



Optimization of castor bean (*Ricinus communis* L.) cultivation methods using biostimulants in an arid climate

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Abstract

In arid areas, low soil fertility, an imbalance of nutrients, and the inability of crops to absorb some nutrients are among the main constraints on crop production. The use of biological fertilizers and biostimulants can be a suitable solution. A 2-year field experiment was conducted as a factorial experiment based on a randomized complete block design with three replications to study the response of castor bean to arbuscular mycorrhizal fungi (AMF) and amino acid biostimulant (AAB) (foliar, soil, and combined application methods). Findings showed that plants inoculated with AMF had better performance: the biological and seed yields were increased by an average of 20.9 and 26.4% over the 2 years of the experiment. The yield components showed a 14.0 to 18.6% increase, and the water productivity (WP) was improved by 13.9%. The seed oil content was increased by 5.1%: linoleic acid, an oil fatty acid, showed the highest response, with an increase of 10.0%. Among the AAB treatments, the combined application method (CAM) brought the best results. The highest increases in biological and seed yields (68.4 and 63.2%, respectively) were obtained from the CAM treatment. The WP was improved by 67.8% and the seed oil content showed an increase of 9.4%. Among the fatty acids, the highest increase (24.4%) belonged to linoleic acid. The results show a positive and significant response of castor beans to biostimulants, which indicates that replacing chemicals with biological fertilizers could be a promising approach to the cultivation of castor beans in arid climates.

Keywords Arid climate · Biostimulants · Principal component analysis · *Ricinus communis* · Symbiotic associations

Introduction

The castor bean (*Ricinus communis* L.) is an important industrial and medicinal plant from the Euphorbiaceae family. This crop is particularly cultivated in arid and semi-arid regions for oil production (Kallamadi et al. 2015). Its high

oil content may vary between 37.2 and 60.6% (Wang et al. 2013). Due to its unique properties, the plant has a high medicinal value and many applications in the pharmaceutical industry (Jyothsna et al. 2009; Severino et al. 2012). The plant residues—including leaves, stems, and those remaining after processing (seed cake)—are potential sources of bioethanol and biogas (Bateni et al. 2014). The castor plant has more than 700 types of industrial applications (Anjani et al. 2018), so interest in developing the cultivation of this crop is increasing day by day (McKeon et al. 2016). For this reason, it has recently been proposed as an alternative plant for sustainable agriculture in arid and semi-arid regions with harsh climatic conditions (Vasconcelos et al. 2017).

The ecological, environmental, and biological effects of the application of chemical fertilizers in agroecosystems have been investigated in numerous studies, and the harmful effects of applying chemical inputs have been discussed. In recent decades, due to the capacity of chemical fertilizers to enhance crop yields and farmers' incomes, there has been a tendency of Iranian farmers to overuse these agrochemicals (Ullah et al. 2023). However, the history of agriculture in

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Iran shows that the ecological principles of crop production, such as improving soil organic matter, recycling nutrients through plant residues, and increasing biodiversity through strategies like multiple cropping, were an integral part of agriculture in this country before the introduction and the development of fertilizers and pesticides (Rodrigo-Comino 2018; Esmailian et al. 2022; Mirzaei et al. 2022, 2023).

As mentioned above, it is very important to choose crop management strategies that consider the ecological principles of and designs based on sustainable and organic agriculture, i.e., environmentally friendly agricultural activities. Solutions to this problem include reducing or eliminating synthetic and chemical inputs, making use of the mutual relationships between microorganisms, and applying biological amendments to supply the environmental needs of the plants, especially nutrients.

Biofertilizers, also known as microbial inoculants or plant biostimulants, are biological compounds used in crop cultivation in different ways, including as a seed treatment or via foliar or soil application. By interacting with the plant and improving the plant's environment, these compounds increase root growth and nutrient uptake and, ultimately, improve crop growth and yield (Vessey 2003). Biostimulants can help reduce the use of agrochemicals, including chemical fertilizers and pesticides, which have the potential to present high environmental risks (Hamza and Suggars 2001; Kolomazník et al. 2012). Although most microorganisms in soil are parasites that produce toxic substances with negative effects on plant growth processes, some microorganisms are symbiotic and cooperate with plants. For example, the relationship between mycorrhizal fungi and plants is a symbiotic relationship that is established with plants belonging to different families (Pereg and McMillan 2015; Eskandari et al. 2017). These fungi are also able to interact with other beneficial soil microorganisms (Miransari 2011). Various studies have shown that the use of mycorrhizal fungi can, directly and indirectly, improve the productivity and sustainability of agricultural ecosystems due to the many advantages they have for agricultural agroecosystems. They are especially important to farmers in crop management decision-making (Ryan and Kirkegaard 2012).

Amino acids, another family of biostimulant compounds, not only improve the growth and the quantitative and qualitative traits of the plants directly and indirectly by affecting plant physiological processes, but they also have positive effects on the plant's ability to overcome adverse effects of environmental stresses (Hammad and Ali 2014; Yu and Yang 2020). These compounds stimulate the metabolic processes of plants (Hildebrandt et al. 2015) and have special effects on the biosynthesis of secondary metabolites and phytohormones (Rodríguez et al. 2015). Therefore, their application can improve the growth, yield, and quality of medicinal plants (Poorghadir et al. 2020). Today, the trend

toward using natural compounds and biostimulants such as amino acids to increase crop yields and improve crop nutritional levels is expanding worldwide (Haghighi et al. 2020).

