Exogenous application of medicinal plant extracts improved productivity and qualitative traits of cotton (*Gossypium hirsutum* L.) under drought stress

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Abstract

Drought is a major factor that limits agricultural productivity worldwide. Several approaches have been used to help plants improve their performance under drought stress. However, the external application of medicinal plant extracts as biostimulants is a promising strategy that is still in its early stages. To mitigate the adverse effects of drought on cotton (*Gossypium hirsutum* L.), a field experiment was conducted using a split-plot design based on a randomized complete block design with three replications. The first factor was the application of 0%, 10%, and 15% *Camellia sinensis* (Cs) and *Rosmarinus officinalis* (Ro) methanolic extracts. The second factor was the frequency of the irrigation cycle, which was conducted every 10, 15, and 20 days (W10, W15, and W20). The best outcomes were observed with the 10% Ro extract under the W10 irrigation cycle, which resulted in the highest plant height (99.99 cm), fresh (3.32 g) and dry weight (0.46 g) of leaves, fiber strength (7.86%), elasticity (26.06 g·tex^{-1}), and color brightness (80.76 Rd). The best results for the total number of bolls (26.77), wool (5.24 g), boll weight (14.10 g), fiber length (32.10 mm), length uniformity (87.33%), and the minimum electrolyte leakage (30.33%), leaf particles in fibers (0.52), and short fiber index (10.14%) were found with 15% Ro extract under the W10 irrigation cycle. Both methanolic extracts, particularly 15% Ro, have shown promising results in most measured traits and could be a superior strategy for sustainable cotton production under drought stress.

Keywords: Antioxidants; Biostimulant; Cotton fiber quality; Drought stress; Fiber length

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Introduction

Cotton (*Gossypium hirsutum* L.) is a perennial plant belonging to the Malvaceae family. It is the most commercially important crop, providing around 35% of the total fiber used worldwide. The seeds of the plant are used as a supplement and a source of protein for both human and animal nutrition. Cotton is typically grown in warm climates, such as tropical and subtropical regions, and can be cultivated in more than 80 countries, most of which are developing nations (Abdelraheem et al., 2019; Chaudhry, 2010). As the most widespread fiber crop, cotton forms a significant link between the agricultural and industrial sectors (Ahmadi and Aghaalikhani, 2012).

According to the United Nations in 2017, the world population is increasing rapidly and is expected to reach 9.8 billion by 2050. However, this

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population growth will cause climate changes that will lead to more severe abiotic and biotic stress conditions, creating major challenges in crop production ((Nakashima et al., 2014; Shaar-Moshe et al., 2017). Abiotic stresses are a significant threat to agricultural production, resulting in a 73% reduction in cotton production worldwide (Mahmood et al., 2019). Drought and heat are the most critical abiotic stresses affecting the phenology, growth, fiber yield, and quality of cotton. This problem will become more severe in the future due to the frequent occurrence of high temperatures and water shortages (EL Sabagh et al., 2020). Although cotton is commonly cultivated in arid and semi-arid regions, it is not considered a drought-resistant crop (Penna et al., 1998). The cotton industry faces the challenge of drought and heat stress, which can reduce fiber yield by up to 34% (Ullah et al., 2017).

Drought and other abiotic stresses cause excessive production of reactive oxygen species (ROS) that damage plant cells and inhibit physiological processes. In cotton and other plants, ROS such as hydroxyl radical (OH) and singlet oxygen ($^1O_2$) can oxidize various biochemical compounds, including lipids, proteins, DNA, and RNA (Ratnayaka et al., 2003; Tripathy and Oelmüller, 2012). However, plants have developed antioxidant mechanisms to cope with ROS production and accumulation. These mechanisms include enzymatic antioxidants like catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase (GPX), and dehydroascorbate reductase (DHA), as well as non-enzymatic antioxidants like reduced glutathione, ascorbic acid, α-tocopherol, phenols, flavonoids, and carotenoids.

Plants use antioxidant mechanisms to counteract the production of ROS. However, according to Zhang et al. (Zhang et al., 2014), the balance between ROS production and antioxidant activity in cotton under drought stress determines whether damage will occur or the plant will be able to cope with the stress. Due to the toxic effects of synthetic antioxidants, there has been a growing trend toward using natural antioxidants, which are polyphenol compounds found in various parts of plants, such as their bark, leaves, stems, fruits, roots, and seeds (Stoilova et al., 2007).

Various studies have shown that green tea (*Camellia sinensis*) extract is a viable alternative to synthetic antioxidants because of its high phenolic content, including catechins, flavanols, flavonols, phenolic acids, and caffeine (Nag and Das, 2013). Additionally, rosemary (Rosmarinus officinalis) extract is widely used in various industries as a food preservative due to its potent antioxidant properties (Al-Sereiti et al., 1999).

We hypothesized that the extracts of these two medicinal plants can serve as promising candidates to enhance the growth, physiology, and fiber quality of cotton while reducing the adverse effects of drought stress due to their proven high antioxidant properties.

