

## The influences of Bio-stimulator Compounds on growth, essential oil and chemical composition of chamomile plants grown under water stress

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**Abstract:** This study was conducted at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, during the two successive seasons of 2013/2014 and 2014/2015. This work aimed to investigate the effect of water stress and bio-stimulator on the vegetative growth, flower yield, essential oil production and chemical compositions of Chamomile (*Matricaria chamomilla* L.). Water stress was imposed by three different irrigation intervals (4, 8 and 12 days). The plants received different irrigation intervals were sprayed every 2 weeks with **bio-stimulator** of either Amino Suam or Setter-2 at concentration of 500, 1000 and 2000 ppm for each one, while the control plants were sprayed with tap water. The experimental units were designed in randomized complete block design (RCBD) with 21 treatments and three replications for **everyone** treatment. The results showed that, highest values of plant height, number of branches/plant, diameter of flower heads, essential oil percentage, total chlorophylls content and total carbohydrates percentage were obtained from irrigation interval of 8 days as compared to 4 or 12 days. Increasing irrigation intervals from 4 to 8 or 12 days resulted in a significant reduction in **the stem** diameter, fresh and dry weights of the herb, number of flower heads/plant, fresh and dry weights of flower heads, essential oil yield, as well as N, P and K% in dried herb while proline content was increased with increasing intervals. On the other hand, in most cases all the studied parameters were increased significantly as a result of spraying **plants** with either **concentration** of Amino Suam or Setter-2 compared to control plants. Regarding the effect of interaction between the two studied factors, the results indicate that in most cases, plants irrigated every 4, 8 and 12 days and sprayed with any concentration of bio-stimulators (Amino Suam or Setter-2) had the values for all the studied parameters which were significantly higher than those of control plants.

Concerning essential oil composition,  $\alpha$ -bisabolol oxide A,  $\alpha$ -bisabolol oxide B, farnesene, bisabolone oxide,  $\alpha$ -bisabolol and Chamazulene were the six major compounds of Chamomile oil. The highest percent of  $\alpha$ -bisabolol oxide A (53.50%) were obtained from plants irrigated every 12 days and sprayed with Setter-2 at concentration of 2000 ppm and the highest percent of  $\alpha$ -bisabolol oxide B (16.99%), farnesene (12.55%) and bisabolone oxide (12.03%) were resulted from plants irrigated every 8 days and sprayed with Amino Suam at 1000 ppm, while the highest percent of  $\alpha$ -bisabolol (6.36%) and Chamazulene (4.37%) were obtained from plants irrigated every 4 days and sprayed with Amino Suam at 2000 ppm.

**Key words:** *Matricaria chamomilla*, water stress, Bio-stimulator, Amino suam, Setter-2.

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## 1. Introduction:

German chamomile (*Matricaria chamomilla* L., syn. *Chamomilla recutita* (L.) Rauschert), belongs to the family Asteraceae. Chamomile is an annual herbaceous plant, native of south and east Europe and it has spread through most of Europe and has extended further to North Africa, Asia, north and South America, Australia and New Zealand. Chamomile is one of the important medicinal plant species; its dried flowers have been used in herbal remedies for thousands of years as an old age remedy, known in ancient Egypt, Greece and Rome. The flowers accumulate blue essential oil from 0.2 to 1.9% which finds a variety of uses. The chamomile drug is included in the pharmacopeia of 26 countries. Pharmacological properties include anti-inflammatory, antiseptic, carminative, healing, sedative, antispasmodic and mildly sudorific (Singh *et al.*, 2011).

Recently, water scarcity or limited water resources is among the reasons that force many countries to reduce irrigation applications, so the farmer in such conditions of reduced water supply, tends to increase the irrigation interval which creates water stress (Jain *et al.*, 2000). Water stress or drought is the major environmental factors, as abiotic stress, limiting productivity of crop and medicinal plants (Abdelmajeed *et al.*, 2013). Water supply at adequate amount plays vital roles for the different physiological and biochemical processes such as photosynthesis, respiration, transpiration, translocation, stomatal closure, ion uptake, carbohydrates, nutrient metabolism, enzyme reaction and cell turgidity occurs simultaneously. However, water stress reduces plant growth promoters by its effect on such processes (Flexas and Medrano, 2002, Jaleel *et al.*, 2008, and Abdelmajeed, 2013). Water stress induces several changes in various physiological, biochemical and molecular components of photosynthesis either through pathway regulation by stomatal closure and decreasing flow of CO<sub>2</sub> into mesophyll tissue (Flexes *et al.*, 2004). It has been found that the water deficit may lead to cause significant physiological and biochemical changes in different species of medicinal and aromatic plants, such as a reduction in the growth and yield (Aziz *et al.*, 2008, Razmjoo *et al.*, 2008 and Moosavi *et al.*, 2014), reductions in total chlorophyll and carbohydrate contents, as well as increasing in prolin content (Pirzad *et al.*, 2011 and El-Mekawy *et al.*, 2013). Moreover, prolonging the irrigation intervals also may affect the volatile oil composition, oil percentage and oil yield.

Natural bio-stimulators has recently become extensively used in modern agricultural production, carried out to intensify the quantity and improve the quality of crop yield, in addition to ensure safe both for human beings and for the environment (Poincelot, 1993,

Dhargalkar and Pereira, 2005). The active ingredients of most currently used bio-stimulators are amino acid substances compounds of elements, vitamins, hydrolyzed proteins, triacotanol, humic acids, alga extracts and brassinolides (Mandal *et al.*, 2002). It was found that, applied bio-stimulators containing amino acids, vitamins and/or macro and micro elements had a positive effect on physiological and biochemical changes in some medicinal and aromatic plants such as an increase in the growth and yield (Jelačić *et al.*, 2007, Sarojnee *et al.*, 2009 and Golzadeh *et al.*, 2012), higher chlorophyll content (Nia *et al.*, 2014), higher carbohydrates content (Rafiee *et al.*, 2013), higher uptake and accumulation of nutrients (Gomaa, 2008), increasing in prolin content (Smolik *et al.*, 2013), as well as increasing oil percentage and oil yield (Haj Seyed Hadi and Darzi, 2014). Additionally, there are some reports that application of bio-stimulant compounds can be increasing the quantitative and qualitative yield of crops in addition to alleviate the adverse effect of environmental stresses such as salinity (Gawronska *et al.*, 2008, Omer *et al.*, 2013) and water stress or drought (Hammad and Ali, 2014).

There are no enough available data as regards evaluating the effect of bio-stimulator compounds and its possibility using for alleviating the adverse impact of water stress on chamomile plants. Therefore, the objective of this research was to determine the influence of Amino Suam and Setter-2 as neutral bio-stimulator substances on the performance of chamomile plants grown under different irrigation intervals.

## 2. Materials and methods:

This study was conducted at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, during the two successive seasons of 2013/2014 and 2014/2015. This work aimed to investigate the effect of different irrigation intervals and foliar application of two commercial bio-stimulators (Amino Suam and Setter-2) on Chamomile (*Matricaria chamomilla* L.).

### 2.1. Experimental procedure

Seeds of *Matricaria chamomilla* L. plants were kindly produced from the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Egypt. On 1<sup>th</sup> of September (in the first and second seasons, respectively), the seeds were sown in the nursery at the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza. After 45 days old

from seeds sowing (on the 15<sup>th</sup> of October, 2013/2014 and 2014/2015, in both seasons, respectively), uniform seedlings, with an average plant height of 13-15 cm, were transplanted to 30 cm diameter plastic pots filled with a mixture of clay + sand (2:1: v/v), each pot had one seedling. The physical and chemical characteristics of soil mixture are presented in Table 1.

Table (1): The physical and chemical characteristics of soil mixture used for growing *Matricaria chamomilla* during 2013/2014 and 2014/2015 seasons.

