

Evaluating the potential of natural and chemical water retainers on barley (*Hordeum vulgare*) morphological and biochemical aspect

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Abstract

Climate change has increased water demand while increasing water resources. To enhance water absorption and nutrient retention in soil, hydro-retainers are widely used in agriculture; however, most are synthetic, non-biodegradable acrylate-based products. This research was conducted to evaluate the impact of a hydro-retentive synthetic potassium polyacrylate and natural cladode powder from *Opuntia ficus-indica*, on germination of three barley (*Hordeum vulgare*) varieties Kounouz, Artorio and Rihane. Using natural cladode powder, potassium polyacrylate, and their combination at different concentrations (0.2%, 0.5%, and 1%), to test the germination of Kounouz and Artorio *in vitro* over a 15-day treatment period and Rihane *in vivo* over a one-month treatment period. This work focuses on the physiological and biochemical behaviour of the barley varieties in response to the application of synthetic hydrogel and natural cladode powder and their combination. The measurements included germination rate, plant height, root length, total protein, chlorophyll content, oxidative stress markers, and hydrogen peroxide levels. The findings reveal varietal-specific responses to hydro-retentive treatments, with potassium polyacrylate significantly enhancing germination rates, especially at higher concentrations. Conversely, *Opuntia ficus-indica* cladode powder showed positive effects at lower concentrations but inhibited germination at higher doses. Improvements in chlorophyll content and antioxidant enzyme activities at specific concentrations suggest an optimization pathway for stress resilience in barley seedlings. Mixed hydrogel treatments, particularly at a 0.5% concentration, affected positively the evaluated parameters.

1. INTRODUCTION

The global population has increased from 1.7 billion in 1910 to nearly 7.7 billion in 2019, and projections by ONU in 2019 anticipate a rise to 9.7 billion by 2050. Considering this global scenario, meeting the escalating demand for food needs not only high productivity but also optimized use of limited resources such as arable land and water (Spiertz, 2012). Considering that agriculture is mainly dependent on water, a resource currently facing limitations, the need to use it efficiently becomes evident. Tunisia faces water scarcity and high spatial and temporal climate fluctuations. Its water resources are very limited (Habaieb and Albergel, 2001). The challenge of achieving sustainable agriculture in arid and semi-arid environments requires innovative strategies to save water and improve irrigation management techniques.

Within this context, the use of Superabsorbent polymers (SAP) is a promising strategy to mitigate high water demands and enhance crop yield in arid and semi-arid regions (Farrell et al., 2013; Islam et al., 2011; Koupai et al., 2008). Hydro-retentive agents could absorb and retain substantial amounts of water, mitigate water stress and improve crop resilience to drought. SAP are materials that have the ability to absorb and retain large quantities of liquid in comparison to their own size. With the potential of holding water up to 1000 times of their dry weight, superabsorbent polymers are suitable for application in agriculture as a soil conditioner to improve the crop yield. SAP can absorb water when it is available in excess, and release it gradually to the roots of the plant in times of deficit. In addition to the water absorption capacity, the superabsorbent material can also act as a water management tool for the efficient use of water and fertilizers (L. Krasnopeevea et al., 2022). The agricultural use of acrylate-based super absorbent polymers largely began in the 1990s to reduce the environmental consequences of non-point pollution by retaining water and nutrients, improving crop yield. However, the majority of these products are typically prepared by cross-linking acrylic acid esters, and the remaining unreacted acrylate monomers can threaten soil integrity and the long-term success of these goals. Additionally, conventional acrylate-based SAPs have an environmental impact and are classified as toxic. The products exhibit severe toxicity towards the health of humans and animals, affecting all major organ systems (L. Krasnopeevea et al., 2022). It is widely recognized that toxic acrylate-based SAPs degrade and persist in agricultural soils and the environment as far longer time than previously thought. Given the intensification of food crop cultivation and the growing demand for productivity, it is crucial to limit the agricultural use of these detrimental SAPs (Elshafie and Camele, 2021). In addition, agricultural activities generate huge amounts of organic waste or crop residues, which represent an environmental problem regarding its accumulation. Several alternatives have been proposed to deal with this issue, often offering benefits for the environment and the agricultural practices. Managing the natural dry vegetation, which grows wildly in certain areas, presents an additional challenge. In this context, the cladodes of the *Opuntia ficus-indica* can be dried and used in several applications. The powder obtained after this preparation can be stored and used in different ways. These peculiar characteristics have been exploited in recent times as a tool for increasing soil or pot growth medium fertility and water retention and for decreasing its pH. Some of these properties have been obtained from chemical procedures; others depend on the mucilage content present in the milled powder. (Shoukat et al., 2023)

This research focuses on evaluating the impact of using potassium polyacrylate, as a synthetic superabsorbent polymer, and *Opuntia ficus-indica* cladode powder, derived from the *Opuntia ficus-indica*, as a sustainable and biodegradable alternative, on seed

germination and early plant growth of different varieties of barley *Hordeum vulgare*. By testing water retention capacities, germination rates, growth metrics, and biochemical responses under the effect of these agents.

2. MATERIAL AND METHODS

This study aims to characterize the impacts of two types of hydro-retentive agents on barely germination and growth. Potassium polyacrylate (PAP) was used as a synthetic superabsorbent and *Opuntia ficus-indica* cladode powder (Bio) as a biological water-retaining agent.

2.1. Plant material

Three varieties of *Hordeum vulgare* barley seeds, "Kounouz" and "Artorio", were used to conduct this study.

2.2. Hydro-retainers used in the experiment: *Opuntia ficus-indica* cladode powder and Potassium polyacrylate

Opuntia ficus-indica cladode powder: The prickly pear cladode powder used in this study was supplied by a local company (NOPAL, Tunisia) located in Kasserine (35°12'27.4"N 8°46'09.9"E), Tunisia specialized in the production of organic *Opuntia ficus indica* products. The collected cladodes were sun dried then ground into very fine particles. The powder was used without any pre-treatment.

Potassium polyacrylate: The polymer chains of potassium polyacrylate are composed of monomeric units of potassium acrylate (potassium prop-2-enoate) and acrylic acid (prop-2-enoic acid) in a proportion of approximately 65% and 34.7%, respectively. These polymer chains are cross-linked with 0.3% of pentaerythritol triallyl ether. The final structure of the resulting material consists of a cross-linked network of flexible polymer chains with potassium salt groups of carboxylic and carboxylate acids.

2.3. Water retention measurements

For each treatment, the respective polymer and the prickly cladode powder masses were accurately measured, 0.1g, 0.25 g, and 0.5g, and then immersed in 50 ml of rainwater for an hour to achieve swelling equilibrium. Subsequently, the suspensions were filtered using a filter over a period of 2 hours. The quantity of water retained by the swollen samples was determined using the following calculation:

$$WRP = (m_1 - m_0) / m_0$$

WRP = Water Retention Power, expressed in g of water per g of product

m0 = mass, expressed in g of dry hydrogel

m1 = mass, expressed in g of swollen hydrogel after filtration

2.4. *In vitro* culture: seeds pretreatments and germination

Barley seeds were stored at 4°C, and then washed with tap water and subsequently sterilized using a commercial sodium hypochlorite solution under sterile conditions. The sterilization process involved immersing the seeds in a 50% sodium hypochlorite solution for 3 minutes, followed by a 20% sodium hypochlorite solution for 10 minutes and finally in a 10% sodium hypochlorite solution for 30 minutes. Following disinfection, the seeds were rinsed with distilled water.

For each treatment, every quantity of PAP (Potassium Polyacrylate) and *Opuntia ficus-indica* cladode powder were weighed and placed in petri dishes, followed by the addition of 10 ml of water (as indicated in Table 1). After a 15-minute period, 10 sterilized barley seeds were placed in each dish, which was then sealed with cellophane paper. Then, the dishes were kept in darkness for 48 hours and later transferred to a culture chamber set at a constant temperature of 24°C, with a photoperiod of 14 hours of daylight and 8 hours of darkness, spanning a total duration of 15 days. This *in vitro* germination was designed with three replicates.

