

## The effect of bio-fertilizers and a super-absorbent on the quantitative and qualitative characteristics of castor bean (*Ricinus communis* L.) under water-deficit conditions

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### Abstract

The effect of bio-fertilizers and super-absorbents on the modification of water-deficit stress was studied in castor. A field experiment was conducted with a split-plot design based on a completely randomized block design with three replications. The first factor included four levels (70, 100, 130, and 160 mm of evaporation pan A Class) in the main plot; the sub-factor was the application of four bio-fertilizer treatments (*Azospirillum*, *Citrobacter*, *Azospirillum* + *Citrobacter*, and control); and the superabsorbent at two levels (superabsorbent consumption and control) as a factorial was placed in the sub-plots. With the intensification of the water-deficit stress from 70 to 160 mm of evaporation, the number of seeds per plant, the 1,000-kernel weight, and the oil percentage significantly dropped. All three bio-fertilizer treatments significantly increased the number of grains, 1,000 kernel weight, and oil percentage compared to the control. *Citrobacter* in all four irrigation levels significantly increased the content of photosynthetic pigments, grain yield, oil yield and proline content and decreased the catalase activity and malondialdehyde content. The application of the super-absorbent under the stress condition of 160 mm significantly raised the content of chlorophyll b, soluble sugar, grain yield, oil yield, and proline compared to the control treatment. Among the combinations of bio-fertilizer and super-absorbent treatments, the highest photosynthetic pigments' content, grain yield, and oil yield and the lowest catalase enzyme activity belonged to *Azospirillum* + superabsorbent consumption. However, the maximum soluble sugars and the minimum malondialdehyde content belonged to *Citrobacter* + superabsorbent.

**Keywords:** antioxidant; drought; grain yield; microorganism; proline

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## Introduction

Castor bean (*Ricinus communis* L.) is one of the most important medicinal plants of the Euphorbiaceae family (Marwat *et al.*, 2017). This plant is widely used in pharmaceutical, cosmetic, and health industries in developed countries (Liv *et al.*, 2012). This annual plant grows in cold climates, and its height may reach 2 to 3 m. Castor seeds contain oil, which is responsible for their medicinal properties and fatty acid compounds. The content of seed oil in castor is estimated at 40-60% based on the variety (Iqbal *et al.*, 2012). In 2020, the area under castor bean cultivation and its seed production in the world was estimated to be approximately 1.22 million hectares and 2.02 million tons, respectively. In Iran, 16 hectares have been dedicated to the cultivation of castor beans which had a production of 26 tons (FAO, 2022). As with other crop plants, water is one of the most important factors affecting the growth, the content of effective substances, and essential oils of medicinal plants; therefore, droughts can cause a lot of damage to the body of medicinal plants and the effective substances produced in them.

Similar to other stresses, drought stress induces the production of reactive oxygen species (ROS) and other non-radical agents such as hydrogen peroxide and singlet oxygen in plant tissues and cells (Singh-Gill and Tuteja, 2010). Although RPS at low concentrations can act as regulators of some plant reactions or as secondary messengers, they are usually identified as destructive agents in plants and cause oxidative damage to various molecules such as lipids, proteins, and nucleic acids in cells (Kruk *et al.*, 2005).

The reaction of cultivars and genotypes differs to the stress of water shortage, and some cultivars and genotypes have acceptable performance thanks to their drought resistance; however, plant growth environment conditions and plant management can significantly shape the quantity and quality of plant yield under water-deficit conditions (Tefamariam *et al.*, 2010). Drought stress harms grain yield and yield components in castor (Sadeghi-Bakhtavari and Hazrati *et al.*, 2018). In investigating the effect of foliar spraying of chemical fertilizers on castor properties under different irrigation treatments, it was observed that the highest plant height, number of pods, biological yield, and grain yield belonged to normal irrigation (20 mm from the evaporation pan) and foliar spraying of K+S+N fertilizers (Osati *et al.*, 2022). The management role of some modifying additives such as superabsorbent hydrogel polymers for optimal use of water in agriculture has recently received global attention and applications on a large scale; In Iran, too, some researchers have turned their attention to this topic (Islam *et al.*, 2011).

The advantages of super-absorbents include increasing the capacity to hold water and food for a long time, reducing the number of irrigation times, uniform water consumption for plants, rapid and favorable root growth, decreasing the leaching of nutrients in the soil, reducing the cost of irrigation, optimal use of chemical fertilizers, better soil aeration, the possibility of cultivation in desert areas and steep surfaces, improving the activity and proliferation of mycorrhizal fungi and other soil micro-organisms, and enhancing the porosity and stability of the soil structure (Abobatta, 2018).

Another method recently adopted to deal with drought and adjust the water shortage stress is the use of beneficial soil microorganisms. One of the most important of these microorganisms is the growth-promoting bacteria (Fasusi *et al.*, 2021).

The recent concept of eco-technology, the rise in the price of chemical fertilizers, and the destructive environmental impacts of these residual fertilizers on the soil have promoted the use of bio-fertilizers for sustainable production and environmental protection. Furthermore, bio-fertilizers will lead to the development and prosperity of agriculture due to environmental adaptation (El-Naggar *et al.*, 2015). These bacteria can produce and secrete active biological substances in the root environment, thereby contributing to the development of the root system, promoting the absorption of nutrients and biological fixation of nitrogen, and eventually increasing the economic yield of the product (Kumar *et al.*, 2017). Therefore, the utilization of biological sources of macro elements such as nitrogen fixation and sulfur solubilizing microorganisms will minimize the use of chemical fertilizers and production costs (Smitha *et al.*, 2019).

The most important mechanisms of bio-fertilizers for plant growth promotion include the biosynthesis of phytohormones, reduced membrane potential root wall cells, increased synthesis of enzymes modulating plant hormone levels, solubility and mineralization of organic and non-organic substances that make nutrients available to plants (Hellal *et al.*, 2011; Nejat-zadeh-Barandozi and Gholami-Borujeni, 2014).

