

Effect of water regime on the growth, flower yield, essential oil and proline contents of *Calendula officinalis*

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Abstract. Metwally SA, Khalid KA, Abou-Leila BH. 2013. Effect of water regime on the growth, flower yield, essential oil and proline contents of *Calendula officinalis*. *Nusantara Bioscience* 5: 65-69. The effects of water regime on the growth, content of essential oil and proline of *Calendula officinalis* L. plants were investigated. Water regimes of 75% of field water capacity increased certain growth characters [i.e., plant height (cm), leaf area (cm²), flower diameter (cm) and spike stem diameter] and vase life (day). Water regime promoted the accumulation of essential oil content and its main components as well as proline contents.

Key words: *Calendula officinalis*, essential oil, flower yield, growth, proline, vase life, water regime

Abstrak. Metwally SA, Khalid KA, Abou-Leila BH. 2013. Pengaruh tata air terhadap pertumbuhan, hasil panen bunga, kandungan minyak atsiri dan prolin pada *Calendula officinalis*. *Nusantara Bioscience* 5: 65-69. Pengaruh tata air terhadap pertumbuhan, kandungan minyak atsiri dan prolin dari tanaman *Calendula officinalis* L. diteliti. Pengaturan tata air sebanyak 75% dari kapasitas air lapangan meningkatkan karakter-karakter pertumbuhan tertentu [yaitu: tinggi tanaman (cm), luas daun (cm²), diameter bunga (cm) dan diameter tangkai bulir] dan masa hidup bunga (hari). Pengaturan tata air menyebabkan akumulasi kadar minyak atsiri dan komponen utamanya serta kandungan prolin.

Kata kunci: *Calendula officinalis*, minyak atsiri, hasil panen bunga, pertumbuhan, proline, tata air

INTRODUCTION

Calendula officinalis L. (English marigold, pot marigold; Figure 1) belongs to the Asteraceae (Compositae) family; it is an annual with bright or yellow-orange daisy-like flowers which are used for ornamental and medicinal purposes (Bcerentrup and Robbelen 1987; Cromack and Smith 1988). *Calendula officinalis* can be broadly applied as an antiseptic, anti-inflammatory and cicatrizing (Correa Júnior 1994) as well as a light antibacterial (Chiej 1988) and antiviral (Bogdanova and Farmakol 1970) agent. Many *Calendula* species have a characteristic scent or taste caused by mono and sesquiterpenes within the essential oil, which in many cases are the reason for their application in folk medicine (Yoshikawa et al. 2001). Recently, many attempts have been made to characterize their therapeutic properties better and to enhance the production of these useful compounds within their essential oils. Selected *Calendula* chemo-types growing in soil or in vitro, for example, flowers of the cadinol chemo-type, are very important in European and western Asian folk medicines and are used to treat inflammatory conditions (Yoshikawa et al. 2001). Distinct subspecies of *C. officinalis* have been reported from various countries (Chalchat et al. 1991; Nicoletta et al. 2003), i.e. Herbaria, Mecsek, Melius,

Golden Dragon and Adamo (Bakó et al. 2002). *Calendula officinalis* can be used as a colorant because it primarily contains two classes of pigments, the flavonoids and carotenoids, which can be used as yellow and orange natural colors, respectively. Natural colors are gaining considerable attention since several synthetic colorants have given rise to allergic, toxic and carcinogenic effects (Lea 1988). Flavonoids have antioxidant activities which play an important role in food preservation and human health by combating damage caused by oxidizing agents (Meda et al. 2005). Carotenoids are important to humans and other animals as precursors of vitamin A and retinoids. In addition, they act as antioxidants, immune-enhancers, inhibitors of mutagenesis and transformation, inhibitors of premalignant lesions, screening pigments in primate fovea, and non-photochemical fluorescence quenchers (Castenmiller and West 1998).