Physical characteristics							
Soil texture	Clay (%)	Coarse sand (%)	Fine sand (%)	Silt (%)	Field capacity (% V)		
Clay loam	38.9	15.7	26.3	19.1	50.7		
Chemical characteristics							
Macro-nutrients (ppm)			Organic matter	CaCO <sub>3</sub>	EC	CEC (meq/100 g)	
N	P	K	(%)	(%)	(dS/m)		
66.22	12.67	59.64	1.74	1.70	1.72		
						39.40	pH
							7.15

The treatments were initiated after 15 days from the transplanting; the plants were irrigated every 4, 8 and 12 days for imposing water stress. On the 31<sup>st</sup> of October till 15<sup>th</sup> March (in both seasons), the plants received different irrigation intervals were sprayed every 2 weeks (about 10 times) with either Amino Suam<sup>®</sup> [commercial name, consists of amino acids (10000 mg/100 mL Amino Suam) including (glycine, alanine, valine, methionine, isoleucine, threonine, cysteine, phenylalanine, serine, lysine, glutamic, aspartic acid, arginine, hydroxyproline, proline, hydroxylysine and tryptophan) as well as Mg (6000 mg/100 mL Amino Suam) ], or Setter-2 [commercial name, consists of 0.5% ascorbic acid, 0.5% citric acid, 5% total N, 0.1% Mn, 0.1% Cu, 9% chelated Ca<sup>++</sup> and 1.5% chelated Boron]. The concentrations of Amino Suam and Setter-2 were 500, 1000 and 2000 ppm for each bio-stimulator. In addition, the untreated control plants continued to be sprayed with tap water. Both of commercial products were obtained from Union for Agriculture Development Co., UAP, Egypt. Wetting agent (Bio-new film at 1 ml /L) was added to freshly prepared solutions of bio-stimulator and the plants foliage were sprayed until run off point using plastic atomizer. The common cultural practices, including hand picking of weeds, as well as fertilization using life green (NPK, 20-20-20), which was applied monthly at the rate of 2.5 g/pot was also performed.

The layout of the experiment was a randomized complete block design with 21 treatments [3 irrigation intervals x 7 plant bio-stimulators concentrations (including the control)], with 3 blocks (replicates), each block consisting of 84 plants (4 plants/treatment).

## 2.2. Measurement of vegetative growth and flowering parameters:

Vegetative growth parameters were recorded once at about 105 days from transplanting (On 1<sup>st</sup> February in both seasons respectively), Two samples of plants were randomly taken from each replicate to determine the following parameters: plant height (cm), number of branches/plant, stem diameter (mm, at 5 cm above the soil surface) and fresh and dry weights of the herb (g/plant). Whereas, flowering parameters were continuously recorded every 15 days (about 8 times), during the flowering stage (from 15<sup>th</sup> of December till 30<sup>th</sup> of March in both seasons, respectively). The flower heads were collected at early morning, and the flowering parameters including, number of flower heads/plant, fresh and dry weights of flower heads (g/plant) as well as the diameter of flower heads (mm) were determined with each harvest/replicate and the cumulative measurements were used to calculate means values of studied parameters. Diameter of flower heads was measured by recording the diameter average of 20 flowers/plant at each flower harvesting.

## 2.3. Extraction and analysis of essential oil:

For determination of essential oil, collected flower heads air- dried in the shade, then they were combined together to determine the essential oil content for each replicate by steam hydro-distillation according to Guenther (1961) and British Pharmacopeia (1980). The extracted essential oil was measured and oil percentage as well as oil yield (g/plant) was calculated as follows:

$$\text{Oil \%} = \text{oil volume in the graduated tube} / \text{dry weight of flower heads (25 g)} \times 100$$

$$\text{Oil yield (g/plant)} = (\text{Oil percentage} \times \text{dry weight of flower heads}) / 100$$

The obtained oil was dehydrated by anhydrous Na<sub>2</sub>SO<sub>4</sub> and stored in the freezer until analysis its constituents. Samples of the essential oil obtained in the second season were analyzed using DsChrom 6200 Gas Liquid Chromatography equipped with a flame ionization detector for separation of volatile oil constituents. The analysis conditions were as follows.

The chromatograph apparatus was fitted with capillary column BPX-5, 5% phenyl (equiv.) polysilphenylene- siloxane 30m x 0.25mm ID x 0.25µm film. Temperature program ramp increase with a rate of 1° C / min from 70° to 80° C, rate of 5° C / min from 80° to 120° C and rate of 10° C / min from 120° to 190° C. Flow rates of gases were nitrogen at 1 ml / min, hydrogen at 30 ml / min and 330 ml /min for air. Detector and injector temperatures were 300° C and 250° C, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of the main components of volatile oil.

## 2.4. Determination of chemical constituents:

Total chlorophylls in fresh leaf samples were determined by using chlorophyll meter (Model SPAD 502) as described by Netto *et al.* (2005). In addition, the total carbohydrates content (% of dry matter) was determined in dried herb samples block the procedure of Dubois *et al.* (1956). Other dried herb samples were digested to extract nutrients (as described by Piper, 1947), and the extract was chemically analyzed to determine its contents of nitrogen, phosphorus and potassium (% of dry matter) according to the methods described by Cottenie *et al.* (1982). Proline content in dried herb ( $\mu$  moles /g dry matter of herb) was also determined to use the method recommended by Bates *et al.* (1973).

**2.5. Statistical analysis:** The average of the obtained data of the two seasons was subjected to statistical analysis according to the procedure of Steel and Torrie (1980). Least Significant Difference (L.S.D.) test at the 5% level was calculated to compare the mean values of determined criteria for the different treatments.

### 3. Results and discussion

#### 3.1. Vegetative growth and flowering parameters

The results recorded on *Matricaria chamomilla* L. plants (Tables 2 - 4) show that the studied growth and flowering parameters were significantly affected by the duration of irrigation intervals. In both seasons, plant height, number of branches/plant as well as the diameter of flower heads tended to increase with increasing irrigation intervals till reached its maximum significant values under the moderate irrigation interval (8 days), followed by significant decrease under the longest irrigation interval (12 days) which resulted in the lowest values, compared with the shortest irrigation interval (4 days). These results are agreement with the findings Khalil *et al.* (2010) on *Ocimum basilicum* L. However, the recorded data indicate that prolonging the irrigation intervals from 4 to 8 or 12 days resulted in a steady significant reduction in stem diameter, fresh and dry weights of herb, number of flower heads/plant as well as fresh and dry weights of flower heads. Accordingly, the highest mean values were obtained from plants irrigated with the shortest intervals (4 days), whereas the lowest values were obtained from plants irrigated with the longest intervals (12 days). Similar reductions in the growth and flowering parameters a result of prolonging irrigation intervals were obtained by Fatima *et al.*, (2002) on *Cymbopogon martini* L. and *C. winterianus* J., Narayanappa *et al.* (2004) on *Artemisia pallens* W., Aziz *et al.* (2008) on *Thymus vulgaris* L., Razmjoo *et al.* (2008) on *Matricaria chamomile* L., Said-Al Ahl *et al.* (2009) on oregano (*Origanum vulgare*



L.), Said-Al Ahl and Abdou (2009) on dragonhead (*Dracocephalum moldavica* L.), Asrar and Elhindi (2011) on *Tagetes erecta* L., Kharadi *et al.* (2011) on *Plumbago zeylanica* L.,

**Table 2.** Effect of irrigation intervals and bio-stimulator treatments on plant height (cm), number of branches/plant and stem diameter (mm) of *Matricaria chamomilla* during the 2013/2014 and 2014/2015 seasons.