Table 1
The different concentrations and combinations of hydro-retainers used

	Treatment name and composition	Treatment composition
Bio : <i>Opuntia ficus-indica</i> cladode powder	Bio 0.2%	Bio 0.2% (w/v)
	Bio 0.5%	Bio 0.5%(w/v)
	Bio 1%	Bio 1% (w/v)
PAP : Potassium polyacrylate	PAP 0.2%	PAP 0.2% (w/v)
	PAP 0.5%	PAP 0.5% (w/v)
	PAP 1%	PAP 1% (w/v)
Mix : Bio + PAP	Mix 0.2%	Bio 0.2% (w/v) + PAP 0.2%(w/v)
	Mix 0.5%	Bio 0.5%(w/v) + PAP 0.2%(w/v)
	Mix 1%	Bio 1%(w/v) + PAP 0.2%(w/v)

Table 2
Cumulative rate of *in vitro* germination of the seeds of both barley varieties over a 15-day period of exposure to hydro-retainers.

		Day 3			Day 6			Day 9			Day 12			Day 15		
Kounouz	Positive control	26.7	±	4.4	50.0	±	6.7	56.7	±	4.4	66.7	±	8.9	66.7	±	8.9
	Bio 0.2%	40.0	±	20.0	53.3	±	15.6	63.3	±	11.1	66.7	±	4.4	66.7	±	4.4
	Bio 0.5%	16.7	±	4.4	20.0	±	6.7	26.7	±	4.4	26.7	±	4.4	26.7*	±	4.4
	Bio 1%	16.7	±	4.4	16.7	±	8.9	16.7	±	8.9	40.0	±	6.7	40.0*	±	6.7
	PAP 0.2%	23.3	±	15.6	40.0	±	13.3	36.7	±	8.9	53.3	±	15.6	53.3	±	15.6
	PAP 0.5%	33.3	±	17.8	40.0	±	20.0	43.3	±	22.2	50.0	±	13.3	50.0	±	13.3
	PAP 1%	36.7	±	4.4	70.0	±	6.7	73.3	±	4.4	86.7	±	4.4	86.7	±	4.4
	Mix 0.2%	43.3	±	4.4	50.0	±	0.0	50.0	±	0.0	56.7	±	4.4	56.7	±	4.4
	Mix 0.5%	26.7	±	17.8	50.0	±	6.7	40.0	±	26.7	56.7	±	4.4	56.7	±	4.4
	Mix 1%	23.3	±	15.6	43.3	±	4.4	26.7	±	17.8	30.0	±	6.7	33.3*	±	4.4
Artorio	Positive control	53.3	±	11.1	80.0	±	6.7	80.0	±	6.7	90.0	±	6.7	90.0	±	6.7
	Bio 0.2%	33.3	±	11.1	73.3	±	11.1	83.3	±	11.1	90.0	±	6.7	90.0	±	6.7
	Bio 0.5%	50.0	±	13.3	60.0	±	20.0	63.3	±	24.4	63.3	±	24.4	60.0	±	26.7
	Bio 1%	23.3	±	11.1	26.7	±	15.6	26.7	±	15.6	40.0	±	6.7	40.0*	±	6.7
	PAP 0.2%	50.0	±	6.7	66.7	±	4.4	73.3	±	11.1	70.0	±	6.7	70.0	±	6.7
	PAP 0.5%	50.0	±	6.7	76.7	±	4.4	83.3	±	4.4	73.3	±	4.4	73.3	±	4.4
	PAP 1%	93.3	±	4.4	96.7	±	4.4	96.7	±	4.4	96.7	±	4.4	96.7	±	4.4
	Mix 0.2%	56.7	±	11.1	70.0	±	13.3	83.3	±	11.1	90.0	±	13.3	90.0	±	13.3
	Mix 0.5%	23.3	±	11.1	30.0	±	20.0	33.3	±	17.8	50.0	±	13.3	50.0*	±	13.3
	Mix 1%	0.0	±	0.0	6.7	±	4.4	10.0	±	6.7	10.0	±	6.7	10.0*	±	6.7

* indicates a significant mean difference between treatments positive control and each treatment (Bio 0.2%. Bio 0.5%. Bio 1%. PAP 0.2%. PAP 0.5%. PAP 1%. Mix 0.2%. Mix 0.5% and Mix 1%) at $p < 0.05$.

2.5. *In vivo* culture: seeds pretreatments and germination

A soil substrate was prepared, consisting of wet sand mixed with peat (1/3 v:v). 50 g of this mixture were placed in perforated pots to ensure drainage. Different treatments were applied to each pot, and three barley seeds were sown per pot. The experiment was conducted in a greenhouse at a controlled day-temperature of 28°C during a one-month period. Irrigation was performed using rainwater only on the first day. This *in vivo* germination was designed with three replicates.

2.6. Measurement of morphological parameters

The average cumulative germination percentage is determined by calculating the ratio of the cumulative number of seeds germinated on each day to the total initial number of seeds, expressed as a percentage up to 100.

Cumulative germination percentage (%) = (seeds germinated / Total seeds) x 100

Germination index: The Germination Index (GI), as defined by Zucconi et al. (1981), is expressed as the ratio:

$GI = (\text{Average number of germinated treated seeds} \times \text{Treated root length}) / (\text{Average number of germinated control seeds} \times \text{Root length of control})$.

2.7. Biochemical parameters

Biochemical parameter measurement was carried out on the 15th day of *in vitro* cultivation across all the two barley varieties to assess the potential toxicity of the hydro-retainers.

Soluble protein content by the Bradford method: The proteins were quantified using the Bradford method (Bradford, 1976) This method is based on a colorimetric assay that detects the color change of Coomassie blue at 595 nm after complexation with the aromatic amino acids (tryptophan, tyrosine, and phenylalanine) and the hydrophobic residues of the amino acids present in the solution. The change in absorbance is directly proportional to the amount of dye bound, and consequently, to the protein concentration in the sample. A calibration curve was established using a bovine serum albumin solution (Sigma) ranging from 0 to 0.18 µg/µl.

Chlorophyll pigments: The contents of chlorophyll a, chlorophyll b, and carotenoids are assessed following the methodology established by Lichtenthaler (1987). Cold extraction is performed using 25 ml of acetone for 1 g of fresh leaf material. After centrifugation at 5000 g for 10 minutes at 4°C, absorbance readings were taken at wavelengths of 470 nm, 662 nm, and 645 nm using a spectrophotometer. The pigment concentrations, expressed in µg/ml, were then calculated using the following equations:

$$Ca = 12.70A_{663} - 2.69A_{645}$$

$$Cb = 22.90A_{645} - 4.68A_{663}$$

$$Ca + b = 20.21A_{645} + 8.02A_{663}$$

$$Cx + c = 1000 A_{470} - 1.90 Ca - 63.14 Cb$$

(Ca and Cb: Concentration of chlorophylls a and b; Cx + c: Concentration of carotenoids)

Dosage of the free radical of H₂O₂: The quantification of H₂O₂ in germinated seeds was conducted using the methods outlined by Sagisaka (1976). Hydrogen peroxide (H₂O₂) was extracted by homogenizing 500 mg of tissue with 5 ml of trichloroacetic acid (TCA) at a concentration of 0.1%. Subsequently, the homogenate was centrifuged. Then, 0.5 ml of potassium phosphate (10 mM, pH = 7) and 1 ml of potassium iodide (1 M) were added to the resulting supernatant in order to determine H₂O₂ levels. The absorbance was measured at 390 nm.

Measurement of Superoxide Dismutase (SOD) activity: Superoxide dismutase (SOD) activity is measured using the method of Beauchamp & Fridovich (1971), which involves spectrophotometrically determining the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm. An SOD activity unit is defined as the amount of enzyme needed to inhibit 50% of NBT reduction. The reaction mix contains 50 mM phosphate buffer (pH 7.5), 10 mM methionine, 2 µM riboflavin, 0.1 mM EDTA, 70 µM NBT, and the extract, performed at 25°C in light for 10 minutes. The reaction is halted by dark storage at 4°C for 10 minutes before measuring absorbance.

