Rahbari A *et al.* (2021)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 49, Issue 4, Article number 12003
DOI:10.15835/nbha49412003
Research Article

Castor bean (*Ricinus communis* L.) responses to drought stress and foliar application of Zn-nano fertilizer and humic acid: grain yield, oil content, antioxidant activity, and photosynthetic pigments

Ali RAHBARI[1], Jafar MASOUD SINAKI[2]*, Ali DAMAVANDI[2], Shahram REZVAN[2]

[1]*Department of Agriculture, Damghan Branch, Islamic Azad University, Damghan, Iran; ali.rahbari62@gmail.com*
[2]*Department of Agriculture, Production and tTchnology of Herbal Medicines Research Centre, Damghan Branch, Islamic Azad University, Damghan, Iran; jmsinaki2020@gmail.com; damavandi@yahoo.com; rezvanshahram92@gmail.com*

Abstract

Castor bean is considered as an important non-edible oilseed crop and source of castor oil, which has many applications ranging from cosmetics to the biofuels industry. Humic acid (HA) results from organic matter decomposition and is beneficial to plant growth and development. In the present study, a two-year experiment was conducted in Damghan, Iran, to study the physiological responses of castor bean to foliar application of zinc nano-chelate (Zn-nano) and HA under drought stress. The drought stress was used as the main treatment in three levels: normal irrigation as control, irrigation up to 75 BBCH scale (Biologische Bundesantalt, Bundessortenamt und Chemische Industrie) (mild stress), and irrigation up to 65 BBCH (severe stress). Foliar application of HA in three levels (non-application, application of the recommended rate and two times more than the recommended rate), as well as Zn-nano fertilizer in two levels (application at 1.5 part per thousand (ppt) and non-application) as subplots. The drought stress, HA, and Zn-nano fertilizer could significantly affect the number of capsules, the number of seeds, 100-seed weight, seed yield, oil yield, protein percentage and yield, activities of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD), and chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (total Chl) contents. In addition, severe drought stress resulted in reducing the number of capsules (33.9%), the number of seeds (32.7%), 100-grain weight (16.0%), as well as seed (43.0%), oil (59.3%), and protein (29.9%) yield. Based on the results, the highest yield components, oil and protein contents, and photosynthetic pigments were achieved in the foliar application of HA (recommended rate) and Zn-nano fertilizers under normal irrigation during the second year. Further, the foliar application of Zn-nano fertilizer led to a decrease in the activities of CAT, SOD, and POD enzymes. According to partial regression analysis, the recommended rate of HA application the changed the nature of relationships governing the characteristics, especially under drought stress conditions. Finally, the foliar application of HA (recommended rate) and Zn-nano fertilizers could create an excellent resistance to drought stress in castor under dry and semi-arid climate conditions by improving yield and yield components and physiological traits.

Keywords: BBCH scale; castor bean; chlorophyll; humic acid; oil content; Zn-nano fertilizer

Received: 21 Jul 2020. Received in revised form: 22 Aug 2021. Accepted: 09 Sep 2021. Published online: 02 Nov 2021.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Castor bean is an indeterminate, non-edible industrial oilseed crop belonging to the Euphorbiaceae family, which is found in most tropical and subtropical parts of the world (Anjani *et al.*, 2018). The oil content of castor bean accessions was reported to vary between 45.7 to 54.0% (Roman-Fiueroa *et al.*, 2020). The oil of the plant is regarded as the only commercial source of ricinoleic acid (over 85% of the oil), which has many industrial and pharmaceutical applications such as aviation fuels, fuel additives, paints, dyes, biopolymers, perfumes, and biodiesel (Liv *et al.*, 2012; Ramanjaneyulu *et al.*, 2013). Planting and researching castor bean have attracted a lot of attention due to an increased demand for its oil in the world, along with its growth in marginal lands (Sujatha *et al.*, 2008). India, Mozambique, China, and Brazil are the major castor bean producing countries. The results of surveying planting statistics of this plant in Iran indicated that its production and cultivation increased (Sadeghi-Bakhtavari and Hazrati, 2021).

In Iran, most of the land under planting is located in arid and semi-arid. In these areas, quality and quantity yields are severely decreased due to the lack of water resources and stress conditions for plants (Gholinezhad, 2017). Drought stress is considered one of the most important abiotic stress, which negatively affects plants' quality and quantity yield worldwide (Gomes Neto *et al.*, 2018). In addition, castor bean is reportedly drought-tolerant or semi-drought tolerant (Babita *et al.*, 2010; Ostadi *et al.*, 2020). The responses of yield and yield components of castor bean to drought stress rely on environmental and genetic characteristics (Severino and Auld, 2013). Further, the response to drought stress between determinate and indeterminate plants is very different. Regarding determinate plants, anthesis provides a clear division between the vegetative and reproductive phases. The plant responds with a reduced seed number if drought stress occurs before anthesis or reduced seed weight after anthesis (particularly during seed filling). In castor, the response to drought stress is complicated because the plant initiates racemes at different times. Each raceme can adjust the seed number and weight according to environmental conditions and plant source-sink status (Severino and Auld, 2018).

The drought stress enhances the generation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals, which can result in peroxidation lipid, degrading protein, damaging DNA, increasing antioxidant activity enzymes, and ultimately cell death (Zhang *et al.*, 2015; Barros *et al.*, 2017). Enzymatic and non-enzymatic scavenging mechanisms control ROS in plant cells. Numerous studies have shown that the activity of antioxidant enzymes is correlated with plant tolerance to abiotic stresses, including responses to drought stress in wheat (*Triticum aestivum* L.), alfalfa (*Medicago sativa* L.), rice (*Oryza sativa* L.), and chickpea (*Cicer arietinum* L.) (Wang *et al.*, 2009; Qin *et al.*, 2010; Lotfi *et al.*, 2015).

Nutrition management is important in the large-scale cultivation of castor bean or other oilseed crops under drought stress conditions (Machado and La Rovere, 2017). The decrease in nutrient uptake in plants under drought stress may be attributed to the reduced root characteristics and physiological responses such as ABA synthesis (Rouphael *et al.*, 2012). The use of organic and nano fertilizers can be regarded as one of the solutions to this problem. Humic acid (HA) is known as a natural compound resulting from decaying organic matter in soil, peat, and lignin. Furthermore, it can be used in sustainable agriculture (Nardi *et al.*, 2004; Tursun, 2019). Before acting as a fertilizer, HA acts as a soil amendment, which helps increase the number of microorganisms in the soil, thereby improving the physical conditions of the soil. In addition, it can adjust soil pH and affect the activity and/or the concentration of enzymes and hormones. Further, HA can regulate plant growth and increase water use efficiency, leading to an increase in plant resistance to drought and salinity stress, as well as increasing nutrient absorption, germination, and root growth. Finally, all of these attributes can improve the quantity and quality of products (Eneji *et al.*, 2013; Gholami *et al.*, 2018). Karakurt *et al.* (2009) reported that HA application could significantly influence the total Chl content of pepper.

Zinc (Zn) is a vital micronutrient for growing and developing plants and human beings, and Zn deficiency is common in many crops (Ojeda-Barrios *et al.*, 2014). Anderson *et al.* (2018) indicated the significant effect of soil and foliar-applied Zn forms on the growth and yield attributes of lentils. Zinc is necessary for activating several enzymes like dehydrogenase, tryptophan synthetase, and superoxide dismutase (SOD) (Rajiv *et al.*, 2018). Further, Zn fertilization could significantly increase the rice grain yield compared with the unfertilized (Slaton *et al.*, 2005). Prado *et al.* (2008) reported the positive effect of various zinc sources like zinc sulfate and zinc oxide on rice growth. Furthermore, Rajiv *et al.* (2018) observed the highest growth characteristics and grain yield in zinc oxide nanoparticle treatment.

In 1989, Bleiholder *et al.* developed a two-digit decimal coding system for angiosperms, the BBCH-scale (Biologische Bundesan - talt, Bundessortenamt und Chemische Industrie). This scale uses 10 principal stages (0-9), divided each one into 10 secondary (0-9) growth stages; a three digits "extended BBCH-scale" was proposed for certain crops (Delgado *et al.*, 2011). The BBCH scale (Biologische Bundesantalt, Bundessortenamt und Chemische Industrie) has been used to describe the phenological growth stages of numerous horticultural important plant and tree species (Ramírez and Kallarackal, 2015). Attibayeba *et al.* (2010) and Bagheri *et al.* (2013) investigated the phenology of oilseed crops and indicated the significant role of irrigation treatments based on BBCH scale on grain yield, oil percentage, and oil yield of sesame plant.

Due to many applications of castor bean oil, high demand worldwide is estimated at 270,000-360,000 tonnes per annum, among which only 60% can be met with the current production estimates around the world (Mutlu and Meier, 2010). The management of agronomy operations such as fertilization with eco-friendly fertilizers and irrigation methods is considered as one of the strategies to overcome the demand and supply gap (Babita *et al.*, 2010). On the other hand, the production of medicinal plants using organic and nano-fertilizers is a very important and essential factor in obtaining high-quality medicinal crops and free of chemical residues. By considering all of the studies mentioned above, the present study aimed to evaluate the grain yield, oil content, antioxidant activity, and photosynthetic pigment responses of castor bean to foliar application of Zn-nano fertilizer and HA under drought stress conditions.

Materials and Methods

Experimental site, plant materials, and treatments

A two-year experiment was conducted by determining the rate of castor bean tolerance to drought stress, as well as evaluating the effect of Zn-nano chelate and HA fertilizers under different irrigation treatments based on BBCH scale. The experimental design was a split-plot factorial based on a completely randomized block design with three replications in Semnan Province, Iran (Damghan city; lat. 34° 45́, long. 53° 55́ E, altitude 117 m, rainfall 140 mm) during two consecutive years including 2015-2016 and 2016-2017. Climatic parameters such as temperature and precipitation during the two years of the experiment are presented in Table 1.

