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RESEARCH ARTICLE

The effect of Azolla extract on vegetative growth indicators of three varieties of Barley (*Hordeum vulgare* L.)

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ABSTRACT

The field experiment was carried out on land belonging to a farmer in the Al Bandar area (3 km from the center of Samawah) during the 2022-2023 season to determine the effect of four levels of Azolla extract (45, 30, 15,0 liters ha-1) on three varieties. From barley (AXAD, Ibaa 99, Ibaa 265), the experiment was applied using a completely randomized block design according to the split-plot method and with three replications, with the varieties occupying the main panels and the Azolla levels in the secondary panels. The results showed that the level of 30 liters of ha-1 was significantly superior to the flag leaf area, the flag leaf content of chlorophyll, the length of the spike, and the number of branches per square meter, as it gave averages of (24.48 cm2), (44.63 SPAD), (5.82 cm), and (509.40 beaches m). 2) Sequentially. The results also showed that the Aksad variety was superior in the nitrogen content of the plant, the flag leaf's chlorophyll content, the length of the spike, and the number of branches per square meter, and gave averages of (2.47%), (45.22 SPAD), (6.17 cm), and (512.50 Shatam-2), respectively, and the variety was superior. Fathers 265 in plant height and leaf area and gave two averages of (104.05 cm) and (23.33 cm2), respectively.

Keywords: *Barley; Azolla; vegetative growth indicators*

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INTRODUCTION

The barley crop is considered an important strategic grain crop. It is one of the oldest crops known to man, and it contributes mainly to achieving human food security. It ranks fourth in importance after wheat, corn, and rice. It is considered the main source of bread in the ancient world, as its grains contain Amino acids and proteins important for human nutrition and carbohydrates, as well as a high level of fiber, fats, selenium, and vitamins, especially vitamin B (Gani and Salman, 2011). Due to the lack of production in the barley crop, there was a need to increase the production of varieties with good genetic characteristics and high production that are suitable for the environmental conditions in the region, as barley varieties vary in their production capacity depending on the variation in their genetic makeup and the extent of their influence on environmental conditions on the one hand, and the lack of control over production techniques on the other. For this reason, many researchers were interested in searching for new ways to raise productivity while improving quality by exploiting the biodiversity found in varieties and genetic compositions with high yields (Al-Sahuki, 2006; Al-Nasr-Allah and Alhasany, 2023). Azolla extract is one of the important organic nutrients because it contains nutrients, amino acids, and growth regulators that have an important role in physiological processes as well as improving the growth and production of plants

(Altai et al., 2019). The Azolla plant is a fern that reproduces sexually and asexually and belongs to the Azollaceae family. Which lives on the surfaces of drains and stagnant water and is characterized by rapid growth and spread, as its biomass can be doubled significantly every 2-3 days (Cohen et al., 2002).

Azolla contains 3-4% dry matter and is considered a rich source of proteins, essential amino acids, and vitamins such as A and B12. It contains many elements such as calcium, phosphorus, potassium, iron, magnesium, and copper. Blue-green algae (*Anabaena azolla*) that coexist symbiotically with Azolla fixes nitrogen. At a higher rate than legumes, ranging between 100-170 kg nitrogen ha^{-1}, Azolla is a rich source of protein, as it constitutes 25-30% protein, 10-15% minerals, and 7-10% a mixture of amino acids, biologically active materials, and polymers (Pillai et al., 2005). Our study aims to determine the best combination of interaction between varieties and Azolla extract, which gives the best growth and yield of barley crops.

