

Effect of Water Stress at Different Growth Stages and Amino Acid Foliar Application on Seed Yield and Water Use Efficiency of Castor Bean (*Ricinus communis* L.)

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Abstract

A field experiment was conducted at the Field Crops Research Station, College of Agriculture, University of Diyala, during the 2025 season to study the effects of water stress and amino acid Foliar Application on seed yield and water-use efficiency in castor bean plants. A randomized complete block design (R.C.B.D.) with a split-plot arrangement and three blocks was used. The first factor was the application of water stress stages, which included the first water stress stage, implemented after the plant had six vegetative leaves on May 11, 2025, and lasting for 30 days of vegetative growth; the second water stress stage, implemented after the first stage on June 10, 2025, at the same water stress level (60% of the prepared irrigation), also lasting for 30 days of vegetative growth; and a control irrigation treatment (100% of the prepared irrigation) without water stress. The second factor was the application of four amino acid concentrations (0, 1, 2, and 3 ml L⁻¹) in two applications, 15 days apart. The results showed that the irrigation treatment without water stress was superior in leaf area, fresh weight of the plant, number of capsules, weight of 300 seeds, seed yield, and water use efficiency, with values of (20.34 cm², 88.90 g, 55.25 capsules per plant, 309.08 g, 117.54 g, and 0.040 kg ha⁻¹) respectively. In contrast, the spray treatment with a concentration of 3 ml L⁻¹ of amino acids was superior in leaf area, fresh weight of the plant, number of capsules, seed yield per plant, and water use efficiency, with values of 113.76 cm², 300.11 g, 54.00 capsules per plant, 20.26 g, and 0.042 kg ha⁻¹ respectively, except for the weight of 300 seeds, which was superior with a 2 ml L⁻¹ spray, reaching 87.81 g. The interaction between irrigation without water stress and Foliar Application with a concentration of 3 ml L⁻¹ of amino acids also showed a significant improvement in seed yield, reaching 25.00 g. plant⁻¹.

Keywords: *Ricinus communis* L., Water stress, Foliar application, Bio-stimulant

Introduction

The castor bean plant (*Ricinus communis* L.) is a summer annual plant belonging to the family (Euphorbiaceae). It is widely distributed in tropical and subtropical regions of the world due to its ability to adapt to diverse environmental conditions and its increasing economic importance [18]. India is the leading producer of castor beans, accounting for 90%, followed by China, Brazil, and North America [15]. The oil content in castor beans is 40-45%. The

oil is important in medical and industrial uses due to its unique properties, high percentage of unsaturated fatty acids, and high content of bioactive compounds, which give it medicinal properties in pharmaceutical uses as a laxative, moisturizer, and purgative [13]; for treating some ulcer diseases and diabetes; as an antioxidant for dangerous compounds; and for developing sustainable synthetic medicines as an alternative to traditional non-natural medicines [19].

Water stress is a major obstacle to the availability and absorption of nutrients by plants, especially during critical stages of plant development, to enhance their resistance to environmental stresses such as drought, heat, and soil salinity and their negative effects on the growth, metabolism, and production of active compounds of medicinal plants. Bio-stimulants, such as Foliar Application with amino acids, are considered strategies and techniques for improving biological processes [7], [22], as well as enhancing photosynthetic efficiency, chlorophyll formation, and increasing the plant's ability to withstand harsh stresses in the growing environment. This, in turn, impacts the synthesis of essential carbohydrate and protein compounds for plant growth and production. Both [11],[10] showed that Foliar Application amino acids on plants increases the nitrogen content and its vital role in the synthesis of secondary metabolites and improves plant growth and production. Exposure of plants to water stress, especially in the growth stages of plants most sensitive to water deficiency, constitutes a factor that hinders plant growth, activates harmful compounds and reactive oxygen species (ROS), and disrupts proteins, DNA molecules, and the enzymatic

activity of various vital reactions in cells. [12] indicated that, in addition to the effect of water stress, it reduces the availability and absorption of nutrients and prevents the development and expansion of the root system in the soil. Additionally, it disrupts the balance of nutrients and hormones in the plant [8]. Due to the medical importance of the castor plant, this study was conducted with the aim of determining the optimal concentration of the amino bio-stimulant under two growth stages of water stress on seed yield and water-use efficiency.

