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Effect of *Atriplex halimus* extract as a biostimulant on physiological and biochemical regulation in faba bean plants.

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Abstract

This study investigated the effect of *Atriplex halimus* extract on faba bean plants (*Vicia faba*). Three different concentrations (0.1% 0.25%, and 0.5%) of *Atriplex halimus* extract were applied. The obtained results indicated that the tested extract had a positive effect on several physiological and biochemical parameters of the tested plants, which led to enhancing their growth. A significant ($p < 0.05$) increase in plant metabolites and photosynthetic pigments content was observed in the treated plants. Furthermore, *Atriplex halimus* extract significantly improved the activities of enzymes involved in the activation of the carbon-nitrogen enzymatic system; including, phosphoenolpyruvate carboxylase (EC 4.1.1.31), isocitrate dehydrogenase (EC 1.1.1.42), Glutamine Synthase (EC 6.3.1.2) glutathione-S-transferase (EC 2.5.1.18), glutathione reductase (EC 1.8.1.7). This effect was more pronounced in plants treated with 0.25% of *Atriplex halimus* extract. Altogether, our results suggest that the application of the tested extract could be used as a promising plant growth biostimulant to improve faba bean plants' growth and yield.

1. Introduction

The development of sustainable and environmentally friendly agricultural systems in order to fulfill the needs of the world's growing population is, indeed, the major challenge faced by global agriculture. The pressure is increasing on different levels such as climate change, soil erosion, biodiversity loss, and also from consumers' changing tastes in food and concerns about how it is produced. Moreover, many natural obstacles generated from plants themselves and pests (insects, phytopathogens, and weeds) continually pose their own challenges as well to farming systems (Naboulsi et al., 2022; Procházka et al., 2022).

Therefore, modern agriculture provides a large number of solutions that could potentially enhance the quality and quantity of crops, which means higher incomes for farmers, and more nutritious food for consumers (Eckardt et al., 2009; Mahari et al., 2021). With that being said, one of the most promising solutions found to solve and meet the faced challenges is the use of bio-stimulants that are now available in the market, making agriculture more sustainable. (Naboulsi et al., 2022; Latique et al., 2021)

The concept and definition of plant biostimulants are still under evolution, which is partly a reflection of the diversity of inputs that can be considered to be bio-stimulants. As defined by European Biostimulant Industry Council (www.biostimulants.eu), the content of certain substance(s) and/or micro-organisms in plant bio-stimulants results in the stimulation of the natural processes destined to enhance/benefit crop quality, nutrients uptake, and use efficiency, as well as tolerance to biotic and abiotic stresses when they are applied to plants or the rhizosphere. However, the mechanisms by which plants' bio-stimulants work is still not clear, and this is mainly due to the complex set of bioactive molecules and their proportions present in these plants (Du Jardin, 2015).

Plant bio-stimulants based on natural materials have received considerable attention from both the scientific community and commercial enterprises, especially in the last two and a half decades (Yakhin et al., 2016).

There is a potentially novel approach to the regulation/modification of physiological processes in plants to stimulate their growth. This approach is offered by bio-stimulants in order to mitigate stress-induced limitations, to increase plant yield through their rich content of active compounds (amino acids, polyphenols, flavonoids, etc.), and through their ability to stimulate different enzymatic activities that enhance plant growth and help overcome stressful conditions. In addition, plants are known to cope with different stresses through the stimulation of the secondary metabolism that leads to the synthesis of phenolic compounds (Ouyang et al. 2007).

In the present study, *Atriplex halimus* plants have been chosen as a source of bio-stimulant for the treatment of faba bean plants *(Vicia faba)*, the beneficial effect of this extract application is a result of many components that work synergistically at different concentrations.

The bio-stimulant was used in different concentrations (0.1%, 0.25%, and 0.5%). These plants were collected from the south area of Morocco called Akka near the city of TATA. It is noteworthy that, although they grow wildly in this southern area of Morocco, they have never been valorized in the country.

The main objective of this investigation is the obtainment of insight into the amounts of valuable components such as bioactive compounds present in *Atriplex halimus* plants by carrying out different dosages, mainly; soluble sugar, amino acid, indole acetic acid, polyphenol, flavonoid, and chlorophyll content. The same approach was made for *Vicia faba* plants treated with this bio-stimulant.

We also aimed in this study to evaluate the impact of soil irrigation by the different concentrations of *Atriplex halimus* plant extract on chlorophyll content and on the activation of the carbon-nitrogen enzymatic system (PEPc, GS, GDH, MDH GST, and GR) to understand and test if this plant extract could give promising and positive plant growth results on faba bean plants by improving their yield and quality.

2. Materials And Methods

2.1. *Atriplex halimus* material and extract preparation

In this study, *Atriplex halimus* plants were collected from the south area of Morocco, called Akka, near the city of TATA. *Atriplex halimus* plants were hand-picked and thoroughly transported to the laboratory. Thereafter, samples were placed at ambient temperature until the plant was fully dried.

