

ORIGINAL ARTICLE

Biochemical Responses of Two Soybean (*Glycine max*) Varieties to Aluminum Stress in Nutrient Solution

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KEYWORDS

Aluminium;
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Flavonoid; Anthocyanin

ABSTRACT: Aluminum toxicity is the most widespread form of metal toxicity to plants in soil acids, initially causing inhibition of root elongation and blocks absorption of water and nutrients. According to this fact that soybean has been widely used in industry, this study investigated the effects of aluminum toxicity on biochemical factors in two varieties of Williams and Katoul of soybean plant. The study was carried out in a randomized design with aluminium (0, 200, 500, 700 μM) treatments and four replications in hydroponic culture. Results of biochemical tests showed that aluminum reduced the content of photosynthetic pigments, flavonoids, phenolic compounds, anthocyanins and reduced sugars in both cultivars of soybean. The proline content decreased with increasing aluminum in var. williams, but at var. katoul increased. It seems that *G. max* var. katoul suffers less than var. Williams. As regards, proline accumulation under Al stress to be generally higher in *G. max* var. katoul; hence, these results suggest that var. katoul is more resistant than var. Williams.

INTRODUCTION

Environmental pollution is a major problem faced by human societies with it. Development of industry, urban development, population growth and excessive human intervention in nature, leads to pollution of water, soil and air. The toxic metals pollution is a major problem around the world because these metals are almost impossible to destruction, and many of them have toxic effects on living organisms [1]. Metals toxicity has an inhibitory effect on plant growth, enzymatic activity, performance stomata, photosynthetic activity and

nutrient accumulation, and damage to the root system [2]. Aluminum (Al) ranks third in abundance among the Earth's crust elements, after oxygen and silicon, and is the most abundant metallic element. Aluminosilicate soils have high levels of aluminum metal, but small amounts of aluminum appear in the form of soluble which can affect biological systems [3]. Al bioavailability, and in consequence, toxicity, is mainly restricted to acid environments. Acid soils (with a pH of 5.5 or lower) are among the major factors limiting plant growth.

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Major agricultural production, especially grain product, is negatively influenced by acid soils [4].

When pH decline below 5.5, aluminosilicate clays and aluminum hydroxide minerals begin to dissolve, releasing aluminium-hydroxy cations and Al^{3+} then it exchange with other cations. In this kind of circumstances, Al^{3+} constitute the mononuclear species contains AlOH^{2+} , $\text{Al}(\text{OH})^{2+}$, $\text{Al}(\text{OH})_3$, and $\text{Al}(\text{OH})_4$ [5]. The mononuclear Al^{3+} species and Al_{13} are considered as the most toxic forms [6]. The main effect of Al stress can be prevented the root growth [7, 8]. When plants are exposed to many abiotic stresses including Al toxicity, reactive oxygen species (ROS) production is enhanced that leading to oxidative stress in the cells of plants [9,10]. The reactive oxygen species (ROS) can oxidize cellular components such as lipids, proteins, enzymes, and nucleic acids, which ultimately lead to cell death. Metals can act as catalysts in ROS production and encourage oxidative damage in plants. Aluminium cannot directly catalyze reduction–oxidation reactions, but oxidative stress can occur in the presence of Al [11].

Aluminum toxicity symptoms are easily recognizable. Foliar symptoms of Al in plants are similar to phosphorus deficiency (overall stunting, small, dark green leaves and late maturity, purpling of stems, leaves, and leaf veins, yellowing and death of leaf tips). In some circumstance, Al toxicity is caused calcium (Ca) deficiency or decreased Ca transport problem [12, 13]. Mechanisms of aluminum resistance in plants can be noted are composition and permeability of plasma membrane, making rhizosphere alkaline, aluminum division in vacuole, production of organic acid and secretion of phosphoric compounds [14].

In this research, we tried to investigate the effects of Al toxicity on some biochemical properties in two varieties of Williams and Katoul in soybean plant.