Table 1. Climate conditions (temperature and precipitation) during the growing season 2015-2016 and 2016-2017

Year	Climate parameters	Dec	Nov	Oct	Sep	Aug	Jul	Jun	May	April	Mar	Feb	Jan	Average
2015-2016	Minimum temperature (°C)	4.0	6.7	15.1	24.8	28.8	29.8	27.0	22.4	16.1	10.0	2.4	2.4	15.8
	Total monthly precipitation (mm)	9.5	9.3	1.1	1.1	1.1	1.9	3.4	10.2	7.1	15.9	11.6	3.6	6.3
2016-2017	Minimum temperature (°C)	5.5	7.2	14.3	26.9	28.6	29.5	26.6	24.2	17.9	12.3	3.9	3.5	16.7
	Total monthly precipitation (mm)	7.11	0.8	6.2	6.2	6.2	6.2	5.4	5.7	5.8	7.13	4.11	1.3	6.6

Drought stress treatment was conducted at three levels, including normal irrigation (control), irrigation cut-off at 75 BBCH stage (mild stress) and irrigation cut-off at 65 BBCH stage (severe stress), which were located in the main plot. In addition, foliar application of HA compounds in three levels such as non-application (control), recommended rate, and two times more than that of recommended rate, as well as Zn-nano fertilizer in application and non-application levels were conducted in the sub-plots.

In order to analyse the data and provide the required soil elements, the sample from different parts of the farm soil was considered (Table 2). As shown, the soil was clay loam (29% sand, 42% silt, and 29% clay). Then, the field was ploughed, and accordingly the two discs were perpendicularly disrupted. Each plot (5 × 3 m) consisted of 4 rows of planting with 30 × 70 cm intra- and inter-row distances. The intervals of the plot and replicating each other were 1.5 and 2.5 m, respectively. Before planting, 100 kg of superphosphate triple and urea, 50 kg sulfur for increasing the solubility of micro and macronutrients, and 50 kg potassium sulfate per hectare were used based on soil analysis and reference review (Valadabadi *et al.*, 2010).

Table 2. Some physical and chemical properties of soil in the experimental area (depth of 0-30 cm)

Texture	pH	Electrical conductivity ($dS.m^{-1}$)	Organic matter (%)	Total nitrogen (%)	Phosphorus ($mg.kg^{-1}$)	Potassium ($mg.kg^{-1}$)
Loam clay	7.9	4.56	0.29	1.02	8.60	160

Seed planting (castor bean 'Damghan' cv.) was conducted in late May for two consecutive years. Bulk density (BD), field capacity (FC), and permanent wilting point (PWP) for root development depth were 1.81, 29.54, and 12.6, respectively (Pansu and Gautheyrou, 2007). Through regular irrigation, the moisture content of all tested plots was maintained before flowering at the FC level. Irrigation cut-off was considered for 65 BBCH treatment (equivalent to severe stress) in 50% flowering and 75 BBCH treatment (equivalent mild stress) in 50% seed formation while ripening was performed based on phenological stages. Regarding HA treatment, Humabon source (brand of Bonasia produced in Iran) including 72% HA, 15.5% fulvic acid and 12% potassium dioxide was used at the beginning of flowering and two weeks later. The recommended rate of the company was 250 g per 1000 L water. Further, like HA, Zn-nano fertilizer was used in two stages from Zinc-nano chelate and 12% source was produced by Khazra Co. with 1.5 part per thousand (ppt) concentration. The spraying was conducted in the evening due to the lower temperature and humidity of the evening hours, the reduction of evaporation, and better penetration of the solution into epidermis cells.

Physiological traits

Furthermore, the sampling (leaves) was cut off two weeks after the last irrigation and all of the plots were taken simultaneously (in 3 replicates) in order to measure some physiological traits such as photosynthetic pigments and antioxidant enzyme activity.

Photosynthetic pigments content

Then, the chlorophylls a, b, and total chlorophyll were determined based on the method of Lichtenthaler (1987). According to this method, 0.25 g of fresh sample was extracted by using 5 mL 80% acetone. In addition, the extract was centrifuged at 11000 rpm for 10 min. Accordingly, the optical density (O.D.) of the extract was measured at the wavelengths of 646.8 and 663.2 nm in order to estimate chlorophyll a (Chl a) and chlorophyll b (Chl b), respectively, by using a spectrophotometer (Hitachi-U2001, Tokyo, Japan). The amount of pigment available in each sample was calculated according to the following equations:

$$\text{Chl a (mg/gr FW)} = 12.7\ (\text{O.D of } 663) - 2.69\ (\text{O.D of } 645) \times \frac{v}{w \times 1000}$$

$$\text{Chl b (mg/gr FW)} = 22.9\ (\text{O.D of } 645) - 4.68\ (\text{O.D of } 663) \times \frac{v}{w \times 1000}$$

$$\text{Total Chl (mg/gr FW)} = 20.2\ (\text{O.D of } 645) + 8.02\ (\text{O.D of } 663) \times \frac{v}{w \times 1000}$$

Whereas W: the fresh weight by grams for extracted tissue; V: the final size of the extract in 80% acetone; O.D: optical density at a specific wavelength.

Antioxidant enzyme activities

Regarding the assay of activities for POD, SOD, and CAT, 0.25 g of the fresh leaf samples were homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 1% soluble polyvinylpyrrolidone. Then, the homogenate was centrifuged at 13.000 × g for 21 min at 4 °C and the supernatant was used for assaying the activities of antioxidant enzyme (Noman *et al.*, 2015) as described in the next procedures.

Peroxidase (POD) activity

The POD activity was assayed by the method proposed by Chance and Maehly (1995). An aliquot of the tissue extract (100 µL) was added to the assay solution including 3 mL of reaction mixture containing 13 mM guaiacol, 5 mM H_2O_2, and 50 mM sodium (Na)-phosphate (pH 6.5). An increase in the optical density at 470 nm for 1 min at 25 °C was recorded using a spectrophotometer.

Superoxide dismutase (SOD) activity

The total SOD activity was determined by Beauchamp and Fridovich's (1971) method. The 1.5 mL reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 µM EDTA, 13 mM methionine, 75 µM NBT, 2µM riboflavin, and 50 µL enzyme extract. Finally, riboflavin was added, and the tubes were shaken and illuminated with two 20-W fluorescent tubes. The reaction was allowed to proceed for 15 min after which the lights were turned off and the tubes were covered with a black cloth. The absorbance of the reaction mixture was read at 560 nm (Ahmadi *et al.*, 2010).

Catalase (CAT) activity

The total CAT activity was measured based on the method proposed by Chance and Maehly (1995). The reaction mixture (1.5 mL) consisted of 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, 20 mM H_2O_2, and 50 µL enzyme extract. The reaction was started by adding the enzyme extract. Based on the results, a decrease in H_2O_2 was monitored at 240 nm (Ahmadi *et al.*, 2010).

Yield attributes

Based on the harvest conducted in September during the physiological maturity stage, the capsules were brownish-yellow. It is worth noting that this stage occurred a few days earlier than non-stress and moderate stress treatments for severe stress treatment. Further, five plants per plot were selected to measure the number of capsules per plant, number of seeds per capsule, and 100-seed weight. Accordingly, two central rows of each plot were harvested at the physiological maturity stage after eliminating marginal effects (0.5 m) in order to determine grain yield (Gislum *et al.*, 2018).

Seed oil and protein content

In addition, the seed oil percentage was determined by using the Soxhlet method according to the following details Leiboritz *et al.* (1987). The grains were first dried by using the autoclave, and then powdered. Accordingly, the samples were placed in the upper part of the Soxhlet apparatus by using a cellulose cartridge. The volatilization of the solvent diethyl ether in the bottom of the apparatus resulted in solubilizing the oil, which was isolated, collected, and weighed following the evaporation of the solvent. Seed protein percentage was estimated by Bradford's (1976) method by using a spectrophotometer in the wavelengths of 590 nm. Finally, the seed oil and protein percentages were multiplied in the seed yield in order to calculate the oil and protein yield.

Statistical analysis

All of the data obtained during two years were analysed by SAS software (Statistical Analysis Software, 9.2). A split-plot factorial based on completely randomized blocks design was performed to estimate the variance components of the effects of drought stress, HA, Zn-nano fertilizer, and their interactions. Further, the differences among the treatments were evaluated by LSD (least significant difference) only when the ANOVA F-test indicated the significance level of 0.05. Partial regression coefficients were calculated in Microsoft Excel software based on the method described by Akintunde (2012) and stepwise regression was calculated by Minitab 19 software.

Results

Yield and yield components

Based on the ANOVA results (Table 3), the effects of year (Y), drought stress (D), HA, and Zn-nano fertilizer were significant on the number of capsule and seed, 100-seed weight, and seed yield. During the second year, yield and yield components were significantly superior to those in the first year. Furthermore, severe drought stress led to a reduction in the number of capsule and seed (33.9 and 31.22%, respectively), 100-seed weight (16.0%), and seed yield (43.0%). Regarding yield and yield component characteristics, the results indicated that mild stress (irrigation up to 50% seed ripening equals 75 BBCH scale) with normal irrigation treatment (control) had insignificant effects. In addition, the application of HA, recommended or two times more than that of the recommended rates, could significantly improve the number of capsules and seed, 100-seed weight, and seed yield. However, the most favourable mean of these traits was observed in the application of HA at the recommended level. On the other hand, the foliar application of Zn-nano fertilizer resulted in increasing the number of capsules and seeds (13.35 and 13.35%, respectively), 100-seed weight (8.78%), and seed yield (21.46%), compared to the non-application treatment. Regarding the interaction of Y in D, the highest means for the number of capsule and seed (365 and 1095 no. per m^2, respectively), 100-seed weight (24.1 g), and seed yield (2673.8 kg ha^{-1}) were observed in normal irrigation (non-stress condition) during the second year (Figure 1). Based on the results, the foliar application of Zn-nano fertilizer led to the moderate reduction of 100-seed weight under severe drought stress compared to those in the non-application treatment. Under non-application of Zn fertilizer on 100-seed weight, 25.01% reduction occurred due to severe drought stress, compared to that of the normal irrigation (Figure 2).