In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water deficit (Burbott and Loomis 1969; Sabih et al. 1999). Solinas and Deiana (1996) reported that secondary products of plants can be altered by environmental factors and that water deficit is a major factor affecting the synthesis of natural products. Water deficit resulted in a significant reduction of fresh and dry matter, and essential oil yield of mint (*Mentha* sp.) plants (Misra and Strivastava



Figure 1. *Calendula officinalis* L. (English marigold, pot marigold)

2000). Fresh and dry weights of *Ocimum basilicum* L. decreased as plant water deficit increased while the linalool and methyl chavicol contents increased (Simon et al. 1992). The essential oil yield and proline contents of basil (*Ocimum* sp.) increased by subjecting plants to water deficit just before harvesting (Baeck et al. 2001). Khalid (2006) reported that fresh and dry weights of *Ocimum* sp. were significantly decreased by water deficit. Meanwhile, essential oil percentage, as well as the main constituents of the essential oil, proline content increased. Baher et al. (2002) showed that water deficit reduced the fresh and dry weights of *Satureja hortensis* L. plants, while severe water deficit increased essential oil content more than moderate water deficit. The main constituents, such as carvacrol, increased under moderate water deficit, while α -terpinene content decreased under moderate and severe water deficit. Hendawy and Khalid (2005) showed that essential oil, and proline contents showed a pronounced increase by increasing the water stress levels of *Salvia officinalis* L. plants. On the other hand, Petropoulos et al. (2007) noted that water deficit had relatively little effect on the essential oil composition of parsley (*Petroselinum crispum*).

The Egyptian climate is mostly arid and semi-arid, where water availability is a major problem for crop production (Abou El-Fadl et al. 1990). In such conditions cultivation of resistant plants is one way to utilize these lands and therefore the selection of suitable crops, which could cope with these conditions, is a necessity. In arid and semi-arid regions, where water availability is a major limitation in crop production, using alternative water resources. The major challenge facing water management is the availability of water. Its amount is fixed, but its demand will continue to increase steadily into the foreseeable future. Reclamation of desert lands has been a

top priority and challenge for the Egyptian government over the last few decades. In this study, we investigate the possible effect of water deficit on the flower yield, essential oil composition and proline content of *C. officinalis* flowers, an economically important medicinal and ornamental plant in Egypt.

MATERIALS AND METHODS

Experiments were carried out in a greenhouse at the National Research Centre, Egypt, during 2010/2011 and 2011/2012. *Calendula officinalis* seedlings were obtained from the Medicinal and Aromatic department, Agriculture Research Centre, Egypt. Uniform seedlings were transplanted into plastic pots (30 cm diameter and 50 cm height). In the first week of November during both seasons, the pots were transferred to a greenhouse adjusted to the natural conditions. Each pot was filled with 10 kg of air-dried Typic Torrifluvents soil (USDA 1999), with a field water capacity (FWC) of 62.5% based on the weight of the soil. Physical and chemical properties of the soil used in this study were determined according to Jackson (1973) and Cottenie et al. (1982) and are presented in Table 1. Three weeks after transplanting, the seedlings were thinned to three plants per pot. *Calendula officinalis* plants were divided into four main groups were subjected to different levels of water regime: 25, 50, 75, or 100% (the control) corresponding to the FWC determined in the soil by weight. All agricultural practices, other than the experimental treatments were done according to the recommendation of the Ministry of Agriculture, Egypt.

Table 1. Physical and chemical properties of the soil used

Soil texture	pH	EC*	Org-C	OM	Total N %	Total P	Total K
Sandy loam	7.2	0.6	0.9	1.9	0.3	0.1	0.1

Note: EC* = electronic conductivity (salinity), Org-C = organic C, OM = organic matter

Harvesting

Fresh flowers were collected from each treatment at three flowering stages, start flowering or flower bud initiation (25 days after bud formation), full flowering (86 days after bud formation) and end of flowering (119 days after bud formation) in both seasons, all of which were air dried. Yield (dry weights of flower) was recorded (g plant⁻¹). On the other hand, the vegetative growth characters [Plant height (cm), leaf area (cm²), flower diameter (cm) and spike stem diameter] and vase life (day) were recorded during the start flowering stage.