Materials and Methods

Experimental Design

The research was conducted at the research field of the Faculty of Agriculture, Ilam University, located at 46°22' E longitude and 33°39' N latitude. The field is situated at an altitude of 1446 meters above sea level. The experiment was designed as split plots using a randomized complete block design with three replications, where each replication contained 10 plants. The experiment had two factors. The first factor involved the treatment with *Camellia sinensis* methanolic extracts (0%, 10%, and 15%), denoted as control, Cs 10%, and Cs 15%, respectively, and *Rosmarinus officinalis* (0%, 10%, and 15%), denoted as control, Ro 10%, and Ro 15%, respectively. The second factor involved different levels of the irrigation cycle (every 10, 15, and 20 days), denoted as W10, W15, and W20, respectively.

Preparation of Methanolic Extracts

To prepare the methanolic extracts, 160 g of each dried medicinal plant was completely powdered and soaked in 1 liter of 50% methanol. After 48 hours of shaking, the solutions were filtered using Whatman No. 1 paper. To separate the methanol, the extracts were left to rest in the open air for 24 hours. These extracts were then used as stock solutions. Different treatments and irrigation cycles were applied to the cotton plants during the vegetative growth phase, starting from the stage of four true leaves. The plants were sprayed four times to ensure optimal results.

Soil Preparation and Planting the Seeds

The process of planting cotton seeds involved several steps. First, the research field was plowed, and a drip irrigation system was installed. Next, the field was disinfected with Mancozeb fungicide. Then, delinted cotton seeds of the Khorshid variety were sown in each hole at a depth of 3 cm. The plants were spaced 80 cm apart in rows that were 1 meter apart. Once the seeds had germinated, the seedlings were thinned out, leaving only one plant every 80 cm.

Morphological Traits

Plant Height, Fresh and Dry Weight of Leaves, Leaf Area, Total Number of Bolls, Number of Un bloomed Bolls, Wool Weight, and Boll Weight After the plants had finished flowering and the cotton bolls had fully opened, various morphological traits were measured, and samples were taken to perform quality tests on cotton fibers. For measurement purposes, 5 plants were randomly selected from each experimental unit, and their characteristics were studied. The height of the plants at the time of maximum growth and when the bolls started to open was measured in centimeters. To determine the weight of the leaves, an equal number of leaves were taken from the middle part of the labeled plants. After measuring the fresh weight of the leaves, the samples were kept in an oven at 70 °C for 48 hours, and their dry weight was measured using a scale with an accuracy of 0.01 g. The surface area of the leaves was measured with checkered paper. Wool and boll weight were also measured using a scale with an accuracy of 0.01 g during the peak of cotton production.

Physiological Traits

To measure the relative water content (RWC), healthy, fully developed leaves were cut and weighed using a digital scale with an accuracy of 0.01 g. The cut leaf discs were then placed in Petri dishes with lids containing dH2O and stored in the dark at 4°C for 24 hours. The leaf samples were then removed from dH_2O, dried between two layers of paper towels, and weighed again. The leaf discs were then transferred to an oven at 70 °C for 24 hours, and the dry weight was determined. Finally, the relative leaf water content was calculated as a percentage using the following formula (Galmés et al., 2007):

$$\mathbf{RWC = [(FW - DW) / (TW - DW)] \times 100}$$

where FW represents the leaf fresh weight, DW is the leaf dry weight, and TW is the leaf turgor weight.

To determine the electrolyte leakage (EL), an equal number and size of leaf discs were taken from each treatment and immediately placed into dH2O in equal volume. After 24 hours, the initial electrical conductivity (EC1) was measured with an EC meter (Conductometer, GLP 31). The samples were then placed in a Bain-marie (70 °C), and the final EC2 was measured. The EL of the samples was calculated using the following formula (Valentovic et al., 2006):

$$\mathbf{EL = (EC1 / EC2) \times 100}$$

In this equation, EL represents membrane electrolyte leakage, EC1 is the electrical conductivity of the samples after 24 hours, and EC2 is the electrical conductivity of the samples after the Bain-marie.

Fiber Qualitative Traits

Fiber Length, Average Length, Uniformity, Elasticity, Strength, Fineness, Moisture, Color Lightness, Yellowness, Number of Trashes in the Sample, Surface Covered with Debris, Leaf Particles in Fibers, Maturity of Fibers, and Index of Short Fibers

To determine the qualitative characteristics mentioned above, cotton fiber samples were collected from each experimental unit and sent to

Table 1
Effect of methanolic extracts (*Camellia sinensis* and *Rosmarinus officinalis*) and different irrigation cycle levels on cotton's morphological traits (*Gossypium hirsotum*).

