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Research Article

Effect of irrigation intervals on growth of annual flowers under climatic conditions of South Punjab, Pakistan

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Abstract

Irrigation is one of the major factors to control the growth of annual flowers. Optimization of plant water requirement is important in the current scenario of climate change under limited water availability. Present research was conducted to determine the effect of irrigation intervals on vegetative, reproductive and physiological growth of two annuals, *Calendula officinalis* L. and *Dianthus barbatus* L. The seedlings of both annuals were grown in pots containing garden soil as growing media. At first, seedlings were grown for 15 days under regular irrigations. Then four irrigation intervals (T_1; daily, T_2; 2 days interval, T_3; 4 days interval, T_4; 6 days interval) were applied. At four days of irrigation intervals, calendula produced maximum plant height (42 cm), stem diameter (5.63 mm), shoot fresh weight (18.40 g), shoot dry weight (4.04 g), root dry weight (1.52 g), root length (21 cm), no. of flower buds (15.67), no. of opened flowers (12.67), flower diameter (35 mm), total antioxidants (79.45) and total phenolic contents (314.58 mg/100g GAE). Dianthus also produced maximum plant height (41 cm), stem diameter (5.85 mm), shoot fresh weight (27.88 g), shoot dry weight (4.86 g), root dry weight (2.76 g), root length (25 cm), no. of flower buds (11.33), no. of opened flowers (6.67), flower diameter (34.90 mm), total antioxidants (86.08) and total phenolic contents (331.87) at four days of irrigation intervals. But increasing irrigation interval from 4 days to 6 days significantly decreased the vegetative and reproductive growth of both the annuals. Hence, it is concluded that irrigation interval after seedling establishment improved the vegetative, reproductive and physiological growth of annual flowers.

Keywords: Calendula; Dianthus; Drought; Irrigation; Yield parameters

Introduction

Annuals are those plants which complete their whole life cycle in one growing season, after that all plant parts (root, stem and leaves) dies except seeds that survives as dormant for a longer period due to

unfavorable climatic conditions. As compared to the perennials flowers, annuals flower have a single transition stage from vegetative to reproductive growth. Morphological characteristics of annuals are mainly depends on the growing climatic conditions [1], which divide annuals as summer annuals (germinates and grows in summer season), winter annuals (germinates and grows in winter) [2]. Annuals are used to decorate the garden and any other place with their variety of colorful flowers. They can be grown in variety of containers and flowers bed. These plants are generally easy to grow and produce higher yields [3] and improves the nutrient status of the soil [4].

Dianthus (*Dianthus barbatus*) is well established across the world for its diversity of colors, beauty, wide flower range and excellent keeping quality [5]. It has more than three hundred species and belongs to family, Caryophyllaceae [6]. Calendula (*Calendula officinalis;* Asteraceae family) is a winter annual herb with flower and leaves that are pinnately divided and used in horticultural industry as a decorative plant [7]. However, it is also used as medicinal plant and its flowering is usually used in food items to give both flavor and color to foods [8-10].

Irrigation is an important activity of agricultural operations for optimum plant growth and maximum crop production [11]. Water is among most restricting factors for crop production and its importance has been increased due to occurrence of its shortage [12, 13]. In arid areas water and nutrients are limiting factors for growth of annuals and their photosynthetic activities increases at high temperature along with sufficient water availability [14]. Their root to shoot ratio also increases as the availability of water decreases [15].

In Pakistan, various water sources (rainfall, glaciers, rivers and dams, surface water, ground water) are used for irrigation. Less rainfall and water unavailability are the main factors that limits the production of crops in desert areas [16]. Water reservoirs also reached at very low level due to water unavailability and reservoir capacity has reduced. Like various other countries, Pakistan is also consuming large amount of ground water as compared the demanding rate of replenishing groundwater at a worse condition [17] that can change into a disaster. Demand of water supply is increasing along with growing human population and economic growth of countries in the [18]. With increasing demand the supply of water is decreasing that is adversely affecting biodiversity in the world, which may be due to rainfall, temperature, evaporation rate, soil quality, vegetation type and water runoff [19]. Decreased water supply and low water use efficiency to crops are the main hurdles in decreasing per acre yield.

