Asian Journal of Crop Science

ISSN 1994-7879

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http://ansinet.com

Asian Journal of Crop Science 5 (4): 338-359, 2013
ISSN 1994-7879 / DOI: 10.3923/ajcs.2013.338.359

Response of *Achillea santolina* L. To Fertilizers under Different Irrigation Intervals

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ABSTRACT

Most of promising lands in El-Arish are situated near the Mediterranean Sea, as insufficient available water irrigation with high concentration of salinity from wells water is generally used for irrigation. Production of medicinal and aromatic plants using microorganisms in biofertilization to reduce or replace the chemical fertilizers became an essential process to ensure safety for human and environment. The effect of three irrigation intervals (7, 14 and 21 days) and half or full dose of recommended rate inorganic NPK fertilizer as compared with control ones (recommended rate of inorganic NPK fertilizer without microbial inoculation) on vegetative growth, chemical composition and essential oil productivity of *Achillea santolina* L. Plants was studies at the Experimental Farm of the Faculty of Environmental Agriculture Science, El-Arish, during two seasons (2010/2011 and 2011/2012). Results showed that the moderate irrigation interval every 14-days was the prior for producing higher percentage and yield of volatile than the intervals every 7 and 21 days. The growth and yield parameters of the plants increased as a result of inoculation of symbiotic N_2-fixers+phosphobacteria with inorganic fertilizer followed by the same mixture given half dose NPK fertilizer and then control. The interaction between the two factors of study, it could be noticed that growth and yield parameters considerably improved as a result of watering interval at 14 days and inoculation with biofertilizeres and supplemented with half or full dose of inorganic NPK-fertilizer in the two seasons.

Key words: Irrigation intervals, biofertilization, oil yield, *Achillea santolina* L.

INTRODUCTION

Achillea santolina L. (Fam. Asteraceae). Common names: Lavender cotton (English); Qaysûm (Arabic). Plants are white-wooly, with erect stem which attain up to 1 m height, leaves are small, estipulate thick white to grayish-green, oblong, serrate with undivided lamina. Fragrant perennial herbs, yellow corymbose heads (Tackholm, 1974). The part which used is the fresh or dry whole plant, the fresh herb contains volatile oil that reaches about 1% which consists of 59 components of which α-pinene, β-pinene, limonene, 1, 8-cineole, linalool, carvacrol, eugenol, artemesia ketone, palustrol sabinen hydrate α- and β-thujones, santolina alcohol and α-terpineol. The plant also contains tannin, flavonoids fatty acids and bitter substances (Mustafa *et al*., 1995). *Achillea santolina* was used by Bedouin for the treatments of caught, aromatic bitter stomachic and anthelmintic.

Nowadays, there is the National Project for Sinai Development to increase the National income of Egypt. For this purpose, the answer could be partly found in medicinal plants cultivation which occupies a prominent economic position. *Achillea santolina* L. is among the medicinal plants that can be cultivated in the new land.

The effective role of water supply on the growth and production of several medicinal plants was observed by many investigators. Silva *et al.* (2010) on *Aloe vera*; Al-Kayssi *et al.* (2011) on black cumin; Sidika *et al.* (2012) on purple basil; Rebey *et al.* (2012) on Cumin; Vazin (2013) on Cumin; Lal *et al.* (2013) on lemon grass found that providing the plants with suitable water amounts resulted in better growth and yield than those grown under drier conditions.

Biofertilizers are very important to produce medicinal and aromatic plants for drugs extraction and manufacturing as a main substance for human disease healing and health care. Producing such drugs has to be clean having no or the least chemical residues of any harmful kinds Additionally, biofertilizers are substitution of chemical fertihzers for healthy and cheap production. (Subba Rao, 1981).The effect of biofertilization on the growth and productivity of medicinal plants Hassan *et al.* (2010) on khella; Hassan (2012) on roselle; Harb *et al.* (2011) on black cumin with used chemical fertilizer combined with biofertilzer resulted in higher values of volatile oil yield/plant and per fed.

This investigation aimed to determine of the suitable water interval and biofertilizer on the growth and yield parameters of *Achillea santolina* L. plants under North Sinai conditions.

MATERIALS AND METHODS

This investigation was conducted at the Experimental Farm of the Faculty of Environmental Agriculture Science at El-Arish, Suez Canal University during two successive seasons of 2010/2011 and 2011/2012 to study the effect of three irrigation intervals of 7, 14 and 21 days and inoculation with nitrogen fixing bacteria “NFB” and phosphate dissolving bacteria “PDB” alone and/or in the presence of half or full dose of recommended rate inorganic N, P and K fertilizers 150 kg fed.$^{-1}$. ammonium sulfate (20.5%N)+200 kg fed.$^{-1}$. calcium superphosphate (15.5) P_2O_5+50 kg fed.$^{-1}$. potassium sulfate (48% K_2O) as compared with control (recommended rate of inorganic N P K fertilizer without microbial inoculation) on vegetative growth, chemical composition and essential oil productivity of *Achillea santolina* L. plants under North Sinai conditions.

This study included 18 treatments as follows:

Irrigation every 7 days:

- N.P.K
- NFB+PDB mixture
- 50% N.P.K+NFB+PDB mixture
- N.P.K+NFB
- N.P.K+PDB
- N.P.K+NFB+PDB mixture

Irrigation every 14 days:

- N.P.K
- NFB+PDB mixture
- 50% N.P.K+NFB+PDB mixture
- N.P.K+NFB
- N.P.K+PDB
- N.P.K+NFB+PDB mixture

Irrigation every 21 days:

- N.P.K
- NFB+PDB mixture
- 50% N.P.K+NFB+PDB mixture
- N.P.K+NFB
- N.P.K+PDB
- N.P.K+NFB+PDB mixture

The mechanical and chemical analyses of the used soil were determined before cultivation and chemical analyses of organic manure sources are shown in Table 1. The well water was used to obtain water irrigation treatment every 7, 14 and 21 days in the two seasons by using drip irrigation system, as the plants were irrigated by sufficient water to maintain soil moisture at 65-70% of the field capacity and chemical analyses of this water is shown in Table 1.

Layout of the experiment: On 20th October during the two seasons seeds of *Achillea santolina* L. plants were sown in the nursery, on 5th February the seedling of about 10 cm height were transplanted on pipe lines from plastic material of 16 mm diameter. The space among them was 60 cm and the distance among hills was 50 cm (one plant/hill).The plants were

Table 1: Some initial chemical and physical characteristics of soil, well water and used organic manures during 2010-2011 and 2011-2012 seasous

Parameters Soluble ious meq L^{-1} (soil paste extract) meq L^{-1}	Soil		Well water		Organic manure source		
	1st	2nd	1st	2nd	Chicken manure (ChM)	1st	2nd
Ca^{++}	3.04	2.10	10.60	10.11	Total N (%)	1.92	1.77
Mg^{++}	2.11	2.20	5.80	5.19	Total P (%)	0.80	1.68
Na^{+}	1.18	4.49	32.70	33.74	Total K (%)	1.02	1.20
K^{+}	0.47	0.31	0.98	0.95	Fe (ppm)	512.00	508.00
Cl^{-}	1.02	2.30	40.50	38.43	Cu (ppm)	360.00	352.00
CO_3^{--}	-	-	-	-	Zn (ppm)	265.00	244.00
HCO_3^{-}	2.00	2.40	5.68	6.24	Mn (ppm)	241.00	175.00
SO_4^{--}	3.78	4.40	3.90	4.63	Ni (ppm)	58.30	36.00
ECe (dS m^{-1})	0.68	0.91	5.00	5.06	Organic matter (%)	56.90	53.73
Concentration (ppm)	-	-	3200	3400	Organic carbon (%)	30.55	28.15
F.C (%)	7.50	7.71	-	-	C/N (%)	15.91	16.46
pH (1:2.5)	8.10	8.20					
Particular size distributiou (%)							
Clay	0.16	0.17					
Silt	0.33	0.35					
Fine sand	79.88	79.87					
Coarse sand	19.63	19.61					
Soil texture	Sandy soil	Sandy soil					

1st: 2010/2011, 2nd: 2011/2012

irrigated every 7, 14 and 21 days by drip irrigation system was used with droppers of 2.0 L h^{-1} beside every hill. Organic manure was added to the growing soil before cultivation at rate of 20 m^3 $fed.^{-1}$.