*Bio-stimulator Treatments (B)	First season (2013/2014)				Second season (2014/2015)			
	Irrigation intervals (I)			Mean (B)	Irrigation intervals (I)			Mean (B)
	4 days	8 days	12 days		4 days	8 days	12 days	
Plant height (cm)								
Control	55.33	56.67	47.67	53.22	52.00	54.00	47.00	51.00
A1	59.67	66.00	52.00	59.22	55.00	63.67	52.67	57.11
A2	66.00	72.33	52.00	63.44	62.33	72.67	55.67	63.56
A3	61.33	67.67	57.33	62.11	63.33	70.00	56.33	63.22
S1	64.00	72.00	58.67	64.89	65.33	77.00	58.00	66.78
S2	71.00	72.00	61.67	68.22	67.67	73.00	63.33	68.00
S3	71.67	76.00	67.00	71.56	67.00	76.67	63.00	68.89
Mean (I)	64.14	68.95	56.62	-----	61.81	69.57	56.57	-----
L.S.D. (0.05)								
I	1.10				1.13			
B	1.69				1.72			
I X B	2.92				2.98			
Number of branches/plant								
Control	7.33	8.33	6.67	7.44	7.67	8.00	7.00	7.56
A1	13.00	11.67	9.33	11.33	13.00	12.00	8.67	11.22
A2	11.33	14.33	10.67	12.11	11.67	14.33	9.67	11.89
A3	12.33	13.67	9.00	11.67	12.33	14.67	9.33	12.11
S1	12.33	13.67	8.33	11.44	12.33	15.00	8.67	12.00
S2	12.67	14.33	9.00	12.00	12.33	15.00	9.00	12.11
S3	14.00	14.33	8.00	12.11	13.00	14.33	8.33	11.89
Mean (I)	11.86	12.90	8.71	-----	11.76	13.33	8.67	-----
L.S.D. (0.05)								
I	0.41				0.32			
B	0.62				0.49			
I X B	1.07				0.84			
Stem diameter (mm)								
Control	6.60	7.24	6.56	6.80	7.53	7.37	6.22	7.04
A1	7.95	8.87	7.41	8.08	8.46	7.69	7.32	7.82
A2	8.87	8.86	9.54	9.09	10.23	9.91	8.53	9.56
A3	12.69	13.00	11.62	12.44	13.17	12.25	11.77	12.39
S1	12.33	10.67	11.15	11.39	12.34	11.76	11.99	12.03
S2	12.30	11.27	11.61	11.73	11.84	12.08	11.69	11.87
S3	13.74	12.42	11.70	12.62	12.98	11.58	11.71	12.09
Mean (I)	10.64	10.33	9.94	-----	10.94	10.38	9.89	-----
L.S.D. (0.05)								
I	0.28				0.22			
B	0.43				0.34			
I X B	0.75				0.59			

\*A1= Amino Suam at 500 ppm A2= 1000 ppm A3= 2000 ppm  
S1= Setter-2 at 500 ppm S2= 1000 ppm S3= 2000 ppm

**Table 3.** Effect of irrigation intervals and bio-stimulator treatments on fresh and dry weights of the herb (g/plant) and diameter of flower heads (mm) of *Matricaria chamomilla* during the 2013/2014 and 2014/2015 seasons.

*Bio-stimulator Treatments (B)	First season (2013/2014)				Second season (2014/2015)			
	Irrigation intervals (I)			Mean (B)	Irrigation intervals (I)			Mean (B)
	4 days	8 days	12 days		4 days	8 days	12 days	
Fresh weights of herb (g/plant)								
Control	45.3	38.5	34.6	39.5	46.6	41.5	32.3	40.1
A1	70.7	62.4	57.5	63.5	65.4	55.7	46.5	55.9
A2	91.7	81.2	67.6	80.2	99.1	83.9	75.8	86.3
A3	90.4	85.6	70.9	82.3	105.2	90.7	78.5	91.5
S1	91.7	101.2	69.8	87.6	97.9	106.1	85.4	96.5
S2	118.6	100.9	73.4	97.6	79.9	98.6	94.0	90.8
S3	113.5	100.4	75.1	96.3	107.6	96.3	91.4	98.4
Mean (I)	88.8	81.5	64.1	-----	86.0	81.8	72.0	-----
L.S.D. (0.05)								
I	1.9				5.7			
B	2.9				8.7			
I X B	5.1				15.0			
Dry weights of herb (g/plant)								
Control	10.1	7.8	6.5	8.2	10.6	7.6	6.4	8.2
A1	18.4	16.5	13.1	16.0	17.9	16.5	14.4	16.3
A2	24.9	20.7	15.9	20.5	25.5	20.4	15.9	20.6
A3	25.9	22.1	17.6	21.9	26.5	19.6	16.6	20.9
S1	25.5	29.2	18.4	24.4	26.6	28.1	17.8	24.1
S2	33.4	28.5	20.5	27.5	33.9	29.1	18.8	27.2
S3	30.8	26.5	18.2	25.2	31.1	28.0	18.2	25.8
Mean (I)	24.1	21.6	15.7	-----	24.6	21.3	15.4	-----
L.S.D. (0.05)								
I	0.6				0.4			
B	0.9				0.6			
I X B	1.6				1.0			
Diameter of flower heads (mm)								
Control	10.52	9.95	9.15	9.87	9.88	10.21	9.45	9.85
A1	11.28	11.86	11.45	11.53	10.93	11.78	11.31	11.34
A2	16.85	18.04	17.91	17.60	18.61	19.22	19.77	19.20
A3	23.89	22.05	21.21	22.38	20.01	21.45	21.81	21.09
S1	17.64	15.91	17.66	17.07	17.72	20.77	18.85	19.12
S2	15.60	24.03	18.61	19.41	17.57	18.91	16.52	17.67
S3	21.94	21.53	21.70	21.72	25.46	21.77	21.45	22.89
Mean (I)	16.82	17.62	16.81	-----	17.17	17.73	17.02	-----
L.S.D. (0.05)								
I	0.43				0.43			
B	0.66				0.65			
I X B	1.14				1.12			

\*A1= Amino Suam at 500 ppm A2= 1000 ppm A3= 2000 ppm

S1= Setter-2 at 500 ppm S2= 1000 ppm S3= 2000 ppm



**Table 4.** Effect of irrigation intervals and bio-stimulator treatments on a number of flower heads/plant, fresh and dry weights of flower heads (g/plant), of *Matricaria chamomilla* during the 2013/2014 and 2014/2015 seasons.

*Bio-stimulator Treatments (B)	First season (2013/2014)				Second season (2014/2015)			
	Irrigation intervals (I)			Mean (B)	Irrigation intervals (I)			Mean (B)
	4 days	8 days	12 days		4 days	8 days	12 days	
	Number of flower heads/plant							
Control	37.67	36.13	24.47	32.76	38.93	38.87	26.93	34.91
A1	44.13	42.47	35.00	40.53	43.87	44.33	34.67	40.96
A2	50.20	43.07	37.87	43.71	45.67	45.80	38.60	43.36
A3	68.47	57.07	49.00	58.18	60.00	54.13	45.33	53.16
S1	47.67	44.33	37.27	43.09	45.67	40.53	38.60	41.60
S2	56.20	49.60	37.20	47.67	61.53	42.53	37.60	47.22
S3	74.07	60.67	43.20	59.31	73.73	58.53	42.73	58.33
Mean (I)	54.06	47.62	37.71	-----	52.77	46.39	37.78	-----
L.S.D. (0.05)								
I	1.14				0.80			
B	1.75				1.23			
I X B	3.02				2.12			
Fresh weight of flower heads (g/plant)								
Control	66.9	55.1	34.7	52.2	69.2	57.8	35.1	54.0
A1	149.4	86.1	57.6	97.7	139.2	96.0	52.4	95.9
A2	178.0	107.0	79.5	121.5	160.4	115.2	77.1	117.6
A3	214.8	145.4	99.3	153.1	198.9	136.0	91.5	142.1
S1	147.5	88.1	75.2	103.6	146.2	81.6	59.5	95.8
S2	197.0	120.8	78.2	132.0	213.0	112.7	76.3	134.0
S3	257.9	152.2	80.8	163.6	258.3	149.7	85.0	164.3
Mean (I)	173.1	107.8	72.2	-----	169.3	107.0	68.1	-----
L.S.D. (0.05)								
I	4.0				2.3			
B	6.2				3.6			
I X B	10.7				6.2			
Dry weight of flower heads (g/plant)								
Control	18.3	15.3	9.6	14.4	21.1	17.0	10.4	16.2
A1	40.3	23.7	15.9	26.6	41.1	28.6	15.4	28.4
A2	48.9	29.3	21.8	33.3	48.3	34.1	22.8	35.0
A3	61.4	39.9	27.1	42.8	54.5	40.2	25.2	40.0
S1	41.5	24.2	20.9	28.9	43.2	24.2	17.4	28.3
S2	54.7	33.5	22.4	36.9	60.2	33.5	22.7	38.8
S3	71.6	42.2	22.5	45.4	76.2	44.5	25.3	48.6
Mean (I)	48.1	29.7	20.0	-----	49.2	31.7	19.9	-----
L.S.D. (0.05)								
I	1.3				1.0			
B	1.9				1.6			
I X B	3.3				2.7			