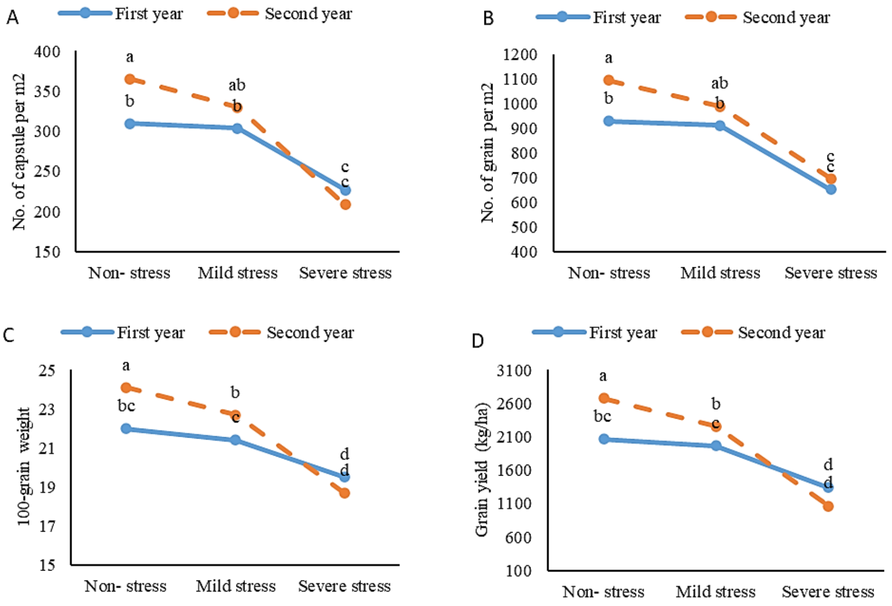

Figure 1. Effect of drought stress (normal irrigation as control, irrigation up to 50% seed ripening equal 75 BBCH as mild stress, and irrigation up to 50% flowering equal 65 BBCH as severe stress) on number of capsule (A), number of grain (B), 100-grain weight (C), and grain yield (D) during two consecutive years
Different letters on top of column indicate significant different at P ≤ 0.05 according to the LSD test.

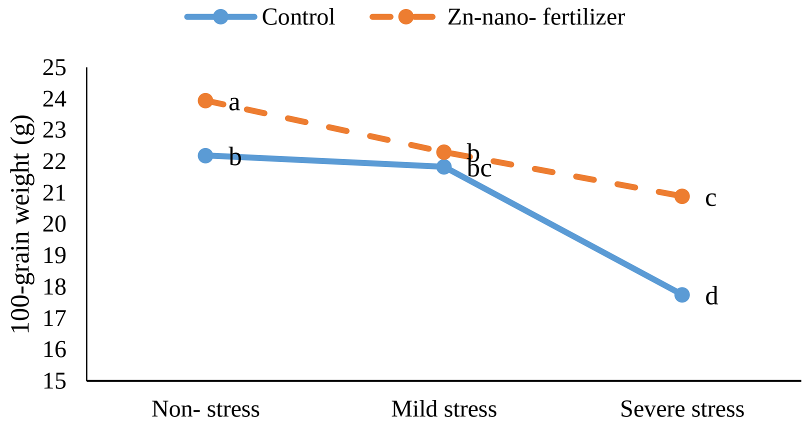

Figure 2. Effect of drought stress (normal irrigation as control, irrigation up to 50% seed ripening equal 75 BBCH as mild stress, and irrigation up to 50% flowering equal 65 BBCH as severe stress) on 100-grain weight under Zn-nano fertilizer treatment

Different letters on top of column indicate significant different at P ≤ 0.05 according to the LSD test.

Table 3. Analysis of variance indicating the effects of drought stress (D), humic acid (HA), and Zn-nano fertilizer (Zn) on yield and yield components and protein and oil contents of castor bean (*Ricinus communis* L.)

Treatments	No. of capsule per m^2	No. of seed per m^2	100-seed weight (g)	Seed yield (kg ha^{-1})	Seed oil percentage	Seed oil yield (kg ha^{-1})	Seed protein percentage	Seed protein yield (kg ha^{-1})
Year (Y)								
First	280.2±75.6 b	840.6±226.8 b	21.0±2.3 b	1790.4±594.1b	39.0±6.3 a	718.9±307.5 b	23.9±2.3 b	424.0±130.4 b
Second	304.6±81.3 a	914.0±244.0 a	21.9±3.1 a	2065.8±767.8 a	40.2±8.6 a	873.6±439.4 a	26.2±4.3 a	528.5±183.9 a
LSD (*p* =0.05)	21.5	64.6	0.58	162.3	1.75	77.4	0.68	39.9
Drought stress (D)								
Normal irrigation	337.4±64.7 a	1012.3±194.1 a	23.0±2.7 a	2369.1±655.2 a	45.9±3.5 a	1097.2±341.8a	22.1±1.7 c	527.6±160.8 a
Mild stress	316.8±48.2 a	950.5±144.8 a	22.0±2.5 a	2110.4±365.7 a	39.6±7.5 b	845.9±197.3 b	24.9±3.1 b	531.9±114.9 a
Severe stress	223.0±70.5 b	696.0±211.5 b	19.3±1.5 b	1350.0±537.7 b	33.33±4.9 c	445.7±276.0 c	28.1±3.0 a	369.4±169.4 b
LSD (*p* =0.05)	36.8	110.6	1.29	270.1	2.31	125.7	0.69	64.8
Humic acid (HA)								
Control	257.8±75.8 b	773.5±227.4 b	20.1±2.9 c	1602.0±640.9 c	36.2±6.6 c	604.6±314.3 c	23.0±2.3 b	363.0±135.6 c
Recommended	322.7±75.1 a	968.1±225.3 a	22.6±2.2 a	2227.1±673.8 a	43.2±7.8 a	983.2±386.1 a	25.7±3.7 a	599.1±142.2 a
Twice to that of recommended	296.7±74.5 a	890.3±223.6 a	21.6±2.7 b	1955.2±647.1 b	39.4±6.6 b	801.0±363.9 b	26.4±3.7 a	506.8±160.0 b
LSD (*p* =0.05)	24.4	79.2	0.72	198.7	2.14	94.8	0.83	48.8
Zn-nano-fertilizer (Zn)								
Non-application	274.1±76.8 b	822.4±230.4 b	20.5±2.9 b	1741.3±675.1b	39.2±6.7 a	714.1±357.8 b	24.8±3.4 a	424.1±151.5 b
Application	310.7±77.8 a	932.2±233.4 a	22.3±2.4 a	2114.9±674.0 a	40.0±8.3 a	878.4±397.6 a	25.3±3.8 a	528.4±167.0 a
LSD (*p* =0.05)	21.5	64.6	0.58	162.3	1.75	77.4	0.68	39.9
Statistics								
Year (Y)	*	**	*	**	NS	**	**	**
Drought stress (D)	**	**	**	**	*	**	**	**
Humic acid (HA)	*	**	*	**	*	*	*	**
Zn-nano-fertilizer (Zn)	*	**	**	*	NS	**	NS	*
Y × D	*	*	**	**	NS	**	**	*
Y × HA	NS	NS	NS	NS	NS	NS	**	NS
Y × Zn	NS	NS	NS	NS	**	*	**	NS
D × HA	NS	NS	NS	NS	NS	NS	*	NS
D × Zn	NS	NS	**	NS	NS	NS	NS	NS
HA × Zn	NS	NS	NS	NS	NS	NS	NS	NS
Y × D × HA	NS	NS	NS	NS	NS	NS	NS	NS
Y × D × Zn	NS	NS	NS	NS	NS	NS	NS	NS
Y × HA × Zn	NS	NS	NS	NS	*	NS	NS	NS
D × HA × Zn	NS	NS	NS	NS	NS	NS	NS	NS
Y × D × HA × Zn	NS	NS	NS	NS	NS	NS	NS	NS

(Means ± SD); LSD: least significant difference; NS: non-significant; * and **, significant at probably levels 5 and 1%, respectively

Grain oil and protein contents

As shown in Table 3, the effect of Y, D, HA, and Zn-nano fertilizer on oil and protein yield was significant. In addition, a significant effect of D and HA was observed on oil and protein percentage. Further, the protein and oil contents (percentage and yield) were higher in the second year than those in the first year (Table 3). The results indicated that severe drought stress treatment (irrigation up to 65 BBCH) resulted in decreasing oil percentage and increasing protein percentage. On the other hand, severe drought stress led to a reduction in oil and protein yield (59.3 and 29.9%, respectively), compared to that of the control treatment. Additionally, the highest oil percentage, and oil and protein yield were achieved in the foliar application of HA (recommended rate) and Zn-nano fertilizers under normal irrigation during the second year. Interaction effect of Y × D was significant on oil yield, protein percentage, and protein yield (Table 3). In addition, the highest grain oil yield (1251.1 kg ha^{-1}) and grain protein yield (600.7 kg ha^{-1}) were observed in the normal irrigation (non-stress condition) during the second year (Figure 3). Regarding grain protein percentage, a significant increase occurred under severe drought stress condition (irrigation up to 65 BBCH) during two years of the experiment (Figure 3). As for the interaction D in HA, the highest protein percentage was observed in the foliar application of the recommended rate and two times more than that of the recommended rate in HA under severe drought stress condition (28.94 and 30.01%, respectively). The lowest amount for this trait was achieved in non-application of HA under normal irrigation treatment (Figure 4).

