Open Access J. Glob. Innov. Agric. Sci., 2024, 12(x): xxx ISSN (Online): 2788-4546; ISSN (Print):2788-4538 DOI: https://doi.org/10.22194/JGIAS/24.1125 http://www.jgiass.com



Effects of Brassinosteroid Hormone on Chemical Composition of Okra (Abelmoschus esculentus L.) under Salt Stress

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This study investigated how two okra cultivars (Khenissari and Petra) responded to salinity and Brassinosteroid hormone treatments during a greenhouse season. Plants were exposed to varying levels of salinity in irrigation water (0 to 7.5 dS m-1) and sprayed with different Brassinosteroid concentrations (0 to 1 mg l-1). Higher salinity significantly reduced key nutrients in leaves (nitrogen, phosphorus, potassium) and the potassium/sodium ratio while increasing sodium and chlorine levels. Khenissari generally performed better than Petra, retaining more nutrients and showing lower sodium/chlorine levels. Spraying with Brassinosteroid, particularly at 1 mg l-1, significantly increased nutrient levels (especially potassium) and the potassium/sodium ratio while decreasing sodium and chlorine. Most interactions between salinity and Brassinosteroid treatments significantly affected the studied traits. This research suggests that Brassinosteroid could potentially mitigate the negative effects of salinity on okra plants, particularly the Khenissari cultivar, by improving nutrient uptake and reducing sodium/chlorine accumulation. Further research is needed to optimize the timing and dosage of Brassinosteroid application for optimal salinity tolerance in okra.

Keywords: Okra cultivars; Abelmoschus esculentus L. Salty; Brassinosteroid Hormone; Salt Stress.

INTRODUCTION

Water salinity is one of the most significant issues affecting agriculture worldwide, particularly in dry and semi-arid countries (Munns and Tester, 2008). Desert regions depend on well water to irrigate their plants because there is a paucity of surface water and indiscriminate use. Irrigation of water leads to abiotic stress. Salinity is the primary cause of abiotic stress, which has a detrimental effect on crop germination, growth, and yield as it contains dissolved salts to grow okra plants in lands that are salinized or irrigated with highly salinized water, such as well water; therefore, it is necessary to use some techniques at present in order to increase salt tolerance.

Among these alternative technologies is treatment with Brassinosteroid C28H48O6, a plant steroidal hormone that stimulates growth and is considered the sixth of the growth regulators (Abe, 1989). Because of its recent appearance as a new plant hormone and the numerous and varied impacts it has on physiological processes, including germination, growth, flowering, and aging, many crops can tolerate it when exposed to various abiotic challenges, encourages cell response such as stem elongation, increase leaf epinasty and ethylene, and regulates photosynthesis (Sasse, 2003). Under stressful circumstances, it regulates stomata closure and opening, transpiration, and maintaining the water content of plant tissue (Huttner and Strasser 2012).

One of the most significant summer crops in both Iraq and the rest of the world is okra. It is a member of the Malvaceae family, which originated in Ethiopia and Sudan. Others are produced for their green pods, used in cooking, drying, canning, and freezing. It is necessary. Okra pods are sometimes used as a coffee substitute in several nations. The stems and pods of okra are also used to extract it. The paper industry uses ripe fibers. Okra is a vegetable crop high in calcium, niacin, and riboflavin. It has respective concentrations of 0.6 mg, 0.9 mg, and 82 mg per 100 gm. Protein, carbs, phosphate, ascorbic acid, and vitamin A levels are moderate. Few soluble solids are present (Hijazi *et al.*, 2001).

MATERIALS AND METHODS

Experimental Design: It regulates the stomata's opening and closure. The investigation was carried out in the 2019–2020 growing year in an unheated greenhouse with dimensions of

Saihood, Z.A., A.N. Gerry and A.A. Abdulla. 2024. Effects of Brassinosteroid hormone on chemical composition of okra (*Abelmoschus esculentus* L.) under salt stress. Journal of Global Innovations in Agricultural Sciences 12:xxxxx.