Controls are set in the dark to establish the maximum NBT reduction (A max). SOD inhibition percentage is calculated as: $(A_{\text{max}} - A_{\text{sample}}) / A_{\text{max}} \times 100$.

Catalase activity measurement (CAT): Catalase activity was determined by the method of Aebi (1984) using the extinction coefficient $39.4 \text{ mM}^{-1} \text{ cm}^{-1}$.

2.8. Statistical analysis

The Student's t-test was used to test hypotheses, using Microsoft Excel. The data represents the means of three replicates, accompanied by the $\pm$ SE (standard error). Statistically significant differences at the $P < 0.05$ level have been indicated. Graphical representations were generated using Microsoft Excel (Microsoft corp., USA).

3. RESULTS AND DISCUSSION

3.8. Water retention power

The comparison of water holding capacity of different hydrogels is shown in Figure 1, Bio, (PAP), and mix (Mix) at 0.2%, 0.5%, and 1% concentrations. Among these, bio hydrogel showed the lowest potency, holding only 4.895 grams of water per gram of hydrogel.

Mixed hydrogels (Mix), investigated at different concentrations exhibited decreasing water holding capacity with increasing concentration, reaching 51.1g per gram of hydrogel at a concentration of Mix 0.2% to 10.13g water/g hydrogel for Mix 1%. However, PAP has the highest water retention capacity, yielding 160.665 g of water per gram of hydrogel. These results are similar to those obtained by Zhang et al. (2020).

3.9. Effect of hydro-retainers on morphological parameters

3.9.1. Effect of hydro-retainers on cumulative germination rates: *In vitro*

Barley seeds of the varieties Kounouz and Artorio were treated with different concentrations of Bio, PAP, and Mix (0.2%, 0.5%, and 1%), respectively. Cumulative germination rates were recorded on days 3, 6, 9, 12, and 15. This was compared with a positive control (no hydro-retainer treatment). The control group showed that Artorio had the highest germination rate (90%) on day 15 compared to Kounouz. In Kounouz, Bio treatment enhanced germination from the day 9 at lowest level (0.2 %) and recorded a percentage of germination rate equivalent to the control table (2). However, higher concentrations adversely affected germination, indicating possible toxicity or other damaging effects at these concentrations. In contrast, Artorio seeds showed high germination rates in 0.2% concentration of bio-treatment, resulting in comparable germination levels to the control for up to 15 days, while higher levels had a negative impact. The application of PAP hydrogel enhanced germination rates, especially at the 1% concentration; the germination rates of Kounouz and Artorio seeds were 86.7% and 96.7%, respectively. Mix hydrogel treatments' effects depend on their concentration applied and varietal characteristics. Kounouz seeds germination was slightly improved at lower concentrations (0.2% and 0.5%) and a decrease was observed at higher concentrations (1%). In contrast, the growth rate of Artorio seeds significantly increased at a 0.2% concentration but significantly decreased at 1%. This means that the effectiveness of the mixed hydro-retainers depends on their concentration and barley genotype. In general, lower concentrations enhance germination, whereas higher concentrations may affect the process due to possible factors such as increased osmotic pressure. Figure 2 illustrates the germination's morphological aspect after 15 days. Potassium polyacrylate, especially at 1% concentration, positively enhanced the germination rates. However, it has been found to cause toxic symptoms such as leaf chlorosis and root tip necrosis. This suggests that its water-holding capacity plays an important role in maintaining an optimal moisture content for enzymatic activity essential for germination but with accumulation of toxic molecules in plant tissue. Research indicates that plants treated with superabsorbent polymers can better resist drought. Su et al. (2017) demonstrated that using superabsorbent polymers, such as polyacrylamide, sodium polyacrylate, and other synthetic hydrogels, improved the germination and growth of *Caragana korshinskii* seeds.

3.9.2. Effect of hydro-retainers on cumulative germination rates: *In vivo*

Barley seeds of the variety Rihane were treated with different concentrations of Bio, PAP, and Mix (0.2%, 0.5%, and 1%), respectively. Cumulative germination rates were recorded on days 7, 14, 21 and 28 (table 4). The positive control maintained high germination rates ($83.3 \pm 16.7\%$) since day 7. Low doses (0.2%) of Bio and PAP performed similarly to the control, while higher doses reduced germination, with Bio decreasing to 50% and 33.3% at 0.5% and 1%, respectively. PAP dropped to 33.3% at 0.5% but partially recovered

to 50% at 1%. The mixture at 0.2% improved germination from 66.7% to 83.3%, while at higher concentrations (0.5% and 1%), it led to significant inhibition, with 1% completely blocked germination. According to Kumar et al. (2021), adding *Ficus indica* cladode to soil reduces its pH and boosts organic carbon levels. This effect is due to cation exchange and organic matter interactions in humid tropical soils. Moreover, dried cladode treatment facilitates a rapid, stable release of N/P/K nutrients, and compost made with 20% or more wet cladode meets conventional N/P/K standards which may enhance germination and growth.

3.9.3. Germination index: *In vitro*

The evaluation of *in vitro* germination indices (GI) in Kounouz and Artorio barley varieties revealed that seed germination is affected by different treatments and concentrations (Figure 3). Among the bio hydrogel treatments, the application of Bio at a concentration of 0.2% increased the germination index (GI) of Kounouz varieties to 1.62, which was higher than that of Artorio's GI 1.23, indicating that Kounouz may tolerate this dose better. This result was confirmed by the dose 1% of Bio. The GI of Kounouz was still relatively high at 1.15, while the GI of Artorio decreased significantly to 0.14, highlighting the high sensitivity of Artorio to Bio treatments. PAP at lower concentrations (0.2% and 0.5%) reduced the GI in both varieties in comparison to the control, with Artorio shows a better tolerance than Kounouz. In contrast, applying 1% of PAP reduced the GI of both varieties with much less severe impact in Kounouz (0.95) than in Artorio (0.42), indicating varietal resilience in Kounouz to higher PAP concentrations. In the combined treatments, Mix 0.2% showed an improved GI in both varieties, Kounouz (1.06) was less affected than Artorio (1.1), suggesting a positive effect of this concentration on germination. However, the GI significantly decreased with higher concentrations (Mix 0.5% and 1%), with Artorio particularly affected at 1%, where the GI dropped to 0, indicating a complete inhibition of germination under this condition. These observations highlight a variable varietal response to different hydrogel concentrations and emphasize the importance of careful consideration when implementing hydrogel treatments in barley cultivation. In the combined hydrogel treatments, Mix 0.2% improved GI was observed in each variety, Kounouz (1.06) was less affected than Artorio (1.1). However, the GI drastically decreased with Mix 0.5% and 1% for Artorio in particular at 1%, where the GI dropped to 0, indicating a complete inhibition of germination. These data show that Artorio is more sensitive to higher concentrations of hydrogels, especially Bio 1% and Mix 1%, where the GI of Artorio decreased significantly or was completely inhibited. These results are aligned with those of Sarvaš et al. (2007), showing that excessive concentrations of superabsorbent polymers can cause plant mortality. In contrast, Kounouz was more tolerant to higher concentrations of hydro-retainers, especially at 1% PAP, where its germination index remained quite high despite the increased concentration. These observations indicate a variable varietal response to hydrogel concentrations.

3.9.4. Effect of hydro-retainers on seedlings growth: *In vitro*

The *in vitro* growth of two barley varieties, Kounouz (Figure 4a) and Artorio (Figure 4b), was significantly impacted by hydrogel treatments and its concentration. A baseline is established through positive control, which shows that Kounouz and Artorio have similar root and stem lengths, which suggests similar growth patterns under standard conditions. With hydrogel treatments, the barley varieties Kounouz and Artorio responded differently depending on the type and concentration of hydrogel. Lower concentrations of Bio treatment increased the root and stem length of Kounouz variety at 0.5% concentration. whereas Artorio showed significant growth reduction, especially in root length, at the 1% concentration. PAP treatments generally slowed growth in Kounouz and Artorio, though Artorio was slightly more resistant at lower concentrations but showed major growth declines at 1%. Mixed treatments had different effects, Artorio variety was more sensitive than Kounouz since its growth was completely stopped at 1% concentration.