For the extract preparation, 100g of dry crushed plant material was mixed with 1L of distilled water and boiled for one hour. During that time, 1M of NaOH powder was added, then the mixture was filtered to remove debris (Sivasankari et al., 2006). The obtained filtrate represented the 100% crude extract. The treatment concentrations; 0.5% 0.25% and 0.1%, were subsequently prepared by adding distilled water.

2.2. Estimating the concentration of soluble sugar

The determination of soluble sugars was carried out according to the method of Yemm and Willis (1954). The plant leaves were placed in an oven at 80°C for 48 hours. Thereafter, they were ground in a mortar at

4°C in the presence of 1.3ml of 80% ethanol. The ground materials were transferred into Eppendorf tubes to be vortexed and centrifuged at 4°C for 15 minutes at 5000g. Finally, the obtained supernatants were used to determine the amount of soluble sugars. To do so, 1ml of the supernatant was mixed with 2ml of a reagent consisting of a 1g/l anthrone solution (dissolved in 95% sulfuric acid). After vortexing, the mixture was placed in a water bath at 100°C for 10 minutes.

After cooling on ice, the optical density was measured at 630 nm, and the amount of sugar was determined using a calibration curve produced by different concentrations of anhydrous glucose.

2.3. Estimation of amino acids concentration

To determine the amino acid content, 200µl of the supernatant obtained from extraction used for estimation of soluble sugars was added to 1.8ml of ninhydrin reactive (63% glycerol(v/v), 37% 0.5M citrate buffer pH 6.5 content 7.1 g/l of ninhydrin). The mix was boiled in water for 15 minutes. After cooling at room temperature, the OD was measured at 570 nm, and the amount of amino acid was determined using a calibration curve produced by different concentrations of glycine.

2.4. Estimation of flavonoid and phenolic compounds concentration

The flavonoid content was assessed following the method of Ben Mrid et al, (2019). Briefly, 40 µL of each sample was mixed with 10 µL of acetate potassium (1M) and 10 µL of aluminum chloride (10%). Thereafter, 100 µL of 50% methanol was added and the total volume was made up to 400 µL with distilled water. The absorbance of the mixture was taken at 415 nm. Quercetin was used as standard. The flavonoid content was expressed as a milligram of Quercetin equivalence (QE) per gram of extract.

The total phenolic content was determined by the method of the Folin–Ciocalteu, following the Ben Mrid et al, (2019) To 100 µL of the sample, 400 µL of Folin Ciocalteau's reagent and 1 mL of saturated Na2CO3 (7%) were added and the final volume was made up to 1.6 mL with distilled water. The tubes were left to stand in the dark for 30 min, after which its absorbance was read at 725 nm against a blank. The total phenolic content of plant extracts was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid.

2.5. Plant Material and Growth Conditions

Faba bean plants (*Vicia faba*) were grown under controlled conditions (16 h light regimes and 8 h dark regimes at 18°C). After disinfection with sodium hypochlorite (5%) for 3 min and rinsing with distilled water, the seeds were planted in plastic pots filled with soil. The different pots were watered every three days. A week after, plants under study were treated with *Atriplex halimus* extract at various concentrations (0, 5%, 0.25%, and 0.1%).

All the tested plants were harvested after 8 weeks, and morphological characteristics such as shoot length and fresh weight were measured. All experiments and enzyme preparations were performed with freshly harvested plants.

Parts of these plants were incubated at 80°C for 48 hours in order to be used for soluble sugar, amino acid, and polyphenols dosages.

2.6. Determination of Chlorophyll Content

To determine the chlorophyll content, 200 µl of soluble pellets of plant homogenate in water was added to 800 µL of 80% acetone. After 72 hours of incubation at 4°C, the chlorophyll content was determined in three independent replicates. The method of Arnon (1949) was used to estimate chlorophyll a and chlorophyll b contents is as follows:

Chlorophyll a $mg.ml^{-1}$ = (12.7*OD663nm) – (2.69*OD645nm)

Chlorophyll b $mg.ml^{-1}$= (22.9*OD645nm) – (4.86*OD663nm)

2.7. Preparation of plant extracts for enzyme activity assays

Using a pre-coled mortar and pestle, leaves were homogenized in ice cold 50 mM phosphate buffer (PH 7.6) containing 14mM β-mercaptoethanol 1mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), 9.4 µM leupeptin, and 10% (w/v) glycerol. The homogenates were centrifuged at 12,000 g for 20 min, and the supernatants were used for the determination of enzyme activities. All procedures were performed at 0 – 4°C.

For PEPC and NADP-ICDH, the supernatant was saturated (60%) with solid ammonium sulfate for 30 min. The saturated supernatant was centrifuged again in the same conditions, and the resulting pellet was resuspended in the extraction buffer and used for enzyme assays.

