Growth Responses of Calendula (*Calendula officinalis* L.) to Temperature Regimes and Different Soil Types

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ABSTRACT

Temperature stress is the major environmental regime that affects agricultural production worldwide, especially in arid and semi-arid regions. Arid and semi-arid regions are characterized by highly variable environmental conditions were temperature goes extremely low to extremely high and affects the performance, physiology and biochemistry of the plants. This research investigated the effect of temperature regimes on sowing seeds of *Calendula officinalis* L. at (1, 15 September and 1, 15 October, 2013 and 2014) grown in different soil types (clay, loamy, sand and mix between them). Significant increases in vegetative growth characters were observed for plants cultivated in sandy soil compared to those cultivated in clay loamy or mixed soils. The best result was obtained with 15 September treatment sandy soil plants. This result included higher branches number and leaf area, higher shoot fresh and dry weights, as well as higher number of flowers. Physiological traits include transpiration rates, stomatal conductance, intercellular CO_2 concentration and sample cell H_2O, were highly significant in first October planting date with clay loamy soil type in both seasons. Environmental regimes and different soil type's showed clear effects on the biochemical contents (total soluble protein, H_2O_2 and catalase activity fractions of the leaves) of three week old seedlings. It is concluded that sowing in September in sandy soil is recommended for Calendula production in arid and semi-arid regions.

Keywords: Arid and semi-arid environment, Biochemical contents, Calendula, Gas exchange, Growth, Water potential.

INTRODUCTION

Calendula (*Calendula officinalis* L.) (pot marigold, English marigold) belongs to the Asteraceae (Compositae) family; it is an annual plant with bright or yellow orange daisy-like flowers which are used for ornamental, medicinal or culinary purposes (Cromack and Smith, 1988).

Selected Calendula chemotypes growing in vitro or in soil, flowers of the cadinolchemotype, are very important in European and western Asian folk medicines and are used to treat inflammatory conditions (Masayuki *et al.*, 2001). Distinct subspecies of *C.officinalis* have been reported from various countries (Nicoletta *et al.*, 2003), i.e. Herbaria, Mecsek, Adamoand Golden Dragon (Bakô *et al.*, 2002). Calendulacan be used as a colorant species because it primarily contains two classes of pigments, the carotenoids and flavonoids, which can be used as orange and yellow natural colors, respectively. Natural colors are gaining considerable attention since several synthetic colorants have given rise to toxic, carcinogenic and allergic effects (Lea, 1988).

Environmental constraints are major factors limiting crop productivity in several regions of the world (Shah and Paulsen, 2003). Abiotic stresses such as drought and high temperature frequently limit the growth and productivity of major crop species including Calendula. In additional concern is global climate change, with the expected increase in global temperatures, intensification of drought, changed distribution of precipitation in arid and semiarid areas (Wigley and Raper, 2001), and the combination among those factors with growth stage, leading to reductions in productivity (Bai *et al.*, 2004). It was found that a combination of heat stress and drought had a significantly greater harmful effect on the growth and productivity of crops compared with a stress applied alone (Savin and Nicolas, 1996;Wang and Huang, 2004). For example, during heat stress, plants open their stomata to cool their leaves by transpiration. Nevertheless, if heat stress is combined with drought, the plants have to keep their stomata closed to reduce water loss, so the leaf temperature leftovers high (Rizhsky *et al.*, 2002). Such combined stresses often have the largest negative effects on flowers yield.

The objective of this study is to investigate the impact of seasonal dynamics of temperature on an important medicinal plant Calendula grown under different soil types. A number of growth parameters were determined under temperature regimes, including vegetative growth and flower yield components. The gas-exchange measurements, water potential and enzymatic activities were also under consideration.

MATERIALS AND METHODS

1. Field location and climate

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The field study was carried out in the College of Food and Agricultural Sciences of the King Saud University, Riyadh, Saudi Arabia, 24°44'12.66"N and 46°37'13.32"E. The climate of the region was arid with very little precipitation through the year, except some flash rains in March and April. In winter, the temperatures were mild with some few winds and sand storms. Monthly averages of the temperature, solar radiation, relative humidity, evapotranspiration, rainfall, and wind speed are shown in Table (1).

2. Plant materials

Calendula seeds (*Calendula officinalis* L. cv. Calypso Yellow) provided by Sakata's Reliable Seeds Company (USA), were sown on different planting dates; September 1, 15 and October 1, 15, 2013 for the first season and 2014 for the second season. Seedlings were planted in plastic trays. Thirty-day-old seedlings (healthy and uniform in size) were transplanted into 15 cm diameter plastic pots (one seedling pot^{-1}) containing different soil types (sand, clay loamy and mix between them 1:1, v:v) as shown in Table (2).

3. Experimental design

The experiment layout was a spilt plot system in random complete block design (RCBD), with three replicates for the first and second seasons (Steel and Torrie, 1986). Every replicate contained 12 treatments for the first season [four times sown September (1 (T1), 15 (T2) and October 1(T3), 15 (T4) x three different soil types]. The main plots were devoted for the four times sowing date sand the sub-plots included the three different soil types. Fifty seeds were used for each treatment in each replicate; the total number of seeds used in the experiment was 1800 seeds season^{-1}.