Water can be saved by decreasing loss of water through various alternative irrigation methods e.g. enhancing irrigation interval that will save water without producing adverse effects on vegetative and reproductive growth of plants [20, 21]. Hence, the plants have evolved various defense mechanisms to overcome the oxidative damage due to the drought stress including over and increased production of antioxidants that stopped the circulation of oxidative chain cycles [22]. Phenolic compounds have various physiological properties other than reducing the damaging impacts of drought including antiatherogenic, anti-inflammatory, antiallergic, antithrombotic, antimicrobial ones [23]. They also have defensive purpose in plant protection from biotic damage by herbivores and pathogens [24].

Use of ornamental plants is increasing with increasing aesthetic gratification of the communities in south Punjab. These seasonal plants are mostly used at different occasions for beautification and growers have to

manage their flowers according to functions. In this case, early and late flowering is common need of growers, while irrigation water is one of the important effective limiting factor for plant growth and yield [25]. Therefore, present research work was planned to determine the effects of various irrigation intervals on morphological, reproductive and physiological growth of two annuals (calendula and dianthus).

Materials and methods

Experimental location

A pot experiment was carried out at the nursery of MNS-University of Agriculture, Multan, in which effect of irrigation intervals was observed on two flowering plants calendula (*Calendula officinalis* L.) and Dianthus *(Dianthus barbatus).* Climate of study area is sub-tropical with cold winters and hot summers, the average weekly temperature and humidity of study area is mentioned in (Figure 1).

Seedlings were obtained from the Shahdab Nursery Multan. Uniform seedlings were transplanted into pots on 13 February, 2018. Physical and chemical properties of the soil used in this study are presented in (Table 1).

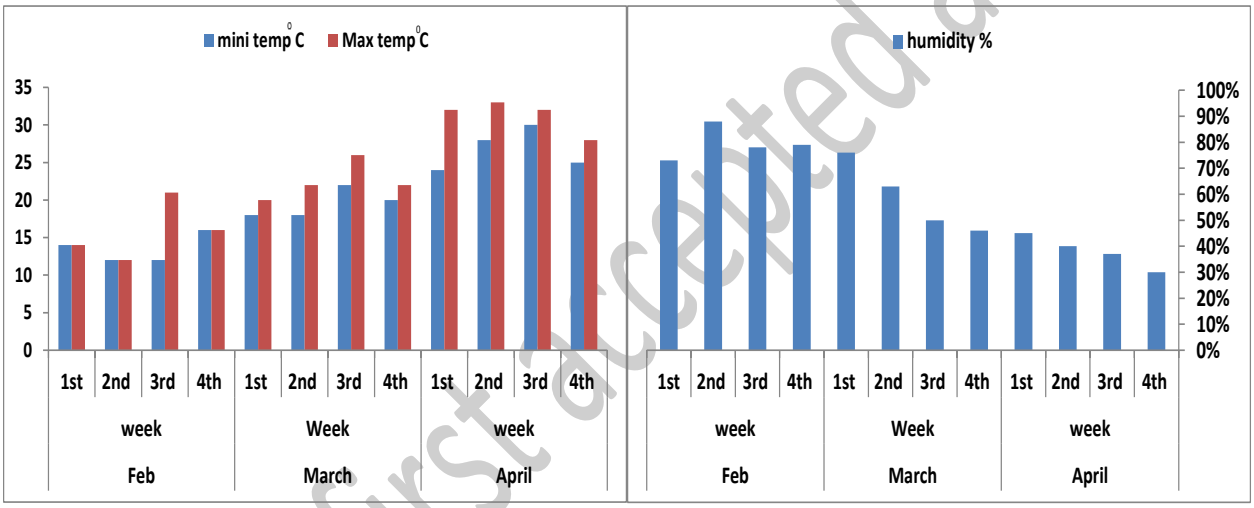

Figure 1. Average weekly data of temperature and humidity

Table 1. Physical and chemical properties of the soil and some other parameters

Soil texture	Soil EC (dS/m)	Soil PH	Soil weight/pot (kg)	Size of pots (inches)
loamy soil	2.61	6.46	3.640	9

Experimental design and treatments

The seedlings were grown in pots of 9 inches size filled with garden soil as growing media and irrigated with tap water. Experiment was designed and arranged in randomized complete block design (RCBD) with four treatments and three replications that containing three plants. Seedlings were allowed to establish for 15 days with common cultural practice. Four irrigation intervals were (T_0, daily Irrigate, T_1, Irrigation after 2 days, T_2, Irrigation after 4 days, T_3, Irrigation after 6 days) were maintained in both the crops throughout the experiment.

