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Effect of Different Levels of a Growth Regulator on Morphological Indicators and Biomass of Sprouted Barley

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ABSTRACT

Objective: *The research was conducted in the laboratories of the Department of Life Sciences//College of Education for Women/University of Tikrit to study the response of sprouted barley Hordeum vulgare L. to 4 concentrations of the commercial plant growth regulator Atonik. The treatments included a control T1(with no additive), T2 (0.25 ml/L), T3 (0.50 ml/L), and T4 (0.75 ml/L).* ***Method:*** *The experiment followed a Completely Randomized Design (CRD) with 3 replicates / treatment. Growth was monitored on days 3, 6, 9, 12, and 15, assessing the following parameters: shoot length, root length, number of leaves, fresh weight, and dry weight.* ***Results:*** *The results, analyzed using the Least Significant Difference (LSD) test, revealed significant differences (P≤0.05) in favor of treatment T3. In 14th day the T3 record highest growth rates in shoot length of 17.50 cm and a root length of 14.20 cm also 2.60 leaves /plant compare with control which recorded 11.20 and 8.40 cm and 1.10 leaves / plant respectively. That morphological superiority is significant reflecte in the biomass. Significant higher in fresh weight and dry weight record in T3 comper to the control.* ***Novelty:*** *The application of growth regulator Atonik at concentration of 0.50 ml/L is the optimal dosage to stimulating vegetative and root growth as well as enhancing biomass productivity in sprout barley.*

INTRODUCTION

Barley Hordeum vulgare L. its high nutritional value and significant content of proteins and enzymes and consider one of the most important forage and food crops globally [1]. Yiblet et al. reported that hydroponic barley sprouting had emerged as a sustainable alternative to forage production aligned with global research trend toward sustainability that technique enables the production of high quality green fodder within a short time-frame requiring minimal water and space and eliminating need for soil [2]. Sreenivasulu et al. reported that percentage rate of germination and acumulation of biomass in both root and shoot systems directly influence the quality and physiological characteristics of the sprouted barley [3].

Przybysz et al., indicate plant growth regulators and stimulants are employed to enhance growth efficiency during the relatively short cultivation period also commercial product Atonik composed primarily of sodium nitrophenolates serves as an effective biostimulant playing a fundamental role in activating metabolic and biological processes within plant cells [4]. While Ruzicka et al., (2009) indicate that Atonik functions by increasing cell membrane permeability stimulating the activity of respiratory enzymes and accelerating cytoplasmic streaming in addition to promoting cell division and elongation within meristematic tissues [5]. And Haghighi et al., (2019) report that a local and international studies indicate that determining the optimal concentration of Atonik

is a key factor in achieving the best possible physiological response and supporting vegetative characteristics [6].

Stoilova et al., (2025) said that assessment of morphological traits like root and shoot length and number of leaves provides accurate indicator of plants response to biological stimulants and development patterns of vegetative components [7]. Munser et al., (2025) said the biomass of the cultivated plant measured in terms of fresh and dry weight serves as a primary metric for evaluating the efficiency with stored seed reserves are converted in functional vegetative and root tissues and the proportion of dry matter is a key determinant of the final nutritional value of the product [8]. Achieving a balance between vegetative and root growth is essential for maximizing productive within a short cultivation cycle the use of the growth regulator Atonikat specific to determined concentrations within closed plastic tray cultivation systems has not been sufficiently studied in the local environment, despite the existence of research on barley sprouting. Consequently, the study was conducted to evaluate the impact of varying concentrations of Atonik 0.25, 0.50, and 0.75 ml/L on the growth rates of sprouted barley and to identify the optimal concentration for enhancing morphological traits and biomass indicators [9].