The Kounouz and Artorio varieties, both treated with 1% PAP, showed signs of toxicity, including complete inhibition of growth and necrosis of seeds. Furthermore, the roots of seeds treated with 1% PAP exhibited necrotic spots and browning, which could be due to the accumulation of insoluble phenolic compounds such as lignin in the xylem vessels, which could decrease the upward transport of water and nutrients. However, other studies reported that superabsorbent polymers act as water reservoirs during water stress conditions in *Raphanus sativus* plants. It was shown that plants grown in soil with hydrogels had a yield and shoot height that was approximately 10% higher than those grown in soil without hydrogels (Rychter et al., 2019).

Seedlings treated with PAP initiated more absorbent hairs farther from the apex than controls, leading to cell degradation in the area responsible for elongation. This could be due to the disturbance of the balance of certain growth hormones, in particular auxin (Voothuluru et al., 2024). This response is considered an adaptation of plants to escape the toxicity of the product.

3.9.5. Effect of hydro-retainers on seedlings growth: *In vivo*

The *in vivo* germination of Rihane barley seeds for over 28 days was influenced by varying hydrogel concentrations (Figure 9 and 10). On Day 7, Bio 0.2% showed growth statistically similar to the control, suggesting that moderate hydrogel doses provide sufficient moisture without oversaturation. In contrast, seeds treated with Mix 1% did not germinate. This negative effects of high hydrogel concentrations, likely due to excessive water retention and poor aeration. By day 14, the positive control stopped its growth, and the seedlings began wilting due to water scarcity. Mix 0.2% outperformed the control, while Bio 1%, Mix 1% continued to struggle. On day 21, Bio 0.2% and Mix 0.2% significantly surpassed the control (both around 30.5 cm), indicating a better balance of moisture retention. By day 28, Bio 0.2% (34 cm) and Mix 0.2% (33.17 cm) continued to outperform the control, while Mix 0.5% and PAP 1% also showed higher growth this might be due to the application of cladode powder that can be a valuable soil amendment because its influence on water regulation, organic matter improvement, pH beneficial effect, and all biotechnical properties is an essential factor in maintaining the maximum productivity of agricultural land. (Bacchetta et al. 2024). Conversely, Bio 1% remained significantly low and Mix 1% did not germinate, indicating the detrimental effects of high hydrogel concentrations, such as waterlogging and oxygen deprivation. Overall, moderate hydrogel concentrations (Bio 0.2% and Mix 0.2%) consistently promoted optimal growth. This may be due to the rich composition of the cladodes powder which according to Silva et al (2023) it contains about 1.9-5.28% crude protein, 20% crude fiber, up to 2% acetic acid, saponins, phytate, tannin, sterols, vitamin C, tocopherol, lower pH, ammonia N, acid detergent lignin, vitamins B1, B6, niacin, iron, calcium, magnesium. However, higher concentrations of Bio 1% delayed development, underscoring the importance of balanced moisture levels for barley seedling growth. The findings of Frantz et al. (2005) suggest that the potential benefit of using SAP is significant only in the early stages of plant growth, with little or no benefit for production parameters at other stages. SAPs did not have any significant effect on the wilting point of plants (Ali et al., 2023). In accordance with these results, it was found that the height of wheat plants decreased significantly as the rate of SAP applied was increased according to Ahmed (2024).

3.10. The impact of hydro-retainers on biochemical parameters

3.10.1. Effects of hydrogels on total protein content

In the control groups, Artorio showed a higher baseline total protein content in the roots (75.33 ± 0.94 mg prot/ml/gMF) than Kounouz (61.33 ± 0.47 mg prot/ml/gMF), which indicates varietal differences in protein synthesis or storage capacities (table 3). Bio hydrogel treatment resulted in a decrease in root protein content for both varieties of barley as the concentration increased. PAP hydrogel treatment indicated that Artorio had a high protein content, particularly at 0.2% (95.33 ± 1.89 mg prot/ml/gMF for Artorio vs. 44.00 ± 0.47 mg prot/ml/gMF for Kounouz). This result could reflect a differential varietal response to PAP hydrogel, which may be linked to differences in root architecture or hydrogel absorption efficiency. Under treatment with Mixed hydrogel, Artorio was reacting more positively, mainly at higher concentrations. Artorio started with a higher reference stem protein content (150 ± 0.94 mg prot/ml/gMF) than Kounouz (142 ± 0.94 mg prot/ml/gMF). Moreover, treatments with hydrogel showed that Artorio maintained or increased stem protein levels, particularly with Bio and Mix at lower concentrations. Our findings are in agreement with the results reported by Wang et Nii (2000) and Parida et al. (2002), who reported that the application of SAPs reduces the content of soluble proteins in leaves particularly under conditions of deficient irrigation.

Table 3. Effects of treatments on total protein content (mg protein/ml/gMF) in the two varieties of barley over a 15-day treatment period.

Total root protein content (mg prot/ml/gMF)										
Treatment										
		Positive control	Bio 0.2%	Bio 0.5%	Bio 1%	PAP 0.2%	PAP 0.5%	PAP 1%	Mix 0.2%	Mix 0.5%
Kounouz	root	61.33±	61.33±	52.00*±	40.67*±	44.00*±	67.33*±	66.67*±	64±	82.00*±
		0.47	0.94	0.47	0.94	0.47	0.94	0.94	0.94	0.94
	stem	142±	162.67*±	121*±	92.33*±	143.33±	120.67*±	113.33*±	135.3±	151.33*±
		0.94	0.94	0.47	1.41	0.94	1.89	1.89	1.89	0.94
Artorio	root	75.33±	63±	72.67±	55±	95.33*±	84.33±	76.33±	107*±	120.0*±
		0.94	2.36	2.83	0.47	1.89	1.41	1.41	1.41	0.94
	stem	150±	189.00*±	178.33*±	170.00*±	175.33*±	127.00*±	134.33*±	175.3*±	191.0*±
		0.94	2.36	2.36	0.94	1.89	1.41	2.36	1.89	1.41

* indicates a significant mean difference between treatments positive control and each treatment (Bio 0.2%, Bio 0.5%, Bio 1%, PAP 0.2%, PAP 0.5%, PAP 1%, Mix 0.2%, Mix 0.5% and Mix 1%) at p<0.05.

Table 4. Cumulative rate of *in vivo* germination of the seeds of barley Rihane over a 30-day period of exposure to hydro-retainers.

Treatment	Day 7	Day 14	Day 21	Day 28
Positive Control	83.3 ± 16.7	83.3 ± 16.7	83.3 ± 16.7	83.3 ± 16.7
Bio 0.2%	83.3 ± 16.7	83.3 ± 16.7	83.3 ± 16.7	83.3 ± 16.7
Bio 0.5%	50.0 ± 16.7	50.0 ± 16.7	50.0 ± 16.7	50.0 ± 16.7
Bio 1%	33.3 ± 0.0	33.3 ± 0.0	33.3 ± 0.0	33.3 ± 0.0
PAP 0.2%	83.3 ± 16.7	83.3 ± 16.7	83.3 ± 16.7	83.3 ± 16.7
PAP 0.5%	33.3 ± 0.0	33.3 ± 0.0	33.3 ± 0.0	33.3 ± 0.0
PAP 1%	50.0 ± 16.7	50.0 ± 16.7	50.0 ± 16.7	50.0 ± 16.7
Mix 0.2%	66.7 ± 0.0	83.3 ± 16.7	83.3 ± 16.7	83.3 ± 16.7
Mix 0.5%	33.3 ± 0.0	33.3 ± 0.0	33.3 ± 0.0	33.3 ± 0.0
Mix 1%	0.0* ± 0.0	0.0* ± 0.0	0.0* ± 0.0	0.0* ± 0.0

* indicates a significant mean difference between treatments positive control and each treatment (Bio 0.2%, Bio 0.5%, Bio 1%, PAP 0.2%, PAP 0.5%, PAP 1%, Mix 0.2%, Mix 0.5% and Mix 1%) at p<0.05.