Treatments	Irrigation cycle (day)	Plant height (cm)	Leaf FW (g)	Leaf DW (g)	Leaf area (cm^2)
Control	W10	73.11^{d}	2.66^{abc}	0.39^{abc}	79.50^{cd}
Control	W15	73.22^{d}	2.47^{abc}	0.34^{abc}	69.00^{cd}
Control	W20	61.22^{e}	1.71^{c}	0.23^{c}	51.03^{d}
Cs 10%	W10	89.55^{abc}	3.26^{a}	0.43^{ab}	92.83^{bc}
Cs 10%	W15	78.22^{cd}	2.96^{ab}	0.4^{ab}	86.40^{bc}
Cs 10%	W20	72.33^{de}	2.34^{abc}	0.32^{abc}	76.43^{cd}
Cs 15%	W10	92.33^{ab}	3.21^{a}	0.44^{ab}	102.00^{abc}
Cs 15%	W15	83.66^{bcd}	2.65^{abc}	0.37^{abc}	90.13^{bc}
Cs 15%	W20	71.66^{de}	2.35^{abc}	0.33^{abc}	77.83^{cd}
Ro 10%	W10	99.99^{a}	3.32^{a}	0.46^{a}	113.00^{ab}
Ro 10%	W15	82^{bcd}	2.57^{abc}	0.34^{abc}	89.33^{bc}
Ro 10%	W20	75.88^{d}	2.12^{abc}	0.31^{abc}	81.55^{bcd}
Ro 15%	W10	96.66^{a}	2.43^{abc}	0.35^{abc}	125.07^{a}
Ro 15%	W15	81.66^{bcd}	2.48^{abc}	0.34^{abc}	100.01^{abc}
Ro 15%	W20	72.38^{de}	1.89^{bc}	0.28^{bc}	83.87^{bc}

*Common letters in each column indicate non-significance at the 5% probability level based on Tukey's test. In this table, Cs is *Camellia sinensis*, Ro is *Rosmarinus officinalis* and W is the irrigation cycle (day).

the Department of Cotton and Fibrous Plants Research, Agricultural Research and Training Center, Tehran, Iran. An HVI device (Premier Evolvics PVT. LTD., India) was used to evaluate these samples.

Data Analysis

The data were analyzed using SAS statistical software (version 9.1, SAS Institute Inc., Cary, NC, USA). To compare the means, Tukey's test with a significance level of 0.05 was used. Additionally, Microsoft Office Professional Plus Excel (2013) was utilized to create the diagrams.

Results

Morphological Traits

Plant Height, Fresh and Dry Weight of Leaves, Leaf Area, Total Number of Bolls, Number of Unbloomed Bolls, Wool Weight, and Boll Weight

The results of the experiment indicated that plant height, as well as fresh and dry weight of leaves, were highest (99.99 cm, 3.32 g, and 0.46 g, respectively) in the 10% Ro treatment with a W10 irrigation cycle. Conversely, the control at the W20 irrigation cycle (without extract application) showed the lowest values for these traits. In

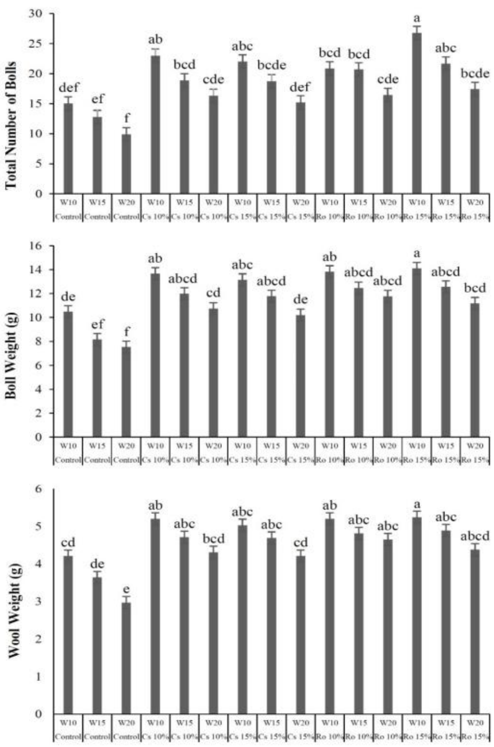

Fig. I. Interaction effect of methanolic extracts and different levels of irrigation cycle on the total number of bolls, boll weight, and wool weight of cotton (*Gossypium hirsotum*). In this figure, Cs is *Camellia sinensis*, Ro is *Rosmarinus officinalis*, and W is the irrigation cycle (day).

contrast, the highest leaf area (125.07 cm²), total number of bolls (26.77), wool weight (5.24 g), and boll weight (14.10 g) were observed in the 15% Ro treatment with a W10 irrigation cycle. The lowest values for leaf area (51.03 cm²), boll number (9.91), wool weight (2.97 g), and boll weight (7.53 g) were recorded in the control at the W20 irrigation cycle (Table 1, Figs. I and II).

Relative Water Content (RWC) and Electrolyte Leakage (EL)

The highest RWC (87.53%) was observed in the 15% *Camellia sinensis* (Cs) extract treatment with an irrigation cycle of W10. The lowest RWC (65.57%) was found in the control at the W20 irrigation cycle (Fig. III). In terms of EL, the maximum (49.01%) was recorded in the control at the W20 irrigation cycle, while the minimum (30.33%) was found in the 15% *Rosmarinus officinalis* (Ro) extract treatment with the W10 irrigation cycle (Fig. III).

