

## **Original Research Article**

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

#### **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, chemical constituents 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 inflorescences ethanolic extracts and essential oil obtained from chamomile plants treated with safflower and red beet extracts showed the highest scavenging activities on DPPH radical and lowest IC<sub>50</sub> 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*

#### **Introduction**

Recently, public health and environmental safety encourage the use of plant extracts for improving growth, chemical composition and productivity of plant especially medicinal plants. Chamomile is one the important medicinal and aromatic plant belong to *Asteraceae* family, has a sweet, grassy and lightly fruity aroma. Chamomile has many medicinal uses because of its calming, carminative and spasmolytic properties. It has antimicrobial and anti-inflammatory effects. The main chemical components of the chamomile extracted oil, ~~are as:~~  $\alpha$ -pinene,  $\beta$ -pinene, camphene, sabinene, myrcene, 1,8-cineole  $\gamma$ -terpinene, caryophyllene, propyl angelate, butyl angelate, chamazulene, a-bisabolol, bisabolol oxide A, bisabolol oxide B and bisabolone oxide A. Also, flavone glucosides (apigenin 7-O-glucoside and various acylated derivatives of apigenin 7-O-glucoside) and flavonols (luteolin

glucosides, quercetin glucosides and isohamnetin) were identified in chamomile (Berry, 1995).

However, plant extract has recently become an increasing more common treatment in modern agricultural production, among such substances are roselle, turmeric, safflower and red beet water extracts.

Roselle calyces (*Hibiscus sabdariffa* L.) is a member of the family *Malvaceae* (Anonymous, 1970). Roselle is a tropical shrub with red or green inflated edible calyces (Seck, 1997). The calyces groups are red, dark red and green types (Schippers, 2000). The calyces have been found to be rich in vitamin C and other antioxidants such as flavonoids (Wong *et al.*, 2002) and also minerals (Babalola *et al.*, 2001).

Turmeric, *Curcuma longa* L. (*Zingiberaceae*) rhizome commonly used as a spice (Sivananda, 1958). The higher content of turmeric from K, amino acids, different nutrients, vitamins, antioxidants and plant pigments especially curcumin and volatile oils encourage the pomologists to undertake many attempts for using it as an important plant extract (Peter, 1999).

Safflower (*Carthamus tinctorius* L.) contains water soluble yellow dye and carthamidin. Safflower is world's oldest crop belonging to the *Compositae* family which originated in the Middle East. Safflower is an annual oil seed crop. It has been used traditionally as a medicinal herb and as a natural dye source for coloring food and textile (Camas *et al.*, 2007).

Beet root (*Beta vulgaris* L.) is botanically classified as an herbaceous biennial from *Chenopodiaceae* family and has several varieties with bulb colors ranging from yellow to red. Deep red-colored beet roots are the most popular for human consumption. Beet roots are rich in valuable active compounds such as carotenoids (Dias *et al.*, 2009), glycine betaine (De Zwart *et al.*, 2003), saponins (Atamanova *et al.*, 2005),  $\beta$ -cyanines (Patkai *et al.*, 1997), folates (Jastrebova *et al.*, 2003), betanin, polyphenols and flavonoids (Vali *et al.*, 2007). Vinson *et al.* (1998) and Žitňanová *et al.* (2006) illustrated that red beet is one of the most potent vegetables with respect to antioxidant activity.  $\beta$ -cyanins are a group of compounds exhibiting antioxidant and radical-scavenging activities (Escribano *et al.*, 1998; Pedreno & Escribano, 2000).

The purpose of this study is to investigate the biological activity of roselle, turmeric, safflower and red beet aqueous extracts as foliar sprayers on 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:

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

### Preparation of plant water extracts

Calyces of roselle (*Hibiscus sabdariffa* L.), rhizomes of turmeric (*Curcuma longa* L.), flowers of safflower (*Carthamus tinctorius* L.) and roots of red beet (*Beta*

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*vulgaris* L.) were brought from the local market, then dried, grinded and macerated 2g powder in 100 ml of distilled water for 24h then filtered and used freshly.

#### **Chemical analysis of plant water extracts**

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

Total titratable acidity (TA) of plant aqueous extracts (mg citric acid 100 g<sup>-1</sup> d.wt.) was determined according to **A.O.A.C. (2000)**.

Soluble phenolic compounds were estimated by the method of Folin-Ciocalteu as described by **Shahidi and Naczk, (1995)** using gallic acid as a standard-.

Total flavonoids concentration was determined by the aluminum chloride colorimetric assay according to **Marinova et al. (2005)** using quercetin as a standard.

Anthocyanin concentration in the plant water extracts was colourimetrically proceeded as g 100g<sup>-1</sup> d.wt. according to **Du and Francis (1973)**.

Plant aqueous extracts digested using H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> according to the method described by **Piper (1950)** to determine N, P and K percentage according to the method described by **Black et al. (1965)** and **Wilde et al. (1985)**.