MATERIALS AND METHODS

An experiment was carried out during the agricultural season (2022-2023) in a farmer's field in the Al Bandar area, which is located southwest of Al-Muthanna Governorate (3 km from the center of the city of Samawah), to study the role of Azolla extract at four levels (0, 15, 30 and 45 liters) on the growth and yield of three barley varieties (Aksad, Abaa 99, Abaa 265). Plowing, smoothing, and leveling operations were carried out, and the land was divided according to the design used. After that, the experimental field was planned, and the seeds were planted on November 3 (Al-Jayashi et al., 2020). The land was divided into 36 experimental units. The area of the experimental unit was 4 m2 (2 x 2 m), containing 10 lines between two lines and the last 20 cm, and a seed rate of 120 kg ha^{-1} was adopted (General Authority for Agricultural Research, 2011). The nitrogen fertilization process was carried out in an amount of 120 kg ha-^{1} in the form of urea fertilizer (46% N) in two batches, and it was sufficient to add the first batch only after (15) days of planting after calculating the amount of nitrogen present in the soil (Table 1). The phosphate fertilization process was carried out in the amount of (100 kg ha^{-1}) in the form of triple superphosphate fertilizer (P2O5 46 %) in one batch at planting as described Al-Abedi, (2011) and potassium fertilizer in the amount of (80 kg ha^{-1}) in the form of potassium sulphate (K2O%50) in one batch at planting (Ali et al., 2014). Irrigation and weeding were also carried out whenever necessary. The Azolla plant was grown in the home garden's 4 m cement basin. A mixture of clay and cow manure was placed inside the basin, then fresh water was added from the tap, and then placed 2 kilograms of live Azolla plants obtained from one of the farms that produced it. The Azolla plant needed 3-4 days to double its biomass. The Azolla was harvested to make the extract after 7 days. Random samples were taken from different places of each replicate and mixed together to take a composite sample representing the experimental field and from depth (0-30), and some physical and chemical analyzes of the experimental field were conducted on it before planting, which are shown in Table (1).

Table (1) Physical and chemical characteristics of the experimental field before planting

Units	Values	Attribute	
	7.20	pH	
ds/m^{-1}	4.50	E.c (1:1)	
Mg/kg dry weight	26.60	Nitrogen(availble)	
Mg/kg dry weight	13.20	phosphorus(availble)	
Mg/kg dry weight clay	118.70	potassium(availble)	
	22.33	Clay	
g/kg^{-1}	29.12	Silt	Soil separators
	48.55	Sand	
Sandy loam		Soil texture	

Studied attributes

1- Nitrogen concentration in plants (%)

Samples were taken from the shoots of each experimental unit, at the 75% flowering stage, and the nitrogen concentration was calculated according to the method described by Cresser and Parsons, (1979) in a nitrogen distillation device (Macrokjeldhal).

2- Plant height (cm)

It was measured using a ruler from the soil surface to the end of the spike, without cutting, for ten plants taken randomly for each experimental unit at harvest.

3- Area of the flag leaf (cm2)

It was calculated at the stage of completion of flowering, and ten plants were taken randomly from the midlines of each experimental unit, according to the equation (Thomas, 1975).

(Area of flag paper = length of paper x maximum width x 0.75).

4- Chlorophyll (SPAD) content of leaves

The total chlorophyll content of leaves was estimated using the SPAD Chlorophyll Meters (Spectrum Technologies Inc.) as an average of ten plants, taken randomly from the midlines of each experimental unit at the stage of complete flowering.

5- Spike length (cm)

It was calculated as an average of ten spikelets taken randomly from the harvested midlines. The length of the spike was calculated from the base of the basal terminal spikelet to the top of the upper terminal spikelet without the stem.

6- Number of tillers per square meter (tillers m^{-2})

The numbers of tillers were calculated from harvesting two middle lines in the fully mature stage, and the harvested area represented 80 cm, which was then converted to square metres.

