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

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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-grain weight, 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 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
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 Bundesanstalt, 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 Bundesanstalt, 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° 45’ E, long. 53° 55’ 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 | May | 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 | 1.1 | 1.4 | 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).

| 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:

\[
\text{Chl a (mg/gr FW)} = 12.7 \left( \text{O.D. of 663} \right) - 2.69 \left( \text{O.D. of 645} \right) \times \frac{V}{w \times \frac{1000}{p}}
\]

\[
\text{Chl b (mg/gr FW)} = 22.9 \left( \text{O.D. of 645} \right) - 4.68 \left( \text{O.D. of 663} \right) \times \frac{V}{w \times \frac{1000}{p}}
\]

\[
\text{Total Chl (mg/gr FW)} = 20.2 \left( \text{O.D. of 645} \right) + 8.02 \left( \text{O.D. of 663} \right) \times \frac{V}{w \times \frac{1000}{p}}
\]

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$_2$O$_2$, 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$_2$O$_2$, and 50 µL enzyme extract. The reaction was started by adding the enzyme extract. Based on the results, a decrease in H$_2$O$_2$ 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 m², 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 equals 75 BBCH as mild stress, and irrigation up to 50% flowering equals 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 $P \leq 0.05$ 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±75.6a              | 840.2±222.6b          | 21.0±2.3b           | 1790.4±594.1b          | 39.0±6.3a           | 718.2±307.5b               | 23.9±2.3b               | 424.0±130.4b             |
| Second                           | 304.2±83.3a              | 914.0±244.0a          | 21.9±5.1a           | 2065.8±767.8a          | 42.0±8.6a           | 876.2±459.4a               | 26.2±3.3a               | 528.2±153.9a             |
| LSD ($p=0.05$)                   | 21.5                     | 64.4                  | 0.58                | 162.3                   | 1.75                | 77.4                       | 0.68                    | 39.9                      |
| **Drought stress (D)**           |                          |                       |                     |                          |                     |                            |                         |                           |
| Normal irrigation                | 337.8±64.7a              | 1015.3±194.3a         | 23.0±2.7a           | 2450.2±384.3a          | 45.9±5.3a           | 1097.2±341.8a              | 22.1±1.7a               | 527.2±160.8a             |
| Mild stress                      | 316.8±46.2a              | 950.2±144.3a          | 22.0±2.5a           | 2110.4±365.7a          | 39.6±7.5b           | 849.2±197.3b               | 24.9±5.1b               | 531.9±140.9a             |
| Severe stress                    | 223.0±75.5b              | 696.0±211.5b          | 19.3±3.1b           | 1560.5±257.7b          | 33.3±5.9c           | 445.7±276.0c               | 28.1±3.0a               | 569.4±149.4b             |
| LSD ($p=0.05$)                   | 36.8                     | 110.6                 | 0.58                | 162.3                   | 1.75                | 77.4                       | 0.68                    | 39.9                      |
| **Humic acid (HA)**              |                          |                       |                     |                          |                     |                            |                         |                           |
| Control                          | 257.8±75.8b              | 775.5±227.4a          | 20.1±2.9a           | 1602.0±404.9c          | 36.2±8.6a           | 604.6±154.3c               | 23.0±2.3b               | 563.0±135.6c             |
| Recommended                      | 322.7±51.4a              | 908.1±235.3a          | 21.6±2.3a           | 2227.1±453.8a          | 43.2±7.8a           | 983.2±286.4a               | 25.7±3.7a               | 649.1±244.2a             |
| Twice to that of recommended     | 296.7±45.5a              | 890.3±232.6a          | 21.6±2.7a           | 1955.2±467.4a          | 39.4±6.6b           | 801.0±263.9b               | 26.4±5.7a               | 506.8±160.0b             |
| LSD ($p=0.05$)                   | 24.4                     | 79.2                  | 0.72                | 198.7                   | 2.31                | 125.7                      | 0.99                    | 48.8                      |
| **Zn-nano-fertilizer (Zn)**      |                          |                       |                     |                          |                     |                            |                         |                           |
| Non-application                  | 274.1±78.8a              | 822.4±230.4a          | 20.5±2.9b           | 1743.5±375.7b          | 39.2±6.7a           | 714.1±357.8b               | 22.4±2.3a               | 424.2±151.5b             |
| Application                      | 310.7±37.8a              | 932.2±235.3a          | 22.3±2.4a           | 2114.9±510.4a          | 40.9±9.3a           | 878.4±397.6a               | 25.3±3.8a               | 528.2±210.5a             |
| LSD ($p=0.05$)                   | 31.3                     | 64.6                  | 0.58                | 162.3                   | 1.75                | 77.4                       | 0.68                    | 39.9                      |