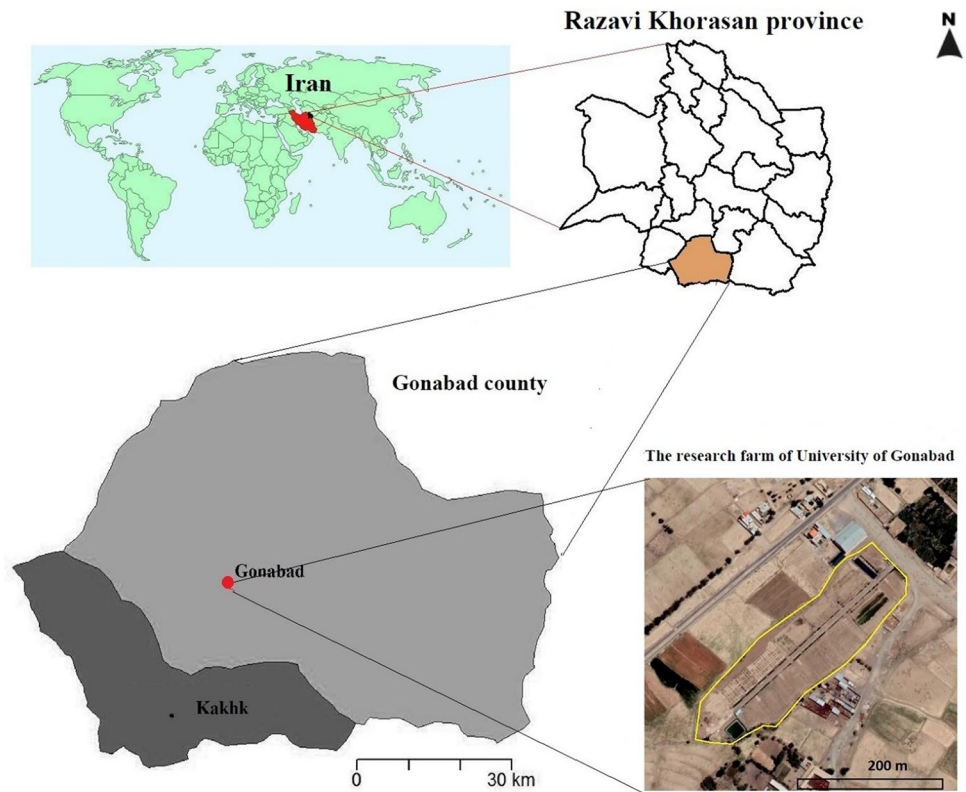
As mentioned, castor bean is a valuable crop that has many uses in industry, food, and medicine, and its applications are increasing. This crop, due to its special characteristics, high adaptability to different climatic conditions, and low environmental requirements, can play an important role in the implementation of sustainable agricultural strategies in arid areas such as the study area, but limited research (especially into its response to biological nutrition systems and organic farming techniques) has been done in this regard. In addition, no similar research to the current study has been carried out. Therefore, the objective of the present study was to investigate the response of castor bean to biofertilizers and the replacement of chemicals with organic fertilizers in an arid region with a long history of cultivating this crop. A 2-year field experiment was conducted as a factorial experiment based on a randomized complete block design with three replications to study the response of castor bean to arbuscular mycorrhizal fungi and amino acids (foliar, soil, and combined application methods were used).

Materials and methods

Site description

A field experiment was conducted to investigate the effects of the plant-growth-promoting (PGP) properties of an amino acid biostimulant (AAB) and arbuscular mycorrhizal (AM) fungi on the growth, yield, and oil characteristics of castor bean at the Research Farm of the University of Gonabad, Iran (58° 43' E; 34° 20' N; 1085 m.a.s.l.) during two consecutive growing seasons (2018 and 2019). The map of the study area is presented in Fig. 1.

The annual average temperature in the area is 18 °C and the minimum and maximum temperatures are 10 °C and 23 °C, respectively. The number of frost days during the cropping year was 33 (IRIMO 2018). The area has an arid climate according to the Köppen climate classification, and is characterized by high temperatures during mid-spring to late summer and low temperatures during mid-fall to late winter, with an annual average precipitation of 146 mm, mostly concentrated in winter and early spring, and a potential pan evaporation of 2021 mm (IRIMO 2018). The weather data for the experimental period that were obtained from Gonabad Meteorological Station are given in Table 1. The average temperatures during the growing seasons of castor bean were 24.41 °C and 26.08 °C in 2018 and 2019, respectively. The rainfall during the crop growth periods was 57.66 mm in 2018 and 38.7 mm in 2019. The sunshine hours in the growing season were 1922 h in 2018 and 1965 h in

Fig. 1 Experimental site location**Table 1** The meteorological data for the experimental site during the two growing seasons of castor bean

Parameter	T_{\min} (°C)		T_{\max} (°C)		T_{mean} (°C)		Rainfall (mm)		Mean relative humidity (%)		Mean sunshine (h)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Month												
April	12	12	25	24	18.5	18	50.3	33.3	44	52	229	243
May	16	17	29	31	22.5	24	7.3	5.4	30	32	283	303
June	23	21	36	35	29.5	28	0	0	18	21	352	369
July	23	25	38	39	30.5	32	0	0	17	19	376	367
August	21	21	36	36	28.5	28.5	0	0	21	20	364	358
September	16	18	32	34	17.0	26.0	0	0	19	21	318	325

2019. The average relative humidity (RH) was 24.83% in 2018 and 27.5% in 2019. Before sowing, the soil was sampled randomly from 0 to 30 cm depth and sent to the laboratory. The physicochemical properties of the experimental field soil are presented in Table 2.