\*A1= Amino Suam at 500 ppm    A2= 1000 ppm    A3= 2000 ppm  
 S1= Setter-2 at 500 ppm        S2= 1000 ppm    S3= 2000 ppm

Moussavi *et al.* (2011) on *Trachyspermum Ammi* L., Hassan *et al.* (2012) on *Coriandrum sativum* L., Khalil and El-Noemani (2012) on *Lepidium sativum* L., Aloghareh *et al.* (2013) on *Pimpinella anisum* L., Amirjani (2013) on *Catharanthus roseus* L., El-Mekawy *et al.* (2013) on *Achillea santolina* L., Hendawy *et al.* (2013) on *Silybum marianum* L., Hussein *et al.* (2013) on Jojoba, Hussein *et al.* (2014) on *Mentha longifolia* L., Metwally *et al.* (2014) on *Ricinus communis* L., Moosavi *et al.* (2014) on *Calendula officinalis* L. and Mohamed *et al.* (2014) on *Curcuma* spp.

The reduction in plant growth parameters (such as plant height and stem diameter) in response to water stress may be either due to the blocking up of xylem and phloem vessels, thus hindering any translocation through them (Lovisolo and Schuber, 1998), or due to decrease in cell elongation resulting from the inhibiting effect of water shortage on growth-promoting hormones which, in turn, led to a decrease in each of cell turgor, cell volume and eventually cell growth (Boyer, 1988 and Banon *et al.* 2006). Also water stress may be reduced cyclin-dependent kinase activity results in slower cell division which in turn affect in number of branches/plant (Schuppler *et al.*, 1998). In addition water stress effect on plants photosynthetic efficiency mainly due to the closing of stomata which limits CO<sub>2</sub> diffusion into the leaf, or by its effect on inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), a non-stomatal factor (Lawlor and Cornic, 2002 and Flexas *et al.*, 2004). The adverse effect of water stress by the longest watering intervals (12 days) on reducing flowering parameters may be due to the trough its effect on plant vegetative biomass, the reducing water availability around the roots may be resulting in the lower absorption of nutrients and water thus leading to decrease in biosynthesis accumulation and production of lower vegetative biomass (Bouton *et al.*, 1985 and Singh *et al.*, 1997), since the flowering can be considered as the final resultant of all physiological and metabolic processes in the plant, so the reduction of vegetative biomass maybe consequently lead to decreasing number of flower heads/plant as well as fresh and dry weights of flower heads (El-Mekawy *et al.*, 2013). Concerning the effect of bio-stimulator treatments, the data presented in Tables (2 - 4) indicate that application of bio-stimulator treatments had a favorable effect on studied vegetative and flowering parameters of the plants. In both seasons, application of any rate of the two types of bio-stimulators (Amino Suam or Setter-2) significantly increased the values of all studied vegetative and flowering parameter compared to control plants (which sprayed with tap water). In most cases, with any one of the two types of bio-stimulators, raising the

application rate resulted in a steady increase in the recorded mean values compared to control. Moreover, at the same rates of two bio stimulators types, Setter-2 was generally more effective than Amino Suam for increasing of studied parameters. Among the different concentrations of Setter-2, the highest rate (2000 ppm) was more effective one, since giving the highest mean values in most cases. Similar increases in growth and flowering parameters as a result of bio-stimulator treatments have been reported by Jelačić *et al.* (2007) and Nia *et al.* (2014) on *Rosmarinus officinalis* L., Gomaa (2008) on dragonhead, Sarojnee *et al.* (2009) on *Capsicum annum* L., Haj Seyed Hadi *et al.* (2011), Golzadeh *et al.* (2012) and Omer *et al.* (2013) on *Matricaria chamomile* L., Kwiatkowski (2011) on *Thymus vulgaris* L., Hafez (2013) on *Helianthus tuberosus* L., Rahimi *et al.* (2013) and Haj Seyed Hadi and Darzi (2014) on *Ocimum basilicum* L., Rafiee *et al.* (2013) on *Calendula officinalis* L., Nia *et al.* (2015a) on *Gazania rigens* L. and Nia *et al.* (2015 b) on *Viola tricolor* L., They found that application of commercial products of bio-stimulators containing amino acids, vitamins and/or macro and microelements enhanced growth and flowering traits of studied plants compared with the control. Also, significant increases in the growth and flowering criteria as a result of the application of amino acids, ascorbic acid and /or micronutrient chelates have been obtained by Gamal EL-Din and Abdel-Wahed (2005) and Ranjbar *et al.* (2014) on *Matricaria chamomile* L., El-Awadi, and Hassan (2010) on *Foeniculum Vulgare* M., Khalil *et al.* (2010) on *Ocimum basilicum* L., Datir *et al.* (2012) on *Capsicum annum* L.].

The favorable effect of the bio-stimulator treatments on the vegetative growth and flowering parameter (compared to the control) can be explained by the important role of amino acids (which it is the main component of Amino Suam), ascorbic acid (vitamin c), citric acid, micro and macro-elements (existing in bio-stimulant formulation of Setter-2) on the different physiological processes within the plant, which in turn affect the plant growth. Amino acids are important for growth regulation and as modulators of growth and cell differentiation, which may be affecting general metabolism and consequently morphogenesis (Basu *et al.*, 1989). Amino acids are of special interest to plant producers due to their wide range of roles in plant metabolism. Amino acids are not only building blocks of proteins but also precursors for a myriad of other molecules that serve important functions in plants. Amino acids are involved in the synthesis of other organic compounds, such as protein, amines, alkaloids, vitamins, enzymes, terpenoids and plant hormones that control various plant processes (Glawischnig *et al.*, 2000; Ibrahim *et al.*, 2010). Amino acids such as tryptophan, methionine, proline and arginine contribute to the tolerance of plants against

biotic and abiotic stresses, either directly or indirectly by serving as precursors to secondary compounds and hormones (Muller *et al.* 1995). Ascorbic acid existing in Setter-2 is very important antioxidant which protects plants by suppressing oxidative injury by affecting many enzymes activities (Smirnoff, 1995). Also, nitrogen is present in the structure of protein molecules (Devlin, 1975). Mn participates in important processes such as photosynthesis, and metabolism of both nitrogen and carbohydrate. Boron (B) plays an important role in sugar translocation and carbohydrate metabolism, also involved with cell division, differentiation, growth, and respiration (Taiz and Zeiger, 2002).

Regarding the interaction effect between irrigation intervals and bio- stimulators treatments, the data recorded Tables (2-4) show that in both seasons, plants irrigated every 4, 8 and 12 days and sprayed with any concentration of the tested bio-stimulators (Amino Suam or Setter-2) had the values for vegetative and flowering parameters which were significantly higher than those of untreated control plants. In both seasons, the lowest values for vegetative and flowering parameters were obtained from plants irrigated with the longest interval (12 days) and sprayed with tap water. On the other hand, the highest values for flowering parameters (in most cases) were obtained from plants irrigated with the shortest interval (4 days) and sprayed with the highest concentration of Setter-2 (2000 ppm).

