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Effect of Azolla Extract on Yield, Its Components and Protein Content in three Cultivars of Barley (*Hordeum vulgare* L.)

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Abstract:

The experiment was conducted on a farm in the AL-Bandar region, southwest of Al-Muthanna governorate (3 km from Samawah center), during the 2022-2023 agricultural season. To determine the effect of four levels of Azolla extract (0, 15, 30, 45 liters ha^{-1}) on three barley Cultivars (Axad, IPA 99, IPA 265). The study used a split-plot design with three replications using a complete randomized block design (C.R.B.D) where Azolla inhabited the secondary plots while the Cultivar occupied the primary ones. The results of the experiment showed that the 30 liters ha^{-1} level was significantly superior in spikes number per m^2, 1000 grain weight, and grain yield, giving averages of (484.60 spikes m^2), (45.91 g), and (6.01 t ha^{-1}) respectively. The 45 liters ha^{-1} level was superior in grain protein content, giving an average of (12.37%). Also, the Axad Cultivar was greater in spikes number of m^2, grains number in the spike, grain yield, and grain protein content, giving averages of (492.50 spikes m^2), (47.97 grains $spike^{-1}$),(6.05 t ha^{-1}), and (12.06%) respectively. The IPA 265 Cultivar was superior in 1001 grain weight, giving an average of (46.02 g). The results showed that the 45 liters ha^{-1} level of Azolla extract with the Axad Cultivar for the combination ($V_1 \times A_3$) was significantly superior in the spikes number and grain protein content, giving averages of (528.20 spikes m^2) and (12.86%) respectively.

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Keywords: Barley; Azolla extract; *Hordeum vulgare* L.; protein content.

*Part of PhD dissertation of the first author.

Interaction

One of the earliest cultivated cereals was barley, and it is considered one of the most important cereal crops that contribute significantly to food security. Barley ranks fourth in terms of importance after wheat, maize, and rice. It was the primary grain used to make bread in prehistoric societies. Essential amino acids and proteins can be found in barley grains, carbohydrates at a rate of 60-70%, fiber, fat, selenium, and vitamins, particularly vitamin B, are all abundant, and so are other nutrients (Gani and Salman, 2011). Globally, barley is grown on over 1.158 mil ha, with production of 134 mil t. In Iraq, barley is the second most important cereal crop after wheat. In the 2022 winter season, the area planted with barley was 2309 thousand dunums, a decrease of 25.30% from the previous season. The total production was 144 thousand tons, a decrease of 45.80% from the previous season. Al-Qadisiyah Governorate ranked first with the production of 44.80 ha, representing 31.00% of the total production, followed by Muthanna Governorate by production of 24.8 ha, representing 17.2% of total production (Central Statistical Organization, 2022).

To increase the productivity of any Cultivar, regardless of its good specifications, it is necessary to adopt new and modern technologies in soil and crop management operations that are capable of increasing production in terms of quantity and quality after the old technologies become unviable. One of these technologies is the use of plant extracts that have the same natural properties as the substances found in these plants. Organic fertilizers are materials put into the soil or sprayed directly onto plants to promote growth; they are not intended to replace synthetic fertilizers but rather to work in tandem with them (Abdul et al., 2012). It is known that these natural

substances contribute to most of the important physiological functions of the plant. They improve root growth and increase water efficiency and nutrient absorption from the soil. Adding these extracts to the soil improves the upper layer, stimulates root growth, delays aging, and also increases the vegetative mass and leaf area. Plant extracts have been used as soil fertilizers (Abdul-Jabbar et al., 2012).

Azolla extract is one of the important organic nutrients due to its content of many nutrients, amino acids, and growth regulators play an important role in physiological processes and improve plant growth and yield (Altai et al., 2019). Azolla is a fern plant that reproduces sexually and asexually. It belongs to the Azollaceae family and lives on the surface of canals and stagnant water. It is characterized by rapid growth and spread, as its biomass can be doubled every 2-3 days (Cohen et al., 2002).