#### **Experiment set up**

Pot experiment was carried out under field conditions in the farm of Botany Department, Faculty of Agriculture, Ain Shams University, Shoubra El Kheima, Qalyubia, Egypt, (30° 06' 42" N 31° 14' 46" E) during the successive winter seasons of 2016/2017 and 2017/2018. Seeds were sown on 15<sup>th</sup> of September in nursery beds. On 1<sup>st</sup> of November of both seasons, when the grown seedlings reached about 10-15 cm in length, ten transplants were transferred in plastic pots (30 cm in diameter) filled with clay/sand (2:1 v/v) soil. 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 grown 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 as control. The volume of extracts was consequently increased with increasing plant growth. The foliar applications were applied four times, the first was 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.

#### **Chamomile plant samples**

Three vegetative 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).

Two other samples (at 45 and 120 days after transplanting) were randomly collected to measure shoot fresh weight (g) plant<sup>-1</sup>, plant height (cm), number of branches plant<sup>-1</sup> and dry weight (g) plant<sup>-1</sup>. 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<sup>-1</sup>, total inflorescences fresh and dry weights plant<sup>-1</sup>.

Total soluble phenolic compounds, flavonoids and carotenoids were determined in chamomile inflorescences. The essential oil yield plant<sup>-1</sup> (ml 100g<sup>-1</sup>), its chemical components and scavenging activity on DPPH radical in both oil and inflorescences were estimated.

#### Chemical analyses of chamomile samples

—Total soluble phenols, flavonoids, reducing sugars and free amino acids were extracted from chamomile shoots according to **Ackerson (1981)** using 80% ethanol.

Total flavonoids concentration was determined by the aluminum chloride colorimetric assay according to **Marinova et al. (2005)** using quercetin as a standard.

Soluble phenolic compounds were determined by the method of Folin-Ciocalteu as described by **Shahidi and Naczki, (1995)** using gallic acid as a standard.

-Reducing sugars were ~~colourimetrically~~ colorimetrically determined by using 3,5 di-nitro-salicylic acid solution according to **Miller (1959)** and glucose was used as a standard.

Free amino acids were ~~colorimetrically~~ colorimetrically determined by using ninhydrin solution according to **Jayeraman (1985)** using glycine as a standard.

Total soluble phenolic compounds, flavonoids and carotenoids were determined in chamomile inflorescences as described before.

Inflorescences essential oil was distilled using a micro distilling apparatus and oil volume was measured as ml of oil 100g<sup>-1</sup> d.wt. inflorescences according to **Guenther (1961)**.

Chemical constituents of the essential oil of chamomile 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.

Scavenging activity of essential oil (2, 4, 6, 8 and 10 µg ml<sup>-1</sup>) and ethanolic extract (0.66, 1.33, 2.00, 2.66 and 3.33 mg ml<sup>-1</sup>) of inflorescences on DPPH radical were measured from the bleaching of a purple colored methanolic solution of DPPH according to **Gulluce et al. (2004)**. This spectrophotometric assay uses the stable radical 2, 2'- diphenyl-1-picryl hydrazyl (DPPH) as a reagent. Scavenging activity was calculated as percentage. Inhibition of free radical DPPH calculated according to the following equation: % Scavenging activity = [(A<sub>control</sub> - A<sub>sample</sub>)/A<sub>control</sub>]x 100

#### Statistical analysis

All experiment data was analyzed by analysis of variance (ANOVA) using the General Linear Models procedure of CoStat. Significance between means was tested by "F" test and the value of LSD (p=0.05) was calculated (**Snedecor and Cochran, 1982**). Significant differences of means at  $P \leq 0.05$  were compared by different letters as described by Duncan test of **Gomez and Gomez (1984)** and expressed as mean ± standard deviation (SD).

## Results

#### Chemical analysis of plant aqueous extracts

Data in **table (1)** showed significant differences ( $P \leq 0.05$ ) in the physical-biochemical analyses 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, total acidity (TA) % showed the opposite direction where roselle

extract contained the highest TA% (15.66%) while turmeric extract had the lowest value (0.96%).

Safflower aqueous extract contained the highest concentrations of total soluble phenols ( $2.55 \text{ g } 100\text{g}^{-1} \text{ d.wt.}$ ), reducing sugars ( $15.47 \text{ g } 100\text{g}^{-1} \text{ d.wt.}$ ) and P ( $0.80 \text{ g } 100\text{g}^{-1} \text{ d.wt.}$ ) in addition to its high concentration of amino acids, whereas, the highest concentration of free amino acids ( $1.17 \text{ g } 100\text{g}^{-1} \text{ d.wt.}$ ), flavonoids ( $8.46 \text{ g } 100\text{g}^{-1} \text{ d.wt.}$ ) and K ( $7.85 \text{ g } 100\text{g}^{-1} \text{ d.wt.}$ ) were recorded in red beet extract. Rosella extract was found to contain a high concentration of N compared to other extracts as shown in Table (1). 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  $0.09 \text{ mg } 100\text{g}^{-1} \text{ d.wt.}$ ), respectively, while turmeric and safflower extracts recorded anthocyanin free aqueous extracts.

**Table 1: Some physical properties and biochemical constituents of roselle, turmeric, safflower and red beet aqueous extracts**

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

Data were presented as mean  $\pm$  SD of three replicates (n=3) for each treatment. Means in the same column with different letters are significantly different at  $P \leq 0.05$ .