**Statistics**

| Treatments                        | Year (Y) | Drought stress (D) | Humic acid (HA) | Zn-nano fertilizer (Zn) | Y × D | Y × HA | Y × Zn | D × HA | D × Zn | Y × D × HA | Y × D × Zn | Y × D × HA × Zn | Y × D × HA × Zn |
|----------------------------------|----------|--------------------|-----------------|-------------------------|-------|-------|--------|--------|--------|------------|------------|-----------------|-----------------|
|                                  |          | **NS**             | **NS**          | **NS**                  | **NS**| **NS**| **NS** | **NS** | **NS** | **NS**     | **NS**     | **NS**          | **NS**          |
| **Year (Y)**                     |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |
| **Drought stress (D)**           |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |
| Normal irrigation                |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |
| Mild stress                      |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |
| Severe stress                    |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |
| **Humic acid (HA)**              |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |
| Control                          |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |
| Recommended                      |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |
| Twice to that of recommended     |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |
| **Zn-nano-fertilizer (Zn)**      |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |
| Non-application                  |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |
| Application                      |          |                    |                 |                         |       |       |        |        |        |            |            |                 |                 |

(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 75 BBCH). Additionally, the highest POD activity was achieved in mild stress (irrigation up to 65 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±0.9 a                | 1.66±0.2 c           | 3.40±1.0 c               | 22.22±3.4 b      | 12.59±2.9 a      | 34.88±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      | 36.84±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                       | NS               | NS               | NS                   |
| Y × D                                  | NS                        | NS                   | NS                       | NS               | NS               | NS                   |
| Y × Zn                                 | NS                        | NS                   | NS                       | NS               | NS               | NS                   |
| D × HA                                 | NS                        | NS                   | NS                       | NS               | NS               | NS                   |
| D × Zn                                 | NS                        | NS                   | NS                       | NS               | NS               | NS                   |
| Y × Zn                                 | NS                        | NS                   | NS                       | NS               | NS               | NS                   |
| D × HA                                 | NS                        | NS                   | NS                       | NS               | NS               | NS                   |
| Y × D × HA                             | NS                        | NS                   | NS                       | NS               | NS               | NS                   |
| Y × D × Zn                             | NS                        | NS                   | NS                       | NS               | NS               | NS                   |
| Y × D × HA × Zn                        | NS                        | NS                   | NS                       | NS               | 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 FW) | Total chlorophyll (mg/g FW) |
|--------------|----------------|------------|--------------------|----------------------|---------------------------|--------------------------|---------------------------|
| **First year** |                |            |                    |                      |                           |                          |                           |
|              | Control        | Control    | 1.66±0.1 g.j       | 4.09±1.6 d.k         | 10.80±0.03 c.i           | 29.89±1.9 l.i            |                           |
|              |                | Application| 1.69±0.3 g.j       | 2.5±0.6 mn           | 10.56±1.9 c.i           | 27.99±2.8 j.n            |                           |