Field experiment setup

The experiment was conducted as a factorial experiment based on a randomized complete block (RCB) design with three replications. The first factor consisted of the control (no AM) and arbuscular mycorrhiza application (AM). The second factor included the control (no AAB), soil

Table 2 Soil properties of the experimental site during the 2 years of the experiment

Soil properties	Unit	2018	2019
Texture	USDA soil classification	Sand-loam	Sand-loam
pH	–	7.9	8.2
Organic carbon	%	0.13	0.11
Total N	%	0.03	0.02
Available P	mg kg ⁻¹	8.2	7.4
Available K	mg kg ⁻¹	153	140
EC	dS m ⁻¹	3.9	4.0

application (SA), foliar application (FA), and combined application of amino acids.

The layout of the experiment was as follows: the experimental field was plowed and leveled in early April, and then the plots were constructed manually with dimensions of 3×2 m. The distances between the blocks and the plots were 1.5 and 1.0 m, respectively. The castor bean seeds (*Ricinus communis* L., local variety) were sowed manually in rows with distances of 50 cm and 30 cm between two plants in the row at 5 cm soil depth on 20 and 22 April in 2018 and 2019, respectively. Before sowing, the recommended amount of mycorrhizae (100 g m^{-2}), consisting of colonized root fragments, sand, fungi hyphae, and spores, was poured into the furrows of each row of the respective plots. Immediately after planting, the first irrigation (drip irrigation) was done and, to ensure uniform seed germination and emergence, the second irrigation was performed after 3 days.

At the six-leaf stage, a thinning operation was performed to achieve the desired density. During the growth periods of the crop, two-hand weeding took place. Other field operations were carried out as per the recommendations of local farmers. Amino acid fertilizer was applied at two stages (the eight-leaf stage and the flowering stage).

Data collection and measurements

Before harvesting, four plants were selected randomly, and their traits, including plant height, branch number, ear number, ear length, and capsule number, were measured. At the physiological maturity stage, the plants of two middle rows of each plot were harvested (on 2 and 5 September in 2018 and 2019, respectively). The samples were air-dried for 7 days and then the above-ground dry weight was recorded. After that, the biological yield was calculated. Then, the seeds were separated from the straw and the seed yield and the 100-seed weight was recorded.

The total volume of water used for irrigation was measured by a volumetric meter connected to irrigation pipes so that the total amounts of water used during the crop growing period were 5540 and $4700 \text{ m}^3 \text{ ha}^{-1}$ in 2018 and 2019, respectively.

The water productivity (WP) index was calculated using the following equation (Ali et al. 2007):

$$\text{WP} = \frac{\text{Seed yield (kg)}}{\text{Irrigation water applied (m}^3\text{)}} \quad (1)$$

To measure the oil contents and fatty acids of the castor seeds, the samples were transferred to the laboratory collection of the Medicinal Plants Research Center of Shahed University. The Soxhlet extraction technique was used to determine the seed oil content. To determine the oil fatty acids (stearic acid, palmitic acid, oleic acid, and linoleic acid), a

gas chromatograph (Varian CP-3800) equipped with a flame ionization detector (FID) and a capillary column (BPX70; $50 \text{ m} \times 0.25 \text{ mm} \times 0.20 \text{ }\mu\text{m}$ film thickness) was used. The measured fatty acid contents are given as percentages of the total fatty acid content of the castor bean.

Data analysis

Analysis of variance (ANOVA) and comparison of means using Duncan's multiple range tests at the 5% probability level were performed with SAS software version 9.1 (SAS, Cary, NC, USA).

Principal component analysis

Principal component analysis (PCA) is a technique for reducing the dimensionality of datasets, increasing interpretability, but, at the same time, minimizing information loss. It does so by creating new uncorrelated variables that successively maximize variance (Jolliffe and Cadima 2016). In other words, it aims to reduce the dimensionality of multivariate datasets by extracting information in the form of a small number of principal components while representing typical features of the environment (Fatima et al. 2022). Therefore, by reducing the removal of information, it is possible to extract the main principal factors that contain all the required information (Abdi and Williams 2010). The Kaiser criterion of the eigenvalue of the scree plot was used to extract the principal components of the studied parameters (Bryant and Yarnold 1995). The Kaiser–Meyer–Olkin (KMO) and Bartlett tests were used to measure the appropriateness of the data for factor analysis, which assesses the sample's adequacy for each individual variable in the model. KMO levels of between 0.8 and 1, 0.5 and 0.8, and less than 0.5 were considered as adequate, fairly adequate, and undesirable or inadequate, respectively (Patil et al. 2020).

Results

Effect of mycorrhiza application

The findings showed that the inoculation of castor plants with AM during both years of the experiment caused significant variation in the seed yield (SY), biological yield (BY), capsule number (CN), and ear length (EL). Moreover, AM affected the WP and the seed oil (SO) and linoleic acid (LA) percentages of the castor seed oil, while it had no significant effect on the oleic acid (OA), stearic acid (SA), and palmitic acid (PA) contents. The average of the 2 years of data showed that, compared to the control, the SY increased by 15.6% to 2115 kg ha^{-1} with AM application. With a 20.9% increase, the BY showed an even greater response than the

SY. Likewise, the application of AM significantly increased the CN, EL, plant height (PH), branch number (BN), and ear number (EN), with mean values of 19.5, 21.6 cm, 155.8 cm, and 5.10, respectively (Table 3).

While the SY of castor bean plants treated with AM was 2775 kg ha⁻¹ in 2018, it was 1455 kg ha⁻¹ in 2019. The BY under the effect of AM was 6411 kg ha⁻¹ in 2018, but it was 4605 kg ha⁻¹ in 2019. Moreover, with AM treatment, the CN was 23.2 in 2018, which was 15.0% higher than that of the control. The increase in 2019 due to AM inoculation was 11.3%. In 2018, AM application increased the EL from 19 to 23 cm as compared to the control (Table 4).