Iran is located in an arid and semi-arid region where water is a fundamental factor limiting crop production. Still, little research has been conducted on the effect of seed inoculation with bio-fertilizers and the use of superabsorbent polymers to modulate the impact of water in castor plants. Therefore, this study aimed to investigate the effect of bio-fertilizers and polymer super-absorbents on quantitative and qualitative characteristics of castor at different levels of irrigation.

## Materials and Methods

The present experiment was carried out from the 2018 to 2019 crop season at the Saatlo Agricultural Research Station in Urmia, West Azerbaijan Province, Iran. This area is located at 45° 15' east longitude and 37° 43' north latitudes and an altitude of 1345 m above sea level.

The average annual rainfall of this area is 296 mm, and the average annual temperature is 11.5 °C based on 35-year data from the Meteorological Station of the Agriculture and Natural Resources Research Station of Urmia (Table 1).

The experiment was conducted as a factorial split-plot design based on a randomized complete blocks design with three replications.

The main factor was irrigation treatment at four levels (70, 100, 130, and 160 mm from the evaporation pan); the first sub-factor was the use of biological fertilizers treatments (*Azospirillum*, *Citrobacter*, *Azospirillum* + *Citrobacter*, and the control without any fertilizer); the second sub-factor was superabsorbent use at two levels (the use of superabsorbent (100 kg ha<sup>-1</sup>) and non-use of superabsorbent), which were factorially placed in sub-plots. Before the experiment, plowing, land preparation, and seedbed preparation were carried out.

In this study, a level of 70 mm of evaporation was considered as normal irrigation conditions and a level of 160 mm of evaporation was considered as severe stress of water shortage (Sadeghi-Bakhtavari *et al.*, 2018). In addition, samples were taken from the experimental farm soil at two depths of 0-30 and 30-60 cm to determine the physical and chemical properties of the soil (Table 2).

**Table 1.** Mean meteorological parameters 2018 to 2019 crop season at the Saatlo Agricultural Research Station in Urmia

Month	Monthly rainfall (mm)	Minimum temperature (°C)	Maximum temperature (°C)	Minimum humidity (%)	Maximum humidity (%)
March	43.2	17.1	4.1	78.8	33.7
April	33.7	18.9	7.4	79.1	35.4
May	15.7	25.5	12.2	77.9	29.6
June	15.0	20.3	16.5	65.6	25.7
July	3.01	31.3	17.0	63.5	25.6
August	10.9	29.6	15.4	55.6	18.3
September	15.6	20.2	5.8	68.8	25.9
October	52.5	13.6	3.8	88.7	49.6

Planting in both years of the experiment was performed on June 29. The seed to be planted was a local variety of Isfahan obtained from the Pakan Seed Company of Isfahan. The growth period length of this variety in West Azerbaijan conditions is about 120 days; the 1,000-kernel weight is 165 to 180 grams; and it is an oily type with small seeds.

Plots with dimensions of 10 m (length) and 4 m (width) were prepared. The density used was 3800, in which the distance between the rows and the distance between the plants on the row were considered equal to 35 and 75 cm, respectively. The distance between the blocks was 2 m, and the distance between the plots was 1 m. Then, castor seeds were planted on the prepared stacks at a depth of 3 cm.

**Table 2.** Physical and chemical characteristics of the soil at the test site

N %	P (ppm)	K (ppm)	Organic carbon %	Sand %	Silt %	Clay %	T.N.V	Sp %	pH	Saltines (103×Ec)	Soil texture	Depth (cm)
6	10.4	250	0.6	39	35	26	13	43	8	1.1	loam	0-30

The seeds were treated with thiram (tetramethyl thiuram disulfide) (5 per 1,000) before sowing because there is a risk of low spring temperature and high soil moisture immediately after sowing. The plots were irrigated by the drip method. Irrigation water was estimated using type III flumes (Washington State College). The mouth width and head of the type III flumes were 304.8 and 30 mm, respectively.

Treatment with biological fertilizers was applied in three stages. In the first step, the seeds were dipped in the bacterial suspension mixture of *Azospirillum lipoferum* and *Citrobacter* for 30 minutes. The inoculated seeds were dried in the shade and then planted. The second and third parts of the bio-fertilizers were applied at a rate of 20 ml liter<sup>-1</sup> in the flowering stage until seed formation with the irrigation system.

During the growing season, weed control was performed by manual weeding on two occasions in the 4th and 8th leaf stages of castor growth. During the growth of the plant, no specific pests and diseases were observed in the field. Harvesting was carried out on November 11.

Chlorophyll-a, -b, and carotenoids were determined based on Arnon's (1949) method. Fresh leaves were taken from the plants and triturated in 80% acetone. The absorbance of the extracts was measured at 663, 645, and 480 nm for chlorophyll-a, -b, and carotenoid, respectively, using a spectrophotometer. The concentration of soluble proteins was determined by the Bradford method (Bradford, 1976).

The proline content of leaves was estimated spectrophotometrically based on the ninhydrin method described by Bates *et al.* (1973). Total soluble sugars were extracted and determined by Dubois *et al.* (1956) method. Catalase activity was estimated as described by Prasad *et al.* (1999). Malondialdehyde (MDA) content, an index of lipid peroxidation, was estimated based on the method described by De Vos (1989).

After removing the marginal effects, 10 plants from each plot were used as a sample to measure grain yield components such as the number of capsules per plant, number of seeds per capsule, and 1,000 kernel weight. The total plot was used to measure grain yield after removing the marginal effects. Furthermore, seed oil (%) was measured by the Soxhlet method (10 g per sample), and oil yield was calculated by multiplying seed yield by the crude oil ratio (AOAC, 1984). Data were collected during the field trial and were statistically analysed via one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) at the 5% level in SAS software.

## Results

### *Analysis of variance*

A combined analysis of variance (ANOVA) for the studied treats revealed a significant irrigation and bio-fertilizer effect for almost all traits ( $P > 0.01$ ). The effect of super-absorbent was also significant on all the investigated traits, except for oil percentage ( $P > 0.01$ ). Significant irrigation with bio-fertilizer interaction was detected for the content of chlorophyll a, chlorophyll b, carotenoids, grain yield, oil yield, soluble sugar, proline, catalase, and malondialdehyde ( $P > 0.01$ ). The results indicated chlorophyll a, chlorophyll b, grain yield, oil yield, soluble sugar, and proline effects by irrigation with superabsorbent interaction ( $P > 0.01$ ). Finally, the

effect of bio-fertilizer with super-absorbent interaction on chlorophyll b content, grain yield, soluble sugar, proline, and malondialdehyde was significant at a 1% probability ( $P > 0.01$ ) and on chlorophyll a, carotenoids, and the number of seeds per plant at a 5% probability ( $P > 0.05$ ) (Table 3).

