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Research Article
Improvement of Anti-malarial Artemisinin and Essential Oil Production in Response to Optimization of Irrigation and Nitrogen Supply to *Artemisia annua* L. Plant

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Abstract

Background and Objective: Malaria is a major health problem in many developing countries. Artemisinin-based combination therapies are the highly effective against the most prevalent and lethal malaria parasites. *Artemisia annua* plant is the only source of anti-malarial drug artemisinin. There is no research effort with respect to effect of irrigation and chemical fertilization on this modern medicinal plant in Egyptian agriculture. The objective of study was to describe how plant biomass, essential oil production and anti-malarial artemisinin accumulation can be enhanced through irrigation and nitrogen fertilization. **Materials and Methods:** A field experiment was carried out during 2012 and 2013 seasons to determine biomass yield, essential oil and artemisinin content of German *Artemisia* under water stress (2, 3 and 4 weeks irrigation intervals) and nitrogen fertilization (30, 45 and 60 kg N/fed = 0.42 ha). Irrigation intervals and nitrogen rates were laid out in strip-plot design with three replicates. The obtained data were used to determine optimal irrigation interval and nitrogen rate. Statistical analysis was performed based on a strip-plot arrangement in a randomized complete block design. **Results:** Prolonging irrigation intervals up to 4 weeks significantly decreased ($p<0.05$) biomass yield, essential oil content, artemisinin content, total carbohydrates content and leaves minerals content. Nitrogen nutrition enhanced biomass yield, artemisinin content; total carbohydrates and leaves mineral contents up to 60 kg N. Applying 45 kg N under 2 weeks irrigation interval produced the highest essential oil content, while prolonging irrigation intervals to 3 weeks and applying 60 kg N significantly increased ($p<0.05$) artemisinin production. Camphor, *Artemisia* ketone and 1,8-cineole were the major constituents of the essential oil profile. **Conclusion:** *Artemisia annua* can be grown as an economically viable crop under Egyptian conditions. Water stress and extensive nitrogen fertilization had adverse effect on essential oil production. Moderate water stress enhanced artemisinin accumulation in leaves of artemisia plants.

Key words: *Artemisia*, malaria, irrigation, nutrition, volatile oil composition, artemisinin

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Competing Interest: The authors has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Artemisia annua L. is an annual medicinal and aromatic herb that belongs to the family Asteraceae[1]. It is known in China as qinghao (green herb) and has been used to treat symptoms associated with fever and malaria for over 2,000 years. It is known in the United States as sweet Annie, annual or sweet wormwood[2]. The leaves contain an essential oil with artemisinin as main component. Essential oil and its various components of *A. annua* L. plants are most promising natural plant protection compounds[3-7].

Malaria is a major health problem in many developing countries, mostly in Africa and South-East Asia[8]. The conventional anti-malaria drugs such as chloroquine and fansidar have become almost ineffective because of the development of resistance by plasmodium species to these drugs[9]. According to the report of the World Health Organization (WHO) on malaria[10], there were 214 million new cases of malaria worldwide mostly in Africa (88%) followed by the South-East Asia Region (10%) and the Eastern Mediterranean Region (2%). However, there were an estimated 438 000 malaria deaths worldwide, mostly occurred in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean region (2%). The aerial parts of *Artemisia annua* L. plants contain many biologically active compounds; the most important is a sesquiterpene lactone with an endoperoxide bridge called artemisinin[11,12]. Artemisinin-based Combination Therapies (ACTs) are highly effective against Plasmodium falciparum, the most prevalent and lethal malaria parasite affecting humans. Globally, the number of ACT treatment courses procured from manufacturers increased from 11 million in 2005 to 337 million in 2014. The African region accounted for most (98%) manufacturer deliveries of ACTs in 2014[10].