Fiber Qualitative Traits

The 15% *Rosmarinus officinalis* (Ro) extract treatment with the W10 irrigation cycle resulted in the highest fiber length (32.10 mm), average length (30.73 mm), length uniformity (87.33%), and fiber moisture (7.90%). Conversely, the control with the W20 irrigation cycle (without extract application) showed the lowest values for these traits: fiber length (21.26 mm), average length (16.83 mm), uniformity (69.66%), and moisture (6.73%). The highest fiber strength (7.86%) was observed in the 10% Ro extract with the W10 irrigation cycle, while the control with the W20 irrigation cycle had the lowest fiber strength (6.06%). Additionally, the 15% *Camellia sinensis* (Cs) extract treatment with the W10 irrigation cycle exhibited the highest elasticity (26.06 g.tex^{-1}) and fineness (5.40 µg.inch). The lowest values for

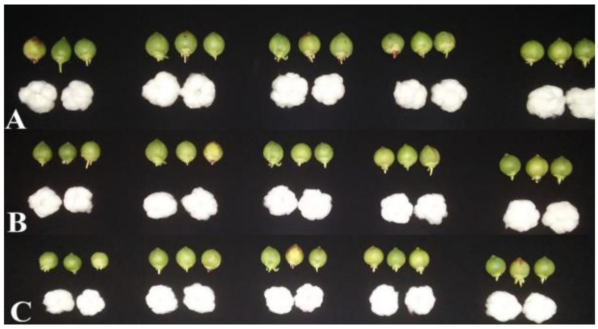

Fig. II. Comparison of the different treatment effects at the irrigation cycle once every 10 days (W10) (A), once every 15 days (W15) (B), and once every 20 days (W20) (C) on boll (above) and wool (below) of cotton fibers (*Gossypium hirsotum*). In the figure from left to right, control treatment, 10% and 15% *Rosmarinus officinalis* extracts, and 10%, and 15% *Camellia sinensis* extracts are shown respectively.

Table 2
Effect of methanolic extracts (*Camellia sinensis* and *Rosmarinus officinalis*) and different levels of irrigation cycle on quality attributes of cotton fibers (*Gossypium hirsotum*).

Treatments	Irrigation cycles (Day)	Length (mm)	Average length (mm)	Length uniformity (%)	Elasticity (g.tex^{-1})	Strength (%)	Fineness (µg.inch)	Moisture (%)
Control	W10	26.48^{cdefg}	24.16^{def}	76.50^{c}	24.60^{ab}	7.1^{b}	4.82^{abcde}	7.67^{ab}
Control	W15	24.04^{fgh}	21.70^{efg}	74.36^{cd}	21.36^{cd}	6.73^{b}	4.60^{cdef}	7.31^{b}
Control	W20	21.26^{h}	16.83^{h}	69.66^{d}	19.66^{d}	6.06^{c}	4.00^{f}	6.73^{c}
Cs 10%	W10	29.83^{abc}	28.83^{ab}	85.53^{ab}	26.06^{a}	7.80^{a}	5.23^{abcd}	7.88^{a}
Cs 10%	W15	27.62^{bcde}	24.33^{cde}	80.16^{bc}	24.43^{ab}	7.10^{b}	4.70^{abcdef}	7.74^{ab}
Cs 10%	W20	24.64^{efg}	20.25^{fgh}	75.46^{cd}	22.96^{bc}	6.70^{b}	4.51^{ef}	7.49^{ab}
Cs 15%	W10	28.93^{abcd}	26.66^{bcd}	85.00^{ab}	25.53^{a}	7.60^{a}	5.40^{a}	7.86^{a}
Cs 15%	W15	27.40^{bcdef}	24.33^{cde}	81.13^{abc}	22.83^{bc}	7.13^{b}	4.67^{bcdef}	7.76^{ab}
Cs 15%	W20	23.79^{hg}	19.83^{gh}	76.83^{c}	21.76^{c}	6.83^{b}	4.44^{ef}	7.72^{ab}
Ro 10%	W10	30.22^{ab}	28.33^{abc}	86.33^{ab}	25.93^{a}	7.86^{a}	5.30^{abc}	7.80^{ab}
Ro 10%	W15	26.57^{cdefg}	24.40^{cde}	79.96^{bc}	23.03^{bc}	7.06^{b}	4.53^{def}	7.53^{ab}
Ro 10%	W20	24.60^{efg}	19.54^{gh}	75.76^{cd}	22.80^{bc}	6.83^{b}	4.36^{ef}	7.48^{ab}
Ro 15%	W10	32.10^{a}	30.73^{a}	87.33^{a}	25.33^{a}	7.76^{a}	5.33^{ab}	7.90^{a}
Ro 15%	W15	28.83^{abcd}	27.00^{abcd}	81.00^{abc}	22.86^{bc}	7.06^{b}	4.76^{abcde}	7.77^{ab}
Ro 15%	W20	25.56^{defg}	21.06^{efg}	75.93^{cd}	22.00^{c}	6.86^{b}	4.44^{ef}	7.64^{ab}

*Common letters in each column indicate non-significance at the 5% probability level based on Tukey's test. In this table, Cs is *Camellia sinensis*, Ro is *Rosmarinus officinalis* and W is the irrigation cycle (day).