N-fertilizer as ammonium sulphate (20.5%N) added at recommended rate 150 kg $fed.^{-1}$. Addition to the soil was in 2 equal doses. The first addition was 15 days after transplanting, it was added to all treatments except for the treatment which was contain (nitrogen fixing bacteria "NFB"+phosphate dissolving bacteria "PDB" only). The second doses were added 45 days after transplanting, it was added to all treatments except for the treatments which was contain (nitrogen fixing bacteria "NFB"+phosphate dissolving bacteria "PDB" only) and 50% N.P.K+NFB+PDB mixture). P-fertilizer as super phosphate (15.5 P_2O_5) was added during soil preparation at recommended rate for all treatment except (nitrogen fixing bacteria "NFB"+phosphate dissolving bacteria "PDB" only) while treatment 50% N.P.K+NFB+PDB mixture were had half of recommended dose. K-fertilizer as potassium sulphate (48%K_2O) were added in 2 equal doses, the first addition was during soil preparation at recommended rate for all treatments except (nitrogen fixing bacteria "NFB"+phosphate dissolving bacteria "PDB" only), the second dose side dressing after 45 days from transplanting for all treatment except (nitrogen fixing bacteria "NFB"+phosphate dissolving bacteria "PDB" only and treatment 50% N.P.K+NFB+PDB mixture).

Experimental design and statistically analyses: Experiment was implemented in randomized complete block design with 3 replicates for every one treatment, each replicate contained 18 plants, the area of each plot was 6 m^2 (2×3 m), the three irrigation intervals (7, 14 and 21 days) were assigned for the main plots while the six levels of fertilizer were the sub-plots treatments. Statistical analysis of the obtained data was carried out according to statistical analysis of variance according to Sendecor and Cochran (1981). Duncan's multiple range tests was used for comparison among means (Duncan, 1955). All the plants received normal agricultural practices whenever needed.

Source of biofertilizer: Cerealin and phosphorine are commercially produced by the General Organization and Equalization Agric. Fund (G.O.A.E.F.) Ministry of Agriculture, Egypt cerealin contain (nitrogen fixing bacteria "NFB") while phosphoren contain (row phosphate dissolving bacteria "PDB" *B. megatherium*). The inoculated with cerealin and phosphorine liquid 2 L was diluted two times and roots of *Achillea santolina* seedlings were dipped into liquid.

Measurements

Vegetative growth: Samples of three plants were randomly taken from each sub-plot to determine the following parameters:

- Plant height (cm)
- Number of branches/plant
- Fresh weight of root/plant (g)
- Fresh weight of herb/plant (g)
- Dry weight of root/plant (g)
- Dry weight of herb/plant (g)

Vegetative growth parameters were recorded one times at 100 days from transplanting. Three plants were randomly taken from each sub-plot.

Plant chemical analysis:
Photothytinthectic pigments:

- Chlorophyll a
- Chlorophyll b

Chlorophyll a and b were determined (mg g^{-1} F.W) on fresh leave sample according to Wettstein formula (Von Wettstein, 1957). After 100 days from transplanting were extracted from representative samples of the fresh leaves using 85% acetone. The concentration of Chlorophyll a and b was determined by using spectrophotometer.

Carbohydrate: Total Carbohydrate percentage in dried leaves was determined according to Herbert *et al*. (1971).

Plant chemical compositions: Total nitrogen, phosphorus and potassium were determined dry matter of leaves at 100 days after transplanting. All plant parts were dried at 70°C till constant weight:

- Total nitrogen was determined by Neslar method described by Bremner and Mulvaney (1982)
- Phosphorus content was determined using the method described by Piper (1950)
- Potassium was determined photometrical according to Brown and Lilliand (1946)

Yield and its components:

- Inflorescences fresh weight $plant^{-1}$ (g)
- Number inflorescences $plant^{-1}$
- Inflorescences dry weight $plant^{-1}$ (g)

Flowering tops were collected 5 times every 2 weeks during the flowering season, unexpanded flower heads were collected. Also, dry flowers were air-dried yield of five cuttings flowering tops of individual plant was estimated. Air-dried flower tops in each treatment carefully hand crushed to measure the percentage of essential oil (Guenther, 1961) was distilled to determined volatile oil according to Egyptian Pharmacopoeia method.

RESULTS AND DISCUSSION

Vegetative traits

Effect of irrigation intervals

Plant height (cm): Data in Table 2 indicated that irrigation every 7 days generally increased plant height compared to irrigation every 14 and 21 days; the plant height was decreased as the water intervals increased in the two seasons; highly significant between treatments were observed; in this respect the values were 44.50, 41.17 and 39.22 cm for irrigated plants every 7, 14 and 21 days, respectively in the first season while they were 46.11, 43.11 and 40.17 cm for irrigated plants every 7, 14 and 21 days in the second season.

These results may be due to the effect of used short watering intervals on increasing the growth of root system, consequently, increasing the nutrients uptake needed for plant growth. Besides,

Table 2: Effect of irrigation intervals and biofertilization on some vegetative growth parameters of *Achillea santolina* L. in two seasons 2010/2011 and 2011/2012