With increasing concern about declining water resources, it has become mandatory conserving irrigation water and reducing chemical inputs in the field of plant production in line with sustainable agricultural practice. Irrigated agriculture is the largest water consuming sector and it faces competing demands from other sectors[13]. Water and nitrogen stress in plants influences many metabolic processes, therefore, the optimization of irrigation and nitrogen fertilization for the production of fresh herbs, essential oils and artemisinin of *Artemisia annua* L. is important. Physiological disorders in plants, such as a reduction in photosynthesis may be occurred by water stress[14]. Water deficit in medicinal and aromatic plants may cause changes in growth parameter, essential oil yield, artemisinin content and composition of essential oils. Water deficit in rosemary plants[15] and in anise plants[16] decreased the essential oil production but water deficit in thyme plants[17] and citronella grass[18] caused an increase in essential oil production. Water deficits in *A. annua* plants up to 62 hours (Ψw = -2.51 MPa) increased leaf artemisinin content, however, moderate water deficit prior to harvest reduced time and costs of drying the crop with inducing artemisinin accumulation[19]. Water stress decreased plant height and shoot weight of German chamomile (*Matricaria recutita* L.) but it had no significant effect on essential oil content or composition[20].

Plants need nitrogen (N) in large content compared to other nutrients, since it is the main constituent of protein and nucleic acid, which influences cell division and cell enlargement[21]. Badawy *et al.*[22] reported that *Artemisia annua* plants must become deficient in N prior to harvest to attain the maximum essential oil content. Fresh biomass and essential oil yields of *A. annua* had been increased with addition of nitrogen and the greatest values (35 t ha^{-1} and 85 kg ha^{-1}, respectively) were obtained with 67 kg N ha^{-1} [23]. However, the step to maximize artemisinin yields is to achieve high biomass before the onset of flowering[24]. Nitrogen deficiency was associated with great reduction in artemisinin and leaf biomass yield[25]. The highest fresh whole biomass yield of 3880 kg ha^{-1} and artemisinin yield of 40.4 kg ha^{-1} was recorded by increasing nitrogen rates up to 97 kg N ha^{-1} [26]. The highest and significant artemisinin content (27.50 mg 100 g^{-1}) was obtained by Ozguven *et al.*[27] from the dried leaves of *A. annua* plants fertilized with 80 kg N ha^{-1}. Nitrogen nutrition of *A. annua* enhanced its nitrogen content and biomass production successively up to 106 mg N L^{-1} for biomass and 206 mg N L^{-1} for leaf nitrogen; further increases in nitrogen had no influence[28]. Artemisinin concentration in dried leaf was maximal at a nitrogen application of 106 mg L^{-1} but artemisinin concentration declined beyond an optimal point with increasing plant nitrogen concentration. Maximization of artemisinin yield requires optimization of plant biomass via control of nitrogen nutrition. Plant height, number of branches per plant leaf yield and artemisinin yield responded of artemisia responded to N rates up to 90 kg N ha^{-1}, while the highest artemisinin content was obtained at 45 kg N ha^{-1} [29].

There is lack of research efforts to study the effect of abiotic stresses on the sole source of anti-malarial drug artemisinin, *Artemisia annua* L., under the Egyptian conditions. Therefore, this experiment was carried out to evaluate the effect of nitrogen fertilization on biomass production, essential oil content, oil composition, artemisinin accumulation and chemical composition of *Artemisia annua* plants under different irrigation intervals.