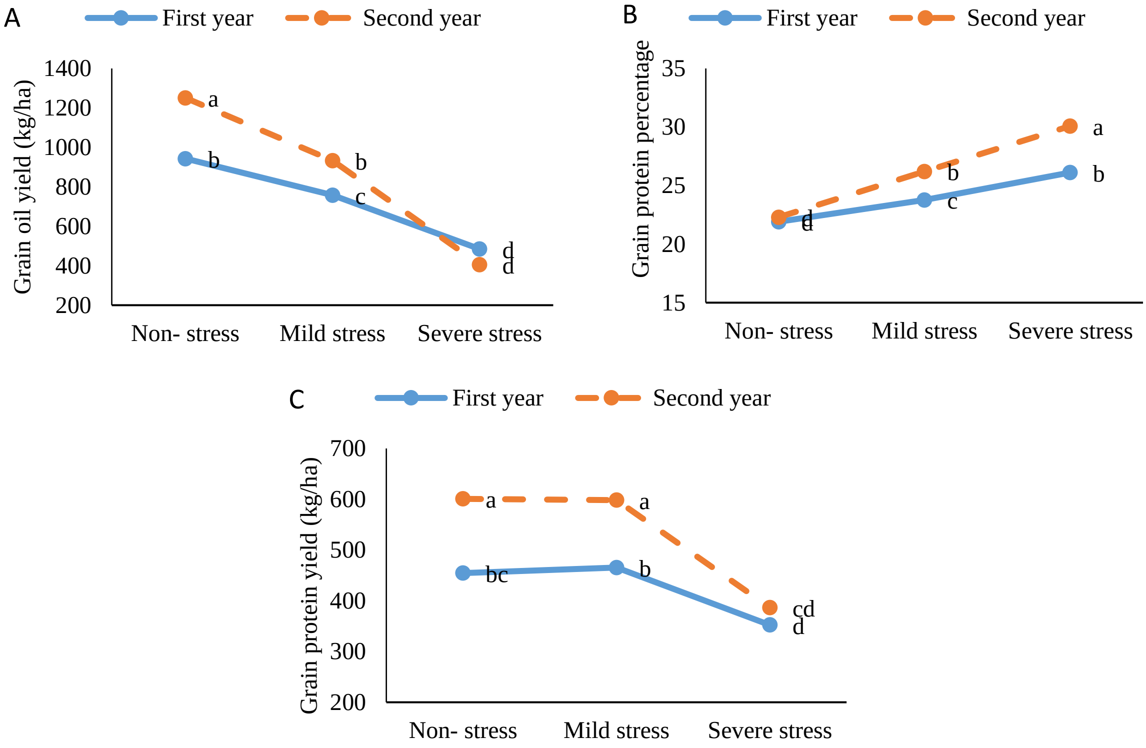

Figure 3. Effect of drought stress (normal irrigation as control, irrigation up to 50% seed ripening equal 75 BBCH as mild stress, and irrigation up to 50% flowering equal 65 BBCH as severe stress) on grain oil yield (A), grain protein percentage (B), and grain protein yield (C) during two consecutive years
Different letters on top of column indicate significant different at P ≤ 0.05 according to the LSD test.

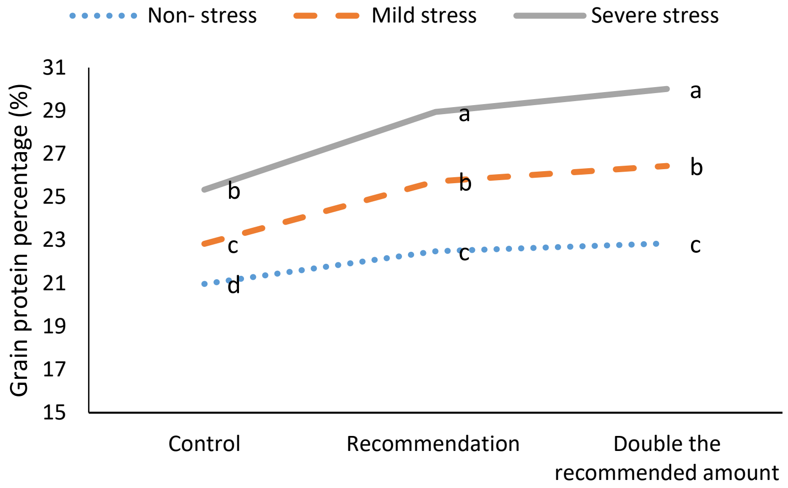

Figure 4. Effect of drought stress (normal irrigation as control, irrigation up to 50% seed ripening equal 75 BBCH as mild stress, and irrigation up to 50% flowering equal 65 BBCH as severe stress) and foliar application humic acid (control, recommended rate, and twice to that of recommended rate) on grain protein percentage

Different letters on top of column indicate significant different at P ≤ 0.05 according to the LSD test.

Antioxidant enzymes activity

The results of ANOVA indicated the significant effects of Y and D on catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) activities (Table 4). Regarding the year of the experiment, the first year had the highest activity for CAT, SOD, and POD enzymes (Table 4). Further, the antioxidant enzymes activity increased under drought stress. Furthermore, the highest CAT and SOD activities (3.74 U min. mg protein^{-1} and 2.36 U mg protein^{-1}, respectively) were obtained in severe stress conditions (irrigation up to 65 BBCH). Additionally, the highest POD activity was achieved in mild stress (irrigation up to 75 BBCH) while the lowest activity for all three enzymes was observed in the control treatment (normal irrigation) (Table 4). In addition, the application of HA in the recommended rate resulted in decreasing CAT and POD activities significantly (33.4 and 27.8%, respectively). As for the non-application (control) and two times more than that of the recommended level in HA, an increase occurred in CAT, SOD, and POD activities, compared to the recommended treatment (Table 4). Based on the results, the foliar application of Zn-nano fertilizer led to a reduction in the activities related to CAT, SOD, and POD enzymes (Table 4). Further, the interaction of Y × D × HA × Zn significantly affected SOD and POD (Table 4). The highest SOD enzyme activity was achieved in the co-application of HA (recommended rate) and Zn-nano fertilizers under severe drought stress during the first year (2.91 U mg protein^{-1}). Furthermore, non-application of Zn with the application of two times more than that of the recommended treatment for HA (2.79 U mg protein^{-1}) and foliar application of Zn-nano fertilizer with non-application of HA (2.88 U mg protein^{-1}) indicated a high SOD activity under severe drought stress during the first year (Table 5). The highest POD activity was observed in the application or non-application of Zn fertilizer with non-application of HA under severe drought stress during the first year (5.92 and 5.91 U min. mg protein^{-1}, respectively). Additionally, the co-application of HA and Zn-nano fertilizers under mild stress led to the high activity of the POD enzyme (5.88 U min mg protein^{-1}) during the first year (Table 5).

Table 4. Analysis of variance indicating the effects of drought stress (D), humic acid (HA), and Zn-nano fertilizer (Zn) on antioxidant enzyme activity and photosynthetic pigments of castor bean (*Ricinus communis* L.)

Treatments	CAT (U.min^{-1} .mg protein)	SOD (U.mg protein^{-1})	POD (U.min^{-1} .mg protein)	Chl a (mg.g^{-1} FW)	Chl b (mg.g^{-1} FW)	Total Chl (mg.g^{-1} FW)
Year (Y)						
First	3.59±1.3 a	2.14±0.4 a	4.46±1.2 a	20.17±2.7 b	10.85±2.8 a	31.02±4.1 b
Second	2.30±1.08 b	1.83±0.2 b	3.82±1.2 b	23.74±2.9 a	10.27±4.9 a	34.01±6.8 a
LSD (*p*=0.05)	0.36	0.10	0.29	0.69	0.90	0.86
Drought stress (D)						
Normal irrigation	1.78±0.4 c	1.66±0.2 c	3.40±1.0 c	22.22±3.4 b	12.59±2.9 a	34.82±5.7 a
Mild stress	3.30±0.8 b	1.93±0.6 b	4.77±1.1 a	23.71±3.1 a	6.91±3.2 b	35.89±2.8 a
Severe stress	3.74±0.6 a	2.36±0.4 a	4.25±1.0 b	19.93±2.1 c	12.17±3.1 a	26.84±3.5 b
LSD (*p*=0.05)	0.28	0.11	0.24	1.19	1.67	1.15
Humic acid (HA)						
Control	3.31±1.3 a	2.05±0.4 a	4.50±1.5 a	21.28±3.2 b	8.69±3.6 b	29.97±5.0 b
Recommended	2.48±1.0 b	1.94±0.5 a	3.52±1.0 b	22.33±4.0 a	11.88±4.2 a	34.21±7.1 a
Twice to that of recommended	3.04±1.1 a	1.96±0.5 a	4.40±1.0 a	22.26±2.5 a	11.10±3.5 a	33.37±4.1 a
LSD (*p*=0.05)	0.44	0.12	0.36	0.84	1.10	1.05
Zn-nano-fertilizer (Zn)						
Non-application	3.15 ±1.2 a	2.00±0.4 a	4.22±1.4 a	21.82±2.8 a	10.11±3.7 b	31.93±4.7 b
application	2.74±1.1 b	1.97±0.5 a	4.06±1.2 a	22.09±3.7 a	11.01±4.2 a	33.10±6.7 a
LSD (*p*=0.05)	0.36	0.10	0.29	0.69	0.90	0.86
Statistics						
Year (Y)	*	*	**	*	NS	*
Drought stress (D)	**	**	*	*	**	*
Humic acid (HA)	*	NS	*	**	**	*
Zn-nano-fertilizer (Zn)	*	NS	NS	NS	*	*
Y × D	**	**	NS	NS	**	**
Y × HA	NS	*	NS	NS	**	**
Y × Zn	NS	**	*	**	*	**
D × HA	NS	NS	**	**	NS	**
D × Zn	NS	NS	NS	NS	NS	*
HA × Zn	NS	NS	NS	NS	*	*
Y × D × HA	NS	NS	NS	NS	NS	NS
Y × D × Zn	NS	NS	NS	NS	NS	NS
Y × HA × Zn	NS	*	NS	NS	*	*
D × HA × Zn	NS	NS	NS	NS	NS	*
Y × D × HA × Zn	NS	*	**	NS	**	**