Experimental data

Data was collected regarding different morphological and physiological parameters after application of treatments. Morphological parameters including plant height (cm), diameter of stem (mm), numbers of buds, numbers of flowers/ plant, flower

diameter (mm), number of days for flower induction, fresh and dry shoot weight (g) and root weight (g) and root length (cm) were recorded. For the analysis of phenolics and antioxidants, leaf samples were stored at -80°C. 1 g of frozen leaf samples were grinded in solution mixture of Methanol: Acetone: HCL at 45:40:1 ratio. Then extracted samples were centrifuged at 10000 rpm for 4 minutes and supernatant was collected in separate eppendorf tubes. Total phenolics contents were measured according to protocol of [26]. 100 µl of supernatant of each sample was added in separate reaction tubes then 200ul of 10% F-C reagent was added in the reaction tubes. After that 800 µl of 700mM Na_2CO_3 was added in the reaction tubes. Reaction mixture was incubated at room temperature for 2 hours. The absorbance was recorded at 765 nm by using ELISA plate reader. For antioxidants 50µl supernatant and 5 ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) added and incubate them for 30 minutes and checked the absorbance at 517 nm by using ELISA plate reader. Total antioxidants were measured according to protocol of [27].

Statistical analysis

The data were statistically analyzed using Statistical software (SAS Institute Inc., Cary. NC, USA) and the comparisons of mean among treatments were determined by Least Significant Difference (LSD) test.

Results

Morphological parameters

Maximum plant height of 42cm and 41cm were produced in daily irrigated plants of calendula and dianthus, respectively. While minimum plant height of 18.41 cm and 13.37 cm were produced in plant of calendula and dianthus, respectively, irrigated at six days interval (Table 2). Daily irrigation produced maximum stem diameter of calendula (5.63 mm) and dianthus (5.85 mm) while six days irrigation interval produced minimum stem diameter of calendula (1.63 mm) and dianthus (1.92 mm).

According to the (Table 2), calendula produced maximum shoot fresh weight (18.40 g) and shoot dry weight (4.04 g) at daily irrigation followed by two days irrigation interval that produced 18.09 g and 3.89 g of shoot fresh weight and shoot dry weight, respectively. Minimum shoot fresh (5.30 g) and dry weight (0.73 g) produced in plant irrigated at six days interval. Dianthus also produced maximum shoot fresh (27.88 g) and dry weight (4.86 g) at daily irrigation while minimum shoot fresh (4.88 g) and dry weight (1.37 g) were produced at six days irrigation intervals.

Root dry weight was maximum in calendula (1.52 g) and dianthus (2.76 g) at four days of irrigation interval but minimum 0.73 g and 0.78g, respectively, at six days of irrigation interval (Table 2). In both, calendula and dianthus, maximum root length 21.00 cm and 25.00 cm was observed at four days irrigation interval followed by 18.50 cm and 23.83 cm length in calendula and dianthus, respectively, at two days of interval.

Irrigation intervals also affected the flowering parameters in both cultivars. According to the (Table 3), calendula and dianthus produced flower in minimum number of days 22.00 and 22.67 respectively, at four days of irrigation interval while daily irrigated plants of calendula and dianthus took maximum days 28.67 and 28.67 to induce flowers. Maximum number of opened flowers of calendula (12.67) and dianthus (6.67) were observed at four days of irrigation interval and minimum number of opened flowers, 3.67 and 3.33, were recorded in calendula and dianthus, respectively, at six days of irrigation interval.

Maximum number of flower buds (15.67 and 11.33) of calendula and dianthus were produced at four days of irrigation interval followed by 12.67 (calendula) and 9.67 (dianthus) at two days of irrigation interval, while six days interval produced minimum 5.00 and 4.67 number of flower buds in

calendula and dianthus, respectively. Calendula (35.00 mm) and dianthus (34.90 mm) produced maximum flower diameter at four days of irrigation interval while minimum 22.00 mm (calendula) and 20.33 mm (dianthus) flower diameter was recorded at irrigation interval of six days (Table 3).

