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RESEARCH ARTICLE

Matricaria chamomilla L. (German chamomile) flower yield and essential oil affected by irrigation and nitrogen fertilization

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ABSTRACT

Matricaria chamomilla is one of the most important medicinal herbs. The aim of this study was to investigate irrigation and N-fertilization effect on German chamomile yield and essential oil production. For the purposes of this study, field experiments were established at the Experimental Farm of the University of Thessaly, Larissa, Greece, in 2016 and 2017. The experimental design was a factorial split-plot design with main factor irrigation (I_1: rainfed, I_2: 100% ETo) and sub factor N-fertilization (F_1: 0, F_2: 80, and F_3: 160 kg ha^{-1}) with four replications. Flower yield measured by samplings at the ideal collection stage (2/3 of the rounds of tubes have been opened on the elevated flowering plant). Essential oil content was determined, using a Clevenger-type distillation apparatus and essential oil analysis by a GC-MS on a fused silica DB-5 column. Irrigation had a significant effect on yield, with irrigated treatments producing higher yield in comparison with rainfed treatments (5,250 and 2,200 kg ha^{-1}, fresh and dry flowers, respectively). Furthermore, N-application had also a significant effect on yield, with the higher N-supply producing higher yield. The highest yield and essential oil production were found for the irrigated and fertilized treatment with the higher N-dressing (I_2F_3, 3,800 and 25 kg ha^{-1} dry flowers and essential oil yield, respectively). Moreover, it was found that the irrigated treatments produced lower amount of essential oil, while N-fertilization had a positive effect on α-bisabolol and chamazulene and a negative effect on bisabolol oxide A. Although irrigation had a negative impact on the content of essential oils, the high increase in yield led to a maximization of the essential oil production for the irrigated cultivation with maximum nitrogen fertilization. Therefore, chamomile seems to be a promising annual cultivation in Greece which can be established in areas of similar environmental conditions producing satisfactory yields.

Keywords: Chamomile; Yield; Irrigation; N-fertilization; Essential Oil Content

INTRODUCTION

Chamomile (*Matricaria chamomilla* L.) is one of the world's leading medicinal products used in a variety of food and cosmetics sectors (Jamshidi, 2000), of the *Asteraceae* (*Compositae*) family.

Chamomile, recognized in ancient Egypt, Greece and Rome, has been used in herbal medicines for thousands of years. The Anglo-Saxons claim that this herb is one of the nine sacred herbs provided by God to man. Chamomile extract is an integral part of many traditional medical and homeopathic remedies (Mann and Staba, 2002).

The blue essential oil from *M. chamomilla* flowers has many uses and its concentration ranges between 0.2 and 1.9% volatile oil. Chamomile is used primarily as an anti-inflammatory, antiseptic, anticonvulsant, stimulant, and spasmolytic while could also be taken as a relaxing, mild sleep promoting tea or tincture (Gawde et al., 2014; Khaki et al., 2012; Murti et al., 2012; Srivastava et al., 2010).

International demand for chamomile is growing rapidly, resulting in the plant being commonly cultivated in Europe. Chamomile is an annual herb, with upright growth, smooth and multi-branched stem, bright green color long petri-dishes leaves, short but widespread roots, narrow and long

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leaves and heterogamous flowers (Singh et al., 2011). The flowers are collected from May to July, and mainly grows indigenously in Europe, NW Asia, North Africa, North America, and in other parts of the world (Upadhyay and Patra, 2011; Applequist, 2002).

Optimal growth requires cool, temperate conditions and temperatures from 7 to 26°C. Chamomile is a tolerant plant which can survive under cold winter nights even at temperatures of -12°C, but needs hot and light long days (Zalecki, 1971). It is reported that temperature affects all plant traits, except for root length and dry weight (Bagheri et al., 2019).

Chamomile' shallow roots do not help the plant to pump moisture from the lower wet soil horizon and therefore needs frequent irrigation to maintain an optimum moisture level. Irrigation during the blooming stage is useful in increasing the flower yield.

Baghalian et al. (2008) have observed that irrigation at the rosette stage significantly increased yield. In alkaline soils, the crop should be irrigated more frequently and about 6–8 irrigations are required during the crop cycle (Chandra et al., 1979). High yield is achieved if soil has adequate moisture, but flooding should be avoided. Sharafi et al., (2002) as well as Wagner (1993) reported that water deficit is one of the most important limiting factors on crop production in arid and semi-arid regions. Furthermore, drought stress reduces water content of tissues and limits the growth of the plants (French and Turner, 1991).

