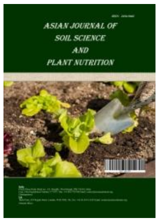

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Azolla as an Alternative of Mineral Nitrogen for Chamomile Plant (*Matricaria chamomilla* L.) Fertilization in Sandy Soil

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Two pot experiments were carried out during the two successive seasons of 2010/2011 and 2011/2012 to apply mineral nitrogen and/or *Azolla* at different methods of addition with a reduction of mineral nitrogen fertilizer and its effect on vegetative growth, the yield of inflorescences, total phenolic concentration, antioxidant activity, nitrate and nitrite concentrations and the volatile oil concentrations and composition of chamomile inflorescences in sandy soil. Data indicated that dry *Azolla* (DA) treatments increased significantly plant height, number of branches/plant, shoot fresh and dry weights and number of inflorescences/plant against the rest treatments. It is clear that DA singly induced maximum growth parameters in both seasons and the reverse was true with fresh *Azolla* (FA) alone. It is obvious that DA achieved the highest total phenolic concentrations, antioxidant activity and volatile oil (VO) concentrations, and the opposite response was true for nitrate & nitrite concentrations. Addition of 50% ammonium nitrate to 50% FA or 50% *Azolla* extract as foliar (spray *Azolla*) SA have positive effect on chemical constituents except nitrate and nitrite concentrations. It is evident that the highest chamazulene and α–bisabolol oxide–B were achieved

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with DA and the reverse was true for α–bisabolol oxide–A. Dry *Azolla* treatment alone was the most efficient one in increasing the yield production with increment in active substances of chamomile plant in sandy soil.

Keywords: Mineral nitrogen fertilizer; Azolla; chamomile; sandy soil.

1. INTRODUCTION

Chamomile (*Matricaria chamomilla* L.) belonging to the Asteraceae family, is one of the important medicinal plants. The biological activity of chamomile is mainly due to the phenolic compounds and principal components of the essential oil of the flowers like alpha-bisabolol and its oxides and azulenes, including chamazulene. These compounds are influential in mediating the aroma of the essential oil and contribute to the therapeutic properties (anti-inflammatory, antibacterial, insecticidal and antiulcer) of this species [1].

Sandy soils are characterized by poor macro and micronutrients which negatively affect growth and productivity of chamomile plants [2]. Plant nutrition is one of the most important factors that increase plant production. Nitrogen is the most effective element in improving yield of the plants [3]. Nitrogen is found in important molecules which are essential for protein synthesis. In addition, N plays an important role in synthesis of the plant constituents through the action of different enzymes. Continuous usage of inorganic fertilizer affects soil structure. In addition, pollution with chemical fertilizers arose as an aim of health cure. Hence, organic manures can serve as alternative to mineral fertilizers for improving soil structure [4] and microbial biomass [5].

Sustainable resources have further enhanced the value of *Azolla*, particularly in agriculture, either alone or in combination with chemical nitrogenous fertilizers. *Azolla* is a small aquatic fern that can grow without combined nitrogen due to nitrogen fixation by symbiotic cyanobacteria and it has been used as a green manure for many crops [6]. The symbiont is able to release nutrient into the soil availing them for plant uptake [7]. Nour-El Din [8] noticed that N_2-fixation combined with a high growth rate can enable *Azolla* to accumulate more than 10 kg nitrogen/ha/day with an *Azolla* biomass of about 25 tons fresh weight/ha/day.

The main objective of the present investigation was to apply *Azolla* (as organic manure) by different methods of application with a reduction of mineral nitrogen fertilizer and its effect on growth, the yield of flower heads, total phenolic concentration, antioxidant activity, nitrate & nitrite concentrations and the volatile oil of chamomile plant.

2. MATERIALS AND METHODS

2.1 Plant Material and Planting

Two pot experiments were conducted during 2010/2011 and 2011/2012 seasons. Seeds of chamomile (*Matricaria chamomilla* L.) were kindly obtained from the Medicinal and Aromatic Plants Department, Ministry of Agriculture, Egypt. Seeds were sown on 26th September in the nursery beds and seedling were transplanted on 1st December when the seedling were about 10-15 cm in length in both seasons in the experimental farm of Faculty of Agriculture, Ain Shams University, Shoubra El. Khema, Kalubeia Governorate, Egypt. Transplanting was done in pots (40 cm diameter) filled with 20 Kg sandy soil (Mechanical and chemical analyses of the used soil were made before sowing in both seasons). Soil analysis was determined according to Page et al. [9] in Central Lab., Faculty of Agric., Ain Shams Univ. (Table 1). The plants were thinned to 3 plants for each pot after 7 days from transplanting. The pots were irrigated with tap water when needed. All agriculture practices were done as usual according to the recommendations of Ministry of Agric., Egypt.