4. Experimental data

Seed germination percentage after four weeks from sowing was recorded. Plants were grown until flowers maturity and harvested (about 180 days) from planting. At harvest, the plant height, diameter of flower and root length was measured using a tape measure and calipers. Leaf area was measured using a leaf area meter (LI-Cor, Lincoln, NE, USA). Number of branches, leaves and flowers plant^{-1} were counted. Flowering date (days from seed sowing) in each treatment was determined. Shoot and root fresh and dry masses (FM and DM) were also estimated. DM was recorded after fresh shoots and roots were oven-dried at 70°C for 48 h.

Leaf chlorophyll and anthocyanin contents were determined using a hand-held chlorophyll content meter (CCM-200 and ACM-200, Optic science, USA, respectively). The chlorophyll and anthocyanin contents determination were measured on the fifth youngest leaves.

Table 1. Meteorological data at Riyadh, Saudi Arabia during the two growing seasons

Months		1st season (2013/2014)							2nd season (2014/2015)						
		T (°C)		Rs (MJm^{-2} d^{-1})	RH (%)	ETp (mm d^{-1})	U_2 m/s	P mm/M^{-1}	T (°C)		Rs (MJm^{-2} d^{-1})	RH (%)	ETp (mm d^{-1})	U_2 m/s	P mm/M^{-1}
		Max.	Min.						Max.	Min.					
Sept.	-15	40.4	32.8	22.46	18.8	4.15	0.37	0.00	41.6	31.1	21.13	15.8	4.10	0.40	0.00
	6-30	38.5	29.7	21.25	19.5	3.90	0.35	0.00	38.1	27.3	20.80	17.3	3.92	0.38	0.08
Oct.	-15	34.6	26.3	20.12	21.1	3.61	0.34	0.00	35.9	26.1	19.51	22.4	3.55	0.35	0.11
	6-31	33.4	22.1	18.44	30.9	3.43	0.31	0.10	33.1	24.1	18.23	35.1	3.16	0.33	1.20
Nov.		25.4	14.8	16.20	39.4	2.84	0.28	9.60	27.4	14.3	15.10	44.3	2.41	0.29	5.60
Dec.		22.6	10.1	12.42	37.9	1.52	0.30	8.30	21.7	9.4	12.77	43.7	1.71	0.30	10.70
Jan.		19.9	8.9	12.95	46.1	1.97	0.49	21.50	20.2	8.2	13.85	50.7	1.83	0.48	11.30
Feb.		23.4	9.4	15.88	39.4	2.64	0.62	4.90	22.9	10.3	16.61	47.9	2.41	0.59	10.10
Mar.		29.3	13.7	18.00	28.9	3.45	0.56	15.40	27.6	14.4	18.40	36.9	3.18	0.60	24.00
Apr.		38.1	26.3	21.15	19.3	19.11	0.45	18.30	32.3	18.9	20.45	33.0	20.45	0.50	29.40

Monthly average. T: temperature; Rs: solar radiation; RH: relative humidity; ETp: potential evapotranspiration; U_2: wind speed; P: precipitation. Data from the Presidency of Meteorology & Environment Protection, Riyadh, Saudi Arabia.

Table 2. Physical and chemical properties of the soil

soil type	sand %	Silt %	clay %	SAR	pH	EC (ds/m^{-1})	Soluble cations and anions (mequiv./l)								
							Na+	Mg++	Ca++	K+	HCO_3^{1-}	$CaCo_3$	Cl^{1-}	SO_4^{2-}	P (av.P)
Sand	93	5	2	1.41	8.23	0.96	1.13	0.29	1.03	0.05	0.25	21.38	1.25	1.04	8.81
Clay loamy	38	32	30	3.15	8.39	049	5.67	1.46	5.13	0.16	1.23	2.94	5.98	4.88	10.6

5. Physiological measurements

5.1. Gas exchange and leaf water potential measurements

Leaf photosynthesis rate (*Pn*), transpiration rate (E), stomata conductance(g_s), intercellular CO_2 concentration (C_i) and sample cell H_2O (S_H_2O) were measured on the flag leaf at anthesis using the portable LI-6400 photosynthesis system (Li-Cor, Lincoln, USA) with the following settings: flow rate 500 μmol s^{-1}, leaf/block temperature 20 °C, photosynthetically active radiation (PAR) 1000 μ mol m^{-2} s^{-1} and ambient CO_2 concentration at 400 mg kg^{-1} (ppm). Four measurements were taken per leaf of all 3 replicates at 15 sec. intervals after the CO_2/H_2O parameters became relatively stable. The leaf water potential was measured on the leaf at a thesis using the portable PSYPRO water potential system with a pressure chamber (Wescor, Inc., Utah., USA).

5.2. Biochemical contents

5.2.1. Total soluble protein content

The total protein content was determined according to Lowry *et al.* (1951) by taking 20 seedling + 25 ml of Tris-Hcl pH 7.6 from each treatment. Plant tissue (0.5 gm) + small amount of solution were placed in mortar and crushed till no tissues appear. The mixture was centrifuged at 5000 rpm under freezing for five minutes, and then transferred to keep it under freezing to be used.