Received 2 Jun 2023; Accepted 23 Jul 2023; Published (online) 15 Feb 2024]

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9 x 50 m in the Tomato Development Project affiliated with Basrah's Directorate of Agriculture. The response of two okra cultivars (Khenissari and Petra) was one of three parameters that the study considered irrigated by water of different salinity levels (RO, 2.5, 5, 7.5 dS m-1) and treatment with growth hormone Brassinosteroid at concentrations (0, 0.5, 1 mg l-1). It was applied to the vegetative system in three sprays, the first one coming two weeks after germination and the second and third sprays coming three weeks apart. R.C.B.D., or Randomised Complete Block Design, was used in a two-time split-split plot design with 4 reduplicates.

Plant Cultivation: The plow vertically plowed the plastic house's soil; then it was leveled, smoothed, and divided into eight lines, each 47 meters long and 40 cm broad, and the distance between one line and the last 1 m. Okra seeds were sown on 12.02.2019 in the middle of the line after soaking them with water for 12 hours in holes 35 cm apart, placed in one hole 4-5 seeds at a depth of 3-5 cm. The drip irrigation system was used, and after two weeks of planting, the plants were thinned to two plants in one hole. One line was divided into 12 experimental units, 3.85 m long and 1 m wide. The number of plants was 22; spacers were left between plots of 80 cm. The number of experimental units was 96, resulting from two cultivars, four salt concentrations, three treatments, and four replicates.

Treatment Application: The leaf nutrients were estimated by taking the fourth leaf from the growing top of several plants from each experimental unit (Walsh and Beaton, 1973) after 60 days of germination. The following were estimated as the percentage of nitrogen based on the method (Page *et al.*, 1982). The method of Murphy and Riley (1962) determined the proportion of phosphorus and the percentage of potassium and sodium according to the method (Page *et al.*, 1982). The percentage of chlorine (%) in the leaves was calculated using the (Furman, 1962) method, and the ratio between the two ions Na / K. Statistics were used to analyze the outcomes by the plan. The Least Significant Difference (L.S.D.) test was used to compare the following arithmetic means with a significant level of 0.05 (Al-Rawi and Khalaf Allah, 1980).

RESULTS AND DISCUSSION

Table 1 demonstrated that the study variables and their interactions significantly impacted the levels of nitrogen and phosphorus in leaves, as the levels of the two elements significantly decreased as irrigation water salinity increased. With an increase in concentration, the content of the leaves decreased dramatically (9.59, 22. 85, 32.58%) for the nitrogen element and (4.94, 14.82, 28.00%) for the phosphorous element, respectively, compared to the comparison treatment.

Salinity	Cultivar	Pe	ercentage	of nitrog	en in leaves	Phosphorous				
DSm ⁻¹		Growth hormone (mg l⁻¹)			Interaction	Growth hormone (mg l ⁻¹)			Interaction	
		1.0	0.5	0	between salinity	1.0	0.5	0	between salinity	
					& cultivar				& cultivar	
R0	Khenissari	5.183	4.360	3.220	4.254	0.461	0.432	0.393	0.429	
	Petra	5.127	4.450	3.493	4.357	0.443	0.437	0.385	0.422	
2.5	Khenissari	5.753	3.630	3.097	4.160	0.520	0.443	0.330	0.431	
	Petra	4.140	3.587	3.150	3.626	0.419	0.384	0.332	0.378	
5.0	Khenissari	3.853	3.453	2.923	3.410	0.497	0.403	0.302	0.400	
	Petra	3.640	3.130	2.933	3.234	0.353	0.319	0.298	0.323	
7.5	Khenissari	4.387	3.043	1.523	2.984	0.497	0.306	0.244	0.307	
	Petra	3.520	2.923	2.023	2.822	0.387	0.333	0.209	0.310	
LSD 0.05		0.327			0.216	0.009			0.005	
interaction					Average salinity				Average salinity	
between	0	5.155	4.405	3.357	4.306	0.452	0.435	0.389	0.425	
salinity and	2.5	4.947	3.608	3.123	3.893	0.469	0.413	0.331	0.404	
hormone	5.5	3.747	3.292	2.928	3.322	0.425	0.361	0.300	0.362	
	7.5	3.953	2.983	1.773	2.903	0.381	0.318	0.227	0.308	
LSD 0.05		0.241			0.183				0.004	
					Average salinity				Average salinity	
	Khenissari	4.794	3.622	2.691	3.708	0.463	0.395	0.317	0.392	
	Petra	4.107	3.523	2.900	3.510	0.400	0.368	0.306	0.358	
LSD 0.05		0.161			0.108				0.002	
Average hormone		2.795	3.572	4.450	LSD 0.05	0.311	0.382	0.432	LSD 0.05	
					0.112				0.003	

Table 1. Effect of Brassinosteroid growth hormone on ratio of both nitrogen phosphorus elements in leaves of two okra cultivars grown under salt stress of irrigation water.