RESEARCH METHOD

The study was conducted in the laboratories of the Department of Life Sciences at the College of Education for Women, University of Tikrit, under controlled environmental conditions. Pure and uniform barley *Hordeum vulgare* L. seeds were prepared, and a germination test was performed to ensure their viability. Completely Randomized Design (CRD) with four treatments/ three replicate in this experiment. A total of 12 plastic containers were used, with each container having a surface area of 1000 cm^2; all treatments consisted of 4 concentrations of the growth regulator Atonik prepared based on the recommended field dosage of 150 ml./dunum. These included: a control (T1, with no additions), and three experimental treatments T2 (0.25 ml/liter), T3 (0.50 ml/liter), and T4 (0.75 ml/liter). Barley seeds were surface sterilized by soaking them in a 1% sodium hypochlorite (NaOCl) solution for 5 minutes. They were then rinsed several times with distilled water to remove any residual sterilizing agent, followed by soaking in distilled water for 12 hours to activate the embryos. The seeds were distributed at equal weights and uniform density within plastic containers. Irrigation was carried out via spraying, using the prepared plant growth regulator solutions; each container received an equal volume of the solution daily. The germination process continued for two weeks (15 days) at laboratory temperature.

To track growth dynamics over time, measurements were taken periodically throughout the cultivation period specifically on days 3, 6, 9, 12, and 15. Morphological traits, including shoot length (cm), root length (cm), and the number of leaves (leaves /plant) were measured using a graduated ruler. Biomass indicators were assessed by collecting a representative 5 cm^2 sample from each container at the specified time points; the fresh weight g/5 cm^2 was recorded, followed by the dry weight g/5 cm^2 after drying the sample in an electric oven at 70°C until a constant weight was achieved. Based on the

standard methods issued by the Association of Official Agricultural Chemists [10], the collected data were subjected to statistical analysis using a software program, following a Completely Randomized Design (CRD). Differences between means were compared using the Least Significant Difference (L.S.D.) test at a significance level of (P≤0.05).

RESULTS AND DISCUSSION

The results in Table 1 show a continuous cumulative increase in the shoot length of the sprouted barley across all treatments as the experiment progressed from the third to the fifteenth day.

Table 1. Effect of different concentrations of the growth regulator Atonik on the stem length (cm) of cultivated barley over various time periods (3, 6, 9, 12, and 15 days) (mean ± standard error).

Treatment	Concentration (ml/liter)	Day 3	Day 6	Day 9	Day 12	Day 15
T1 (control)	0.00	1.10±0.08 c	3.20±0.15 c	5.80±0.22 c	8.90±0.31 c	11.20±0.42 c
T2	0.25	1.40±0.09 b	4.10±0.18 b	7.30±0.25 b	11.00±0.30 b	13.80±0.35 b
T3	0.50	2.10±0.12 a	5.80±0.20 a	9.90±0.29 a	14.20±0.33 a	17.50±0.28 a
T4	0.75	1.70±0.10 ab	4.90±0.16 b	8.40±0.27 b	12.30±0.32 b	15.10±0.31 b
LSD value (P≤0.05)	–	0.35	0.72	01.05	1.28	1.45

Means within the same column (for each time period) that are followed by different letters are significantly different at the (P≤0.05) probability level, according to the Least Significant Difference (L.S.D.) test.

By day 3, T3 (0.50 ml/L) demonstrated an early advantage statistically significant compared to the other treatments by producing the longest shoot, measuring 2.10 cm while the control treatment recorded the lowest growth at 1.10 cm. On days 6 and 9, treatment T3 continued to show a clear, statistically significant superiority, reaching a value of 5.80 cm on the sixth day and 9.90 cm on the ninth day significantly outperforming treatments T4 and T2, which ranked second. Between days 12 and 15 a percentage of vegetative elongation accelerated reaching their peak by the end of the experiment on day 15; treatment T3 achieved the highest final mean stem length reaching 17.5 cm followed by treatments T4 and T2 with means of 15.1 and 13.80 cm respectively. the control T1 showed the lowest values throughout the growth period; recording 11.2 cm; the increased stem elongation observed over-time is attributed to the role of Atonik nitrophenols in stimulating cytoplasmic movement and cell division within the meristematic apices during the early period days 3 to 6 [4]. As the culture period progresses (days 9–15), the compound promotes the synthesis of plant hormones that accelerate cell elongation; the superior performance of concentration T3 over T4 is due to the former being the optimal physiological dose for activation; higher concentrations lead to the saturation of hormonal receptors or partial inhibition [11].