5.2.2. Catalase activity

Briefly, the reaction solution (3 mL) for catalase enzyme was comprised of 50 mM phosphate buffer (pH 7.0), 15 mM H2O2, and 50L enzyme extracts. Reaction was initiated by the addition of H2O2 to the reaction solution. The catalase activity was determined by following the decomposition of H2O2 and measuring the absorbance decrease at 240 nm for 1 min (Cakmak et al.,1993). The Catalase activity was expressed as μkat mg^{-1} protein min^{-1}.

5.2.3. Hydrogen peroxide concentration

Fresh leaves (0.1g) were homogenized in 5mL of 0.1% trichloroacetic acid (TCA). After centrifugation at 12,000 rpm for 15 min, the supernatant (0.5mL) was then mixed with 0.5mL of phosphate buffer (pH 7) and 1mL of 1MKI. After incubation, the absorbance was taken at 390 nm. The content of H2O2 was determined using a calibration curve constructed using a series (1–50μM) of analytic grade H2O2 (Velikova et al., 2000).

RESULTS

1. Effect of temperature regimes

It is evident from the results that Calendula plants, sown in four different times in Riyadh region, exhibited a diverse pattern of growth, physiological and biochemical attributes. Germination, leaf number, leaf area, shoot and root fresh and dry masses, and flowers yield component (flowering date, flower diameter and flower number) were affected by temperature regimes treatments (T1-T4). Temperature treatment (1 September) gave the highest values of germination on both seasons (Table 3).Plants sown in 15 September in both seasons exhibited highest values for plant height (16.79 and 18.24 cm) respectively, whereas the significant reduction in plant height was recorded in 1 September (13.82 and 14.30 cm) which was the least value among the four times in the both seasons. However, the plants collected in 15 September and 15 October showed at par values for plant height. Large leaf area was significantly reduced in plants, but the reduction in leaf area due to the growth delay times from T2 to T4 (Table 3).

The increases in treatments from T1 to T4 led to delay flowering date and decrease flower number (Table 3). Temperature significantly reduced ($p<0.05$) the flowering date in plants under both seasons. The increases in flowering date with decrease temperature led to delay flowering date. However, a significant reduction in most flower traits occurred only when sown on 15 Oct. Continuous late in agriculture significantly reduced flower components. Generally, as with some vegetative growth traits, T2 treatment gave higher flower number $plant^{-1}$.

2. Response to different soil types

According to the results presented in table (3), increases were recorded with respect to germination, plant height and flower number per plant traits with sand soil type compared with other soil types. Leaf area was affected by clay loamy soil treatment. However, the highest value of flowering date per plant was found at mix soil treatment.

3. Interaction effect between temperature and different soil types

3.1. Plant growth characters

The results indicate that the temperature regimes treatments affected significantly Calendula development. Increasing germination percentage was in 1 September with sand, mix than clay loamy soil type. Significant differences were found in most plant growth traits, except for root length, chlorophyll and anthocyanin contents (Tables 4 and 5). Generally, 15 September under clay loamy soil type showed higher plant height, larger leaf area and heavier root fresh and dry masses.

Table 3. Mean performance of vegetative and flower parameters of *Calendula officinals*L.plants as affected by temperature stress and different soil types during the two seasons

Main effect	Germination (%)		Plant height (cm)		Leaf area plant^{-1} (cm^2)		Flowering date (days)		Flower number plant^{-1}	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
					Effect of temperature regimes					
1 Sept. (T1)	49.78a	45.25a	13.82a	14.30b	160.77b	169.51a	180.35c	183.65c	3.01a	4.11b
15 Sept. (T2)	30.18b	26.47b	16.79a	18.24a	182.61a	200.45a	191.00b	196.97b	4.22a	5.34a
1 Oct. (T3)	32.47b	33.64ab	14.12a	14.57b	174.20ab	173.78a	201.02a	202.98a	2.99a	3.32c
15 Oct. (T4)	40.80ab	43.56a	15.80a	16.36ab	156.67b	126.79b	205.31a	205.43a	2.68a	1.88d
Sub-Main effect					**Response to different soil types**					
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Clay	24.02c	21.40c	15.83b	16.78a	181.13a	195.96a	188.25b	192.03b	2.93a	4.08a
Sand	54.61a	54.92a	18.31a	18.57a	161.49ab	157.23b	195.85b	196.67b	3.75a	4.25a
Mix	36.29b	35.36b	11.26c	12.26b	163.08b	149.70b	199.17a	203.08a	3.00a	2.67b

Values in each column followed by the different letter(s) are significantly different at $P \leq 0.05$.

Table 4. Vegetative growth parameters of different soil types and *Calendula officinals*L. plants grown under temperature stress during the two seasons