The Khenissari cultivar showed significant superiority in the content of its nitrogen and phosphorus elements leaves, with an increase of (5.64, 9.49%), respectively, compared to the Petra cultivar.

The amount of the two components nitrogen and phosphorus in the leaves significantly increased after spraying with the growth hormone Brassinosteroid, and the effect increased significantly by increasing the concentration, with an increased rate of (27.79, 59.212%) for the nitrogen element and (22.82, 38.90%) for the phosphorus element, The bilateral interaction between salinity and the cultivar had an impact when compared to the control treatment, respectively. Interestingly, plants of the Petra cultivar irrigated with RO water excelled in giving the highest values for the nitrogen *element, reaching 4.357%, while plants of the Khenissari cultivar irrigated with RO water gave the highest values for the phosphorus element, amounting to 0.429%, while the plants of the Petra cultivar irrigated with a salinity level of 7.5 dSM-1 gave the lowest nitrogen percentage amounting to 2.822, while The plants of Khenissari irrigated at a salinity level of 7.5 des m⁻¹ had the lowest concentration of phosphorus (0.307%). The interaction between salt and the hormone considerably impacted both factors. A salinity of 2.5 dsm⁻¹ had the highest percentage of phosphorus, amounting to 0.469%. At the same time, the plants irrigated at a salinity

level of 7.5 dm⁻¹ that were not sprayed with the hormone gave the lowest percentage for both elements, amounting to (1.773, 0.227%), respectively.

The Khenesri cultivar's plants exposed to the hormone at a concentration of 1 mg l-1 yielded the highest values, demonstrating the strong interaction between the cultivar and the hormone for nitrogen and phosphorus amounting to (0.463, 4.794%), respectively. In contrast, the plants of the Petra variety that were not sprayed gave the lowest percentages, amounting to (0.306, 2.90%) and, respectively. The three-way interaction showed a significant effect, as the plants of the Khenissari variety, irrigated at a salinity level of 2.5 dS.m⁻¹ and excelled by delivering the highest percentage after being sprayed with the hormone at a concentration of 1 mg l-1 of nitrogen and phosphorus (0.520,5753%), respectively. In contrast, the plants of the Khenissari cultivar irrigated at a salinity level of 7.5 dSm⁻¹ gave The non-sprayed and non-sprayed plants the lowest nitrogen percentage, amounting to 1.523%. In contrast, the irrigated Petra plants with a salinity level of 7.5 dSm⁻¹ and the non-sprayed plants gave the lowest phosphorus percentage of 0.209%. Table 2 demonstrates that the research variables and their interactions substantially impacted the potassium content of the leaves and the ratio of potassium/sodium, as the increase in salinity caused a significant decrease in these two characteristics. %

Table 3. Effect of Brassinosteroid growth hormone on a ratio of elements chlorine and sodium in leaves of two okra cultivars grown under salt stress of irrigation water.