2.2 Fertilization

The fertilization with calcium superphosphate (16-18% P_2O_5) at 300 kg/fed was added pre transplanting and potassium sulphate (48-52% K_2O) at 75 kg/fed was added at two equal amounts after one and three months from transplanting. These nutrients were added to all pots. As for nitrogen fertilization treatments, ammonium nitrate (33.5% nitrogen) at 120 units of N and/or *Azolla* were added at four equal amounts every one month commencing 7 days after transplanting as recommended by Ministry of Agric., Egypt.

Table 1. Mechanical and chemical analysis of cultivated soil

	Mechanical analysis				Chemical analysis meq/l								
	Sand%	**Silt%**	**Clay%**	**Soil texture**	**PH**	**Ec**	**Ca**	**Mg**	**Na**	**K**	**Cl**	**HCO_3**	**SO_4**
Season 1	96.61	3.39	0	Sandy	7.77	0.56	2.0	0.5	1.64	0.39	1.15	1.2	1.82
Season 2	97.26	2.74	0	Sandy	8.27	0.69	2.88	0.67	2.92	0.32	2.23	1.7	2.14

Azolla was kindly provided from Agric. Microbiol. Dept., Soil, Water and Environmental Res. Inst. (SWERI), Agric. Res. Center, Giza, Egypt. Cultivation of *Azolla* was carried out in plots of 1×2 m and 15 cm in depth, covered with plastic sheet and layer of clay soil thickness of 3 cm. Water is manipulated at a depth of 10 cm, the plots were inoculated with standard inoculum under normal conditions. *Azolla* cultures were kept at a constant volume throughout the experiment by frequent irrigation with water to compensate water loss by evaporation. Fifteen days after inoculation**,** *Azolla* is harvested from water spread area. *Azolla* was collected and incorporated into 0.01 mercuric chloride for 1 min and washed gently in running tap water for several times by using screen of 0.2 mech. Application of *Azolla* was carried out by three methods: dry *Azolla* (air dried on tissue paper) DA, fresh *Azolla* (as a green manure) FA and *Azolla* extract as a foliar application (spray *Azolla*) SA.

2.3 Treatments

The mineral nitrogen fertilization (ammonium nitrate) at recommended rate was taken as a base for *Azolla* N. The amount of added *Azolla* either dry or fresh was calculated on bases that dry *Azolla* contains 3% N and fresh *Azolla* has 95% moisture content. Fresh or dried materials of *Azolla* and / or ammonium nitrate were applied as follow:

T1– Ammonium nitrate at recommended dose (Control).
T2–Dry *Azolla* (air dried).
T3–75%dry *Azolla* + 25% ammonium nitrate
T4–50% dry *Azolla* + 50% ammonium nitrate
T5–Fresh *Azolla* (as a green manure).
T6–75% fresh *Azolla* + 25% ammonium nitrate
T7–50% fresh *Azolla* + 50% ammonium nitrate
T8–*Azolla* extract as foliar
T9–75% *Azolla* extract + 25% ammonium nitrate
T10– 50% *Azolla* extract + 50% ammonium nitrate

2.4 Growth Parameters

Six plants were randomly taken from each treatment, the 1st sample at 60 days (the beginning of flowering), and the 2nd sample at 100 days (the middle of flowering) after transplanting. The following growth parameters were determined in both samples: Plant height (cm), number of branches/plant, shoot fresh and dry weights (g/plant) as well number of inflorescences/plant at the end of picking (140 days after transplanting).

The experiment was established as completely randomized design with six replicates, each replicate has three plants.

The results were subjected to statistical analysis of variance as described by Snedecor and Cochran [10]. Means were compared by LSD method. Data was performed using (SAS V.9.1) computer software program [11].

2.5 Chemical Analyses

Chamomile inflorescences were collected within February to April when the ligulate flower are still blooming and 1/2 – 3/4 of tubular flowers are opened (when the ray flowers are in mood). According to the growth characters and inflorescences yield of chamomile plant, the following treatments were selected for chemical analyses (total phenolic compounds, antioxidant activity, nitrate & nitrite concentrations and the volatile oil) of inflorescences: T1, T2, T7 and T10.