Plants nutritional management under drought stress conditions is one of the most important factors in crop production because drought stress affects the availability of nutrients in the soil (Munns, 1993). Moreover, crops that receive a sufficient quantity of nutrients through improved fertilization management show a better resistance to drought (Lal et al., 1993, Solinas et al., 1996).

A few studies have been conducted on the effect of N fertilization to the yield and the essential oil of chamomile. It was found that the increase in N-fertilization results to higher flower yield and the indicated optimum N fertilization level for the yield of chamomile was 60 kg ha^{-1} (Johri *et al*., 1992). Singh et al., (2011) observed that application N in the form of ammonium sulphate at 40 kg ha^{-1} significantly increased the yield of fresh flowers while the essential oil content decreased from 0.64 to 0.59%. Misra and Kapoor (1978) found that the optimum dose of N is 50–60 kg N ha^{-1}, while Balak et al. (1999) found that the highest growth and yield indices observed in treatments with 120 kg N ha^{-1}. Later on, Andrzejewska and Woropaj-Janczak (2014) reported that 90 kg N ha^{-1} resulted to higher yield. El-Hamidi et al. (1965) claimed that an increase N-application causes a remarkable decrease in hamazulene concentration.

Chamomile's major oil constituents are α-bisabolol (A and B oxides), β-farnesene and chamazulene (Orav et al., 2010; Raal et al., 2012). Moreover, it is reported (Singh 1997) that N-fertilization significantly increased the content of α-bisabolol and chamazulene, but significantly decreased the content of bisabolol oxides A and B in the essential oil. It is reported that the quantity of essential oil in chamomile was inversely related to its quality in terms of α-bisabolol and chamazulenes (El-Hamidi et al., 1965). Moreover, Hamzeii (2003) concluded that the best fertilization treatment for fresh and dry flower yield, number of flowers per plant and essential oil content was 150 kg ha^{-1} while the best for flower diameter was 75 kg ha^{-1}.

Taking into account the aforementioned information, the aim of the present study was to investigate the effect of irrigation and nitrogen fertilization on the flower yield of *Matricaria chamomilla* L. (German chamomile) and the essential oil production which is adapted to the climatic conditions of Central Greece and Mediterranean in general, in order to increase the harvested yield without reducing the quality of the product.

MATERIALS AND METHODS

Experimental design

German chamomile (type C: α-*bisabolol* > α-*bisabolol oxide B* > α-*bisabolol oxide A*) which is the most known species (Hansen and Christensen, 2009), was grown in a typical soil-climatic environment of Thessaly plain, in central Greece. The experimental site is located at the Experimental Farm of the General Department of the University of Thessaly, (Larissa plain, 39°62'69" N, 22°38'14" E, 84m ASL).

The soil texture of the experimental site is characterized as loam and its properties at a depth of 30 cm are presented in Table 1.

Table 1: Soil chemical properties of the field experiment

Property	Soil depth (0-30) cm
Texture	Loam
pH (1:5 soil to H_2O)	7.81±0.16
Electrical conductivity, extract (dSm^{-1}) (1:5 soil to H_2O)	0.11±0.01
Organic matter (%)	0.93±0.05
N-inorganic (ppm)	44.8±4.07
K-exchangeable (ppm)	373.3±7.45
P –Olsen (ppm)	13.1±1.87
$CaCO_3$ (%)	0.63±0.07

Data represent average means and SE deviation (n) = 4.

Sowing took place by hand on November 2016 and 2017, and the harvest during May-June of 2017 and 2018, respectively. Due to the fact that chamomile's seeds are very small, the seeds before the sowing were mixed with well sieved ash and mixed very well in a ratio of 1:2 and the row distances were 30 cm.

A 2x3 split plot experimental design was used with four replications (blocks) and six plots per replication (6x4 = 24 plots). Irrigation comprised the main factor (I_1: rainfed, I_2: 100% ETo) and sub factor the three N-fertilization levels (F_1: 0, F_2: 80, and N_3: 160 kg ha^{-1}). Plot size was 9 m^2 (3 m width x 3 m length). All plots were hand weeded and there was not used any pesticide.

Complete weather data were recorded on a daily basis by an automated meteorological station, which was installed next to the experimental field. The area is characterized by a typical Mediterranean climate with hot and dry summers and cool-humid winters.