MATERIALS AND METHODS

This study was conducted at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza (30°01' 39.36" N latitude and 31°12' 36.50" E Longitude) during 2012 and 2013 seasons to study the effect of water stress and nitrogen fertilization on growth parameters, essential oil content, oil composition, artemisinin accumulation and chemical composition of *Artemisia annua* L. plants. The soil textural class of the experimental area is loamy clay soil with, pH (7.4 and 8.10), Electric conductivity; EC (0.88 and 0.95 dS m^{-1}), organic matter (1.75 and 2.20%), available N (15.60 and 18.20 ppm), available P (6.15 and 7.65 ppm) and available K (200 and 250 ppm) in the first and second season, respectively. *Artemisia annua* seeds that were originally imported from Germany were sown in a glasshouse on seedbeds on 25th and 27th February in the first and second season, respectively to obtain the seedlings. At the age of 60 days, the seedlings of 15-20 cm height were transplanted into the experimental field on 28th and 30th April in the first and second seasons, respectively. The experimental design was a randomized complete block in a strip-plot arrangement with three replications. A respective spacing of 2m was maintained between blocks. Irrigation intervals (2, 3 and 4 weeks) represent the horizontal factor and nitrogen levels (30, 45 and 60 kg N/feddan = 0.42 ha) represent the vertical factor. Artemisia seedlings were transplanted on ridges 60 cm apart and 50 cm between seedlings to ensure 14000 plants per fed. Each plot included 5 ridges each was 4 m in length. Therefore, plot area was 12 m^2. Irrigation treatments started 15 days after transplanting in which the plants were irrigated every 5 days. Nitrogen was added in the form of ammonium nitrates (33.5% N) in two equal doses, the first was applied one month after transplanting, while the second one was applied at one month later. Phosphorous in the form of super phosphate (15.5% P_2O_5) at the rate of 200 kg per fed was added before sowing and during soil preparation. Potassium in the form of potassium sulfate (48% K_2O) was added at the rate of 100 kg per fed with the first dose of N. Controlling of weeds was by hoeing whenever required. Fifteen plants of each plot were harvested above ground level in 5th September (onset of flowering) during both seasons. Data collected on plant height (cm), number of branches per plant, fresh leaves yield (ton per fed), dry leaves yield (ton per fed), essential oil content, essential oil yield (kg per fed), essential oil composition, artemisinin content, artemisinin yield (kg per fed) and chemical composition. The fresh leaves were dried at room temperature and the dry weight was recorded. Leaves yield (ton per fed.) was calculated on plot basis. Essential oil content was determined according to British Pharmacopoeia[30] by water distillation of 100 g of air dried herb for 3 h. The essential oil percentage was estimated as follows:

$$\text{Essential oil (\%)} = \frac{\text{Reading measured pipette}}{\text{Sample weight}} \times 100 \quad (1)$$

$$\underset{\text{(kg per fed)}}{\text{Essential oil yield}} = \text{Leaf dry yield (kg per fed)} \times \text{Oil percentage} \quad (2)$$

The essential oil samples obtained from different treatments were dried over anhydrous sodium sulfate and were subjected to GC/MS analysis according to Adams[31] to determine their main constituents at the Central Laboratory of Faculty of Agriculture, Cairo University.

Artemisinin content had been measured using High Pressure Liquid Chromatography (HPLC) according to Acton and Klayman[32] and later modified by Charles *et al.*[33] and Hao *et al.*[34]. The content of artemisinin was determined in 5 g air-dried leaves of each plant sample.

$$\underset{\text{(kg per fed)}}{\text{Artemisinin yield}} = \text{Leaf dry yield (kg per fed)} \times \text{Artemisinin percentage} \quad (3)$$

Total carbohydrates content was determined in homogenized samples (0.2 g) from dried leaves according to DuBois *et al.*[35], nitrogen content was determined by the modified micro-Kjeldahl method as described by AOAC[36], phosphorus content was determined according to the method of Jackson[37]. Potassium was determined using Model SP 1900 atomic absorption spectrophotometer with a boiling air-acetylene burner and recorded read out.

Statistical analysis: Data recorded on vegetative growth traits, essential oil content and yield, artemisinin content and yield were analyzed using analysis of variance based on a strip-plot arrangement in a randomized complete block design according to procedures outlined by Steel *et al.*[38] using MSTAT-C computer package[39]. Treatment mean comparisons were performed using Least Significant Difference (LSD) at 5% level of probability.

RESULTS AND DISCUSSION

Growth traits: Prolonging irrigation intervals had adverse effect on plant height and number of branches per plant, as well as leaves fresh and dry yields during the two growing

Table 1: Effect of irrigation intervals and nitrogen rates on some growth parameters of *Artemisia annua* plants during 2012 and 2013 growing seasons