2.6 Determination of Phenolic Compounds

0.1 g of dry samples of flower heads was macerated in 10-20 ml 80% ethanol for at least 24 hours at 0° C, the alcohol was collected, and the remained tissue re-extracted with 10-20 ml 80% ethanol about three times. At the end, the collected extract was completed to 100 ml using 80% ethanol. The colorimetric method of Folin-Denis as described by Shahidi and Naczk [12] was employed for phenolic compounds determination.

2.7 Determination of Antioxidant Activity (DPPH Assay)

The free Radical scavenging activity using the 1,1-diphenyl-2-picryl-hydrazil (DPPH) reagent was determined according to Brand-Williams et al. [13]. The powdered flower heads (0.1 g) were extracted with 50% methanol, 50% water. To 0.75 ml of the extract sample 1.5 ml of freshly prepared methanolic DPPH solution (20 ug ml^{-1}) was added and stirred. The decolorizing process was recorded after 5 min of reaction at 517 nm and compared with a blank control.

Antioxidant activity = control absorbance – sample absorbance / control absorbance x 100%.

2.8 Determination of Nitrate and Nitrite Concentrations

Chamomile sampled from the flower heads was dried by air to determine nitrate and nitrite concentrations. Nitrate and nitrite levels in the dry flower heads were determined according to the method of Nrisinha and Donald [14].

2.9 Determination of Volatile Oil

Twenty five grams of air dried powdered plant materials (flower heads) were extracted by the hydro distillation method during 3 hours in an all–glass Clevenger type apparatus according to Egyptian Pharmacopoeia [15]. The extracted volatile oils were dried over anhydrous Na_2SO_4 and stored in sealed glass vials, covered with aluminum foil to protect the contents from photo-conversion and kept under refrigeration until analysis. Pooled volatile oil from samples was evaluated for its components by GC/EI-MS.

2.10 Gas Chromatography – Mass Spectrometry (GC-MS)

The GC-Ms analysis of the volatile oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadruple Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml / min and a split ratio of 1:10 using the following temperature program: 40°C for 1 min; rising at 4.0°C / min to 160°C and held for 6 min; rising at 6°C / min to 210°C and held for 1 min. The injector and detector were held at 210°C. Diluted samples (1:10 hexane, v/v) of 0.2 μ / l of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

2.11 Identification and Quantification of Volatile oil Components

Relative percentage amounts of the volatile oil constituents were evaluated from the total peak area (TIC) by apparatus software. The components of the volatile oil were identified by comparing their mass spectral fragmentation patterns with those of similar compounds from the database (NIST and WILEY library) as well as by comparing their Kovats gas chromatographic retention indices with those of the literature.

3. RESULTS

3.1 Growth Parameters

3.1.1 Plant height

Data illustrated in Table 2 showed the effect of ammonium nitrate and/or *Azolla* on chamomile plant height. It is clear that dry *Azolla* (DA), either alone or with 25% or 50% ammonium nitrate increased plant height significantly against the rest treatments. Dry *Azolla* singly induced maximum plant height in both seasons. On the other hand, fresh *Azolla* (FA) or spray *Azolla* extract (SA) alone detected significant decrement against the control and there is no significant difference in plant height between the two methods of application in both seasons. Regarding the 1st sample, addition 25% or 50% ammonium nitrate to FA or SA led to no significant differences in chamomile plant height as compared to the control (T1) in the 1st season. Meanwhile, no significant differences were achieved between FA treatments and the same treatments of SA either alone or with different concentrations of ammonium nitrate with a reduction in plant height against the control in the 2nd season. As for the 2nd sample, addition 25% or 50% ammonium nitrate to SA (T9 or T10) led to significant increment in plant height as compared to the same treatments with FA (T6 or T7) in the 1st season, while its reach to the same level of significance in the 2nd season.

3.1.2 Number of branches

Data in Table 2 showed that dry *Azolla* (T2) achieved the highest increment in the number of branches/plant above the rest of treatments to reach the 5% level of significance during the two sampling dates of the two seasons. Fresh *Azolla* alone (T5) significantly diminished the number of branches/plant below the control (T1) in both seasons except the 2nd season at the middle of flowering period which reach to the same level of significance with the control. On the other hand, addition 25% or 50% ammonium nitrate to both FA or SA as well SA alone led to no significant differences in the number of branches / plant than the control (T1) in the two seasons at the beginning of flowering while, the same treatments gained significant increase against the control at the 2nd sample. Meantime, Dry *Azolla* treatments (T2, T3 and T4) stimulated number of branches/plant at the two sampling dates in both seasons.

