Response of Two Cowpea Cultivars to Spraying with Moringa Leaf Extract and Jasmonic Acid in Growth Yield Components

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ABSTRACT
A field experiment was conducted at the Research Station of the College of Agriculture, University of Kufa/Najaf, for the spring season of 2023, to determine the growth and yield response of two cowpea varieties to spraying with Moringa leaf extract and jasmonic acid (JA). The experiment included three factors, the first factor was two varieties of cowpea: American Ramshon and Dutch variety, the second factor was foliar spray with Moringa leaf extract at three concentrations of 0.2 and 4 g L-1, and the third factor was foliar spray with three concentrations of JA at 0, 10 and 20 mg L-1. The spraying was applied three times during the growing season, with an interval period of 14 days. The experiment was split-split-plots based on Randomized Complete Blocks Design (R.C.B.D) and three replicates. The results showed that the Dutch variety was significantly superior in the vegitative growth indicators, while the American cowpea Ramshon had the highest response in quantitative and chemical indicators, especially the total plant yield of green pods recording 7.470 tons ha-1 compared to 5.886 tons ha-1 for the Dutch variety. Spraying with Moringa leaf extract at a concentration of 4 g L-1 recorded significantly higher indicators in the shoot dry weight 65.59 g plant-1, number of pods 25.61 pods plant-1, and leaf and pods content of carbohydrates and protein 2.65 and 12.95%. On the other hand, foliar spray with JA at 20 mg L-1 recorded a significant increase in plant length 45.88 cm. Most of the studied indicators in the experiment recorded higher rates in the interaction treatment of the Dutch variety and spraying 4 g L-1 Moringa leaf extract and 10 mg L-1 of JA compared to all individual and interaction treatments.

1. Introduction

Cowpea (Vigna unguiculata L.) belongs to the legume family Leguminosea. Its original habitat is mostly Central Africa. Globally, its cultivation is widespread in hot and temperate regions. Cowpea is a plant that can tolerate hot and dry environmental conditions, as well as its tolerance to salinity. It is cultivated for its green pods and dry seeds. It is a crop rich in nutrients, containing 86% water, 3.3% protein, 9.5% carbohydrates, and 44 calories per 100 g of its green pods. It is fresh green or cooked and preserved in cans and in different ways (Boras et al., 2006). From the medical side, fresh green bean pods help reduce blood sugar, while dry ones can be consumed as a tea to treat sciatica, chronic rheumatism, uric acid accumulation, and cell renewal (Mustafa, 2010). The total area planted with cowpea worldwide reached 2,684,296 hectares and its production reached 6,144,394.66 tons, while the total area planted in Iraq reached 677 hectares and its production reached 1,894 tons (FAO, 2024). The quantitative yield of cowpea also varies according to the cultivated varieties, including hybrid varieties with a deep root system that give a plentiful yield (Odedina et al., 2014), in addition to the fact that its yield depends on the method of growth, whether that variety is upright or climbing (Elnaim, 2010).

The nutritional and medicinal importance of the cowpea plant requires the search for modern agricultural methods to improve plant growth and increase the quantitative and qualitative yield. (Boras et al., 2006) indicated the use of many plants in preparing plant extracts and using them as foliar fertilizers on vegetable crops, including Moringa oleifera L. It is one of 13 species of the genus Moringa belonging to the Moringnance family. Moringa leaves contain vitamins A, B and C in addition to nutrients such as potassium, calcium, magnesium, zinc, iron, copper and amino acids, as well as growth hormones, auxins and cytokines. In recent years, some researchers have turned to using the growth regulator jasmonic acid, as studies have shown the positive effect of the acid on the growth of flowers of some garden plants, in addition to its important effect on cellular organization and plant development processes and stimulating the plant against environmental stresses such as heat, drought and salinity (Al-Asadi and Al-Khaikani, 2019). Therefore, the experiment aimed to determine the response of growth and yield to foliar spray with Moringa leaf extract and/or jasmonic acid in single treatments or combination in two cowpea varieties. And to determine the best spray concentration of both experimental factors.

2. Methodology

Experiment site, cultivation practices and plant service were carried out during the spring growing season of 2023 at the research station of the College of Agriculture / University of Kufa. The field soil was analyzed and the soil properties were determined chemically and physically before implementing the experiment in a representative sample of ten random samples from several different locations of the field soil at a depth of 0-30 cm (Table1). The Meteorological Authority in Najaf Governorate was consulted to take data on the monthly maximum and minimum temperature rates (Table2).