Water was applied using a drip irrigation system every ten days period (drippers at 50 cm with drip flow 4 l/hour), while the total amount of applied water was 130 mm for 2016 and 110 mm for 2017, respectively. The irrigation schedule was determined according to the Class A evaporation pan method (FAO 1986). The observed evaporation from the A pan was multiplied by the pan coefficient (Kpan) and the daily crop coefficient Kc (due to the lack of previous studies under Greek climatic conditions, the Kc of grass was used).

Flower harvest

Flower yield measured on final samplings at the ideal collection stage (when 2/3 of the rounds of tubes have been opened on the elevated flowering plant), start of the flowering stage where the concentration of essential oils is maximized (Marquard and Kroth, 2001). In the case of irrigated treatments sampling took place three times while only twice in the case of the rainfed treatments during May-June.

Photograph 1. Chamomile field experiment.

In case to avoid any border effect, 1 m^2 in the inner plot was harvested in each sampling. The samples were weighed at the field and then a sub-sample was taken for air drying and further laboratory measurements.

Soil determinations

The texture of the soil was determined by the bouyoucos hydrometer method (Bouyoucos, 1962). The samples were analyzed by Atomic Absorption, Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia, with the use of flame and air-acetylene mixture (Varian, 1989). The methodology of the soil determination is referred by (Page et al., 1982).

Essential oil content measures

In a Clevenger-type distillation apparatus twelve and a half grams of dried inflorescences of each treatment were subjected to hydro distillation (250 ml of water) for 2 h under three replications, in case to measure the essential oil content. The estimation of the essential oil content is on DW plant material (ml 100 g^{-1}) basis. The distilled essential oils were stored at a temperature of 4°C until further analysis (Tsivelika et al., 2018).

Essential oil analysis

A GC-MS on a fused silica DB-5 column, using a Gas chromatograph interfaced with a mass spectrometer was used to analyze the distilled essential oils. The relative content of each compound was calculated as a percentage (%) of the total chromatographic area and the results are expressed as the mean percentage (%) of three replicates (Sarrou et al., 2017; Tsivelika et al., 2018).

Statistical analysis

The analysis of variance (ANOVA) within sample timings for all measured and derived data carried out with the use of GenStat (7th Edition) statistical package, where $LSD_{0.05}$ was the test criterion for assessing differences between the means (Steel and Torrie, 1982) of the main and/or interaction effects.

RESULTS AND DISCUSSION

Climatic conditions

The study area is characterized by a typical Mediterranean climate with cold humid winters and hot-dry summers. In fact, for both experimentation years, average air temperature ranged from approximate 14°C during spring (Fig. 1), while precipitation varied between 99 mm (2016) and 131 mm (2017) during the same period. Precipitation during the first half of June was almost similar for both years (46.5 in 2016 and 48.4 in 2017).

Therefore, it seems that prevailing weather conditions in 2017 are more favorable for chamomile cultivation due