Due to the low production of barley, there is a need to increase the production of Cultivars with good genetic traits and high yields that are suitable for the environmental conditions in the region. Barley Cultivars vary in their productivity potential depending on the variation of their genetic composition and how much environmental factors play a role in shaping them on the one hand and the lack of control over production techniques on the other hand. For this reason, many researchers have been interested in searching for new ways to increase productivity while improving quality by exploiting the existing biodiversity in Cultivars and high-yielding genetic compositions (Al-Sahoki, 2006).

In this context, this study aimed to determine the most suitable level of Azolla extract at which the best growth and yield of barley is achieved, Assess the response of the studied barley Cultivars to Azolla extract, and Identify the best combination of Azolla extract and barley Cultivars for maximum growth and yield.

Materials and Methods

Design of the experiment and location

During the 2022–2023 growing season, an experiment was run in a farmer's field in the Al-Bandar area which is located southwest

of Al-Muthanna Governorate (3 km from the center of Samawah city), which is located at longitude 45.18 east and latitude 31.19 north. To study the role of Azolla extract at four levels (0, 15, 30, 45 liters ha^{-1}), which are denoted by (A_0, A_1, A_2, A_3) sequentially, in some of the growth and the yield of three barley Cultivars (Axad, IPA99, IPA265), which are denoted by (V_1, V_2, V_3) sequentially. Some physical and chemical analyses of the experimental field before planting were carried out, as shown in Table (1), and random samples were selected from various locations within each repetition and combined to create a composite sample reflecting the experimental field and the depth of (0-30).

Table (1) some physical and chemical characteristics of the experimental field

Attribute		Values	Units
Ph		7.20	
E.c (1:1)		4.50	ds/m^{-1}
Nitrogen (available)		26.60	mg/kg^{-1} dry weight
Phosphorus (available)		13.20	mg/kg^{-1} dry weight
Potassium (available)		179.70	mg/kg^{-1} dry weight Clay
content of soil	Clay	22.33	g/kg^{-1}
	Silt	29.12	
	Sand	48.55	
Soil texture		Sandy loam	

*Analyzes conducted in a fertility laboratory, Faculty of Agriculture, Al-Muthanna University

Field operations

Tillage, smoothing, and leveling operations were carried out, and the land was divided according to the design used. The experimental field was then planned, and the seeds were planted on November 3, 2022 (Al-Giashi, 2020). Each of the 36 experimental units had an area of 4 m^2, with 10 rows spaced 20 cm apart. The seeding rate was 120 kg ha^{-1} (General Authority for Agricultural Research, 2011). 1000 seeds weight for each Cultivar was calculated, and the seeding rate was determined based on weight.

Nitrogen fertilization was carried out at 120 kg ha^{-1} in urea fertilizer (N%46) in two batches. the one was added after 15 days of planting after calculating the amount of nitrogen present in the

soil (Table 1). Potassium fertilizer was applied at 80 kg ha^{-1} in the form of (K_2O%50) in one batch at planting (Al-Abedy, 2011). Phosphorus fertilizer was applied at 100 kg ha^{-1} in the form of (P_2O_5%46) in one batch at planting (Ali et al., 2014). Irrigation and weeding operations were also carried out whenever necessary.

The Azolla plant was grown in a 4-meter cement basin in the home garden. A mixture of clay and cow manure was placed inside the basin, then fresh water was added from the tap, then 2 kilograms of live Azolla plants were obtained from one of the farms that produced it. The Azolla plant needed 3- 4 days to double its biomass. After 7 days, the Azolla was harvested to make the extract.