					Plant fresh weight (g)				Plant dry weight (g)			
	Plant height (cm)		Branches No./plant		Herb		Root		Herb		Root	
Treatments	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Watering intervals												
7-days	44.50^{a}	46.11^{a}	19.67^{a}	19.61^{a}	28.37^{a}	29.87^{a}	12.10^{a}	13.97^{a}	15.49^{a}	16.26^{a}	6.57^{a}	7.35^{a}
14-days	41.17^{b}	43.11^{b}	17.22^{b}	18.11^{b}	23.98^{b}	24.91^{b}	9.46^{b}	10.51^{b}	12.06^{b}	12.96^{b}	4.91^{b}	5.68^{b}
21-days	39.22^{c}	40.17^{c}	14.72^{c}	15.28^{c}	20.21^{c}	20.97^{c}	8.10^{c}	8.75^{c}	9.23^{c}	10.13^{c}	3.76^{c}	4.43^{c}
Fertilization treatment												
N.P.K	39.11^{b}	40.22^{bc}	15.78^{bc}	17.00^{bc}	20.41^{cd}	21.29^{cd}	8.45^{ab}	9.52^{ab}	9.13^{b}	9.97^{c}	3.77^{ab}	4.61^{ab}
(NFB+PDB) mixture	36.22^{e}	37.89^{e}	12.55^{e}	13.56^{d}	17.72^{e}	18.66^{e}	6.55^{d}	7.61^{d}	7.98^{c}	8.22^{d}	3.47^{b}	4.20^{c}
½ N.P.K+ (NFB+PDB) mixture	37.67^{bc}	39.00^{bc}	14.55^{cd}	16.22^{c}	19.07^{cd}	19.94^{cd}	8.03^{bc}	8.95^{bc}	9.50^{bc}	9.55^{bc}	3.76^{ab}	4.47^{b}
N.P.K+NFB	43.3^{ab}	44.67^{a}	17.78^{b}	19.44^{b}	25.21^{b}	25.73^{b}	9.27^{ab}	10.24^{ab}	11.92^{ab}	12.70^{ab}	4.43^{ab}	5.06^{ab}
N.P.K+PDB	40.78^{bc}	41.11^{b}	17.11^{bc}	17.78^{bc}	21.75^{bc}	22.21^{c}	8.85^{ab}	9.77^{ab}	10.27^{b}	10.9^{bc}	4.18^{ab}	4.83^{ab}
N.P.K+(NFB +PDB) mixture	44.62^{a}	45.56^{a}	21.44^{a}	22.00^{a}	28.97^{a}	29.64^{a}	10.18^{a}	10.93^{a}	13.77^{a}	14.32^{a}	4.86^{a}	5.74^{a}
Interaction between watering interval and treatment fertilization												
7-days												
N_0	41.67^{b}	40.33^{ef}	16.67^{b-f}	18.68^{d}	21.86^{e-g}	22.30^{d-f}	8.66^{bc}	9.80 a-d	10.07^{c-f}	10.77^{c-f}	4.36^{a-d}	5.23^{a-d}
N_1	37.33^{fg}	39.67^{e-g}	14.00^{e-g}	15.33^{f-h}	19.66^{f-i}	20.20^{e-h}	7.43^{c-d}	8.40^{d-f}	8.70^{d-g}	9.90^{d-f}	4.00^{a-d}	4.80^{a-e}
N_2	38.67^{d-g}	41.00^{de}	14.67^{c-g}	18.00^{d-f}	21.07^{e-h}	21.63^{d-g}	8.16^{b-d}	8.93^{c-e}	9.80^{c-g}	10.63^{c-f}	4.30^{a-d}	4.93^{a-e}
N_3	44.33^{ab}	46.00^{b}	18.33^{b}	21.33^{bc}	27.23^{bc}	27.57^{bc}	9.86^{ab}	10.67^{a-c}	13.47^{b}	14.00^{b}	4.96^{a}	5.70^{a-c}
N_4	42.00^{a-d}	43.33^{cd}	18.00^{bc}	19.33^{cd}	22.60^{d-f}	22.93^{de}	9.40^{a-c}	10.40^{a-c}	10.60^{cd}	11.33^{c-e}	4.63^{a-c}	5.30^{a-d}
N_5	45.00^{a}	48.33^{a}	24.33^{a}	25.00^{a}	33.83^{a}	34.57^{a}	11.10^{a}	11.63^{a}	16.30^{a}	16.90^{a}	5.20^{a}	6.13^{a}
14-days												
N_0	38.00^{d-g}	40.33^{ef}	16.33^{b-f}	17.67^{d-f}	20.00^{f-i}	20.90^{e-g}	8.46^{b-d}	9.53^{b-d}	8.86^{d-g}	9.73^{d-f}	3.60^{b-d}	4.36^{c-e}
N_1	36.67^{fg}	38.33^{fg}	13.33^{f-h}	14.00^{h}	17.50^{hi}	18.60^{gh}	6.40^{de}	7.53^{ef}	7.83^{e-g}	9.13^{ef}	3.23^{d}	3.96^{de}
N_2	37.67^{e-g}	38.67^{e-g}	16.33^{b-f}	17.00^{d-g}	18.20^{g-i}	19.30^{f-h}	8.00^{b-d}	9.00^{c-e}	8.13^{d-g}	9.20^{ef}	3.56^{b-d}	4.33^{c-e}
N_3	43.67^{a-c}	43.33^{cd}	18.00^{bc}	19.33^{cd}	26.03^{b-d}	26.67^{c}	9.43^{a-c}	10.37^{a-c}	12.03^{bc}	12.90^{bc}	4.20^{a-d}	4.86^{a-e}
N_4	40.33^{b-f}	40.33^{ef}	17.67^{b-d}	18.33^{de}	21.60^{e-h}	22.00^{d-g}	8.70^{bc}	9.60^{b-d}	10.30^{c-e}	10.90^{c-f}	4.03^{a-d}	4.70^{b-e}
N_5	44.67^{a}	44.67^{bc}	21.67^{a}	22.33^{b}	28.56^{b}	29.97^{b}	9.80^{ab}	11.07^{ab}	13.17^{b}	13.87^{b}	4.86^{ab}	5.86^{ab}
21-days												
N_0	37.67^{e-g}	40.00^{ef}	14.33^{d-g}	14.67^{gh}	19.36^{f-i}	20.67^{e-g}	8.23^{b-d}	9.23 b-e	8.46^{d-g}	9.43^{ef}	3.36^{cd}	4.23^{de}
N_1	34.67^{g}	35.67^{h}	10.33^{h}	11.33^{i}	16.00^{i}	17.17^{h}	5.83^{e}	6.90^{f}	7.40^{g}	8.63^{f}	3.20^{d}	3.83^{e}
N_2	36.67^{fg}	37.33^{gh}	12.67^{gh}	13.67^{hi}	17.96^{g-i}	18.90^{f-h}	7.93^{b-d}	8.93^{c-e}	7.56^{fg}	8.83^{ef}	3.43^{cd}	4.16^{de}
N_3	42.00^{a-d}	43.67^{bc}	17.00^{be}	17.67^{d-f}	22.36^{d-f}	22.97^{de}	8.53^{b-d}	9.70^{b-d}	10.27^{c-e}	11.20^{c-f}	4.13^{a-d}	4.63^{b-e}
N_4	40.00^{c-f}	39.67^{e-g}	15.67^{b-g}	15.67^{e-h}	21.06^{e-h}	21.70^{d-g}	8.46^{b-d}	9.33^{b-e}	9.90^{c-g}	10.50^{c-f}	3.90^{a-d}	4.50^{c-e}
N_5	44.33^{ab}	44.67^{bc}	18.33^{b}	18.67^{d}	24.53^{c-e}	24.40^{cd}	9.63^{a-c}	10.10^{a-d}	11.83^{bc}	12.20^{b-d}	4.53^{a-d}	5.23^{a-d}

1st: 2010/2011 season, 2nd: 2011/2012 season

enhancing the rates of physiological processes and increasing the hydrostatic pressure on the cell wall, this is necessary for the enlargement of cell. Hence, enhancement of the assimilated food and increase the cell elongation and division consequently, the whole growth of plant could be increased (Bouton *et al*., 1985). These results are similar to those obtained by Farooq *et al*. (2009) reported that reduced water uptake results in a decrease in tissue water contents and turgor. Therefore, under drought stress conditions, cell elongation in plants is inhibited by reduced turgor pressure. Likewise, drought stress also trims down the photo-assimilation and metabolites required for cell division as a consequence, impaired mitosis, cell elongation and expansion result in reduced plant height and growth. Eiasu *et al.* (2012) on purple basil plant, Vazin (2013) on Cumin fond that water stress negatively affected plant growth.

Number of branches/plant: Data in Table 2 showed in both seasons that irrigation every 21 days highly significant decreased number of branches/plant compared to irrigation every 7 and 14 days. Also, the number of branches/plant was decreased as the water irrigation intervals increased in the two seasons. Maximum values in this respect were 19.67 and 19.61 for the lowest irrigation interval (7 days) while the minimum values were 17.22, 18.11, 14.72 and 15.28 at the medium longest irrigation intervals (14 and 21 days), respectively for both seasons similarly. These results were found by El-Mekawy (2006) on *Achillea santolina* L. plants. Alvarez *et al.* (2011) found that moderate deficit irrigation fore carnations plants showed a slightly reduced total dry weight, plant height and leaf area. While, severe deficit irrigation (SDI) had clearly reduced all the plant size parameters, lower number of shoots. Asrar and Elhindi (2011) on marigold they found that drought stress reduced growth vigor i.e., plant height, fresh and dry weights of shoot and plant.

Fresh and dry weighs of herb/plant: The results given in Table 2 showed that irrigation every 7 days highly significant increase in fresh and dry weights of herb/plant while irrigation every 14 and 21 days gave low values in this respect in the two seasons. The maximum values were at irrigated every 7 days (28.37 and 29.87 g plant^{-1}) for fresh weight in both seasons while dry weight of herb was (15.49 and 16.26 g plant^{-1}) in both seasons. On the other hand the minimum values in this respect were for irrigation every 14 and 21 days, such intervals gave the values of fresh weight of herb (23.98, 24.91, 20.21 and 20.97 g plant^{-1}), respectively while the dry weight were (12.06, 12.96, 19.23 and 10.13 g plant^{-1}), respectively, in this respect.

These results are in harmony with those reported by Khattab *et al.* (2002) on *Salvia splendens* plants, Lal *et al.* (2013) on lemon grass. These results may be due to the role of water availability on increasing the biosyntheses accumulated, consequently increased the dry matter of herb and the role of water availability on increasing the biosynthates accumulated, consequently increased the dry matter of herb (Bouton *et al.*, 1985). Also, Ninou *et al.* (2013) showed that under higher irrigation regimes more photosynthetically active leaves (higher net assimilation rate, transpiration rate and stomatal conductance gas) compared with lower irrigation rates.

Fresh and dry weights of root/plant: Data in Table 2 showed that using irrigation every 7 days gave highly significant increase fresh and dry weight of roots/plant, compared with irrigation every 14 and 21 days which gave the lowest values in the two seasons. The maximum values in this respect for irrigated plants every 7 days followed by irrigated every ones 14 days and then those irrigated every 21 days which gave minimum values in this respect for both seasons, Similar results were reported by El-Makawy (1999) on *Calotropis procera*, *Peganum harmala* and *Marrubium vulgare* plants and Shahin *et al.* (2006) on sisal.

Effect of fertilization

Plant height: Data in Table 2 clearly showed that during the two seasons plant height significantly affected by chemical fertilization and dual inoculation by two bacterization. The maximum values were recorded with the treatment of 150 kg fed.$^{-1}$ of NPK fertilizer plus inoculation with the mixture of N_2-fixing bacteria (cerealin) and phosphate dissolving bacteria(phosphorine), followed by full NPK fertilizer plus inoculation with N_2-fixers (*Azotobacter* and *Azospirillum*) and then by NPK fertilizer plus inoculation with phosphobacteria and inoculation with non-symbiotic N_2-fixers (*Azotobacter* and *Azospirillum*) supplemented with half dose of inorganic N-fertilizer in the two seasons, respectively. The obtained results indicated also that the alone inoculation with the mixture of biofertilizer without inorganic N-fertilizer remarkably decreased the plant height compared with the control. On the other hand, it was noticed that adding 75 kg fed.$^{-1}$ of NPK fertilizer to biofertilizeres (*Azotobacter*+phosphate solubilizing bacteria) inoculation gave low values compared with control, without significant difference between them. These results may be due to the influence of the biofertilizer on enhancing soil acidity (pH-values) and water retention in the rooting medium hence the availability and absorption of nutrients could be enhanced, leading to more initiation and elongation of cells Mostafa (2002). Similar trend of results were cleared by Parakhia *et al.* (2000) on fenugreek found that the inoculation of *Azotobacter* and *Rhizobia* increased shoot height.

