids, ß-cyanins and ß-xanthins beside the presence of sugars and protein that naturally exist in red beet. Also, **Al Surmi *et al.* [33]** recorded high concentrations of soluble phenols, amino acids and N, P, K ratios in safflower extract while roselle leaves and calyces contained phenols, flavonoids and anthocyanins that act as antioxidants **[34]**. **Okereke *et al.* [35]** reported that roselle extract contained a high value of glycosides. The main ingredients in roselle extracts are vitamins C, A, D, B1 and B2, antioxidants, anthocyanins, Fe, Mg and omega 3-ß-carotene **[36]**.

Spraying apple trees with turmeric extract increased leaf nitrogen, phosphorus and potassium concentrations **[37].** They attributed this increase to the high concentration of turmeric extract in potassium salt as found in this study. **Ibrahim *et al.* [38]** mentioned that both pH and titratable acidity **were** physical properties. The value of pH and titratable acidity in the present study were compatible with that obtained by **Ibrahim *et al.* [38]** who found that roselle extract has a comparatively high acidic to cause lower pH in the extract. They attributed the high acidity of roselle to its natural constituents of organic acids such as citric acid, mallic acid and 3-indolyl acetic acid.

The physical properties and chemical constituents of the four studied aqueous extracts showed various economic traits in chamomile growth and productivity. Roselle and turmeric extracts showed a clear increase in plant height while safflower

and red beet extracts markedly provided large numbers of branches compared to other extract treatments. Similar findings were reported with alfa alfa, clover, red clover and landino aqueous extracts when influenced the growth of various legumes and grasses species **[39]**. Foliar spraying of pear tree "Le-Conte cv." with roselle, cinnamon and ginger water extracts gave the best fruits weight and fruits number per tree and increase total soluble solids and total fruit sugars % in comparison to control **[40]**. Based on the previous findings, it was cleared that using roselle extract improved the nutritional status, yield and physical-biochemical characteristics of Valencia orange fruits **[41]**. The higher content of K, vitamins, amino acids, curcumin and volatile oils in turmeric extract encourage researchers to interest in using it as an important plant extract [42]**.** The positive effect of turmeric extract on enhancing growth and productivity could be due to their higher content of protein, carbohydrates, amino acids, Ca, K, P, Fe, ascorbic acid, thiamine, riboflavin, niacin, curcumin and other pigments **[8]**. Also**, Armanious [43]** revealed that using turmeric extract at 0.05% was preferable than garlic and onion extracts in improving the leaf area, yield, nutrients status and fruit quality of Thompson seedless grapevines.

The significant increase in growth and inflorescences production of plant sprayed with the aqueous extracts of safflowers and red beet may be related by their high concentrations of reducing sugars and free amino acids which induced the highest values of growth parameters and gave higher yield components. These results were in agreement with **Rolland *et al.*[44]** who indicated that total sugars and amino acids serve as a storage sink involved in carbon and nitrogen pathways to modulate plant growth and development. Amino acids play various roles in plant physiological processes such as nitrogen source, hormone precursors, regulate nitrogen uptake that improved plant growth and yield **[45]**. Genetic analyses have approved extensive interaction between total sugars and plant hormones signaling activation**[44]**. Also, it was proved that accumulation of high levels sugars in many plant species promotes vegetative phase and increase number of leaves which resulted in elevating the canopy and the final outcome becomes increasing in the number of flowers, while low concentration of sugars slightly inhibited the flowering in arabidopsis **[46]**.

Applied plant aqueous extracts as foliar spraying alleviated the concentrations of chl a, chl b and carotenoids in addition to increase reducing sugars and amino acids in treated chamomile plants in the present study. The explanation of this increase is that the active compounds in the chemical composition of the studied aqueous extracts especially in safflower and beet root extracts display potent antioxidant and osmoregulator properties under environmental conditions. In this context, **Zonouri *et al.*, [47]** reported that increasing antioxidants in plant cells have a potential strength as free radical scavengers that prevent the degradation of chlorophylls and protect chloroplast membranes. Antioxidants can neutralize the H_2O_2 formation in the cell, which involved in abscisic acid (ABA) transmitting signals. ABA accelerated stomatal closure that limits the assimilation of CO_2 and affected photosynthesis process [48]. External application of antioxidants can reserve the stomatal closure **[49**.