2.8. Enzyme activity assays

The GR activity was determinated by the oxidation of NADPH at 340nm as described by Bouchmaa et al, (2019).

The activity of GST was estimated according to the method of Ben Mrid et al, (2018).

The NADP-ICDH activity was measured according to Bouchmaa et al, (2018). The ICDH activity was measured spectrophotometrically by monitoring the oxidation of NADH at 340 nm for 5 min. The assay mixture contained 50 mM potassium phosphate buffer (pH 7.5), 1 mM MnCl2, 1 mM NADP+, and 4 mM isocitrate.

The PEPC activity was determined according to the method described by Ben Mrid et al, (2017). The activity was assayed by coupling to NAD-malic dehydrogenase (MDH), and monitoring NADH oxidation at 340 nm spectrophotometrically in a 1 mL assay mixture containing 100 mM Hepes—KOH (pH 7.3), 5 mM MgCl2, 0.2 mM NADH, 3 U of MDH, 2.5 mM PEP, 5 mM NaHCO3 and leaves extract. One unit of PEPC is the amount of enzyme extract, which catalyzes the transformation of 1 µmol substrate per minute at 30 ◦C.

The GDH activity was measured in the aminating direction, as described by Ben Mrid et al, (2018). The activity was performed in the amination direction at 30°C in a reaction buffer containing 100 mM Tris-HCl (pH 8), 1 mM CaCl2, 13 mM α-ketoglutarate, 50 mM (NH4)2SO4 and 0.25 mM NADH. Kinetic activity was determined spectrophotometrically by monitoring NADH at 340 nm. The activity of NAD + malate dehydrogenase was assayed by monitoring NADH at 340 nm. The reaction buffer contained 50 mM potassium phosphate buffer (pH 7.5), 1 mM oxaloacetic acid, 0.25 mM NADH, and the enzyme solution.

The GS activity was measured based on the protocol used by Ben Mrid et al, (2016)

2.9. Statistical analysis

SPSS 25 package for Windows, version 10.0.1 was used for all statistical analyses. one-way ANOVA, followed by the Student Newman—Keuls post hoc test, were used to compare differences in the means ($p < 0.05$). Different letters indicate significant differences.

3. Results And Discussion

3.1. Composition of *Atriplex halimus* US extract and its effect on shoot length and fresh weight of cultivated faba bean plants

To determine the richness of bioactive compounds present in *Atriplex halimus* extract, the content of several molecules such as soluble sugars, amino acids, indole acetic acid, polyphenol, and flavonoid was dosed. Data in Table 1 represents the concentration of each of these molecules in 100% *Atriplex halimus* extract. For instance, flavonoids represent 27.65 mg. g-1 DW, while polyphenol content was 5.72 mg. g-1. In addition, soluble sugars, amino acids, and IAA contents were 3.104, 0.303, and 0.224 mg. g^{-1}DW, respectively (table 1).

Furthermore, the importance of different concentrations of *Atriplex halimus* extract on the growth of faba bean plants was investigated. Data in Table 2 revealed that shoot length increased by 8.48%, 10.62%, and 17.75% with 0.1%, 0.25%, and 0.5% of *Atriplex halimus* extract, respectively, compared to control plants. The plants' fresh weight also slightly increased with the *Atriplex halimus* extract application compared to the control plants.

From the present results, it can be seen that the irrigation with different concentrations of *Atriplex halimus* extract improved the shoot's length in all treatment conditions (Table 2). Our findings are in accordance with Rathinapriya et al. (2020) who showed the capacity of a liquid algal biostimulant in improving vegetative growth parameters of foxtail millet plants, such as, shoot length and fresh weight. Along the same lines, Kunicki et al. (2010) proved that biostimulants have been gaining interest in sustainable agriculture thanks to their ability to activate several physiological processes that enhance nutrient use efficiency, to stimulate plant development and their ability to rationalize fertilizers consumption, which eventually leads to the increase in the overall plant growth (Kunicki et al., 2010).

These beneficial effects on the plants' vegetative growth parameters and physiological processes could also help counteract the effect of biotic and abiotic stresses and enhance the quality and yield of the crops as well (Ziosi et al., 2012).

These results could also be explained by the richness of *Atriplex halimus* plants with compounds that have stimulatory effects such as sugars, and which represent 3.104 mg. g-1 DW in the tested extract (table 1). Indeed, sugars have an impact on cell division; this was reflected upon the increase of the treated plants' biomass compared to the control. Additionally, many researches have shown that sugars affect plant growth and development by activating different pathways. Sugar has participated in plant growth by increasing cell volume, cell division, and the development of programs that specify the identity of tissues and organs. In addition, sugar can improve biomass and provide energy in the form of carbohydrate (Bläsing et al., 2005; Osuna et al., 2007; Price et al., 2004; Usadel et al., 2008).