Soil preparations were carried out (ploughing, sterilization and leveling), then the seeds of the American and Dutch experimental varieties of cowpea were planted directly in the field on 3/20/2023 for the spring season on both sides of the line alternately and with a planting distance of 25 cm. The area of the experimental unit was 1.25 m^2 (1.25 m length x 1 m line distance), which contained eight plants. A distance of 0.5 m was left between the experimental units and a distance of 1 m was left at the beginning and end of the field as guard plants. Agricultural service operations were applied due to their importance to the experimental plants after planting, including irrigation by the drip system according to the plant's need, and manual weeding was carried out several times during the season (Matloub et al., 1989). The treatments were carried out in the early morning by foliar spray of cowpea plants until completely wet using a 16-liter backpack sprayer and according to the concentrations for each treatment. Before that, the plants were watered to increase their efficiency in absorbing the sprayed material (Al-Sahaf, 1989). Spraying was done three times during the growing season: the first after the plant reached the stage of forming four true leaves, the second spray at the beginning of flowering, and the third spray two weeks after the second spray, while the control plants were sprayed with water only.

Moringa leaf extract and Jasmonic acid

For preparation of Moringa *Moringa oleifera* leaf extract, 100 g of Moringa leaf powder was placed in a beaker, 1 liter of distilled water was added to it, then it was placed on an electric shaker for one hour. The resulting solution was filtered using several layers of medical gauze (gauze) and the volume was completed to 1 liter, considering the result at a concentration of 100% (raw). From which the concentrations required in the experiment were prepared, the concentrations used were placed in plastic bottles and kept in the refrigerator until use (Al-Marsoumi, 1999).

Jasmonic acid was prepared by taking 0.1 of jasmonic acid C12 H18O3 (the one package content) with adding 100 ml of one standard HCL acid, placed in a 100 ml flask (Stock's solution) 1000 ppm, which is the original (raw) concentration. Then the amount was diluted with distilled water according to the concentrations used in the experiment (0, 10 and 20 mg L^{-1}).

Table 1. Some chemical and physical properties of field soil before planting

Property	**Unit**	**Value**
pH		**7.19**
EC	**dSm m^{-2}**	**1.47**
OR	**%**	**0.96**
N	**mg Kg^{-1} soil**	**40.3**
P		**58.11**
K		**84.85**
Soil particles		
Clay	**%**	**56**
Silt		**70**
Sand		**874**
Soil texture		Sandy loam

Table 2. Monthly temperatures (maximum and minimum) for the 2023 growing season

Months	Temperature °C	
	Max	Min

March	**26**	**15**
April	**32**	**18**
May	**38**	**24**
June	**43**	**29**
July	**47**	**30**
August	**47**	**32**
September	**44**	**31**

Table 3. Moringa leaf extract content of some chemicals

Macro-nutrients	%	Micro-nutrients	%	Plant hormones	%
N	**3.29**	**Fe**	**147**	**Auxin**	**0.25**
P	**2.35**	**Mn**	**0.17**	**Cytokinin**	**0.20**
K	**4.42**	**B**	**0.04**		
Mg	**0.61**	**Zn**	**0.73**		
Ca	**0.4**				

The experiment was carried out using the split-split-plot design according to the Randomized Complete Block Design (R.C.B.D.) with three replicates and 54 experimental units, two varieties of cowpea, the American Ramshorn and the Dutch variety in the main plots, Moringa plant extract at three concentrations of 0, 2 and 4 g L^{-1} in the sub-plots, and the growth regulator Jasmonic acid at concentrations of 0, 10 and 20 mg L^{-1} for the sub-sub-plots.

Analysis of variance (A.N.O.V.A) was performed to test the differences between the means according to the Least Significant Difference (L.S.D.) at the probability level of 0.05 (Al-Rawi and Khalaf Allah, 2000). The experimental data were analyzed using the VSN International Gen Stat 12.1 (2009) program in conducting its analysis.

The studied experiment indicators included the vegetative growth indicators for five random plants from each experimental unit for the three replicates: plant length (cm), dry weight of the vegetative mass (g/plant^{-1}) (Al-Sahaf, 1989). Quantitative yield indicators: number of pods (1 pod/plant^{-1}) from the beginning of harvesting until the last harvest cumulatively, total yield (tons/ha^{-1}) for 10 harvests starting from 1/6 to 15/8: Experimental unit yield = yield of one plant * No of plants in the experimental unit. Where: Total yield (tons/ha^{-1}) = $\frac{\text{Experiment unit yield} * 1000\text{m2}}{\text{Experiment unit area m2}}$

Qualitative yield indicators were also evaluated including: percentage (%) of carbohydrates in leaves (Herbert, 1971) and protein in pods (%) based on dry weight.