Materials and Methods

Experimental location

A field experiment was conducted during the spring of 2025 in the fields of the College of Agriculture, University of Diyala, located northeast of Baghdad, at a longitude of 44.64° and latitude of 33.75°, in clay-loam soil.

Random soil samples were taken from various areas of the field at a depth of 0-30 cm prior to planting. The soil was mixed, air-dried, ground, and then passed through a sieve with 2 mm openings. A mixed sample was then taken to determine its physical and chemical properties at the laboratories of the Diyala Agriculture Directorate, as detailed in Table 1.

Table 1. Physical and chemical properties of soil before planting

Properties	Values	Units
Organic matter	1.6	%
EC	8.4	D.S.m ⁻¹
pH	7.5	
Available nitrogen	189	mg/kg ⁻¹
Available potassium	276	mg/kg ⁻¹
Phosphorus	3.1	mg/kg ⁻¹
Calcium carbonate CaCO ₃	27.5	%
Clay	39.2	
Silt	23.2	
Sand	37.6	
Texture	Clay loam	

Design the experiment

A experiment was conducted according to the Randomized Complete Block Design (R.C.B.D.) with a split-plot arrangement to study the effect of water stress and Foliar Application with an amino Bio-stimulant on seed yield and water-use efficiency of a common local castor bean cultivar with three replications. The experimental land was prepared by plowing it twice and smoothing, then divided into rows according to the irrigation system applied using surface drip irrigation in the field. All treatments were distributed on the rows in each replication. The length of the treatment (experimental unit) was 5 m, the distance between the holes was 60 cm, the distance between the sprayed treatments with the Bio-stimulant was 80 cm, and the distance between the water stress treatments was 1 m. Thus, the number of plants in each experimental unit became 8 castor bean plants, and thus the plant density became 16666 plants per hectare. All experimental units were fertilized with phosphate fertilizer at a rate of (N18:P48 NP 100 kg) in a single application during plowing. Nitrogen fertilizer (urea) was added at a rate of 100 kg urea ha⁻¹ in two applications: the first at plowing and the second two weeks after germination. Castor seeds were sown on 26/3/2025 after being soaked in water for 12 hours, with three seeds per hole at a depth of 3 cm and covered with soil. The plants germinated two weeks after sowing and were thinned to one plant per hole after the development of two true leaves. Crop maintenance, including the removal of annual weeds, was carried out as needed. The fruits ripened and were harvested on 25/8/2025.

Experimental Treatments

Water Stress

Two water stress treatments were implemented when the plant had six leaves, using 60% of the prepared irrigation, at two stages of castor bean growth, as follows: the first water stress stage was implemented on May 11, 2025, and continued for 30 days of vegetative growth, ending on June 9, 2025. The second water stress stage was implemented after the first water stress stage ended on June 10, 2025, using the same 60% of the prepared irrigation, and continued for 30 days of vegetative growth before flowering, ending on July 10, 2025, besides the irrigation treatment without water stress (100% of ready-made irrigation). The irrigation continued in the field until August 6, 2025. A treatment without water stress provides 32 liters of water per treatment, while an estimated 60% of the available irrigation for the two stages of water stress in the plant is as follows:

= Percentage of water available for water stress treatments × Amount of water available per treatment and without water stress

The irrigation method included a surface drip system installed with 16 mm plastic pipes, equipped with drainage drippers 60 cm apart and connected to a main pipe with a diameter of 1.5 inches.