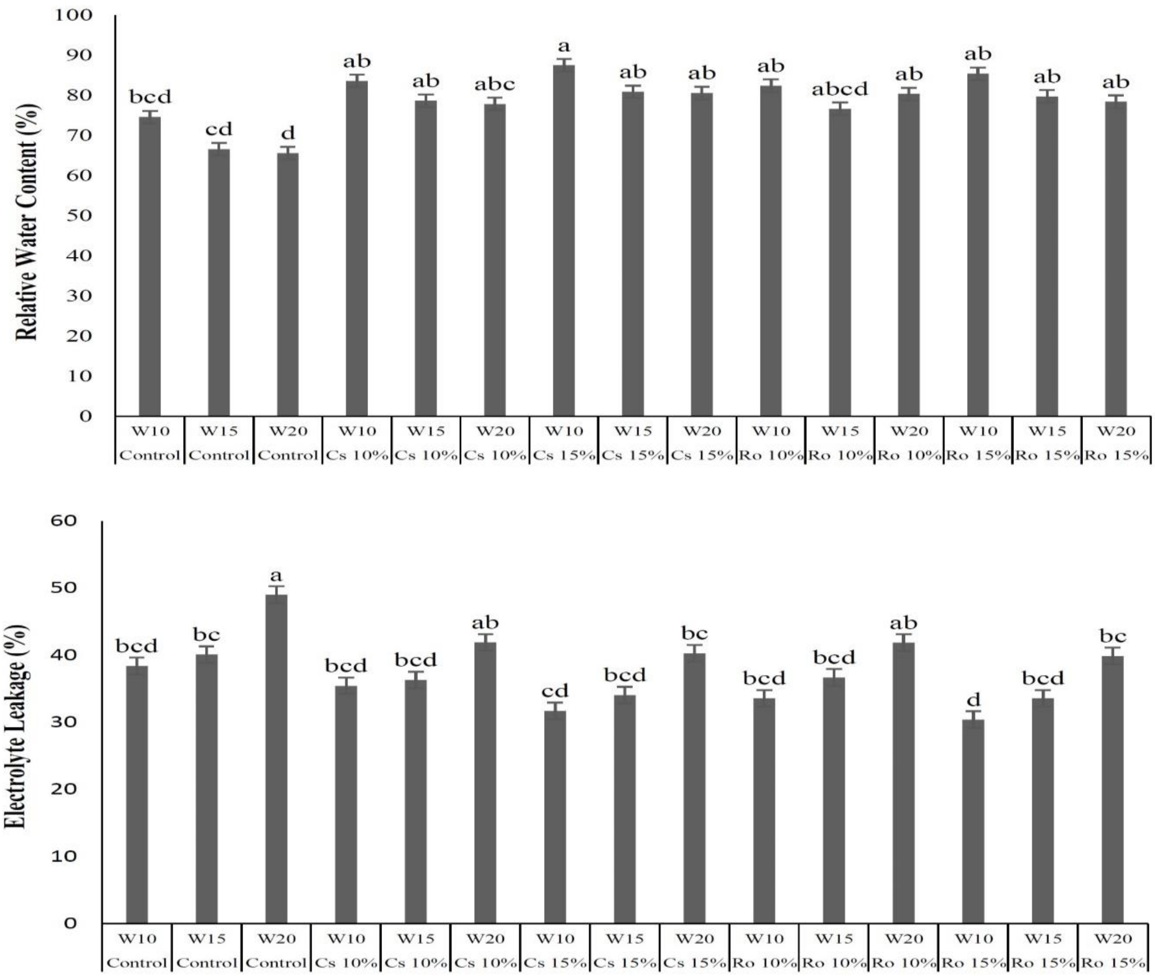

Fig. III. Effect of methanolic extracts and different levels of irrigation cycle on RWC and EL of cotton. In this figure, Cs is *Camellia sinensis*, Ro is *Rosmarinus officinalis*, and W is the irrigation cycle (day).

these traits were recorded in the control with the W20 irrigation cycle (Table 2).