Time	soil	Germination (%)		Plant height (cm)		branches number plant^{-1}		leaf number plant^{-1}		Leaf area plant^{-1} (cm^2)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
1 Sept. (T1)	Clay	41.31bc	37.02cde	15.63bcd	18.03abc	3.02abc	3.67abc	20.04cd	21.32ab	167.73bcd	189.46abc
	Sand	64.65a	61.03a	15.83a-d	16.43bc	2.66abc	3.35abc	23.65a-d	27.01ab	151.12cdc	156.23c
	Mix	43.33b	37.70bcd	10.00de	8.43e	4.01abc	3.02abc	26.31abc	27.71a	163.47bcd	162.85bc
15 Sept. (T2)	Clay	20.05d	16.33f	22.03a	22.10a	2.34bc	5.02a	21.00bcd	26.00ab	202.87a	228.64a
	Sand	49.03ab	45.38ad	18.20ab	18.73ab	5.03a	5.67a	32.03a	28.03a	177.67abc	218.16ab
	Mix	21.45d	17.70ef	10.13de	13.90cd	1.35c	1.64c	18.29cd	14.32b	167.31bcd	154.54c
1 Oct. (T3)	Clay	15.67d	14.59f	13.96b-e	16.07bc	2.29bc	3.36abc	15.02d	20.01ab	163.01bcd	212.62ab
	Sand	55.04ab	57.29ab	19.97ab	19.26ab	4.68ab	4.65ab	30.33ab	25.67ab	186.84ab	165.90bc
	Mix	26.70cd	29.05def	8.43e	8.37e	1.32c	1.31c	20.36bcd	16.33ab	172.75bcd	142.81c
15 Oct. (T4)	Clay	19.03d	17.66ef	11.70cde	10.92de	1.65c	2.01bc	15.31d	15.64ab	190.92ab	153.13c
	Sand	49.72ab	56.02abc	19.23ab	19.83ab	1.36c	1.59c	20.70bcd	18.02ab	130.31e	88.63d
	Mix	53.66ab	57.00ab	16.47abc	18.33abc	2.68abc	1.68c	19.34cd	16.00ab	148.80de	138.60cd

Values in each column followed by the different letter(s) are significantly different at $P \leq 0.05$.

But the same treatment (T2) under sandy soil type showed higher branch number, leave number, fresh and dry masses plant^{-1} traits.

3.2. Flower characters

The clay loamy dependency value for flower diameter of Calendula plants was significantly higher under T1 followed by T2 treatments, but the T2 with sand soil type apparent increasing flower number of plant^{-1} (Table 6). On the other hand, mix soil type under T2 (15 September) led to delay flowering date compared with all other treatments.

3.3. Measurement gas exchanges and leaf water potential

High temperature significantly affected all leaf gas exchange parameters (Pn, E, g_s, C_i and S_H_2O) (Fig.1). The effects of temperature on leaf gas exchange were only observed under the different soil types. However, leaf photosynthesis rate showed conflicting in growth stage (Fig. 1A). Stomata conductance, intercellular CO_2 concentration, transpiration rate and sample cell H_2O increased with treatment of clay loamy soil type in 1 October (Fig. 1B, C, D and E).On the other hand, g_s, E and S_H_2O were decreased with decrease temperature and planting date in clay loamy soil type, while they were showed decrease with all the treatments uses mix soil type.

Leaf water potential at morning (Ψ_l) had significantly different values of −2.86, −2.63 and −2.63 MPa in the first season, but values of −3.09, −2.75 and −2.01 MPa in the second season for the sand, clay loamy and mix, respectively. The results indicate that interaction effect water potential between the temperature regimes treatments and different soil types significantly affected water potential of Calendula plants. The leaf water potential (Ψ_l) also decreased in sand soil plants at 15 September (Fig. 1F).

3.4.Biochemical contents

Data in Figure 2A showed that Calendula plants cultivated in the mix soil type under all treatments had the lowest mean value for total soluble protein, while the highest mean for total soluble protein were detected at September for clay loamy than sand soil types. The comparison between temperatures, data indicated that 1 September had the highest average total soluble protein content.

Data presented in Figure 2B, growing conditions significantly affected the catalase activity enzyme in leaves. Plants collected from sandy soil type in 15 September showed more catalase activity enzyme, but in the low concentrations with late sown seeds. The minimum catalase activity was observed in the leaves of plants grown in 15 October treatment. Changes in environmental conditions significantly affected H_2O_2 concentration in all plants (Figure 2C). Nonetheless, clay loamy had more H_2O_2 concentration followed by sand and mix soil types under different growing conditions. Clay loamy soil caused greater H_2O_2 accumulation in the Calendula at15 September when compared with all temperature regimes in both seasons.

DISCUSSION

Temperature is an important determinant of morphology, physiology and biochemistry of plants. In this research, we studied growth, morphological, physiological and biochemical attributes of an important medicinal plant Calendula in response to varying temperature conditions of arid and semi-arid land. The plants investigated in September in both seasons with higher temperature (38.5- 29.7 and 38.1- 27.3°C) respectively, showed optimum growth (branch number, leaf number, leaf area, shoot and root fresh and dry masses, flower diameter and flower number) whereas the same parameters showed a significant decrease in October. Prakash *et al.* (2011) observed an increase in morphological characteristics of Plantago plants in suitable environmental conditions and a decrease beyond a certain limit. Sudden and extreme decrease in temperature is accompanied with more stressful conditions which affect the growth and development of plant species. Growth patterns of Calendula plants, fresh and dry mass accumulation were studied as they indicate primary productivity in response to temperature regimes (Smith, 2007). Temperature not only acts as an important limiting factor for enzymatic activities and metabolism of plants, but also regulates cell division, translocation of food and photosynthesis. A temperature of 30^{o}C is optimal for most biochemical processes (Devlin and Witham, 1986) in plants. The raise growth characteristic in the studied plants at higher temperature can also be explained on the basis of the fact that plants grown in September are exposed to greater irradiance, large diurnal fluctuations of temperature, reduced partial pressure of gases, increased rate of transpiration due to high wind velocity, limited water and nutrient supply and a narrow time window for growth and development of plants (Streb *et al.*, 1998).