Essential oil isolation

Fresh flowers were collected from each treatment and the three flowering stages in both seasons, air dried and weighed to extract the essential oil. Dry flowers (500 g) from each of these treatments were hydro-distilled for 3 h using a Clevenger-type apparatus (Clevenger 1928). The essential oil content was calculated as a percentage. Also g essential oil plant⁻¹ was calculated according to the dry weight of flowers per plant.

Gas Chromatography-Mass Spectrophotometric (GC-MS) analysis

The ADELISGLC MS system, equipped with a BPX5 capillary column (0.22 mm id × 25 m, film thickness 0.25 µm) was used. Analysis was carried out using He as the carrier gas, with a flow rate of 1.0 mL/min. The column temperature was programmed from 60 to 240°C at 3°C/min. The sample size was 2 µl, the split ratio 1: 20; injector temperature was 250°C; ionization voltage applied was 70 eV, mass range m/z 41-400 amu. Kovat's indices were determined by co-injection of the sample with a solution containing a homologous series of *n*-hydrocarbons in a temperature run identical to that described above.

Identification of essential oil components

The separated components of the essential oil were identified by matching with the National Institute of Standards and Technology (NIST) mass spectral library data, and by comparison with Kovat's indices of authentic components and with published data (Adams 1995). Quantitative determination was carried out based on peak area integration.

Proline determination

Proline content was determined in fresh leaves during three flowering stages using the method of Bates et al. (1973).

Statistical analysis

In this experiment, 2 factors were considered: water deficit (100, 75, 50 and 25 % FWC) and flowering stages. For each treatment there were 4 replicates, each of which had 8 pots; in each pot 3 individual plants were planted. The experimental design followed a complete random block design. According to Snedecor and Cochran (1990) the averages of data for two seasons were statistically analyzed using 2-way analysis of variance (ANOVA-2) for flower yield (g plant⁻¹), Proline (µm mg⁻¹) and

Table 2. Effect of water regime on the vegetative growth characters and vase life

Water stress treatment	Plant height (cm)	Vegetative growth characters			Vase life (day)
		Leaf area (cm ²)	Flower diameter (cm)	Spike stem diameter (cm)	
100	36.3	79.3	5.0	0.3	8.3
75	39.3	95.0	5.7	0.5	10.0
50	38.3	85.3	5.5	0.4	9.3
25	35.7	52.7	4.5	0.2	6.7
F. values	0.3	0.1 ***	0.1	0.1 **	0.1 **
LSD at 0.05	NS	6.4	NS	0.04	1.4

Note: *P<0.05, **P<0.01, ***P<0.001 according to F-values of the 2-way analysis of variance (ANOVA-1).

Table 3. Effect of water regime, flowering stages and their interactions on flower yield, proline, and essential oil content

Treatments Stages	Water regime	Flower yield (g plant ⁻¹)	Proline (µm mg ⁻¹)	Essential oil	
				%	mL plant ⁻¹
Start	100	9.3	2.1	0.1	0.009
	75	113.1	2.5	0.2	0.226
	50	50.4	3.8	0.2	0.101
	25	44.1	8.6	0.3	0.132
	Overall	54.2	4.3	0.2	0.117
Full	100	135.7	3.4	0.2	0.271
	75	233.6	3.9	0.2	0.467
	50	222.9	5.8	0.3	0.669
	25	100.3	10.9	0.4	0.401
	Over all	173.1	6.0	0.3	0.452
End	100	69.1	2.4	0.2	0.138
	75	152.6	2.9	0.3	0.458
	50	103.1	4.8	0.4	0.412
	25	58.8	9.8	0.5	0.294
	Overall	95.9	5.0	0.4	0.326
Overall water regime	100	71.4	2.6	0.2	0.139
	75	166.4	3.1	0.2	0.384
	50	125.5	4.8	0.3	0.394
	25	67.7	9.8	0.4	0.276
F values					
Water regime		2466.2***	2555.9***	9.727***	31606.9***
Stages		15248.6***	247.4***	7.364***	140934.9***
Water regime X stages		3796.1 ***	5.7 ***	0.455	5553.3***
LSD at 0.05					
Water stress		38.8	0.6	0.04	0.04
Stages		22.8	0.3	0.03	0.03
Water stress X stages		21.7	0.2	NS	0.02