**Table 3.** Combined analysis of variance of castor traits under irrigation bio-fertilizers and super-absorbents treatment

Ms										
SOV	Df	Chlorophyll a	Chlorophyll b	Carotenoid	Capsules number per plant	Seeds number per plant	Thousand kernel weight	Grain yield	Oil percentages	Oil yield
Year(Y)	1	68.84 <sup>ns</sup>	42.24 <sup>ns</sup>	20.17 <sup>ns</sup>	6167.60 <sup>ns</sup>	31678.35 <sup>ns</sup>	1408.33 <sup>ns</sup>	58730.02 <sup>ns</sup>	31.82 <sup>ns</sup>	4600.32 <sup>ns</sup>
Repeat (year)	4	44.11	7.16	34.17	4159.32	20408.43	807.16	27054.92	27.72	1028.26
Irrigation (I)	3	12.57 <sup>**</sup>	112.21 <sup>**</sup>	46.98 <sup>**</sup>	1230.99 <sup>**</sup>	11285.92 <sup>**</sup>	1524.13 <sup>**</sup>	101987.2 <sup>**</sup>	9.98 <sup>**</sup>	279776.78 <sup>**</sup>
Y×I	3	1.33 <sup>ns</sup>	0.85 <sup>ns</sup>	0.82 <sup>ns</sup>	127.16 <sup>ns</sup>	522.67 <sup>ns</sup>	278.16 <sup>ns</sup>	5977.90 <sup>ns</sup>	3.76 <sup>ns</sup>	4429.18 <sup>ns</sup>
E <sub>b</sub>	12	0.60	0.65	0.69	105.12	405.73	76.13	4439.64 <sup>ns</sup>	25569	2631.7
Bio-fertilizer(B)	3	45.87 <sup>**</sup>	54.15 <sup>**</sup>	5.75 <sup>**</sup>	2253.85 <sup>**</sup>	16234.71 <sup>**</sup>	29731.6 <sup>**</sup>	339057.39 <sup>**</sup>	109.37 <sup>**</sup>	152591.14 <sup>**</sup>
I × B	9	3.43 <sup>**</sup>	5.38 <sup>**</sup>	2.37 <sup>**</sup>	73.47 <sup>ns</sup>	312.1 <sup>ns</sup>	464.37 <sup>ns</sup>	3486.50 <sup>ns</sup>	1.68 <sup>ns</sup>	194605.80 <sup>**</sup>
Y×B	3	0.24 <sup>ns</sup>	0.07 <sup>ns</sup>	0.30 <sup>ns</sup>	46.63 <sup>ns</sup>	51.72 <sup>ns</sup>	72.62 <sup>ns</sup>	1356.81 <sup>ns</sup>	0.88 <sup>ns</sup>	1784.49 <sup>ns</sup>
Y×I× B	9	0.49 <sup>ns</sup>	0.49 <sup>ns</sup>	0.71 <sup>ns</sup>	75.08 <sup>ns</sup>	603.14 <sup>ns</sup>	700.04 <sup>ns</sup>	5088.68 <sup>ns</sup>	2.02 <sup>ns</sup>	30099.43 <sup>ns</sup>
Super absorbent(S)	1	8.29 <sup>**</sup>	13.13 <sup>**</sup>	14.29 <sup>**</sup>	4926.82 <sup>**</sup>	38264.63 <sup>**</sup>	3350.02 <sup>**</sup>	129636.04 <sup>**</sup>	6.90 <sup>**</sup>	48230.28 <sup>**</sup>
Y×S	3	0.0002 <sup>ns</sup>	1.05 <sup>ns</sup>	0.56 <sup>ns</sup>	3.36 <sup>ns</sup>	79.43 <sup>ns</sup>	28.52 <sup>ns</sup>	969.30 <sup>ns</sup>	0.20 <sup>ns</sup>	231.39 <sup>ns</sup>
I × S	3	3.31 <sup>**</sup>	2.31 <sup>**</sup>	0.49 <sup>ns</sup>	147.58 <sup>ns</sup>	292.04 <sup>ns</sup>	99.56 <sup>ns</sup>	2370.43 <sup>ns</sup>	2.26 <sup>ns</sup>	15592.77 <sup>**</sup>
Y×I×S	3	0.33 <sup>ns</sup>	0.81 <sup>ns</sup>	0.54 <sup>ns</sup>	9.66 <sup>ns</sup>	542.48 <sup>ns</sup>	217.56 <sup>ns</sup>	5224.23 <sup>ns</sup>	0.29 <sup>ns</sup>	7329.75 <sup>ns</sup>
B×S	3	1.31 <sup>**</sup>	2.87 <sup>**</sup>	2.78 <sup>**</sup>	148.19 <sup>ns</sup>	1443.81 <sup>*</sup>	292.18 <sup>ns</sup>	20438.15 <sup>**</sup>	2.70 <sup>ns</sup>	28322.90 <sup>*</sup>
Y×B×S	3	0.24 <sup>ns</sup>	0.15 <sup>ns</sup>	0.10 <sup>ns</sup>	63.64 <sup>ns</sup>	55.55 <sup>ns</sup>	60.27 <sup>ns</sup>	1530.14 <sup>ns</sup>	0.25 <sup>ns</sup>	1388.96 <sup>ns</sup>
I×B×S	9	0.46 <sup>ns</sup>	0.29 <sup>ns</sup>	0.66 <sup>ns</sup>	37.96 <sup>ns</sup>	340.37 <sup>ns</sup>	522.72 <sup>ns</sup>	2168.23 <sup>ns</sup>	1.38 <sup>ns</sup>	13848.46 <sup>ns</sup>
Y×I×B×S	9	0.49 <sup>ns</sup>	0.41 <sup>ns</sup>	0.80 <sup>ns</sup>	114.25 <sup>ns</sup>	596.36 <sup>ns</sup>	761.47 <sup>ns</sup>	5325.41 <sup>ns</sup>	3.37 <sup>ns</sup>	36200.49 <sup>ns</sup>
E <sub>c</sub>	112	0.48	0.44	0.84	7672.42	401.83	66.84	4406.75	4.45	11283.7
Coefficient of variation		3.37	6.10	3.25	7.84	6.12	14.01	6.38	5.84	10.51

ns, \* and \*\* not significant and significant at the level of five and one percent statistical probability, respectively