Number of branches: The Data in Table 2 show the effect of NKP level and biofertilizer on *Achillea santolina* L. plant during two seasons, whereas application of fertilization exerted significant increases on number of branches. Increasing the applied NPK-levels from 75-150 kg fed.$^{-1}$ mixture with biofertihzer markedly increased number of branches in both seasons. Regarding the effect of biofertilizer, it is clear from such data in Table 1 that number of branches was markedly increased with biofertilizer treatments than with the untreated ones. Furthermore, application of biofertihzer with NPK was more useful than (NFB+PDB) or NPK alone, all levels of NPK fertilizer were generally more effective in the presence than in the absence of biofertilizer. In this regard, plants receiving 150 kg NPK/fed. with mixture of biofertilizer gave the highest values of number of branches in both seasons. Similar results were reported by Sheikh *et al.* (2000) on Dutch iris, Attia and Saad (2001) on *Catharanthus roseus* plants, Kandeel and Sharaf (2002) on *Ocimum bassilicum* plants and El-Mekawy and Mohamed (2005) on *Nigella sativa*.

Increasing the number of branches/plant resulted from nutrients fertilization might be due to the indirect participation of those nutrients in reducing the apical dominance of steam, resulting in more branching. Besides that, these nutrients might play a direct role in plant metabolism, resulting in enhancing branches growth these results may be due to the influence of the biofertilizer on enhancing soil acidity (pH-values) and water retention in the rooting medium hence the availability and absorption of nutrients could be enhanced, leading to more initiation and elongation of stem cells (Cacciari *et al.*, 1989).

Fresh and dry weights of herb/plant: The Data given in Table 2 indicated that fresh and dry weights of herb/plant significantly increased under the used recommended fertilization plus mixture of biofertilizer compared to other fertilization treatments which gave the heights values. While, the lowest values of fresh and dry weight of herb were resulted from bacterization only in both seasons. These results are in line with those reported by Harridy *et al.* (2001) on lemongrass they concluded that application of NPK-fertilizer to the plants significantly increased fresh and dry weights compared with the without mineral fertilizer treatment. Moreover, all values were markedly increased with increasing the applied NPK-levels from 75-150 kg fed.$^{-1}$.

On the other hand, application of biofertilizer exerted significant increase in both fresh and dry weight of *Achillea santolina* plant than with the untreated ones. Also, fresh and dry weight was generally increased with bioferilizer mixture. These increases in plant growth ascribed to biofertilizer application might be due to the vital role of bacteria which is present in the applied biofertilizer and capable of contributing some hormone substances, such as gibberellins, auxins and cytokinins (Tien *et al*., 1979; Bouton *et al*., 1985). These phytohormones may stimulate the cell elongation and division of plant which hence plant growth (Paleg, 1965) Moreover, the increment in bacteria population and its activity into the absorption zone of plant roots might improve soil fertility and plant development by phosphobacteria and N_2-fixation and release of certain other nutrients, i.e., Fe, Zn and Mn (Bhonde *et al*., 1997). The positive interactions between the applied NPK-fertilizer levels and bioferitilizer on plant vegetative growth may be due to the effects of N-element and biofertilizer together on the absorption of established plant roots and nutrient uptake (Wange *et al*., 1995; Chugh and Dhaliwal, 2013).

Such increase in fresh and dry weights of herb might due to the increase in CO_2 fixation and/or enhancing cell division and/or cell enlargement as well as the anabolic processes; leading to more weight. Fertilization might play a direct or indirect role in plant anabolism through activating the photosynthetic processes as well as the accumulation of their products in plant organs, resulting in more plant materials. The enhancing effect of NPK-fertilizer on plant growth may be due to the positive effects of NPK-element on activation of photosynthesis and metabolic processes of organic compounds in plants. Which in turn, encourage the plant vegetative growth. Furthermore, N and P is an essential component of many organic compounds in plant, such as proteins, enzymes, chlorophylls, vitamins, carotenoids, hormones and nucleic acids Gardner *et al*. (1985).

Fresh and dry weight of root: Data given in Table 2 indicated in both seasons that the used mixture fertilization treatment gave highly significant increment of fresh and dry weights of roots/plant compared to chemical fertilizer alone. The maximum values were obtained due to fertilizing the plants with full NPK dose under the mixture bioferilization treatments, no significant differences were observed among most treatments in this respect. These results are in harmony with those found by Kandeel and Sharaf (2002) on *Ocimum basilicum*. The increase in fresh and dry weight of roots due to fertilization treatments might be attributed to the enhancing effects of it on root elongation and growth. Also, Harb *et al*. (2011) on *Nigella sativa* indicated that the biofertilization (Glomus macrocarpus fungus or Nitrobein bacteria) or organic manure alone or in combination with half or full NPK fertilizer increased plant height (cm), No. of branches and leaves, root length (cm) as well as herb and root dry weight when compared with uninoculated plants and the best significant results of herb and root dry weight were found with mycorrhizal fungus and Azotobacter with full NPK fertilizers treatment as compared to the other treatments.

Effect of the interactions between irrigation intervals and fertilization

Plant height: Data of both seasons in Table 2 revealed that the treatment of fertilization at full dose in combination with biofertilization and watering every 7 days gave significant increase in plant height comparing with irrigation with chemical or biofertilizer only. The used chemical fertilization, under the same irrigation interval increased plant height. But, increasing watering interval under fertilizer treatment decreases it. These results are in line with those of El-Mekawy (2009) noticed the effect of the interaction between the used different watering intervals and fertilization that the growth and yield parameters considerably improved as a result of watering interval at 10-days and inoculation with symbiotic N_2-fixers (*Azotobacter* and *Asospirillum*) supplemented with half or full dose of inorganic N-fertilizer in the two seasons, against the lowest values resulted from the irrigated plants every 20 or 30-days and inoculated with biofertilizer only of *Thymus capitatus* L. plant.

The improvement in plant height due to the additions of some chemical fertilization under increase watering intervals might be due to that the used chemical fertilizer overcame the harmful of stress water on plant height (Schaik *et al*., 1997).

Number of branches/plant: Data of both seasons in Table 2 showed that the treatments of fertilization and irrigation every 7 days gave the maximum values of number branches/plant compared to other treatments. The treatments of mixture fertilization under the used irrigation intervals increased number of branches comparing with those watering chemical fertilizer treatment or biofertilizer treatment alone. Using both fertilizer kinds, under the same irrigation interval increased number of branches/plant. But, increasing watering intervals under the same levels (50 or 100%) of chemical fertilization decreased it. The highest number of branches/plant was obtained by using 100% chemical fertilization level+mixture biofertilizer under irrigation every 7 days comparing to the other ones in the two seasons. Similar results were reported by El-Mekawy (2009) they indicated that plant height and number of branches/plant and fresh and dry weights of *Thymus capitatus* L. plant increased at the treatment of short irrigation intervals with the mixture of bio and NPK-fertilization and Maheshwari *et al*. (1998) who recorded that high biomass yields of palmarosa were result by using *Azotobacter* alone and *Azotobacter* with 40-80 kg N/ha under rainfed. and irrigated condition, respectively.

Fresh and dry weights of herb/plant: Table 2 indicate that the interaction treatments between mixture fertilization and irrigation every 7 days caused an increase in fresh and dry weights of herb/plant compared to biofertilizer alone or those of other watering intervals. Using biofertilizer treatment, under the same irrigation interval increase fresh and dry weights of herb/plant. But, increasing watering interval under biofertilizer or the same level of NPK fertilizer (half or full) decreased them. Generally, the fresh weight of herb values for different treatments was highly significant among them in the first season and in the second season. Also, the differences among treatments used in case of dry weight were significant in both seasons. Similar results were reported by El-Mekawy and Mohamed (2005) on *Nigella sativa*. Gholamhoseini *et al*. (2013) results irrespective of the mycorrhizal and the drought stress intensity that inoculated plants produced more dry matter than did non-inoculated of sunflower under each irrigation regime.