Irrigation intervals	Nitrogen rates (kg per fed)	Plant height (cm)		Branches No. per plant		Fresh leaves yield (ton per fed)		Dry leaves yield (ton per fed)	
		2012	2013	2012	2013	2012	2013	2012	2013
2 Weeks	30	172.22	188.39	49.67	50.00	6.35	6.64	2.56	2.59
	45	198.33	204.55	51.33	52.55	8.27	8.67	3.34	3.38
	60	222.89	236.78	52.44	53.44	9.75	10.56	3.93	4.01
Mean		197.81	209.91	51.15	52.00	8.12	8.62	3.28	3.33
3 Weeks	30	168.44	172.78	48.44	49.35	4.63	5.04	2.02	2.21
	45	183.11	190.33	50.55	51.34	6.43	6.84	2.92	3.10
	60	206.11	208.66	51.28	52.32	7.78	8.40	3.27	3.72
Mean		185.89	190.59	50.09	51.00	6.28	6.76	2.74	3.01
4 Weeks	30	138.89	145.22	46.78	48.78	3.59	3.68	1.61	1.71
	45	150.56	160.00	48.11	49.22	4.68	4.85	2.17	2.21
	60	190.56	195.00	50.22	51.72	4.86	4.93	2.49	2.58
Mean		160.00	166.74	48.37	49.91	4.38	4.49	2.09	2.17
Mean of N rates	30	159.85	168.80	48.30	49.38	4.86	5.12	2.07	2.17
	45	177.33	184.96	50.00	51.04	6.46	6.79	2.81	2.90
	60	206.52	213.48	51.31	52.49	7.46	7.96	3.23	3.44
LSD at 5%									
Irrigation (I)		9.03	6.10	0.32	0.15	0.30	0.32	0.13	0.27
Nitrogen (N)		12.11	9.60	0.53	0.76	0.13	0.22	0.20	0.43
I X N		n.s	n.s	0.97	n.s	0.49	0.56	0.30	0.30

n.s: Non significant

seasons (Table 1). The highest mean values for plant height, branches number, fresh and dry yield of leaves were measured in plants irrigated each two weeks in both seasons, thereafter further prolongation in irrigation intervals significantly decreased ($p<0.05$) mean values of all studied growth traits. Dry yield of leaves was decreased by about 16.46 and 36.28% by prolongation of irrigation intervals from 2 weeks to 3 weeks and 4 weeks, respectively in the first season, corresponding to 9.61 and 34.83%, respectively in the second season. These results are in line with that reported by Baghalian *et al.*[20], who obtained reduction in the plant height and shoot weight of German chamomile grown under water stress. However, water deficit in plants may lead to physiological disorders, such as a reduction in photosynthesis[14].

Raising N rates from 30 kg to 45 and 60 kg per fed significantly increased ($p<0.05$) vegetative growth traits, viz., plant height and number of branches per plant as well as fresh and dry yield of leaves in both seasons (Table 1). A gradual increase in dry leaf yield as N rate increased up to 60 kg per fed was recorded. The increase amounted to 35.96 and 56.54% in the first season and 33.49 and 58.33% in the second season as N rate increased from 30 to 45 and 60 kg per fed, respectively. This increasing in vegetative growth traits is mainly due to the role of N in stimulating the meristematic growth activity which contributes to the increase in number of cells in additions to cell enlargement. Nitrogen is the main constituent of protein and nucleic acid, which influences cell division and cell enlargement[21]. This explains the response of plant height, number of branches per plant and leaf yield of *Artemisia annua* plants to high N rates[29,40]. The interaction between irrigation intervals and N rates was significant for fresh and dry yields of leaves in both seasons (Table 1). The highest values of fresh yield of leaves (9.75 and 10.56 ton per fed) and dry yield of leaves (3.93 and 4.01 ton per fed) resulted from irrigation artemisia plants every 2 weeks and fertilized it with 60 kg N per fed in the 1st and 2nd season, respectively.

Essential oil and artemisinin productivity: Data presented in Table 2 revealed that a supply artemisia plants with water every two weeks was the optimum for essential oil production in both seasons. Prolonging irrigation intervals from 2 weeks to 3 weeks and 4 weeks significantly decreased ($p<0.05$) essential oil content and yield during the two seasons. Essential oil content decrease amounted to 0.02 and 0.08% in the first season and 0.03 and 0.09% in the second one, respectively. Essential oil yield decrease amounted to 17.13 and 45.30% in the first season and 16.58 and 45.28% in the second one, respectively. Decrease in essential oil yield accompanying high water deficit has been due to the decrease in essential oil content and dry leaf yield. The essential oil reduction accompanying water deficit was reported by Singh and Ramesh[15] on rosemary plants and Zehtab-Salmasi *et al.*[16] on anise plants. Irrigation of artemisia

Table 2: Effect of irrigation intervals and nitrogen rates on essential oil and artemisinin production of *Artemisia annua* plants during 2012 and 2013 growing seasons