### Photosynthetic pigments

The highest chlorophyll a, chlorophyll b, and carotenoid contents, respectively with an average of 23.00, 14.93, and 30.49 mg/g of fresh weight, were recorded from the treatment with *Citrobacter* along with irrigation treatment after 70 mm of evaporation. The lowest amounts of the above-mentioned pigments were assigned to the bio-fertilizer control treatment along with the irrigation level after 160 mm of evaporation, respectively with an average of 18.35, 9.20, and 25.56 mg g of fresh weight<sup>-1</sup> (Table 6). The application of biological fertilizers, especially *Citrobacter*, at all irrigation levels improved the content of photosynthetic pigments compared to the control.

The use of the super-absorbent in the irrigation after 70 mm of evaporation had the highest chlorophyll a and b content with an average of 21.29 and 12.78 mg g of fresh weight<sup>-1</sup>, respectively. Moreover, the lowest mentioned pigments' content was observed in super-absorbent non-application (control) with the irrigation level after 160 mm of evaporation (Table 7).

The use of the super-absorbent significantly increased the chlorophyll content compared to the control treatment at the irrigation level after 100 mm of evaporation.

Among the bio-fertilizers with super-absorbent interaction treatments, treatment with *Azospirillum* along with the application of the super-absorbent with an average of 22.25, 13.79, and 29.02 mg g fresh weight<sup>-1</sup>, respectively, showed the highest, while the control of both treatments (no use of super-absorbent and bio-

fertilizer) had the lowest content of chlorophyll a, chlorophyll b, and carotenoid pigments with an average of 18.56, 9.83, and 27.27 mg g weight<sup>-1</sup>, respectively (Table 8). The application of the superabsorbent and bio-fertilizer demonstrated a synergistic effect on the concentration of photosynthetic pigments.

#### *Yield components and grain yield*

##### Number of capsules

In this experiment, the irrigation treatment after 70 mm of evaporation had the highest number of capsules per plant with an average of 234.81. Water deficit had a negative effect on the number of capsules per plant, such that in the irrigation treatment, after 160 mm of evaporation, it reached its minimum with an average of 223.08 capsules (Table 4).

Among the bio-fertilizer treatments, the highest number of capsules per plant belonged to the *Citrobacter* + *Azospirillum* treatment with an average of 233.17 capsules, and the lowest number was assigned to the control treatment with an average of 219.51 capsules (Table 5).

**Table 4.** Mean comparison of effect of irrigation levels on studied traits of castor

Irrigation (mm from the evaporation pan)	Capsules number per plant	Grains number per plant	Thousand kernel weight (g)	Oil (%)
70 mm	234.81a	633.17a	209.12a	51.00a
100 mm	227.84b	615.47b	203.21b	53.23b
130 mm	225.14c	607.31c	200.58c	50.20b
160 mm	223.08d	601.51d	197.54d	49.81c

In each column, averages with common character do not have a significant difference at the 5% level.

**Table 5.** Mean comparison of effect of bio-fertilizer on studied traits of castor

Bio-fertilizer	Capsules number per plant	Thousand kernels weight	Oil (%)
Control	219.51d	185.37d	48.44d
<i>Azospirillum</i>	227.81c	195.38c	49.15c
<i>Citrobacter</i>	230.60b	217.41a	52.56a
<i>Azospirillum</i> + <i>Citrobacter</i>	233.17a	212.05b	50.07b

In each column, averages with common character do not have a significant difference at the 5% level

##### Number of grains per plant

The mean comparison of irrigation levels revealed that with the intensification of water shortage, the number of grains significantly decreased, such that the highest and lowest values of the mentioned trait (respectively 633.17 and 601.51 grains) were produced in irrigation conditions after 70 and 160 mm of evaporation (Table 4).

Among the interaction treatments, the highest number of grains per plant with an average of 641.17 grains belonged to the interaction treatment of *Azospirillum* + *Citrobacter* with the use of superabsorbent. The lowest number of grains per plant was assigned to the control of both treatments (Table 8).

##### Thousand kernel weight

Water stress had a decreasing effect on the thousand kernel weight. Irrigation after 100, 130, and 160 mm of evaporation reduced the thousand kernel weight compared to normal conditions (irrigation after 70 mm of evaporation) by 2.90, 4.25, and 5.86%, respectively (Table 4).

The mean comparison of biological fertilizer treatments showed that the highest thousand kernel weight with an average of 217.41 g was related to the *Citrobacter* treatment, while the lowest value with an average of 185.37 g was assigned to the control treatment (no application of biological fertilizer) (Table 5).

Grain yield

The grain yield decreased with the intensification of water deficit stress, but the application of biological fertilizers, especially *Citrobacter*, at all irrigation levels had a positive effect on grain yield. The use of *Citrobacter* at irrigation levels after 70, 100, 130, and 160 mm of evaporation increased the grain yield compared to the control treatment at each irrigation level by 10.60, 9.19, 10.62, and 11.80%, respectively (Table 6). Treatment with *Citrobacter* at irrigation conditions after 70 mm of evaporation with an average of 1263.9 kg ha<sup>-1</sup> had the highest, whereas the control treatment at the irrigation level after 160 mm of evaporation with an average of 775.2 kg ha<sup>-1</sup> had the lowest grain yield.