### 3.2. Essential oil percentage and oil yield (g/plant)

It is evident from data in Table (5) that in both seasons, the essential oil percentage in flower heads of chamomile plants tended to increase with increasing irrigation intervals till reached its maximum significant values under the moderate irrigation interval (8 days), followed by significant increase under the longest irrigation interval (12 days) compared with irrigation intervals of 4 days which recorded the lowest values. The obtained results of oil percentage increments under water deficient are in agreement with those of Singh *et al.* (1997) on *Cymbopogon flexuosus* S., Fatima *et al.* (2006) on palmarosa ( *Cymbopogon martinii* (R.) var. motia), Rahmani *et al.* (2008) *Calendula officinalis* L., Farahani *et al.*, (2009) on *Melissa officinalis* L., Said-Al Ahl *et al.* (2009) on oregano (*Origanum vulgare* L.), Khalil and El-Noemani (2012) on *Lepidium sativum* L., Aloghareh *et al.* (2013) on *Pimpinella anisum* L. and El-Mekawy *et al.* (2013) on *Achillea santolina* L.

The increase in oil present with longer irrigation intervals (8 and 12 days) compared to the shortest interval was explained by Hossein *et al.* (2009) who mentioned that water stress

maybe increased oil percent of more medicinal and aromatic plants because in case of stress, more metabolites are produced in the plant cells and substances prevented from oxidization in these stressed cells. Moreover, [Penka \(1978\)](#) mentioned that essential oils are the product of the respiratory catabolic processes which increase under the dry conditions of the growing site of the plant. On the other hand, the noticed decrease in oil % under the highest irrigation interval (12 days) compared to 8 days may be due to increasing severity of water above certain limits caused reduction in photosynthesis and carbohydrate production. ([Chanirar et al, 1989](#) and [Flexas and Medrano, 2002](#)).

**Table 5.** Effect of irrigation intervals and bio-stimulator treatments on the essential oil percentage (%) and oil yield (g/plant) of *Matricaria chamomilla* during the 2013/2014 and 2014/2015 seasons.

*Bio-stimulator Treatments (B)	First season (2013/2014)				Second season (2014/2015)			
	Irrigation intervals (I)			Mean (B)	Irrigation intervals (I)			Mean (B)
	4 days	8 days	12 days		4 days	8 days	12 days	
	<u>Oil percentage (%)</u>							
Control	0.47	0.52	0.52	0.50	0.56	0.95	0.56	0.69
A1	0.68	0.67	0.60	0.65	0.75	1.08	0.89	0.91
A2	0.67	0.59	0.67	0.64	0.65	1.16	1.01	0.94
A3	0.60	0.89	0.67	0.72	0.75	1.13	0.97	0.95
S1	0.75	0.67	0.67	0.69	0.95	1.15	0.80	0.96
S2	0.68	0.89	0.88	0.82	0.85	1.13	0.85	0.95
S3	0.68	0.96	0.96	0.87	0.95	1.28	0.95	1.06
Mean (I)	0.65	0.74	0.71	-----	0.78	1.13	0.86	-----
L.S.D. (0.05)								
I		0.06				0.07		
B		0.09				0.11		
I X B		0.16				0.19		
	<u>Oil yield/plant (g/plant)</u>							
Control	0.08	0.08	0.05	0.07	0.12	0.16	0.06	0.11
A1	0.27	0.16	0.10	0.18	0.31	0.31	0.14	0.25
A2	0.32	0.17	0.14	0.21	0.31	0.40	0.23	0.31
A3	0.37	0.35	0.18	0.30	0.41	0.46	0.25	0.37
S1	0.31	0.16	0.14	0.20	0.40	0.28	0.14	0.27
S2	0.37	0.30	0.20	0.29	0.52	0.38	0.19	0.36
S3	0.49	0.41	0.22	0.37	0.73	0.57	0.24	0.51
Mean (I)	0.32	0.23	0.15	-----	0.40	0.37	0.18	-----
L.S.D. (0.05)								
I		0.02				0.03		
B		0.03				0.05		
I X B		0.05				0.08		

\*A1= Amino Suam at 500 ppm    A2= 1000 ppm    A3= 2000 ppm  
 S1= Setter-2 at 500 ppm        S2= 1000 ppm    S3= 2000 ppm

The data recorded in Table (5) also indicate that in both seasons, essential oil yield was reduced gradually as irrigation intervals were increased from 4 to 8 or 12 days. This

reduction in the recorded mean values was statistically significant, in both seasons, compared the treatments to each other. The obtained reduction of the essential oil yield as a result of increasing watering intervals could be explained through its effect on decreasing the corresponding dry flower heads, increase the essential oil percentage but reduced yield of dry flower heads by the water stress, lead to reduce essential oil yield. (El-Mekawy *et al.* 2013). Similar reductions in essential oil yield as a result of increasing water stress have been reported by Mohamed, (2000) on *Carum carvi* L., Pirzad *et al.* (2006) on *Matricaria chamomilla* L., Kumar *et al.* (2008) on *Coriandrum sativum* L., Hassan *et al.* (2012) on *Coriandrum sativum* L., Vazin *et al.* (2013) on *Cuminum cyminum* L., and Hussein *et al.* (2014) on *Mentha longifolia* L.

As for the effect of bio-stimulator treatments, the data presented in Table (5) indicate that bio-stimulator treatments were very beneficial in terms of increasing essential oil percentage and oil yield of chamomile plants. In both seasons, spraying plants with any rate of the two studied bio-stimulator treatments caused significant increase in essential oil percentage and oil yield compared to plants sprayed with tap water (control). The results are consistent with those obtained by Gomaa (2008) on dragonhead, Haj Seyed Hadi *et al.* (2011) and Golzadeh *et al.* (2012) on *Matricaria recutita* L., Haj Seyed Hadi and Darzi (2014) on *Ocimum basilicum* L. and Nia *et al.* (2014) on *Rosmarinus officinalis* L. Also, significant increases in essential oil percent and oil yield were obtained by the application of amino acids and/or ascorbic acid [Gamal EL-Din and Abdel-Wahed (2005) and Ranjbar *et al.* (2014) on *Matricaria chamomile* L., Khalil *et al.* (2010) on *Ocimum basilicum* L.]. The data in both seasons also show that bio-stimulator of Setter-2 appeared to be more effective than Amino Suam for increasing essential oil percentage and oil yield. Moreover, application of the highest rate of Setter-2 (2000 ppm) was the most effective since gave the highest values compared to control plants or plants receiving any other bio-stimulators treatment.

Regarding the interaction effect between the irrigation intervals and bio-stimulator treatments the data in Table (5) show that in both seasons, plants irrigated every 4, 8 and 12 days and sprayed with any concentration of Amino Suam or Setter-2 had values of essential oil percentage and oil yield which were significantly higher than those of plants sprayed with tap water (control). In both seasons, in most cases, the lowest values were resulted from plants irrigated with the longest interval (12 days) and sprayed with tap water. On the other hand, the highest values for essential oil percentages were resulted from plants irrigated with the



moderate interval (8 days) and sprayed with the highest concentration of Setter-2 (2000 ppm), whereas the highest values for oil yield were obtained from plants irrigated with the shortest interval (4 days) and sprayed with the highest concentration of Setter-2 (2000 ppm).

### 3.3. Chemical constituents

#### 3.3.1. Contents of total chlorophylls and total carbohydrates

Data presented in Table (6) indicate that irrigation intervals had considerable effect on chlorophylls and carbohydrates contents in herb of *Matricaria chamomilla* L. In both seasons, in most cases, plants irrigated with the moderated irrigation intervals (8 days) had significantly higher values of total chlorophylls content and total carbohydrates percentage in their herb than those irrigated every 4 and 12 days. The only one exception to this general trend was detected in the first season with plants irrigated every 8 days which had insignificantly higher total carbohydrates percentage than plants received irrigation every 4 and 12 days. With prolonging irrigation intervals to reach at 12 days the values of total chlorophylls content and total carbohydrates percentage were significantly decreased compared to other treatments. The results for decreasing total chlorophylls as a result increasing water stress are in agreement with the findings of [Narayanappa et al. \(2004\)](#) on *Artemisia pallens* W., [Khalil et al. \(2010\)](#) on *Ocimum basilicum* L. [Asrar and Elhindi \(2011\)](#) on *Tagetes erecta* L., [Pirzad et al. \(2011\)](#) on *Matricaria chamomilla* L., [Aloghareh et al. \(2013\)](#) on *Pimpinella anisum* L., [Amirjani \(2013\)](#) on *Catharanthus roseus* L., [El-Mekawy et al. \(2013\)](#) on *Achillea santolina* L. Whereas the results for decreasing total carbohydrates percentage as a result increasing water stress are consistent with those of [Mohamed et al. \(2014\)](#) on *Curcuma* spp., [Metwally et al. \(2014\)](#) on *Ricinus communis* L., they attributed this decline in carbohydrates to soil water deficiency as it helps certain chemical stimulus abscisic acid (ABA) through xylem vessels to leaves of stressed plants that led to stomatal closure which in turn leading to decline in photosynthesis and carbohydrate accumulation.