The means of the 2 years of data showed that the WP for castor bean under AM application was 0.41 kg m⁻³, while its value in the control was 0.36 kg m⁻³ (Table 3). The SO also increased significantly due to AM application, so the oil content in non-inoculated plants was 37.1% while it was 39% in the inoculated ones. Among the seed oil fatty acids, only LA showed significant variation under AM inoculation: a 10.0% increase with mycorrhiza application (Table 3). The WP of plants affected by AM was 0.51 kg m⁻³ in 2018, but its value in non-inoculated plants was 0.43 kg m⁻³. The corresponding values in 2019 were 0.31 and 0.28 kg m⁻³, respectively. The results also showed that the castor bean WP in the first experimental year was significantly higher in comparison to the second year (Table 4). In both of the years of the experiment, SO increased significantly under the effect of mycorrhiza inoculation, so the application of biofertilizer resulted in oil percentages of 39.4 and 38.6% in 2018 and 2019, respectively. The corresponding values in the control were 37.5 and 36.7%, respectively (Table 4).

Effect of amino acid application

Analysis of the variance of the data for the 2 years of the experiment showed that all amino acid biostimulant (AAB) application methods led to increases in the SY and BY of castor bean. Among the AAB treatments, the highest value of SY, a mean of 2382 kg ha⁻¹, was obtained in the combined application method (CAM) treatment, and the lowest value (1459 kg ha⁻¹) was observed in the control. However, there was no significant difference between the CAM and FA methods. Also, the CAM treatment yielded the highest BY, 6184 kg ha⁻¹, while the lowest one (3672 kg ha⁻¹) was observed in the control.

The combined analysis of the measured data showed that the yield component parameters improved significantly upon AAB application (Tables 3 and 4). Based on the mean comparison results, the CN increased by 41.0% due to the CAM treatment as compared to the control. The increase in EL was 60.0%. There was no significant difference between the FA and CAM treatments for this trait. Whereas the CAM treatment resulted in the highest PH

Table 3 Quantitative and qualitative traits of castor bean in response to mycorrhiza and amino acid application (mean values for each year of the experiment are shown)

Treatment	Year	Seed yield (kg ha ⁻¹)	Biological yield (kg ha ⁻¹)	Capsule no.	Ear length (cm)	Plant height (cm)	Branch no.	Ear no.	WP	Seed oil (%)	Linoleic acid (%)	Oleic acid (%)
Mycorrhizae	2018	2573 ^a	5705 ^a	21.6 ^a	21.0 ^a	158.3 ^a	5.42 ^a	5.62 ^a	0.48 ^a	38.5 ^a	3.25	3.88
	2019	1371 ^b	4359 ^b	15.0 ^b	19.2 ^b	144.6 ^b	3.85 ^b	3.87 ^b	0.29 ^b	37.4 ^b	3.29	3.89
Control	2018	1829 ^b	4555 ^b	17.1 ^b	18.5 ^b	147.2 ^b	4.24 ^b	4.39 ^b	0.36 ^b	37.1 ^b	3.10 ^b	3.81
	2019	2115 ^a	5508 ^a	19.5 ^a	21.6 ^a	155.8 ^a	5.03 ^a	5.10 ^a	0.41 ^a	39.0 ^a	3.41 ^a	3.96
Amino acids	Control	1459 ^c	3672 ^c	15.1 ^d	15.0 ^c	140.4 ^b	3.51 ^c	3.75 ^c	0.28 ^c	36.2 ^c	2.87 ^c	3.53 ^b
	SA	1849 ^b	4621 ^b	17.0 ^c	19.0 ^b	146.2 ^b	4.50 ^b	4.58 ^b	0.36 ^b	37.4 ^b	3.19 ^{bc}	3.84 ^{ab}
	FA	2197 ^a	5650 ^a	19.7 ^b	22.3 ^a	156.0 ^a	5.12 ^{ab}	4.96 ^b	0.43 ^a	39.0 ^a	3.44 ^{ab}	4.08 ^a
	CAM	2382 ^a	6184 ^a	21.3 ^a	24.0 ^a	163.3 ^a	5.42 ^a	5.70 ^a	0.47 ^a	39.6 ^a	3.57 ^a	4.09 ^a

Control no fertilizer, AM arbuscular mycorrhizae, SA soil application of amino acids, FA foliar application of amino acids, CAM combined application of amino acids

Columns with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range tests

Table 4 Quantitative and qualitative traits of castor bean in response to the application of mycorrhizae and amino acids during the 2 years of the experiment