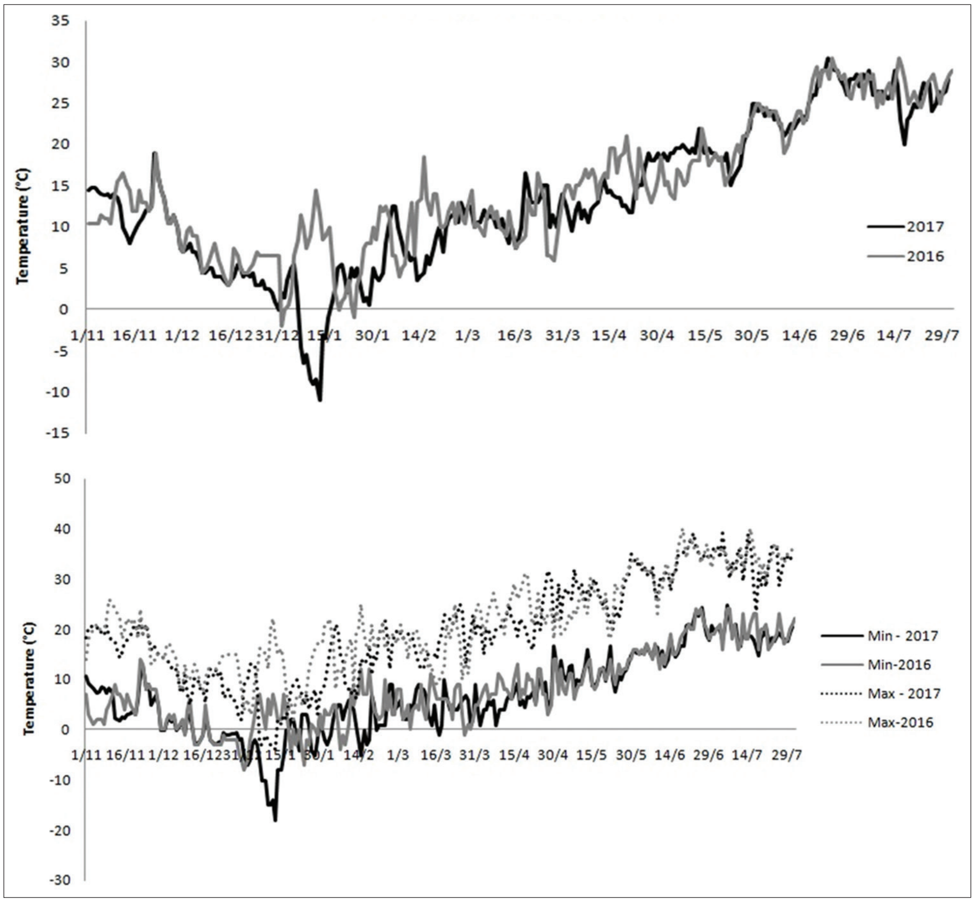

Fig 1. Average, minimum and maximum temperature occurring in studied site during the growing periods of chamomile in the years 2016 and 2017.

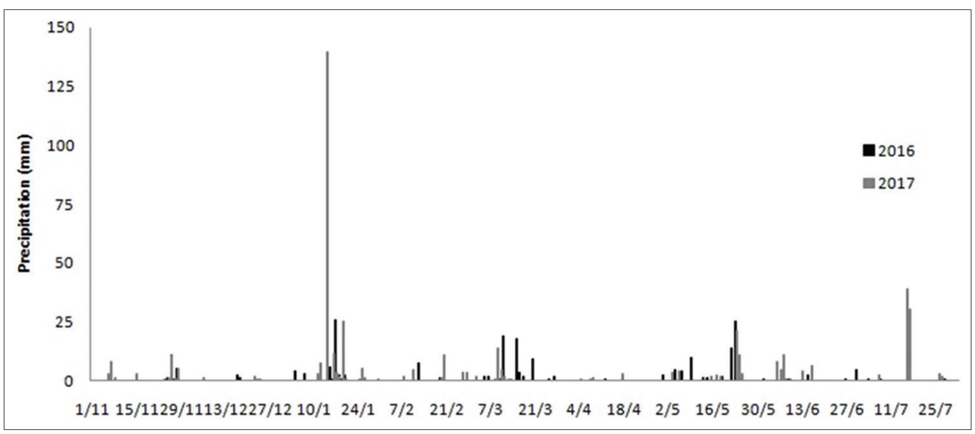

Fig 2. Precipitation occurring in studied site during the growing periods of chamomile in 2016 and 2017.

to the higher precipitation occurred during crop growth period and especially during April-May which is the blooming period (see Fig.2). As it has been mentioned, chamomile has shallow roots and is unable to pump moisture from lower soil horizon.

Soil characteristics

The soil of the experimental field at depth of 0-30 cm was characterized as loam; the pH at (1:5) soil/water extract was 7.81, while the electrical conductivity measured in the same extract was 0.11 dSm^{-1}. Organic matter and $CaCO_3$ soil content was 0.93% and 0.63%, respectively. The $CaCO_3$ soil content was 0.63% and the levels of available mineral nutrient elements N, P and K were found to be 44.8, 13.1 and 373.3 ppm, respectively.

Chamomile flower yield

Average fresh and dry biomass (2 cuts per growing season for the rainfed treatments and 3 cuts for the irrigated) was higher during the second year of the study (Table 2).