#### Effect of foliar application of plant water extracts on growth parameters of chamomile plants.

Tables (2 and 3) revealed significant differences in growth parameters of chamomile shoot was detected by foliar applications of plant water extracts at 45 and 120 days after transplanting during the two seasons 2016/2017 and 2017/2018.

Obtained results in Table (2) 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 sprayed by safflower and red beet, 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 2: Growth parameters "at 45 DAT" of chamomile plants sprayed with four different aqueous extracts during 2016/2017 and 2017/2018 successive seasons.**

Seasons	Growth parameters	Foliar applications				
		Control (distilled water)	Roselle extract	Turmeric extract	Safflower extract	Red beet extract
2016/2017	Plant height (cm)	21.17 <sup>c</sup> ±2.31	42.23 <sup>b</sup> ±3.46	56.77 <sup>a</sup> ±0.64	35.67 <sup>c</sup> ±3.05	27.30 <sup>d</sup> ±2.99
	No. of branches	27.33 <sup>d</sup> ±2.08	70.33 <sup>c</sup> ±8.08	157.33 <sup>b</sup> ±6.81	177.00 <sup>a</sup> ±6.24	160.67 <sup>b</sup> ±6.66

	Shoot f.wt.	12.73 <sup>c</sup> ±1.48	36.80 <sup>d</sup> ±4.29	40.57 <sup>c</sup> ±1.17	68.73 <sup>a</sup> ±3.69	55.17 <sup>b</sup> ±2.77
	Shoot d.wt.	2.38 <sup>d</sup> ±0.30	6.84 <sup>c</sup> ±0.65	7.61 <sup>c</sup> ±0.37	12.93 <sup>a</sup> ±1.18	10.38 <sup>b</sup> ±0.74
2017/2018	Plant height (cm)	21.63 <sup>d</sup> ±3.45	41.85 <sup>b</sup> ±1.13	53.80 <sup>a</sup> ±3.93	37.90 <sup>b</sup> ±1.44	26.77 <sup>c</sup> ±1.75
	No. of branches	26.67 <sup>d</sup> ±1.15	68.67 <sup>c</sup> ±6.66	156.67 <sup>b</sup> ±7.09	170.33 <sup>a</sup> ±7.09	159.67 <sup>b</sup> ±4.04
	Shoot f.wt.	13.38 <sup>c</sup> ±0.32	36.57 <sup>d</sup> ±4.30	42.60 <sup>c</sup> ±2.08	67.52 <sup>a</sup> ±5.93	54.65 <sup>b</sup> ±3.75
	Shoot d.wt.	2.45 <sup>c</sup> ±0.12	6.90 <sup>d</sup> ±0.73	7.94 <sup>c</sup> ±0.32	12.78 <sup>a</sup> ±0.94	10.26 <sup>b</sup> ±0.69

Data were presented as mean ± SD of three replicates (n=3) for each treatment. 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), at 120 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 2- folds more than control with all different aqueous extracts at both seasons of experiment (Table 3).

**Table 3: Growth parameters "at 120 DAT" of chamomile plants sprayed with four different aqueous extracts during 2016/2017 and 2017/2018 successive seasons.**

Seasons	Growth parameters	Foliar applications				
		Control	roselle extract	turmeric extract	safflower extract	red beet extract
2016/2017	Plant height (cm)	53.67 <sup>c</sup> ±2.31	66.60 <sup>ab</sup> ±1.68	70.50 <sup>a</sup> ±5.0	60.50±2.18	59.17 <sup>bc</sup> ±8.61
	No. of branches	53.67 <sup>e</sup> ±2.52	141.00 <sup>d</sup> ±2.65	153.33 <sup>c</sup> ±6.66	236.00 <sup>a</sup> ±7.81	166.00 <sup>b</sup> ±7.0
	Shoot f.wt.	79.75 <sup>e</sup> ±3.33	121.37 <sup>d</sup> ±4.87	131.47 <sup>c</sup> ±3.62	160.87 <sup>a</sup> ±6.82	143.00 <sup>b</sup> ±5.24
	Shoot d.wt.	18.61 <sup>c</sup> ±1.01	27.70 <sup>b</sup> ±2.26	29.65 <sup>b</sup> ±1.71	34.73 <sup>a</sup> ±0.48	32.67 <sup>a</sup> ±0.57
2017/2018	Plant height (cm)	52.47 <sup>c</sup> ±0.75	66.50 <sup>b</sup> ±2.75	73.50 <sup>a</sup> ±1.25	61.80 <sup>c</sup> ±2.29	56.97 <sup>d</sup> ±2.30
	No. of branches	53.00 <sup>e</sup> ±2.0	141.67 <sup>d</sup> ±9.5	159.67 <sup>c</sup> ±1.53	219.00 <sup>a</sup> ±6.0	177.00 <sup>b</sup> ±3.46
	Shoot f.wt.	78.18 <sup>d</sup> ±5.47	118.23 <sup>c</sup> ±2.14	133.57 <sup>b</sup> ±9.41	158.80 <sup>a</sup> ±7.55	142.43 <sup>b</sup> ±0.61
	Shoot d.wt.	17.89 <sup>c</sup> ±1.02	27.19 <sup>b</sup> ±1.01	28.33 <sup>b</sup> ±2.22	35.88 <sup>a</sup> ±1.83	34.35 <sup>a</sup> ±1.14

Data were presented as mean ± SD of three replicates (n=3) for each treatment. 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, this increase reached the significant level when compared to control (Table 4).