Table 2. Effect of different concentrations of the growth regulator Atonik on root length (cm) of sprouted barley over various time periods (3, 6, 9, 12, and 15 days) (mean ± standard error).

Treatment	Concentration (ml/liter)	Day 3	Day 6	Day 9	Day 12	Day 15
T1 (control)	0.00	0.80±0.05 c	2.50±0.12 c	4.80±0.19 c	6.90±0.25 c	8.40±0.30 c
T2	0.25	1.20±0.07 b	3.60±0.15 b	6.20±0.21 b	8.40±0.22 b	10.10±0.25 b
T3	0.50	1.80±0.10 a	5.10±0.18 a	8.70±0.24 a	11.80±0.31 a	14.20±0.38 a
T4	0.75	1.40±0.08 b	4.20±0.14 b	7.30±0.22 b	10.10±0.27 b	12.30±0.29 b
LSD value (P≤0.05)	–	0.28	0.55	0.85	1.12	1.82

Means within the same column (for each time period) that are followed by different letters are significantly different at the (P≤0.05) probability level, according to the Least Significant Difference (L.S.D.) test.

The results in Table 2 show a continuous, significant development in the root length of the sprouted barley throughout the experimental period, spanning from the third to the fifteenth day. By day 3, the third treatment had already emerged as the superior one, recording the longest root at 1.80 cm a result that was significantly different from the fourth and second treatments, which recorded 1.40 cm and 1.20 cm, respectively. the control T1 yielded the shortest root-length at 0.80 cm but at days 6 and 9 root system continued its rapid elongation under the influence of the treatments. On the hand maintaine a significant advantage recording 5.10 cm on the 6^{th} day and 8.70 cm on 9^{th} day there by outperforming treatments T4 and T2. By days 12 and 14, root elongation reached its peak, culminating at the end of the 15-day germination period. Treatment T3 achieved the highest final mean root length of 14.20 cm, followed by treatments T4 and T2 with mean lengths of 12.30 cm and 10.10 cm, respectively; meanwhile, the control (T1) recorded the lowest mean root length throughout the experimental period, at 8.40 cm. The stimulatory effect on root system elongation throughout the growth stages is attributed to the role of the active nitrophenols the constituents of the plant growth regulator Atonik in inducing and activating root primordia and root apical meristems at an early stage. Atonik enhances cytoplasmic streaming and facilitates the active transport of nutrients and water across root cell membranes, thereby accelerating cell division and cell elongation within root tissues [12]. The results of the study also showed that the root system responds more effectively to the concentration in the T3 treatment because the roots are more sensitive to hormonal changes compared to the stems. Therefore, the higher concentration of 0.75 ml/liter in the T4 treatment led to a slight and significant decrease in the root growth rate compared to the optimal concentration of T3, which is explained by the phenomenon of hormonal saturation of cellular receptors, which causes partial inhibition of the elongation rate when the recommended physiological limit is exceeded [13].

Table 3. Effect of different concentrations of the growth regulator Atonik on the number of leaves (leaves/plant) of sprouted barley over various time periods (3, 6, 9, 12, and 15 days) (mean ± standard error).

Treatment	Concentration (ml/liter)	Day 3	Day 6	Day 9	Day 12	Day 15
T1 (control)	0.00	0.50±0.03 b	1.00±0.04 c	1.10±0.05 c	1.10±0.05 c	1.10±0.05 c
T2	0.25	0.80±0.04 a	1.20±0.05 bc	1.50±0.07 b	1.70±0.08 b	1.80±0.08 b
T3	0.50	1.00±0.05 a	1.60±0.08 a	2.00±0.10 a	2.30±0.11 a	2.60±0.12 a
T4	0.75	0.90±0.05 a	1.40±0.06 ab	1.80±0.08 ab	2.00±0.09 ab	2.10±0.10 ab
LSD value (P≤0.05)	–	0.22	0.28	0.31	0.38	0.48

Means within the same column (for each time period) that are followed by different letters are significantly different at the (P≤0.05) probability level, according to the Least Significant Difference (L.S.D.) test.