3.1.3 Shoot fresh and dry weights

Data presented in Table 3 represent the effect of different treatments on top fresh and dry weights of chamomile plant at the beginning and middle of flowering period. It is clear that dry *Azolla* treatments achieved the maximum fresh and dry weights of shoot system above the rest treatments to reach the 5% level of significance during flowering stage in both seasons. It is evident that FA alone (T5) reduced significantly plant fresh and dry weights below the control (T1), while there is no significant difference with spraying *Azolla* extract (T8) singly against T1 for the two sampling dates in both seasons. Application of 50% FA + 50% ammonium nitrate (T7) recorded significant increment in fresh and dry weights against FA (T5) in the 2nd sample, while the increase did not reach the 5% level of significance in the 1st sample in both seasons. Spray *Azolla* treatments have positive effect on fresh and dry weights than FA treatments in both seasons.

Table 2. Effect of ammonium nitrate and/or *Azolla* on plant height and number of branches of chamomile during the two sampling dates of the two seasons

Treatments	Plant height (cm)				Branches number/ plant			
	1st season		2nd season		1st season		2nd season	
	1st sample	2nd sample	1st sample	2nd sample	1st sample	2nd sample	1st sample	2nd sample
T1	23.16 d	55.16 c	27.66 d	51.33 d	9.66 c	15.16 f	9.83 cd	12.50 e
T2	62.83 a	88.33 a	71.33 a	95.00 a	20.83 a	33.00 a	22.16 a	30.50 a
T3	53.16 b	83.66ab	65.50 b	90.83 b	18.50 b	30.83 b	20.16 b	28.83ab
T4	49.66 c	82.83 b	57.16 c	87.66 c	16.50 b	26.16 c	20.16 b	26.83 b
T5	18.33	37.00 gf	18.66 f	31.33 g	7.16 d	13.16 g	6.00 e	12.00 e
T6	21.16de	41.33 ef	22.33 e	36.50 ef	8.16 cd	17.16 e	9.00 cd	16.50 d
T7	23.66 d	42.00 e	24.33 e	38.50 e	9.16 cd	18.16 e	10.00 cd	16.66 d
T8	19.16 e	34.16 g	21.33 ef	33.50 gf	8.33 cd	14.83 f	8.33 d	15.16 d
T9	24.33 d	50.33 d	22.33 e	37.50 e	8.33 cd	17.83 e	10.16 c	17.66cd
T10	23.33 d	48.83 d	23.50 e	37.00 e	9.66 c	20.00 d	10.50 c	19.66 c
LSD 5%	3.49	4.68	3.31	3.11	2.27	1.56	1.74	2.51

Means with the same letter are not significantly different

Table 3. Effect of ammonium nitrate and/or *Azolla* on shoot fresh and dry weights of chamomile during the two sampling dates of the two seasons

Treatment	Fresh weight (g / plant)				Dry weight (g / plant)			
	1st season		2nd season		1st season		2nd season	
	1st sample	2nd sample	1st sample	2nd sample	1st sample	2nd sample	1st sample	2nd sample
T1	7.00 c	12.20fde	8.43 cd	10.16ed	3.66 bc	5.90 dc	3.93 ed	6.20 cd
T2	19.10 a	30.43 a	14.96 a	35.66 a	8.06 a	16.16 a	7.30 a	19.20 a
T3	17.60ab	25.80 b	14.80 a	31.03 b	7.20 a	14.10 a	6.76 ab	17.50ab
T4	16.30 b	21.80 c	12.00 b	30.03 b	7.13 a	9.83 b	5.70 cb	16.20 b
T5	3.23 e	7.86 g	5.23 e	6.23 f	1.86 d	3.53 e	2.46 f	2.50 e
T6	4.20 ed	8.46 g	7.20 d	7.20 ef	2.40 dc	4.26 de	3.36 ef	4.10 ed
T7	5.46 ecd	9.86 fg	7.66 cd	9.43 e	2.90 dc	4.50 de	4.03 ed	5.30 cd
T8	5.90 cd	11.23 fe	8.20 cd	13.00 cd	3.46 dbc	5.63 de	3.33 ef	6.60 c
T9	6.50 cd	12.36de	8.53 cd	15.10 c	3.56 bc	6.30 dc	3.93 ed	7.26 c
T10	7.90 c	14.20 d	9.20 c	15.43 c	4.66 b	8.03 bc	5.16 cd	7.40 c
LSD 5%	2.44	2.42	1.85	3.14	1.64	2.20	1.31	2.33