**Table 4: Inflorescences yield "at 150 DAT" of chamomile per plant sprayed with four different aqueous extracts during 2016/2017 and 2017/2018 successive seasons.**

Seasons	Yield	Foliar applications				
		Control	Roselle extract	Turmeric extract	Safflower extract	Red beet extract
2016/2017	no. of inflorescences plant <sup>-1</sup>	118.00 <sup>e</sup> ±6.0	184.00 <sup>d</sup> ±4.58	246.00 <sup>c</sup> ±6.0	279.33 <sup>a</sup> ±4.73	258.67 <sup>b</sup> ±3.79
	Inflorescences f.wt. plant <sup>-1</sup>	12.16 <sup>d</sup> ±0.43	19.56 <sup>c</sup> ±1.34	26.12 <sup>b</sup> ±0.83	29.51 <sup>a</sup> ±0.36	28.28 <sup>a</sup> ±0.60
	Inflorescences d.wt. plant <sup>-1</sup>	2.90 <sup>c</sup> ±0.2	4.46 <sup>b</sup> ±0.38	6.27 <sup>a</sup> ±0.09	7.14 <sup>a</sup> ±0.51	7.02 <sup>a</sup> ±0.89
2017/2018	no. of inflorescences plant <sup>-1</sup>	121.00 <sup>e</sup> ±2.65	181.67 <sup>d</sup> ±4.51	245.67 <sup>c</sup> ±5.03	281.00 <sup>a</sup> ±2.0	261.67 <sup>b</sup> ±3.06
	Inflorescences f.wt. plant <sup>-1</sup>	11.85 <sup>e</sup> ±1.10	19.96 <sup>d</sup> ±0.15	26.48 <sup>c</sup> ±0.56	31.57 <sup>a</sup> ±0.43	28.68 <sup>b</sup> ±1.18
	Inflorescences d.wt. plant <sup>-1</sup>	2.39 <sup>d</sup> ±0.02	3.68 <sup>c</sup> ±0.17	4.79 <sup>b</sup> ±0.2	6.60 <sup>a</sup> ±0.78	6.20 <sup>a</sup> ±0.15

Data were presented as mean ± SD of three replicates (n=3) for each treatment. 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 (5 and 6) showed significant increase ( $P \leq 0.05$ ), in most cases, in the tested chemical constituents of chamomile shoots sprayed with the four plant water extracts.

#### Chl a, Chl b and carotenoids

Foliar applications with red beet and safflower extracts induced markedly increase in Chl a (0.92 and 0.94), 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 season (Table 6).

Table (5): 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 <sup>-1</sup> f.wt.)	0.79 <sup>c</sup> ±0.001	0.85 <sup>c</sup> ±0.001	0.89 <sup>d</sup> ±0.001	0.94 <sup>b</sup> ±0.001	0.92 <sup>a</sup> ±0.009
Chl b (mg g <sup>-1</sup> f.wt.)	0.27 <sup>c</sup> ±0.01	0.30 <sup>b</sup> ±0.001	0.30 <sup>b</sup> ±0.001	0.34 <sup>a</sup> ±0.001	0.35 <sup>a</sup> ±0.001
Carotenoids (mg g <sup>-1</sup> f.wt.)	0.22 <sup>d</sup> ±0.001	0.23 <sup>c</sup> ±0.002	0.23 <sup>c</sup> ±0.003	0.24 <sup>b</sup> ±0.001	0.25 <sup>a</sup> ±0.001
Flavonoids mg (100g <sup>-1</sup> f.wt.)	25.65 <sup>d</sup> ±3.85	92.74 <sup>b</sup> ±1.005	115.4 <sup>a</sup> ±0.82	45.45 <sup>c</sup> ±2.75	42.39 <sup>c</sup> ±1.04
Phenolic compounds mg (100g <sup>-1</sup> f.wt.)	88.03 <sup>bc</sup> ±12.37	92.29 <sup>b</sup> ±5.59	114.07 <sup>a</sup> ±9.01	73.64 <sup>d</sup> ±3.37	77.47 <sup>cd</sup> ±1.03
RS (mg 100g <sup>-1</sup> f.wt.)	220.23 <sup>c</sup> ±1.05	295.28 <sup>d</sup> ±1.009	305.14 <sup>c</sup> ±0.95	429.22 <sup>a</sup> ±1.105	339.45 <sup>b</sup> ±1.05
AA (mg 100g <sup>-1</sup> f.wt.)	76.44 <sup>c</sup> ±0.09	77.44 <sup>cd</sup> ±1.098	78.25 <sup>c</sup> ±0.02	87.78 <sup>a</sup> ±1.10	82.57 <sup>b</sup> ±0.28
Data were presented as mean ± SD of three replicates (n=3) for each treatment. Means in the same column with different letters are significantly different at $P \leq 0.05$ .					