Note: *P<0.05, **P<0.01, ***P<0.001 according to F-values of the 2-way analysis of variance (ANOVA-2).

Table 4. Effect of water regime on the chemical constitution of essential oil

Constituents (%)	RI	Water regime treatments				F. values	LSD
		100	75	50	25		
γ-Cadinene	1514	2.5	2.6	2.7	2.8	1.3	NS
Δ-Cadinene	1523	18.8	18.9	19.3	19.8	8.9**	0.2
β-Calacorene	1564	4.4	4.5	4.7	4.9	1.3	NS
Nerolidol (E)	1565	6.9	7.1	7.8	7.9	11.9***	0.2
β-Acorenol	1635	4.9	5.2	5.3	5.5	1.4	NS
α-Eudesmol	1653	10.8	10.9	11.8	12.9	36.9***	0.1
α-Cadinol	1663	33.9	34.8	34.9	35.2	1.6	NS
Pentacosane	2501	3.8	4.2	4.4	4.9	6.3*	0.2
Total identified		86.0	88.2	90.9	93.9		

Note: *P<0.05, **P<0.01, ***P<0.001 according to F-values of the 2-way

essential oil (% and mL plant⁻¹); while using 1-way analysis of variance (ANOVA-1) for plant height (cm), leaf area (cm²), flower diameter (cm), Spike stem diameter, Vase life (day) and essential oil constituents. The applications of that technique were according to the STAT-ITCF program (Foucart 1982).

RESULTS AND DISCUSSION

Effect of water regime on the vegetative growth characters and vase life

Vegetative growth characters [Plant height (cm), leaf area (cm²), flower diameter (cm) and spike stem diameter] and vase life (day) of *Calendula* plants were affected by changes of soil moisture. The highest values of these measurements were recorded when plants subjected to 75% of FWC with the values of 39.3, 95.0, 5.7, 0.5 and 10.0 respectively. On the other hand, the lowest values were recorded when the plants were subjected to 25% of FWC. ANOVA indicated that the changes in plant height and flower diameter were insignificant but highly significant for leaf area while more significant for pike stem diameter and Vase life (Table 2). The inhibition of plant growth characters and flower yield under water deficit treatment (25% of FWC) may be due to exposure to injurious levels of drought causing a decrease of turgor which would result in a decrease of growth and development of cells, especially in stems and leaves (Merrill and Eckard 1971). Cell growth is the most important process and is affected by water stress. Plant size is indicated by a decrease in height or smaller size of leaves when there is a decrease in the growth of cells (Hsiao 1973). When leaf size is smaller, the capacity to trap light decreases too and the capacity of total photosynthesis decreases, i.e., photosynthesis is restricted in water shortage conditions, with a subsequent reduction in plant growth and performance (Hsiao 1973). Water stress resulted in significant reductions in CO₂ exchange rate, total assimilatory area, fresh and dry matter and chlorophyll in Japanese mint (*Mentha arvensis* L. cv. MS 77) (Misra and Srivastava 2000). The loss of photosynthesis in drought stress conditions results in a loss of dry matter production at the leaf level of mungbean, bean, topiary bean, *Sesuvium portulacastrum* (ambiguously) and *Pesquisa agropecuaria* (Embrapa) plants (Cox and Jolliff 1987; Abdul-Hamid et al. 1990; Castonguay and Markhart 1991; Nunez-Barrios 1991; Viera et al. 1991; Slama et al. 2007). However, the decrease in flowers vase life under stress condition may be due to loss of turgidity (Hirt and Shinozaki 2003).