Year	Treat- ment	Seed yield (kg ha ⁻¹)	Biological yield (kg ha ⁻¹)	Harvest index (%)	Capsule no.	Ear length (cm)	Plant height (cm)	Branch no.	Ear no.	100- seed weight (g)	WP (kg m ⁻²)	Seed oil (%)	Linoleic acid (%)	Oleic acid (%)	Stearic acid (%)	Palmitic acid (%)	
2018	Mycorrhizae																
	Con- trol	2371 ^b	4999 ^b	47.9	20.0 ^b	19.0 ^b	155.4	5.0	5.4	16.9	0.43 ^b	37.5b	3.06b	3.83	1.24	1.28	
	AM	2775 ^a	6411 ^a	45.4	23.2 ^a	23.0 ^a	161.2	5.8	5.9	17.1	0.51 ^a	39.4a	3.45a	3.93	1.35	1.31	
	Amino acids																
	Con- trol	1885 ^c	3871 ^c	49.7	17.2 ^b	16.1 ^b	147.2 ^b	3.8 ^b	4.5 ^c	16.3	0.34 ^c	35.9b	2.89c	3.49b	1.12	1.05b	
	SA	2480 ^b	5205 ^a	48.0	19.4 ^b	18.7 ^b	150.6 ^b	5.2 ^a	5.6 ^b	17.2	0.46 ^b	37.3b	3.08bc	3.75ab	1.30	1.28ab	
FA	2823 ^{ab}	6430 ^a	44.6	23.6 ^a	23.0 ^a	163.0 ^{ab}	6.2 ^a	5.9 ^{ab}	17.2	0.52 ^{ab}	40.4a	3.43ab	4.12a	1.31	1.35ab		
CAM	3103 ^a	7315 ^a	44.3	26.3 ^a	26.1 ^a	172.4 ^a	6.3 ^a	6.5 ^a	17.3	0.58 ^a	40.4a	3.61a	4.17a	1.43	1.51a		
2019	Mycorrhizae																
	Con- trol	1287 ^b	4112 ^b	31.2	14.2 ^b	18.1	140.0 ^b	3.4 ^b	3.4 ^b	16.6	0.28 ^b	36.7b	3.14	3.79	1.40	1.16	
	AM	1455 ^a	4605 ^a	31.5	15.8 ^a	20.3	150.3 ^a	4.3 ^a	4.3 ^a	16.1	0.31 ^a	38.6a	3.43	4.00	1.41	1.30	
	Amino acids																
	Con- trol	1034 ^c	3475 ^b	29.7	13.1 ^c	13.8 ^b	133.7 ^c	3.2 ^b	3.0 ^c	16.0	0.22 ^c	36.6	2.87b	3.58b	1.26	1.39	
	SA	1219 ^b	4037 ^b	30.3	14.7 ^b	19.4 ^a	141.7 ^{bc}	3.7 ^{ab}	3.6 ^{bc}	16.9	0.26 ^b	37.6	3.30ab	3.93a	1.43	1.03	
FA	1570 ^a	4870 ^a	32.3	15.9 ^a	21.6 ^a	149.0 ^{ab}	4.0 ^{ab}	4.0 ^b	16.0	0.34 ^a	37.6	3.45a	4.04a	1.47	1.20		
CAM	1661 ^a	5052 ^a	33.0	16.4 ^a	21.9 ^a	154.2 ^a	4.5 ^a	4.9 ^a	16.3	0.36 ^a	38.8	3.53a	4.02a	1.45	1.29		
Year		17,335,244 ^{**}	21,752,708 ^{**}	2826 ^{**}	524.7 ^{**}	38.34 [*]	2246 ^{**}	29.14 ^{**}	36.75 ^{**}	5.03 [*]	0.39 ^{**}	8.84 [*]	0.01 ^{ns}	0.001 ^{ns}	0.15 ^{ns}	0.05 ^{ns}	
Error		43,805	656,730	93.31	3.54	7.03	74.57	0.71	0.43	0.76	0.0016	4.87	0.19	0.29	0.16	0.11	
Mycorrhizae		983,269 ^{**}	10,891,838 ^{**}	14.87 ^{ns}	66.03 ^{**}	114.4 ^{**}	882.4 [*]	7.52 ^{**}	6.02 ^{**}	0.14 ^{ns}	0.04 ^{**}	43.7 ^{**}	1.38 ^{**}	0.27 ^{ns}	0.04 ^{ns}	0.08 ^{ns}	
Amino acids		1,987,191 ^{**}	14,903,006 ^{**}	3.84 ^{ns}	91.63 ^{**}	190.8 ^{**}	1245 ^{**}	8.55 ^{**}	7.95 ^{**}	1.74 ^{ns}	0.08 ^{**}	27.2 ^{**}	1.13 ^{**}	0.81 ^{**}	0.14 ^{ns}	0.13 ^{ns}	
M-A		9939 ^{ns}	838984 ^{ns}	50.90 ^{ns}	6.42 ^{ns}	1.45 ^{ns}	25.72 ^{ns}	0.42 ^{ns}	0.20 ^{ns}	0.92 ^{ns}	0.0003 ^{ns}	1.47 ^{ns}	0.29 ^{ns}	0.11 ^{ns}	0.01 ^{ns}	0.15 ^{ns}	
Y-M		166852 ^{ns}	2,527,713 [*]	24.70 ^{ns}	7.92 ^{ns}	10.36 ^{ns}	94.08 ^{ns}	0.02 ^{ns}	0.52 ^{ns}	1.39 ^{ns}	0.005 ^{ns}	0.007 ^{ns}	0.02 ^{ns}	0.03 ^{ns}	0.03 ^{ns}	0.03 ^{ns}	
Y-A		186,688 [*]	1,821,806 [*]	52.36 ^{ns}	22.07 ^{**}	12.30 ^{ns}	42.50 ^{ns}	1.38 ^{ns}	0.18 ^{ns}	0.60 ^{ns}	0.005 ^{ns}	8.32 [*]	0.05 ^{ns}	0.07 ^{ns}	0.01 ^{ns}	0.22 ^{ns}	
Y-M-A		25414 ^{ns}	768289 ^{ns}	52.17 ^{ns}	6.77 ^{ns}	3.45 ^{ns}	10.70 ^{ns}	0.06 ^{ns}	0.17 ^{ns}	0.21 ^{ns}	0.0009 ^{ns}	0.18 ^{ns}	0.16 ^{ns}	0.07 ^{ns}	0.01 ^{ns}	0.06 ^{ns}	

Control no fertilizer, AM arbuscular mycorrhizae, SA soil application of amino acids, FA foliar application of amino acids, CAM combined application of amino acids

ns, *, and ** indicate not significant, significant at the 5% level, and significant at the 1% level of probability, respectively. Columns with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range tests

(163.3 cm), the FA treatment did not produce a significant difference (156.0 cm). The BN showed a 54.4% increase due to the CAM treatment as compared to the control. The increase in the EN was 52.2%. Also, the castor bean WP showed a 67.8% increase due to the CAM treatment. The highest increases in SO, LA, and OA (9.4, 24.4, and 15.9%, respectively) were observed in the CAM treatment. The FA treatment was in second place in terms of improving these traits (Table 3).