#### Flavonoids and Soluble phenolic compounds

Tables (5 and 6) showed also that exogenous applied turmeric extract elevated flavonoids (115.40 and 101.20 mg 100g<sup>-1</sup> f.wt.) and soluble phenolic compounds concentrations (114.07 and 103.76 mg 100g<sup>-1</sup> f.wt.) more than control ~~plants-plants~~ 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 both seasons when compared with control.

#### Reducing sugars and amino acids

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

Table (6): 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 constituents	Control	roselle extract	turmeric extract	safflower extract	red beet extract
Chl a (mg g <sup>-1</sup> f.wt.)	0.554 <sup>c</sup> ±0.01	0.602 <sup>d</sup> ±0.001	0.649 <sup>c</sup> ±0.003	0.853 <sup>a</sup> ±0.85	0.709 <sup>b</sup> ±0.002
Chl b (mg g <sup>-1</sup> f.wt.)	0.224 <sup>c</sup> ±0.002	0.245 <sup>d</sup> ±0.003	0.250 <sup>c</sup> ±0.001	0.291 <sup>a</sup> ±0.002	0.276 <sup>b</sup> ±0.003

Carotenoids (mg g <sup>-1</sup> f.wt.)	0.196 <sup>c</sup> ±0.001	0.205 <sup>d</sup> ±0.004	0.215 <sup>c</sup> ±0.003	0.235 <sup>a</sup> ±0.004	0.225 <sup>b</sup> ±0.002
Flavonoids mg (100g <sup>-1</sup> f.wt.)	15.32 <sup>c</sup> ±0.03	89.63 <sup>b</sup> ±0.08	101.2 <sup>a</sup> ±0.1	22.40 <sup>d</sup> ±0.05	39.66 <sup>c</sup> ±0.02
Phenolic compounds mg (100g <sup>-1</sup> f.wt.)	85.96 <sup>c</sup> ±0.025	89.12 <sup>b</sup> ±0.025	103.76 <sup>a</sup> ±0.23	55.76 <sup>c</sup> ±0.02	64.33 <sup>d</sup> ±0.02
RS (mg 100g <sup>-1</sup> f.wt.)	198.36 <sup>c</sup> ±0.03	215.46 <sup>d</sup> ±0.08	221.34 <sup>c</sup> ±0.01	295.24 <sup>a</sup> ±0.04	253.63 <sup>b</sup> ±0.08
AA (mg 100g <sup>-1</sup> f.wt.)	65.48 <sup>c</sup> ±1.22	71.67 <sup>d</sup> ±1.27	77.39 <sup>c</sup> ±1.73	99.74 <sup>a</sup> ±0.03	87.73 <sup>b</sup> ±1.12
Data were presented as mean ± SD of three replicates (n=3) for each treatment. 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<sup>-1</sup> d.wt.) and flavonoids (1282 and 773 mg 100g<sup>-1</sup> d.wt.) in chamomile inflorescences followed by roselle or safflower extracts in comparing with control, while spraying with turmeric extract led to reduce both phenols (483 and 420 mg 100g<sup>-1</sup> d.wt.) and flavonoids concentrations (485 and 296 mg 100g<sup>-1</sup> d.wt.) than control at both seasons, respectively (Tables 7 and 8).

Carotenoids concentration in chamomile inflorescence highly increased when safflower extract sprayed on plants which reached (40.64 and 37.48) mg 100g<sup>-1</sup> d.wt. followed by red beet extract (36.78 and 34.01) mg 100g<sup>-1</sup> d.wt. which also showed significantly increase in carotenoids concentration more than control (28.94 and 27.55) mg 100g<sup>-1</sup> d.wt. at both seasons, respectively.

**Table (7): 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 <sup>b</sup> ±10	569 <sup>b</sup> ±10	483 <sup>d</sup> ±9	538 <sup>c</sup> ±1	601 <sup>a</sup> ±5.29
Flavonoids (mg /100g d.wt.)	574 <sup>d</sup> ±4.36	712 <sup>c</sup> ±10	485 <sup>c</sup> ±2	942 <sup>b</sup> ±1	1282 <sup>a</sup> ±9.5
Carotenoids (mg/100g d.wt.)	28.94 <sup>c</sup> ±0.01	32.56 <sup>d</sup> ±0.01	33.45 <sup>c</sup> ±0.01	40.64 <sup>a</sup> ±0.01	36.78 <sup>b</sup> ±0.01
Oil yield (ml/100g d.wt.)	0.73 <sup>c</sup> ±0.01	2.07 <sup>b</sup> ±0.01	1.19 <sup>d</sup> ±0.01	2.43 <sup>a</sup> ±0.01	1.22 <sup>c</sup> ±0.01
IC 50 of oil (µg ml <sup>-1</sup> )	14.36 <sup>b</sup> ±0.03	35.82 <sup>d</sup> ±0.02	37.87 <sup>c</sup> ±0.026	20.74 <sup>c</sup> ±0.025	0.638 <sup>a</sup> ±0.002
IC 50 of ethanolic extract of inflorescences (mg ml <sup>-1</sup> )	1.465 <sup>d</sup> ±0.01	1.049 <sup>c</sup> ±0.01	2.205 <sup>c</sup> ±0.001	0.349 <sup>a</sup> ±0.01	0.875 <sup>b</sup> ±0.01