RESULTS AND DISCUSSION

1- Nitrogen concentration in plants (%)

The results of Table (2) indicated the superiority of the fourth level, A3, which gave an average of (2.72%) and did not differ significantly from the third level, A2, which gave an average of (2.71%). They differed significantly from the comparison treatment A0, which gave an average of (1.64%). The reason for increasing the nitrogen content by increasing the levels of addition may be due to the Azolla extract's content of nutrients and amino acids, which helped the plant to grow in general, and thus this is reflected in good root growth when some amino acids are available. This helps directly in stimulating the roots, such as the amino acid methionine, which contributed to absorbing the largest amount of nitrogen, which led to an increase in its concentration in the plant. This is consistent with (Al-Jabri and Al- hasany, 2022), as he indicated an increase in the concentration of nitrogen in the plant. As shown, the results Table (2) indicates the superiority of the Aksad variety, as it gave an average of (2.47%) and did not differ significantly from the variety Aba 265, which gave an average of (2.27%). They differed significantly from the variety Aba 99, which recorded the lowest average of (1.90%). The reason may be due to the difference. The genetic composition of the varieties in the nature of root growth and their ability to absorb nitrogen from the soil, as well as the nature of the vegetative growth of the varieties and their nutritional requirements, and this is consistent with (Al-Hassany, 2021), as he indicated the difference in the concentration of nitrogen in the plant according to the varieties.

The data in Table (2) indicated that the interaction was significant between the Azolla extract and the varieties, as the fourth level of extract A3 with the variety Oxad, the combination (V1 × A3), gave the highest average for this trait, amounting to (2.92%), while the combination represented by the second level of extract A1 with the variety Aba gave 99 (V2 × A1) The lowest average was (1.29%).

Table (2) The effect of Azolla extract, varieties, and their interaction on plant nitrogen concentration (%)

Azolla Cultivars	Axad	IPA99	IPA265	Azolla average
A0	1.94	1.51	1.48	1.64
A1	2.16	1.29	1.94	1.80
A2	2.86	2.48	2.78	2.71
A3	2.92	2.34	2.89	2.72
Cultivars average	2.47	1.90	2.27	
L.S.D (0.05)	**Cultivars**	**Azolla**	**Interaction**	
	0.24	0.18	0.32	

2- Plant height (cm)

The results of Table (3) indicated that the comparison treatment A0 was significantly superior and gave an average of (104.48 cm), with a significant difference from the third and fourth levels, A2 and A3, which gave averages of (100.33 and 100.32 cm), respectively. This may be due to the role of some amino acids in Azolla extract helps in early crop growth, thus prompting the plant to flower and not wasting nutrients manufactured in increasing plant height, such as the amino acids isoleucine, lysine, and glutamic acid. It was noted in the results of Table (3) that there was a significant superiority for the variety Aba 265, as it gave an average of 104.05 cm. It did not differ significantly from the variety Aba 99, which gave an average of 102.35 cm. It differed significantly from the variety Aksad, which gave the lowest average of 98.62 cm. The reason for the variation of varieties in plant height is due to the difference in genetic composition between the varieties, and this is consistent with Al-Hassany, (2021). The results of Table (3) also showed that the interaction is significant between the Azolla extract and the varieties, as the first level A0 with the variety Aba 265, the combination (V3 × A0), gave the highest average for this trait, amounting to (107.72 cm), while the combination represented by the fourth level A3 and the variety Kassad (V1 × A3) had the lowest average (97.85 cm).

Table (3) The effect of Azolla extract, varieties and their interaction on plant height (cm)

Azolla Cultivars	Axad	IPA99	IPA265	Azolla average
A0	99.78	105.93	107.72	104.48
A1	98.85	102.00	103.83	101.56
A2	98.00	100.80	102.20	100.33
A3	97.85	100.68	102.43	100.32
Cultivars average	98.62	102.35	104.05	
L.S.D (0.05)	**Cultivars**	**Azolla**	**Interaction**	
	3.82	0.89	3.74	

3- Area of the flag leaf (cm^2)

The results of Table (4) indicated the superiority of the third level, A2, as it gave an average of (24.48 cm^2) and did not differ significantly from the fourth level, A3, which gave an average of (23.30 cm^2), and they differed significantly with the comparison treatment A0, which gave the lowest average of (19.06 cm^2). The reason for the increase in the area of the flag leaf when treated with Azolla extract may be due to the nutritional elements and amino acids it contains, which help in increasing vital activities, and the increase in the nitrogen content of the leaves (Table 2), which increases the process of

cell division and elongation and thus increases the area of the flag leaf consistent with (Saudi, 2017).