3. Results and discussion

The results showed that plant length and shoot dry weight differed among the cultivars due to the effect of treatments (Tables 4 and 5). The Dutch cultivar of cowpea recorded higher rates of plant length 44.29 cm and shoot dry weight 58.55 g than the American Ramshor cultivar which recorded 42.46 cm and 55 g, respectively. Also, foliar spraying with Moringa extract at 4 g/L recorded higher values for plant length and shoot dry weight than lower concentrations. Also, spraying with JA led to higher plant length and shoot dry weight, which increased with increasing its concentration to 10 mg/L. The interaction treatment of the Dutch cultivar and 4 g/L Moringa extract in the presence of JA at 10 mg/L recorded the highest values not only for plant length but also for vegetative dry weight with significant differences from most interaction treatments and individual ones.

Table 4. Effect of cultivar, foliar spray with Moringa leaf extract, Jasmonic acid and their interactions on cowpea plant length (cm)

Cowpea	**Moringa leaf extract**	**Jasmonic acid mg L^{-1}**			**Average**
		0	10	20	

Ramshon var.	0	41.49	44.35	43.12	42.03
	2	42.59	42.30	41.19	42.99
	4	42.79	49.75	43.50	45.35
Dutch vae.	0	40.58	44.79	44.06	43.14
	2	42.54	44.30	45.08	43.97
	4	44.41	49.76	43.08	45.75
L.S.D 0.05		4.23			3.44
Average		42.4	45.88	43.34	
Interaction var/JA	Ramshor	41.29	44.47	41.61	42.46
	Dutch	42.51	46.28	44.08	44.29
L.S.D 0.05		1.77			1.38
Interaction Plant ext./JA	0	40.04	43.57	42.59	42.07
	2	42.57	43.30	43.14	43.00
	4	43.6	49.76	43.29	45.55
L.S.D 0.05		3.38			2.95

Table 5. Effect of cultivar, foliar spray with Moringa leaf extract, Jasmonic acid and their interactions on cowpea shoot dry weight (g plant^{-1})

Cowpea	**Moringa leaf extract**	**Jasmonic acid mg L^{-1}**			**Average**
		0	10	20	
Ramshon var.	0	44.19	44.88	55.54	48.2
	2	48.2	62.3	58.01	56.17
	4	54.13	66.99	69.77	63.63
Dutch vae.	0	49.04	58.57	57.33	54.98
	2	51.31	57.04	51.02	53.13
	4	60.34	72.51	69.82	67.56
L.S.D 0.05		5.112			3.206
Average		51.2	60.38	60.25	
Interaction var/JA	Ramshon var.	47.84	57.06	60.11	55.00
	Dutch vae.	53.56	62.71	59.39	58.55
L.S.D 0.05		2.863			2.887
Interaction Plant ext./JA	0	46.61	51.72	56.43	51.59
	2	49.76	59.67	54.52	54.65
	4	57.23	69.75	69.8	65.59
L.S.D 0.05		3.734			2.541

The cowpea variety in the experiment had a positive effect on the vegetative growth indicators, including plant length and dry weight of the vegetative group. The Dutch variety outperformed the other American experiment variety, Ramshorn, by giving the highest rates of vegetative indicators. This is attributed to the genetic nature of the variety and its interference in the course of the plant's internal vital activities and its effect on controlling the nature of plant growth. Or the reason may be due to the variety's suitability to the prevailing environmental conditions in Najaf Governorate, resulting in a difference in the efficiency of the photosynthesis process, which had a positive effect on increasing the strength of vegetative growth (Al-Mufarji and Al-Jabouri, 2017) Mfeca et al. (2019). Moringa leaf extract showed an effect when sprayed on the vegetative growth indicators, plant length and dry weight of the vegetative group, due to its nitrogen content (N) and its essential role in the physiological processes within the plant, especially the construction of many organic compounds, the most important of which are chlorophyll pigment, DNA and RNA nucleic acids, proteins, hormones and enzymes that enter into the process of protoplasm construction, and the construction of cytochromes important in the processes of photosynthesis and respiration (Al-Sahaf, 1989). Accordingly, it increases the plant's ability for nutrients production through the process of