Spraying with the amino Bio-stimulant Use the amino Bio-stimulant (Amino Star 20), which contains a number of plant amino acids (Table 2), at four Foliar Application levels (Foliar Application with distilled water only, 1, 2, and 3 ml per litre of distilled water) in the early morning, in two Foliar Application batches on 5/8 and 5/22/2025, on the plants after adding (Zahi) 1 ml per litre of distilled water to reduce the surface tension of the solution with the plant.

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Table 2. Components of the amino Bio-stimulant for (Amino Star 20 solution, produced by Delta Chemical Industries

Components	Percentages %
Amino Acids of Plant Origin Methionine, Tryptophan, Glycine, Glut Acid, Valine, Theanine	20
Zinc EDTA	4
Organic Nitrogen	10

Climate data for the agricultural site was taken from the Meteorological and

Seismological (Table 3).

Department-Baghdad

Table 3. Climate data for the agricultural site

Months	Relative humidity	Maximum temperature	Minimum temperature
April	37	32.52	17.1
May	24	39.46	21.9
June	19	43.46	23.61
July	22	45.42	26.40
August	23	45.19	26.20

Studied Traits

The studied traits below were calculated using the average of five plants taken from each experimental unit.

1. Leaf Area (cm²): The average of [7] leaves taken from each experimental unit was used, and their leaf area was measured using a metal ring of known area before flowering of the plants according to the following equation:

$$\text{Leaf area} = \frac{\text{Disc area (cm}^2\text{)} \times \text{dry leaf weight (g)}}{\text{dry disc weight (g)}} \quad [20].$$

2. Fresh weight of plant (g plant⁻¹): Estimate the fresh weight of the mature plant for five plants in each experimental unit and calculate their average weight using a sensitive electronic balance.

3. Number of capsules/plant: Calculate the number of dry fruits for five plants from each experimental unit at full maturity.

4. Weight of 300 seeds (g): Randomly select 300 seeds from the seed yield of each experimental unit and weigh them

using a sensitive electronic balance Sartorius species (German).

5. Seed yield per plant (g): Record the average seed yield per plant at full maturity after averaging the weight of five plants from each experimental unit using a sensitive electronic balance.

6. Water use efficiency (kg(m³)⁻¹): Measured according to the method described in [1].

$$\text{Water use efficiency} = \frac{\text{Seed yield kg ha}^{-1}}{\text{Water used (m}^3\text{)}}$$

Statistical analysis

The statistical analysis of the field experiment data was performed using the split-plot arrangement according to the Randomized Complete Block Design (R.C.B.D.) and the ready-made statistical analysis program S.A.S [14] was used. The Duncan Multiple Range Test [5] was also used to compare the means at a significance level of (0.05).

Results

The effect of water stress and Foliar Application with amino acids on leaf area (cm²)

The results in Table (4 and 10) showed There are highly significant differences

between the average treatments of water stress and Foliar Application with amino acids in the leaf area characteristic, as the treatment without water stress gave the highest average, reaching 117.54 cm², compared to the second stress treatment, which was 84.89 cm². The results of the same table also showed significant differences between the treatments of Foliar Application with amino acids on castor plants in the leaf area characteristic, and the Foliar Application treatment of 3 ml L⁻¹ had the highest average of 113.76 cm² compared to the treatment

of not Foliar Application with amino acids 90.652 cm². There was a significant interaction between the two factors of water stress and Foliar Application with amino acids, and the interaction treatment (Foliar Application 3 ml L⁻¹ of amino acids and without water stress) gave the highest average in leaf area, which reached 149.48 cm² compared to the interaction treatment (not Foliar Application with amino acids and the second water stress), which gave the lowest average of 78.30 cm².