The data in Table (4) indicated the superiority of the variety Aba 265, as it gave an average of (23.33 cm^2) and did not differ significantly from the variety Axad, which gave an average of (22.03 cm^2), and they differed significantly from the variety Aba 99, which gave an average of (20.13 cm^2), perhaps due to the reason The superiority of the cultivar Abaa 265 in flag leaf area is due to the difference in its genetic composition from the rest of the varieties under study. The reason for the increase in flag leaf area in the cultivars Abaa 265 and Aksad may be due to an increase in the nitrogen content of the plant (Table 2), which helped in increasing the vital processes in the plant and was reflected positively in increasing the area of the flag leaf, and this is consistent with (Al-Jayashi et al., 2020). The data in Table (4) also indicated that the interaction was significant between the Azolla extract and the cultivars, as the third level of the extract A2 with the cultivar Aba 265, the combination (V3 × A2), gave the highest average for this trait, amounting to (25.71 cm^2), and it did not differ significantly from many of the combinations, while the comparison treatment gave A0 with variety Aba 99 (V2 × A0) had a lower average of (17.98 cm^2). This may be explained by what was discussed in the effect of individual factors and this was reflected in the interaction.

Table (4) The effect of Azolla extract, varieties, and their interaction on flag leaf area (cm^2).

Azolla Cultivars	**Axad**	**IPA99**	**IPA265**	**Azolla average**
A0	18.56	17.98	20.63	19.06
A1	18.66	20.70	22.09	20.48
A2	25.39	22.34	25.71	24.48
A3	25.52	19.48	24.90	23.30
Cultivars average	22.03	20.13	23.33	
L.S.D (0.05)	**Cultivars**	**Azolla**	**Interaction**	
	1.58	1.66	2.73	

4- Science leaf content of chlorophyll (SPAD)

It was noted in the results of Table (5) that there was a significant superiority for level A2, as it gave an average of (44.63 SPAD) for this characteristic, and it did not differ significantly with levels A3 and A1, which gave averages of (44.08 and 43.02 SPAD), respectively, and it differed significantly with the comparison treatment A0. It gave the lowest average (41.72 SPAD). The reason for the increase in the flag leaf's chlorophyll content may be due to the nutritional elements and amino acids contained in the Azolla extract, as there is an important role for some amino acids that contribute to the formation of the chlorophyll molecule, such as the amino acid alanine, which contributed In increasing the leaf content of chlorophyll, this is consistent with (AL-Hasany et al., 2019). The data in Table (5) indicated that the Aksad variety was significantly superior, as it gave an average of (45.22 SPAD) and did not differ significantly with the variety Abaa 265, which recorded an average of (43.45 SPAD), but it differed significantly with the variety Abaa 99, which gave the lowest average of (41.43 SPAD). This may explain the discrepancy in the chlorophyll content of the flag leaf due to the genetic nature of the Aksad variety and the extent of its response to the surrounding environmental conditions better than the rest of the varieties included in the study, and this is consistent with (Al-Jayashi et al., 2020).

Table (5). The effect of Azolla extract, varieties, and the interaction between them on the chlorophyll content of the flag leaf (SPAD)

Azolla Cultivars	Axad	IPA99	IPA265	Azolla average
A0	43.25	40.03	41.89	41.72
A1	44.93	40.64	43.50	43.02
A2	46.35	42.57	44.98	44.63
A3	46.34	42.47	43.43	44.08
Cultivars average	45.22	41.43	43.45	
L.S.D (0.05)	**Cultivars**	**Azolla**	**Interaction**	
	2.65	1.68	N.S	

5- Spike length (cm)

The results of Table (6) showed a significant superiority for the third level, A2, as it gave an average of (5.82 cm). It did not differ significantly with the fourth level, A3, which gave an average of (5.42 cm). They differed significantly with the comparison treatment A0, which gave the lowest average of (5.11 cm). The reason for the increase in the length of the spike may be due to the increase in the area of the flag leaf, Table (4), resulting from increasing the levels of addition to Azolla extract, thus increasing carbon metabolism and providing the largest amount of nutrients, which led to an increase in the length of the spike, and this is consistent with (Altai et al., 2019), as They indicated that the length of the spike was increased by adding plant extracts.