The results presented in Table (3) show that, by day 3 the growth regulator treatments T2, T3, and T4 demonstrated a significant early advantage in accelerating the emergence of the first leaf, compared to the control treatment, which recorded the lowest rate of leaf emergence at 0.50 leaves/plant.By days 6 and 9, significant differences between the concentrations became clearly apparent; the third treatment T3recorded the highest mean number of leaves 1.6 (leaves/plant) on day 6 and 2.00 (leaves/plant) on day 9 significantly outperforming both the control treatment and the low concentration treatment T2, while showing no significant difference from treatment T4, which recorded 1.80 (leaves/plant). By days 12 and 15, leaf development in the control group had effectively ceased at the stage of the first leaf—averaging 1.10 leaves per plant—due to the depletion of the seed's nutrient reserves and the absence of hormonal stimulation. In contrast, leaf formation continued in the treated groups; treatment T3 recorded the highest final average of 2.60 leaves per plant, followed by treatments T4 and T2, with averages of 2.10 and 1.80 leaves per plant, respectively.The significant increase and accelerated development in the number of fully formed leaves on the plant are attributed to the direct role of the active nitrophenols the constituents of the product Atonik in stimulating the shoot apical meristems. Atonik promotes cell division and the differentiation of leaf primordia by triggering the biosynthesis of growth-promoting hormones, particularly endogenous cytokinins, which are responsible for cell division and apical dominance [4].Early stimulation with Atonik contributes to increased synthesis of chlorophyll pigments by enhancing photosynthetic efficiency in developing leaf tissues, thereby accelerating vegetative morphological development compared to untreated plants [12]. As observed in the aforementioned growth characteristics, the T3 concentration maintained an optimal physiological response increasing both leaf area and leaf number without reaching the state of physiological inhibition induced by the higher concentration, T4.

Table 4. Effect of different concentrations of the growth regulator Atonik on the fresh weight (g/5 cm²) of sprouted barley over various time periods (3, 6, 9, 12, and 15 days) (mean ± standard error).

Treatment	Concentration (ml/liter)	Day 3	Day 6	Day 9	Day 12	Day 15
T1 (control)	0.00	1.25±0.03 c	2.40±0.06 c	4.25±0.10 c	6.00±0.15 c	7.25±0.18 c
T2	0.25	1.40±0.03 b	2.80±0.08 b	5.10±0.12 b	7.50±0.18 b	9.10±0.21 b
T3	0.50	1.60±0.04 a	3.40±0.09 a	6.25±0.14 a	9.25±0.20 a	11.25±0.23 a
T4	0.75	1.50±0.04 a	3.05±0.07 b	5.65±0.11 b	8.40±0.19 b	10.05±0.20 b
LSD value (P≤0.05)	–	0.11	0.24	0.38	0.55	0.66

Means within the same column (for each time period) that are followed by different letters are significantly different at the (P≤0.05) probability level, according to the Least Significant Difference (L.S.D.) test.

The results presented in Table (4) indicate significant differences in the accumulation of fresh weight for 5 cm² tissue samples between the Atonik growth regulator treatments and the control treatment across all measurement intervals 3, 6, 9, 12, and 15 days. On day 3, treatments T3 and T4 showed a superior early response, recording 1.60 g/5 cm² and 1.50 g/5 cm², respectively, with no significant difference between them, in contrast, the control treatment recorded the lowest sample weight at 1.25 g/5 cm². At 6, 9, and 12 days, the significant advantage clearly shifted in favor of treatment T3, which recorded the highest cumulative values 3.40 g, 6.25 g, and 9.25 g/5 cm², respectively significantly outperforming the other treatments. On day 15, it was observed that treatment T3 achieved the highest cumulative fresh weight, reaching 11.25 g/5 cm² an increase of 55.2% compared to the control treatment, which recorded 7.25 g/5 cm². Treatments T4 and T2 ranked second, with mean values of 10.05 g/5 cm² and 9.10 g/5 cm², respectively. The significant increase in the fresh-weight of the 5 cm² sampled area; is linked to the direct physiological role of the sodium-based compounds that constitute Atonik. These compounds stimulating the cytoplasmic stream and enhance permeability the cell membranes facilitate the rapid and efficient-uptake of water and nutrients. A cording this elevates osmotic pressure and cell turgor pressure within the vegetative and root-tissues developing within the specified area [14][12]. Furthermore, the superior observed in morphological development in T3 characterized by increased stem and root-elongation and a higher density of leaf formation resulted in a greater accumulation of green biomass and total water content within the 5cm² sample. Relative decline in T4 compared to T3 suggest that a concentration of 0.50 mL/L represents a optimal physiological dosage for nutrient uptake and the accumulation of fresh biomass conversely; higher concentrations may limit the efficiency of cellular activation due to the saturation of hormonal receptors [4].