Fresh and dry weights of roots/plant: Data presented in Table 2 show that the interaction treatments between fertilization and irrigation at every 7 days gave the maximum values of fresh and dry weights of root/plant compared to without chemical fertilizer or those of other irrigation intervals. The interaction treatments of chemical+biofertilization and the used irrigation intervals caused an increase the weights of fresh and dry weight of root/plant comparing with that chemical fertilizer alone under used watering intervals. Using application of NPK fertilizer either alone or combined with biofertilization treatment, under the same watering interval increase the fresh and dry weight of roots/plant. But, increasing irrigation intervals under the same level of fertilizer decrease it. The highest weights of fresh and dry of roots/plant were obtained by using NPK fertilization with two bacterization under irrigation every 7 days comparing to other interaction ones or those of irrigation every 14 or 21 days in the two seasons. Maheshwari *et al.* (1998) on palmarosa, Gholamhoseini *et al.* (2013) on sunflower. The biofertilizer (BF) improved carbon and nitrogen mineralization by promoting soil microbial activities and narrowed down C:N ratio. Effect of BF on cation exchange capacity of the soil became evident with time. Physical structure of the soil was influenced due to BF as indicated by significant decline in bulk density and increase in water holding capacity, hydrauhc conductivity and mean weight Plant growth and yield (Nisha *et al.*, 2007).

CHEMICAL CONSTITUENTS

Effect of irrigation intervals

Chlorophyll a and b: Data in Table 3 showed that chlorophyll a and b highly significant decreased with increase irrigation intervals. The irrigation every 7 days gave the highest values of chlorophyll a and b followed by moderate values when irrigation every 14 days while irrigated plants every 21 days gave the minimum values in this respect. However, with increasing irrigation intervals chlorophyll a and b were decrease in both seasons. Similar results were found by Kandeel (2004), on *Ocimum basilicum*, Salman (2004) on basil plant.

Total carbohydrate content: Results in Table 3 indicated that with increase irrigation intervals the total carbohydrate content in leaves were increased, moderate irrigation interval 14 days gave the highest results followed by irrigation every 21 days and irrigation every 7 days which gave the lowest values in this respect in the two seasons. Similar results were found by Dos Santos *et al.* (2013) suggest that during the dry season in the semi-arid region, dynamic photoinhibition occurred in the leaves of Jatropha curcas around mid-day, with a rapid recovery during the afternoon. However, chronic photoinhibition may have occurred in some plants with a severe water deficit. A reduction in the effective quantum efficiency was also observed during the driest months. The increase of catalase activity and the accumulation of proline, total soluble sugars and amino acids during periods of low water availability in the soil. Bolla *et al.* (2010) suggest on rose that mild water stress caused a decline in leaf chlorophyll and chlorophyll fluorescence, negative effect on photosynthetic rate and stomatal conductance leading to a limited supply of metabolic energy and therefore to plant growth restriction. Ninou *et al.* (2013) showed that under higher irrigation regimes more photosynthetically active leaves (higher net assimilation rate, transpiration rate and stomatal conductance gas) compared with lower irrigation rate.

Total N, P and K percentage: With regard to the effect of irrigation intervals on nitrogen, phosphorus and potassium, the results in Table 3 showed that highly significant differences among treatments, however short irrigation interval gave the highest values of total nitrogen and phosphorus percentage while potassium gave lowest values in both seasons. Similar findings were also reported by Khattab *et al.* (2002) found the superiority of increasing water supply on the content of N, P and K elements in the leaves of *Salvia splendens* plants due to that increasing soil moisture content caused a marked effect on increasing the solubility of such elements in the soil which led to promote the absorbing efficiency of such elements by the plants.

Effect of fertilization

Chlorophyll a and b: The Data were recorded in Table 3 show that chlorophyll a and b exhibited a significant increase by using both mixture biofertilization and chemical fertilization treatments

Table 3: Effect of irrigation intervals and fertilization on volatil of *Achillea santolina* L. in two seasons (2010/2011 and 2011/2012)

	Chlorophyll ($mg\ g^{-1}$ F.w)											
	a		b		Total carbohydrate		N%		P%		K%	
Treatments	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Watering intervals												
7-days	11.14^{a}	11.57^{a}	4.96^{a}	4.87^{a}	7.58^{c}	8.65^{c}	2.99^{a}	3.21^{a}	0.68^{a}	0.69^{a}	3.30^{b}	3.36^{b}
14-days	10.16^{b}	10.78^{b}	4.28^{b}	4.32^{b}	9.95^{a}	10.81^{a}	2.58^{b}	2.76^{b}	0.41^{b}	0.54^{b}	5.41^{a}	5.58^{a}
21-days	6.78^{c}	7.90^{c}	3.58^{c}	3.69^{c}	8.78^{b}	9.58^{b}	2.15^{c}	2.27^{c}	0.39^{c}	0.40^{c}	2.72^{c}	2.78^{c}
Fertilization												
N.P.K	8.73^{b-d}	9.30^{b}	4.31^{bc}	6.01^{ab}	6.96^{bc}	9.84^{b}	2.60^{b}	2.61^{b}	0.47^{b}	0.48^{b}	3.30^{b}	3.36^{b}
(NFB+PDB) mixture	6.89^{d}	8.22^{c}	2.80^{c}	4.36^{b}	5.62^{c}	7.03^{c}	1.25^{c}	1.26^{d}	0.36^{c}	0.37^{b}	2.26^{c}	0.26^{c}
½ N.P.K+(NFB+PDB) mixture	7.83^{cd}	8.89^{bc}	3.02^{bc}	4.53^{b}	6.03^{bc}	8.75^{b}	1.89^{b}	1.68^{b}	0.45^{b}	0.47^{b}	2.29^{b}	3.35^{b}
N.P.K+NFB	10.80^{ab}	11.35^{ab}	4.57^{b}	6.43^{ab}	8.40^{ab}	10.98^{ab}	2.83^{ab}	2.84^{ab}	0.51^{a}	0.53^{ab}	3.39^{ab}	4.42^{ab}
N.P.K+PDB	9.48^{bc}	10.24^{a-c}	3.58^{bc}	6.41^{ab}	7.17^{bc}	9.97^{b}	2.58^{b}	2.60^{b}	0.46^{ab}	0.49^{b}	3.33^{b}	3.37^{b}
N.P.K+(NFB+PDB) mixture	12.42^{a}	12.51^{a}	6.63^{a}	8.45^{a}	9.80^{a}	11.16^{a}	2.91a	2.94^{a}	0.57^{a}	0.58^{a}	4.43^{a}	4.49^{a}
Interaction between watering interval and treatnent fertilization												
7-days												
N_0	10.73^{b-d}	10.79^{c-e}	5.87^{cd}	6.82^{b-d}	5.09^{cd}	7.24^{e-g}	2.62^{b}	2.64^{bc}	0.38^{b}	0.37^{b}	3.34^{b}	3.35^{bc}
N_1	9.23^{b-e}	9.23^{d-f}	3.59^{f}	5.65^{cd}	4.61^{de}	5.83^{fg}	1.27^{d}	1.30^{e}	0.36^{b}	0.37^{b}	2.24^{e-g}	2.24^{g-i}
N_2	9.73^{b-d}	9.73^{c-f}	4.49^{cd}	6.11^{cd}	4.64^{de}	7.12^{e-g}	1.70^{c}	1.7 2d	0.36^{b}	0.36^{b}	2.29^{b-e}	2.29^{b-g}
N_3	15.19^{ab}	15.94^{a}	7.40^{b}	8.37^{a-c}	6.71^{bc}	7.84^{d-f}	2.73^{ab}	2.74^{ab}	0.45^{a}	0.46^{a}	3.32^{bc}	3.34^{b-d}
N_4	11.35^{b}	14.86^{cd}	6.30^{bc}	7.09^{bc}	5.46^{cd}	7.42^{e-g}	2.61^{b}	2.62^{bc}	0.47^{a}	0.47^{a}	3.33^{b}	3.33^{b-e}
N_5	17.53^{a}	16.02^{a}	8.18^{a}	10.79^{a}	8.19^{ab}	9.88^{bc}	2.86^{a}	2.86^{a}	0.48^{a}	0.47^{a}	3.34^{b}	3.35^{bc}
14-days												
N_0	9.12^{bc}	8.12^{ef}	4.33^{de}	5.19^{cd}	7.91^{ab}	9.87^{bc}	2.60^{b}	2.62^{bc}	0.36^{b}	0.36^{b}	3.36^{b}	3.36^{bc}
N_1	4.57^{gh}	6.82^{f}	3.42^{de}	2.52^{ef}	5.73^{cd}	7.87^{d-f}	1.25^{d}	1.26^{e}	0.36^{b}	0.35^{b}	2.25^{e-g}	2.27^{d-h}
N_2	6.71^{e-h}	7.19^{ef}	3.65^{de}	4.83^{d-f}	6.70^{bc}	8.24^{cd}	1.68^{c}	1.69^{d}	0.37^{b}	0.37^{b}	2.30^{g}	2.29^{b-g}
N_3	11.53^{b}	12.67^{bc}	5.58^{bc}	7.57^{b-d}	9.57^{a}	11.15^{ab}	2.72^{ab}	2.74^{ab}	0.44^{a}	0.44^{a}	3.36^{b}	3.46^{b}
N_4	10.06^{b}	11.70^{cd}	4.40^{cd}	6.41^{b-d}	8.14^{ab}	10.62^{b}	2.58^{b}	2.60^{c}	0.47^{a}	0.46^{a}	3.34^{bc}	3.37^{bc}
N_5	17.01^{a}	15.29^{ab}	6.04^{b}	9.27^{ab}	9.62^{a}	12.03^{a}	2.81^{a}	2.84^{a}	0.47^{a}	0.46^{a}	4.42^{a}	4.43^{a}
21-days												
N_0	6.80^{e-g}	7.29^{ef}	3.33^{de}	4.96^{de}	6.90^{b-d}	9.64^{bc}	2.58^{b}	2.59^{c}	0.36^{b}	0.36^{b}	2.24^{fg}	2.25^{hi}
N_1	4.22^{h}	6.70^{f}	1.50^{f}	2.03^{f}	5.61^{cd}	8.59^{cd}	1.23^{d}	1.24^{e}	0.35^{b}	0.35^{b}	2.23^{d-g}	2.24^{d-g}
N_2	5.61^{f-h}	6.89^{f}	3.29^{de}	4.64^{d-f}	6.55^{b-d}	8.65^{cd}	1.64^{c}	1.65^{d}	0.35^{b}	0.36^{b}	2.28^{b-f}	2.29^{b-g}
N_3	7.46^{d-f}	8.28^{ef}	3.77^{de}	5.21^{de}	8.20^{ab}	10.05^{b}	2.71^{ab}	2.74^{ab}	0.44^{a}	0.44^{a}	2.28^{b-f}	2.29^{bc}
N_4	7.85^{d-f}	8.09^{ef}	3.48^{de}	5.19^{de}	7.29^{a-c}	9.87^{bc}	2.57^{b}	2.59^{c}	0.45^{a}	0.45^{a}	2.24^{d-g}	2.27^{bc}
N_5	8.73^{c-e}	8.92^{d-f}	5.14^{bc}	6.99^{b-d}	8.35^{ab}	10.95^{ab}	2.81^{a}	2.82^{a}	0.45^{a}	0.45^{a}	3.34^{b}	3.34^{bc}