Azolla extraction methods: -

The first Method - Cold pressure:
A method was used to extract Azolla using the extraction method, where fresh plant parts of Azolla are prepared and then washed with tap water to remove suspended impurities and then with distilled water. After that, they are placed in bags containing distilled water in a ratio of 1:1 and kept in the freezer for 12 hours. After that, they are extracted and placed at room temperature to dissolve (cold pressure), then placed in a household blender for 5 minutes, after which the mixture is filtered using a double cotton cloth. After that, a centrifuge is used with a number of 12000 cycles for 10 minutes, after which it is sterilized and filtered using a 0.22-micron filter paper. The solution is stored at a temperature of 5 °C until it. Crude extract is considered to be 100% concentrated (Wilson et al., 1997).

The second method - Powder:
The plant is prepared and washed with distilled water to remove impurities and dirt. It is then dried in air by placing it in an electric oven at 40 °C to stabilize the weight. The dry plant is then ground into powder. 10 g of dry plant powder is mixed with 80% ethanol (99% ethanol is taken, 80 ml + 20 ml distilled water = 80% ethanol). The mixture is mixed using a magnetic stirrer for 15 minutes. The mixture is then left at room temperature for 24 hours (in a sealed container) and filtered using filter paper with a pore size of 0.1. It is then placed in a centrifuge for 5 minutes to remove any remaining impurities.

It is then transferred to glass petri dishes and placed in an electric oven at 35 °C until dry. A powder is obtained, which is stored at a temperature of (0-5 °C) until use. This powder represents an extract with a concentration of 100%. (It is important to avoid direct exposure to light in all the steps above to prevent photo-oxidation of some light-sensitive substances in the plant (Kadum et al., 2019).

The first batch of Azolla extract was added as a ground application with irrigation water a month after emergence, and two weeks after the ground application, the plant was sprayed with the extract.

Table (2): Contents of Azolla extract of nutrients, amino acids, and hormones

Contents of Azolla extract	**Extract of the first method**	**Extract of the second method**	**Unit**
N	0.89	1.33	%
P	0.04	0.07	%
K	0.18	0.24	%
Mg	28	41	(mg/gm)
Fe	45	57	(mg/gm)
Mn	178	266	(mg/gm)
Zn	175	250	(mg/gm)
Cu	8.8	12.6	(mg/gm)
Histidine	35.11	39.25	(µg / gm)
Alanine	40.15	49.58	(µg / gm)
Arginine	39.88	45.25	(µg / gm)
Asparagine	43.65	51.28	(µg / gm)
Aspartic Acid	45.98	54.08	(µg / gm)
Cysteine	55.66	63.29	(µg / gm)
Glutamic Acid	59.25	66.08	(µg / gm)
Glutamine	53.65	62.49	(µg / gm)
Glycine	48.97	55.08	(µg / gm)
Isoleucine	47.98	52.65	(µg / gm)
Leucine	58.98	63.29	(µg / gm)
Lysine	62.56	69.04	(µg / gm)
Methionine	50.88	57.58	(µg / gm)
Phenylalanine	55.26	63.65	(µg / gm)
Proline	63.65	70.11	(µg / gm)
Serine	52.64	65.89	(µg / gm)
Threonine	60.11	69.08	(µg / gm)
Tyrosine	65.98	72.56	(µg / gm)

Nutrition Laboratory - Department of Environment and Water - Ministry of Science and Technology – Baghdad

Results and Discussion

Spikes number per m^2 (spikes m^2)

Table (3) showed a significant superiority of A_2 of Azolla extract, as it recorded an average of 484.60 spikes m^2, which resulted in an average of 482.60 spikes m^2 and did not significantly differ from the treatment with the fourth level A_3. They were notably different from the A_0 control group, which produced the fewest spikes on average (438.80 m^2). The reason for an increase in spikes number at the third level of Azolla extract may be due to the amino acids, nutrients, and growth regulators contained in the extract, which caused an increase in the general growth of the plant, the production of vegetative tillers, and the development of those vegetative tillers into fruit. This is consistent with (AL-Hasany et al., 2919), who indicated an increase in the number of spikes per m^2 when adding seaweed extract.