Biochemical Parameters

At four days irrigation interval highest leaf antioxidants content 79.42 and 86.08 (% Inhibition of DPPH) were obtained calendula and dianthus respectively, while at daily irrigation, lowest leaf antioxidants 64.63 and 72.68 (% Inhibition of DPPH) were obtained in calendula and dianthus respectively (Table 4).

In calendula and dianthus, maximum leaf phenolic content 314.58 and 331.87 (mg/100g GAE) were recorded in plants irrigated at four days of interval (Table 4). Minimum value of leaf total phenolic 203.42 and 286.52 (mg/100g GAE) were recorded in calendula and dianthus respectively, in plants irrigated on daily basis (Table 4).

Table 2. Morphological parameters of *calendula* and dianthus subjected to irrigation intervals

Annuals	Irrigation Intervals (Days)	Plant Height (cm)	Stem diameter (mm)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Weight (g)	Root Length (cm)
Calendula	0	42.00a ± 2.88	5.63a ± 0.73	18.40ab ± 1.81	4.04a ± 0.97	1.26bc ± 0.65	14.40bc ± 2
	2	37.83a ± 4.66	5.41a ± 1.04	18.09ab ± 6.34	3.89ab ± 0.81	1.47bc ± 0.31	18.50ac ± 0.76
	4	19.10b ± 1.75	2.03b ± 0.21	9.88b ± 8.19	3.32ac ±1.19	1.52bc ± 0.07	21.00ac ± 3.28
	6	18.41b ± 6.65	1.63b ± 0.22	5.30b ± 8.39	1.58bc ±0.44	0.73c ± 0.29	13.27c ± 2.64
Dianthus	0	41.00a ± 1.37	5.85a ± 0.74	27.88a ± 0.76	4.86a ± 0.65	0.89bc ± 0.23	23.33ab ± 1.51
	2	35.90a ± 1.16	5.70a ± 0.14	19.24ab ± 1.81	4.23a ± 0.09	1.99ab ± 0.53	23.83a ± 3.66
	4	16.60b ± 3.43	3.22b ± 0.30	7.41b ± 1.26	2.58ac ± 0.64	2.76a ± 0.10	25.00a ± 6.77
	6	13.37b ± 1.13	1.92b ± 0.07	4.88b ± 1.53	1.37c ± 0.51	0.78c ± 0.43	14.03bc ± 2.88

Means sharing the same letter do not differ significantly at p≤0.05 value ± show the standard error

Table 3. Flowering parameters of *c*alendula and dianthus subjected to irrigation intervals

Annuals	Irrigation Intervals(Days)	No. of day to flower	No. of flowers buds	No. of opened flowers	Flower diameter (mm)
Calendula	0	28.67a ± 1.20	11.33ab ± 1.20	6.00bc ± 0.11	32.67a ± 0.49
	2	26.00ab ± 2.02	12.67ab ± 2.66	6.67b ± 0.66	33.90a ± 0.84
	4	22.00c ±1.76	15.67a ± 2.40	12.67a ± 0.57	35.00a ± 1
	6	25.33bc ± 0.88	5.00cd ± 2.18	3.67c ± 0.88	22.00b ± 1.15
Dianthus	0	28.67a ± 1.15	5.67cd ± 1.20	3.67c ± 1.20	20.00b ± 2
	2	28.33a ± 0.57	9.67bc ± 0.88	5.00bc ± 1.85	32.00a ± 3.33
	4	22.67bc ± 0.57	11.33ab ± 0.33	6.67b ± 0.33	34.90a ± 2
	6	27.00a ± 0.88	4.67d ± 1	3.33c ± 0.33	20.33b ± 2.33

Means sharing the same letter do not differ significantly at p≤0.05 value ± show the standard error

Table 4. Total antioxidants and total phenolics of c*alendula* and dianthus subjected to irrigation intervals

Annuals	Irrigation Intervals (Days)	Total antioxidants (% Inhibition of DPPH)	Total phenolic (mg/100g GAE)
Calendula	0	64.63d ± 0.96	203.42b ± 32.90
	2	68.92cd ± 3.05	312.49a ± 24.23
	4	79.45ab ± 0.24	314.58a ± 7.59
	6	71.42bcd ± 0.83	286.36a ± 16.64
Dianthus	0	72.68bcd ± 3.07	286.52a ± 6.60
	2	80.21ab ± 7.95	287.53a ± 6.20
	4	86.08a ± 2.72	331.87a ± 17.50
	6	77.90abc ± 1.30	317.45a ± 25.53