**Table 6.** Mean comparison of interaction effects of irrigation of and Bio-fertilizer on studied traits of castor

Irrigation (mm from the evaporation pan)	Bio-fertilizer	Chlorophyll a (mg g fresh weight <sup>-1</sup> )	Chlorophyll b (mg g fresh weight <sup>-1</sup> )	Cartonoid (mg g fresh weight <sup>-1</sup> )	Grain yield (Kg h <sup>-1</sup> )	Oil yield (Kg h <sup>-1</sup> )	Prolin (micromole g fresh weigh <sup>-1</sup> )	Soluble sugar (micromole g fresh weight <sup>-1</sup> )	Catalase (ΔA240/mg protcin)	Maloddi aldehyde (nmol g FW <sup>-1</sup> )
70 mm	Control	20.94cf	12.04c	28.99bc	1142.7d	557.74d	23.83k	107.50k	39.22k	133.41g
	<i>Azospirillum (A)</i>	19.26jk	11.74c	29.41b	1190.6c	588.97c	25.16jk	111.42j	35.98n	134.13g
	<i>Citrobacter (C)</i>	23.00a	14.93a	30.49a	1263.9a	663.61a	25.91j	113.33i	32.97o	132.44g
	A+ C	22.54ab	11.89c	30.55a	1232.5b	620.73b	27.41i	115.92h	37.96m	130.48g
100 mm	Control	19.40ijk	10.18gh	28.11def	1015.5g	503.71f	29.58h	119.33fg	44.51g	144.70f
	<i>Azospirillum (A)</i>	19.62hij	10.42fg	28.62cde	1033.4g	518.42e	30.83gh	120.33cf	41.30j	145.07f
	<i>Citrobacter (C)</i>	21.74cd	12.43b	28.69cd	1108.9e	591.05e	31.83g	118.83g	38.55l	143.75f
	A+ C	20.59f	11.20d	28.87bc	1066.0f	545.30d	34.91f	119.75efg	43.28h	146.57ef
130 mm	Control	20.46fg	9.81hi	27.42fg	894.5j	433.91i	40.66e	120.83c	48.50d	156.73ab
	<i>Azospirillum (A)</i>	20.02gh	9.99hi	27.92ef	910.8j	447.13hi	41.16e	124.50d	45.29f	150.12cde
	<i>Citrobacter (C)</i>	22.14bc	10.70ef	28.13def	989.5h	514.20ef	46.66d	126.25c	42.54i	148.78de
	A+ C	20.80cf	10.83e	28.02def	945.2i	473.21g	47.00d	126.33c	47.27e	150.59cd
160 mm	Control	18.35l	9.20j	25.56i	775.2m	381.20k	47.66d	125.33cd	54.20a	159.65a
	<i>Azospirillum (A)</i>	19.78hi	9.70i	27.12gh	799.8l	397.35j	53.50c	128.83b	50.77c	154.71b
	<i>Citrobacter (C)</i>	18.88k	10.58cf	27.11gh	866.7k	453.06h	61.50b	129.00b	47.71e	153.75bc
	A+ C	21.30de	10.40fg	26.45h	867.6k	439.50i	65.91a	131.42a	53.16b	149.99de

In each column, averages with common character do not have a significant difference at the 5% level

Among the irrigation and super-absorbent interaction treatments, the application of super-absorbent under irrigation conditions after 70 and 160 mm of evaporation significantly increased grain yield compared to the control treatment. In this experiment, the use of super-absorbent under irrigation treatment after 70 mm of evaporation with an average of 1226.3 kg ha<sup>-1</sup> showed the highest, and the super-absorbent non-application under irrigation after 160 mm of evaporation with an average of 808.0 kg ha<sup>-1</sup> showed the lowest grain yield (Table 7).

**Table 7.** Mean comparison of interaction effects of irrigation of and Super-absorbent on studied traits of castor

Irrigation (mm from the evaporation pan)	Super absorbent	Chlorophyll a (mg g fresh weight <sup>-1</sup> )	Chlorophyll b (mg g fresh weight <sup>-1</sup> )	Cartonoid (mg g fresh weight <sup>-1</sup> )	Grain yield (Kg h <sup>-1</sup> )	Oil yield (Kg h <sup>-1</sup> )	Prolin (micromole g fresh weigh <sup>-1</sup> )	Soluble sugar (micromole g fresh weight <sup>-1</sup> )	Catalase (ΔA240/mg protein)
70 mm	Control	20.55b	11.27b	29.57a	1188.5b	598.32b	24.66h	111.58e	37.08f
	Use	21.29a	12.78a	30.15a	1226.3a	617.20a	26.50g	112.50e	35.99g
100 mm	Control	20.83b	10.89c	28.57a	1050.4c	536.49c	31.20f	119.83d	42.38d
	Use	21.35a	11.23b	28.57a	1061.5c	542.76c	32.37e	119.29d	41.44e
130 mm	Control	20.16cd	10.53d	27.84a	928.9d	464.45d	42.66d	124.38c	46.37b
	Use	20.52bc	10.80c	27.9a	941.1d	469.78d	45.08c	124.58c	45.43c
160 mm	Control	20.03de	10.06e	26.25a	808.0f	406.53f	55.58b	128.17b	51.64a
	Use	19.70e	10.46d	26.88a	846.6e	429.02e	58.70a	129.12a	51.29a

In each column, averages with common character do not have a significant difference at the 5% level

In this experiment, the simultaneous application of the bio-fertilizer and super-absorbent had a positive synergistic effect on increasing grain yield; treatment with *Azospirillum* and the super-absorbent with an

average of 1075.3 kg ha<sup>-1</sup> had the highest grain yield and significantly increased the grain yield compared to the separate treatments with the bio-fertilizer and super-absorbent (Table 8).