According to Table 3, the Axad Cultivar is superior because it produced an average of 492.50 spikes per m^2, whereas the IPA265 Cultivar produced an average of 474.60 spikes per m^2. The IPA99 Cultivar had the fewest spikes overall, with an average of 425.60 per m^2, so these plants were quite different. Variation in the genetic aptitude of the types to produce tillers and produce nutrients that assist the development of these vegetative tillers into fruit may explain the rise in the number of tillers. This is consistent with (Al-Giashi, 2020), who showed that spike numbers vary with the Cultivars.

Table (3) indicates that interaction is significant between Azolla extract and Cultivars. The fourth level A_3 of Azolla extract with the Axad Cultivar for the combination ($V_1 \times A_3$) gave the highest mean the trait of 528.20 spikes m^2, while the comparison treatment A_0 and the IPA99 Cultivar for the combination ($V_2 \times A_0$) gave the lowest of 396.30 spikes m^2.

Table (3): Effect of Azolla extract, Cultivars, and their interaction on spikes number per m^2 (spikes m^2)

Azolla / Cultivars	**Axad**	**IPA99**	**IPA265**	**Azolla average**
A_0	497.60	396.30	422.50	438.80

A_1	462.00	438.30	452.50	450.90
A_2	482.20	452.50	519.10	484.60
A_3	528.20	415.40	504.30	482.60
Cultivars average	492.50	425.60	474.60	
L.S.D 0.05))	**Cultivars**	**Azolla**	**Interaction**	
	34.71	31.97	54.03	

Grains number per spike (spikes^{-1})

According to the results in Table 4, the Axad Cultivar is superior, yielding an average of (47.97 grains spike^{-1}) compared to the IPA99 Cultivar (46.32 grains spike^{-1}). In stark contrast, the IPA265 Cultivar produced the lowest average (44.93 grains spike^{-1}) for this characteristic. Consistent with (Abdulla et al., 2018), who indicated the variation in the number of grains per spike with the variation in the Cultivars. The superiority of the Axad Cultivar in terms of the quantity of grains per spike may be attributable to the Cultivars genetic ability. who indicated the variation in the number of grains per spike with the variation in the Cultivars.

Table (4): Effect of Azolla extract, Cultivars, and their interaction on grains number per spike (spikes^{-1})

Azolla / Cultivars	**Axad**	**IPA99**	**IPA265**	**Azolla average**
A_0	48.38	46.73	45.93	47.02
A_1	47.50	45.50	46.50	46.50
A_2	48.05	46.65	43.25	45.98
A_3	47.95	46.40	44.05	46.13
Cultivars average	47.97	46.32	44.93	
L.S.D 0.05))	**Cultivars**	**Azolla**	**Interaction**	
	2.20	N.S	N.S	

1000 grains Weight (g)

Table (5) shows that the third level A_2 had a significant superiority, as it gave an average for this trait of 45.91 g and did not significantly differ from A_3, which gave 45.67 g. They differed significantly from the control treatment, which gave the lowest mean of 43.89 g. The reason may be due to the nutrients and amino acids

contained in the extract (Table 2), which helped to increase cell growth and division, thereby increasing the weight of 1000 grains. this is consistent with (Altai et al., 2019) who showed an increase in 1000-grain weight with the addition of plant extracts.

Table (5) showed that on average, the IPA265 Cultivar was significantly better than the others, by an amount of 46.02 g. It was not noticeably different from the IPA99 Cultivar, which averaged 45.45 g for this characteristic. The Axad Cultivar, which produced the lowest average yield, was different. The reason for the superiority of the IPA 265 Cultivar in the weight of 1000 grains may be due to the decrease in the number of grains per spike (Table 4), increased the weight of a thousand grains because there was less competition for resources within the spike. This agrees with the findings of (Gill et al., 2017) who found that different types of rice resulted in significantly varying weights for a thousand grains.