|              | 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  | Control    | 1.66±0.1 g.j       | 3.92±0.4 e.l         | 13.03±1.8 b.g           | 32.71±2.1 f.j            |                           |
|              | of recommended| Application| 1.8±0.2 e.j        | 3.91±0.9 e.l         | 14.13±1.3 b.c           | 34.64±1.7 d.g            |                           |
|              |                | Control    | 2.2±0.1 bcd        | 5.92±0.7 a           | 7.47±0.3 i.n            | 26.09±1.6 mno            | 30.57±1.3 h.k             |
|              |                | Application| 2.8±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 c.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 c.l            | 26.00±2.8 mno            |                           |
|              | Twice to that  | Control    | 2.79±0.4 a         | 5.04±0.3 a.e         | 9.38±3.4 f.l            | 30.59±3.1 h.k            |                           |
|              | of recommended| Application| 2.52±0.4abc        | 4.3±0.7 c.j          | 8.04±1.8 h.m            | 27.03±1.8 k.n            |                           |
|              |                | Control    | 2.0±0.1 d.i        | 5.31±1.1 a.d         | 12.94±2.2 kg            | 36.35±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    | 2.0±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  | Control    | 1.89±0.1 d.j       | 5.76±0.3 ab          | 12.92±1.6 b.g           | 34.02±1.1 c.h            |                           |
|              | of recommended| Application| 2.2±0.4 b.e       | 5.88±0.5 a           | 13.35±1.9 b.e           | 35.10±0.4 d.g            |                           |
|              |                | Control    | 1.7±0.2 g.j        | 2.7±1.1 lmn          | 9.24±0.3 g.l            | 30.24±2.8 l.i            |                           |
|              |                | 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         | 12.5±1.0 c.g            | 38.14±3.4 c.d            |                           |
|              |                | Application| 1.5±0.1 i          | 2.57±0.1 mn          | 19.39±0.3 a             | 49±2.1 ± i.a             |                           |
|              | Twice to that  | Control    | 1.65±0.1 g.j       | 3.64±1.0 h.n         | 13.88±1.8 bcd           | 37.8±1.7 c.d             |                           |
|              | of recommended| Application| 1.54±0.1 j         | 3.64±0.6 h.n         | 12.52±3.4 c.g           | 36.25±2.1 c.f            |                           |
|              |                | Control    | 2.54±0.1 ab        | 5.19±0.5 a.e         | 2.2±0.3 o              | 22.9±0.7 o              |                           |
|              |                | Application| 1.7±0.09 f.j       | 4.4±0.3 c.j          | 4.06±1.5 n.o            | 25.89±1.6 mno            |                           |
|              | Recommended    | Control    | 2.12±0.1 c.f       | 2.46±0.02 n          | 7.8±3.6 h.n             | 28.39±2.8 j.m            |                           |
|              |                | Application| 1.85±0.09 c.j      | 2.97±0.4 k.n         | 6.69±1.8 k.n            | 26.81±1.9 l.mn           |                           |
|              | Twice to that  | Control    | 2.04±0.1 d.g       | 3.52±0.5 h.n         | 4.62±3.4 mno            | 26.84±1.8 l.mn           |                           |
|              | of recommended| Application| 2.19±0.09 b.e     | 3.91±0.2 c.l         | 5.97±1.1 l.o            | 30.40±4.1 h.l            |                           |
|              |                | Control    | 2.54±0.1 ab        | 4.93±1.1 a.g         | 7.25±1.2 i.n            | 32.49±1.3 g.hi           |                           |
|              |                | Application| 1.89±0.08 d.j     | 5.01±0.7 a.f         | 13.05±1.5 b.g           | 39.19±2.6 b.c            |                           |
|              | Recommended    | Control    | 1.62±0.02 hji      | 3.19±0.6 i.n         | 13.11±1.5 b.f           | 37.38±2.8 c.d.e          |                           |
|              |                | Application| 1.5±0.1 i         | 4.16±0.3 c.k         | 16.39±3.8 a.b           | 42.08±5.5 b              |                           |
|              | Twice to that  | Control    | 1.71±0.1 f.j       | 4.58±0.2 b.i         | 13.43±1.6 b.e           | 37.95±0.4 c.d            |                           |
|              | of recommended| Application| 1.59±0.1 j         | 4.7±0.1 a.h          | 11.98±1.2 c.g           | 37.06±1.1 c.d.e          |                           |

(Means ± SD); Different letters on each column indicate significantly different at P ≤ 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 the 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 activity; 12: 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.

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