The stimulatory effect may also be related to the presence of the IAA in our extract, which represents 0.224 mg. g-1DW (table 1). This phytohormone was reported to be likely responsible for the increase in roots and shoots length of wheat seedlings (Egamberdieva, 2009).

3.2. Effect of *Atriplex halimus* extract on the contents of photosynthetic pigments, soluble sugars, amino acids, flavonoids, and polyphenols of faba bean plants

A high increase in soluble sugars, total chlorophyll, chlorophyll a, and chlorophyll b contents were observed in faba bean plants, regardless of the concentration of *Atriplex halimus* extract used.

Application of 0.5%, 0.25%, and 0.1% of *Atriplex halimus* extract stimulated sugar's biosynthesis in faba bean leaves. These three concentrations increased the sugar content by 35.07%, 26.46%, and 9.23% compared to the control, respectively (Figure 1A). The result from our experiment has shown an important effect of *Atriplex halimus* extract on sugar biosynthesis in faba bean leaves. This is in accordance with the results of Ferrini and Nicese (2002); Amanda et al. (2009); Ertani and Nardi (2013), who reported an increase in sugars biosynthesis in several plant species treated with biostimulants. These authors proved that higher sugar content in leaves usually speeds up nitrogen incorporation through the nitrate assimilation pathway. The carbohydrates represent the carbon skeletons for incorporating reduced nitrate (ammonia) in amino acids and increases the protein biosynthesis (Schiavon et al., 2008).

The tested *Atriplex halimus* extract at various concentrations increased chlorophyll a and b contents in faba bean leaves. Chlorophyll content increased significantly following *Atriplex halimus* extract application. The concentration ranged from 8.59 µg g−1 FW (untreated plant) to 16.97 µg g−1 FW and 19.80 µg g−1 FW in plants treated with 0.1% and 0.25% of *Atriplex halimus* extract, respectively. However, the maximum values of chlorophyll a were found in plants treated with 0.5% of *Atriplex halimus* extract (24.71 µg g−1 FW) (Figure 1D). Likewise, chlorophyll b content ranged from 6.02 µg g−1 FW to11.69 µg g−1 FW and 10.72 µg g−1 FW at 0.1%, 0.25% of *Atriplex halimus* extract, respectively. Additionally, the

highest chlorophyll b value was recorded with 0.5% of *Atriplex halimus* extract (21.04 µg g–1 FW) (Figure 1E).

Therefore, our results demonstrated a positive effect of this biostimulant on chlorophyll contents. This was evidenced by an intensification of the leaves' color, which indicates an increased chlorophyll content. This effect was observed in cowpea seeds pre-soaked in carrot extract (Abbas and Akladious, 2013). In the same context, Abdalla (2013) reported a significant increase in chlorophyll levels in rocket *(Eruca sativa*) plants treated with *Moringa oleifera* extract. This is in accordance with results from our experiments in which the chlorophyll a and b content increased significantly after *Atriplex halimus* extract application, especially at 0.5%. The same stimulatory effects resulting from biostimulant application on rocket plants' chlorophyll content were also reported by Vernieri et al. (2006, 2005). It is of note that chlorophyll content reflects the photosynthetic activity in the plants' leaves, which in turn report the health of the treated plants (Zhang et al., 2017). Thus, we could suggest that our plant extract could be used to ensure healthy and normal plant growth.

More interestingly, amino acids content decreased by 0.07 mg. g-1 DW and 0.11 mg. g-1 DW in faba bean plants treated with 0.25% and 0.5% of *Atriplex halimus* extract, respectively, compared to the control (Figure 1B). In contrast, the concentration of amino acids (0.303 mg. g-1 DW) in *Atriplex halimus* plants was higher than that of the treated faba bean plants. The difference in amino acids concentration between the two plants could be linked to the observed positive effect on the chlorophyll content. Indeed, many studies showed that there is a relation between amino acids and chlorophyll biosynthesis in plants. In line with this, Marschner (2012); Taiz and Zeiger (2013) showed that among amino acids, glutamic acid and glycine are known as precursors of chlorophyll synthesis, which enhance the photosynthetic activity. However, amino acids may also have been previously used to form other photosynthetic structures since they all are considered as parts of proteins (Hildebrandt et al., 2015), which explains the decreased concentration of amino acids, and the increased chlorophyll content in the treated plants.

Biostimulants are known to affect several chemical properties in plants, and many molecules can be dosed to learn more about these physiochemical changes. Results in Figures 1F and 1G represent flavonoids, and polyphenols concentrations in faba bean leaves treated with different concentrations of *Atriplex halimus* biostimulant. Flavonoid and polyphenol contents were both high regardless of the concentration used. At 0.25% and 0.1%, *Atriplex halimus* extract stimulated the expression of flavonoids in plant leaves. In fact, these two concentrations increased the flavonoids content by 58.07% and 17.37%, compared to the control, respectively. (Figure 1F). However, the application of 0.5% of *Atriplex halimus* extract increased the flavonoids content in faba bean leaves only by 11.54% compared to the control.