The separate findings for each year of the experiment demonstrated that in 2018 the CAM treatment resulted in the highest increase in SY, with a mean SY of 3103 kg ha⁻¹, while the lowest value, a mean of 1885 kg ha⁻¹, was observed in the control. This trend was also observed in 2019: the highest SY (1661 kg ha⁻¹) was observed in the CAM treatment and the lowest value (1034 kg ha⁻¹) was observed in the control. The highest BY value (7315 kg ha⁻¹) occurred as a result of the CAM treatment, while the lowest value (3871 kg ha⁻¹) was observed in the control. In 2019, the highest (5052 kg ha⁻¹) and lowest (3475 kg ha⁻¹) values were obtained in the mentioned treatments, respectively. The experimental results showed that there was no statistically significant difference between the CAM and FA treatments regarding the SY and the BY of castor bean (Table 4). The CAM treatment caused 52.9 and 25.2% increases in the CN as compared to the control in 2018 and 2019, respectively. The CAM treatment resulted in the highest increase in the EL in both years of the experiment, with 62.1 and 58.7% increases in the first and second year, respectively. According to the results of the mean comparison, there was no significant difference between the CAM and FA treatments in the case of CN and EL. In 2018, the tallest plants (172.4 cm) were recorded in the CAM treatment, while the shortest plants (147.2 cm) were recorded in the control. In 2019, the highest and lowest values of PH (154.2 and 133.7 cm) were obtained in the CAM and control treatments, respectively. The EN showed 44.4 and 63.3% increases due to the combined method of AAB application in 2018 and 2019, respectively. In both years, the BN increased significantly due to AAB application. The highest values in 2018 and 2019 (6.3 and 4.5, respectively) were observed in the CAM treatment. No significant difference was observed with the FA treatment. However, the lowest values, 3.8 and 3.2 for 2018 and 2019, respectively, were observed in the control.

The WP of castor bean improved more in the first year than in the second year of the experiment upon AAB application. The CAM treatment caused the highest increase in this parameter. The WP increased by 70.6 and 63.6% as compared to the control in the first and second year, respectively. In 2018, the CAM and FA treatments both resulted in a 12.5% increase in the SO as compared to the control. Though no significant difference was observed between the AAB treatments in the case of SO in 2019, the CAM

treatment increased the SO by 6.0% as compared to the control (Table 4).

In 2018, the CAM treatment resulted in the highest value of LA (3.61%). For the FA treatment, this value was 3.43%, and for the SA treatment, it was 3.08%, taking the second and third places, respectively. Similar results were observed in the second year of the experiment. As presented in Table 4, the CAM, FA, SA, and control achieved 3.53, 3.45, 3.30, and 2.87% LA, respectively. The highest percentage of OA in 2018, a mean of 4.17%, was observed in the CAM treatment. There was no significant difference in this regard from the FA treatment (4.12%). However, the lowest value (3.49%) was obtained in the control. In 2019, the percentages for OA upon applying the CAM, FA, and SA treatments were 4.02, 4.04, and 3.93%, respectively. However, there was no significant difference among these treatments. Our results revealed that there was no significant difference between the AAB treatments in the variation of SA in both years of the experiment. The CAM treatment, which produced a 43.8 increase in the PA percentage, showed the best result, and the FA and SA treatments, with no significant difference, were in second place (Table 4).

Principal component analysis (PCA)

The correlation matrix and Bartlett's test of sphericity were used to determine if the data could be used for PCA (Gad et al. 2023). Table 5 shows the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy. The KMO value (0.649) obtained was greater than 0.5 and Bartlett's test of sphericity value (0.000) was less than 0.05. The KMO and Bartlett tests are measures of how appropriate data are for factor analysis, which measures the sample's suitability for each

Table 5 Correlations of the various parameters and factors

Parameter	Factor 1	Factor 2
Seed oil	0.936	0.142
Palmitic acid	0.404	0.850
Stearic acid	0.980	-0.158
Oleic acid	0.943	0.119
Linoleic acid	0.896	0.248
Water productivity	0.977	0.165
100-seed weight	0.525	-0.480
Ear number	0.928	0.289
Branch number	0.939	0.294
Plant height	0.902	0.393
Ear length	0.970	0.223
Capsule number	0.911	0.341
Harvest index	-0.194	-0.850
Biological yield	0.912	0.404
Seed yield	0.980	0.154

individual variable in the model (Gad et al. 2023). Correlation coefficients above 0.3 were obtained for the retained items. As suggested by Mustapha et al. (2012), any correlation coefficients less than 0.3 and Bartlett's test values above 0.05 were not used.

Two of the original components were kept after the PCA (F1, F2). F1 accounted for 78.95% of the data set's variability, whereas F2 accounted for 11.56% (Fig. 2).

Table 5 shows the variables' loading values. There were strong connections between the factors and the variables, as indicated by values that are near to 1. Following Hinge et al. (2022), these loadings were further divided into three categories: high (> 0.75), moderate (from 0.75 to 0.50), and weak (from 0.50 to 0.30).

F1 shows that strong positive relationships exist between seed oil, stearic acid, oleic acid, linoleic acid, water productivity, seed yield, yield components (ear number, branch number, ear length), plant height, and biological yield, which illustrates that all traits related to the yield and traits related to the oil quality are included in factor 1. If the yield and yield components are important for the selection of experimental treatments, factor 1 will play a very important role due to its high correlation with these traits, and the use of combined amino acid and mycorrhiza treatments will improve all these traits together. The high correlation between yield components means that the use of combined treatments will ultimately increase the seed yield. High positive correlations were observed between the seed yield and the yield components except for 100-seed weight, harvest index, and palmitic acid, which had low correlations with seed yield (Fig. 3).