3.10.2. Effect of Bio and PAP hydrogels on the chlorophyll content

Analysis of chlorophyll and carotenoid levels in barley varieties Kounouz and Artorio under different hydrogel treatments reveals an interaction between hydrogel composition, concentration and physiological responses of plants. For the Kounouz variety (Figure 5a), Bio treatments showed moderate increase, especially at the 0.5% concentration. Responses to PAP treatments were varying, showing both increases and decreases in carotenoid and chlorophyll levels. Mixed treatments (Mix 0.2% and 0.5) increased chlorophyll levels.

This suggests that potassium polyacrylate combined with cladode powder could work together to improve water retention, nutrient availability and photosynthetic efficiency. A better photoprotection mechanism, which is essential to reduce oxidative stress in a variety of environmental contexts, is suggested by the increase in carotenoids that has been found in most treatments, especially in those with a higher chlorophyll content. Biological treatments have shown mixed results for Artorio barley (Figure 5b) but at 0.5 % have significantly enhanced chlorophyll content at 0.5%. PAP treatments in Artorio had mixed results as well as Kounouz, affecting chlorophyll and carotenoid levels depending on concentration. Mixed treatments generally had a positive effect, particularly at concentrations of 0.5% and 1%, significantly increasing chlorophyll and carotenoid content. This suggests that Bio 0.5% and Mix treatments could improve photosynthesis and plant growth. However, Bio 1% treatment decreased these parameters, probably due to toxicity at higher concentrations (Parida et al., 2002), confirming the leaf discoloration observed in Figure 2a. These findings highlight that Kounouz and Artorio genotypes react differently to hydrogel treatments; but both types generally show benefits. Artorio responded favorably to various hydrogel kinds and concentrations.

3.10.3. Superoxide dismutase (SOD) activity

An essential component of plants' antioxidant defense mechanism, superoxide dismutase scavenges superoxide radicals and reduces oxidative stress. SOD is the first line of defense against reactive oxygen species (ROS), converting the superoxide radical into hydrogen peroxide. The figure 6 presents the activity of Superoxide Dismutase (SOD) in the stems of two barley varieties, Kounouz and Artorio, under different hydrogel treatments. Compared to Kounouz, Artorio showed a greater baseline SOD activity in the positive control group, suggesting possible varietal variations in the antioxidant defense systems. This could indicate that Artorio has a stronger starting ability to scavenge superoxide radicals, indicating a greater antioxidant activity (Figure 6). Both barley varieties demonstrated steady SOD (superoxide dismutase) activities after Bio treatments at all evaluated doses. Bio had a minor impact on SOD activity. SOD activity was affected differently by PAP hydrogel treatments. All PAP concentrations led to a decrease in Kounouz SOD activity, while Artorio SOD was not significantly affected. Using mixed hydrogel treatments, SOD activity was slightly altered. Kounouz showed a decline, while in Artorio, an increase of SOD was observed in mixing hydrogel concentrations.

3.10.4. Dosage H₂O₂ free radical

The control treatment of Kounouz showed a higher concentration of H₂O₂ (0.57 mM) compared to Artorio (0.41 mM), indicating varietal differences in oxidative stress responses (Figure 7). In Bio treatments, the concentration of H₂O₂ decreased from 0.2% to 1% in Kounouz and Artorio. PAP treatments showed variable responses. Kounouz and Artorio showed a significant decrease in their H₂O₂ levels for 0.5% and 1% respectively.

3.10.5. Catalase (CAT) activity

Catalase, an essential antioxidant enzyme, plays a central role in mitigating oxidative stress by breaking down hydrogen peroxide into water and oxygen, thus protecting cells from oxidative damage. The core activities for both varieties under control conditions establish a comparative standard for assessing the impact of hydrogel treatments. The application of Bio treatment increased the catalase activity with higher concentrations, suggesting that Bio treatment positively affected antioxidant defense mechanisms in barley. This may be due to natural antioxidants present in the natural powder including betaine, carotenoids, riboflavin and folic acid (Silva et al. 2023). The application of PAP hydrogel with a concentration of 0.5% and 1% increased catalase activity in both varieties. Bio and PAP treatments have increased the catalase content of Artorio, thus improving plant resistance to oxidative stress. In addition, hydrogel Mix treatments improved catalase activity for Kounouz and had a reverse effect on Artorio (Figure 8). The main function of SOD is to convert the superoxide radical into H₂O₂. Unless, an increase or stability in SOD activity would normally correspond to an increase in H₂O₂ levels. The continued increased level of SOD activity of both varieties during Bio hydrogel treatments results in a decrease in H₂O₂ concentration rather than an increase, indicating active treatment by other mechanisms, probably catalase. By breaking down H₂O₂ into oxygen and water, catalase directly reduces the potentially harmful effects of H₂O₂ that SOD produces, and the increased activity of CAT is correlated with the observed decrease in H₂O₂ levels throughout treatments, especially in situations where hydrogel treatments are used to stimulate CAT activity.

This demonstrates that high levels of CAT activate an effective detoxification process.

The data show that a compensatory regulation occurs when SOD activity decreases, resulting in a decrease in H₂O₂ production. This could decrease oxidative stress and, therefore, reduce CAT demand, unless H₂O₂ accumulates from other sources (elsayed et al., 2023). PAP and Mix treatments are particularly characterized by a decrease in SOD activity and an increase in CAT activity.

The differential responses observed, suggest that the effectiveness of hydrogel treatments to improve tolerance to oxidative stress is influenced by the specific composition of the hydrogel, the applied dose and barley variety. This complexity highlights the need for tailor-made approaches in the application of hydrogels, aiming to optimize the defense mechanisms against oxidative stress in crops.

4. CONCLUSION

This study highlights the significant impact of the hydro-retentive agent: potassium polyacrylate applied individually or mixed with cladode powder *Opuntia ficus-indica*, on the germination, growth and stress tolerance of two barley varieties. Potassium polyacrylate showed higher water retention than cladode powder *Opuntia ficus-indica*, approving its potential to improve agricultural efficiency under appropriate environmental conditions. To achieve optimal results, the need to optimize the choice and concentration of hydro-retainers is highlighted in the various responses of the two varieties of barley Kounouz, Artorio and Rihane. Minimizing concentrations is essential for potassium polyacrylate, since higher concentrations led to inhibit growth, while cladode powder was more effective at lower doses.

The *in vitro* experiment revealed that hydrogel concentrations at the right levels could significantly enhance photosynthetic efficiency and stress resilience, indicating a promising approach to enhance crop productivity. Indeed, combining hydrogel treatments with cladode powder at a concentration of 0.5% resulted in synergistic effects that improved various growth parameters and have great potential to maximize plant growth and tolerance. The *in vivo* experiment revealed that low-to-moderate hydrogel concentrations (0.2–0.5%) improve moisture retention and promote plant growth without causing oxygen deprivation. In contrast, high concentrations (1%) negatively affect growth due to waterlogging, oxygen depletion, or possible toxicity. Since plants were only watered on the first day, hydrogel treatments showed their ability to store and slowly release water. The decline of the control group after day 14 further indicates the importance of hydrogels in improving growth under limited watering. Overall, Bio 0.2% and Mix 0.2% perform best, suggesting that lower hydrogel concentrations effectively maintain soil moisture without harmful effects and that Bio treatment seems to have fertilizing properties.

In general, the study identifies the importance of using hydro-retentive agents as a solution to sustainable agricultural practices, urging more research to refine their use to maximize agricultural productivity.