Likewise, the application of 0.25% and 0.1% of *Atriplex halimus* biostimulant stimulated the expression of polyphenols in faba bean leaves. The two concentrations increased polyphenol content up to 25.6 mg. g-1 DW compared to the control (22.51 mg. g-1DW), while this concentration slightly increased when 0.5% of *Atriplex halimus* extract was applied (25.03 mg. g-1DW) (Figure 1G).

The accumulation of plant secondary metabolites, particularly flavonoid and phenolic compounds, is usually a consistent feature of plants grown under stress, as it represents a defensive mechanism to cope with multiple abiotic stresses (Cheynier et al., 2013). Indeed, plant phenolics and flavonoids play an essential role in several physiological processes to improve plants' tolerance and adaptability under suboptimal conditions (Andersen, 2003; Lattanzio et al., 2009). In particular, many secondary metabolites endowed with antioxidant properties belong to these two groups (Sharma et al., 2019), and they were found able to ameliorate plant performance under stressful growth conditions. For instance, polyphenols are found to be involved in signal transduction from the root to the shoot and help in nutrients mobilization (Halvorson et al., 2009). In fact, these molecules are very important in the case of oxidative stress as they play an important role in the neutralization of ROS generated by abiotic stresses.

The results from our experiment are of great importance for increasing plants' ability to withstand stressful conditions. Particularly for the enhancement of metal chelation, a process that is favored by plant flavonoids. Thus, it helps in reducing the levels of harmful hydroxyl radical in plant cells (Mira et al., 2002; Williams et al., 2004); this fits well with the observed results by Handa et al. (2018) and Kaur et al. (2017) who revealed that the levels of flavonoids in plants were found to be enhanced by metal excess. Indeed, under metal toxicity, the accumulation of specific flavonoids, including anthocyanins and flavanols, which are involved in aiding the plant's defense mechanism, are also enhanced (Handa et al., 2019; Kısa et al., 2016; Trejo-Tapia et al., 2001).

3.4. Effect of *Atriplex halimus* extract on the enzyme activities of cultivated faba bean plants

The phosphoenolpyruvate carboxylase activity (PECP) in faba bean plant leaves' subjected to different *Atriplex halimus* extract concentrations is shown in Figure 2A. The activity of PEPC enzyme in faba bean plants was 10.91% and 11.62% higher than the control when 0.1% and 0.5% of *Atriplex halimus* extract were used, respectively. In addition, the application of 0.25% of *Atriplex halimus* extract gave the highest results, and the PECP activity increased by 24.75% compared to non-treated plants. PEPC is a cytosolic enzyme widely distributed in most plant tissues. Moreover, this enzyme plays a vital role in carbon metabolism. For instance, in C4 species, the first carboxylation reaction of C4 photosynthesis in mesophyll cells is catalyzed by phosphoenolpyruvate carboxylase (PEPC). To catalyze the carboxylation of phosphoenolpyruvate, the enzyme PEPC utilizes bicarbonate ions to yield Pi and 4-C acid oxaloacetate, which is readily converted to malate by NAD(P)-malate dehydrogenase (Chollet et al., 1996). Hence, our result showed that *Atriplex halimus* extract enhanced the PEPC activity in the faba bean plants. These results are in accordance with those of Jaleel et al., 2017 who used chitosan oligosaccharide as a biostimulant, which elevated the PEPC activity in lemongrass by about 53.2% (Jaleel et al., 2017). Along the same lines, (Liu et al, Zhang et al. 2007, 2017) revealed that PEPC activity in wheat was significantly improved when chitosan oligosaccharide was used as a biostimulant; this was evidenced by the increment of oxaloacetate content in the treated plants. Thus, our biostimulant enhanced the synthesis and accretion of organic acids, which provides a skeleton for the production of amino acids during nitrogen assimilation.

The activity of malate dehydrogenase (MDH) in leaf extracts of faba bean plants subjected to different concentrations of *Atriplex halimus* is shown in Figure 2B. The activity of this enzyme in faba beans increased up to 65.68 µmol min-1 mg-1 protein and 66.07 µmol min-1 mg-1 protein when 0.1% and 0.25% of *Atriplex halimus* extract were applied. However, the application of 0.5% of *Atriplex halimus* extract decreased MDH-NAD activity compared to the non-treated plants (38.21 µmol min-1 mg-1 protein) (Figure 2B).

The beneficial effect of *Atriplex halimus* extract treatments varied with the extract's concentrations. The highest GDH activity levels were detected in plants treated with 0.25% of *Atriplex halimus* extract (19.23 µmol. min^{-1} mg^{-1} protein). Whereas the observed increase in GDH activity level was statically insignificant when 0.1% and0.5% of *Atriplex halimus* treatment was used. However, they were slightly higher (17.17 µmol. min^{-1} mg^{-1} protein) compared to the control (14.97 µmol. min^{-1} mg^{-1} protein) (Figure 2C).