Table (5): Effect of Azolla extract, Cultivars, and their interaction on 1000 weight (g)

Azolla / Cultivars	**Axad**	**IPA99**	**IPA265**	**Azolla average**
A_0	43.17	43.93	44.57	43.89
A_1	43.97	44.80	45.40	44.72
A_2	43.83	47.67	46.23	45.91
A_3	43.70	45.40	47.90	45.67
Cultivars average	43.67	45.45	46.02	
L.S.D 0.05))	**Cultivars**	**Azolla**	**Interaction**	
	1.53	1.36	N.S	

Grain yield (t ha^{-1})

Table (6) showed a significant superiority of the third level A_2 in grain yield, giving an average of 6.01 t ha^{-1}, and did not differ significantly from the fourth level A_3, which gave an average of 5.72 t ha^{-1}. They were quite different from the baseline A_0 therapy, which only produced an average of 5.19 t ha^{-1}. Grain yield may have gone up because of an increase in yield components such as spikes per m^2 (Table 3)

and 1000-grain weight (Table 5), which reflected positively and contributed to the increase in grain yield. It is agreed with (Altai et al., 2019) who indicated that Azolla extract led to an increase in total yield.

Table (6) showed the Axad Cultivar had a significant superiority, giving an average grain yield of 6.05 t ha^{-1}. It differed significantly from the IPA 265 and IPA 99 Cultivars, which gave averages of 5.58 and 5.24 t ha^{-1}, respectively. The increased grain production shown with the Axad Cultivar may be attributable to the Cultivar's relative superiority in two key yield components: spikes per m^2 (Table 3) and grains per spike (Table 4). It is consistent with (Noaema et al.,2023) who indicated the difference between Cultivars in grain yield.

Table (6): Effect of Azolla extract, Cultivars, and their interaction on grain yield (t ha^{-1})

Azolla / Cultivars	**Axad**	**IPA99**	**IPA265**	**Azolla average**
A_0	5.69	4.95	4.92	5.19
A_1	5.98	5.36	5.36	5.56
A_2	6.22	5.52	6.30	6.01
A_3	6.29	5.12	5.75	5.72
Cultivars average	6.05	5.24	5.58	
L.S.D 0.05))	**Cultivars**	**Azolla**	**Interaction**	
	0.37	0.31	N.S	

Protein percentage in grain (%)

According to Table 7, the fourth-level A_3 typically performs 12.37% better. The average of 12.25% was not drastically different from the results of the third level, A_2. They were drastically different from the control group's results (9.45% on average), which were the lowest. The amino acids found in the Azolla extract (Table 2) may be responsible for the increased protein content of the grains, which were transferred from the leaves to the grains, resulting in an increase in protein content in the grains. It is consistent with (Al-Jabri, 2022), who indicated that the addition of Azolla extract increased protein content in grains.

Table (7) shows that the Axad Cultivar had a significant superiority, giving an average of 12.06%. The average yield of 11.30% was not significantly different from that of the IPA 99 Cultivar. It differed significantly from the IPA 265 Cultivar, which gave the lowest average of 10.09%. The reason for the variation in protein content in grains among Cultivars may be due to genetic differences between Cultivars, which are reflected in this trait. This is consistent with (Al-Ziyadi, 2020).

Table (7) also indicated a significant interaction between Azolla extract and Cultivars. The fourth level of extract A_3 with the Axad Cultivar gave the highest mean of (12.86%). The control treatment A_0 with the IPA 265 Cultivar gave the lowest average of (8.51%). It may be due to the effects, as mentioned above, of the factors individually, which were reflected in the interaction between them.

Table (7): Effect of Azolla extract, Cultivars, and their interaction on protein percentage in grains (%)

Azolla / Cultivars	**Axad**	**IPA99**	**IPA265**	**Azolla average**
A_0	10.82	9.02	8.51	9.45
A_1	12.16	10.85	8.57	10.53
A_2	12.39	12.49	11.86	12.25
A_3	12.86	12.82	11.42	12.37
Cultivars average	12.06	11.30	10.09	
L.S.D 0.05))	**Cultivars**	**Azolla**	**Interaction**	
	0.93	0.76	1.33	

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