photosynthesis and its positive reflection in increasing plant growth rates compared to plants sprayed with water only. This is consistent with the results by Khabar (2019) in his experiment on the fava bean plants. The plant extract contains phosphorus (P) and its effective role in stimulating enzymatic reactions to build vital and energy compounds and increasing the effectiveness of photosynthesis in the plant, which has a positive effect on increasing the production of dry matter and its accumulation in the plant due to the increases achieved in vegetative growth indicators (Abu Dahi and Al-Younes, 1988). In addition to the extract containing potassium (K), which affects the process of opening and closing the stomata and thus increasing the absorption of water and nutrients that activate the photosynthesis process and increase its outputs and its effect on cell division and then increase vegetative growth (Nassim et al., 2019). Among the other essential elements contained in the plant extract is boron (B), which contributes to plant growth and development, which affects most of the plant's vital processes, including the transfer of sugars, the formation of carbohydrates, the growth of the pollen tube, increasing fertilization and maintaining the water balance in the cell (Abu Dahi and Al-Younes, 1988). As for the zinc element (Zn) found in the plant extract of Moringa leaves, it has an important effect in the synthesis of the amino acid Tryptophan, which is the initiator of the synthesis of auxin Indole Acetic Acid and increases the process of cell division, in addition to increasing the number of leaves through the process of meristematic cell division in the formation of the leaf primordial and then increasing the leaf area and producing good vegetative growth (Al-Sahhaf, 1989). The clear improvement in growth indicators is attributed to the content of the extract of growth regulators auxin and cytokinin present in the contents of the Moringa leaf extract and their positive effect Thomas (1982) (Amar, 2003). The increase in vegetative growth is due to foliar nutrition with JA, which works to increase the concentration of nitrogen and potassium and their necessity in building cells and increasing the efficiency of the photosynthesis process. This enables the plant to retain sufficient water, which is reflected in the increase in the number of cells and thus the increase in the plant height index. The increase in vegetative growth indicators may also be due to the role of JA in protecting the plant and improving growth by stimulating the photosynthesis process and increasing pigments, increasing the permeability of membranes to the necessary nutrients, which leads to improving vegetative indicators, including the dry weight of the vegetative plant parts (Imran, 2023).

As for the yield indicators, number of pods (pod plant^{-1}) and total yield (ton/ha^{-1}), the results showed significant differences between the two experimental varieties in number of pods and yield of the plant (Tables 6 and 7). Where, the American variety being superior with 22.50 pods/plant^{-1} and total yield of 7.470 tons/ha^{-1} compared to 17.98 pods/plant^{-1} and 5.886 tons/ha^{-1} for the Dutch variety. The spraying treatment with Moringa leaf extract at a concentration of 4 g/L^{-1} was significantly superior with a number of pods of 25.61 pods/plant^{-1} and a total yield of 13.343 tons/ha^{-1} compared to the control sprayed with water only 15.09 pods/plant^{-1}. It was also found that spraying with JA had a significant effect on the number of pods in the plant, especially at 20 mg L^{-1}. The highest number of pods reached 22.00 pods/plant^{-1} and a total yield of 9.622 tons/ha^{-1}. The results (Tables 6 an7) show that the highest yield indicators for the number of pods and the total yield were recorded in the interaction treatment of the American variety with spraying at a concentration of 20 mg L^{-1} JA and 4 g/L Moringa extract, which recorded 30.96 pods/plant^{-1} and a total yield of 13.976 tons/ha^{-1}, with a significant difference from most other treatments.

Table 6. Effect of cultivar, foliar spray with Moringa leaf extract, Jasmonic acid and their interactions on cowpea yield of No. of pods (pod plant^{-1})

Cowpea	Moringa leaf extract	Jasmonic acid mg L^{-1}			Average
		0	**10**	**20**	
Ramshon var.	**0**	**14.26**	**14.32**	**18.92**	**15.83**
	2	**17.39**	**15.4**	**29.04**	**20.61**
	4	**17.85**	**24.4**	**23.77**	**22.01**
Dutch vae.	0	**10.66**	**15.71**	**16.64**	**14.34**
	2	**16.64**	**16.66**	**20.49**	**17.93**
	4	**20.49**	**21.41**	**23.11**	**21.67**

L.S.D 0.05		**10.358**			**6.707**
Average		**16.22**	**17.98**	**22.00**	
Interaction var/JA	Ramshon var.	**17.50**	**19.04**	**30.96**	**22.50**
	Dutch vae.	**15.93**	**17.93**	**20.08**	**17.98**
L.S.D 0.05		**5.36**			4.46
Interaction Plant ext./JA	0	**12.46**	**15.01**	**17.78**	**15.09**
	2	**17.02**	**16.03**	**23.44**	**18.83**
	4	**19.17**	**22.91**	**34.76**	**25.61**
L.S.D 0.05		**7.77**			**5.61**