Color Lightness, Yellowness, Number of Trashes, Surface Covered with Debris, Leaf Particles in Fibers, Maturity of Fibers, and Index of Short Fibers

The 10% *Camellia sinensis* (Cs) extract treatment with a 10-day irrigation cycle (W10) exhibited the lowest fiber yellowness (7.10 +b). Conversely, the control with a 20-day irrigation cycle (W20) displayed the highest fiber yellowness (8.45 +b). The 15% *Rosmarinus officinalis* (Ro) extract treatment with a W10 irrigation cycle showed the lowest surface covered with debris (0.07%), leaf particles in the fiber (0.52), and short fiber index (10.14%). The highest values for these traits were observed in the control with the W20 irrigation cycle. Additionally, the lowest fiber maturity (0.61 micronaire) was observed in the 15% Cs extract at the W15 irrigation cycle, while the highest fiber maturity (0.83 micronaire) was recorded in the control at the W20 irrigation cycle. The maximum color brightness (80.76 Rd) was obtained in the 10% Ro treatment with the W10 irrigation cycle, and the minimum (70.33 Rd) was observed in the control with the W20 irrigation cycle. The lowest number of trashes in the sample (11.66) was noted in the 15% Cs extract at the W10 irrigation cycle, while the highest (23.00) was found in the control at the W20 irrigation cycle (Table 3). Lastly, the highest number of unbloomed buds (10.77) was observed in the 15% Ro treatment with the W10 irrigation cycle, whereas the lowest number (2.36) was recorded in the control at the W20 irrigation cycle (Fig. IV).

Table 3
Effect of methanolic extracts (*Camellia sinensis* and *Rosmarinus officinalis*) and different levels of irrigation cycle on quality attributes of cotton fibers (*Gossypium hirsotum*).

Treatments	Irrigation cycles (Day)	Color brightness (Rd)	Yellowness (+b)	No. of trashes	Surface covered with debris (%)	No. of leaf particles	Maturity (Micronaire)	Short fiber index (%)
Control	W10	75.86^{bcd}	7.76^{bcd}	16.66^{ab}	0.12^{abc}	1.66^{bcd}	0.81^{a}	13.53^{bcd}
Control	W15	72.90^{de}	8.23^{ab}	18.33^{ab}	0.19^{ab}	2.33^{ab}	0.82^{a}	15.23^{b}
Control	W20	70.33^{e}	8.45^{a}	23.00^{a}	0.20^{a}	3.00^{a}	0.83^{a}	17.76^{a}
Cs 10%	W10	79.33^{ab}	7.10^{e}	12.66^{b}	0.09^{bc}	0.83^{cd}	0.81^{a}	10.33^{ef}
Cs 10%	W15	76.96^{bc}	7.76^{bcd}	16.00^{ab}	0.16^{abc}	1.33^{bcd}	0.81^{a}	11.43^{def}
Cs 10%	W20	75.76^{cd}	8.13^{abc}	18.00^{ab}	0.13^{abc}	2.00^{abc}	0.82^{a}	13.40^{bcd}
Cs 15%	W10	80.46^{a}	7.20^{de}	11.66^{b}	0.08^{bc}	1.00^{cd}	0.80^{a}	11.56^{def}
Cs 15%	W15	77.56^{abc}	7.53^{cde}	14.66^{ab}	0.15^{abc}	1.43^{bcd}	0.61^{b}	12.63^{cd}
Cs 15%	W20	76.76^{b}c	8.03^{abc}	15.66^{ab}	0.13^{abc}	1.66^{bcd}	0.82^{a}	13.00^{cd}
Ro 10%	W10	80.76^{a}	7.23^{de}	12.00^{b}	0.076^{c}	0.68^{d}	0.81^{a}	10.33^{ef}
Ro 10%	W15	74.76^{cd}	7.90^{abc}	18.00^{ab}	0.15^{abc}	1.26^{bcd}	0.82^{a}	12.33^{cde}
Ro 10%	W20	74.90^{cd}	8.16^{abc}	20.66^{ab}	0.16^{abc}	1.66^{bcd}	0.82^{a}	14.10^{bc}
Ro 15%	W10	80.53^{a}	7.20^{de}	12.00^{b}	0.07^{c}	0.52^{d}	0.80^{a}	10.14^{f}
Ro 15%	W15	77.56^{abc}	7.76^{bcd}	15.66^{ab}	0.15^{abc}	1.33^{bcd}	0.82^{a}	12.26^{cde}
Ro 15%	W20	75.30^{cd}	7.93^{abc}	18.66^{ab}	0.15^{abc}	1.50^{bcd}	0.82^{a}	13.46^{bcd}

*Common letters in each column indicate non-significance at the 5% probability level based on Tukey's test. In this table, Cs is *Camellia sinensis*, Ro is *Rosmarinus officinalis* and W is the irrigation cycle (day).

Discussion

(Iqbal et al., 2019)Plants possess the ability to resist damage caused by abiotic stresses through various physiological and biochemical mechanisms, such as changes in morphology and metabolism (Bakhsh and Hussain, 2015). When a plant senses a stress signal through the plasma membrane, it initiates the production of abscisic acid (ABA), which predominantly occurs in plastids (Bakhsh and Hussain, 2015). ABA synthesis begins in the roots and is transported through vascular tissues, leading to stomatal closure in various cells, including guard cells (Kuromori et al., 2010). However, stomatal closure results in a decrease in CO_2 intake and net photosynthesis (Iqbal et al., 2019; Kuromori et al., 2010) , ultimately causing a significant reduction in crop productivity and dry matter production (Awan et al., 2021; Li et al., 2022; Zhang et al., 2016).