Means with the same letter are not significantly different

3.1.4 Number of inflorescences

Data illustrated in Table 4 showed the effect of different treatments on inflorescences number/ plant during the two successive seasons. It is clear that dry *Azolla* treatments induced the highest significant numbers of flowers heads/ plant above the other treatments in both seasons. The results showed that there is no significant difference of the flower heads number /plant between T5 and T6 against the control (T1) in the two seasons. While, the addition of 50% ammonium nitrate to 50% FA (T7) led to significant increase above the control in both seasons. SA treatments achieved significant increments above the control (T1) in the two growing seasons. Meanwhile, addition of 50% ammonium nitrate to SA (T10) led to significant increment against SA alone (T8) in both seasons.

3.2 Chemical Constituents

3.2.1 Total phenolic concentration

The effect of different treatments on total phenolic concentration of chamomile inflorescences during the harvesting stage in the two successive seasons are shown in Fig. 1. The highest total phenolic concentration was gained with DA (54.7 and 49.9 µg / g d.w.) while, the lowest concentration was recorded with the control (32.5 and 34.7 µg / g d.w.) in the two successive seasons. Addition of 50% ammonium nitrate with 50% SA (T10) increased total phenolic concentration as compared with 50% FA + 50% ammonium nitrate (T7) in both seasons. It is clear that application of DA singly or combination between 50% ammonium nitrate + 50% FA or SA increased total phenols than the mineral nitrogen in the two growing seasons.

3.2.2 Antioxidant activity

The effect of different treatments on antioxidant activity of chamomile inflorescences extracts are shown in Fig. 2. It is evident that DA (T2) induced the highest antioxidant activity above the other treatments. The values were 72.1 and 73.0% in both seasons respectively. On the other hand, mineral fertilization (T1) decreased antioxidant activity below treated plants with *Azolla* (as organic fertilization) in the harvesting stage of both seasons. Addition of 50% ammonium nitrate to 50% SA (T10) stimulated antioxidant activity against the same treatment with 50% FA (T7) in the two growing seasons.

3.2.3 Nitrate and nitrite concentrations

Fig. 3 reveals the effect of different treatments on nitrate and nitrite concentrations of chamomile inflorescences during the harvesting stage in the two successive seasons. It could be noticed that the highest nitrate and nitrite concentrations were achieved in case of inorganic fertilization (T1) and the reverse was true in case of dry *Azolla* (T2) in both seasons. Addition of 50% ammonium nitrate with 50% SA (T10) reduced nitrate and nitrite concentrations than the same treatment with FA (T7) in the two growing seasons.

Generally, Nitrate and nitrite levels in all treatments under investigation are within legal limits, therefore, this type of German chamomile inflorescences extract are safe for human consumption.

3.2.4 Volatile Oil (VO) concentration

Data presented in Table 5 illustrate VO% of chamomile inflorescences through six picking periods in both seasons. It is clear that the control (T1) treatment in both seasons, in addition to T7 in the 2nd season recorded the lowest volatile oil percentage below the rest treatments. DA (T2) achieved the highest VO% through six picking periods. The values were 0.42%, 0.48%, 0.62%, 0.64%, 0.55% and 0.50% in the 1st season and 0.32%, 0.44%, 0.68%, 0.64%, 0.52% and 0.48% in the 2nd season. It is clear that the highest VO% was detected through the picking weeks eight and nine. As for the addition of 50% ammonium nitrate to FA (T7) or SA (T10), T10 recorded higher VO% than T7 in both seasons through three picking periods.

From the aforementioned results VO concentration increased according to apply different treatments, in the following order: DA > 50% SA > 50% FA > control.

3.2.5 Volatile oil composition

The volatile oil (VO) composition of chamomile inflorescences in both seasons was presented in Table 6. The total of five compound representing 86.64 – 98.60% of total detected constituents with different treatments through the two growing seasons. The unknown compounds representing 1.40 – 13.36% of total detected constituents in the two seasons. The main constituents of *M. chamomilla* VO as detected by GC – MS were

Table 4. Effect of ammonium nitrate and/or *Azolla* on number of inflorescences/plant during the harvesting stage of chamomile plant in the two growing seasons

	Treatments										LSD5%
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
1st season	55.00g	169.83a	130.33b	114.83c	52.00g	58.83g	74.00f	75.33ef	84.83e	95.33d	10.20
2nd season	64.16e	187.50a	161.33b	156.16b	65.16e	69.66e	85.66cd	84.16d	91.00cd	97.50c	12.99