Irrigation intervals	Nitrogen rates (kg per fed)	Essential oil (%)		Essential oil yield (kg per fed)		Artemisinin (%)		Artemisinin yield (kg per fed)	
		2012	2013	2012	2013	2012	2013	2012	2013
2 Weeks	30	0.49	0.53	12.63	13.71	0.10	0.11	2.43	2.79
	45	0.56	0.59	19.01	20.05	0.13	0.14	4.23	4.84
	60	0.45	0.47	17.57	18.70	0.14	0.15	5.45	6.16
Mean		0.50	0.53	16.40	17.49	0.12	0.13	4.04	4.60
3 Weeks	30	0.48	0.50	9.98	10.92	0.12	0.13	2.49	2.77
	45	0.50	0.55	15.75	16.84	0.16	0.18	4.57	5.48
	60	0.46	0.44	15.04	16.01	0.17	0.19	5.46	6.94
Mean		0.48	0.50	13.59	14.59	0.15	0.16	4.17	5.06
4 Weeks	30	0.41	0.43	6.54	7.33	0.08	0.09	1.29	1.56
	45	0.46	0.51	10.33	11.30	0.11	0.12	2.45	2.66
	60	0.40	0.39	10.04	10.07	0.11	0.13	2.83	3.36
Mean		0.42	0.44	8.97	9.57	0.10	0.11	2.19	2.53
Mean of N rates	30	0.46	0.49	9.72	10.65	0.10	0.11	2.07	2.38
	45	0.51	0.55	15.03	16.07	0.13	0.15	3.75	4.33
	60	0.44	0.43	14.22	14.92	0.14	0.16	4.58	5.48
LSD at 5%									
Irrigation (I)		0.01	0.02	0.70	1.74	0.01	0.01	0.24	0.45
Nitrogen (N)		0.01	0.04	1.08	2.38	0.01	0.01	0.36	0.77
I X N		0.02	0.03	1.36	1.40	0.02	0.02	0.65	0.70

plants every 3 weeks was the optimum for artemisinin production, since it gave the highest and significant artemisinin content and artemisinin yield in both seasons. Further prolongation of irrigation intervals adversely affected artemisinin production and gave the lowest mean values of artemisinin content and artemisinin yield in both seasons.

Although, water stress proved to alter the secondary metabolite accumulation in medicinal plants[19,41] but a moderate water deficit has sometimes proved beneficial for the accumulation of biologically-active compounds in medicinal and aromatic plants[42,43]. Moderate water deficit (3 weeks) in the present study led to a significant increase ($p<0.05$) in the leaf artemisinin content and that might be attributed to the growth decreases under moderate water deficit, while photosynthesis is still occurring. Thus, the excess photo-assimilates, used in small quantity for growth, would be redirected towards secondary metabolism[44], such as artemisinin biosynthesis.

Essential oil and artemisinin contents in *Artemisia annua* plants as well as their yields were significantly affected ($p<0.05$) by N rates in both seasons (Table 2). Increasing N rate from 30 to 45 kg per fed significantly increased essential oil content by 0.05 and 0.06% and essential oil yield by 54.63 and 50.89% in the first and the second seasons, respectively, thereafter further increase in N rate significantly lowered ($p<0.05$) essential oil content and essential oil yield. Despite the increase in dry leaf yield with increasing N rate up to 60 kg but the yield of essential oil has fallen, suggesting the importance of the essential oil content in estimating essential oil crop harvest compared to herbage yield. The depressive effect of high N rates on essential oil content coincides whit those reported by Badawy *et al.*[22] and Simon *et al.*[23], who obtained high essential oil content of *Artemisia annua* plants fertilized with moderate rates of nitrogen. Increasing N rates from 30 kg to 45 and 60 kg per fed significantly increased artemisinin content by 0.03 and 0.01% and artemisinin yield by 81.16 and 121.26% in the first season, respectively. In the second season, these increases reached to 0.04 and 0.01% for artemisinin content and 81.93 and 130.25% for artemisinin yield, respectively. The lowest values of artemisinin content and artemisinin yield resulted from application of 30 kg N per fed in both seasons. These results agreed with that concluded by Fiqueira[25], Magalhaes and Delabays[26] and Yeboah *et al.*[29]. Agriculture practices greatly affecting essential oil and artemisinin contents in the leaves of *A. annua* plants. Essential oil and artemisinin contents varied from 0.01 to 1.4% and 0.04 to 1.9% respectively, according to the growing conditions of *Artemisia annua* plants[45-47]. So it became necessary to optimize the utilization of water and nitrogen fertilizer to achieve the highest productivity of essential oil and artemisinin.