To fully exploit limited water resources, maximizing high-yielding agriculture is essential. Various metabolites, such as glutathione, ascorbic acid, tocopherol, carotenoids, flavonoids, and anthocyanin's, protect plants against oxidative stress by scavenging free radicals (Sairam et al., 1997). In particular, the role of phenolic compounds in reducing or inhibiting lipid peroxidation, scavenging free radicals, quenching singlet oxygen, or dissociating peroxides makes these extracts crucial antioxidants for protection against oxidative stress damage and defense against reactive oxygen species (ROS) (Ksouri et al., 2007).

Drought can have varying destructive effects depending on the timing and intensity of stress, growth stage, and species. Although drought stress negatively impacts all stages of cotton growth, the seedling, flowering, and boll development stages are particularly sensitive to water deficit (Shi et al., 2021). Recent research suggests that the number of bolls, boll weight, and leaf number can serve as indicators of cotton drought resistance (Chen et al., 2012). Studies on cotton have found that drought stress leads to a decrease in fiber, boll, and wool yield due to reduced net photosynthesis under water deficit conditions, which in turn affects growth and yield (Chastain et al., 2014).

To achieve high-yielding agriculture, it is crucial to fully utilize limited water resources. Many metabolites, such as glutathione, ascorbic acid, tocopherol, carotenoids, flavonoids, and anthocyanins, protect plants against oxidative stress by scavenging free radicals (Sairam et al.,

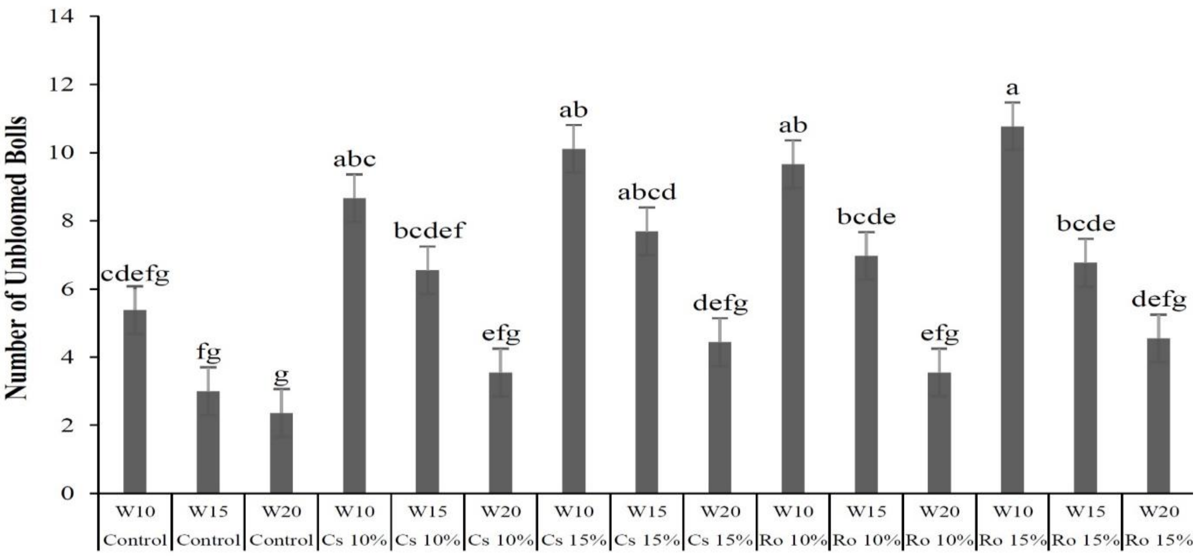

Fig. IV. Effect of methanolic extracts and different levels of irrigation cycle on the number of unbloomed bolls of cotton (*Gossypium hirsotum*). In this figure, Cs is *Camellia sinensis*, Ro is *Rosmarinus officinalis*, and W is the irrigation cycle (day).

1997). Phenolic compounds play a vital role in reducing or inhibiting lipid peroxidation, scavenging free radicals, quenching singlet oxygen, and dissociating peroxides. These extracts act as essential antioxidants, protecting against oxidative stress damage (Ksouri et al., 2007).

Flavonoids are a type of phenolic compound that help prevent oxidative stress in plants. They achieve this by either participating directly in regeneration reactions or indirectly by chelating iron, due to their antioxidant role (Popova et al., 1997). Another important phenolic compound, salicylic acid, regulates the flavonoid content and the activity of antioxidant enzymes such as catalase and peroxidase, which helps mitigate oxidative stress caused by drought. Salicylic acid acts as an internal growth regulator, assisting in various plant processes including resistance to environmental stress, stomatal closure, ion absorption and transport, membrane stability, growth rate, and photosynthesis (El-Tayeb, 2005; Horváth et al., 2007).

Plants rich in antioxidant compounds can protect cells from oxidative damage (Kumaran, 2006). In this study, methanolic extracts of *Camellia sinensis* and *Rosmarinus officinalis*, which contain phenolic antioxidant compounds, helped create a balance between reactive oxygen species (ROS) and antioxidant activity under drought stress conditions. This balance contributed to the improved morphological traits of cotton across different irrigation cycles, consistent with previous scientific findings.