**Table 8.** Mean comparison of interaction effects of bio-fertilizer of and super-absorbent on studied traits of castor

Super absorbent	Bio-fertilizer	Chlorophyll a (mg g fresh weight <sup>-1</sup> )	Chlorophyll b (mg g fresh weight <sup>-1</sup> )	Carotenoid (mg g fresh weight <sup>-1</sup> )	Grains number per plant	Grain yield (Kg h <sup>-1</sup> )	Oil yield (Kg h <sup>-1</sup> )	Prolin (micromole g fresh weigh <sup>-1</sup> )	Soluble sugar (micromole g fresh weight <sup>-1</sup> )	Catalase (ΔA240 mg protein <sup>-1</sup> )	Maloddi aldehyde (nmol g FW <sup>-1</sup> )
Control	Control	18.56e	9.83e	27.27e	554.21 <sup>b</sup>	945.7f	463.55g	34.95e	117.08f	46.77a	150.37a
	<i>Azospirillum (A)</i>	19.64d	10.05de	27.76de	601.00 <sup>f</sup>	968.2e	474.73f	35.91de	119.42e	46.45a	150.33a
	<i>Citrobacter (C)</i>	19.87d	10.17d	28.10cd	613.62 <sup>e</sup>	980.5de	486.18e	36.08d	121.67cd	43.85c	148.82a
	A+C	19.82d	10.23d	28.43bc	621.12 <sup>d</sup>	986.7d	489.75e	39.25c	120.87d	42.83d	143.20bc
Use	Control	21.44b	11.78b	28.19bcd	605.41 <sup>f</sup>	1010.3c	510.41d	39.91c	121.38d	41.31e	143.70bc
	<i>Azospirillum (A)</i>	22.25a	13.79a	29.02a	627.32 <sup>e</sup>	1075.3a	565.32a	43.04b	122.33bc	39.58f	145.65b
	<i>Citrobacter (C)</i>	20.57c	10.97c	28.67ab	633.25 <sup>b</sup>	1039.2b	545.64b	43.16b	123.83a	45.55b	141.82c
	A+C	22.28a	11.19c	28.27bc	641.17 <sup>e</sup>	1045.4b	528.95c	44.45a	122.88b	45.29b	143.54bc

In each column, averages with common character do not have a significant difference at the 5% level

### Oil percentages

Water stress had an adverse effect on the accumulation of oil in castor grain. The irrigation conditions after 70 mm of evaporation with an average of 51.00% had the highest, while irrigation conditions after 160 mm with an average of 49.81% had the lowest oil percentage (Table 4).

In this experiment, the oil percentage showed a positive reaction to seed treatment with the bio-fertilizer. Among the bio-fertilizer treatments, the highest oil percentage with an average of 52.56% belonged to *Citrobacter*, whereas the lowest value of this trait with an average of 48.44% was assigned to the biological fertilizer control treatment (Table 5).

### Oil yield

Inoculation with *Citrobacter* increased the oil yield at all irrigation levels and had a positive effect on improving oil yield under different irrigation conditions.

Among the interaction treatments, treatment with *Citrobacter* under irrigation after 70 mm conditions (with an average of 663.61) and the control of biological fertilizer treatment under irrigation conditions after 160 mm (with an average of 381.20 kg per hectare) had the highest and lowest oil yields, respectively (Table 6).

Based on the results, the use of the super-absorbent significantly increased oil yield only in two irrigation conditions after 70 and 160 mm of evaporation, and there was no significant difference between the treatments using and not using the super-absorbent in other irrigation treatments. In this experiment, the use of the super-absorbent under irrigation conditions after 70 mm (with an average of 1226.3 kg per hectare) showed the highest, and the treatment of not using the super-absorbent under irrigation after 160 mm (with an average of 406.53 kg ha<sup>-1</sup>) had the lowest oil yield (Table 7).

The results revealed that bio-fertilizer treatment increased oil yield in both conditions of super-absorbent application and non-application compared to the control. The results also showed that the simultaneous application of the super-absorbent and bio-fertilizer had an increasing effect on oil yield compared to their separate application (Table 8).

### Proline content

The intensification of water deficit and bio-fertilizers, especially *Citrobacter* and *Azospirillum + Citrobacter*, increased the proline content. The treatment of *Azospirillum + Citrobacter* at the irrigation level



of 160 mm of evaporation (with an average of 65.91 micromols g fresh weight<sup>-1</sup>) gained the highest, while the control treatment of bio-fertilizer at the irrigation level after 70 mm had the lowest proline content (with an average of 23.83 micromols g fresh weight<sup>-1</sup>). Two treatments of *Citrobacter* and *Azospirillum* + *Citrobacter* at all four levels of irrigation significantly raised the proline content compared to the control treatment (Table 6).

The application of the super-absorbent with the irrigation level after 160 mm of evaporation (with an average of 58.70 micromoles g fresh weight<sup>-1</sup>) showed the highest, and the control treatment of super-absorbent with the irrigation level after 70 mm of evaporation (with an average of 24.66 micromols g fresh weight<sup>-1</sup>) had the lowest proline content in leaves. The results demonstrated that the use of the super-absorbent at all irrigation levels elevated the proline content of leaves (Table 7).

Based on the results of mean comparisons, the highest and lowest proline contents with an average of 44.45 and 34.95 (micromol per gram of fresh weight) respectively belonged to using the super-absorbent and treatment with *Azospirillum* + *Citrobacter* bio-fertilizer and the control treatment of both treatments (no use of super-absorbent and no bio-fertilizer) (Table 8).

#### Soluble sugar

The soluble sugar content increased with the intensification of drought stress, and treatment with the biological fertilizer also intensified this elevation. Treatment with *Azospirillum* + *Citrobacter* at the irrigation level after 160 mm of evaporation (with an average of 131.42 micromoles g weight<sup>-1</sup>) showed the highest, whereas the control treatment of bio-fertilizer at the irrigation level after 70 mm of evaporation (with an average of 107.50 micromols g fresh weight<sup>-1</sup>) had the lowest soluble sugar content (Table 6).

The use of the super-absorbent under irrigation conditions after 160 mm of evaporation (with an average of 129.12 micromols g wet weight<sup>-1</sup>) and the super-absorbent control treatment under irrigation conditions after 70 mm of evaporation (with an average of 111.58 micromoles g fresh weight<sup>-1</sup>) had the maximum and minimum amounts of soluble sugar, respectively (Table 7).

The results revealed that the use of the super-absorbent and bio-fertilizers simultaneously increased the soluble sugar content, such that the treatment with *Azospirillum* + *Citrobacter* along with the application of the super-absorbent (with an average of 123.83 micromols g fresh weight<sup>-1</sup>) had the highest content of soluble sugar. The lowest value of the mentioned trait (with an average of 117.08 micromols g fresh weight<sup>-1</sup>) was observed in the control treatment of super-absorbent and not treatment with the biological fertilizer (Table 8).