Table 2: Irrigation and N-fertilization effect on chamomile harvest fresh and dry matter flower yield

Characteristics	Fresh Weight (kg ha^{-1})		Dry Weight (kg ha^{-1})	
Treatments	1st Year	2nd Year	1st Year	2nd Year
I_1	1990	2222	753	845
I_2	5062	5527	2094	2262
$LSD_{.05}$	*1440.0*	*1241.6*	*694.8*	*626.4*
F_1	934	1019	368	401
F_2	3206	3824	1285	1511
F_3	6438	6780	2619	2748
$LSD_{.05}$	*1056.6*	*1125.6*	*393.0*	*417.4*
I_1F_1	558	625	210	230
I_1F_2	1458	1680	540	627
I_1F_3	3955	4360	1510	1677
I_2F_1	1310	1413	525	572
I_2F_2	4955	5968	2030	2395
I_2F_3	8920	9200	3728	3817
$LSD_{.05}$	*1566.9*	*1527.5*	*681.8*	*651.8*
CV (%)	27.5	26.7	25.3	24.7

a. I^1: rainfed and I2: 100% ETo, b. F_1: 0, F_2: 80, and F_3: 160 kg ha^{-1},c. *$LSD_{.05}$*: least significant difference at *P* < 0.05

Irrigation factor had a significant effect on fresh and dry yield (Table 2). The harvested fresh yield varied between 2,000 and 5,000 kg ha^{-1} in 2016 for the rainfed and the irrigated treatments, respectively. On the other hand, due to the higher rainfall and the favorable distribution, every ten days, the yield was increased up to 2,200 and 5,500 kg ha^{-1} for the rainfed and the irrigated treatments in 2017.

Significant effect of the N-fertilization with 160 kg N ha^{-1} was also recorded, resulting to higher yield, exceeding 6,500 and 2,700 kg ha^{-1} for fresh and dry flowers yield, respectively (Table 2).

Statistically significant differences were found for the interaction of the studied factors with the treatment I_2F_3 producing higher yield (about 9,000 and 3,750 kg ha^{-1} fresh and dry, respectively) in both years, almost twice as much as the second best-performing treatment (I_2F_2, Table 2).

In addition, another remarkable observation for both years was the fact that treatment I_1F_2 produced almost the same yield as I_2F_1, while in treatment I_1F_3 recorded a slightly lower compared to I_2F_2 (Table 2). The above observation demonstrates the great importance of nitrogen fertilization but also reveals need for irrigation particularly in regions with corresponding climatic data and unequal distributed rainfall (Fig 2). Therefore, areas where there is not the opportunity for irrigation, N-fertilization may increase the final yield because the availability of nutrients in the soil is affected by drought stress (Munns, 1993, Solinas et al., 1996).

These yields are lower than those reported in previous study of Upadhyay et al. (2016), probably due to the lower number of cuts (2 in the rainfed treatments and 3 in the irrigated compared to 5-6 that is reported). These findings are in agreement with earlier findings (Singh, 1982; Singh, 1970) where it is reported that in clay-loamy soils, a maximum yield of 7,637 kg ha^{-1} fresh flowers was obtained and the average yield ranged between 3,500 and 4,000 kg ha^{-1}. Furthermore, in saline–alkaline soils, a yield of 3,750 kg fresh flowers per hectare was found.

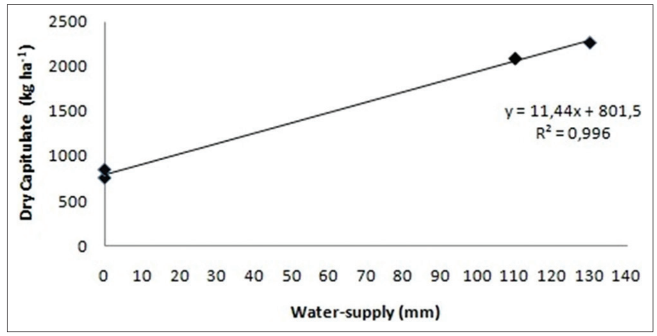

Fig 3. Linear relation between water supply (mm) and dry flower yield (kg ha-1) in the years 2016 and 2017.

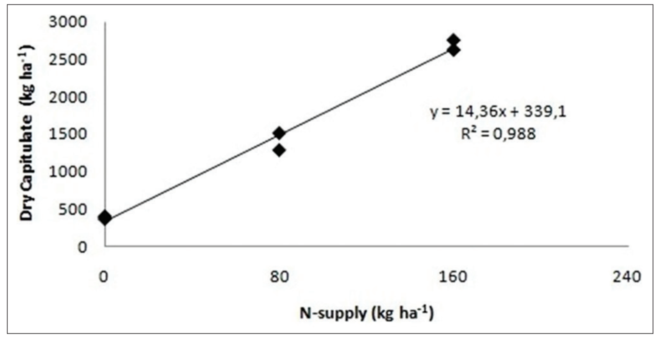

Fig 4. Linear relation between nitrogen supply (mm) and dry flower yield (kg ha-1) in the years 2016 and 2017.