Effect of water regime, flowering stages and their interactions on the flower yield

Water regime and/or flowering stage affected the total flowers (g plant⁻¹) (Table 3). Thus, various characteristics of the flowers decreased under the various water regime levels, especially at 25 % of FWC at the end of flowering stages. Greatest yields were obtained at 75 % of FWC, especially at full flowering stage (Table 3). The decrease in flower heads was highly significant for water regime

treatments and for flowering stages. In addition, the changes in this variable were highly significant for the water regime × flowering stage interaction (Table 3). Our results showed that growth and flower yield of *C. officinalis* plants was clearly affected by the different water regime, where the growth parameters recorded the highest values when plants irrigated after adaptation of 75 % of FWC. This superiority may be due to treatment provide the plant all time of growth with adequate supply of water which accelerates physiological processes and plant growth. In this respect, Tayel and Sabreen (2011) indicated that soil water potential through the growing season is necessary to maintain crop growth. Moreover, addition of adequate water decreased abscisic acid and increased cytokinins, and indole acetic acid hormones, which reflecting good plant growth and finally yield (Hayat 2007).

Effect of water regime, flowering stages and their interactions on proline content

The accumulation of proline in *C. officinalis* leaves was promoted by applying various levels of soil moisture, flowering stages and their interaction (Table 3). The highest proline content resulted from 25 % FWC treatment at full flowering stage (Table 3). The increase in proline content was highly significant for water regime flowering stages and their interaction treatments (Table 3). The results of proline content agree with those of Slama et al. (2007) and Blum and Ebercon (1976) who indicated that proline is regarded as a source of energy, carbon, and nitrogen for recovering tissues under stress conditions.

Effect of water regime, flowering stages and their interactions on essential oil content and its chemical composition

Data in table indicates that the highest percentage of *C. officinalis* essential oil was obtained from flower heads at the full flowering stage, i.e. full flowering stage, therefore, this stage was investigated to identify the essential oil components. Treatment with 25% (FWC) caused the most pronounced increase in the essential oil percentage at all flowering stages; however, this percentage decreased at control treatments. The essential oil yield (g plant⁻¹) increased in the most water regime treatments compared with the control treatment in. The essential oil% and yield of flower heads were greatest at full flowering. Water regime treatments and flowering stage, when assessed separately, had a greater effect on essential % and yield of flowers than their interaction, i.e., water regime treatments × flowering stages. The changes in essential oil yield were highly significant for water regime treatments and flowering stages (Table 3).

A qualitative and quantitative comparison of the main constituents present with the water regime treatments in hydro-distilled *C. officinalis* essential oil were studied (Table 4). A total of 8 compounds, accounting for 86.0-94.3% of the oil, were identified. The effects of soil moisture levels on the chemical composition of essential oil extracted are shown in Table 4. The main components were γ -cadinene, Δ -cadinene, β -calacorene, nerolidol, β -acoreno,

α -eudesmol, α -cadinol and pentacosane. Moreover, the highest percentages of the main components resulted from the treatment of 25% FWC. These percentages decreased as soil moisture levels increased. The changes in nerolidol and α -eudesmol constituents were highly significant for water regime treatments while the changes in Δ -cadinene constituent were more significant. The changes in pentacosane were significant. On the other hand, changes in γ -cadinenem, β -calacorene, β -acorenol and α -cadinol were insignificant for water regime treatments (Table 4). The effect of different treatments on essential oil and its constituents may be due to its effect on enzyme activity and metabolism of essential oil production (Burbott and Loomis 1969).