Concerning, the effect of bio-stimulator treatments the data in Table (6) reveal that foliar application of bio-stimulator treatments was very beneficial in terms of increasing the synthesis and accumulation the contents of both total chlorophylls and total carbohydrates in herb of chamomile plants. In both seasons the values recorded were significantly higher in the plants receiving any concentration of the tested bio-stimulator treatments compared to the control. It is also clear from the data in the table (6) that in most cases, raising the application



rate of any type of the two studied bio-stimulators (Amino Suam or Setter-2) resulted in a steady increase in the recorded mean values compared to control. Similar results were reported by Gomaa (2008) on dragonhead, Haj Seyed Hadi *et al.* (2011) and Omer *et al.*

**Table 6.** Effect of irrigation intervals and bio-stimulator treatments on total chlorophylls, total carbohydrates and proline contents in herb of *Matricaria chamomilla* during the 2013/2014 and 2014/2015 seasons.

*Bio-stimulator Treatments (B)	First season (2013/2014)			Mean (B)	Second season (2014/2015)			Mean (B)
	Irrigation intervals (I)				Irrigation intervals (I)			
	4 days	8 days	12 days		4 days	8 days	12 days	
Total chlorophylls content (SPAD)								
Control	29.90	29.53	29.21	29.55	28.89	29.61	28.53	29.01
A1	31.81	30.54	31.22	31.19	30.52	32.14	31.28	31.31
A2	32.20	33.86	31.25	32.44	34.94	31.48	30.90	32.44
A3	42.91	39.76	37.96	40.21	38.01	33.53	33.23	34.93
S1	30.26	37.21	35.16	34.21	33.96	37.04	32.83	34.61
S2	32.96	37.72	36.73	35.80	34.10	40.74	33.95	36.26
S3	39.25	36.46	33.38	36.36	34.22	35.26	34.66	34.71
Mean (I)	34.19	35.01	33.56	-----	33.52	34.26	32.20	-----
L.S.D. (0.05)								
I	0.62				0.41			
B	0.94				0.63			
I X B	1.63				1.09			
Total carbohydrates (% of dry matter)								
Control	12.87	12.70	11.29	12.28	13.39	13.36	12.68	13.14
A1	13.46	12.69	12.32	12.82	14.74	14.99	13.53	14.42
A2	15.23	14.91	13.53	14.56	14.99	14.98	15.66	15.21
A3	18.17	17.25	16.77	17.39	18.81	19.12	17.74	18.56
S1	17.34	15.09	16.99	16.47	18.45	16.79	16.75	17.33
S2	17.84	18.75	16.02	17.54	18.42	18.72	17.73	18.29
S3	16.65	20.91	16.71	18.09	17.19	20.88	17.82	18.63
Mean (I)	15.94	16.04	14.80	-----	16.57	16.98	15.99	-----
L.S.D. (0.05)								
I	0.55				0.39			
B	0.85				0.60			
I X B	1.46				1.03			
proline content (µ moles/g fresh matter)								
Control	2.32	3.61	5.97	3.97	1.29	2.44	4.01	2.58
A1	3.71	4.37	5.27	4.45	2.67	3.37	5.53	3.86
A2	4.03	5.47	7.57	5.69	3.00	4.55	7.99	5.18
A3	5.06	9.02	3.71	5.93	4.11	8.35	7.18	6.55
S1	3.91	3.91	3.64	3.82	2.65	4.19	6.76	4.53
S2	4.40	5.60	4.48	4.82	3.40	6.23	9.08	6.24
S3	4.27	6.28	8.92	6.49	3.26	5.42	8.25	5.64
Mean (I)	3.96	5.47	5.65	-----	2.91	4.94	6.97	-----
L.S.D. (0.05)								
I	0.40				0.50			
B	0.62				0.76			
I X B	1.07				1.31			

\*A1= Amino Suam at 500 ppm A2= 1000 ppm A3= 2000 ppm

S1= Setter-2 at 500 ppm

S2= 1000 ppm S3= 2000 ppm

(2013) on *Matricaria chamomile* L., Rafiee *et al.* (2013) on *Calendula officinalis* L., Nia *et al.* (2014) on *Rosmarinus officinalis* L., Nia *et al.* (2015 a) on *Gazania rigens* L. , and Nia *et al.* (2015 b) on *Viola tricolor* L.. Also, significant increases in photosynthetic pigments were obtained by the application of amino acids and/or ascorbic acid [El-Awadi, and Hassan (2010) on *Foeniculum Vulgare* M. and Khalil *et al.* (2010) on *Ocimum basilicum* L.].

The favorable effect of bio-stimulators on increasing the total carbohydrates concentration may be indirectly attributed to the increase in the content of total chlorophylls as a result of the treatments. As the synthesis of total chlorophylls was promoted, the rate of photosynthesis increased, leading to an increase in carbohydrate synthesis. The effect of nitrogen or amino acids on the chemical contents could be through biosynthesis of organic compounds such as protein, pigments, amines, vitamins, enzymes, terpenoids, purines, pyrimidines (Goss, 1973 and Kamar and Omar, 1987).

Regarding the interaction effect between irrigation intervals and bio stimulators, the data in Table (6) show that in both seasons, plants irrigated every 4, 8 and 12 days and sprayed with any concentration of the tested bio-stimulators (Amino Suam or Setter-2) had values of total chlorophylls and total carbohydrates that were significantly higher than those of untreated control plants. In both seasons, the lowest values of total chlorophylls and total carbohydrates were obtained from plants irrigated with the longest interval (12 days) and sprayed with tap water. Whereas, the highest values of total carbohydrates (in both the seasons) were obtained from plants irrigated with the moderate interval (8 days) and sprayed with the highest concentration of Setter-2 (2000 ppm). On the other hand, the highest values of total chlorophylls (in the first season) were obtained from plants irrigated with the shortest interval (4 days) and sprayed with the highest concentration of Amino Suam (2000 ppm), whereas the highest values of total chlorophylls (in the second one) were obtained from plants irrigated with the moderate interval (8 days) and sprayed with Setter-2 at concentration of 100 ppm.

### 3.3.2 Proline Contents

As shown in Table (6), the data indicate that increasing irrigation intervals from 4 to 8 or 12 resulted in significant steady increment in proline content in leaves of *Matricaria chamomilla* L. plants. Accordingly, the highest mean values were obtained from plants irrigated with the

longest intervals (12 days), whereas the lowest values were obtained from plants irrigated with the shortest intervals (4 days). These results are in agreement with those obtained by [Baher et al. \(2002\)](#) on *Satureja hortensis* L., [Yamada et al. \(2005\)](#) on *Petunia hybrida* cv. 'Mitchell', [Zhao et al. \(2007\)](#) on *Rehmannia glutinosa* G., [Farahani et al. \(2009\)](#) on *Melissa officinalis* L., [Khalil and El-Noemani \(2012\)](#) on *Lepidium sativum* L., [Khalil et al. \(2012\)](#) on *Jatropha curcas* L. and [Hussein et al. \(2013\)](#) on Jojoba.

Such increases in proline values as a result of water shortage were attributed to one of the defense mechanisms which stressed plants used to reduce the osmotic potential, thus increasing cell water uptake with concomitant increases in both cell activity and turgidity ([Wang et al., 2003](#)). Moreover, [Pushpam and Rangasamy \(2000\)](#) reported that increasing proline content in stressed plant tissues may be due to hydrolysis of protein under stress conditions to different amino acids, especially proline, which intracellular osmotic adjustments to maintain its turgidity.