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

**Table (8): 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 <sup>c</sup> ±2	515 <sup>b</sup> ±10	420 <sup>d</sup> ±4.58	525 <sup>b</sup> ±10	563 <sup>a</sup> ±1
Flavonoids (mg /100g d.wt.)	402 <sup>c</sup> ±10	592 <sup>b</sup> ±10	296 <sup>d</sup> ±3.6	762 <sup>a</sup> ±10	773 <sup>a</sup> ±11
Carotenoids (mg/100g d.wt.)	27.55 <sup>c</sup> ±0.01	31.32 <sup>d</sup> ±0.01	31.98 <sup>c</sup> ±0.01	37.48 <sup>a</sup> ±0.01	34.01 <sup>b</sup> ±0.01
Oil yield (ml/100g d.wt.)	0.58 <sup>e</sup> ±0.01	2.08 <sup>b</sup> ±0.01	1.12 <sup>c</sup> ±0.01	2.25 <sup>a</sup> ±0.01	0.801 <sup>d</sup> ±0.001
IC 50 of oil (µg ml <sup>-1</sup> )	20.61 <sup>b</sup> ±0.02	60.26 <sup>d</sup> ±0.06	88.83 <sup>c</sup> ±0.07	25.52 <sup>c</sup> ±0.15	0.856 <sup>a</sup> ±0.004
IC 50 of ethanolic	1.345 <sup>d</sup> ±0.016	0.939 <sup>c</sup> ±0.01	1.512 <sup>a</sup> ±0.01	0.235 <sup>c</sup> ±0.01	0.778 <sup>b</sup> ±0.01



extract of  
inflorescences (mg  
ml<sup>-1</sup>)

Data were presented as mean  $\pm$  SD of three replicates (n=3) for each treatment. Means in the same column with different letters are significantly different at  $P \leq 0.05$ .

### 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<sup>-1</sup> 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 (7 and 8).

### Biochemical constituents of chamomile essential oil

GLC-MS analysis improved that chamomile essential oil contains 16 compounds *viz*, artimisia ketone, artimisia alcohol, iso-borneol, trans –  $\beta$ - farnesene germacrene D, germacrene B, cadinene, saphthulenol, farnesene epoxide, tau-cadinol,  $\alpha$  bisabolol oxide B, caryphylene oxide, bisbolone oxide,  $\alpha$  bisabolol, chamazulene and bisabolol oxide A (Tables 9 and 10). The main components of chamomile volatile and essential oil as detected by GC-MS were bisabolol oxide A,  $\alpha$  bisabolol oxide –B, trans –  $\beta$ - farnesene, chamazulene, bisabolone oxide organized by concentration for the two growing seasons.

$\alpha$  Bisabolol 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 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 10).

At the first season, artimisia ketone, artimisia alcohol, trans –  $\beta$ - 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.  $\alpha$  Bisabolol was not detected with foliar spray of roselle, turmeric and safflower extracts at the first season, while it detected with roselle and turmeric extracts 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 (9): Effect of foliar application with plant aqueous extracts on the chemical composition of essential oil in chamomile inflorescences and their percentages during the season of 2016 / 2017.**

First season 2016 / 2017						
Foliar application treatment						
Chemical constituents	Retention time ( R <sub>t</sub> )	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- $\beta$ -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	0.2	0.2	0.15
Cadinene	26.84	-	0.18	-	0.22	-
Sapthulenol	29.39	0.55	0.51	0.7	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
$\alpha$ -Bisabolol oxide B	32.20	7.81	1.91	11	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	1.32	1.82
$\alpha$ bisabolol	33.59	0.25	-	-	-	0.27
Chamazulene	35.24	2.71	1.44	0.25	1.09	0.38
$\alpha$ Bisabolol oxide A	35.86	78.64	89.25	80.74	90.87	90.16

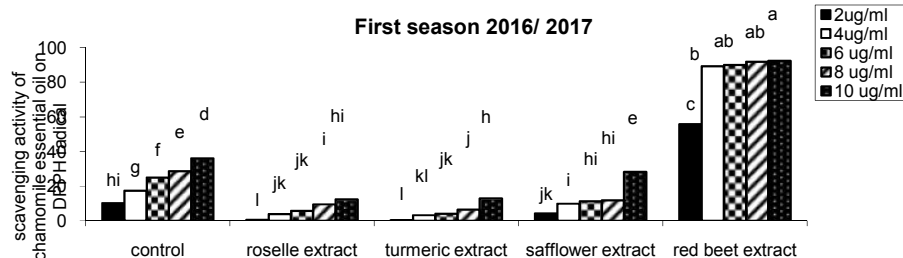
Table (10): Effect of foliar application with plant aqueous extracts on the chemical composition of essential oil in chamomile inflorescences and their percentages during the season of 2017/ 2018.