Means with the same letter are not significantly different

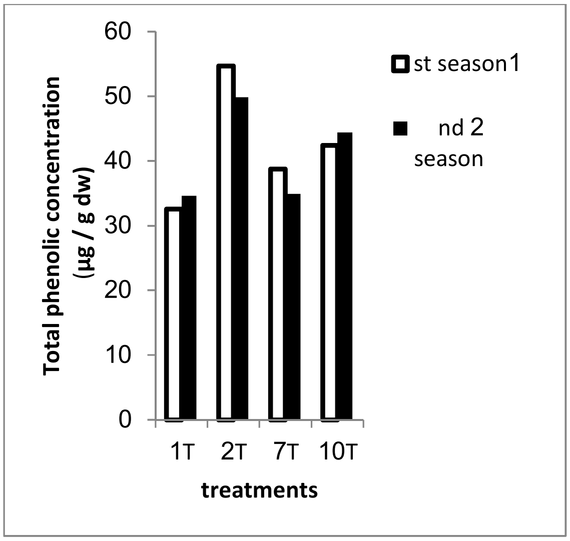

Fig. 1. Effect of ammonium nitrate and/or *Azolla* on total phenols (µg/g d.w.) of chamomile inflorescences during the harvesting stage in the two successive seasons

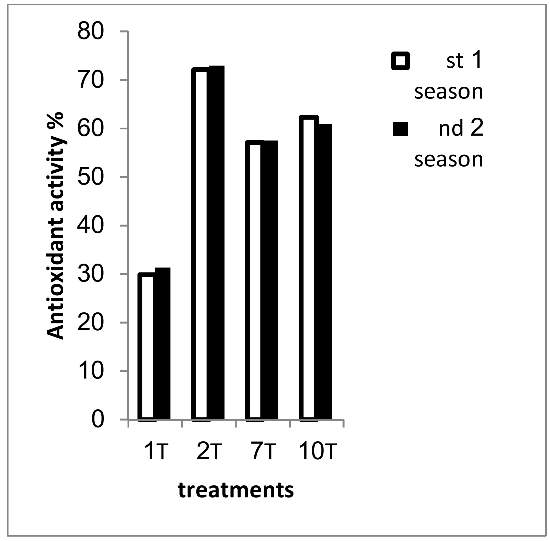

Fig. 2. Effect of ammonium nitrate and/or *Azolla* on the antioxidant activity (%) of chamomile inflorescences during the harvesting stage in the two successive seasons

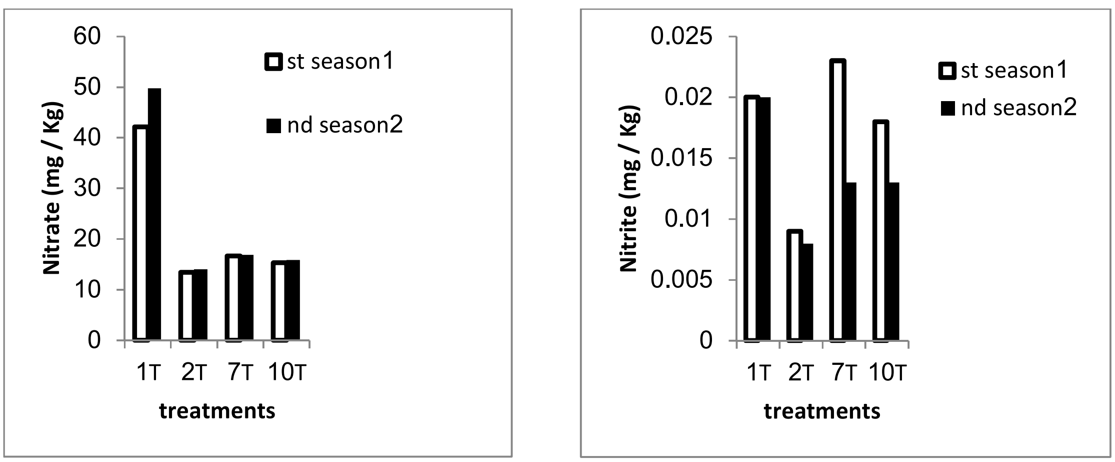

Fig. 3. Effect of ammonium nitrate and/or *Azolla* on nitrate and nitrite concentrations (mg/kg d.w) of chamomile inflorescences during the harvesting stage in the two successive seasons