Table 5. Effect of different concentrations of the growth regulator Atonik on the dry weight (g/5 cm²) of sprouted barley over various time periods (3, 6, 9, 12, and 15 days) (mean ± standard error).

Treatment	Concentration (ml/liter)	Day 3	Day 6	Day 9	Day 12	Day 15
T1 (control)	0.00	0.16±0.005 c	0.31±0.008 c	0.55±0.014 c	0.78±0.018 c	0.94±0.021 c
T2	0.25	0.18±0.006 b	0.36±0.010 b	0.66±0.016 b	0.97±0.022 b	1.18±0.025 b
T3	0.50	0.21±0.007 a	0.44±0.012 a	0.81±0.019 a	1.20±0.026 a	1.46±0.030 a
T4	0.75	0.20±0.006 a	0.39±0.011 b	0.73±0.015 b	1.09±0.024 b	1.31±0.027 b
LSD value (P≤0.05)	–	0.015	0.032	0.049	0.071	0.085

Means within the same column (for each time period) that are followed by different letters are significantly different at the (P≤0.05) probability level, according to the Least Significant Difference (L.S.D.) test.

The results in Table (5) indicate the presence of statistically significant differences among the treatments across all cultivation periods. On day 3, treatments T3 and T4 showed a significant early response, recording 0.21 g/5 cm² and 0.20 g/5 cm², respectively with no significant difference between them and both significantly outperformed the control treatment, which recorded 0.16 g/5 cm² . On days 6, 9, and 12, treatment T3 continued to demonstrate a clear, statistically significant superiority, yielding the highest dry matter values 0.44 g, 0.81 g, and 1.20 g per 5 cm², respectively outperforming the other treatments by a statistically significant margin. On day 14 treatment T3 yielded the highest accumulation of dry matter within a 5 cm² area, reaching 1.46 g an increase of 55.3% compared to the control treatment, which recorded the lowest value of 0.94 g. Treatment T4 followed, with an average of 1.31 g. The significant increase in the dry weight of the sampled section (5 cm²) is attributed to the fact that dry matter represents the actual product of metabolic activity, photosynthesis, and the cumulative accumulation of organic compounds within both the shoot and root systems. Atonik nitrophenols contribute to the activation of key enzymes involved in metabolism such as nitrate reductase and protein synthesis. This increase in efficiency of carbon-compound synthesis directing these compounds toward the formation by walls and new tissues in cell [15][4]. the morphological increase in stem-length and the expansion of root system within the 5cm² area provided larger surface area for metabolic processes and dry matter accumulation. The significant decline observed in T4 compared to T3 confirms that a concentration of 0.50 ml/L represents, in optimal hormonal level for maximizing conversion efficiency without inducing regulatory stress or excessive energy expenditure on the elimination of excess hormones.

CONCLUSIONS

Fundamental Finding : The application of Atonik enhances the growth of sprouted barley and increases biomass accumulation compared to the control group. The T3 treatment with a concentration of 0.50 ml/L produces the best results, achieving the

highest shoot and root lengths, number of leaves, and fresh and dry weights. **Implication :** Atonik at a concentration of 0.50 ml/L is recommended to achieve maximum productivity in sprouted barley cultivation, as this concentration provides the optimal physiological response. **Limitation :** The application of a higher concentration of 0.75 ml/L results in a relative decline in growth and biomass outcomes, likely due to hormonal saturation. **Future Research :** Further research could investigate the physiological mechanisms underlying the decline in growth at higher Atonik concentrations, particularly the effects of hormonal saturation, to determine the optimal concentration range for sprouted barley.

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