MATERIALS AND METHODS

Preparation of seedlings:

Seeds of soybean (*Glycine max*) were sterilized in 10% H_2O_2 (V/V) for 10 min followed by thorough washing in de-ionized water, and then germinated on transplantation tray for 7 d. Then the seedlings were removed from tray that washed carefully under tap water to remove adhering particles. Afterwards, they were transferred to PVC pot containing 1800 mL of Hoagland's half strength Solution containing: 0.5mM KNO_3 , 0.5mM Ca $(\text{NO}_3)_2$, 0.5mM MgSO_4 , 0.1mM KH_2PO_4 , 10 μM FeEDTA, 1 μM ZnSO_4 , 0.5 μM CuSO_4 , 10 μM H_3BO_3 , 0.2 μM Na_2MoO_4 , 0.5 μM CuSO_4 . Soybean plans were planted in pots with four holes, and with one plant in each hole. The nutrient solution was renewed twice a week and aerated continuously. Plants were grown for a minimum one week with condition of 35/25 °C (day/night) temperatures and 13/11 h (light/dark) in a greenhouse of Damghan University, Damghan, Iran.

Treatment

After 7 d of pre-treatment in hydroponics for compatibility the plants, different concentrations of AlCl_3 (0, 200, 500, 700 μM) was added to Hoagland's solution. Experiment was performed in randomized complete block design with 4 replications. After one weeks of treatment, the necessary measures were assessed. To determine the dry weight of the plant samples, initially they were placed for 72 h in the oven at 70 °C, and then the samples were weighed on scales 0.0001.

Photosynthetic Pigments content

In order to measure photosynthetic pigments including chlorophyll a, chlorophyll b, and carotenoid, fresh leaves were used after exerting Al stress period. The concentrations of chlorophyll a, chlorophyll b and carotenoid were calculated by lichtenthaler method (1987) [15]. In this method, absorption rate of photosynthetic

pigments were obtained at wavelengths of 663, 646, 470 nm with spectrophotometer and measured with Lichtenthaler equations as follows:

$$\text{Chla} = (12.25A_{663} - 2.79A_{646})$$

$$\text{Chlb} = (21.21A_{646} - 5.1A_{663})$$

$$\text{Car} = (1000A_{470} - 1.8\text{Chla} - 85.02\text{Chlb}) / 198$$

Flavonoids content

Flavonoids were measured by method of Krizek et al. [16]. In this method, leaf samples were homogenized in a porcelain mortar with 0.1 gram of leaf tissue and 5 ml of acid ethanol. Then, the extract was centrifuged, and the supernatant was incubated in a water bath for 10 min at 80 °C and allowed to cool to room temperature. Then, the amount of flavonoids was determined from the absorbance at 270 nm [16].

Anthocyanin content

Anthocyanin concentration was measured according to the method of Wagner [17]. In this method, fresh plant samples (0.1 g) were homogenized in a mortar with 5ml Acidified methanol, and were kept in the dark for 24 hours at 25 °C. Then it was centrifuged for 10 min at 4000 rpm and absorption of the supernatant was measured at 550 nm. To calculate the concentration of Anthocyanin, extinction coefficient (ϵ) of 33000 cm M^{-1} was used [17].

Phenolic compounds content

In order to measure phenolic compounds, 0.1 g of fresh plant samples was homogenized in a mortar with 3 ml of 95% ethanol, and was placed in the dark for 24 hours. The homogenate was centrifuged at $4000 \times g$ for 5 min. Then the absorption of the supernatant was measured at 725nm [18]. The results of phenolic compounds were calculated and presented according to microgram per gram of fresh weight.

Reducing sugar content

For evaluation of sugar content, Somogy method was used [19]. Accordingly, 0.05 gr of leaf tissue homogenates in a porcelain mortar with 5 ml of distilled water for 5 min and were centrifuged at 4000 rpm. Then to 1ml of the supernatant, 0.5 ml 5% phenol and 1.5 ml of 98% sulfuric acid were added and vortex. After 1-hour absorbance of supernatants were measured at 485 nm.