(Means ± SD); LSD: least significant difference; NS: non-significant; CAT: catalase activity; SOD: superoxide dismutase activity; POD: peroxidase activity; Chl a: chlorophyll a; Chl b: chlorophyll b; Total Chl: Total chlorophyll.

Table 5. Interaction effects of year × drought stress × humic acid × Zn-nano fertilizer on antioxidant enzyme activities and photosynthetic pigments of castor bean (*Ricinus communis* L.)

Year	Drought stress	Humic acid	Zn-nano fertilizer	SOD (U.mg protein^{-1})	POD (U.min^{-1} .mg protein)	Chlorophyll b (mg.g^{-1} FW)	Total chlorophyll (mg.g^{-1} FW)
First year	Normal irrigation (control)	Control	Control	1.66±0.1 g.j	4.09±1.6 d.k	10.80±0.03 c.i	29.86±1.9 i.l
			Application	1.69±0.3 g.j	2.5±0.6 mn	10.56±1.9 c.i	27.99±2.8 j.m
		Recommended	Control	1.83±0.1 e.j	2.96±0.2 k.n	11.69±1.0 c.h	32.87±1.5 f.i
			Application	1.73±0.1 f.j	3.68±0.4 g.n	12.79±3.7 b.g	34.96±3.4 d.g
		Twice to that of recommended	Control	1.66±0.1 g.j	3.92±0.4 e.l	13.03±1.8 b.g	32.71±2.1 f.i
			Application	1.8±0.2 e.j	3.91±0.9 e.l	14.13±0.3 bc	34.64±1.7 d.g
	Severe drought stress (65 BBCH)	Control	Control	2.2±0.1 bcd	5.92±0.7 a	7.47±0.3 i.n	26.09±1.6 mno
			Application	2.88±0.3 a	5.91±0.9 a	6.75±0.3 j.n	24.10±2.8 no
		Recommended	Control	2.5±0.2 abc	3.98±0.3 e.l	10.11±4.6 d.k	27.00±1.9 k.n
			Application	2.91±0.4 a	3.36±0.2 i.n	9.65±1.5 e.l	26.00±2.8 mno
		Twice to that of recommended	Control	2.79±0.4 a	5.04±0.3 a.e	9.38±3.4 f.l	30.59±3.1 h.k
			Application	2.52±0.4abc	4.34±0.7 c.j	8.04±1.8 h.m	27.03±1.8 k.n
	Mild drought stress (75 BBCH)	Control	Control	2±0.1 d.i	5.31±1.1 a.d	12.94±4.2 b.g	36.33±2.6 c.f
			Application	2.19±0.2 b.e	4.3±1.1 c.j	9.32±1.1 f.l	31.50±1.3 g.i
		Recommended	Control	2.01±0.1 d.h	5.4±0.7 abc	9.34±2.2 f.l	33.08±0.6 f.i
			Application	2.12±0.3 c.f	4.11±0.7 d.k	13.02±1.5 b.g	34.53±2.8 d.g
		Twice to that of recommended	Control	1.89±0.1 d.j	5.76±0.3 ab	12.92±1.6 b.g	34.02±1.1 e.h
			Application	2.2±0.4 b.e	5.88±0.5 a	13.35±1.9 b.e	35.10±0.4 d.g
Second year	Normal irrigation (control)	Control	Control	1.7±0.2 g.j	2.71±1.1 lmn	9.24±0.03 g.l	30.24±2.8 i.l
			Application	1.56±0.04 j	3.76±1.1 f.m	10.55±3.7 c.j	33.04±1.9 f.i
		Recommended	Control	1.58±0.05 j	3.41±0.7 i.n	12.55±1.0 c.g	38.14±3.4 cd
			Application	1.59±0.1 ij	2.57±0.1 mn	19.39±0.3 a	49.34±1.5 a
		Twice to that of recommended	Control	1.65±0.1 g.j	3.64±1.0 h.n	13.88±1.8 bcd	37.82±1.7 cd
			Application	1.54±0.1 j	3.64±0.6 h.n	12.52±3.4 c.g	36.25±2.1 c.f
	Severe drought stress (65 BBCH)	Control	Control	2.54±0.1 ab	5.19±0.5 a.e	2.26±0.3 o	22.97±0.7 o
			Application	1.73±0.09 f.j	4.44±0.3 c.j	4.06±1.5 no	25.89±1.6 mno
		Recommended	Control	2.12±0.1 c.f	2.46±0.02 n	7.87±3.6 h.n	28.39±2.8 j.m
			Application	1.85±0.09 e.j	2.97±0.4 k.n	6.69±1.8 k.n	26.81±1.9 lmn
		Twice to that of recommended	Control	2.04±0.1 d.g	3.52±0.5 h.n	4.62±3.4 mno	26.84±1.8 lmn
			Application	2.19±0.09 b.e	3.93±0.2 e.l	5.97±1.1 l.o	30.40±3.1 h.l
	Mild drought stress (75 BBCH)	Control	Control	2.54±0.1 ab	4.93±1.1 a.g	7.25±4.2 i.n	32.48±1.3 ghi
			Application	1.89±0.08 d.j	5.01±0.7 a.f	13.03±1.5 b.g	39.19±2.6 bc
		Recommended	Control	1.62±0.02hij	3.19±0.6 j.n	13.11±1.5 b.f	37.38±2.8 cde
			Application	1.5±0.1 j	4.16±0.3 c.k	16.39±3.8 ab	42.08±5.5 b
		Twice to that of recommended	Control	1.71±0.1 f.j	4.58±0.2 b.i	13.43±1.6 b.e	37.95±0.4 cd
			Application	1.59±0.1 j	4.7±0.1 a.h	11.98±1.2 c.g	37.06±1.1 cde

(Means ± SD); Different letters on each column indicate significantly different at $P \leq 0.05$ according to the LSD test.
SOD: superoxide dismutase activity; POD: peroxidase activity

Correlation, stepwise, and partial regression coefficients

According to the results of correlation coefficients, seed yield had positively correlation with number of capsules, number of seed, 100-seed weight, and total Chl and negatively correlation with CAT and SOD activities (Table 6).

A stepwise regression analysis was computed in order to remove the effect of non-effective traits in the regression model on seed yield. The stepwise regression analysis under no-stress conditions revealed that 99.30% of the seed yield variation was explained by a model which includes the number of seed and 100-seed weight (Table 7). On the other hand, under drought stress conditions revealed that 99.77% of the seed yield variation was explained by a model which includes thr number of seeds, 100-seed weight, SOD and POD activities, and total Chl content (Table 8).

Table 6. Correlation coefficients among physiological and quality attributes of castor bean under drought stress

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1													
2	1	1												
3	0.65	0.65	1											
4	0.96	0.96	0.82	1										
5	0.58	0.58	0.53	0.61	1									
6	0.91	0.91	0.79	0.96	0.8	1								
7	-0.27	-0.27	-0.22	-0.28	-0.22	-0.27	1							
8	0.89	0.89	0.75	0.92	0.56	0.88	0.1	1						
9	-0.42	-0.42	-0.41	-0.45	-0.53	-0.53	0.08	-0.42	1					
10	-0.41	-0.41	-0.46	-0.47	-0.51	-0.53	0.16	-0.43	0.71	1				
11	-0.25	-0.25	-0.23	-0.26	-0.36	-0.32	0	-0.25	0.49	0.33	1			
12	0.43	0.43	0.42	0.48	0.26	0.46	0.06	0.51	-0.38	-0.37	0.03	1		
13	0.53	0.53	0.54	0.57	0.58	0.61	-0.32	0.49	-0.26	-0.41	-0.16	0.25	1	
14	0.62	0.62	0.61	0.67	0.55	0.69	-0.18	0.63	-0.4	-0.5	-0.1	0.74	0.83	1

1: Number of capsules; 2: Number of seed; 3: 100-seed weight; 4: Seed yield; 5: Seed oil percentage; 6: seed oil yield; 7: Seed protein percentage; 8: Seed protein yield; 9: CAT activity; 10: SOD activity; 11: POD activity12: Chl a; 13: Chl b: 14: Total Chl.