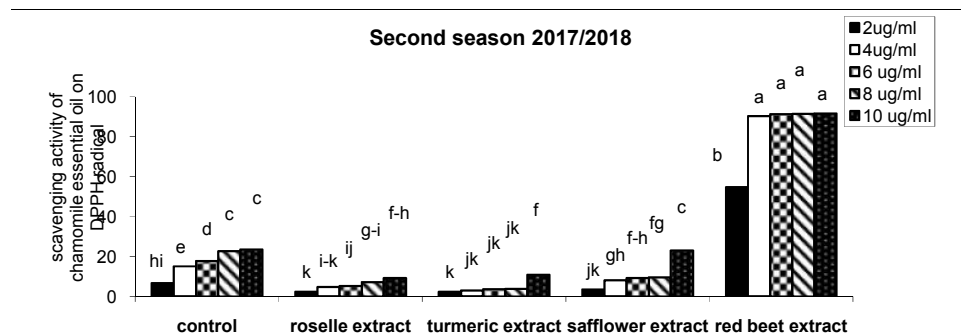
Chemical constituents	Retention time (R <sub>i</sub> )	Foliar application treatment				
		control	roselle extract	turmeric extract	safflower extract	red beet extract
Artimisia ketone	8.4	0.72	0.2	1.55	-	0.4
Artimisia alcohol	9.16	0.32	-	0.39	-	0.5
iso borneol	12.99	0.18	0.06	0.24	0.29	0.49
trans- $\beta$ -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	1.4	0.61
$\alpha$ -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
$\alpha$ bisabolol	33.59	0.12	0.08	0.09	-	0.19
Chamazulene	35.24	2.14	2.23	0.36	0.63	0.19
$\alpha$ Bisabolol oxide A	35.86	84.48	88.48	78.88	90.04	90.02

### Scavenging activity of essential oil and ethanolic extract of chamomile inflorescences

Increasing the concentration of chamomile essential oil accompanied with increasing scavenging activity on DPPH radical in all treatments (Figs. 1-2). Essential oil from plants treated with red beet extract had the highest scavenging activity of DPPH radical and lowest value of IC<sub>50</sub> (0.638 and 0.856  $\mu\text{g ml}^{-1}$  oil) during 2017 and 2018, respectively, in comparison with other treatments (Fig.1 and Tables 9 & 10).

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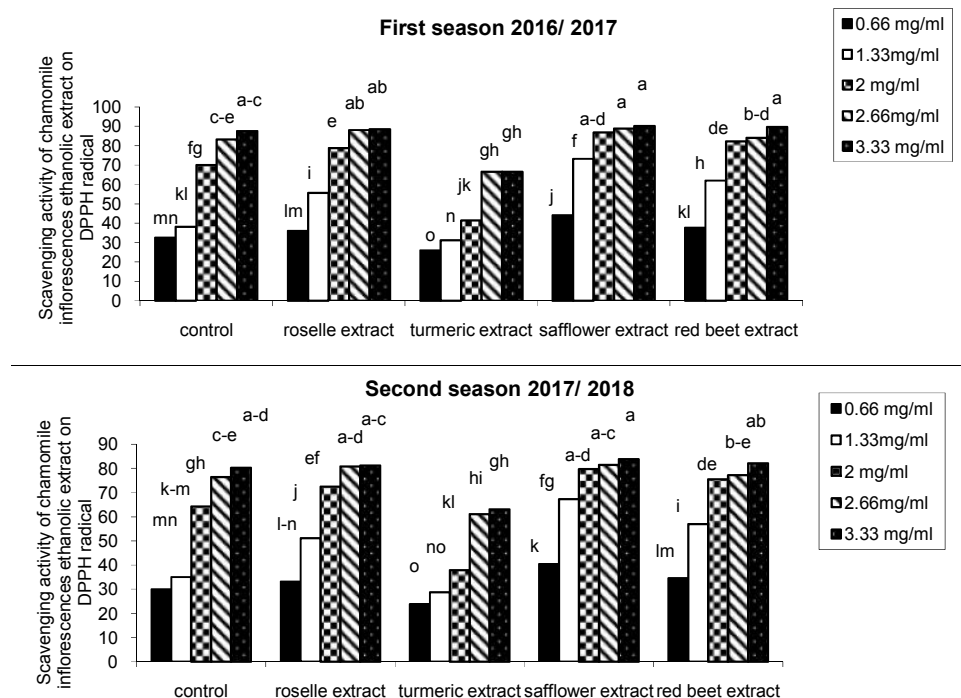


**Figure (1): Effect of foliar application of plant aqueous extracts on scavenging activity of chamomile essential oil on DPPH radical during the two seasons.**

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 water 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 shown in Fig. 2 and Tables 9 & 10.

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**Figure (2): Effect of foliar application of plant aqueous extracts on scavenging activity of chamomile inflorescences ethanolic extract on DPPH radical during the two seasons.**