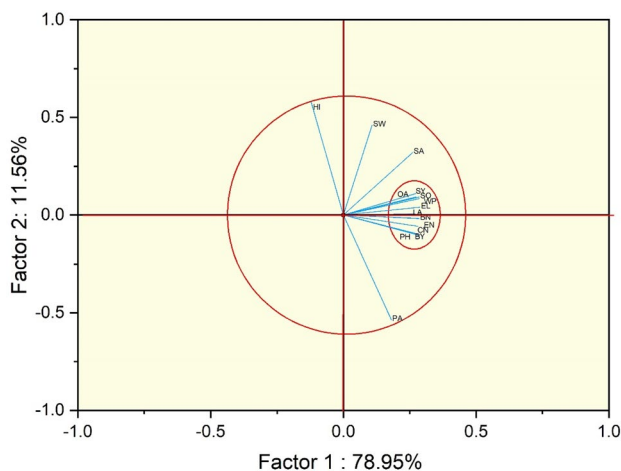


Fig. 2 Plots of PCA scores for F_2 vs. F_1 . The abbreviations for the loading plots are: SY seed yield, BY biological yield, HI harvest index, SW 100-seed weight, SA stearic acid, PA palmitic acid, OA oleic acid, LA linoleic acid, WP water productivity, EN ear number, BN branch number, PH plant height, EL ear length, CN capsule number

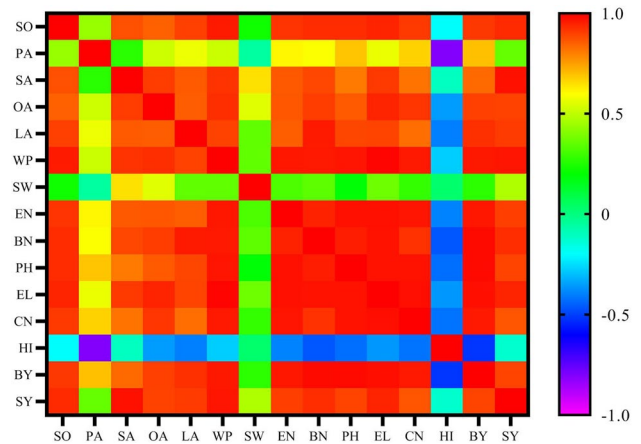


Fig. 3 Correlations among measured parameters, where the abbreviations used in the correlation matrix are: SY seed yield, BY biological yield, HI harvest index, SW 100-seed weight, SA stearic acid, PA palmitic acid, OA oleic acid, LA linoleic acid, WP water productivity, EN ear number, BN branch number, PH plant height, EL ear length, CN capsule number. Dark red highly positive, light red less positive

Among the yield components, ear length had the highest correlation with seed yield (0.94%) and 100-seed weight had the lowest correlation with seed yield (0.47%). Oil quality parameters such as stearic acid, oil percentage, linoleic acid, and oleic acid had the highest correlations with seed yield, with values of 0.97, 0.93, 0.90, and 0.89%, respectively (Fig. 3). Since the palmitic acid content had low correlations with other fatty acids, the low correlation of seed oil content (0.35%) with seed yield seen in Fig. 3 is expected.

As shown in Table 5, factor 2 had a positive loading for palmitic acid, +0.850, and a strong negative association with harvest index (− 0.850). Considering that the harvest index is the ratio of seed yield to biological yield, these results show that whenever the ratio of seed yield to biological yield decreases—i.e., a smaller share of the photosynthetic products is transferred to the reproductive organs, especially the seeds—palmitic acid synthesis increases and vice versa.

Discussion

The findings revealed that AM significantly improved the growth and agronomic parameters as well as the seed oil characteristics of castor bean during the 2 years of the experiment.

The increases in SY in the first and second years of the experiment due to the inoculation of the castor plant with mycorrhizae were about 17 and 13%, respectively. BY increased by about 28 and 12%, respectively. The correlation analysis also showed that the SY of castor bean was positively and significantly correlated with the BY. It has been

mentioned that increasing root biomass and root effective surface or root hairs as indirect mechanisms of mycorrhizal fungi inoculation enhances the absorption of macro- and micronutrients by the plant (Calvo et al. 2014). The absorption of more nutrients leads to an improvement in plant growth and increases the rate of photosynthesis. Consequently, it increases the production of assimilates and results in more and faster translocation from the source (leaves) to the sink (seeds).

Our results showed increases in the SY and BY of castor bean due to amino acid application. In both years of the experiment, the CAM treatment led to the greatest increases in the SY and BY of this crop. Various studies have shown that the use of amino acids as plant growth stimulants improves crop growth and yield by increasing the absorption and fixation of nitrogen, increasing the biosynthesis of plant hormones, and stimulating the metabolism and metabolic processes in the plant (Miller et al. 2007; Hildebrandt et al. 2015; Rodriguez et al. 2015; Khan et al. 2019; Aghaye Noroozlo et al. 2019).

The findings of this study revealed that all the growth traits and yield components of castor bean, such as the PH, BN, EN, CN, and EL, were improved as a result of inoculation with mycorrhizae (Tables 3 and 4). Mycorrhiza inoculation can greatly improve water and nutrient uptake and help avoid their loss from the soil, thereby improving water and nutrient efficiency, plant growth parameters, and yield components. It was reported that mycorrhizal soil had higher extractable N, P, and organic carbon (OC), indicating higher soil fertility. Enhanced nutrient availability, an expanded root zone, and increased photosynthesis due to mycorrhizae were reported by Ziane et al. (2017).