Table 7. Stepwise regression for seed yields as dependent variables and other traits as independent variables under no-stress condition

Attributes entered into the model	Coefficients	SE Coef	T-Value	P-Value
Constant	-2214.4	78.1	-28.36	0.000
Number of seeds (X1)	2.2794	0.0628	36.30	0.000
100-seed weight (X2)	98.66	4.42	22.32	0.000
R-Sq(adj)= 99.30%				
Y= -2214.4 +2.2794 X1 + 98.66 X2				

Table 8. Stepwise regression for seed yields as dependent variables and other traits as independent variables under drought stress

Attributes entered into the model	Coefficients	SE Coef	T-Value	P-Value
Constant	-425	102	-4.15	0.000
Number of seeds (X1)	1.3302	0.0644	20.65	0.000
100-seed weight (X2)	50.89	2.49	-11.42	0.000
SOD activity (X3)	13.53	9.26	1.46	0.149
POD activity (X4)	-9.32	3.72	-2.51	0.015
Total Chl (X5)	2.489	0.950	2.62	0.011
R-Sq(adj)= 99.77%				
Y= -425 +1.3302 X1 + 50.89 X2 +13.53 X3 -9.32 X4 + 2.489 X5				

Partial regression coefficients were estimated to determine the relative importance of traits affecting seed yield (Table 9). Considering that the data were standardized before the regression analysis, therefore, the regression coefficients were comparable with each other and hence, the higher coefficient represents the greater weight of the corresponding traits. According to the results of the current study, the direct effects of traits on seed yield varied when the plants were exposed to foliar HA. At the without drought stress and HA1 level (non-application of HA), the highest positive direct effects on seed yield belonged to number of capsules and seeds while, at mild drought stress and HA2 level (recommended concentration of HA), number of capsules and seeds, Chl a, and total Chl and severe drought stress and HA2 level (Twice to that of recommended of HA), Chl a and total Chl had the highest positive direct effects. According to these results, foliar application of HA seems to increase the photosynthetic pigments which leads to decrease in the intensity of drought stress. Results of correlation, stepwise, and partial regression coefficients indicated that the number of seeds and 100-seed weight were the high importance in seed yield under stress and non-stress conditions, but photosynthetic pigments and antioxidant enzymes activities (especially severe drought stress) were more important under stress conditions. As reported by Sabaghnia *et al.* (2010), number of seed and 100-seed weight were the most important traits related to seed yield under both normal and water-stressed conditions.

Table 9. Partial regression coefficients of seed yield over some agro-physiological traits in castor bean as influenced by different drought stress and foliar humic acid (HA)

Model	Normal irrigation			Mild stress			Severe stress		
	HA1	HA2	HA3	HA1	HA2	HA3	HA1	HA2	HA3
Intercept	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Number of capsules	**0.391**	0.413	0.401	0.320	**0.428**	0.359	0.200	0.305	0.266
Number of seeds	**0.311**	0.385	0.354	0.243	**0.403**	0.315	0.195	0.294	0.243
100-seed weight	0.266	0.351	0.277	0.149	0.355	0.246	0.138	0.222	0.163
CAT	-0.165	-0.199	-0.175	-0.295	-0.208	-0.222	-0.352	**-0.400**	-0.350
SOD	0.082	0.135	0.075	-0.095	0.135	0.100	0.165	0.302	0.247
POD	-0.095	0.053	0.067	-0.200	-0.300	-0.251	-0.253	**-0.387**	-0.305
Chl a	0.258	0.302	0.266	0.295	0.502	0.325	0.195	0.677	0.284
Chl b	0.102	0.158	0. 127	0.123	0.120	0.128	0.068	0.250	0.100
Total Chl	0.281	0.316	0.300	0.324	**0.584**	0.333	0.207	**0.794**	0.280

HA1: non-application as control; HA2: Recommended concentration; HA3: Twice to that of recommended

Discussion

Effect of drought stress on yield attribute and grain oil and protein contents

The reaction of castor bean to drought stress is more complicated because the plant produces inflorescences at different times (indeterminate). Each inflorescence plant can adjust the grain number and grain weight based on environmental conditions, as well as source and reservoir status (Severino and Auld, 2013). In the current study, severe drought stress resulted in decreasing yield and yield components of castor such as the number of capsule and seed, 100-seed weight, and seed yield (Table 3). In addition, the results indicated that the castor bean can tolerate drought stress well. However, no significant difference was found between mild drought stress (irrigation up to seed ripening equal 75 BBCH) and the normal irrigation (free-stress condition). Other researchers reported that the plant has a high tolerance to drought stress. However, its seed yield decreases when the available water decreases (Tadayyon *et al.*, 2017). Further, drought stress changes the photo-assimilation and metabolites required for cell division and affects mitosis by reducing the rate of growth (Farooq *et al.*, 2009). Interrupting the supply of essential trace elements in the soil, as well as reducing or causing an imbalance in the nutritional elements in the plants are considered as the main reasons for the reduction in growth and yield under drought stress (Hu and Schmidhalter, 2005). In general, drought stress or drought stress led to a reduction in dry mass yield and yield components due to the inhibition of the growth processes, photosynthetic rate, and assimilated export from leaf blades to other plant organs, along with the disturbance in transporting and distributing photosynthesis products (Staniak *et al.*, 2017).

Based on the results of the present study, drought stress treatments significantly decreased grain oil percentage, yield and grain protein yield, while increased grain protein percentage (Table 3). Based on the results, Karimi *et al.* (2012) reported that water stress can significantly influence the protein content of castor beans. However, a negative relationship was found between oil and protein percentage under drought stress condition, which is consistent with that of Popovic *et al.* (2012), who reported a negative correlation between protein and oil contents in grain.

Effect of drought stress on physiological traits

Castor bean has long been known to have a drought stress hardy and indicates a high developmental and physiological reaction to drought stress. Plants respond to drought stress by altering photosynthetic pigments such as Chl a, Chl b, and total Chl (Shi *et al.*, 2014). Dai *et al.* (1992) reported that the stomatal limitation may be responsible for the inhibitory effect of drought stress on photosynthesis in castor bean plants under increased vapor pressure deficits. In the present study, Chl a and total Chl contents showed a reduction under severe drought stress, but mild drought stress resulted in increasing Chl a and total Chl (Table 4). In addition, mild drought stress (irrigation up to 50% seed ripening equal 75 BBCH) led to an increase in photosynthetic pigments, compared to normal irrigation treatment (without stress), due to the effect of arousing moderate drought stress and a relatively good castor tolerance to this level of stress. Further, the stimulating effect of water stress and relatively good castor tolerance to this stress level can be highlighted in this regard. The acclimation of castor bean to drought stress condition includes some features such as higher leaf mass per area and lower water loss rate, increased negligible photosynthetic capacity, quick recovery and overcompensation for photosynthesis after re-watering, and increased chlorophyll content (Shi *et al.*, 2014). The reduction of fresh and dry biomass was considered as a common adverse effect of drought stress on plants. Furthermore, a reduction in photosynthesis rate caused by decreasing leaf expansion and impairing photosynthetic machinery is regarded as another major factor (Yan *et al.*, 2015). In addition, drought stress can affect the photosynthetic pigments (Anjum *et al.*, 2003) and reduce Chl a, Chl b, and total Chl contents, which was reported in a wide variety of plants (Yan *et al.*, 2015). According to Terzi *et al.* (2010) drought stress can create some changes in the photosynthetic components and decrease the chlorophyll content, which was in line with the results of the present study.

Effect of HA fertilizer on quality and quantity traits

The induction of drought tolerance in plants through using organic- and nano-fertilizers can have many applications in agriculture due to the impairment of nutrient absorption under osmotic stress (Bakry *et al.*, 2013). The results indicated that the application of organic fertilizers (HA) and nano-fertilizer (Zn) could positively influence the yield and physiological and qualitative traits of castor beans. Castor bean is mainly cultivated in arid and semi-arid conditions, which mostly destroys the soil of these areas due to the lack of organic matter for crop production. Therefore, improving soil properties through using nano, organic and other fertilizers should be considered for producing castor beans (Aghhavani Shajari *et al.*, 2018). In addition, HA is an organic matter without any negative environmental impacts, which plays a positive effect on the growth and yield of plants by improving the soil properties such as physical, chemical and biological structure and having hormonal compounds (Sabzevari *et al.*, 2010). Further, increasing HA rate could positively influence nutrient contents and micronutrients through their chelating and improving soil fertility (Liu and Cooper, 2000). HA application increased the photosynthetic activity by increasing the activity of the enzyme Rubisco (Delfine *et al.*, 2005). Furthermore, the foliar application of HA treatment improved the mobility and efficiency of nutrients and increased the amount of Zn and iron, which resulted in increasing photosynthesis, as well as carbohydrate and protein production (Sanjari Mijani *et al.*, 2015). Based on the results, the application of two times more than the recommended rate for HA resulted in reducing the effect on seed yield (13.9%), seed oil percentage (9.64%), seed oil yield (22.7%), and seed protein yield (10.3%), compared to that of the recommended rate. Thus, the use of HA more than the recommended rate had a toxic effect. Tan (2003) and Khaled and Fawy (2011) reported the application of HA at a high level played a negative effect on the plant growth parameters and yield components of corn.