DPPH= Diphenyl 1-picrylHydrazyl, GAE=Gallic Acid Equivalent
Means sharing the same letter do not differ significantly at p≤0.05 value
± show the standard error

Discussion

Irrigation intervals have significant effect on the vegetative and physiological parameters of annual flowers because irrigation stress causes morphological, physiological, biochemical and molecular changes in the plants [28]. Maximum Plant height 42cm and 41cm, shoot fresh (18.40 g and 27.88 g) and dry weight (4.04 g and 4.86 g) were produced in plant calendula and dianthus plants respectively at daily irrigated plants followed by plant irrigated two days of interval. [29] also reported increase in fresh weight of canation plants with daily irrigation. Higher irrigation intervals (6 days) adversely effected the shoot length, shoot fresh and dry weight as water stress for long intervals reduces cell size due to decrease in turgor pressure in the cells that lowers the CO_2 concentration and ultimately causes stomatal closure and decrease in photosynthesis [30, 31]. Our Results are in line with [32] who reported that adequate irrigation of water enhances plant growth and yield as compared to the lower water supply to plants. Our results are also according to the findings of [33] who stated that stem of carnation increases with sufficient water supply and reduces in limited water supply due to decrease in turgor pressure of cells. If water amount remains constant and irrigation intervals increase at higher degree then total yield will also decrease in carnation, [34] rose [35] and gladiolus [36]. Long irrigation intervals have negative effect on morphology, yield and essential oil contents of oregano plant [37]. [38] also observed that higher irrigation intervals adversely affect the biomass of Khorasan thyme plants.

In present study, at four days of irrigation interval calendula and dianthus produced produce flowers with minimum number of days 22.00 and 22.67 respectively while higher irrigation intervals (6 days) produced flowers with maximum number of days. Shorter irrigation intervals positively enhance the plant growth due to enhancing uptake of nutrients and metabolic activities that ultimately increases the chemical constituents in the cells [39]. Our results are in accordance to [40] who resulted that 4 days irrigation interval in coriander improves the plant yield while higher irrigation interval of 8 days adversely affect the growth and yield of plants. [41] also recorded similar results that shorter irrigation intervals has positive effect on crop growth and yield as compared to longer irrigation intervals.

Calendula and dianthus produced maximum number of flowers buds (15.67 and 11.33), maximum opened flower (12.67 and 6.67) and maximum flower diameter (35.00 mm and 34.90 mm) respectively at four days of irrigation interval. [42] also observed similar

results in case of gerbera. **[29]** also produced maximum yield and quality of carnation plants with irrigation interval. This increase of flower yield of both the annuals may be due to increased water molecules in the soil (saturation), which reduced the air spaces and oxygen supply to the roots in root zone that ultimately adversely affected the yield.

Water is limiting factor for plants of arid and semiarid regions and applied short irrigation interval can activate the defense mechanism, in which plants enhances its metabolic activates **[43, 44]**. According to our observations irrigation interval is very destructive during seed germination and supportive for flowering after establishment of seedling. **[45]** resulted that timing of applying irrigation is important as at early reproductive stage irrigation interval enhances the flowering. Balasio *et al* **[46]** recorded higher grain yield due to irrigation interval at vegetative and reproductive stages of crop growth. **[47]** observed a significant role of water stress on yield and quality of coriander.

Conclusion

Irrigation intervals effect the vegetative, reproductive and physiological growth of winter annual flowers (*Calendula officinalis* L. and *Dianthus barbatus* L.). According to our observations four days irrigation intervals after establishment of seedling of calendula and dianthus flowers enhances the flowering and reduces days to produce flowers that is a common need of ornamental flowers growers. But irrigation intervals more than four days adversely affect the growth of these annual flowers.

Authors' contributions

Conceived and designed the experiments: G Akhtar & Y Sajjad, Performed the experiments: G Akhtar, M Younas & N Farid, Analyzed the data: G Akhtar, S Ullah & K Razzaq, Contributed materials/ analysis/ tools: G Akhtar & IA Rajwana, Wrote the paper: G Akhtar & M Amin.

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