Plants employ osmotic regulation as an effective mechanism to manage abiotic stress responses. This process enables them to tolerate dehydration by accumulating various organic and mineral substances, such as sugars, proline, mannitol, amino acids, alkaloids, and mineral ions. This accumulation increases the concentration of these substances inside the cells and reduces the osmotic potential. Osmotic regulation allows plants to adjust stomatal and photosynthetic functions naturally, improve root system growth for water absorption, delay leaf senescence, and enhance dry matter accumulation and overall performance (Abdelraheem et al., 2019).

Despite these mechanisms, drought conditions can lead to significant reductions in net photosynthesis, transpiration rate, stomatal conductance, carboxylation efficiency, RWC, and leaf water potential in cotton (Kumar et al., 2001; Lv et al., 2007; Noreen et al., 2013). In this study, drought stress led to a significant reduction in leaf RWC due to increased cell sap concentration.

However, the methanolic extracts improved leaf RWC by modulating the stress conditions.

Under drought stress, the production of reactive oxygen species (ROS) increases, which impacts cellular functions. The reduction in CO_2 fixation during the Calvin cycle leads to decreased $NADP^+$ regeneration, thereby affecting the photosynthetic electron transport chain (Cruz de Carvalho, 2008). ROS in cells include hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), superoxide anion radical (O_2^-), and singlet oxygen ($^1O_2$), with •OH and $^1O_2$ being particularly reactive. These ROS can damage various cellular components, such as lipids, proteins, DNA, and RNA. RNA, causing membrane damage, ion leakage, and, in severe cases, cell death (Fang et al., 2015). Our study demonstrated that drought stress increased ion leakage in cotton plants. However, the antioxidant properties of methanolic extracts helped mitigate the destructive effects of ROS on cell membranes and reduced ion leakage.

Abiotic stresses, such as drought, can lead to significant morphophysiological changes in plants, adversely affecting their yield and quality, including fiber length, elasticity, fineness, and strength (Loka et al., 2011). Drought stress has been shown to significantly decrease micronaire and fiber length (Rai, 2011). Cotton fiber development is highly dependent on water availability; under stress conditions, cotton leaves lose turgor pressure, and photosynthesis is constrained. This limitation leads to a reduced supply of carbohydrates to developing bolls, negatively impacting fiber growth (Allen and Aleman, 2011).

Previous research has demonstrated that varying moisture levels can impact cotton fiber quality. Inadequate irrigation practices can negatively affect both the quantity and quality of cotton fibers. Our study aligns with these findings, showing that proper irrigation practices are crucial for maintaining fiber quality (Balkcom et al., 2006). Moreover, different irrigation practices can influence the quality of spun yarn. For example, fibers from cotton grown under drought stress may exhibit desirable spun yarn characteristics but often with reduced strength (Moragne et al., 2009). Drought stress has been reported to cause up to a 34% loss in fiber yield in the cotton industry (Ullah et al., 2017). In this study, we successfully improved cotton fiber quality and performance under drought stress conditions through the use of natural antioxidant extracts.

Plants employ four primary strategies to cope with drought: avoidance, escape, tolerance, and recovery (Fang and Xiong, 2015). Drought avoidance includes physiological adaptations such as stomatal adjustments and enhanced root development under moderate drought conditions. Drought tolerance involves the ability to endure severe dehydration through mechanisms like osmotic regulation and osmotic protectors (Luo, 2010). Plants may also escape drought stress by shortening their life cycle (Manavalan et al., 2009).

Abscisic acid (ABA) plays a crucial role in regulating various physiological processes related to stress responses, growth, and reproduction. During drought stress, ABA can induce premature flowering as a survival strategy (Muhammad Aslam et al., 2022). In this study, cotton plants exhibited a defense mechanism to escape from drought stress when methanolic extracts were not applied. This resulted in increased fiber yellowness, a higher number of trashes, more surface covered with debris, and increased leaf particles and short fiber index due to the adverse effects of drought stress.

By utilizing methanolic extracts, the cotton plants demonstrated enhanced resilience to drought, improving fiber quality and reducing undesirable traits such as yellowness and surface debris. This indicates that the application of these extracts can mitigate some of the negative impacts of drought stress on fiber characteristics.

Conclusion

The results indicate that longer intervals between irrigation cycles lead to a reduction in both the yield and quality of cotton fibers. However, the application of methanolic extracts, particularly the 15% *Rosmarinus officinalis* (Ro) extract, yielded significant improvements in most of the measured traits. This suggests that these extracts can enhance cotton fiber quality under drought conditions. Given the ongoing water crisis and the increasing need for sustainable agricultural practices, medicinal plant extracts present a promising alternative to synthetic antioxidants. These natural treatments effectively mitigate the adverse impacts of abiotic stresses, such as drought, on cotton plants, offering a valuable option for enhancing agricultural resilience and environmental sustainability.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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