1st: 2010/2011 season, 2nd: 2011/2012 season

comparing with biofertilizer only or use chemical fertilization in the two seasons. These results are in similar to those found by Bolla *et al.* (2010) suggest on rose that mild water stress caused a decline in leaf chlorophyll and chlorophyll fluorescence, negative effect on photosynthetic rate and stomatal conductance leading to a limited supply of metabolic energy and therefore to plant growth restriction.

Such increase might be attributed to know function of some elements like nitrogen which was found in such important molecules as metabolically important compounds in the chlorophylls (Schuch *et al.*, 1995).

Total carbohydrate content: Table 3 indicates that biofertilization treatment increased total carbohydrate content in leaves comparing to untreated plants in the two seasons. Similar results were found Hassan *et al.* (2010) showed that applying biofertilizers alone or combined with chemical fertilizers significantly improved growth characters of roselle plant In addition, the best treatment in this respect was bacteria inoculation +1/2 recommended dose of chemical fertilizers. Othewise, there were no significant differences in this concern between this treatment and the full RD of chemical fertilizers Such increase in fresh and dry weights of herb might due to the increase in CO_2 fixation and/or enhancing cell division and/or cell enlargement as well as the anabolic processes; leading to more weight. Fertilization might play a direct or indirect role in plant anabolism through activating the photosynthetic processes as well as the accumulation of their products in plant organs, resulting in more plant materials (Gardner *et al.*, 1985). The positive interactions between the applied N-fertilizer levels and bioferitilizer on plant vegetative growth may be due to the effects of N-element and biofertilizer together on the absorption of established plant roots and nutrient uptake (Wange *et al.*, 1995; Chugh and Dhaliwal, 2013).

Total nitrogen, phosphours and potassium percentage: With regard to the effect of fertilization on NPK percentage, the results in Table 3 showed that mixture of biofertilizer and chemical fertilization treatments increased N, P and K percentage in leaves, comparing with two bacterization or treated by chemical fertilization treatment only in the two seasons.

Such increase in N, P and K percentage in leaves might be due to the enhancing effect of chemical fertilization on the absorption and/or translocation of the NPK from root to the above ground parts. These results are in line with those reported by Chezhiyan *et al.* (2003), Marfa *et al.*(2002) and Khalil *et al.* (2004) who showed that application of nitrogen and potassium fertilizers at the rate of 100 kg N+140 kg K_2O/fed. through irrigation water recorded the maximum values of growth rate and photosynthetic pigments, whereas, application of the balance rate of nitrogen and potassium at the level of (60 kg N+60 kg K_2O/fed.) being the superior treatment regarding plant nutritional status (N, P, K content and their total uptake) on plants.

Effect of the interactions between irrigation intervals and fertilization

Chlorophyll a and b contents: Data in Table 3 showed that the interactions between using fertilization and irrigation intervals were significant for chlorophyll a and b in the two seasons. Generally, there was an increase in chlorophyll a and b with using treatments contain NPK plus biofertilization compared to treatment without biofertilizers. Also, increasing the irrigation intervals under the same level of fertilizer treatment decreased chlorophyll a and b. However, using fertilizer treatment under the same level of irrigation interval increased chlorophyll a and b in fresh leaves. Also, using fertilization at short irrigation interval (7 days) gave the maximum values while without biofertilizer treatment at long irrigation interval (21 days) gave the minimum values in this respect. These results are in line with those of Shahin *et al.* (2006) on sisal.

Total carbohydrate content: Table 3 indicates that the interaction treatments combined between fertilization and irrigation every 14 days caused an increase in total carbohydrate content compared to biofertilizer and irrigated with other watering intervals. Using chemical fertilizer combined with biofertilzer treatment, under the same irrigation interval increased total carbohydrate content. But, increasing watering interval under the same level of NPK fertilizer (half or full) of chemical fertilizer or biofertilizer decreased it. Generally, the differences among treatments were highly significant in the first season and in the second season. Similar results were reported by Gholamhoseini *et al.* (2013) results irrespective of the mycorrhizal and the drought stress intensity that inoculated plants produced more dry matter than did non-inoculated of sunflower under each irrigation regime. Dos Santos *et al.* (2013) suggest that during the dry season in the semi-arid region, dynamic photoinhibition occurred in the leaves of *Jatropha curcas* around mid-day, with a rapid recovery during the afternoon. However, chronic photoinhibition may have occurred in some plants with a severe water deficit. A reduction in the effective quantum efficiency was also observed during the driest months. The increase of catalase activity and the accumulation of proline, total soluble sugars and amino acids during periods of low water availability in the soil.

Total N, P and K percentage: Data in Table 3 show that interactions between fertilization and irrigation every 7 days caused an increase for nitrogen and phosphorus percentage while using chemical and biofertilization treatment with irrigation every 14 or 21 days increased potassium percentage compared to alone fertilizer treatment. Generally, with increasing level of fertilizer from 50-100% NPK kg fed.$^{-1}$ alone or combined with biofertilizer the percentage of total N, P and K were significant among the treatments and also increasing irrigation intervals caused increase values of potassium and decreased values of nitrogen and phosphorus for fertilizer treatment in the two seasons. Similar results were reported in this respect by El-Khateeb and Boselah (1991) on periwinkle.

FLOWERING CHARACTERS AND OIL YIELD

Effect of watering intervals

Number of inflorescence: Data in Table 4 clearly showed that number of inflorescences/plant increased gradually with decreasing watering intervals and reached the maximum values by watering the plants every 7 day in the two seasons. On the other hand, irrigation every 21 day gave the lowest values in this respect. These results may be due to that the short watering intervals was more suitable for forming more number of inflorescences/plant through enhancing the vegetative biomass which gave large number of flowers, consequently the fruits could be increased. These results are supported with the obtained results by Raju and Sinsinwar (2006) on Indian mustard.