Figure 2D represents the glutamine synthetase (GS) activity in the leaves of faba bean plants subjected to different concentrations of *Atriplex halimus* extract. Results from this experiment revealed that the activity of GS enzyme in the treated plants increased and reached 18.01, 30.68, and 18.26 µmol min-1 mg-1 protein when 0.1%, 0.25%, and 0.5% of *Atriplex halimus* extract were applied, respectively, compared to the control (Figure 2B).

It is known that glutamate dehydrogenase (GDH) acts as a nitrogen-fixing enzyme by incorporation of ammonium. which is considered to be the first step in the biosynthesis of chlorophyll and proteins which are responsible for leaf color, crop growth and yield (Fageria, Moreira and Coelho, 2011). Enzymes included in nitrogen fixation system like GDH, GS are used as biochemical indicators of nitrogen content in plants (Ren et al.; Zhang et al., 2017). Our data showed that *Atriplex halimus* extract positively impacted GS and GDH, thus offering N to the plant to synthesize chlorophyll and proteins, giving their importance in improving the crop's biomass.

In the present study, the activity of NADP- ICDH has increased in all of the conditions of plants treatment, and the highest ICDH activity was observed in plants treated with 0.5% of *Atriplex halimus* extract (29,25 µmol min-1 mg-1 protein) compared to the control (12.34 µmol min-1 mg-1 protein).

The nitrogen assimilation pathway is seen as an adjunct to carbon assimilation that provides energy and carbon skeletons. Based on these results, we propose that Atriplex halimus extract may have two major prospects for improving plant growth. The increase in photosynthesis of the treated plants could activate the assimilation of carbon by increasing the activity of the enzymes MDH and GDH. The higher carbon assimilation influences the nitrogen uptake by the GS enzyme.

Carbon and nitrogen metabolisms are responsible for the activation of many pathways, including the assimilation of ammonia to amino acid and the transamination of glyoxylate with serine to produce hydroxyl pyruvate and glycine, in order to facilitate plant growth and development (Kumar et al., 2019).

However, our data indicated a dose-dependent increase in GST activity upon treatment, the highest GST activity was obtained under 0.25% Atriplex halimus extract (87.77 µmol protein min-1 mg-1), compared to all conditions (Figure 2F). the data obtained also showed that treatment with Atriplex halimus extract stimulated GR activity, in particular at 0.25%. This last resulted in the highest GR activity (55.16 µmol min-1 mg-1 protein) compared to the control and other treatments (Figure 2G).

The increase in the activities of both enzymes reflects the beneficial effects of the tested extract in enhancing plants' resistance, as they are involved in plants' defense mechanisms against different stresses. Additionally, they also play a significant role in the regeneration of glutathione (GSH). This molecule plays an important role as a regulator of plant growth. The glutathione-dependent regulation of the plastidic fructose 1,6-bisphosphate aldolase (FBA) is closely linked to glutathione biosynthesis chloroplasts. In chloroplasts, following illumination, the calvin cycle responsible for fixing atmospheric CO2 to sugars is activated (Abbott and Dilley, 1983; Heldt et al., 1973; Werdan et al., 1975). The photosynthetic production of ATP relies on the illumination-dependent formation of a proton gradient across the thylakoid membranes (Makino et al., 2002), restricting glutathione biosynthesis (Ogawa et al., 2004). At the beginning of photosynthesis, the ATP production is accelerated by cyclic electron flows (Makino et al., 2002), which are essential for photosynthesis (Munekage et al., 2004). These facts suggest that, following illumination, the FBA in the Calvin cycle is activated by photosynthesis, dependently synthesized GSH, and stromal alkalization. Under photooxidative conditions, an electron acceptor in photosystem I, NADP+, is apt to run short due to NADPH's restricted consumption in the Calvin cycle (Allen et al., 2000; Asada, 1999; Foyer and Noctor, 2000; Osmond and Grace, 1995). In this respect, the activation of the FBA by GSSG is physiologically important to maintain the Calvin cycle to produce NADP+.

4. Conclusions

In the present study, we observed that 0.1% and 0.25% concentrations of *Atriplex halimus* extracts applied as soil irrigation on faba bean plants showed better vegetative growth compared to the non-treated plants. Indeed, this extract enhanced plant growth and development by modulating the response of enzymes involved in C and N assimilation as well as in the plants' defense mechanisms (PEPc, GS, GDH, MDH GST, and GR). In addition to this, the tested extract also improved the accumulation of plant metabolites and photosynthetic pigments such as soluble sugars, polyphenols, flavonoids, amino acids and chlorophyll a and b, which ultimately led to improving plant growth. *Atriplex halimus* extract was found to be rich in bioactive compounds. Therefore, it represents an important source of plant biostimulants allowing the treated crops to grow fast. In fact, the application of *Atriplex halimus* extract could contribute to protecting plants against peroxidation imposed by abiotic stresses. Particularly through enhancing polyphenols content and activation of antioxidant enzymes such as GST and GR. All things considered, the present findings encourage the application of *Atriplex halimus* plants' extract as a natural fertilizer in the agricultural sector.