Effect of Zn-nano fertilizer on quality and quantity traits

In the present study, foliar application of Zn-nano fertilizer increased the quantity such as the number of capsules and grain, 100-grain weight, and grain yield, as well as the quality such as the traits related to grain oil and protein contents (Table 3). Furthermore, a significant increase in yield and yield components was found for other crops by evaluating the effect of fertilization with various Zn forms (Anderson *et al.*, 2018). It is obvious that Zn and iron are considered important components of many vital enzymes such as CAT and SOD. It participates in the synthesis of chlorophyll, indole-3-acetic acid (Jeong and Connolly, 2009), and a structural stabilizer for proteins, membrane and DNA-binding proteins (Aravind and Prasad, 2004). In addition, Zn ions are regarded as strong inhibitors of enzymes generating oxygen radicals and protecting stress condition from damaging the attack of these compounds (Weisany *et al.*, 2012). In the present study, the foliar application of Zn-nano fertilizer increased photosynthetic pigments content (Chl a, Chl b, and total Chl), while decreased antioxidant enzyme activity (CAT, SOD, and POD). Babaei *et al.* (2017) indicated that the foliar application of Zn-nano fertilizer increased CAT and POD activities. Further, Zn-nano fertilizer application increased about 17.40% from wheat seed yield, compared to the non-application of nano-fertilizer in the highest stress conditions. The researcher reported some positive effects of Zn application under abiotic stress, such as removing the reactive oxygen species, defending chlorophyll content against free radicals, and increasing the activities of CAT and PPO (Arough *et al.*, 2016). Zn is an essential micronutrient which can enhance crop productivity and improve crop quality, although it is involved in various physiological and biochemical reactions (Yuvaraj and Subramanian, 2014).

Based on the results, the highest means for most of the studied traits were observed in the second year due to the appropriate climate conditions such as average temperature and precipitation in the second year, compared to the conditions in the first year.

Conclusions

In the present study, the D, HA, and Zn-nano fertilizer could significantly influence yield and yield components, oil and protein contents, photosynthetic pigment contents, and antioxidant enzyme activity. In general, the severe drought stress resulted in decreasing yield components, oil and protein yield, contents of Chl a, Chl b, and total Chl, while increasing CAT, POD, and SOD activities. On the other hand, the foliar application of HA and Zn-nano fertilizer could improve the means for the traits under stress or free-stress condition. Further, the application of the fertilizers led to a moderate tolerance in castor beans under drought stress conditions. In terms of yield and yield component characteristics, the results indicated no significant difference between mild stress levels (irrigation up to 50% seed ripening equal 75 BBCH scale) and normal irrigation treatment (control). Finally, castor bean is more tolerant to drought stress conditions, which can produce a considerable quantitative and qualitative yield in summer planting.

Authors' Contributions

AR: Writing-original draft; JMS: Supervisor of Thesis; AD and SR: Advisors of Thesis. All authors read and approved the final manuscript.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

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

References

Aghhavani Shajari M, Rezvani Moghadam P, Ghorbani R, Koocheki A (2018). Increasing saffron (*Crocus sativus* L.) corm size through the mycorrhizal inoculation, humic acid application, and irrigation management. Journal of Plant Nutrition 41(8):1047-1064. *https://doi.org/10.1080/01904167.2018.1433835*

Ahmadi A, Emam Y, Pessarakli M (2010). Biochemical changes in maize seedlings exposed to drought stress conditions at different nitrogen levels. Journal of Plant Nutrition 33:541-556. *https://doi.org/10.1080/01904160903506274*

Anderson S, Schoenau J, Vandenberg A (2018). Effects of zinc fertilizer amendments on yield and grain zinc concentration under controlled environment conditions, Journal of Plant Nutrition 41(14):1842-1850. *https://doi.org/10.1080/01904167.2018.1462386*

Anjani K, Raoof MA, Prasad MSL, Duraimurugan P, Lucose C, Yadav P, Prasad RD, Jawahar Lal J, Sarada C (2018). Trait-specific accessions in global castor (*Ricinus communis* L.) germplasm core set for utilization in castor improvement. Industrial Crops & Products 112:766-774. *https://doi.org/10.1016/j.indcrop.2018.01.002*

Anjum F, Yaseen M, Rasul E, Wahid A, Anjum S (2003). Water stress in barley (*Hordeum vulgare* L.). II. Effect on chemical composition and chlorophyll contents. Pakistan Journal of Agriculture Science 40:45-49.

Akintunde A (2012). Path analysis step by step using excel. Journal of Technical Science and Technologies 1(1):9-15. *https://doi.org/10.31578/.v1i1.29*

Sabaghnia N, Dehghani H, Alizadeh B, Mohghaddam M (2010). Interrelationships between seed yield and 20 related traits of 49 canola (*Brassica napus* L.) genotypes in non-stressed and water-stressed environments. Spanish Journal of Agricultural Research 8(2):356-370.

Aravind P, Prasad MNV (2004). Zinc protects chloroplasts and associated photochemical functions in cadmium-exposed *Ceratophyllum demersum* L., a freshwater macrophyte. Plant Science 166:1321-1327. *https://doi.org/10.1016/j.plantsci.2004.01.011*

Attibayeba NE, N`Kounkou JS, Julien GCD, Mandoukou-Yembi F (2010). Description of different growth stages of *Sesamum indicum* L. using the extended BBCH scale. Pakistan Journal of Nutrition 9:235-239. *https://doi.org/10.3923/pjn.2010.235.239*

Babaei K, Seyed Sharifi R, Pirzad A, Khalilzadeh R (2017). Effects of biofertilizer and nano Zn-Fe oxide on physiological traits, antioxidant enzymes activity and yield of wheat (*Triticum aestivum* L.) under salinity stress, Journal of Plant Interactions 12(1):381-389. *https://doi.org/10.1080/17429145.2017.1371798*

Babita M, Maheswari M, Rao LM, Shanker AK, Rao DG (2010). Osmotic adjustment, drought tolerance and yield in castor (*Ricinus communis* L.) hybrids. Environmental and Experimental Botany 69:243-249. *https://doi.org/10.1016/j.envexpbot.2010.05.006*

Bagheri E, Masood Sinaki J, Baradaran Firoozabadi M, Abedini Esfhlani M (2013). Evaluation of salicylic acid foliar application and drought stress on the physiological traits of sesame (*Sesamum indicum*) cultivars. Iranian Journal of Plant Physiology 3(4):809-816.

Bakry BA, Elewa TA, El-Kramany MF, Wali AM (2013). Effect of humic and ascorbic acids foliar application on yield and yield components of two wheat cultivars grown under the newly reclaimed sandy soil. International Journal of Agronomy and Plant Production 4:1125-1133.

Beauchamp C, Fridovich I (1971). Superoxide Dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44:276-287.

Bradford MM (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Annual Review of Biochemistry 72:248-254.

Chance B, Maehly C (1995). Assay of catalase and peroxidases. Methods of Enzymology 11:764-775.

Dai Z, Edwards GE, Ku MSB (1992). Control of photosynthesis and stomatal conductance in *Ricinus communis* L. (castor bean) by leaf to air vapor pressure deficit. Plant Physiology 99:1426-1434. *https://doi.org/10.1104/pp.99.4.1426*

Delfine S, Tognetti R, Desiderio E, Alvino A (2005). Effect of foliar application of N and humic acids on growth and yield of durum wheat. Agronomy for Sustainable Development 25:183-191.

Delgado PH, Aranguren M, Reig C, Galvan DF, Mesejo C, Fuentes AM, Sauco VG, Agustí M (2011). Phenological growth stages of mango (*Mangifera indica* L.) according to the BBCH scale. Scientia Horticulturae 130:536-540. *https://doi.org/10.1016/j.scienta.2011.07.027*

Eneji AE, Islam R, An P, Amalu UC (2013). Nitrate retention and physiological adjustment of maize to soil amendment with superabsorbent polymers. Journal of Cleaner Production 52:474-480. *https://doi.org/10.1016/j.jclepro.2013.02.027*

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009). Plant drought stress: effects, mechanisms, and management. Agronomy for Sustainable Development 29(1):185-212. *https://doi.org/10.1007/978-90-481-2666-8_12*

Gholami H, Saharkhiz MJ, Raouf-Fard F, Ghani A, Nadaf F (2018). Humic acid and vermicompost increased bioactive components, antioxidant activity and herb yield of Chicory (*Cichorium intybus* L.). Biocatalysis and Agricultural Biotechnology 14:286-292. *https://doi.org/10.1016/j.bcab.2018.03.021*

Gholinezhad E (2017). Effect of drought stress and Fe nano-fertilizer on seed yield, morphological traits, essential oil percentage and yield of dill (*Anethum graveolens* L.). Journal of Essential Oil Bearing Plants 20(4):1006-1017. *https://doi.org/10.1080/0972060X.2017.1362999*

Gislum R, Nikneshan P, Shrestha S, Tadayyon A, Deleuran LC, Boelt B (2018). Characterization of castor (*Ricinus communis* L.) seed quality using Fourier transform near-infrared spectroscopy in combination with multivariate data analysis. Agriculture 8:59. *https://doi.org/10.3390/agriculture8040059*

Gomes Neto V, Ribeiro PR, Del-Bem LE, Bernal DT, Cunha Lima ST, Ligterink W, Fernandez LG, de Castro RD (2018). Characterization of the superoxide dismutase gene family in seeds of two *Ricinus communis* L. genotypes submitted to germination under water restriction conditions, Environmental and Experimental Botany 155:453-463. *https://doi.org/10.1016/j.envexpbot.2018.08.001*

Hu Y, Schmidhalter U (2005). Drought and salinity: A comparison of their effects on the mineral nutrition of plants. Journal of Plant Nutrition and Soil Science 168:541-549. *https://doi.org/10.1002/jpln.200420516*

Jeong J, Connolly EL (2009). Iron uptake mechanisms in plants: functions of the FRO family of ferric reductases. Plant Science 176:709-714. *https://doi.org/10.1016/j.plantsci.2009.02.011*

Karakurt Y, Unlu H, Unlu H, Padem H (2009). The influence of foliar and soil fertilization of humic acid on yield and quality of pepper. Acta Agriculturae Scandinavica Section B - Soil and Plant Science 59:233-237. *https://doi.org/10.1080/09064710802022952*

Karimi S, Abbaspour H, Masoud Sinaki J, Makarian H (2012). Effects of water deficit and chitosan spraying on osmotic adjustment and soluble protein of cultivars castor bean (*Ricinus communis* L.). Journal of Stress Physiology & Biochemistry 8(3):160-169.