Table 5. Effect of ammonium nitrate and/or *Azolla* on VO % of chamomile inflorescences through picking periods (from the 4th to 12th weeks) in both seasons

Treatments		Picking periods (week)											
		1	2	3	4	5	6	7	8	9	10	11	12
1st season	T1	—	—	—	—	—	—	—	—	—	—	—	0.48
	T2	—	—	—	—	0.42	—	0.48	0.62	0.64	0.55	—	0.50
	T7	—	—	—	—	—	—	—	—	0.38	—	0.48	0.38
	T10	—	—	—	—	—	—	—	—	0.40	—	0.52	0.42
2nd season	T1	—	—	—	—	—	—	—	0.38	—	0.48	—	0.44
	T2	—	—	—	0.32	—	0.44	—	0.68	0.64	0.52	—	0.48
	T7	—	—	—	—	—	—	—	0.36	—	0.48	—	0.44
	T10	—	—	—	—	—	—	—	0.40	—	0.52	—	0.48

Table 6. Effect of ammonium nitrate and/or *Azolla* on the chemical constituents percentage of volatile oil extracted from chamomile inflorescences in both seasons

Constituents	Treatments							
	1st season				2nd season			
	T1	T2	T7	T10	T1	T2	T7	T10
Chamazulene	2.13	6.78	1.82	1.72	2.01	5.52	1.55	2.09
α-Bisabolol oxide-B	21.82	19.95	19.49	9.01	6.08	34.60	4.23	4.37
Bisabolol oxide	4.95	7.71	5.15	—	—	—	—	—
α-Bisabolol oxide-A	56.74	52.94	60.18	86.29	87.38	57.97	92.16	89.76
Germane	1.11	—	—	1.48	0.97	0.51	0.50	2.38
Total identified	86.75	87.38	86.64	98.50	96.44	98.60	98.44	98.60
Unkown compounds	13.25	12.62	13.36	1.50	3.56	1.40	1.56	1.40

chamazulene (1.55 – 6.78%), α-bisabolol oxide-B (4.23 – 34.60%), bisabolol oxide (4.95 – 7.71%), α-bisabolol oxide-A (52.94 – 92.16%) and germane (0.50 – 2.38%). Other components were present in amounts less than 2%. It is evident that the highest azulene was achieved with DA (T2), the values were 6.78% and 5.52% for the two successive seasons. The same trend was detected with α‒ bisabolol oxide‒ B. The highest percentage of α‒bisabolol oxide‒A was achieved by addition 50% ammonium nitrate to 50% SA (T10). On the other hand, bisabolol oxide appears only in the first season and small amounts of germane were found in some treatments.

The medicinal value of the plant material was evaluated by the concentration of volatile oil. Generally, the oil quality of all treatments is better but regarding to the quantity and quality of chamomile VO, the best treatment was detected with dry *Azolla.*

4. DISCUSSION

Chamomile is a most favored and much used medicinal plant throughout the world. Phyto-therapeutically useful are inflorescences due to its essential oils stored in peltate glandular trichomes, which are mainly situated in flower heads [16,17].

The results obtained from this investigation illustrated that significant increase was achieved with dry *Azolla* (DA) treatments in chamomile plant height, number of branches/plant, fresh & dry weights/plant and number of flower heads/ plant above the rest treatments in both seasons, and DA singly was the highest. The reverse was true with fresh *Azolla* (FA) alone. On the other hand, addition 50% ammonium nitrate to 50% *Azolla* extract as foliar (SA) increased growth parameters than the same treatment with FA at the end of flowering stage in the two growing seasons. In this respect, Borin et al. [18] and Browaldh [19], reported that organic fertilizers is a rich and a slow release which usage leads to a pure product of plants. They added that using organic fertilizer improves the soil texture. The structural improvement can encourage the plant to have a good root development by improving the aeration in the soil, which leads to a higher plant growth. Khalid and Shafei [20] indicated that treated plants with different combination of organic fertilizers and their rates resulted in a significant increase in growth, yield, essential oil and its main components of dill (*Anethum graveolens* L.). Choudhury and kennedy [21] reported that the aquatic biota cyanobacteria and *Azolla* can supplement the nitrogen requirements of rice plants, replacing 30 – 50% of required urea‒N. Changiz‒Dalivand et al. [22] indicated that using of nitrogen fertilizer and *Azolla* compost rate had a significant effect on the grain yield, plant height, number of panicle and number of tillers of rice plants. Also, Rivaie et al. [23] showed that application of *A. pinnata* to the paddy rice field affected plant height, tiller number, grain number per panicle and grain yield. Ahmed [24] reported that the maximum of flowering followed systematically the plant branching, this may be true as chamomile flowers develop singly at the end of stem and branches of the plant. Subudhi and Singh [25] showed that *Azolla* incorporated with different doses of inorganic nitrogen gave higher yield as compared to use of inorganic nitrogen alone. Talley and Rains [26] state that dried *Azolla* increased yield of rice plants to the same degree as application of ammonium sulphate at the rate of 40-50 kg N ha^{-1}.