The changes rate of essential oil, artemisinin contents and their yields among N rates varied significantly from one irrigation interval to another in both seasons (Table 2).

Fertilized artemisia plants with 45 kg N per fed under irrigation interval of 2 weeks produced the highest essential oil content and essential oil yield in both seasons. Prolonging irrigation interval to 3 weeks and fertilized plants with 60 kg N per fed significantly produced the highest artemisinin content and artemisinin yield during the two seasons of the study.

Main constituents of essential oil: The essential oil of *Artemisia annua* plant that obtained by hydrodistillation of dried leaves and analysed with GC-MS, revealed a great variability both in the qualitative and quantitative composition. It contained more than forty components, which were identified under the different conditions of this experiment. The main constituents of essential oil are listed in Table 3. Analysis of *A. annua* essential oil showed the presence of mainly monoterpenoids and sesquiterpenes. Chemical profile of the essential oil is influenced by water stress and nitrogen fertilization. Eight compounds (Table 3) accounted 82.22, 81.36 and 73.96% of the essential oil components under irrigation intervals of 2, 3 and 4 weeks, respectively in the first season, corresponding to 87.63, 86.37 and 77.79%, respectively in the second one. Over all combinations of irrigation intervals and nitrogen rates, camphor, artemisia ketone and 1,8-cineole were the major constituents of the essential oil profile in both seasons. Monoterpenoids compounds such as camphor, artemisia ketone, 1,8-cineole and camphene were decreased with increasing irrigation intervals from 2 weeks up to 4 weeks, while sesquiterpene compounds such as spathulenol, β-caryophyllene and β-farnesene were increased by prolonging irrigation intervals up to 3 weeks, thereafter more prolonging of irrigation intervals decreased sesquiterpene compounds during the two seasons. The main eight constituents of essential oil (Table 3) accounted 77.75, 85.01 and 74.77% of the essential oil components under fertilization with 30, 45 and 60 kg N per fed, respectively in the first season, corresponding to 81.47, 89.99 and 80.32%, respectively in the second season. The monoterpenoids compounds, i.e., camphor, artemisia ketone, 1,8-cineole and camphene were increased with increasing N rates up to 45 kg per fed, thereafter more increase in N rates decreased these compounds during the two seasons. The sesquiterpene compounds, i.e., spathulenol and β-caryophyllene were increased by raising N rates up to 60 kg per fed in both seasons. The highest content of camphor, artemisia ketone, 1, 8-cineole and camphene was measured in the essential oil of plants irrigated each 2 weeks and fertilized with 45 kg N per fed in both seasons. The highest content of spathulenol, β-caryophyllene and β-farnesene was measured in essential oil of plants irrigated

Table 3: Main constituents of the essential oil profile of *Artemisia annua* plant under different irrigation intervals and nitrogen rates during 2012 and 2013 seasons