#### Catalase activity

The amount of catalase activity increased with the intensification of water stress, but the treatment with bio-fertilizer reduced this trait. Treatment with *Citrobacter* bio-fertilizer decreased catalase activity compared to the bio-fertilizer control treatment at each irrigation level by 15.93, 13.39, 12.28, and 11.97%, respectively (Table 6).

In this experiment, the control treatment of biological fertilizer and irrigation after 160 mm (with an average of 54.20  $\Delta A_{240}$ /mg protein) had the maximum, and the *Citrobacter* treatment with irrigation after 70 mm of evaporation (with an average of 32.97  $\Delta A_{240}$  mg protein<sup>-1</sup>) had the minimum catalase activity (Table 7).

The use of the super-absorbent in irrigation treatments of 70, 100, and 130 mm reduced the amount of catalase activity compared to the non-use treatment by 3.02%, 2.26%, and 2.06%, respectively. Under irrigation conditions, after 160 mm of evaporation, the difference between the application and non-application treatments of super-absorbent was not significant.

The simultaneous application of two bio-fertilizer and super-absorbent treatments reduced the amount of catalase activity; not using the super-absorbent and the control of bio-fertilizer treatments and treatments with *Azospirillum* respectively with an average of 46.77 and 45.45  $\Delta A_{240}$  mg protein<sup>-1</sup>) had the highest, while

the treatment of super-absorbent application along with *Azospirillum* (with an average of 39.58  $\Delta$ A240 mg protein<sup>-1</sup>) had the lowest amount of said enzyme activity (Table 8).

#### Malondialdehyde content

Water shortage increased the peroxidation of cell membrane fats (Malondialdehyde). However, treatment with bio-fertilizers, especially under water-deficit conditions, reduced the activity of malondialdehyde. In this study, the control treatment of bio-fertilizers with irrigation after 130 and 160 mm of evaporation had the maximum value, and all four bio-fertilizer treatments with irrigation level after 70 mm of evaporation had the minimum malondialdehyde activity (Table 6).

Among the treatments of using the super-absorbent and bio-fertilizer treatment, the highest amount of malondialdehyde enzyme was recorded for the treatment of not using the super-absorbent and non-treatment with bio-fertilizers. There was no significant difference between this treatment and the treatment with *Azospirillum* and *Citrobacter* in terms of malondialdehyde content (Table 8). The lowest malondialdehyde activity was assigned to the treatment of super-absorbent application along with *Citrobacter* and *Azospirillum* + *Citrobacter* treatments.

### **Discussion**

The application of biological fertilizers, especially *Citrobacter*, at all irrigation levels improved the photosynthetic pigments' content compared to the control treatment. The decrease in the content of photosynthetic pigments under water shortage conditions can be mainly due to the destruction of the chloroplast structure and the photosynthetic apparatus, photooxidation of chlorophylls, their reaction with singlet oxygen, the destruction of the precursors of chlorophyll synthesis, and prevention of the biosynthesis of new chlorophylls and the activation of chlorophyll-decomposing enzymes, including chlorophyllase and hormonal disorders (Naeem *et al.*, 2018). The decrease in the content of carotenoids under water-deficit conditions is also due to the breakdown of beta-carotene and the formation of zeaxanthin in the xanthophyll cycle (Ahmadi and Ceiocemardeh, 2005). The reduction of leaf chlorophyll content due to water-deficit treatments in castor has also been reported by Osati *et al.* (2022). Biological fertilizers due to the presence of nitrogen-fixing bacteria can properly supply the nitrogen required by the plant. Nitrogen is essential for the synthesis of chlorophyll in plants.

One of the reasons for the maintenance of high chlorophyll content in biological fertilizer treatments is the antioxidant properties of these materials. Due to these antioxidant properties, the ROS produced due to water deficiency were cleaned, and the electrolytic leakage of the leaves was reduced; as a result, the stability of the photosynthetic pigments' content increased (Babaei *et al.*, 2017).

The other beneficial effects of bio-fertilizer use included the reduction of ethylene content due to the plant's improved access to fixed nitrogen. Due to environmental stresses, especially drought stress, the ethylene content of the leaves increases; the rise in ethylene accelerates the aging of the leaves and reduces their chlorophyll content. In the presence of bio-fertilizers containing ACC deaminase, ethylene synthesis significantly declines, and this process reduces chlorophyll decomposition and leaf senescence.

The results showed that the irrigation treatment after 160 mm of evaporation reduced the number of capsules per plant and the number of grains per plant, compared with the irrigation treatment after 70 mm. The decrease in grain yield and yield components under water-deficit conditions can be attributed to the decrease in the reduced allocation of the produced photosynthetic substances to the grains and the reduced activity of enzymes involved in sucrose and starch biosynthesis (Srivastava and Suprasanna 2015). By reducing the number of endosperms and amyloplast cells, water shortage that occurs during the grains-filled period

diminishes the potential of plant sinks; therefore, the volume of the sinks (grains) will be reduced due to the decreased capacity of the endosperm for starch accumulation (Saini and Westgate 2000).

The results of our study showed that adequate water supply in different stages of castor growth plays a regulatory role in the grain filling stage and increases grain yield. This aspect may be of greater significance than defense against possible damages caused by abiotic stress (e.g., water stress) during the grain-filling period.

Among the bio-fertilizer treatments, *Citrobacter* increased the number of capsules and 1,000 kernel weight compared to the control treatment. Growth-promoting bacteria lengthen the plant growth period by producing growth-promoting hormones and providing the nutrients required by the plant; in this way, they will have a significant effect on increasing the 1,000-grain weight (Malakouti and Tehrani 2005). Growth-promoting bacteria also increase the absorption of water and minerals by developing the root surface in the soil, improving photosynthesis, and increasing grain weight by transferring photo-assimilating substances to the reproductive parts of the plant. The rise in grain yield under the influence of biological fertilizers has also been reported in fennel (Rezaei Chianeh *et al.*, 2015).