Proline content:

Content of proline was measured [20]. Samples (0.02 gr) were homogenized with 3 mL sulphosalicylic acid (3% w/v) and centrifuged for 10 min, 1 ml of supernatant were mixed with 1ml of glacial acetic acid and 1ml of acid ninhydrin and stay on warm bath for 1 h at 100 °C. Heating caused reaction between ninhydrin and proline. Then, test tubes were kept in an ice bath. One ml of upper part containing proline was separated and the absorption of each sample was read at wavelength of 520 nm by spectrophotometer system.

STATISTICAL ANALYSIS

All experiments were done with 4 independent replications with a completely randomized design. Data means were used for Duncan's multiple range tests after that one-way analysis of variance (ANOVA) with a significance level of 0.05 were used for analyses of data with SPSS 23 (Chicago, IL, USA).

RESULTS AND DISCUSSION

The analysis of variance demonstrated that different concentrations of Al had significant effect ($P \leq 0.05$) on the amount of chlorophyll a, chlorophyll b and carotenoids in williams cultivar. However, had no significant effect on the katoul (Figure 1, 2 and 3). Al stress significantly decreased photosynthetic pigments in williams variety. The reduction in pigment levels as a consequence of toxic metals have been reported in many plant

species such as sunflower [21] and maize [22]. One of the intense effects of Al is disrupting on the biosynthesis of chlorophyll in plants [23]. Decrease in chlorophyll content in presence of toxic metals as Al is probably due to oxidative induction during chlorophyll biosynthesis. Metal toxicity induce the production of oxygen active species that breakdown and decrease photosynthetic pigments [24].

Biotic or abiotic stressors can reduce the levels of photosynthetic pigments, including carotenoids, [25-27]. Depending on metal types, they can enhance or reduce carotenoid production [28, 29]. The anthocyan content in shoots of *Atriplex hortensis* (var. rubra) decrease into soils contaminated with toxic metals [30] that agree with our observations. Non-photochemical breakdown of carotenoid can decrease carotenoid content and cause breaking the structure of carotenoids [31].

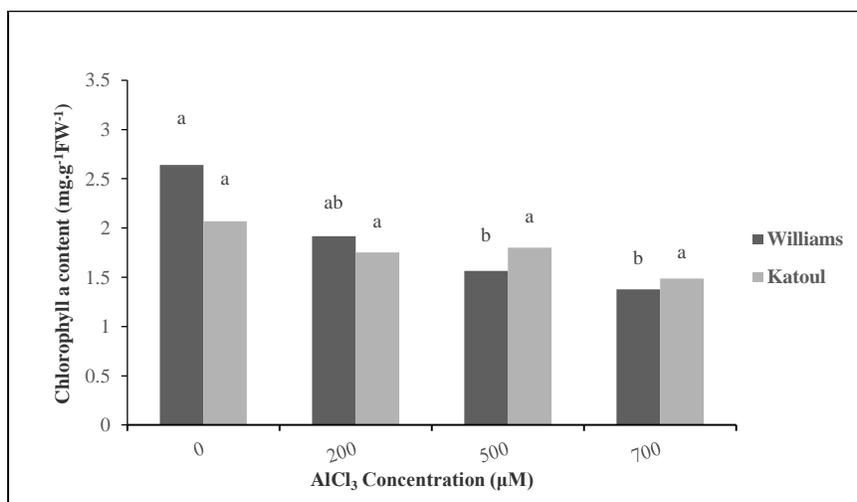


Figure 1. Effect of different AlCl₃ concentrations on Chlorophyll a content of two soybean cultivars (Williams and Katoul), Values are mean of four replicates ± SD. Different letters represent a significant difference $P \leq 0.05$ between treatments.