Table 7. Effect of cultivar, foliar spray with Moringa leaf extract, Jasmonic acid and their interactions on cowpea total yield of pods (Ton h^{-1})

Cowpea	Moringa leaf extract	Jasmonic acid mg L^{-1}			Average
		0	**10**	**20**	
Ramshon var.	**0**	**5.440**	**5.425**	**7.955**	**6.273**
	2	**7.022**	**5.529**	**10.742**	**7.764**
	4	**6.785**	**9.635**	**9.371**	**8.597**
Dutch vae.	**0**	**3.697**	**5.680**	**6.389**	**5.255**
	2	**5.974**	**5.373**	**9.297**	**6.881**
	4	**6.937**	**11.962**	**13.976**	**10.958**
L.S.D 0.05		**2.903**			**2.354**
Average		**5.976**	**7.267**	**9.622**	
Interaction var/JA	**Ramshon var.**	**5.930**	**7.065**	**9.416**	**7.470**
	Dutch vae.	**3.427**	**5.573**	**8.659**	**5.886**
L.S.D 0.05		**1.298**			**1.286**
Interaction Plant ext./JA	**0**	**2.491**	**3.511**	**3.720**	**3.241**
	2	**6.112**	**9.625**	**11.180**	**8.972**
	4	**11.948**	**13.332**	**14.748**	**13.343**
L.S.D 0.05		**2.29**			**1.99**

It is noted from the results that the American variety Ramshon is superior to the Dutch variety in the quantitative yield indicators of the number of pods and the total yield of green pods. This is due to a genetic difference between the two experimental varieties, especially the American variety, which showed a higher response to the environmental conditions (temperatures) prevailing during the growing season, which contributed to the transformation of nutrients through metabolic processes and their reflection on the quantitative indicators. This is consistent with previous studies (Mousa et al., 2017) (Youseph et al., 2017) on the different responses of bean varieties to different growth conditions. The increases in the quantitative yield indicators in the spraying treatment with Moringa extract are mostly due to the extract containing some nutrients such as nitrogen (N), phosphorus (P) and potassium (K) that contribute directly to many physiological and vital plant processes, especially amino acids, nucleic acids, prolines, chlorophyll and organic compounds, in addition to enzymes and the activity of some of them, and increasing the number and size of cells, and thus increasing the vegetative indicators. This led to an impact on securing the necessary food for the plant as a result of reducing the competition between flowers for food, and thus increasing the quantitative indicators, including the number of pods and the total yield of green pods (Al-Sahaf, 1989).

The process of photosynthesis and the transfer of its products of carbohydrates and proteins to flowers during their growth and development stages, thus increasing the total number of flowers and increasing the percentage of nodes. The role of boron (B) in improving pollen germination, fertilization, and increasing the percentage of nodes, in addition to zinc (Zn) in participating in the formation of the amino acid tryptophan and converting it to the natural auxin IAA, and its contribution to early flowering and fruit formation. As for growth regulators, including auxin and cytokinin found in the components

of Moringa leaf extract, they positively affect the growth and development of the plant by increasing the speed and division of cells, in addition to auxins by improving flowering rates in addition to the formation of pollen grains, fertilization, and nodes (Saleh, 1991). This result is consistent with the results reached by Al-Zubaidi and Al-Naimi (2009) and Aziz (2016) in their experiments on various agricultural crops, including peas and broad beans. The increase in quantitative yield indicators may also be due to the content of (plant extract) of essential nutrients in addition to the growth regulators mentioned above, which worked to improve vegetative growth indicators, specifically leaves, and then positively reflected on increasing the production of nutrients and their accumulation in pods. Spraying with jasmonic acid led to a clear effect on the percentage of fruit formation, and increasing the spray concentrations leads to an increase in the activity of the photosynthesis process and an increase in the ability of cells to accumulate sucrose, proteins and amino acids in the plant. In addition to the property of fatty acids that help increase the formation of the total number of flowers and thus increase the percentage of fruit formation. This effect was positively reflected on increasing the number of pods and the total yield (Imran. 2023).

4. Conclusion and future scope

The current study concludes that the American variety plants are significantly superior in most of the studied indicators. It also showed that foliar spraying of cowpea plants with Moringa leaf extract at a concentration of 4 g L-1 and jasmonic acid at a concentration of 10 mg L-1 contributed to improving vegetative growth indicators, which was reflected in a remarkable way in increasing vegetative growth indicators and yield components.

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