Inflorescence fresh and dry weight: Data are presented in Table 4 indicated that the different irrigation intervals were exerted a marked and significant effect on fresh and dry weight of inflorescences/plants. Whereas, application of irrigation water every 7 days was the most effective and favorable treatment, as well as recorded the maximum values in this respect. On contrary, the lowest values of fresh and dry weight of inflorescences resulted from application of the lowest level of irrigation water and/or increasing soil moisture water stress. Similar results were reported by Shalan (2005) on borage plants (*Borago officinalis,* L.). The superiority effect of irrigation water every 7 days during the growing seasons on plants is directly owing to the increase in plant growth rate and its dry matter content, as well as, plant minerals uptake so, the flowering can be considered as the final resultant of all physiological and metabolic processes in the plant. Furthermore water use efficiency, leading to increase the availability and absorption of nutrient elements necessary for flowering precursors of a class of compounds which ultimately forms amino acids and hormones leading to an earlier flowering, gave many lateral branches and number of flowers /plant. Also, increasing in flower dry weight may be due to the increase in flower diameter or flower numbers or both. (Devitt *et al.*, 1991).

Table 4: Effect of irrigation intervals and biofertilization on volatil of *Achillea santolina* L. in two seasons (2010/2011 and 2011/2012)

Treatments	Inflor. No./plant 1st	Inflor. No./plant 2nd	Inflor. fr.wt/plant (g) 1st	Inflor. fr.wt/plant (g) 2nd	Inflor. dry wt./plant (g) 1st	Inflor. dry wt./plant (g) 2nd	Yield/fed. (kg) 1st	Yield/fed. (kg) 2nd	Oil (%) 1st	Oil (%) 2nd	Oil yield fed.$^{-1}$ (kg) 1st	Oil yield fed.$^{-1}$ (kg) 2nd
Watering intervals												
7-days	731.28^{a}	917.20^{a}	13.99^{a}	17.74^{a}	6.11^{a}	7.93^{a}	81.01^{ab}	99.79^{b}	1.35^{c}	1.36^{c}	1.17^{b}	1.60^{ab}
14-days	700.94^{b}	893.20^{b}	13.46^{a}	17.21^{a}	5.78^{ab}	7.69^{a}	85.64^{a}	111.02^{a}	1.43^{b}	1.44^{b}	1.31^{a}	1.70^{a}
21-days	674.33^{c}	863.20^{c}	12.87^{a}	16.61^{a}	5.50^{b}	7.42^{a}	77.05^{b}	89.92^{c}	1.51^{a}	1.52^{a}	1.04^{c}	1.42^{b}
Fertilization												
N.P.K	696.33^{b}	811.70^{b}	13.27^{b}	15.60^{b}	5.71^{b}	6.93^{d}	79.99^{b}	96.96^{d}	1.42^{b}	1.43^{b}	1.14^{d}	1.39^{d}
(NFB+PDB) mixture	576.89^{d}	737.30^{d}	11.0^{d}	14.20^{e}	4.52^{d}	6.17^{e}	63.23^{d}	86.43^{e}	1.30^{c}	1.31^{c}	0.82^{f}	1.13^{e}
½ N.P.K+(NFB+PDB) mixture	654.44^{b}	805.20^{b}	12.41^{c}	15.47^{b-d}	5.29^{c}	6.86^{d}	74.08^{c}	95.99^{d}	1.41^{b}	1.42^{b}	1.05^{e}	1.37^{b-d}
N.P.K+NFB	770.22^{a}	1007.80^{a}	13.82^{ab}	18.07^{b}	6.00^{ab}	8.15^{c}	91.36^{a}	123.20^{a}	1.45^{ab}	1.45^{ab}	1.22^{ab}	1.66^{b}
N.P.K+PDB	762.33^{ab}	935.80^{ab}	14.84^{a}	19.43^{b}	6.53^{a}	8.79^{b}	84.08^{ab}	114.10^{ab}	1.46^{ab}	1.47^{ab}	1.34^{ab}	1.81^{b}
N.P.K+(NFB+PDB) mixture	792.89^{a}	1049.80^{a}	15.32^{a}	20.38^{a}	6.76^{a}	9.20^{a}	94.70^{a}	128.80^{a}	1.55^{a}	1.56^{a}	1.47^{a}	2.01^{a}
Interaction between watering interval and treatnent fertilization												
7-days												
N_0	655.00^{hi}	775.00^{k}	13.87^{d-f}	16.27^{i}	6.04^{d-g}	7.26^{g}	74.01^{ij}	91.84^{i}	1.31^{h}	1.33^{hi}	1.17^{fg}	1.41^{f}
N_1	557.00^{k}	716.00^{m}	11.33^{j}	14.77^{k}	4.76 kl	6.34^{ij}	60.01^{m}	84.00^{k}	1.29^{h}	1.30^{i}	0.82^{kl}	1.13^{hi}
N_2	627.33^{ij}	781.00^{jk}	12.93^{gh}	15.87^{ij}	5.58^{hi}	7.07^{gh}	70.28^{jk}	92.45^{i}	1.30^{h}	1.32^{hi}	1.05^{h}	1.38^{f}
N_3	744.00^{c-e}	980.70^{de}	15.43^{ab}	20.00^{bc}	6.85^{ab}	9.06^{a-c}	87.22^{c-f}	119.80^{d}	1.38^{ef}	1.38^{fg}	1.34^{d}	1.82^{c}
N_4	698.33^{fg}	901.70^{g}	14.33^{c-e}	18.77^{f}	6.29^{c-e}	8.49^{d}	80.55^{gh}	109.60^{f}	1.36^{fg}	1.36^{gh}	1.22^{ef}	1.66^{d}
N_5	764.33^{b-d}	1027.30^{bc}	16.07^{a}	20.80^{a}	7.18^{a}	9.35^{a}	90.25^{b-d}	125.80^{bc}	1.46^{d}	1.47^{c-e}	1.42^{bc}	1.99^{b}
14-days												
N_0	728.33^{d-f}	846.30^{h}	13.47^{e-g}	15.67^{j}	5.81^{f-h}	6.95^{h}	84.61^{d-g}	101.70^{g}	1.43^{de}	1.45^{e}	1.28^{de}	1.54^{e}
N_1	595.33^{jk}	756.30 kl	11.20^{jk}	14.17^{l}	4.50^{lm}	6.18jk	66.69 kl	88.76^{ij}	1.30^{h}	1.31^{hi}	0.88^{jk}	1.20^{gh}
N_2	682.67^{gh}	826.30^{hi}	12.40^{hi}	15.57^{j}	5.27^{ij}	6.90^{h}	78.12^{hi}	98.98^{gh}	1.42^{de}	1.43^{ef}	1.19^{f}	1.49^{e}
N_3	800.33^{ab}	1035.60^{bc}	14.80^{bc}	19.40^{de}	6.49^{bc}	8.78^{cd}	95.95^{ab}	126.80^{a-c}	1.47^{cd}	1.48^{c-e}	1.48^{b}	1.97^{b}
N_4	749.67^{c-e}	972.30^{e}	13.77^{e-g}	18.03^{g}	5.97^{e-h}	8.13^{e}	88.06^{c-e}	118.90^{d}	1.46^{d}	1.46^{de}	1.35^{cd}	1.83^{c}
N_5	831.33^{a}	1066.00^{a}	15.17^{bc}	20.47^{ab}	6.67^{bc}	9.26^{ab}	100.5^{a}	130.90^{a}	1.52^{bc}	1.54^{b}	1.67^{a}	2.19^{a}
21-days												
N_0	705.67^{e-g}	813.70^{i}	12.47^{hi}	14.87^{k}	5.29^{ij}	6.56^{i}	81.34^{f-h}	97.35^{h}	1.52^{bc}	1.52^{bc}	0.96^{i}	1.21^{g}
N_1	578.33^{k}	739.70^{lm}	10.47^{k}	13.67^{l}	4.29^{m}	6.00^{k}	63.00^{lm}	86.52^{jk}	1.31^{gh}	1.32^{hi}	0.88^{l}	1.10^{i}
N_2	653.33^{hi}	808.30^{ij}	11.90^{ij}	14.97^{k}	5.02^{jk}	6.60^{i}	73.83^{ij}	96.55^{h}	1.51^{bc}	1.51^{b-d}	0.91^{ij}	1.22^{g}
N_3	766.33^{b-d}	1007.30^{cd}	14.30^{c-e}	18.90^{ef}	6.23^{c-f}	8.56^{d}	90.91^{bc}	122.90^{cd}	1.54^{b}	1.55^{b}	1.20^{f}	1.65^{d}
N_4	719.00^{e-g}	933.70^{f}	13.37^{fg}	17.40^{h}	5.75^{gh}	7.83^{f}	83.63^{e-h}	113.80^{e}	1.53^{b}	1.54^{b}	1.10^{gh}	1.49^{e}
N_5	783.00^{bc}	1056.30^{ab}	14.73^{b-d}	19.87^{cd}	6.45^{b-d}	8.98^{bc}	93.38^{bc}	129.70^{ab}	1.66^{a}	1.67^{a}	1.32^{d}	1.85^{c}

Oil percentage and yield/plant: It is evident from data in Table 4 that the volatile oil percentage in *Achillea* plants tended to increase with increasing irrigation intervals in both seasons, the highest values of volatile oil percentage in the flower heads were obtained from plants irrigated every 21 days. The results may be explained by the findings of Penka (1978) who 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 production of essential oil yield per plant (unexpanded flower head) increased by reducing the irrigation intervals as shown in Table 3. The highest essential oil yield per plant was obtained by the treatment of watering every

14 days. Meanwhile, the treatment of irrigation every 21 days resulted in the lowest volatile oil yield per plant in the two seasons. The increment of the essential oil yield as a result of reducing watering intervals could be explained through its effect on increasing the corresponding drug yield as shown in Table 4. Similar results were obtained by on *Jasminum grandiflorum*, Vazin (2013) the essential oil of cumin were showed a decreasing under deficient water.