Bio-fertilizers, especially *Citrobacter* and *Azospirillum* + *Citrobacter* under all irrigation treatments significantly increased grain yield compared to the control treatment and moderated the effect of drought stress on grain yield. Therefore, the use of biological fertilizers under normal irrigation conditions and when the plant faces different intensities of water-deficit stress is a suitable solution to promoting grain yield in the castor. The results revealed that the simultaneous use of bio-fertilizers and the super-absorbent significantly increased the number of grains and grain yield in the castor compared to the control treatment. In research on corn, the highest yield and grain yield components were obtained from the combination of super-absorbent and bio-fertilizers (Ebrahimi Chamani *et al.*, 2022). It seems that the combined use of bio-fertilizers and super-absorbent increases the absorption of mineral elements, especially macro elements, the level of photosynthesis, the production of photosynthetic materials, and the transfer of photosynthetic materials to the grains; the sum of these processes increases the number of grains and, ultimately, the grain yield. Zeolite increases nutrient uptake by the plant by enhancing its ability to access nutrients in the roots and eventually improves agronomic traits and grain yield (Ebrahimi Chamani *et al.*, 2022). The application of super-absorbent polymer + EPS-producing bacteria also increases the yield and yield component of maize under drought conditions (Yaseen *et al.*, 2020).

Based on the results, water deficiency harmed the castor seed oil content. The negative effect of drought stress on the seed oil content is due to the adverse effect of water shortage on the seed metabolic processes, disturbed transfer of nutrients to the seeds, and possibly the production of undesirable secondary compounds in oil production. According to Sadeghzadeh and Hazrati (2018), water deficiency decreased the castor oil content, and the maximum oil content was reported in the normal irrigation treatment.

The results showed that treatment with *Citrobacter* increased oil yield at all irrigation levels. The super-absorbent only in two irrigation conditions after 70 and 160 mm of evaporation significantly increased oil yield. The results of the present research revealed increased oil yield in both conditions of super-absorbent application and non-application compared to the control. Oil yield, which is related to grain yield and grain oil content, is one of the most important components of the yield in oil seed crops and decreases under water-deficit stress. As reported by Moghadam *et al.* (2022), the essential oil of *Oncidium ciliatum* was not affected by the application of bio-fertilizers and the super-absorbent, but these treatments induced the synthesis of new compounds.

Accumulation of proline in plants under adverse conditions, including water stress, increases plant resistance due to the various functions of this substance (Hamurcu *et al.* 2020). Under water-deficit conditions, proline and soluble sugars contribute to osmotic regulation, signal transmission, redox balance maintenance, and cell structure stability in plants (Ghafari *et al.* 2019). Proline and soluble sugars play a key role in drought tolerance and adaptation in a wide variety of plant species (Zhang *et al.*, 2021). A study on castor showed that the proline content of the leaves increased with the intensification of water deficiency (Osati *et al.*, 2022).

In the current study, the super-absorbent at all irrigation levels increased the leaf proline content. Drought stress and inoculation with biotics had a synergistic effect on the content of soluble sugar. When the plant is under unfavourable conditions, especially water deficiency, the accumulation of compatible solutions such as sugars inside the organs is intensified. These solutes are organic compounds with low molecular weight and are very soluble. By osmosis regulation, these substances clear ROS, protect membrane stability, and protect the structure of enzymes and proteins, thereby protecting the plant from the adverse effects of water shortage (Farooq *et al.*, 2009). The accumulation of these substances due to abiotic stresses has been confirmed in other studies (Kheirizadeh Arough *et al.*, 2016; Cattivelli *et al.*, 2008).

The increased production of soluble sugars in response to treatment with bio-fertilizers may be due to the rise in the absorption of various food elements, especially nitrogen. The application of bio-fertilizers under stress conditions causes the accumulation of proline and soluble sugars to regulate plant osmosis due to the degradation of proteins (Kheirizadeh *et al.*, 2016). By storing and maintaining moisture in the soil and gradually making it available to the plant, super-absorbent polymer materials prevent the impacts of water shortage, including the reduction of soluble solids. Herein, water stress and bio-fertilizer treatments had a synergistic effect on increasing catalase enzyme activity. Regardless of the bio-fertilizer type, seed treatment raised the catalase enzyme activity at all three levels of irrigation. Besides, the super-absorbent in water-deficit stress treatments increased the catalase enzyme activity.

Considering that environmental conditions and appropriate plant nutrition play a significant role in raising the tolerance level of plants against various stresses (Stamenkovic *et al.*, 2018) In the present study, it is possible that the appropriate and gradual supply of nitrogen, due to the presence of nitrogen-fixing bacteria in bio-fertilizers, has been able to reduce the effects of water deficit on castor by providing more suitable growth conditions for the plant, and as a result, the plant has produced less catalase (Ortiz *et al.*, 2015).

Our results indicated that although the amount of malondialdehyde and peroxidation of cell membrane lipids increased with the intensification of water-deficit stress, treatment with bio-fertilizers reduced the activity of malondialdehyde. Moreover, the super-absorbent reduced the activity of malondialdehyde at different levels of water-deficit stress. Therefore, by improving plant nutritional conditions and regulating the activity of antioxidants, proline, and soluble sugars, biological fertilizers and super-absorbents cleared the destructive free radicals, thereby enhancing the plant's tolerance to drought stress. As such, the plant had an acceptable economic yield under these conditions. The improvement of the antioxidant properties of enzymes due to the use of bio-fertilizers and super-absorbent s under water stress conditions has been proven in other studies (Valipour *et al.*, 2021).

## Conclusions

Water deficiency harmed all the quantitative and qualitative characteristics of castor; however, the use of bio-fertilizers, especially *Citrobacter*, significantly alleviated this effect by improving antioxidant and biochemical properties. The use of super-absorbent s at extreme levels of water shortage stress also had a positive effect on the quantitative and qualitative characteristics of this crop. Thus, the use of bio-fertilizers and super-absorbents in castor cultivation, which encounters periods of drought stress at different intensities, can be a solution to dealing with water-deficit stress.

## Authors' Contributions

All authors collaborated in writing and editing the manuscript. All authors read and approved the final manuscript.

### **Ethical approval** (for researches involving animals or humans)

Not applicable.

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### **Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

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