Calendula plants grown in clay loamy soil significantly enhanced some growth parameters more than those of plants grown in sand and mix soil. The difference between the three soils in growth parameters (leaf area, flower diameter, fresh and dry root masses) may be attributed to the ability of clay soil to support the plants by the favorable water and available macro- and micro nutrients more than sand and mix soil types (AbouHussien *et al.*, 2010).

Table 5. Plant growth parameters of different soil types and *Calendula officinals*L. plants grown under temperature stress during the two seasons

Time	soil	Fresh shoot weight (g)		Dry shoot weight (g)		Fresh root weight (g)		Dry root weight (g)		Root Length (cm)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
1 Sept. (T1)	Clay	13.02bc	12.54abc	3.31c	2.90bcd	11.53a	4.26b	3.46ab	1.70bcd	41.70a	35.83a
	Sand	10.15cd	10.20bc	2.80c	2.52bcd	3.16cde	2.46b	1.58cd	1.28cd	38.13a	40.33a
	Mix	12.14bc	9.65c	3.03c	2.61bcd	5.75bc	1.69b	2.18bcd	0.53d	22.87a	33.40a
15 Sept. (T2)	Clay	14.65b	15.02ab	4.54b	3.12bcd	9.78a	6.98a	3.91a	4.15a	29.23a	44.30a
	Sand	19.35a	16.74a	5.81a	5.28a	6.60b	7.76a	3.42ab	3.83a	39.32a	43.93a
	Mix	10.39cd	8.80c	3.36c	3.28b	2.03c	2.92b	0.82d	1.53bcd	35.73a	25.63a
1 Oct. (T3)	Clay	12.70bc	10.82bc	3.58c	2.78bcd	4.26b-e	2.47b	1.57cd	1.20cd	31.36a	35.77a
	Sand	12.49bc	12.19abc	3.51c	3.24bc	5.07bcd	3.70b	3.16abc	2.91ab	35.83a	41.57a
	Mix	11.07cd	9.15c	2.58c	2.25bcd	2.36de	2.24b	0.83d	0.78cd	25.93a	23.27a
15 Oct. (T4)	Clay	11.08cd	10.70bc	2.72c	3.14bcd	3.38cdc	2.19b	2.33a-d	0.72cd	25.70a	24.30a
	Sand	8.42d	7.59c	2.60c	2.22cd	2.25de	1.52b	1.04d	0.89cd	29.96a	42.03a
	Mix	10.28cd	10.58bc	3.06c	2.18d	4.01b-c	3.81b	2.25bcd	2.04bc	25.86a	28.46a

Values in each column followed by the different letter(s) are significantly different at $P \leq 0.05$.

Table 6. Inflorescences characters parameters of different soil types and *Calendula officinals*L. plants grown under temperature stress during the two seasons

Time	soil	Flowering date (days)		Flower diameter plant^{-1}		Flower number plant^{-1}		Chlorophyll content		Anthocyanin content	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
1 Sept. (T1)	Clay	175.00e	181.67f	5.96a	6.20a	3.01b	4.66b	19.07a	23.03a	5.36a	4.07a
	Sand	171.02e	168.66g	3.35e	3.91cde	2.67b	3.64bcd	15.43a	18.26a	4.23a	4.46a
	Mix	195.03c	200.63cd	4.63b	4.16bcd	3.35b	4.02bc	20.70a	18.77a	5.20a	4.63a
15 Sept. (T2)	Clay	176.67c	186.59cf	4.40bc	5.03b	3.02b	4.68b	26.37a	17.90a	5.76a	4.13a
	Sand	215.01a	217.33a	4.27bcd	4.36bc	6.31a	8.01a	22.03a	20.17a	4.60a	5.47a
	Mix	181.33dc	187.00cf	3.96b-c	4.47bc	3.33b	3.33bcd	23.87a	22.83a	5.83a	4.33a
1 Oct. (T3)	Clay	201.00bc	201.29bcd	3.47cde	4.13bcd	2.65b	4.65b	20.13a	21.50a	4.73a	4.64a
	Sand	193.02cd	196.31de	3.37de	3.57cde	4.01b	3.68bcd	13.20a	19.13a	4.36a	5.10a
	Mix	209.04ab	211.33abc	3.31de	3.00e	2.31b	1.67d	20.57a	20.46a	4.66a	4.87a
15 Oct. (T4)	Clay	200.32bc	198.58de	3.47cde	2.90e	3.03b	2.31cd	12.90a	17.53a	4.59a	4.77a
	Sand	204.34abc	204.36bcd	3.03e	3.22de	2.01b	1.68d	18.83a	20.73a	5.32a	5.20a
	Mix	211.28ab	213.34ab	3.70cde	4.46bc	3.00b	1.66d	23.60a	28.40a	5.36a	5.30a

Values in each column followed by the different letter(s) are significantly different at $P \leq 0.05$.