Effect of fertilization

Number of inflorescences: It is clear from data in Table 4 that number of inflorescences considerably influenced by inoculation with N_2-fixers bacteria and inorganic NPK-fertilizer. The highest number of inflorescence were recorded with the treatment of dual inoculation of *Azotobacter+Azospirillum* supplemented with full dose of inorganic NPK-fertilizer followed by the treatment of control and half dose of inorganic N-fertilizer, with insignificant differences between the later treatments. It is evident that treating plants with bacterial inoculation alone gave the lowest values in this respect. Similar findings were noticed by Wange *et al.* (1995) on tuberose, Sheikh *et al.* (2000) on Dutch iris and Yadav and Khurana (2000) on fennel.

Inflorescences fresh and dry weights: The obtained data in Table 4 indicated that the highest weights of fresh and dry of inflorescences considerably influenced by inoculation with two bacterization and inorganic NPK-fertilizer. The highest values were recorded with the treatment of dual inoculation of (non-symbiotic N_2-fixers+phosphobacteria) supplemented with full dose of inorganic NPK-fertilizer (150 kg fed.$^{-1}$) followed by the treatments of NPK-fertilizer+NFB and NPK-fertilizer+PFB, but with insignificant differences between the later treatments, then the control (150 kg fed.$^{-1}$) and half dose of inorganic N-fertilizer (75 kg fed.$^{-1}$), It is evident that treating plants with bacterial inoculation alone gave the lowest values in this respect. Similar findings were noticed by Attia and Saad (2001) on *Catharanthus roseus* Annamalai *et al.,* (2004) on *Phyllanthus amarus* and El-Mekawy and Mohamed (2005) *Nigella sativa*.

Oil percentage and yield/plant: Data in Table 4 showed that oil percentage and yield/plant considerably influenced by inoculation with two bacterias and inorganic NPK-fertilizer. The highest values of oil percentage and yield/plant were recorded with the treatment of dual inoculation (N_2-fixers plus bio-phosphate) supplemented with high dose of inorganic NPK-fertilizer, followed by the treatments of NPK-fertilizer+NFB and NPK-fertilizer+PFB, with insignificant difference between the later treatments, then the control and half dose of inorganic N-fertilizer, without significant difference between them. It is evident that treating plants with bacterial inoculation alone gave the lowest values in this respect. Similar findings were noticed by Harridy *et al.* (2001) observed that, applying *Azotobacter*+0 NPK, biofertilizers treatments (*Azotobacter*, *Azospirillum* and *Rhizobium*) significantly stimulated the biosynthesis of essential oil in lemongrass plants, the highest oil yield was mostly by the plants inoculated with *Azotobacter* without NPK in all cuts except the last cuts. In the sometime, inoculation with biofertilizers individually or in combination were produced essential oil with a high percentages of citral (a and b) in comparison with oil produced from control plants (without inoculation) and Massoud (2007) on marjoram plant. Al-Kayssi *et al.* (2011) the highest Nigellone volatile oil content of black cumin seeds was obtained when increase the available soil water Singh and Guleria (2013) revealed that produced maximum plant weight, herbage and oil yield of rosemary when use combined application of vermicompost 10 t ha^{-1}+fertilizer NPK (100:25:25 kg ha^{-1}) compared no fertilizer which indicated that 50%

inorganic fertilizer can supplemented with organic manure without affects the oil yield. Yousefzadeh *et al.* (2013) conclude that the application of 50% urea with 50% Azocompost is recommended for optimizing the content and composition of essential oils in dragonhead.

Effect of the interactions between irrigation intervals and fertilization

Number of inflorescences: Data in Table 4 showed that the effect of interactions between using fertilization and irrigation intervals were significant on number of inflorescences in the two seasons. Generally, the interaction between treatments caused an increase in number of inflorescence with using chemical fertilization combined with biofertilizer compared to chemical fertilization or biofertilization treatments alone. Also, increasing the irrigation intervals under the same level of fertilizer treatment decreased number of inflorescence. However, using biofertilizer treatment under the same level of irrigation interval increased number of inflorescence. Also, using fertilization at moderate irrigation interval (14 days) gave the maximum values while biofertilizer treatment at long irrigation interval (21 days) gave the minimum values in this respect. These results are in line with those were attained by Abd EL-Latif *et al.* (2002) on *Matricaria chamomilla* Badran and Safwat (2004) on fennel, Younis *et al.* (2004) on *Jasminum grandiflorum* and El-Mekawy and Mohamed (2005) on *Nigella sativa*.

Inflorescences fresh and dry weights: Table 4 indicates that the interaction treatments between complex fertilization and irrigation intervals caused an increase in inflorescence fresh and dry weights compared to biofertilizer alone under used irrigation intervals . Using chemical fertilizer combined with biofertilzer treatment, under the same irrigation interval increase inflorescence fresh and dry weight. But, increasing watering interval under the same level of NPK fertilizer (half or full) of chemical fertilizer or biofertlizer decreased it. Generally, inflorescence fresh and dry weight was highly significant in the first season and in the second season. Similar results were reported regarding the effects of interaction treatments between fertilizer and irrigation intervals by Shalan (2005) on borage plants *(Borago officinalis,* L.).

Oil percentage and yield/plant: Data in Table 4 showed that the volatile oil percentage and yield/plant tended to increase with the mixture of biofertilzer and chemical fertilizer treatment and increasing irrigation intervals in both seasons. The highest values of volatile oil percentage in the inflorescences were obtained from plants irrigated at long every 21 days. The results may be explained by the findings of Penka (1978), who 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 production of essential oil yield per plant (unexpanded inflorescences) increased by reducing the irrigation intervals as shown in Table 4. Also, using chemical fertilizer combined with biofertilzer treatment, under the same irrigation interval increased values of volatile oil percentage. Also, long watering interval under the same level of NPK fertilizer (half or full) of chemical fertilizer or biofertlizer increased it. The highest essential oil yields per plant and per fed.dan were obtained by the treatment of watering every 14 days. Meanwhile, the treatment of irrigation every 21 days resulted in the lowest volatile oil yield per plant and per fedden in the two seasons. The increment of the essential oil yield as a result of reducing watering intervals could be explained through its effect on increasing the corresponding drug yield as shown in Table 4. Similar results were obtained by Raju and Sinsinwar (2006) on Indian mustard Massoud (2007) on marjoram plant. Gholamhoseini *et al.* (2013) indicated that under drought stress intensity, inoculated plants of sunflower produced more dry matter, heavier seeds and greater seed and oil yields than did non-inoculated plants. Singh and Guleria (2013) revealed that produced maximum plant weight, herbage and oil yield of rosemary when use combined application of vermicompost 10 t ha^{-1}+fertilizer NPK (100:25:25 kg ha^{-1}) compared no fertilizer which indicated that 50% inorganic fertilizer can supplemented with organic manure without affects the oil yield. Yousefzadeh *et al.* (2013) conclude that the application of 50% urea with 50% Azocompost is recommended for optimising the content and composition of essential oils in dragonhead.

CONCLUSION

Finally, from the forgoing results and discussion, it could be recommended to use irrigation every 14-day combined with the mixture of dual inoculation of (symbiotic N_2-fixers+phosphobacteria) in the presence of half or full dose of inorganic NPK-fertilizer to obtain the best growth parameter and oil yield of *Achillea santolina* L. plant under North Sinai conditions.

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