The plant growth parameters and yield components were significantly improved under the influence of amino acid application. The obtained results showed that the CMA and SA treatments led to the greatest improvements in the studied traits. However, in most cases, FA treatment resulted in values equivalent or close to those obtained by the CMA treatment (Table 3 and 4). Plants can absorb amino acids in the form of organic protein compounds as well as the building blocks of proteins (Abd El-Aziz et al. 2009). Amino acids can improve crop growth and yield parameters by improving the biosynthesis of chlorophyll and proteins (Miri Nargesi et al. 2022). Amino acids are known as biostimulants, as they can have positive effects on plant growth by affecting various physiological processes in plants (Kowalczyk et al. 2008). Although plants may be able to synthesize the amino acids they need, the synthesis of these compounds by plants causes the consumption of metabolic energy and thereby a reduction in net photosynthesis. Therefore, the exogenous application of these compounds in the form of biological fertilizers leads to energy storage in plants, and this energy is then used to enhance plant growth and development,

especially in critical phases of plant development (Maini 2006; Paleckiene et al. 2007; Popko et al. 2014).

Water productivity data also showed that castor plants inoculated with mycorrhizae used water more efficiently. Their WP improved significantly, such that this index improved by 18.6 and 10.7% in the first and second years, respectively, when AM was applied (Table 4). Gholamhosseini et al. (2013) reported a positive effect of inoculation with mycorrhizae on the water productivity of sunflower. Low precipitation, droughts, and water shortages are the most important challenges for agriculture in arid and semi-arid areas such as the experimental area in this study. Therefore, the adoption of strategies that reduce irrigation water use and increase crop water productivity is one of the most important issues in such areas. It can be concluded from the obtained results that the inoculation of castor plants with mycorrhizal fungus increases the volume and effective surface of the plant roots in the rhizosphere, making it possible to absorb more water. Therefore, the efficiency of transpiration and photosynthetic activity is increased, and more dry matter is stored in the plant under these conditions (Birhane et al. 2012; Chandrasekaran et al. 2019). On the other hand, it is noted that plants inoculated with mycorrhizae need less water to produce 1 kg of dry matter and maintain more water in their tissues (Kumar et al. 2016; Pirzad and Mohammadzadeh 2018).

The results also indicated a significant improvement in the WP of castor bean due to amino acid application. The FA treatment led to values almost equal to those obtained in the CMA treatment, which indicates a better response of castor plants to the foliar spraying method than to soil application. It seems that the improvement in WP in the castor plant due to amino acid application is more related to the improvement in crop yield through the improvement of physiological aspects; for example, enhanced plant metabolism (Shafeek et al. 2018), nitrogen uptake and translocation (Liu et al. 2008; Souri 2016), and biosynthesis of basic biochemical compounds (El-Awadi et al. 2011), increased cell division, morphogenesis, and delayed senescence (Al-Sahmmari et al. 2018), and increases in the leaf area, chlorophyll content, and photosynthesis rate (Radkowski and Radkowska 2018).

Mycorrhiza inoculation significantly increased the oil content of castor seeds in both of experimental years. It seems that the mycorrhizal fungus improved oil biosynthesis in the plant by increasing the availability and absorption of nutrients, especially phosphorus. Similar results were reported by Heidari and Karami (2014) for sunflower. One of the effective ways to improve the quality of oil seed plants and increase the oil content is plant nutrition management (Ray et al. 2019; Rodrigo-Comino et al. 2022). The application of amino acids also increased the seed oil content. Amino acids play an important role in the uptake and transportation of nutrients in the plant (Anjum et al. 2014). Other

researchers have attributed the increase in crop oil content as a result of amino acid application to the improvement in the assimilation and translocation of photosynthetic products (Zheljzkov et al. 2009; Mohammadi and Rokhzadi 2012).

Although the percentage of fatty acids did not show any statistically significant variation as a result of plant inoculation with mycorrhizae in this study, the fatty acid content was enhanced due to the use of this biofertilizer in all cases. Several researchers have pointed to the stimulating effect of mycorrhizal fungi on the production of plant metabolites (Zouari et al. 2014; Kaur and Suseela 2020; Pellegrino et al. 2022). Some researchers have stated that one of the reasons that the synthesis of fatty acid compounds is increased in host plants is the transfer of these compounds to mycorrhizal fungi, which do not have genes encoding the synthesis of these compounds (Stumpe et al. 2005; Sugiura et al. 2020).

The castor plants treated with amino acid biostimulants had higher seed oil fatty acid contents. Various studies have shown an improvement in crop growth, availability of nutrients, and quality of crops as a result of amino acid fertilization. These compounds not only reduce damage to plants from abiotic stresses, but they also have the same function as plant hormones and play a role as signaling agents in various physiological processes of plants (Khan et al. 2019). One of the main reasons for the increase in the tendency to use amino acids in crop production is their positive effects on crop quality (Haghighi et al. 2020; Poorghadir et al. 2020).

Conclusion

The results of this research reveal the positive and significant effects of mycorrhizae, amino acid biofertilizers, and biostimulants on different traits of castor bean. Overall, mycorrhiza inoculation led to 4.0–26.4% improvements in the studied traits. The biological and seed yields showed the highest response and oil characteristics showed the lowest response to MA application. The response of castor bean to different methods of amino acid application was more noticeable than its response to mycorrhizae. The best results for almost all the studied traits were obtained from the combined method of AAB application. However, in most situations, there was no significant difference in results between this treatment and foliar application treatment. Based on the obtained results, it can be concluded that by using biofertilizers and biostimulants in castor cultivation in agroclimatic areas with arid and semiarid climates, quantitatively and qualitatively acceptable yields can be achieved, and steps can be taken to replace chemical fertilizers with biofertilizers in the transition to organic farming of castor bean.

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Declarations

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