Declarations

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AUTHORS CONTRIBUTION

MR and ZC: Conceptualization, Methodology, Formal analysis, Data curation

MR: Writing – original draft

AA: Writing – review and editing

AE: Supervision

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies with human participants performed by any of the authors.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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Figures

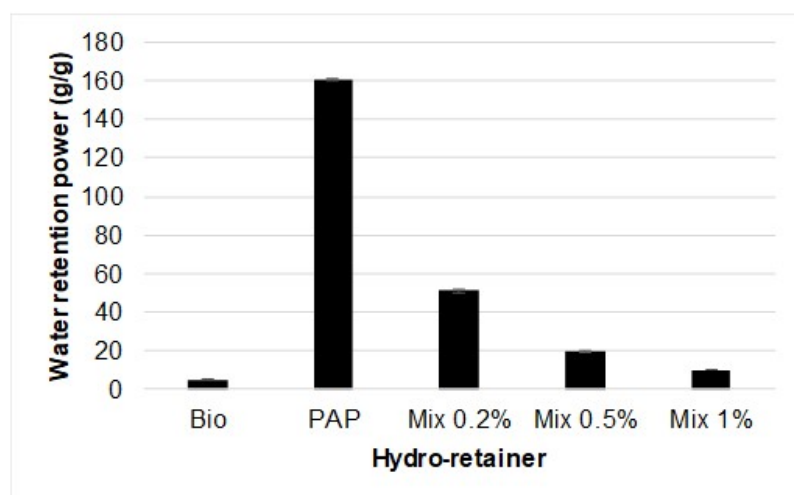


Fig. 1.

Figure 1

Water absorption capacity of tested hydro-retainers (Bio: Cactus powder, PAP: Potassium polyacrylate and Mix: (Bio+PAP))

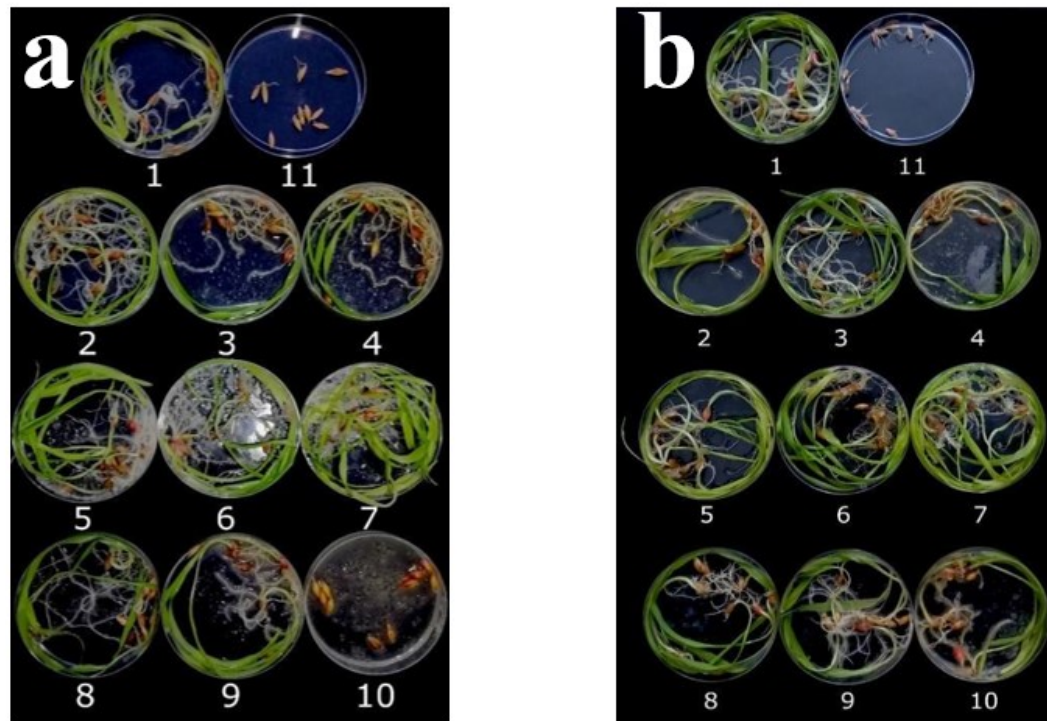


Fig. 2.

Figure 2

Morphological aspect of the seedlings of the two varieties of barley, (a): Kounouz;(b): Artorio after 15 days of treatment, 1: positive control, 2: Bio 0.2%, 3: Bio 0.5%,4: Bio 1%, 5: PAP 0.2%, 6: PAP 0.5%, 7: PAP 1%, 8: Mix 0.2%, 9: Mix 0.5%, 10: Mix 1%, 11: negative control

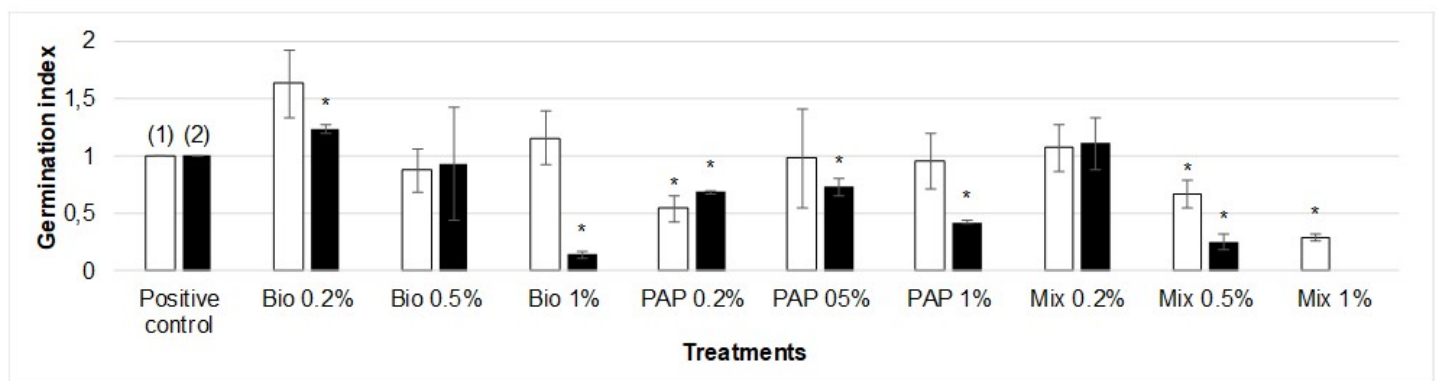


Fig. 3.

Figure 3

Germination index (GI) of the two varieties of barley (1) Kounouz and (2) Artorio amended with hydro-retainers: Bio 0.2%, Bio 0.5%, Bio 1%, PAP 0.2%, PAP 0.5%, PAP 1%, Mix 0.2%, Mix 0.5%, Mix 1%. * indicates a significant mean difference between treatments positive control and each treatment (Bio 0.2%, Bio 0.5%, Bio 1%, PAP 0.2%, PAP 0.5%, PAP 1%, Mix 0.2%, Mix 0.5% and Mix 1%) at $p < 0.05$.