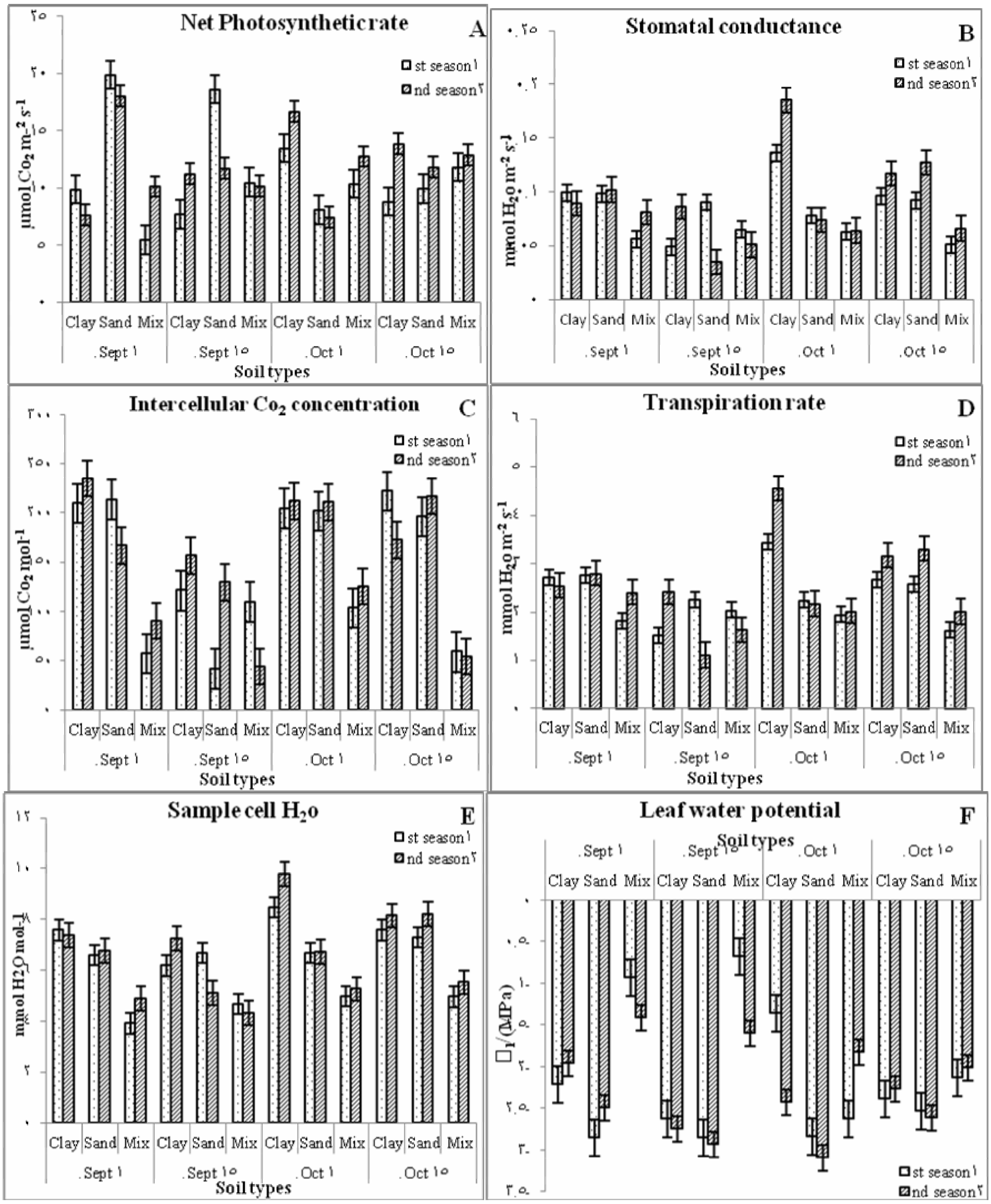

Figure 1. Photosynthetic rate (A), Stomatal conductance (B), Intercellular CO_2 concentration (C), Transpiration rate (D), Sample cell H_2O (E), and Leaf water potential (F) in parameters of different times September 1, 15 and October 1, 15, Calendula plants grown under different soil types, respectively