Table 4. Effect of water stress and Foliar Application with an amino Bio-stimulant on leaf area

Water stress	Bio-stimulant				Means
	0	1 ml	2 ml	3 ml	
No water stress	109.01 b	102.87 b	108.82 b	149.48 a	117.54 a
First water stress	84.64 b	88.48 b	97.69 b	99.61 b	92.60 b
Second water stress	78.30 b	85.98 b	83.10 b	92.21 b	84.89 b
Means	90.65 b	92.44 b	96.53 b	113.76 a	

Effect of water stress and Foliar Application with amino acids on plant fresh weight (g)

The results in Table (5 and 10) showed There are highly significant differences between the treatments of water stress and Foliar Application with amino acids in the trait of plant fresh weight, as the treatment without water stress gave the highest average weight, which reached 309.08 g, compared to the second stress stage, which was 200.05 g. The results of the same table also showed significant differences between the treatments of Foliar Application with amino acids on castor plants in the trait of fresh weight in the plant. The

treatment of Foliar Application with 3 ml L⁻¹ had the highest average of 300.11 g compared to the treatment of not Foliar Application with amino acids 205.83 g. There was a significant interaction between the two factors of water stress and Foliar Application with amino acids, and the interaction treatment (Foliar Application with 3 ml L⁻¹ of amino acids and without water stress) gave the highest average for the trait of fresh weight in the plant, which reached 384.10 g compared to the interaction treatment (not Foliar Application with amino acids and water stress at the second stage), which gave the lowest average of 159.20 g.

Table 5. Effect of water stress and Foliar Application with an amino Bios-timulant on fresh weight

Water stress	Bio-stimulant				Means
	0	1 ml	2 ml	3 ml	
No water stress	265.13 d	270.06 c	317.03 b	384.10 a	309.08 a
First water stress	193.16 h	209.60 g	245.06 e	270.03 c	229.46 b

Second water stress	159.20j	168.10 i	226.73 f	246.20 e	200.05 c
Means	205.83 d	215.92 c	262.94 b	300.11 a	

Effect of water stress and amino acid Foliar Application on the number of capsules per plant

The results in Table (6 and 10) showed There are highly significant differences between the water stress and amino acid Foliar Application treatments in the number of capsules per plant. The treatment without water stress was superior, giving the highest average of 55.25 capsules compared to the stress treatment at the second stage, which was 34.50 capsules. The same table also showed significant differences between the amino acid Foliar Application treatments in the number of capsules per plant, with the 3 ml spraying treatment being superior, giving the highest average of 54.00

capsules compared to the treatment without amino acid spraying, which was 36.00 capsules. A significant interaction occurred between the stress factor and spraying with amino acids. The interaction treatment (Foliar Application with a concentration of 3 ml/L of amino acids and without water stress) gave the highest average number of capsules per plant, reaching 68.00 capsules, compared to the interaction treatment (no Foliar Application with amino acids and water stress at the second stage, which was 26.00 capsules).

Table 6. Effect of water stress and Foliar Application with an amino Bio-stimulant on number of capsules

Water stress	Bio-stimulant				Means
	0	1 ml	2 ml	3 ml	
No water stress	46.00 d	50.00 c	57.00 b	68.00 a	55.25 a
First water stress	36.00 h	38.00 g	46.00 d	50.00 c	42.50 b
Second water stress	26.00 j	29.00 i	39.00 f	44.00 e	34.50 c
Means	36.00 d	39.00 c	47.3 b	54.00 a	

The effect of water stress and Foliar Application with amino acids on the weight of 300 seeds (g)

The results in Table (7 and 10) showed There are highly significant differences between the treatments of water stress and Foliar Application with amino acids in the weight of 300 seeds. The treatment without water stress was Foliar Application in the weight of 300 seeds and gave the highest average of 88.90 g compared to the second stress stage, which was 83.22 g. The results of the same table also showed significant differences between the treatments of Foliar Application with

amino acids on castor plants in the 300-seed weight trait. The Foliar Application treatment of 2 ml L⁻¹ had the highest average of 87.81 g compared to the treatment of not Foliar Application with amino acids, which was 83.40 g. There was a significant interaction between the stress factors and Foliar Application with amino acids, and the interaction treatment (Foliar Application 2 ml L⁻¹ of amino acids without water stress) gave the highest average for the 300-seed weight trait, which was 90.66, compared to the interaction treatment (not Foliar Application with amino acids and water

stress at the second stage, which was 80.21 g).