Finally, plotting the dry flower versus the water supply results in the Yield – Water supply relation illustrated in Fig. 3. This linear relationship might be explained by 99.6% of the existing variation (R^2: 0.996) largely independent of the year, showing that each 1 mm of supplied water results to 19.5 kg ha^{-1} of dry flower increase. On the other hand, plotting the dry flower versus the nitrogen supply results in the Yield – Nitrogen supply relation illustrated in Fig. 4. This linear relationship might be explained by 98.8% of the existing variation (R^2: 0.988) largely independent of the year, showing that each 1 kg of nitrogen supply results to 18.6 kg ha^{-1} of dry flower yield increased.

Previous studies (Baghalian et al., 2008) reported that irrigation significantly increased yield which is in line with the findings of this research. Moreover, there are many references regarding the effect of N-fertilization on dry flower yield (Singh et al., 2011; Hamzeii, 2003; Balak et al., 1999; Franke, 1999; Johri et al., 1992; Letchamo, 1992; Franz and Kirsch, 1974) which are in agreement with the positive effect of nitrogen to the harvested yield of this research.

Essential oil content and quality characteristics

Average essential oil content was statistically significant lower for the irrigated treatments (Table 3) for both studied years, while the higher N-fertilization level produced flowers with higher essential content without statistical significant differences. Therefore, it could be concluded that irrigation has a negative effect on essential oil content, while N-fertilization seems to have a linear relation with the tendency the higher the nitrogen supply the higher the yield and essential oil will be produced.

Specifically, irrigation had a significant effect on essential oil content (Table 3), with average values of 0.76 and 0.64 % in both years for the rainfed and the irrigated treatments, respectively. It was also recorded a superiority of the N-fertilization with the higher amount of nitrogen applied (160 kg ha^{-1}) resulting to higher essential oil content, reaching up to 0.78% (Table 3).

Statistical significant differences were not found regarding to the interaction of the studied factors, while the treatment I_1F_3 produced flowers with higher essential oil content (almost 0.88%) in both years. All irrigated treatments, regardless N-fertilization level, produced flowers with lower essential oil content compared to each of the rainfed treatments (Table 3).

Only few studies exist which demonstrate clearly the effect of N fertilization on the essential oil content of chamomile. Specifically, it was found (Singh et al., 2011) that N-application in the form of ammonium sulphate at 40 kg ha^{-1} decreased the essential oil content from 0.64 to 0.59%, which do not agree with the findings of the present investigation.

Table 3: Irrigation and N-fertilization effect on chamomile (flower) essential oil content and *essential* oil production

Characteristics	Essential Oil Content (%)		Essential Oil Production (kg ha^{-1})	
Treatments	1st Year	2nd Year	1st Year	2nd Year
I_1	0.76	0.77	5.98	6.70
I_2	0.63	0.64	13.16	14.55
LSD$_{.05}$	*0.118*	*0.119*	*3.796*	*3.358*
F_1	0.65	0.65	2.26	2.48
F_2	0.68	0.69	8.11	9. 73
F_3	0.76	0.78	18.34	19.66
LSD$_{.05}$	*ns*	*ns*	*4.230*	*5.084*
I_1F_1	0.69	0.69	1.47	1.63
I_1F_2	0.73	0.74	3.82	4.60
I_1F_3	0.88	0.88	12.64	13.86
I_2F_1	0.62	0.62	3.06	3.32
I_2F_2	0.63	0.64	12.39	14.86
I_2F3	0.65	0.68	24.04	25.46
LSD$_{.05}$	*ns*	*ns*	*ns*	*ns*
CV (%)	22.5	21.6	40.6	43.9

a. I_1: rainfed and I_2: 100% ETo, b. F_1: 0, F_2: 80, and F_3: 160 kg ha^{-1}, c. *LSD*$_{.05}$: least significant difference at $P < 0.05$.

However, the results of the present investigation are in agreement with other studies (Franke, 1999; Franz, 1983; Singh, 1977; Chandra and Kappor, 1971) where was found a positive effect of N-fertilizer on the essential oil content and in particular the maximum contents were recorded at the highest nitrogen levels.