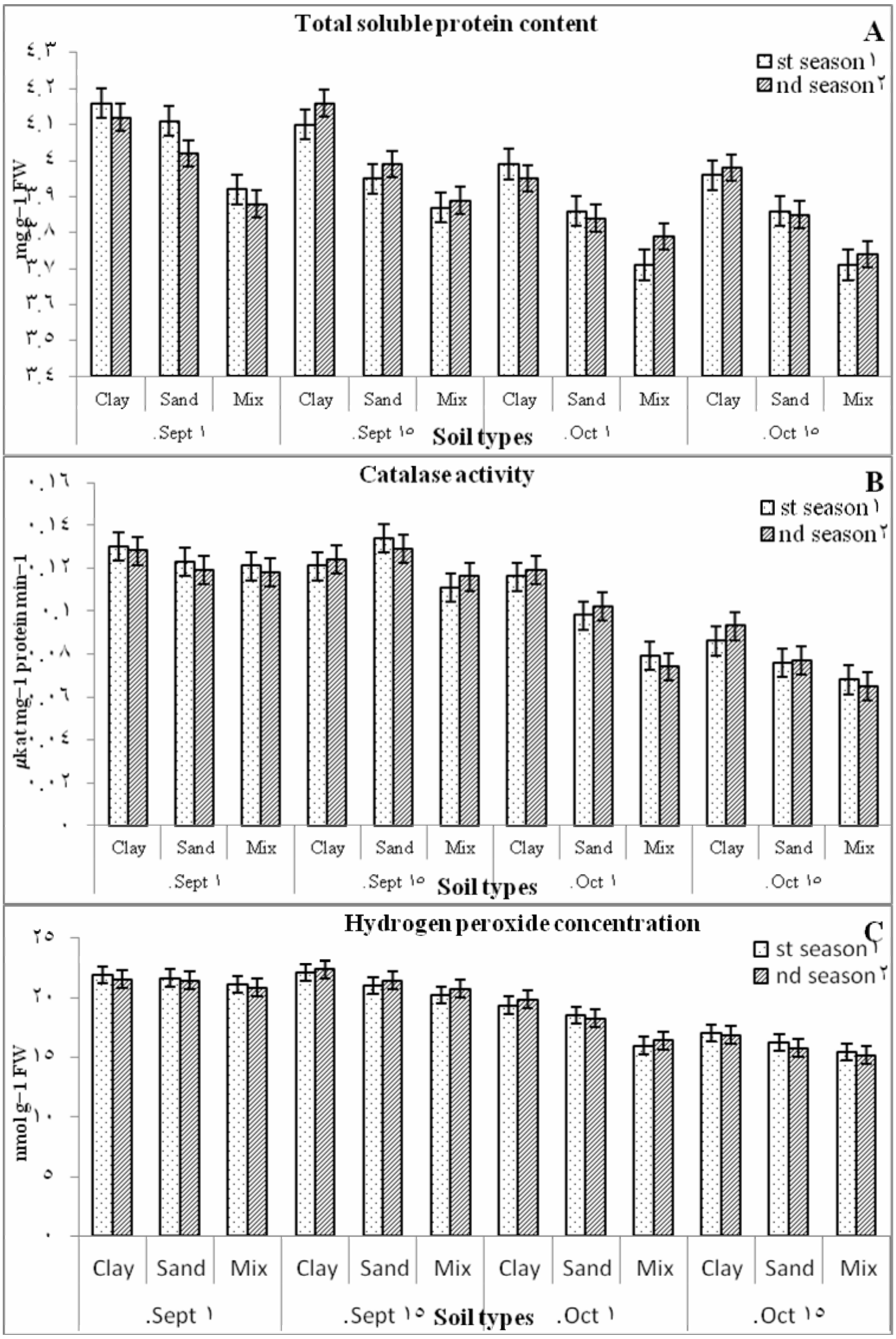

Figure 2. A. total soluble protein, B: catalase enzyme activity and C: Hydrogen peroxide concentration of Calendula as affected by temperature regime (1, 15 September and 1, 15 October) and different soil types of during 2013/2014 – 2014/2015 seasons

The difference in the mean values of growth parameters of the two seasons may be attributed to varying climatic factors (temperature, relative humidity, etc…) and soil conditions. Similar results were mentioned by Muller and Brandes (1997), Singh *et al.* (2002) and Baraldi *et al.* (2008) on Artemisia plants.

Temperature is one environmental factor that profoundly influences plant growth and development. Two common ornamental annual crops, salvia (*Salvia splendens*) and marigold (*Tagetes patula* L.), were grown in glass greenhouses under a mean DLI of 5 to 25 $mol \cdot m^{-2} \cdot d^{-1}$ at temperatures from 14 to 27 °C. The rate of development to flowering of salvia and marigold was primarily influenced by the mean air temperature. For example, time from seedling transplant to flowering of salvia decreased from 42 days to 24 days as temperature increased from 15 to 25 °C. Flower number and plant dry mass on the date of first flowering generally decreased with increasing temperature in both species (Lee and Runkle, 2007).

Data presented in Table 4 and 5 indicated that, plants growing in the sand soil, showed a positive increase in same growth parameters comparing to those growing in clay loamy and mix soils, these differences may be attributable to ecotype, climatic conditions, and stress conditions of the sand soils, cultural practices or a combination of these factors. These results confirmed the preceding works of Kumar *et al.* (2004).

High temperature regimes are known to decrease photosynthesis and productivity of Calendula by different mechanisms. In the present study, the stomata closed when plants were stressed for moisture at moderate or high temperature, which resulted in considerable decline in photosynthesis and transpiration rates (Fig.1). Under different soil type's conditions, high temperature stress decreased the leaf stomatal conductance of all treatments. Overall, the combined effects of different soil types and reduce temperature resulted in higher decline of the different gas exchange parameters. Decline in stomatal conductance was accompanied by corresponding reductions in transpiration and photosynthesis rates, indicating that although stomatal closure conserved water, it also reduced assimilate production. However, both transpiration rate and stomatal conductance declined more than the photosynthesis with mix soil type increased under humidity and high temperature conditions (Anyia and Herzog, 2004).

Concerning physiological and biochemical parameter, Catalase enzyme activity recorded higher value in plant species grown in September and it was lower in plants investigated in October. Catalase is the enzymes which catalyzes the reversible hydration of CO_2 and kept its constant supply to rubisco resulted in an optimum rate of photosynthesis and thus more dry material accumulation. Therefore, increased Catalase enzyme activity might have been one of the reasons for improved growth performance of plants at optimum temperature in September. It was also informed that while transpiration rates increased with lower atmospheric pressure at higher temperature, the agreeing increase in CO_2 uptake was relatively higher than expected (Gale, 1973), thus there is a possibility of greater non-stomatal capability for CO_2 uptake in plants at higher temperature (Li *et al.*, 1993).