Salinity	Cultivar			Cl		Na				
DSm ⁻¹		Growth hormone (mg l ⁻¹) Interaction				Growth	hormone	Interaction		
		1.0	0.5	0	between salinity	1.0	0.5	0	between salinity	
					& cultivar				& cultivar	
R0	Khenissari	0.973	1.777	2.420	1.723	0.3137	0.3523	0.3747	0.3469	
	Petra	1.067	1.567	2.573	1.736	0.2877	0.3093	0.3460	0.3143	
2.5	Khenissari	3.517	4.180	4.593	4.097	0.2877	0.3410	0.4157	0.3570	
	Petra	3.553	4.213	4.887	4.218	0.3097	0.3410	0.4217	0.3592	
5.0	Khenissari	3.660	5.040	5.887	4.862	0.3513	0.4340	0.5127	0.4327	
	Petra	4.660	5.380	5.867	5.302	0.3463	0.4103	0.5310	0.4292	
7.5	Khenissari	4.877	5.930	7.647	6.151	0.4683	0. 6833	0.6833	0.5593	
	Petra	5.490	6.223	7.627	6.447	0.3457	0.4137	0.5677	0.4423	
LSD 0.05		0.368			NS	NS			0.0149	
interaction					Average salinity				Average salinity	
between	0	1.020	1.672	2.497	1.729	0.3007	0.3308	0.3603	0.3306	
salinity and	2.5	3.535	4.197	4.740	4.157	0.3120	0.3410	0.4118	0.3572	
hormone	5.5	4.160	5.210	5.877	5.082	0.3488	0.4222	0.5218	0.4309	
	7.5	5.183	6.077	7.637	6.299	0.4070	0.4700	0.6255	0.5008	
LSD 0.05		0.250			0.209	0.0168			0.0127	
					Average salinity				Average salinity	
	Khenissari	3.257	4.232	5.137	4.208	0.3619	0.4134	0.4966	0.4240	
	Petra	3.692	4.346	5.238	4.426	0.3223	0.3686	0.4666	0.3858	
LSD 0.05		0.201			0.174	NS			0.0073	
Average hormone					LSD 0.05				LSD 0.05	
-		5.187	4.289	3.475	0.104	0.4816	0.3910	0.3421	0.0079	

in potassium element compared to Ro water, respectively, with a decrease of (55.12, 46.79, 28.52%) in the ratio of potassium/sodium compared to Ro water, respectively.

The cultivar showed a significant impact, as plants of the Khenissari cultivar excelled compared to the Petra cultivar. with an increase of 9.25%, and the two cultivars did not differ significantly in the ratio of potassium/sodium. The ratio of (18.53, 14.49%)potassium element and in the potassium/sodium ratio, respectively, while the plants of the Khenissari cultivar that were irrigated with RO gave the greatest potassium ratio of 5.28%, indicating that there was a strong interaction between salinity and the cultivar. The potassium to sodium ratio was 15.392.7.5 dsm m-1 of salinity was used to irrigate the plants in Petra, where the lowest percentage of potassium element was 2.843%, while 7.5 dsm m-1 of salinity was used to irrigate the plants in Khenissari, where the lowest potassium/sodium ratio was 6.119%.

The plants irrigated with Ro and sprayed with the hormone at 1 mg l-1 gave the highest potassium percentage, indicating that salinity and the hormone interacted significantly, reaching 5.343%. The greatest potassium/sodium ratio was 17.73. The plants that were not sprayed and were irrigated at a salinity level of 7.5 dSm-1 had the lowest potassium percentage, 1.945, and the highest K/Sodium percentage, 3.123. The plants of the Khenissari cultivar sprayed with the

hormone at a concentration of 1 mm l-1 gave the highest percentage of potassium, which was equal to 4.427%, while the plants of the Petra variety sprayed with the hormone at a concentration of 1 mg l-1 gave the highest potassium/sodium ratio, which was equal to 12.936. This interaction between the cultivar and the hormone had a significant impact. The triple interaction considerably impacted both cultivars, according to the results. The Khenissari cultivar plants produced the maximum percentage of potassium, 5.917%, and a potassium/sodium ratio of 18.467 when they were watered with Ro water and sprayed with hormone at a concentration of 1 mg l-1. On the other hand, plants of the Petra cultivar that received irrigation at a salinity level of 7.5 produced des m-1, and not the sprayed plants gave the lowest potassium/potassium ratio of 1.945%. In contrast, the irrigated Khenissari plants at a salinity level of 7.5 dsm-1 gave the lowest potassium/sodium ratio of 2.98.

According to Table 3, the study variables substantially impacted the elements present in plant leaves. According to Table 3, the study variables substantially impacted the sodium and chlorine, sodium and chloride, and chloride content of plant leaves, as the increase in water salinity caused a significant increase. Consequently, it grew when salinity levels rose as the percentage increase compared to Ro water was (51.48, 30.33, 8.04%) and (264.3, 193.92, 104.04%) for