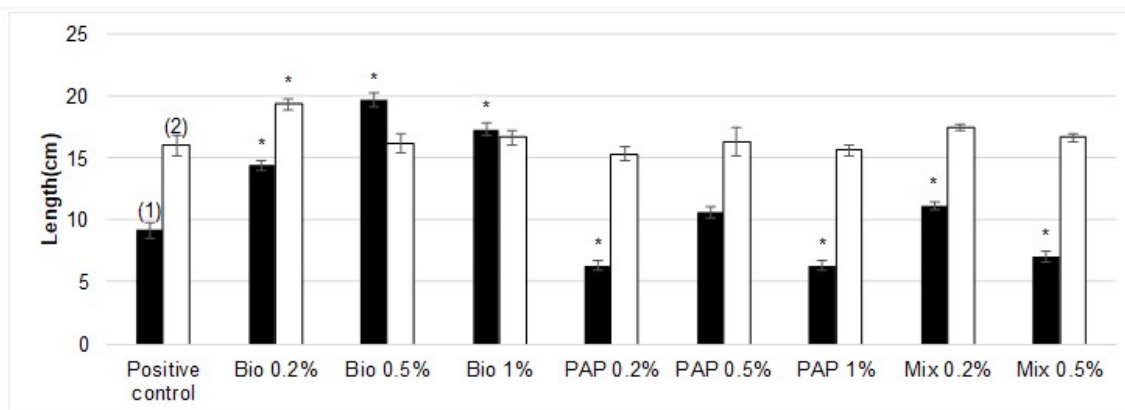


Fig. 4.a

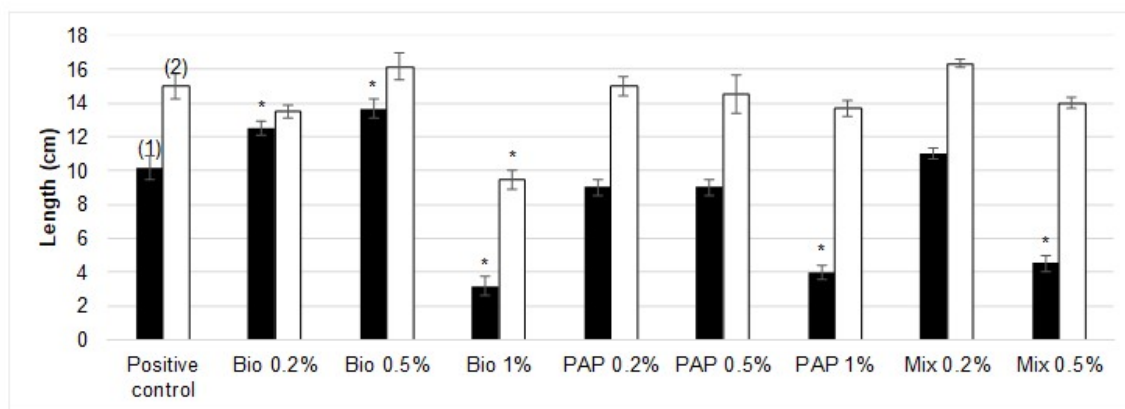


Fig. 4.b

Figure 4

Measurement of the root and leaf length of the seedlings of the barley kounouz after 15 days of germination *in vitro* under the effect of different concentrations of hydro-retainers, (a): Kounouz ;(b): Artorio; (1): root and (2): steam. * indicates a significant mean difference between treatments positive control and each treatment (Bio 0.2%, Bio 0.5%, Bio 1%, PAP 0.2%, PAP 0.5%, PAP 1%, Mix 0.2% and Mix 0.5%) at $p < 0.05$.

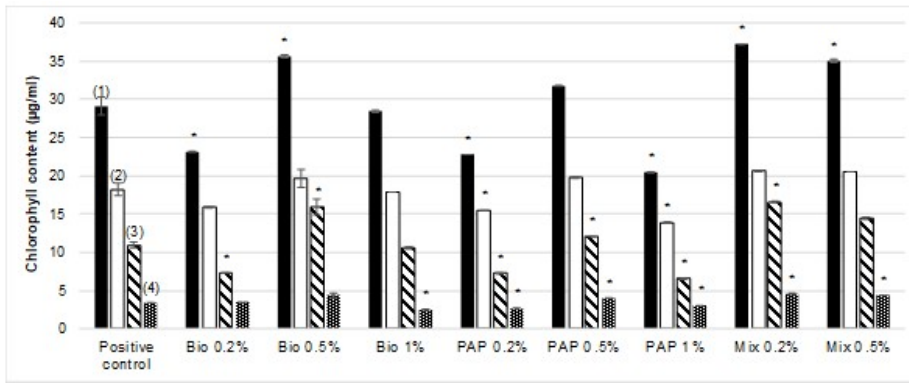


Fig. 5.a

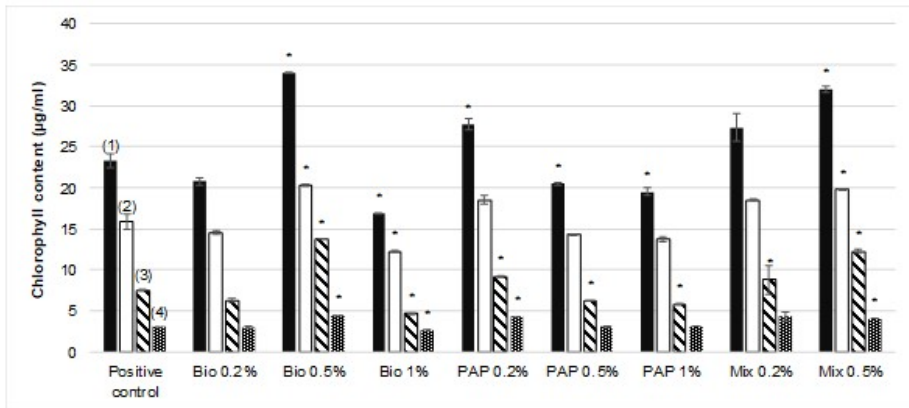


Fig. 5.b

Figure 5

Effect of the treatments on the chlorophyll content of the leaves of the barley seedlings of the two varieties treated for 15 days, (a): Kounouz ;(b): Artorio; (1): chlorophyll a +chlorophyll b; (2): chlorophyll a; (3): chlorophyll b and (3): carotenoids. * indicates a significant mean difference between treatments positive control and each treatment (Bio 0.2%, Bio 0.5%, Bio 1%, PAP 0.2%, PAP 0.5%, PAP 1%, Mix 0.2% and Mix 0.5%) at $p < 0.05$.

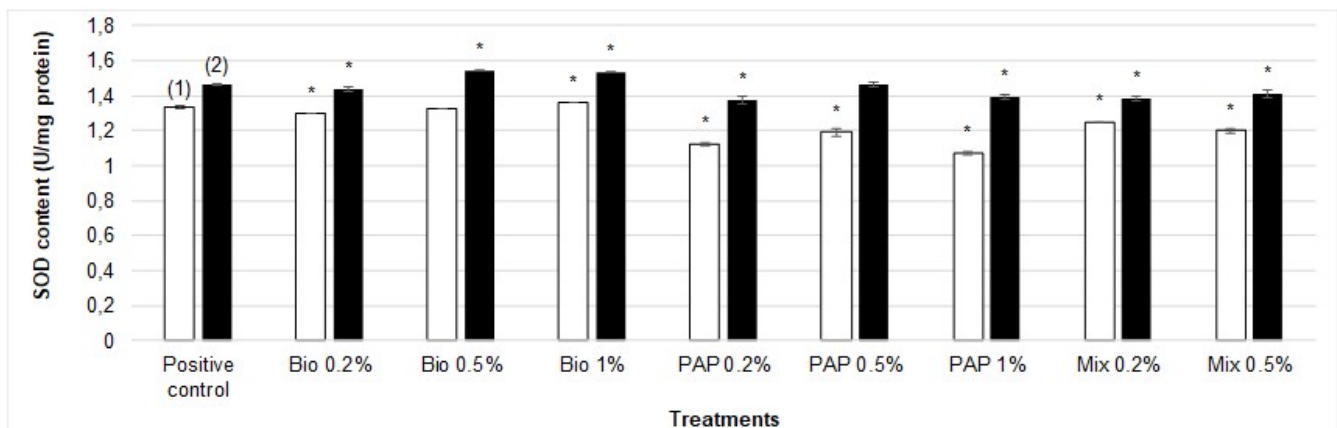


Fig. 6.

Figure 6

Effects of treatments on superoxide dismutase activity in barley seedlings of the two varieties (1) Kounouz and (2) Artorio. * indicates a significant mean difference between treatments positive control and each treatment (Bio 0.2%, Bio 0.5%, Bio 1%, PAP 0.2%, PAP

0.5%, PAP 1%, Mix 0.2% and Mix 0.5%) at $p<0.05$.