		2 weeks				3 weeks				4 weeks				Mean of N rates		
Compounds	Compound types	30 kg N per fed	45 kg N per fed	60 kg N per fed	Mean	30 kg N per fed	45 kg N per fed	60 kg N per fed	Mean	30 kg N per fed	45 kg N per fed	60 kg N per fed	Mean	30 kg	45 kg	60 kg
2012 season																
Camphor	Monoterpene aldehyde	40.07	42.05	37.06	39.73	38.61	39.45	34.28	37.45	36.12	38.75	32.96	35.94	38.27	40.08	34.77
Artemisia keton	Monoterpene aldehyde	17.89	20.07	15.47	17.81	16.60	18.30	14.89	16.60	14.92	16.94	13.95	15.27	16.47	18.44	14.77
Camphene	Monoterpene hydrocarbon	4.48	5.42	3.53	4.48	4.30	4.98	4.00	4.43	4.02	4.18	3.74	3.98	4.27	4.86	3.76
1,8-cineole	Monoterpene oxygenated	6.93	7.45	5.21	6.53	5.03	6.26	4.25	5.18	4.46	6.33	4.31	5.03	5.47	6.68	4.59
Trans-pinocarveol	Monoterpene alcohol	1.65	2.35	3.50	2.50	3.76	3.89	3.92	3.86	3.85	3.34	3.25	3.48	3.09	3.19	3.56
Spathulenol	Sesquiterpene hydrocarbon	3.65	3.74	4.47	3.95	4.55	4.74	6.04	5.11	2.38	3.50	4.96	3.61	3.53	3.99	5.16
β-caryophyllene	Sesquiterpene hydrocarbon	4.12	4.27	4.83	4.41	4.49	5.53	6.07	5.36	3.22	3.59	4.52	3.78	3.94	4.46	5.14
β-farnesene	Sesquiterpene hydrocarbon	2.97	3.05	2.42	2.81	2.81	4.12	3.21	3.38	2.38	2.72	3.48	2.86	2.72	3.30	3.04
2013 season																
Camphor	Monoterpene aldehyde	40.77	42.19	39.06	40.67	38.00	39.62	35.85	37.82	37.18	38.33	34.32	36.61	38.65	40.05	36.41
Artemisia keton	Monoterpene aldehyde	18.35	20.65	16.60	18.53	16.94	18.60	15.16	16.90	15.07	17.16	12.92	15.05	16.79	18.80	14.89
Camphene	Monoterpene hydrocarbon	4.76	5.95	4.41	5.04	4.97	5.83	3.64	4.81	4.50	4.62	3.66	4.26	4.74	5.47	3.90
1,8-cineole	Monoterpene oxygenated	7.26	8.00	6.82	7.36	6.24	8.28	5.78	6.77	5.33	7.65	5.20	6.06	6.28	7.98	5.93
Trans-pinocarveol	Monoterpene alcohol	2.06	3.55	3.95	3.19	4.01	4.35	5.25	4.54	4.50	3.45	3.18	3.71	3.52	3.78	4.13
Spathulenol	Sesquiterpene hydrocarbon	4.10	4.62	5.20	4.64	4.83	5.59	5.90	5.44	3.26	3.57	5.25	4.03	4.06	4.59	5.45
β-caryophyllene	Sesquiterpene hydrocarbon	4.19	5.21	5.87	5.09	5.45	5.68	6.15	5.76	3.36	4.68	5.20	4.41	4.33	5.19	5.74
β-farnesene	Sesquiterpene hydrocarbon	2.08	3.05	4.19	3.11	3.74	5.22	4.02	4.33	3.47	4.11	3.39	3.66	3.10	4.13	3.87

Table 4: Effect of irrigation intervals and nitrogen rates on chemical composition of *Artemisia annua* plants during 2012 and 2013 growing seasons

Irrigation intervals	Nitrogen rates (kg per fed)	Total carbohydrates (g/100 g)		N (%)		P (%)		K (%)	
		2012	2013	2012	2013	2012	2013	2012	2013
2 Weeks	30	13.67	12.50	1.12	1.32	0.82	0.89	3.57	3.61
	45	15.00	15.50	1.43	1.58	0.76	0.84	3.60	3.62
	60	17.33	20.33	1.83	1.92	0.74	0.85	3.73	3.85
Mean		15.33	16.11	1.46	1.61	0.77	0.86	3.64	3.69
3 Weeks	30	14.33	17.17	1.05	1.35	0.56	0.63	2.58	3.33
	45	16.33	19.33	1.37	1.41	0.57	0.62	2.92	3.45
	60	21.33	22.50	1.75	1.85	0.75	0.82	3.68	3.76
Mean		17.33	19.67	1.39	1.54	0.63	0.69	3.06	3.51
4 Weeks	30	8.50	10.67	1.03	1.15	0.16	0.17	2.25	2.35
	45	10.83	12.33	1.22	1.30	0.32	0.35	2.80	2.92
	60	12.17	15.17	1.50	1.55	0.42	0.52	2.92	3.22
Mean		10.50	12.72	1.25	1.33	0.30	0.35	2.66	2.83
Mean of N rates	30	12.17	13.44	1.06	1.27	0.51	0.56	2.80	3.10
	45	14.06	15.72	1.34	1.43	0.55	0.60	3.11	3.33
	60	16.94	19.33	1.69	1.77	0.64	0.73	3.44	3.61
LSD at 5%									
Irrigation (I)		0.51	0.69	0.08	0.09	0.09	0.08	0.14	0.06
Nitrogen (N)		0.49	0.44	0.06	0.08	0.07	0.08	0.15	0.12
I X N		0.45	1.11	0.10	0.17	0.06	0.12	0.20	0.10

each 3 weeks and fertilized with 45 kg N per fed in both seasons. Camphor, artemisia ketone and 1, 8-cineole were the major constituents of *Artemisia annua* essential oil[22,48].