CONCLUSION

It may be concluded that water stress affects on growth, flower yield, vase life, essential oil composition and proline contents of *Calendula officinalis* L. plants.

REFERENCES

- Abdul-Hamid AF, Kubota FA, Morokuma M. 1990. Photosynthesis, transpiration, dry matter accumulation and yield performance of mungbean plant in response to water stress. *J Fac Agric Kyushu Univ* 1 (2): 81-92.
- Abou El-Fadl, IA, Abd-Ella MK, Hussein EH. 1990. Effect of irrigation by saline water on the growth and some principal compounds of peppermint and spearmint in two types of soil. *J Agric Res Tanta Univ* 16: 276-295.
- Adams RP. 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Carol Stream, IL, USA.
- Baeck H, Kuenwoo P, Baeck HW, Park KW. 2001. Effect of watering on growth and oil content of sweet basil (*Ocimum americanum* L.). *Kor J Hort Sci Technol* 19 (1): 81-86.
- Baher ZF, Mirza M, Ghorbanil M, Rezaii MZ. 2002. The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis* L., *Flav Frag J* 17: 275-277.
- Bakó E, Delia J, Tóth G. 2002. HPLC study on the carotenoid composition of *Calendula* products. *J Biochem Biophys Meth* 53: 241-250.
- Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline of water stress studies. *Plant and Soil* 39: 205-207.
- Bcerentrup H, Robbelen G. 1987. *Calendula* and *Coriandrum* new potential oil crops for industrial use. *Fat Sci Technol* 89: 227-230.
- Blum A, Ebercon A. 1976. Genotype responses in sorghum to drought stress. III. Free proline accumulation and drought resistance. *Crop Sci* 16: 379-386.
- Bogdanova X, Farmakol T. 1970. Biological effects of some aromatic plants. *Toksikol* 33: 349-355.
- Burbott AJ, Loomis D. 1969. Evidence for metabolic turnover monoterpene in peppermint. *Plant Physiol* 44: 173-179.
- Castenmiller JJM, West CE. 1998. Bioavailability and bioconversion of carotenoids. *Ann Rev Nutr* 18: 19-38.
- Castonguay Y, Markhart AH. 1991. Leaf gas exchange in water stress common bean and topiary bean. *Crop Sci* 32 (4): 980.
- Chalchat JC, Ph R, Mechet AG. 1991. Chemical composition of essential oil of *Calendula officinalis* L. (pot marigold). *Flav Frag J* 6: 189-192.
- Chiej R. 1988. The Macdonald Encyclopedia of Medicinal Plants. Macdonald & Co., London.
- Clevenger JF. 1928. Apparatus for determination of essential oil. *J Amer Pharm* 17: 346-349.
- Correa Júnior C. 1994. Cultivo de plantas medicinais, condimentares e aromáticas, 2nd ed. FUNEP, Japoticabal.
- Cottenie A, Verloo M, Kiekens L, Velghe G, Camerlynck R. 1982. Chemical Analysis of Plant and Soil. Laboratory of Analytical and Agrochemistry, State University, Ghent, Belgium.
- Cox WJ, Jolhff GD. 1987. Water relations of sunflower and soybean under irrigated and dry land conditions. *Crop Sci* 27: 553-557.
- Cromack H, Smith J. 1988. *Calendula officinalis* production potential and crop agronomy in southern England. *Ind Crops Prod* 7: 223-229.
- Foucart T. 1982. Analyse factorielle, programmation sur micro-ordinateur. Masson, ITCF, Paris. [France]
- Hayat AEH. 2007. Physiological studies on *Hibiscus sabdariffa* L., production I new reclaimed Soils. M Sc Thesis Fac Agric Zagazig Univ., Egypt.
- Hendawy SF, Khalid KA. 2005. Response of sage (*Salvia Officinalis* L.) plants to zinc application under different salinity levels. *J App Sci Res* 1 (2): 147-155.