The data presented in Table (6) show that, in both seasons, proline content in leaves of chamomile plants was increased as a result of spraying plants with any rate of bio-stimulator treatments compared to plants sprayed with tap water (control). In most cases, these increments in the recorded mean values were statistically significant as compared to control. The only exception to this general trend was detected in the first season with the plants sprayed with the lowest concentration of Amino Suam or Setter-2 (500 ppm) which had insignificantly higher values than those of control plants. Similar increases in the proline content in plants treated with bio-stimulator concentrations have been reported by [Omer et al. \(2013\)](#) on *Matricaria chamomile* L. and [Smolik et al. \(2013\)](#) on *Brassica oleracea* L. [Omer et al. \(2013\)](#) stated that this increase may be due to direct added amino acids, which includes composition proline.

Concerning the effect of interaction between the two studied factors the data on (Table 6) show that in most cases, plants irrigated every 4, 8 and 12 days and sprayed with any concentration of the two types of bio-stimulators (Amino Suam or Setter-2) had values of proline content in their leaves which were significantly higher than those of control plants. In both seasons, the lowest values (2.32 and 1.29  $\mu$  moles/g fresh matter in the first and second seasons, respectively) were obtained from plants irrigated every 4 days and sprayed with tap water. On the other, the highest values (9.02  $\mu$  moles/g fresh matter in the first season) were resulted from irrigated every 8 days and sprayed with the highest concentration of Amino

Suam (2000 ppm), while the highest values (9.08  $\mu$  moles/g fresh matter in the second one) were resulted from plants irrigated every 12 days and sprayed with Setter-2 at concentration of 1000 ppm.

### 3.3.3. Contents of N, P and K (% of dry matter)

Results of chemical analysis of dried herb of *Matricaria chamomilla* L. plants (Table 7) show that the concentrations of N, P and K% were generally affected by the different levels of irrigation intervals. In both seasons, three nutrients were reduced steadily with increasing irrigation intervals from 4 to 8 or 12 days. Accordingly, the highest mean values were obtained from plants irrigated every 4 days (shortest irrigation intervals), whereas the lowest values were obtained from plants irrigated every 12 days (longest irrigation interval). The steady reductions in the recorded mean values are similar to those reported by [Hussein and El-Dwieny \(2011\)](#) on *fenugreek* and [El-Mekawy et al. \(2013\)](#) on *Achillea santolina* L. In most cases, the reduction in N, P and K% were statistically significant as a result of rising irrigation intervals from 4 to 12 days compared the treatments to each other. The only two exceptions to this general trend were detected in the second season with plants irrigated every 8 day which had insignificantly lower N and K% than those recorded with plants irrigated every 4 days. The obtained results are in agreement with those obtained by [Asrar and Elhindi \(2011\)](#) on *Tagetes erecta* L., [Hussein et al. \(2013\)](#) on Jojoba.

The decrease in the concentration of the nutrients in herb of chamomile plants as a result of increasing water stress can be easily explained, since the availability of nutrients its solubility in the soil is affected by moisture content, so increasing water stress led to reducing the solubility of the elements in the soil which led to reduce the absorbing efficiency of such elements and their accumulation in plant tissues ([Power, 1990](#)). In this respect [Levitt \(1980\)](#) stated that nutrient uptake by plants is generally decreased under water stress conditions due to a substantial decrease in transpiration rates coupled with impaired active transport and membrane permeability.

The data in Table (7) also show that the uptake and accumulation of N, P and K% in dried herb of chamomile plants were enhanced by using different bio-stimulator treatments. In both seasons spraying of any concentration of the tested bio-stimulator treatments (Amino Suam or Setter-2) resulted in steady increase the three nutrients compared with the control. In both seasons, the increases in the recorded mean values were statistically significant (in most cases) compared to untreated control plants. The only exceptions to these general trends were

detected with plants sprayed with the lowest concentration of Amino Suam (500 ppm) which had insignificantly higher N% (in the first season) and insignificantly higher P and K% (in the

**Table 7.** Effect of irrigation intervals and bio-stimulator treatments on N, P and K concentrations (% of dry matter) in dried herb of *Matricaria chamomilla* during the 2013/2014 and 2014/2015 seasons.

*Bio-stimulator Treatments (B)	First season (2013/2014)				Second season (2014/2015)			
	Irrigation intervals (I)			Mean (B)	Irrigation intervals (I)			Mean (B)
	4 days	8 days	12 days		4 days	8 days	12 days	
	N (% dry matter)							
Control	1.22	1.14	1.31	1.22	0.94	1.04	0.95	0.98
A1	1.47	1.40	1.21	1.36	1.21	1.39	1.15	1.25
A2	1.72	1.49	1.35	1.52	1.53	1.61	1.29	1.48
A3	1.71	1.68	1.45	1.61	1.59	1.61	1.53	1.58
S1	1.91	1.88	1.56	1.78	1.60	1.57	1.63	1.60
S2	2.02	1.74	1.78	1.84	1.87	1.63	1.62	1.71
S3	2.02	1.98	1.77	1.92	2.00	1.80	1.76	1.86
Mean (I)	1.72	1.61	1.49	-----	1.54	1.52	1.42	-----
L.S.D. (0.05)								
I			0.10				0.09	
B			0.15				0.14	
I X B			0.25				0.24	
P (% dry matter)								
Control	0.15	0.13	0.10	0.12	0.15	0.15	0.13	0.14
A1	0.19	0.11	0.11	0.14	0.19	0.18	0.10	0.16
A2	0.19	0.15	0.13	0.15	0.20	0.16	0.16	0.17
A3	0.18	0.16	0.14	0.16	0.20	0.18	0.18	0.19
S1	0.17	0.15	0.14	0.15	0.18	0.19	0.18	0.18
S2	0.21	0.20	0.16	0.19	0.21	0.18	0.17	0.19
S3	0.22	0.20	0.17	0.20	0.24	0.20	0.20	0.22
Mean (I)	0.18	0.16	0.13	-----	0.20	0.18	0.16	-----
L.S.D. (0.05)								
I			0.01				0.01	
B			0.02				0.03	
I X B			0.03				0.05	
K (% of dry matter)								
Control	1.10	0.88	0.82	0.94	1.41	1.32	1.16	1.30
A1	1.29	1.27	1.27	1.28	1.52	1.48	1.51	1.51
A2	1.44	1.33	1.27	1.35	1.66	1.59	1.50	1.58
A3	1.45	1.38	1.28	1.37	1.90	1.48	1.64	1.67
S1	1.45	1.26	1.01	1.24	1.92	1.88	1.65	1.82
S2	1.56	1.50	1.21	1.42	1.95	1.92	1.66	1.85
S3	1.89	1.58	1.47	1.65	2.01	1.86	1.90	1.92
Mean (I)	1.45	1.31	1.19	-----	1.77	1.65	1.57	-----
L.S.D. (0.05)								
I			0.13				0.16	
B			0.20				0.25	
I X B			0.34				0.43	

\*A1= Amino Suam at 500 ppm A2= 1000 ppm A3= 2000 ppm

S1= Setter-2 at 500 ppm      S2= 1000 ppm    S3= 2000 ppm

second one) than those recorded with the control. Similar increases in the N, P and K % in plants treated with bio-stimulator concentrations have been reported by Gomaa (2008) on dragonhead, Rafiee *et al.* (2013) on *Calendula officinalis* L., Nia *et al.* (2015 a) on *Gazania rigens* L. and Nia *et al.* (2015 b) on *Viola tricolor* L. From the data in Table (6) it can be noticed that in most cases, at the same rates of two types of bio-stimulators, Setter-2 appeared to be more effective than Amino Suam for increasing N, P and K%. Among the different concentrations of Setter-2, the highest rate (2000 ppm) was more effective one, since gave the highest mean values in most cases.

The beneficial effects of bio-stimulator treatments in increasing photosynthetic pigments, carbohydrates formation and nutrient accumulation may be explaining its favorable effect on enhancing growth parameters. Also increasing flowering parameters could be attributed to increase photosynthetic rates as a result of using bio-stimulator treatments therefore, translocation of different photosynthetic products from the source (leaves) to the flower heads is being maximized, and thereby increase in the studied flowering traits would be expected.