Khaled H, Fawy H (2011). Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. Soil and Water Research 6(1):21-29. *https://doi.org/10.17221/4/2010-SWR*

Leiboritz HE, Benqrson DA, Mouqle PD, Simpson KL (1987). Effects of Artemia lipid fraction on growth and survival of larval in land liver sides. In: Sorgeloss P, Bengtson DA, Deelier W, Japers E (Eds). *Artemia* Research and its Application. Universal press, Belgium, pp 763.

Lichtenthaler HK (1987). Chlorophylls and carotenoids. Pigments of photosynthetic membranes. Methods in Enzymology 148:350-382

Liu C, Cooper RJ (2000). Humic substances influence creeping bent grass growth. Golf Course Management 1:49-53.

Liv SS, Dick LA, Marco B, Magno JD, Cândido GC, William C, Tan D, … Helge Z (2012). A review on the challenges for increased production of castor. Agronomy Journal 104(4):853-880. *https://doi.org/10.2134/agronj2011.0210*

Lotfi R, Pessarakli M, Gharavi-Kouchebagh P, Khoshvaghti H (2015). Physiological responses of Brassica napus to fulvic acid under water stress: Chlorophyll a fluorescence and antioxidant enzyme activity. The Crop Journal 3(5):434-439. *http://dx.doi.org/10.1016/j.cj.2015.05.006*

Machado LW, Rovere ELL (2017). The Traditional Technological Approach and Social Technologies in the Brazilian Semiarid Region. Sustainability 10(1):25. *https://doi.org/10.3390/su10010025*

Mutlu H. Meier MA (2010). Castor oil as a renewable resource for the chemical industry. European Journal of Lipid Science and Technology 112(1):10-30. *https://doi.org/10.1002/ejlt.200900138*

Nardi S, Morari F, Berti A, Tosoni MGiardini L (2004). Soil organic matter properties after 40 years of different use of organic and mineral fertilisers. European Journal of Agronomy 21:357-367. *https://doi.org/10.1016/j.eja.2003.10.006*

Noman A, Shafaqat A, Fomia N, Qasim A, Mujahid F, Muhammad R, Muhammad Kashif Irshad (2015). Foliar application of ascorbate enhances the physiological and biochemical attributes of maize (*Zea mays* L.) cultivars under drought stress. Archives of Agronomy and Soil Science 61(12):1659-1672. *https://doi.org/10.1080/03650340.2015.1028379*

Ojeda-Barrios DL, Perea-Portillo E, Hernández-Rodríguez OA, Martínez-Téllez J, Abadía J, Lombardini L (2014). Foliar fertilization with zinc in pecan trees. Hort Science 49:562-566. *https://doi.org/10.21273/HORTSCI.49.5.562*

Ostadi A, Javanmard A, Amani Machiani M, Morshedloo MR, Nouraein M, Rasouli F, Maggi F (2020). Effect of different fertilizer sources and harvesting time on the growth characteristics, nutrient uptakes, essential oil productivity and composition of *Mentha piperita* L. Industrial Crops and Products 148:112290. *https://dx.doi.org/10.30495/jcep.2021.679977*

Pansu M, Gautheyrou J (2007). Handbook of soil analysis: mineralogical, organic and inorganic methods, Springer Science & Business Media.

Popovic V, Vidić M, Jocković Đ, Ikanović J, Jakšić S, Cvijanović G (2012). Variability and correlations between yield components of soybean [*Glycine max* (L.) Merr.], Genetika 44(1):33-45. *https://doi.org/10.2298/GENSR1201033P*

Prado R, Rozane D, Simoes R, Romualdo L (2008). Response of rice seedlings to seed application of zinc. Magistra 20:87-94.

Qin J, Wang X, Hu F, Li H (2010). Growth and physiological performance responses to drought stress under non-flooded rice cultivation with straw mulching, Plant, Soil and Environment 56:51-59.

Rajiv P, Vanathi P, Thangamani A (2018). An investigation of phytotoxicity using Eichhornia mediated zinc oxide nanoparticles on *Helianthus annuus*, Biocatalysis and Agricultural Biotechnology 16:419-424. *https://doi.org/10.1016/j.bcab.2018.09.017*

Ramanjaneyulu AV, Vishnuvardhan-Reddy A, Madhavi A (2013). The impact of sowing date and irrigation regime on castor (*Ricinus communis* L.) seed yield, oil quality characteristics and fatty acid composition during post rainy season in South India. Industrial Crops and Products 44:25-31. *http://dx.doi.org/10.1016/j.indcrop.2012.10.008*

Ramírez F, Kallarackal J (2015). Responses of fruit trees to global climate change. Springer, Springer Briefs, New York. *http://dx.doi.org/10.1007/978-3-319-14200-5*.

Roman-Figueroa C, Cea M, Paneque M, Gonzalez ME (2020). Oil content and fatty acid composition in castor bean naturalized accessions under Mediterranean conditions in Chile. Agronomy 10(8):1145. *https://doi.org/10.3390/agronomy10081145*

Rouphael Y, Cardarelli M, Schwarz D, Franken P, Colla G (2012). Effects of drought on nutrient uptake and assimilation in vegetable crops. In: Aroca R (Ed). Plant Responses to Drought Stress. Springer, Heidelberg New York Dordrecht London, pp 171-195.

Sabzevari S, Khazaie HR, Kafi M (2010). Study on the effects of humic acid on germination of four wheat cultivars (*Triticum aestivum* L.). Iranian Journal of Field Crops Research 8(3):473-80.

Sadeghi-Bakhtavari AR, Hazrati S (2021) Growth, yield, and fatty acids as affected by water-deficit and foliar application of nitrogen, phosphorus, and sulfur in castor bean. Journal of Crop Improvement 35(4):453-468. *https://doi.org/10.1080/15427528.2020.1824953*

Sanjari-Mijani M, Siroosmehr A, Fakheri B (2015). The effects of drought stress and humic acid on some physiological characteristics of Roselle. Journal of Agricultural Crop Management 17(2):403-414. *https://doi.org/10.22059/jci.2015.55189*

Severino LS, Auld DL (2013). Seed yield and yield components of castor influenced by irrigation. Industrial Crops and Products 49:52-60. *https://doi.org/10.1016/j.indcrop.2013.04.012*

Shi G, Zhu-Gu F, Liu Z, Le L (2014). Photosynthetic responses and acclimation of two castor bean cultivars to repeated drying-wetting cycles. Journal of Plant Interactions 9(1):783-790. *https://doi.org/10.1080/17429145.2014.945975*

Slaton N, Norman R Ce WJ (2005). Effect of zinc source and application time on zinc uptake and grain yield of flood-irrigated rice. Agronomy Journal 97:272-278. *https://doi.org/10.2134/agronj2005.0272*

Staniak M, Jolanta B, Jerzy K (2017). Changes in yield and gas exchange parameters in *Festulolium* and alfalfa grown in pure sowing and in the mixture under drought stress, Acta Agriculturae Scandinavica, Section B-Soil & Plant Science 68(3):255-263. *https://doi.org/10.1080/09064710.2017.1390149*

Sujatha M, Reddy TP, Mahasi MJ (2008). Role of biotechnological interventions in the improvement of castor bean (*Ricinus communis* L.) and *Jatropha curcas* L. Biotechnology Advances 26:424-435. *https://doi.org/10.1016/j.biotechadv.2008.05.004*

Tadayyon A, Nikneshan P, Pessarakli M (2017). Effects of drought stress on concentration of macro and micronutrients in castor (*Ricinus communis* L.) plant, Journal of Plant Nutrition 41(3):304-310. *https://doi.org/10.1080/01904167.2017.1381126*

TanKH (2003). Humic matter in soil and the environment. Marcel Dekker, New York. Pp 408.

Terzi R, Saglam A, Kutlu N, Nar H, Kadioglu A. 2010. Impact of soil drought stress on the photochemical efficiency of photosystem II and antioxidant enzyme activities of *Phaseolus vulgaris* cultivars. Turkish Journal of Botany 34:1-10. *https://doi.org/10.3906/bot-0905-20*

Valadabadi SA, Yosefi F, Shiranirad AH (2010). Effect of water holding and different nitrogen levels on some of agronomic characteristics of castor bean (*Ricinus communis* L.). Iranian Journal of Agronomy and Plant Breeding 6(1):99-110. *https://doi.org/10.30495/jcep.2021.679977*

Wang WB, Kim YH, Lee HS, Kim KY, Deng XP, Kwak SS (2009). Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stress. Plant Physiology and Biochemistry 47:570-577. *https://doi.org/10.1016/j.plaphy.2009.02.009*

Weisany W, Sohrabi Y, Heidari G, Siosemardeh A, Ghassemi- Golezani K (2012). Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). Plant Omic Journal 5:60-67.

Yan W, Yangquanwei Z, Zhouping S (2015). Evaluation of physiological traits of summer maize under drought stress. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science 66(2):133-140. *https://doi.org/10.1080/09064710.2015.1083610*

Yuvaraj M, Subramanian KS (2014). Controlled-release fertilizer of zinc encapsulated by a manganese hollow core shell. Soil Science and Plant Nutrition 20:1-8. *http://dx.doi.org/10.1080/00380768.2014.979327*

Zhang M, Jin Z, Zhao J, Zhang G, Wu F (2015). Physiological and biochemical responses to drought stress in cultivated and Tibetan wild barley. Plant Growth Regulation 75:567-574. *https://doi.org/10.1007/s10725-014-0022-x*