Furthermore, **Cushman [50]** found that high levels of reducing sugars and amino acids induced an osmotic regulation in cells which improved the water absorbance and translocation that stabilize membranes and inhibit lipid peroxidation. These results also in the same trend with that obtained by **Clifford *et al.* [51]** who reported that the bioavailability of betalains and phenols, the main bioactive components in beet root, helping in protect cellular components in a state of redox balance under the normal metabolic conditions. Moreover, **El Sharony *et al.* [52]** stated that the main ingredients and antioxidant components of roselle extract have been shown to suppress oxide radicals formation and increase total sugars, amino acids and ascorbic acid concentrations in mango fruits. The increasing in carotenoids in plants applied with aqueous extracts in the current study could be attributed to increase the biosynthesis of carotenoids and prevent the conversion of carotenoids into ABA under normal environmental conditions **[48]**.

When biochemical analysis carried out in the inflorescences produced from plants sprayed with the tested plant extracts, it was observed that red beet extract encouraged the increase in soluble phenols, flavonoids and carotenoids in compared to other treatment that also provided these components more than control. Moreover, red beet extract treatment gave less yield of essential oil than treatments of safflower and roselle extracts. It was suggested that the reason of this increasing in yield was due to treatment with safflower or roselle extract stimulated the conversions of phenols and flavonoids, where are considered secondary products, into other secondary metabolites that have an essential role in the composition of volatiles and essential oil of chamomile inflorescences but these conversions were less in red beet extract treatment. This suggestion was compatible with **Figueiredo *et al.* [53]** who reported that the valuable volatile compounds and essential oils consists of multiple phenolic compounds mixed with alkaloids and terpenoids substances. According to these authors, the differences in aroma in the floral oil result from the changeable among the different compounds of phenols and terpenoids which give the oil distinctive characteristics. As well **Zheljazkov *et al.* [54]** showed similar results for the quantity and quality of the essential oil, where the treatments with aqueous extracts of absinthe worm wood, lavender and wild bergamot led to increase the oil yield and the essential oil components of Native spearment.

In the current study, plant extract treatments altered the chemical constituents of chamomile essential oil by increasing or decreasing some component percentage. Some treatments caused disappear in some components, this may be due to plant extract treatments influenced the essential biosynthesis and the conversion of compounds to others as mentioned before. These results were in agreement with many investigators, **McKay and Blumberg [55]** stated that active principle components in chamomile essential oil are α bisabolol oxide A and B and chamazulene . The main constituents in the chamomile essential oil were chamazulene, α bisabolol, bisabolol-A and B oxides and ß-farnesene while the minor constituents were α and ß-caryophylene, caryophylene oxide and spathulenol **[56]**. German chamomile volatile

oil has 5% chamazulene and 50% α bisabolol oxide A [57]. **Zheljazkov [58]** reported that the sage brush and juniper water extracts increased the concentrations of ß-caryophyllene and trans- ß -farnesene in spearmint essential oil relative to the water treatment. The author also reported that the spearmint essential oil content was more valuable when Juniper water extract was applied.

The concentration of antioxidants required to decrease initial DPPH radical concentrations by 50% (IC_{50}) is a measurement widely adopted for evaluating the antioxidant activity, where lower IC_{50} has higher antioxidant power. The present data revealed that chamomile essential oil and inflorescences ethanolic extract obtained from plants sprayed with red beet extract observed the highest free radical inhibitory activity on DPPH, according to their IC_{50} values, followed by safflower and roselle extracts in compared to plants treated with distilled water or turmeric extract. Similar results showed by **Firat *et al.* [59]** who found that chamomile essential oil contains a high level of free radical scavenging capacity through their higher DPPH inhibition and lower value of IC_{50}. They attributed this positive scavenging activity of chamomile to their higher content of α-bisabolol oxide A which exhibited the higher antioxidant potential with lower IC_{50} compared to ß-farnesene and α-bisabolol. This is fully consistent with the results obtained in this study where α-bisabolol oxide A found to be the main compound in the chamomile composition that increased to more than double the concentration in control plants and reached to 90 % in the volatile oil of chamomile with safflower and red beet extract treatments. **Agatonovic-Kustrin *et al.* [59]** reported that chamomile flower heads and leaves had the most prominent antioxidant activities which α bisabolol and its oxide, apigenin and chamazulene being the most effective antioxidants. According to litteratures.

Conclusion

All plant aqueous extract treatments had a noticeable positive effect on growth and oil yield of chamomile. Safflower extract followed by red beet extract exhibited the best growth and shoot biochemical composition which improved the inflorescences yield, essential oil yield and valuable chemical constituents of essential oil.

Therefore, plant extracts as an organic farming could be safer in production and exportation for medicinal plant, cheap and more available to simple farmers than spraying with chemical substance

So plant extract could be recommended as a natural biostimulant application for improving yield of chamomile.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

Ethical: NA
Consent: NA

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