- Hirt H, Shinozaki K. 2003. Plant responses to abiotic stress. *Asian J Pl Sci* 6: 1072-1079.
- Hsiao TC. 1973. Plant responses to water stress. *Annual Review. Plant Physiol* 24: 519-570.
- Jackson M. 1973. Soil Chemical Analysis. Prentice Hall of India Pvt., Ltd., New Delhi.
- Khalid KA. 2006. Influence of water stress on growth, essential oil and chemical composition of herbs (*Ocimum* sp.). *Int Agrophys* 20 (4): 289-296.
- Lea AGH. 1988. HPLC in Food Analysis. Academic Press, London.
- Meda A, Lamien CE, Romito M, Millogo J, Nacoulma GO. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chem* 91: 571-577.
- Merrill RK, Eckard AN. 1971. Evaluation of water stress control with polyethylene glycols by analysis of guttation. *Plant Physiol* 47: 453-456.
- Misra A, Srivastava NK. 2000. Influence of water stress on Japanese mint. *J Herbs Spices Med Plants* 7 (1): 51-58.
- Nicoletta CB, Marongiu PA, Pivetta T, Procceda S. 2003. Extraction, separation, and isolation of volatiles and dyes from *Calendula officinalis* L. And *Aloysia tryphylla* (L'Her) britton by supercritical CO₂. *J Ess Oil Res* 15: 272-277.
- Nunez-Barrios A. 1991. Effect of soil water deficits on the growth and development of dry bean (*Phaseolus vulgaris* L.) at different stages of growth. [Dissertation]. Department of Crop and Soil Sciences, Michigan State University, Michigan.
- Petropoulos SA, Dimitra D, Polissiou MG, Passam HC. 2007. The effect of water deficit stress on the growth, yield, and composition of essential oils of parsley. *Scient Hort* 115: 393-397.
- Sabih F, Abad Farooki AH, Ansari SR, Sharama S. 1999. Water stress on growth and essential oil metabolism in *Cymbopogon martinii* (Palmarosa) cultivars. *J Ess Oil Res* 1: 151-157.
- Simon JE, Reiss BD, Joly RJ, Charles DJ. 1992. Water stress induced alternations in essential oil content of sweet basil. *J Ess Oil Res* 1: 71-75.
- Slama I, Ghnaya T, Hessini K, Messedi D, Savoure A, Abdelly C. 2007. Comparative study of the effects of mannitol and PEG osmotic stress on growth and solute accumulation in *Sesuvium portulacastrum*. *Environ Exp Bot* 61: 10-17.
- Snedecor GW, Cochran WG. 1990. Statistical Methods, 11th ed. Iowa State Univ. Press, Ames, Iowa, USA.
- Solinas V, Deiana S. 1996. Effect of water and nutritional conditions on the *Rosmarinus officinalis* L., phenolic fraction and essential oil yields. *Rivista Italiana EPOSS* 19: 189-198.
- Tayel MY, Sabreen KHP. 2011. Effect of irrigation regimes, phosphorus level and two *Vicia faba* varieties on yield, water, and phosphorus use efficiency. *J app Sci Res* 7 (11): 1518-1526.
- USDA. 1999. Soil Taxonomy. Natural resources conservation service. Washington D.C., USA.
- Viera HJ, Bergamaschi H, Angelocci LR, Libardi PL. 1991. Performance of two bean cultivars under two water availability regimes. II. Stomatal resistance to vapour diffusion, transpiration flux density and water potential in the plant. *Pesquisa Agropeularia Brasileria* 9: 1035-1045. [Portugal]
- Yoshikawa M, Murakami T, Kishi A, Kageura T, Matsuda H. 2001. Medicinal flowers. III. Marigold (1): hypoglycemic, gastric emptying inhibitory and gastroprotective principles and new oleanane-type triterpene oligoglycoside, calenda saponins A, B, C, and D, from Egyptian *Calendula officinalis*. *Chem Pharm Bull* 49: 863-870.