Declarations

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions:

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Tables

Table 1: Flavonoids, polyphenols, IAA, soluble sugars concentration in *Atriplex halimus* extract

mpositions	Flavonoid	Polyphenol	IAA	Soluble sugar	Free amino acid
ncentration	**27,655**±5,380	**5,726**±1,369	**0,224**±0,075	3.104±00.036	0.303±0.015
it	**mg. g^{-1} DW**	**mg. g^{-1} DW**	**mg. g^{-1} DW**	**mg. g^{-1} DW**	**mg. g^{-1} DW**

Table 2: Physiological parameters of faba bean *(Vicia faba L.)* plant stimulated by different concentrations of *Atriplex halimus* extract.

	Cont	0.1% F	0.25% F	0.5% F
nt fresh weight (g)	9.124±0.58^{a}	9.413±1.81^{a}	9.411±1.56^{a}	9.978±1.82^{a}
nt height (cm)	59.3 ±6.63^{a}	64.8±2.3^{ab}	66.35±5.20^{bc}	72.1±6.83^{c}

Figures

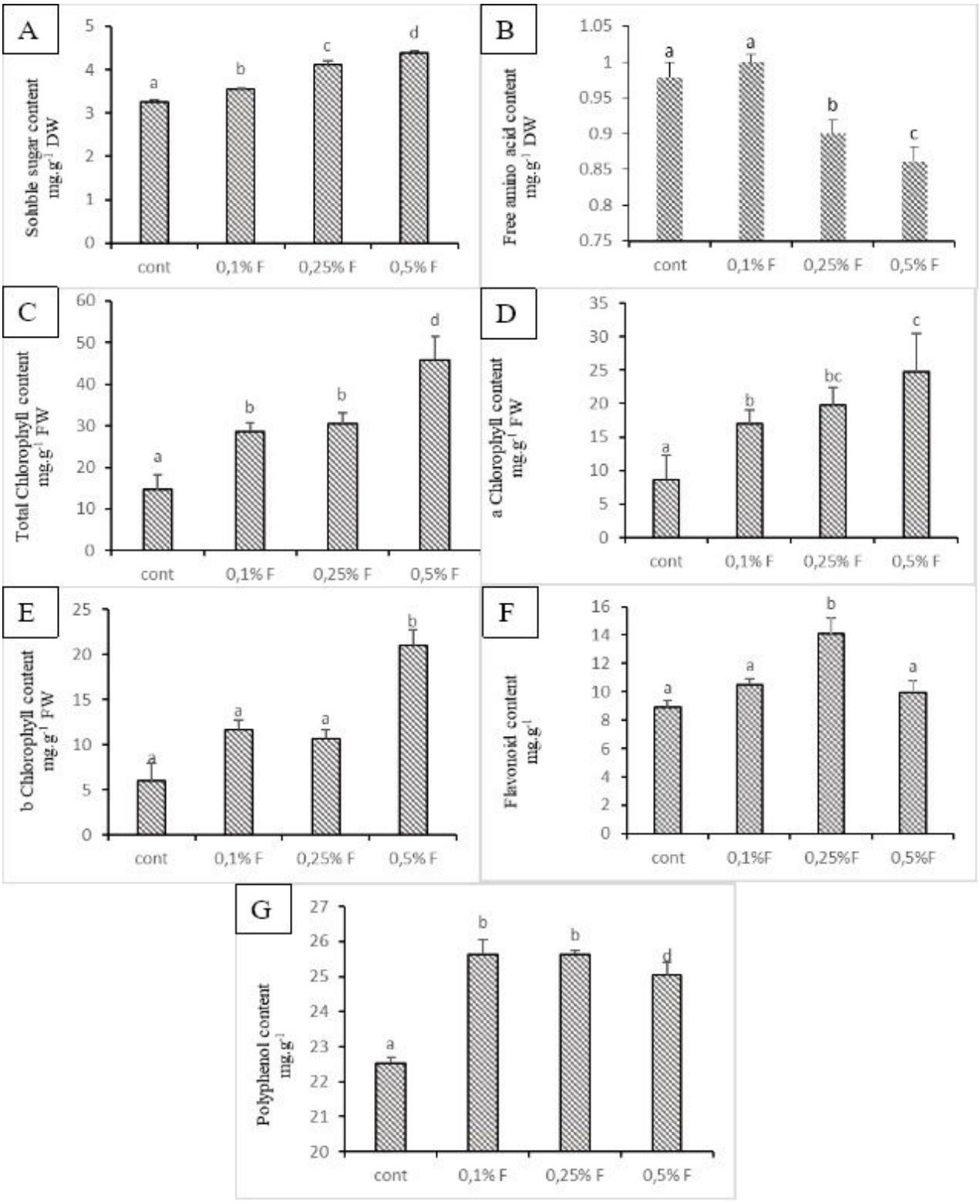


Figure 1

The effect of *Atriplex halimus* extract on chlorophyll content and secondary metabolites in plant leaves of *Vicia faba L*. Results are means ±SD. Different letters for each mean show statistically significant differences at $p < 0.05$.