The data in Table (6) showed that there was a significant superiority of the Aksad variety in spike length, as it gave an average of (6.17 cm), and a significant difference with the two varieties Aba 99 and Aba 265, which gave averages of (5.05 and 5.03 cm), respectively. This discrepancy between the varieties may be due to length, where spike is due to the difference in genetic composition between them, and this is consistent with Al-Hassany (2021), who showed the variation in spike length according to the variety.

Table (6) The effect of Azolla extract, varieties and their interaction on spike length (cm).

Azolla Cultivars	Axad	IPA99	IPA265	Azolla average
A0	5.66	4.62	5.07	5.11
A1	6.41	4.92	4.64	5.32
A2	6.44	5.17	5.84	5.82
A3	6.18	5.49	4.58	5.42
Cultivars average	6.17	5.05	5.03	
L.S.D (0.05)	**Cultivars**	**Azolla**	**Interaction**	
	0.50	0.44	N.S	

6- Number of beaches per square meter (tillers M^{-2})

The data in Table (7) showed a significant superiority of the third level of Azolla A2, which gave an average of (509.40 tillers m^{-2}) for this characteristic, and did not differ significantly from the fourth level, A3, which gave an average of (495.60 tillers m^{-2}), and they differed significantly with the comparison treatment A0, which gave The lowest average was (451.40 tillers m^{-2}). The reason for the increase in the number of strands when adding the Azolla extract due to the extract's content of the growth regulator kinetin, which is a cytokinin that causes breaking the apical dominance and encouraging lateral stranding, which led to an increase in the number of tillers per square meter.

This is consistent with Al-Ghazal et al. (2021), who indicated that the number of fractions increases with increasing concentrations of the additive. The results of Table (7) indicated that the Aksad variety was significantly superior and gave an average number of tillers that amounted to (512.50 tillers m^{-2}) and did not differ significantly from the variety Ibaa 265, which gave an average of (501.60 tillers m^{-2}), and they differed significantly with the variety Ibaa 99, which gave the lowest

average.

For this characteristic, it reached (438.30 tillers m^{-2}), and the reason for the differences between the varieties in the number of tillers may be due to their differences in their genetic composition and the extent of the variety's ability to beach under the environmental conditions of the region, and this is consistent with (Al-Absawi et al., 2023).

Table (7) The effect of Azolla extract, varieties, and the interaction between them on the number of trees per square meter (tillers m^{-2})

Azolla Cultivars	**Axad**	**IPA99**	**IPA265**	**Azolla average**
A0	508.50	403.90	441.70	451.40
A1	488.20	450.40	501.70	480.10
A2	514.70	463.40	550.00	509.40
A3	538.60	435.40	512.90	495.60
Cultivars average	512.50	438.30	501.60	
L.S.D (0.05)	**Cultivars**	**Azolla**	**Interaction**	
	59.62	33.94	N.S	

CONCLUSIONS

The 30 L/ha level of Azolla extract significantly positively affected key barley traits, including flag leaf area, chlorophyll content, spike length, and the number of branches per square meter, making it the most effective treatment. The Aksad variety outperformed the others in nitrogen content; chlorophyll content, spike length, and tillers density, indicating its strong response to Azolla extract application. The Ibaa 265 variety are excelled in plant height and leaf area, suggesting its suitability for conditions requiring taller plants with a larger photosynthetic surface. Azolla extract can be a natural bio-fertilizer to improve barley growth and productivity, reducing reliance on chemical fertilizers and promoting the environment.

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