Table 7. Effect of water stress and Foliar Application with an amino Bio-stimulant on weight of 300 seeds

Water stress	Bio-stimulant				Means
	0	1 ml	2 ml	3 ml	
No water stress	87.60 c	88.70 b	90.66 a	88.65 b	88.90 a
First water stress	82.40 g	86.13 de	88.48 b	86.85 cd	85.96 b
Second water stress	80.21 h	82.69 g	84.29 f	85.70 e	83.22 c
Means	83.40 d	85.84 c	87.81 a	87.07 b	

Effect of water stress and amino acid Foliar Application on seed yield per plant (g plant⁻¹)

The results in Table (8 and 10) show There are highly significant differences between the water stress and amino acid Foliar Application treatments in seed yield per plant. The treatment without water stress gave the highest average yield of 20.34 g, compared to 13.91 g in the second stress stage. The same table also shows significant differences between amino acid Foliar Application treatments on castor bean plants in seed yield per plant. The

treatment with 3 ml L⁻¹ Foliar Application resulted in the highest average yield of 20.26 g, compared to 13.82 g in the treatment without amino acid Foliar Application. A significant interaction was found between the factors of water stress and amino acid Foliar Application. The interaction treatment (Foliar Application 3 ml/L of amino acids without water stress) gave the highest average seed yield, reaching 25.00 g, compared to the interaction treatment (no amino acid Foliar Application and water stress at the second stage), which was 11.01 g.

Table 8. Effect of water stress and Foliar Application with an amino Bio-stimulant on seed yield

Water stress	Bio-stimulant				Means
	0	1 ml	2 ml	3 ml	
No water stress	16.90 e	18.36 c	21.12 b	25.00 a	20.34 a
First water stress	13.56 h	14.72 g	17.01 de	18.50 c	15.94 b
Second water stress	11.01 j	11.61 i	15.71 f	17.29 d	13.91 c
Means	13.82 d	14.89 c	17.95 b	20.26 a	

Effect of water stress and amino acid Foliar Application on water use efficiency (kg seeds (m³)⁻¹ water)

Table 9 shows significant differences between the water stress and amino acid Foliar Application treatments in water use efficiency. The treatment without water stress showed the highest water use efficiency, yielding an average of 0.040 kg seeds (m³)⁻¹ water, compared to the second stress treatment, which yielded 0.030 kg seeds (m³)⁻¹ water. The same table also

shows significant differences between the amino acid Foliar Application treatments. The treatment with 3 ml L⁻¹ showed the highest average water use efficiency, at 0.042 kg seeds (m³)⁻¹ water, compared to the treatment without amino acid Foliar Application, which yielded 0.029 kg seeds (m³)⁻¹ water. A significant interaction occurred between the two factors of water stress and Foliar Application with amino acids. The interaction treatment (Foliar Application 3 ml L⁻¹ amino

acids and without water stress) amounted to 0.049 kg seeds (m³)⁻¹ water, compared to the interaction treatment (no Foliar Application with

amino acids and water stress at the second stage), which was 0.024 kg seeds (m³)⁻¹ water.

Table 9. Effect of water stress and Foliar Application with an amino Bio-stimulant on water use efficiency

Water stress	Bio-stimulant				Means
	0	1 ml	2 ml	3 ml	
No water stress	0.033 gh	0.036 e	0.041 b	0.049 a	0.040 a
First water stress	0.029 i	0.032 h	0.035 ef	0.040 c	0.034 b
Second water stress	0.024 j	0.025 j	0.034 fg	0.037 d	0.030 c
Means	0.029 d	0.031 c	0.037 b	0.042 a	