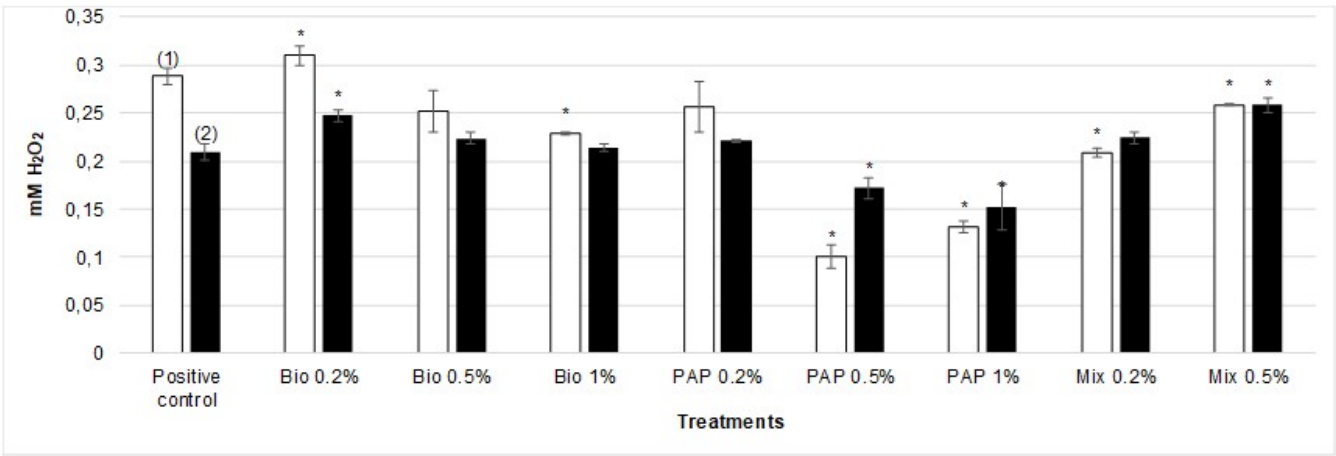


Fig. 7.

Figure 7

Effects of treatments on the H₂O₂ content in barley seedlings of the two varieties (1) Kounouz and (2) Artorio after 15 days of exposure. * indicates a significant mean difference between treatments positive control and each treatment (Bio 0.2%, Bio 0.5%, Bio 1%, PAP 0.2%, PAP 0.5%,

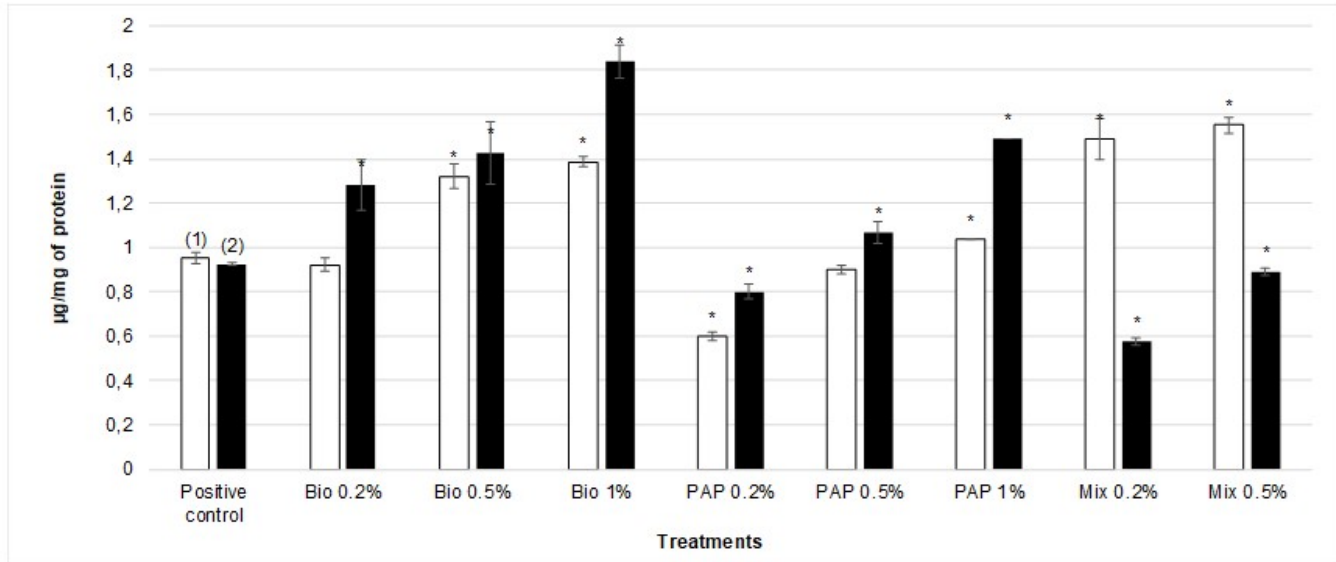


Fig. 8.

Figure 8

Effects of treatments on Catalase content in barley seedlings of the two varieties (1) Kounouz and (2) Artorio. * indicates a significant mean difference between treatments positive control and each treatment (Bio 0.2%, Bio 0.5%, Bio 1%, PAP 0.2%, PAP 0.5%, PAP 1%, Mix 0.2% and Mix 0.5%) at $p<0.05$.

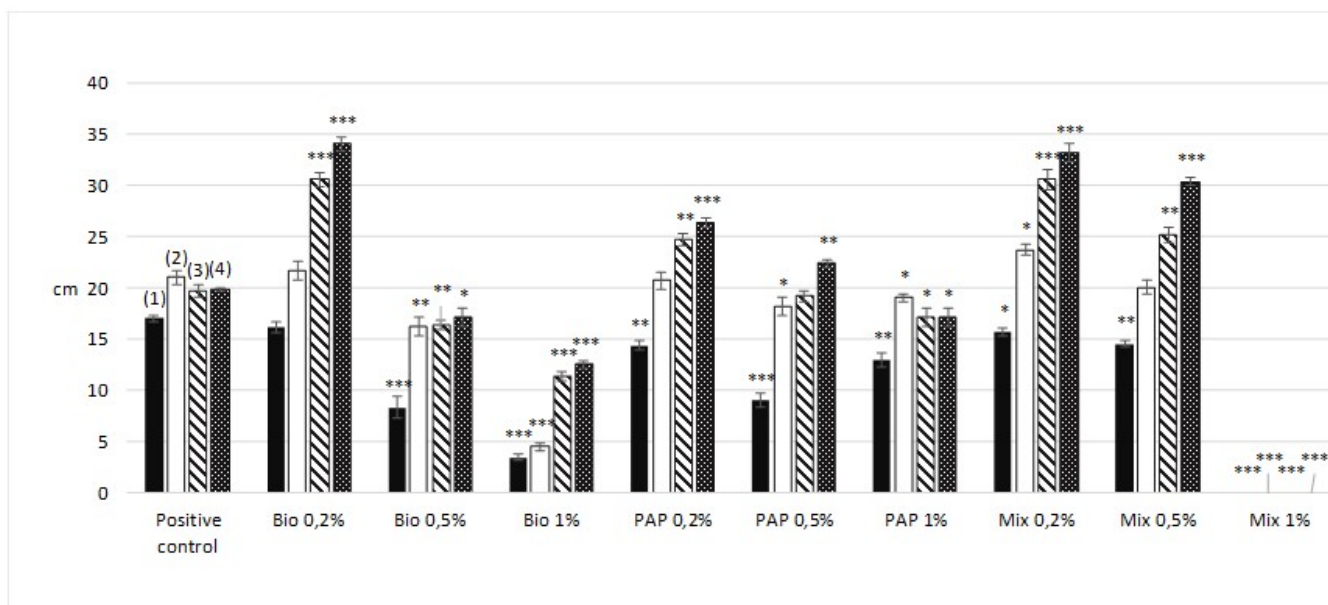


Fig. 9.

Figure 9

Monitoring the effects of treatments on seedling growth after one month. (1) 7 days, (2) 14 days, (3) 21 days and (28) days. * indicates a significant mean difference between treatments positive control and each treatment (Bio 0.2%, Bio 0.5%, Bio 1%, PAP 0.2%, PAP 0.5%, PAP 1%, Mix 0.2% and Mix 0.5%) at $p < 0.05$, ** at $p < 0.01$ and *** at $p < 0.001$.



Fig. 10.

Figure 10

Morphological aspect of the seedlings of the barley variety Rihane 28 days of treatment, 1: positive control, 2: Bio 0.2%, 3: Bio 0.5%,4: Bio 1%, 5: PAP 0.2%, 6: PAP 0.5%, 7: PAP 1%, 8: Mix 0.2%, 9: Mix 0.5%, 10: Mix 1%.