Any change in climatic condition results in enhanced intensity of solar ultraviolet radiation, seasonal and daily variations in temperature (Larcher, 1988) results in accumulation of chemically active molecules and free radicals in plant cells causes alterations in metabolic processes (Asada, 1999). To cope with such detrimental conditions, plants are fortified with a system of antioxidant enzymes, which inhibit free radical processes (Keniya *et al.*, 1993). It is shown that under extreme conditions the protective mechanism of antioxidant system is activated. The higher is the antioxidant activity; the extra resistant is the species toward the stressor.

An extreme increase and decrease in temperature causes generation of several detrimental effects and set the plant sunder stress leading to the generation of reactive oxygen species. However, to cope with such stressful conditions, plants are equipped with antioxidant enzymes such as catalase activity enzyme and H_2O_2. These enzymes prevent or alleviate the damage caused by reactive oxygen species and set the plants to perform normally even under stressful conditions. The results exhibit that catalase activity enzyme and H_2O_2 show a parallel increase in the activities with increasing temperature giving highest values (Vaidyanathan *et al.*, 2003). Thus, increased antioxidant enzyme activities protected the plants from stressful environment which is reflected in terms of improved growth and dry mass and total soluble protein accumulation and catalase activity enzyme in the plants grown in September. On the other hand, plants grown in October showed a decline in antioxidant enzyme activities and physiological, enhanced growth and biochemical parameters including protein accumulation. The inhibition in the activities of antioxidant enzymes during optimum temperature was recorded by Wang *et al.* (2009). Asthir, 2015 mentioned that the group of proteins called heat shock proteins are synthesized following stress and their synthesis is regulated by transcription factors. Under high temperature, reactive oxygen species are often induced and can cause damage to lipids, proteins, and nucleic acids. To scavenge the reactive oxygen species and maintain cell membrane stability, synthesis of antioxidants, osmolytes, and heat shock proteins is of a vital importance.

Any changes in climatic conditions affect growth and development processes in plants as they are depend on many factors, including retention time, season, temperature, relative humidity, wind, soil properties, pH, diversity of species, nutrients availability, hydraulic regimes, plant harvesting and light intensity etc…(Khan and Ansari, 2005; Lu *et al.*, 2010; Abdelmajeed *et al.*, 2013).

CONCLUSION

This study indicated that environmental factors such as temperature and different soil types affect both growth, morphological, physiological and biochemical. Based on the results of this research it is recommended that during September with sand soil the environmental conditions are best suitable for growth and development use of *Calendula officinalis L.* So this interaction can be adopted for optimum growth under arid and semi-arid land conditions.

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الملخص العربي

استجابة نمو نبات الكلانديولا لنظم درجات الحرارة وأنواع تربة مختلفة

ياسر إسماعيل النشار

يعتبر الاجهاد الحراري من النظم البيئية الرئيسية التي تؤثر على الانتاج الزراعي في جميع انحاء العالم، لاسيما في المناطق الجافة والشبة جافة. تتسم المناطق الجافة والشبة جافة بالاختلاف الكبير في الظروف البيئية – فدرجة الحرارة تتراوح ما بين الانخفاض والارتفاع الشديد مما يؤثر على الشكل الظاهري والعمليات الفسيولوجية والكيميائية الحيوية للنباتات. ويقدم هذا البحث دراسة تأثير النظم الحرارية على مواعيد زراعة بذور نبات الاقحوان في الفترة ١، ٩/١٥ و١، ١٠/١٥ خلال عامي ٢٠١٣ و٢٠١٤ المزروعة في انواع مختلفة من التربة(الطينية اللومية– الرملية– الخليط بينهم). وقد لوحظ زيادة في صفات النمو الخضري للنباتات المزروعة التربة الرملية مقارنة بالتربة الطينية اللومية والخليط. وتم الحصول على افضل نمو للنباتات النامية خلال فترة الزراعة ١٥ سبتمبر مع التربة الرملية. وتشمل هذه النتائج عدد فروع اكثر ومساحة ورقة اكبر واثقل في الوزن الرطب والجاف وعدد اكبر من الازهار. تم قياس عدد من الصفات الفسيولوجية المتمثلة في معدل النتح والتوصيل الثغري وتركيز ثاني اكسيد الكربون في الخلايا والمحتوي المائي للخلايا– حيث كانت القيم كبيرة في النباتات المزروعة في اول اكتوبر مع التربة الطينية اللومية في كلاً من الموسمين. لقد اظهرتالنظم البيئية وانواع التربة المختلفة أثار واضحة على محتويات النباتات البيوكيميائية المتمثلة في البروتين الذائب الكلي ونشاط انزيم الكتاليز والبيروكسيد الهيدروجين الموجود في اوراق البادرات بعد ثلاث اسابيع منالزراعة. وبالتالي ينصح بزراعة نبات الاقحوان في المناطق الجافة والشبة جافة خلال شهر سبتمبر في تربة رملية.

الكلمات الافتتاحية: البيئة الجافة والشبة جافة- المحتـوي البيوكيميائية- نبات الاقحوان- التبادل الغـازي- النمـو- الاجهاد المائي.