Multiplying the essential oil content (%) with the dry flower yield results to the final essential oil production per hectare (Table 3). It was found that irrigation and N-fertilization had statistical significant differences (Table 3) with the highest production recorded in the case of the highest N-fertilization. On the other hand, no statistical significant differences were found between the interaction of irrigation and N-fertilization. The most important finding was that the rainfed treatment with the maximum N-fertilization I_1F_3 produced similar essential oil quantity per hectare as in case of the irrigated with low N-fertilization treatment I_2F_2 (Table 3). The essential oil content is also in agreement with previous studies (Weiss and Fintelmann, 2000) and was higher compared to the findings of Rahmati et al. (2011).

The identified essential oil compounds at the different treatments are depicted in Table 4. The main average recorded oil compounds were: chamazulene (11.05), α-bisabolol (25.37), α-bisabolol oxide A (0.70).

Many researchers (Farhoudi and Lee, 2017; Kazemi, 2015; Gosztola et al., 2006; Taviani et al., 2002) found that α-bisabolol varied from 1.11 to 50.58%, and chamazulene from 1.22 to 29.8%, respectively. Another study carried out in Europe for wild populations (Szőke et al., 2004) reported that the content of α-bisabolol ranged between 24 and 41.54% and of chamazulene varied between 8.71 and 9.31%.

Moreover, Salamon et al. (2007) confirmed that chamomile grown under arid climatic conditions with a mean annual precipitation less than 200 mm has very high contents in

Table 4: Identified essential oil compounds as affected from Irrigation and N-fertilization (average values for both cultivating years)

Treatments	I_1F_1	I_1F_2	I_1F_3	I_2F_1	I_2F_2	I_2F_3
Compound	Content (%)					
Chamazulene	10.85	11.20	11.75	10.25	10.85	11.40
α-bisabolol	25.35	26.15	26.85	25.15	24.65	24.05
α-bisabolol oxide A	0.80	0.75	0.70	0.75	0.65	0.60

α-bisabolol oxide A (50-60%), otherwise the content was decreased. These findings are in agreement with the results of the current study where the precipitation was higher than 200 mm.

It was also reported (Singh 1997) that N increased the content of α-bisabolol and chamazulene, but decreased the content of bisabolol oxide A in the essential oil, which is in agreement with the findings of this investigation. Finally it was found that the quantity of essential oil in chamomile is inversely related to its quality in terms of α-bisabolol and chamazulene which was also found in another study (El-Hamidi et al., 1965).

CONCLUSIONS

Irrigation seems to be a crucial factor which strongly affects the final yield and has a negative impact on the essential oil content. In particular, irrigation treatments resulted to increased flower yields, almost equal to three times the production of the rainfed treatments. Essential oil is produced in follicular-glands which is used to reduce the transpiration due to high air temperatures through their diffusion, and provides a kind of "natural cooling". Therefore in the case of the irrigated treatments the environment was cooler which may be the reason of the lower essential oil production.

Nitrogen fertilization had a positive effect on yield and essential oil content, which indicated that the higher the nitrogen supply, the higher the yield and the essential oil is produced. Nitrogen fertilization had a positive effect on α-bisabolol and chamazulene and a negative effect on bisabolol oxide A.

Even though irrigation had a negative impact on essential oil content, the high increase in yield led to a maximization of the essential oil production for the irrigated treatments with maximum nitrogen fertilization. Another remarkable conclusion was the fact that the rainfed treatment with the low nitrogen supply produced almost similar yield with the irrigated unfertilized treatment, indicating the great importance of nitrogen and/or irrigation need for increased yield.

As an overall conclusion could be that chamomile seems to be a very interesting cultivation in Greece and similar Mediterranean environments which may be suggested for cultivation in irrigated areas due to increased yield and high quality of products. Furthermore, a satisfactory yield may be obtained and cultivation can be extended as rainfed crop in areas with rather high precipitation.

Authors' contributions

Kyriakos D. Giannoulis: Conceptualization, Investigation, Methodology, Formal analysis, Validation, Writing – Original – Review & Editing, Visualization. Christina-Anna Kamvoukou: Laboratory Investigation, Resources. Nikolaos Gougoulias: Laboratory Investigation, Methodology, Formal analysis Eleni Wogiatzi: Field Investigation, Methodology, Visualization.

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