Regarding the interaction effect between irrigation intervals and foliar application of bio-stimulator treatments data recorded on (Table 7) show that in both seasons, in most cases, plants irrigated every 4, 8 and 12 days and sprayed with any concentration of the two types of bio-stimulators (Amino Suam or Setter-2) had values of N, P and K%, which were significantly higher than those of untreated control plants. In both seasons, in most cases, the lowest values were obtained from plants irrigated with the longest interval (12 days) and sprayed with tap water. On the other hand, the highest values were obtained from plants irrigated with the shortest interval (4 days) and sprayed with the highest concentration of Setter-2 (2000 ppm).

### 3.3.4. Analysis of oil composition

Results of chemical analysis of chamomile essential oil in the second season (Table 8) indicate that there are 6 identified compounds representing 52.12 - 99.35 % of total detected constituents with different treatments and unknown compounds representing 0.65- 47.88 % of total detected constituents. The main constituents of chamomile oil were  $\alpha$ -bisabolol oxide A (30.09 to 53.50%),  $\alpha$ -bisabolol oxide B (3.51 to 16.99%), farnesene (6.31 to 12.55%), bisabolone oxide (4.68 to 12.03%),  $\alpha$ -bisabolol (1.04 to 6.36%) and Chamazulene (1.05 to 4.37%).

From the data it can be noticed that  $\alpha$ -bisabolol oxide A,  $\alpha$ -bisabolol oxide B, farnesene and bisabolone oxide were increased with increasing irrigation intervals till reached its maximum values under the moderate irrigation interval (8 days) compared with irrigation intervals of 4 and 12 days. On the otherhand,  $\alpha$ -bisabolol and Chamazulene were reduced steadily with

**Table 8.** Effect of irrigation intervals and bio-stimulator treatments on the constituents of essential oil of *Matricaria chamomilla* in the second season (2014/2015)

Treatments/ constituents	Farnesene	$\alpha$ - bisabolol oxide B	bisabolone oxide	$\alpha$ - bisabolol	Chamazulene	$\alpha$ - bisabolol oxide A	Total Identified compounds	unknown compounds
<b>I1+ Cont</b>	6.31	7.98	5.07	2.01	1.98	45.61	68.96	31.04
<b>I1+ A1</b>	7.27	10.79	9.27	4.64	1.27	43.89	77.13	22.87
<b>I1+ A2</b>	9.33	9.99	9.84	5.92	2.32	47.91	85.31	14.69
<b>I1+ A3</b>	8.17	16.35	10.18	6.36	4.37	45.91	91.34	8.66
<b>I1+ S1</b>	7.14	3.51	8.57	5.36	2.31	34.61	61.50	38.50
<b>I1+ S2</b>	7.14	9.97	5.85	4.66	2.37	42.89	72.88	27.12
<b>I1+ S3</b>	10.32	11.32	8.32	5.20	3.49	43.91	82.56	17.44
<b>Mean</b>	7.95	9.99	8.16	4.88	2.59	43.53	77.10	22.90
<b>I2+ Cont</b>	6.94	9.9	4.94	1.99	1.88	35.42	61.07	38.93
<b>I2+ A1</b>	8.53	11.54	8.69	2.35	1.66	48.83	81.60	18.40
<b>I2+ A2</b>	12.55	16.99	12.03	4.02	1.88	50.18	97.65	2.35
<b>I2+ A3</b>	10.23	15.51	11.22	3.61	2.35	48.81	91.73	8.27
<b>I2+ S1</b>	8.13	8.66	9.14	5.71	1.16	40.11	72.91	27.09
<b>I2+ S2</b>	11.77	16.93	12.01	5.51	2.30	50.83	99.35	0.65
<b>I2+ S3</b>	7.36	13.76	8.36	4.23	3.41	38.18	75.30	24.70
<b>Mean</b>	9.36	13.33	9.48	3.92	2.09	44.62	82.80	17.20
<b>I3+ Cont</b>	6.68	5.58	4.68	1.04	1.05	33.09	52.12	47.88
<b>I3+ A1</b>	9.45	8.48	11.50	2.75	1.10	38.53	71.81	28.19
<b>I3+ A2</b>	7.99	12.63	7.21	3.61	1.14	50.5	83.08	16.92
<b>I3+ A3</b>	11.77	11.03	9.88	4.94	1.18	49.39	88.19	11.81
<b>I3+ S1</b>	6.87	7.19	4.92	3.08	1.14	30.09	53.29	46.71
<b>I3+ S2</b>	8.93	8.96	6.83	3.27	1.20	35.41	64.60	35.40
<b>I3+ S3</b>	10.92	12.15	8.92	3.58	2.22	53.50	79.14	20.86
<b>Mean</b>	8.94	8.98	7.71	3.18	1.29	41.50	71.60	28.40
<b>Cont</b>	6.64	7.82	4.90	1.68	1.64	38.04	60.72	39.28
<b>A1</b>	8.42	10.27	9.82	3.25	1.34	43.75	76.85	23.15
<b>A2</b>	9.96	13.20	9.69	4.52	1.78	49.53	88.68	11.32
<b>A3</b>	10.06	14.30	10.43	4.97	2.63	48.04	90.43	9.57
<b>S1</b>	7.38	6.45	7.54	4.72	1.54	34.94	62.57	37.43
<b>S2</b>	9.28	11.95	8.23	4.48	1.96	43.04	78.94	21.06
<b>S3</b>	9.53	12.54	8.53	4.34	3.04	45.20	83.18	16.82

I= irrigation intervals I1= 4 days I2= 8 days I3= 12 days

A= Amino Suam A1= 500 ppm A2= 1000 ppm A3= 2000 ppm

S= Setter-2 S1= 500 ppm S2= 1000 ppm S3= 2000 ppm

increasing irrigation intervals from 4 to 8 or 12 days. In this regard [Pirzad et al. \(2006\)](#) on *Matricaria chamomilla* L. found that  $\alpha$ -bisabolol oxide A,  $\alpha$ -bisabolol oxide B, farnesene and Chamazulene were decreased under water stress.

Regarding the effect of bio-stimulator treatments, in most cases, most of the recorded main constituents were increased with increasing any concentration of studied bio-stimulator types



as compared with control. In this regard Gamal EL-Din and Abdel-Wahed (2005) on *Matricaria chamomile* L. found that application of amino acids led to increases in farnesene,  $\alpha$ -bisabolol oxide B and  $\alpha$ -bisabolol contents, and decreases in chamazulene content while  $\alpha$ -bisabolol oxide A had no clear trend as affected by amino acids applications. Also Omer *et al.* (2013) on *Matricaria chamomile* L. stated that mean contents of bisabolol oxide A, bisabolol oxide B, bisabolone oxide and cis- $\beta$ - farnesene increased by low level of amino acids (125 ppm) compared with other levels of amino acids.

Concerning the interaction effect, it was observed that the highest mean contents of  $\alpha$ -bisabolol oxide A (53.50%) were obtained from plants irrigated every 12 days and sprayed with Setter-2 at 2000 ppm, while the highest mean contents of  $\alpha$ -bisabolol oxide B (16.99%), farnesene (12.55%) and bisabolone oxide (12.03%) were obtained from plants irrigated with the moderate irrigation interval (8 days) and sprayed with Amino Suam at concentration of 1000 ppm. The highest mean contents of  $\alpha$ -bisabolol (6.36%) and Chamazulene (4.37%) were obtained from plants irrigated every 4 days and sprayed with Amino Suam at 2000 ppm. On the otherhand the lowest mean contents of identified compounds were obtained from using the longest irrigation interval (12 days) and spraying with tap water (control).

#### 4. Conclusions

For the best growth, flowering and oil production of *Matricaria chamomilla* grown under irrigation intervals up to 12 days the plants could be sprayed by Setter-2 or Amino Suam at 2000 ppm for alleviating the adverse effect of water stress

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