Chemical composition: Data presented in Table 4 shows that increasing irrigation intervals from 2 to 3 weeks significantly increased ($p<0.05$) total carbohydrates in both seasons. Prolonging irrigation intervals over 3 weeks significantly decreased ($p<0.05$) total carbohydrates in both seasons. Increasing total carbohydrates content at 3 weeks interval may explain the high content of artemisinin under 3 weeks irrigation interval. Prolonging irrigation intervals up to 4 weeks adversely affected on nitrogen, phosphorus and potassium contents in the leaves of artemisia plants in both seasons.

Raising N rates up to 60 kg per fed increased dry leaves content of total carbohydrates, nitrogen, phosphorus and potassium in both seasons (Table 4). Carbohydrates assimilation in *Artemisia annua* plants has been increased with increasing N rates, until it reached the highest value at the rate of 60 kg N per fed in the first and the second season, respectively. Artemisia plants showed increasing uptake of nitrogen, phosphorus and potassium with increasing N rate up to 60 kg per fed. The highest leaves content of nitrogen, phosphorus and potassium was measured in plants fertilized with 60 kg N per fed in both seasons. Gradual increase in total carbohydrates, nitrogen, phosphorus and potassium contents in the leaves of *A. annua* plants with increasing the rate of nitrogen fertilization was reported by Badawy *et al.*[22] and Davies *et al.*[28]. The interaction of irrigation intervals with N rates was significant for the chemical constituents of artemisia leaves in both seasons (Table 4). Total carbohydrates followed the same trend as in artemisinin content, since it reached the highest values in the leaves of artemisia plants irrigated each 3 weeks and fertilized with 60 kg N per fed in both season. The highest leaf content of nitrogen and potassium was measured in plants irrigated each 2 weeks and fertilized with 60 kg N per fed in both seasons. The highest phosphorus content in the dry leaves was measured in plants received 30 kg N per fed and irrigated each 2 weeks but under the two other irrigation intervals, the content of phosphorus in the dry leaves increased with increasing N rates up to 60 kg per fed.

The results showed that the plant of *A. annua* can be grown as an economically viable crop under Egyptian conditions. Water stress and extensive nitrogen fertilization had adverse effect on essential oil production. Moderate water stress enhanced artemisinin accumulation in leaves of artemisia plants. Therefore, essential oil and artemisinin from artemisia plants can be enhanced by optimization growing field conditions. Despite the importance of the study, however, there are some points that should have been taken into consideration, the most important of which is the amount of water that has been given to plants and this is definitely due to the weak financial potential. This can be remedied in future studies.

CONCLUSION AND FUTURE RECOMMENDATION

Artemisia annua L. plant is growing well under the Egyptian conditions. Application of agricultural practices,

especially irrigation and nitrogen fertilization is depending on the purpose of the cultivation of this plant. It might be recommended that to obtain high herbage yield, the plants should be irrigated at two weeks intervals and fertilized with 60 kg N per fed. In case of producing essential oil, the irrigation of the plants at 2 weeks intervals and fertilization with 45 kg N per fed is recommended, while to get high artemisinin yield, irrigation of the plants at 3 weeks intervals and fertilization with 60 kg N per fed is recommended. In general, results of this experiment suggested that *A. annua* can be grown as an economically viable crop under Egyptian conditions and encourage further researches aiming at a possible application of these substances in food, pharmaceutical and cosmetology fields as well as the possibility of using its products as pesticides in the field of agriculture.

SIGNIFICANCE STATEMENT

This study discovers that artemisinin from *Artemisia*, can be extracted economically and enhanced by optimization growing field conditions. This study will help the researcher in advanced studies on malaria control as well as other studies on plant protection with respect to essential oil and its composition.

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