Salinity	Cultivar			Cl		Na				
DSm ⁻¹		Growth hormone (mg l ⁻¹)			Interaction	Growth	hormone	Interaction		
		1.0	0.5	0	between salinity	1.0	0.5	0	between salinity	
					& cultivar				& cultivar	
R0	Khenissari	0.973	1.777	2.420	1.723	0.3137	0.3523	0.3747	0.3469	
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	Petra	3.553	4.213	4.887	4.218	0.3097	0.3410	0.4217	0.3592	
5.0	Khenissari	3.660	5.040	5.887	4.862	0.3513	0.4340	0.5127	0.4327	
	Petra	4.660	5.380	5.867	5.302	0.3463	0.4103	0.5310	0.4292	
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	Petra	5.490	6.223	7.627	6.447	0.3457	0.4137	0.5677	0.4423	
LSD 0.05		0.368			NS	NS			0.0149	
interaction					Average salinity				Average salinity	
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hormone	5.5	4.160	5.210	5.877	5.082	0.3488	0.4222	0.5218	0.4309	
	7.5	5.183	6.077	7.637	6.299	0.4070	0.4700	0.6255	0.5008	
LSD 0.05		0.250			0.209	0.0168			0.0127	
					Average salinity				Average salinity	
	Khenissari	3.257	4.232	5.137	4.208	0.3619	0.4134	0.4966	0.4240	
	Petra	3.692	4.346	5.238	4.426	0.3223	0.3686	0.4666	0.3858	
LSD 0.05		0.201			0.174	NS			0.0073	
Average hori	mone				LSD 0.05				LSD 0.05	
-		5.187	4.289	3.475	0.104	0.4816	0.3910	0.3421	0.0079	

Table 3. Effect of Brassinosteroid growth hormone on a ratio of elements chlorine and sodium in leaves of two okra cultivars grown under salt stress of irrigation water.

sodium and chlorine, respectively. The concentration decreased by (28.96, 18.81%) and (17.31, 33.00%) for the sodium and chlorine elements, respectively, compared to comparison treatment in the percentage of sodium only.

A considerable impact was seen from the salinity and cultivar interaction as the plants of the irrigated Khenissari cultivar at The maximum amount of sodium was found at a salinity level of 7.5 des m-1 amounting to 0.5593%, while the irrigated Petra plants, Ro, gave the lowest sodium percentage, amounting to 0.3145%, as the interaction between salinity and the hormone showed a significant effect, as the plants gave Irrigated at a salinity level of 7.5 des m-1 and not sprayed had the highest percentage of sodium and chlorine, which amounted to (5.877, 0.6255%), respectively. In contrast, Roirrigated and sprayed plants by hormone gave the lowest percentage of these two elements, amounting to (1.02, 0.3007%), respectively.

A considerable impact was seen from the hormone and cultivar relationship on the proportion of the chlorine element if the Petra plant sprayed with the hormone at a dose of 1 mg l-1 yielded the maximum percentage of 5.238%, while the Khenissari cultivar plants sprayed with the hormone at a concentration of 1 mg l-1 had the lowest percentage which amounted to 3.257%. The triple interaction significantly impacted the percentage of chlorine. The Khenissari cultivar plants were not injected with the hormone and were irrigated with 7.5 dsm m-1 salinity, giving the highest rate of 7.647%, while the lowest percentage was in the plants of the Khenissari irrigated Ro and sprayed with the hormone was 0.973% at a dosage of 1 mg l-1.

DISCUSSION

The higher levels of nitrogen, phosphorus, and potassium in the leaves of plants that are irrigated with RO water, as well as plants irrigated with well water diluted to a level of (2.5)and treated with growth hormone 1 mg l of the concentration in the two elements of nitrogen and phosphorus, and this might be due to okra's moderate salt tolerance as a plant (Mass and Hoffman, 1977; Unlukara et al., 2008). Perhaps because the plant possesses a defensive system of enzymatic antioxidants that fight free radicals (ROS) that are induced by salt stress (Gupta and Hugng, 2014), the proportion of elements decreases as the salt stress increases, as the deficiency in nitrogen may be attributed to the lack of total proteins due to Saline conditions that reduced the activity of Jabeen enzyme (Undovenko, 1971) in the leaves of plants irrigated with high salinity levels explain its low percentage. As for the element phosphorus, it can be due to the competitive effect between the two ions -Cl and -H2po4, as the chloride ion works to reduce the absorption of H2po4 by the plant lessen the amount of phosphorus that is transferred from the root system to the vegetative system, causing a decrease in the percentage of phosphorus in the leaves

(Martinez *et al.*, 2018). The salinity of irrigation water leads to the precipitation of phosphorus and a decrease in its readiness for the plant, as well as an increase in salinity leads to poor root growth and penetration into the soil, and since phosphorus is an element with limited movement, this leads to a lack of its absorption (Al-Ta'i *et al.* 2017).