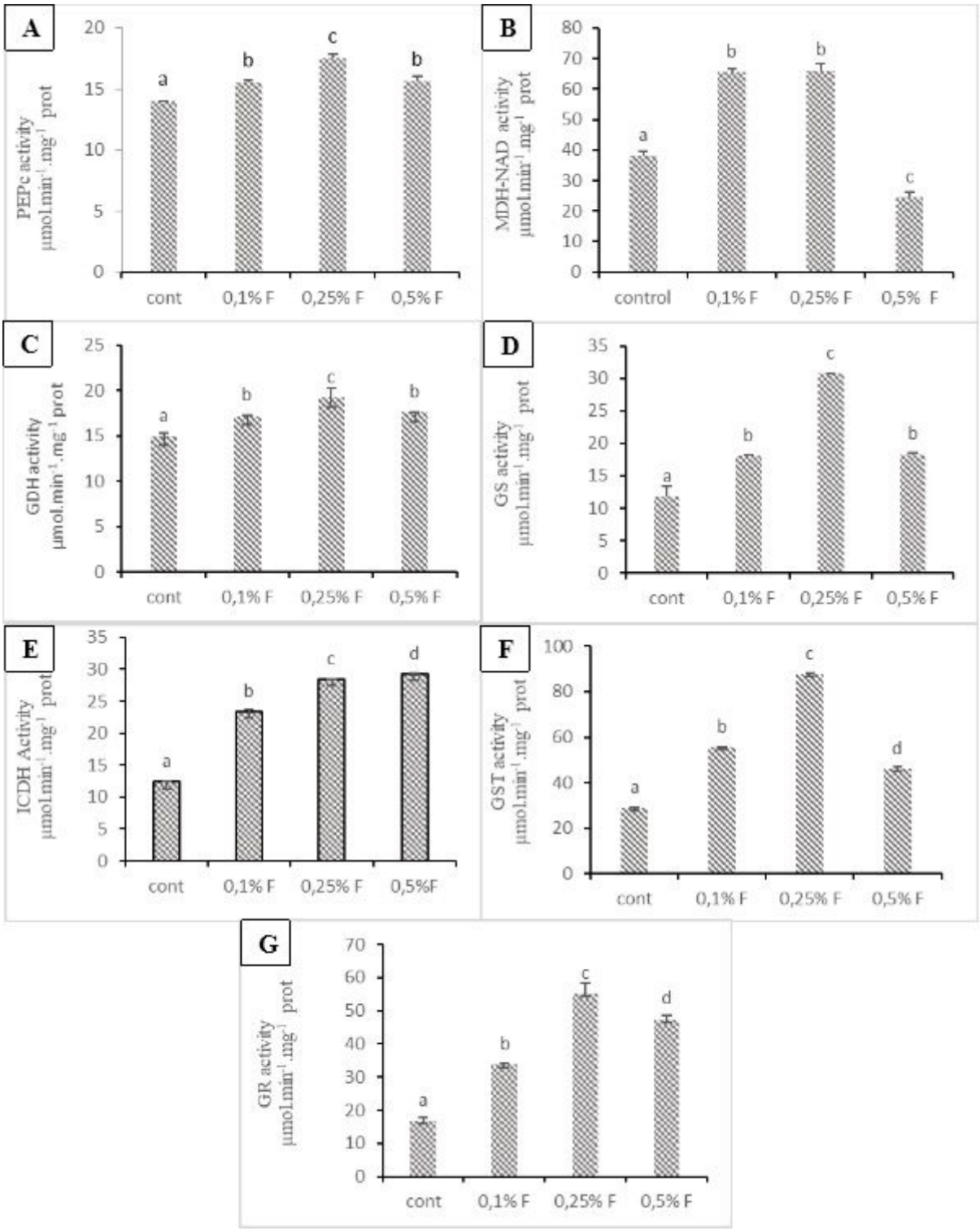


Figure 2

The effect of *Atriplex halimus* extract on enzymes ((A): PEPc, (B): MDH-NAD, (C): GDH, (D): GS, (E): ICDH, (F): GST, (G): GR. Abbreviations are Phosphoenolpyruvate Carboxylase (PEPC), Malate Dehydrogenase (MDH), Glutamate Dehydrogenase (GDH), Glutamine Synthase (GS), Isocitrate Dehydrogenase (ICDH), Glutathione-S-Transferase (GST), and glutathione reductase (GR).