The data showed that the antioxidant activity of chamomile inflorescences extract during the harvesting stage run parallel that detected in total phenolic concentrations in both seasons. The highest total phenolic concentrations and antioxidant activity were obtained with DA alone while, the lowest concentrations were recorded with the control in the two successive seasons. In this regard, Alizadeh et al. [27] showed that chemical fertilizer increased total phenlic concentrations and antioxidant activity in *Satureja hortensis* and apositive correlation between the total phenolic concentrations and antioxidant activity was found. Flavonoids and phenolic compounds exert multiple biological effects such as antioxidant, free radical scavenging and anti-inflammatory properties [28]. Oxidative damage in the human body plays an important causative role in disease initiation and progression [29]. The biosynthesis of secondary metabolites in medicinal and aromatic plants is strongly influenced by environmental factors [30]. Ibrahim et al. [31] observed that the application of organic fertilizer enhanced the production of total phenolics content in *Labisia pumila* compared to the use of inorganic fertilizer.

The obtained result showed that the highest nitrate and nitrite concentrations were achieved in case of mineral fertilization (control) and the reverse was true in case of DA (T2) in both seasons. In this respect, Ibrahim et al. [31] found that nitrate content of *Labisia pumila* was reduced under organic fertilization. Nitrate has been attributed to negative effects of human health. Toxicity of nitrate to human can be manifested by headaches, syncope, vertigo and discoloration that manifest in fingers or lips [32]. Inorganic fertilizer which is absorbed rapidly into the plant usually has higher nitrosamines which have been associated with chronic diseases such as leukemia and gastrointestinal cancer [33].

It is interesting to note that the limits detected for nitrate in all treatments under investigation are within the legal limits. An Average Daily Intake (ADI) for nitrate of 3.7 mg/kg body weight per day was established by the former Scientific Committee on Food (SCF) and was reconfirmed by Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2001 and the European Food Safety Authority (EFSA, 2008). Thus, from the point of view of nitrate, this type of German chamomile inflorescences is safe. Nitrate in non-toxic below maximum residue levels (MRLs), but if reaches above this level, it could be dangerous due to its reduction in nitrites, which can react with amins and amides to produce nitrose compounds responsible for gastric cancer [34].

The data showed that the highest VO% was achieved with DA alone through six picking periods in every season. Meanwhile, the picking weeks eight and nine recorded the highest VO concentration in both seasons. VO percentage increased according to apply different treatments in the following order: DA > 50% SA > 50% FA > control in both seasons. In this regard, Amaral et al. [17] indicated that chamomile has several pharmacological properties due to its essential oils. Besides the major influence of genetic factors, the environment has an important effect on essential oil accumulation and composition [16].

The data clearly showed that the highest chamazulene and α– bisabolol oxide– B were achieved with DA (T2) in both seasons.

Active principles of German chamomile are terpenoids: α–bisabolol, α–bisabolol oxide–A and B, chamazulene and other many components [35]. The essential oil of both German and Roman chamomile has a light blue colour due to the terpenoid chamazulene which is about 5% of the essential oil, bisabolol comprises 50% of German chamomile's essential oil and is a spasmolytic for intestinal smooth muscle [36].

5. CONCLUSION

The data have been concluded that different treatments of *Azolla* as a natural source of nitrogen nutrition could reduce mineral nitrogen fertilizer in sandy soil. Besides, dry *Azolla* significantly enhanced the vegetative growth parameters, yield of inflorescences, total phenolic concentration, antioxidant activity and the volatile oil concentration. Nitrate and nitrite levels in all treatments under investigation are within legal limits, therefore, this type of German chamomile inflorescences extract are safe for human consumption. The highest percentage of chamazulene and α– bisabolol oxide– B were achieved with dry *Azolla* and the reverse was true for α– bisabolol oxide– A in both seasons. Regarding to the quantity and quality of chamomile volatile oil, the best treatment was detected with dry *Azolla*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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