Sources of variation	Degrees of freedom	Leaf area	Fresh weight of plant	Number of capsules	Weight of 300 seeds	Seed yield per plant (g)	Use efficiency
BLOCK	2	322.8**	140.97**	108..00**	69.106**	5.34**	0.00001046
Stress A	2	3494.57**	38180.22*	1314.25**	96.96**	129.92**	0.000029626
ERORR A	4	276.67	1.027	1	0.19	0.1668	0.00000122
Spray B	3	1005.35**	1719,98**	600.25**	33.57**	77.4**	0.00033435
AB	6	317.74**	539.17**	11.25**	3.93**	1.98**	0.00000817
.ERROR	18	265.81	0.4915	0.22	0.23	0.035	0.00000064

Table 10 . Table of variance analysis for the studied traits

Discussion

The decrease in leaf area and fresh weight of the plant during the stress stages (Tables 4 and 5) is attributed to the role of water stress in weakening root growth and water and nutrient absorption. This, in turn, affects the efficiency of photosynthesis in carbohydrate and protein production and their translocation to vegetative parts, thus reducing leaf area [21] and fresh weight [6]. This was confirmed by [23], who stated that water stress during the vegetative growth stage reduces plant weight. The deterioration of physiological activities in the plant and the impaired accumulation of carbohydrate metabolites and nutritional balance during stress stages significantly affect pollination,

fertilization, and the number of seed capsules in the plant [17]. The significant effect of reduced seed weights during the second stress stage is attributed to the plant's physiological deficit during this stage. This deficit impacts the accumulation, translocation, and distribution of nutrients between vegetative and reproductive parts, while simultaneously reducing the activity of enzymes and hormones associated with flower set, fertility, and seed filling. Furthermore, the decrease in seed yield during the second water stress stage (Table 8) may be due to the effect of water stress at this stage on reducing the average number of seed capsules per plant (Table 6). The superior water use efficiency of the non-stress

irrigation treatment is also attributed to the increased seed yield (Table 8). [2] confirmed that water stress stages are a crucial factor in determining crop water use efficiency. The significant improvement observed with amino acid Foliar Application treatment at a concentration of 3 ml L⁻¹ in leaf area and plant fresh weight (Tables 4 and 5) is attributed to their role as nitrogen- and zinc-rich Bio-stimulants. These amino acids play a vital role in chlorophyll synthesis by providing the necessary building blocks and activating enzymes [3]. Furthermore, they enhance nitrate and nutrient uptake, improve the availability of essential amino acids for the synthesis of hormones that promote leaf growth and increase leaf area, and boost carbon metabolism, distributing its bioactive compounds throughout the plant's vegetative parts and increasing plant fresh weight. Furthermore, the significant improvement observed with amino acid Foliar Application at a concentration of 3 ml L⁻¹ in the number of capsules per plant, the weight of 300 seeds, and the plant's seed yield (Tables 6, 7, and 8) is attributed to the role of amino acids in increasing leaf area (Table 4), And its contribution to improving the carbon metabolism process. This, in turn, increases the number of capsules per plant, as well as the accumulation of carbohydrates and proteins necessary for growth, flowering, and fruit set [4]. [9] confirmed that amino acid Foliar Application significantly increases the weight of 300 seeds, the number of capsules in the rhizomatous inflorescence, and the plant's seed yield. Additionally, amino acids act as Bio-stimulants, protecting pigments chlorophyll in plant cells. This improves the efficiency of carbon metabolism, the rate of amino acid translocation to seeds, and seed weight.

The increased water use efficiency observed when amino acids were sprayed at a concentration of 3 ml L⁻¹ on castor bean leaves is attributed to the significant effect of this spray treatment on increasing seed yield per hectare, resulting in superior water use efficiency [16].

Conclusion

We conclude that castor beans are sensitive to water stress during their vegetative growth stages, as it clearly affects their reproductive stage and yield. Furthermore, spraying with amino acids has clearly improved the plant's seed yield.

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