## Discussion

In this study, spraying plant water 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 phenols 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.* (2011)** who stated that red beet contained high concentrations of phenols, flavonoids,  $\beta$ -cyanins and  $\beta$ -xanthins beside the presence of sugars and protein that naturally exist in red beet. Also, **Al Surmi *et al.* (2016)** recorded high concentrations of soluble phenols, amino acids and N, P, K ratios in safflower extract while roselle leaves and calyx contained phenols, flavonoids and anthocyanins that act as antioxidants (**Abdellatif and Ibrahim, 2018**). **Okereke *et al.* (2015)** 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-  $\beta$ -carotene (**Bruneton, 2001**). Spraying apple trees with turmeric extract increased leaf nitrogen, phosphorus and potassium concentrations. **Hadethi *et al.*, 2016**). –They attributed this increase to the importance of turmeric extract which contains potassium salt as found in this study. The physical properties in term of pH and titratable acidity in the present study were recommended with **Ibrahim *et al.* (2013)** who found that roselle extract has a comparatively high acidic to cause lower pH in the extract compared to water without extract. They attributed the high acidity of roselle to its natural constituents of organic acids such as citric acid, mallic acid and 3- andolyl 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 remarkably 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 (**Grant and Sallons, 1964**). Foliar spraying of pear tree "Le-Conte cv." with roselle, cinnamon and ginger water extracts gave the best fruits weight, fruit yield and fruits number per tree and increase total soluble solids and total fruit sugars % in comparison to control (**Abd-El-Latif *et al.*, 2017**). Based on that, using extract of roselle improved the nutritional status, yield and physio-biochemical characteristics of Valencia orange fruits (**Ahmed *et al.*, 2013**). 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. 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 (**Peter, 1999**). The positive action of turmeric extract was shown on Thompson seedless grapevines fruit characters (**Ammon and Wehl, 1991**). They ascribed that to the higher content of eugenol,

limonene, thiamin, niacin, protocatechuic acid, turmerone- caffeic acid, ascorbic acid- carotene- curcumin- coumaric acid- methoxycinnamic acid, vanillic acid- riboflavin- terpineol- cymene zingiberene- squiphellandrene, bomeal and curdioen that act as antioxidants. Also, **Armanious (2014)** 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.* (2006)** 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 (**Kowalczyk and Zielony, 2008**). Genetic analyses have approved extensive interaction between total sugars and plant hormones signaling activation (**Rolland *et al.* 2006**). Also, it was proved that accumulation of high levels sugars in many plant species promote 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 (**Ohto *et al.* 2001**). The rapid pulse of sucrose translocation in phloem increased cell division in shoot apical meristem during the floral evocation (**Bernier *et al.* 1993**).

Applied plant water 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.*, (2014)** 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<sub>2</sub>O<sub>2</sub> formation in the cell, which involved in abscisic acid transmitting signals. ABA accelerated stomatal closure that limits the assimilation of CO<sub>2</sub> and affected photosynthesis process. External application of antioxidants can reserve the stomatal closure (**Chen and Gallie, 2004**). Furthermore, **Cushman (2001)** 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.* (2015)** 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.*(2015) 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 (**Taiz and Zeiger, 2002**).

When biochemical analysis carried out in the inflorescences produced from plants sprayed with 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 is suggested that the reason for this yield increase 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 is compatible with **Figueiredo *et al.* (2008)** 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 **Zheljaskov *et al.* (2010)** showed similar results for the quantity and quality of the essential oil, where the treatments with aqueous extracts of absinth worm wood, lavender and wild bergamot led to increase the oil yield and the essential oil components of Native spearment.

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 (2006)** stated that active principle components in chamomile essential oil are  $\alpha$  bisabolol oxide A and B and chamazulene-. The main constituents in the chamomile essential oil were chamazulene (19.9%),  $\alpha$  bisabolol (20.9%), bisabolol- A and B oxides (21.6% and 1.2% ) and  $\beta$ -farnesene (3.1%), while the minor constituents were  $\alpha$  and  $\beta$ -caryophyllene, caryophyllene oxide- and spathulenol-. Also, there were some monoterpenes such as  $\beta$ -phellandrene (0.8%), limonene (0.8%),  $\beta$ -ocymene (0.4%) and  $\alpha$ -terpinene (0.2%) (**Costescu *et al.*, 2008**). German chamomile volatile oil has 5% chamazulene and 50%  $\alpha$  bisabolol oxide A (**Sharafzadeh and Alizadeh,2011**). Also **Stanojevic *et al.*(2016)** mentioned that chamomile essential oil contains the highest value of  $\beta$  farnesene (29.8%)-,  $\alpha$  bisabolol and its oxide (15.7%)  $\alpha$ -farnesene (9.3%), chamazulene (6.4%) and



germacrene D (6.2%). **Zheljazkov (2013)** reported that the sage brush and juniper water extracts increased the concentrations of  $\beta$ -caryophyllene and trans-  $\beta$  -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.*(2018)** in chamomile essential oil which found to contain 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  $\alpha$ -bisabolol oxide A which exhibited the higher antioxidant potential with lower  $IC_{50}$  compared to  $\beta$ -farnesene and  $\alpha$ -bisabolol. This is fully consistent with the results obtained in this study where  $\alpha$ -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. Previous antioxidant activity studies on chamomile essential oil showed higher antioxidant properties with  $IC_{50}$  value of 2.07mg/l after 90 min of incubation with DPPH free radical (**Stanojevic *et al.*, 2016**). **Agatonovic-Kustrin *et al.* (2015)** reported that chamomile flower heads and leaves had the most prominent antioxidant activities which  $\alpha$  bisabolol and its oxide (15.7%), apigenin and chamazulene being the most effective antioxidants. According to litteratures, **Capuzzo *et al.*- (2014)** stated that it is impossible to estimate chamazulene radical scavenging capacity by reacting with DPPH, owing this to the interference with nitrogen- centered DPPH.

### Conclusion

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

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 most desirable yield of chamomile.

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