The competition between potassium K+ and sodium ions NA+ on absorption sites in the roots and on transport protein proteins that transport the sodium ion instead of potassium is the primary cause of the drop in potassium ion concentration due to the presence of the sodium ion in large quantities in the irrigation water, which it leads to a decrease in the uptake of potassium ions and the fact that ions like NA + and K + have transporters in plants and hydrogen pumps H + pumps that generate energy transporting ions inside the cells (Zhu, 2003). Increasing the concentration of sodium ions due to increased concentration in the irrigation water, and as a result, these substantial amounts are sucked into the Tonoplast vacuole membrane of plant cells via the Na+/H antiport pump, withdrawing water to support plant life (Hasegrawa et al.2001; Chen and Lin, 2000) Because the cell membranes are negatively charged, the sodium ion enters the cell fast. As a result, the sodium ion may accumulate inside the cells more so than in the cell walls (Taiz and Zeiger, 2002).

The increase in the chloride ion in the leaves with an increase in its concentration in the irrigation water may be present in the leaves due to an increase in its concentration in the growth medium, which causes the plant to enhance its absorption. Non-Selective anion channel, leading to its accumulation in the root cells, then moving with the transpiration stream from the roots to the leaves and being held in the vacuoles as it contributes to the salt tolerance with the sodium ion (Flower et al., 1977) and these results are consistent with Al-Motawiri (2014) and Abbas (2018) on the okra plant, with an increase in salinity concentrations, they observed a large decrease in the concentration of nitrogen and potassium ions and a considerable increase in the sodium and chloride ions in the irrigation water that the process of photosynthesis depends to a large extent on balance between elements and mineral nutrition under salt stress (Hainkenne et al., 2014).

The ratio K+/Na+ represents the equilibrium of k+ and Na ions in cells and tissues. Plant salt tolerance is characterized by it (Wu *et al.*, 2018; Hauser and Horie, 2010). The ratio falls as potassium levels fall and sodium levels rise, which has a deleterious impact on the structure. Numerous enzymes, notably the Calvin cycle enzymes, depend on photosynthesis and a high K + / Na + ratio to remain active (Shabala and Pottosin, 2010). Additionally, spraying plants with the hormone EBR-24 causes an increase in the percentage of K+/Na+ and prevents the plastron from oxidation, protecting the second photosynthesis under salt stress (Kalaji *et al.*, 2014; Penella *et al.*, 2016). This superiority results from genetic differences between cultivars' effects on the chemical



composition of leaves and depends on how sensitive these cultivars are to the salt content of irrigation water.

Spraying by hormone at a concentration of (1) mg l-1 revealed a significant increase in the proportion of nitrogen, phosphorus, and potassium and a significant decrease for each of the element's sodium and chlorine and the K/Na ratio. Using EBr improved the plant's tolerance to salt stress by raising the level of proline and ions N, P, CA, Mg, K, and the reduction of Na, Cl ions, which is consistent with previous research.

Conclusion: This summary condenses the key findings while remaining concise and informative. It highlights the negative impact of salinity, the Khenissari cultivar's resilience, the potential of Brassinosteroid, and the need for further research to refine its application.

Authors' contributions: Authors' contributions: Zainab A. Saihood; Awatef N. Gerry; Abdulla A. Abdulla: conceived and designed the experiments, performed the experiments, analyzed the data. and wrote the paper, reviewed the manuscript. All authors read and approved the final manuscript.

Funding: none.

Ethical statement: This article does not contain any studies with human participants or animal performed by any of the authors.

Availability of data and material: We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere

Code Availability: Not applicable

Consent to participate: All authors are participating in this research study.

Consent for publication: All authors are giving the consent to publish this research article in JGIAS

Acknowledgements: This work is a part of the Research in College of Agriculture, University of Basrah, Iraq.

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2024 | Volume 12 | Issue 1 | Page 6



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