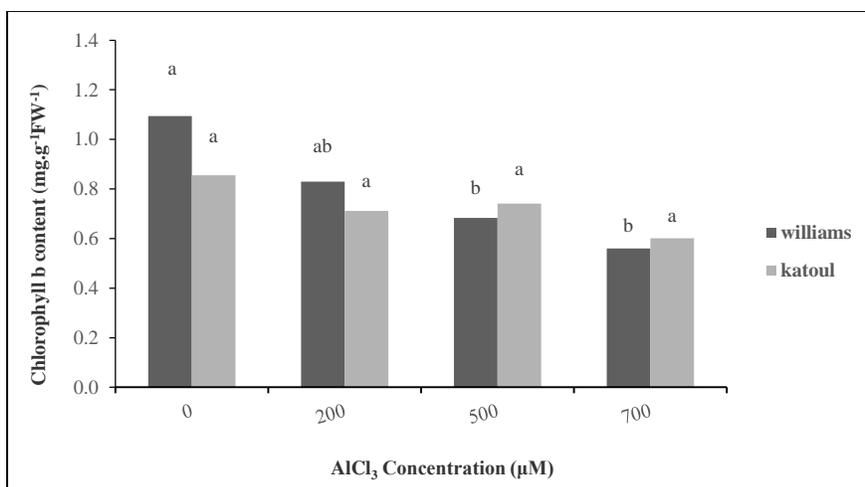


Figure 2. Effect of different AlCl₃ concentrations on Chlorophyll b content of two soybean cultivars (Williams and Katoul), Values are mean of four replicates ± SD. Different letters represent a significant difference $P \leq 0.05$ between treatments.

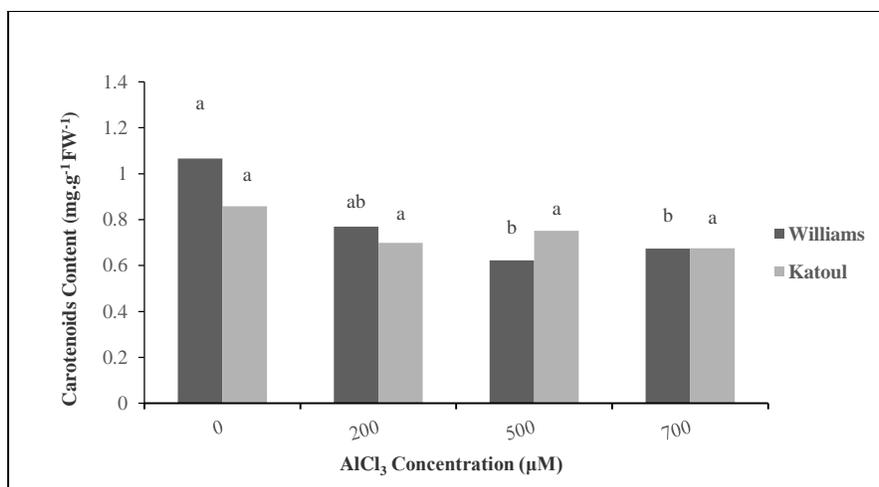


Figure 3. Effect of different AlCl₃ concentrations on Carotenoids content of two soybean cultivars (Williams and Katoul), Values are mean of four replicates ± SD. Different letters represent a significant difference $P \leq 0.05$ between treatments.

Our results showed a decrease in Flavonoids content with gradual increase for aluminum especially in Williams's variety of soybean. A compared with control, Al stress significantly decreased flavonoids content at wavelength of 270 nm by 27.36% in williams variety. Nevertheless, no significant change was observed for flavonoids in katoul (Figure 4).

Flavonoids are introduced as secondary metabolites of

the plant based on phenyl benzopropen structure and as bio-flavonoid. Biosynthesis of the flavonoids is complex and causes involvement of several enzyme phases [31]. Aluminum may affects the enzymes existing in flavonoids path such as Chalkone synthetize and phenyl ammonia leas cause decrease in accumulation of this pigment in plant [32]. Studies on the *Brassica napus* showed that the flavonoids content reduced under aluminum stress that agrees with our results [31].

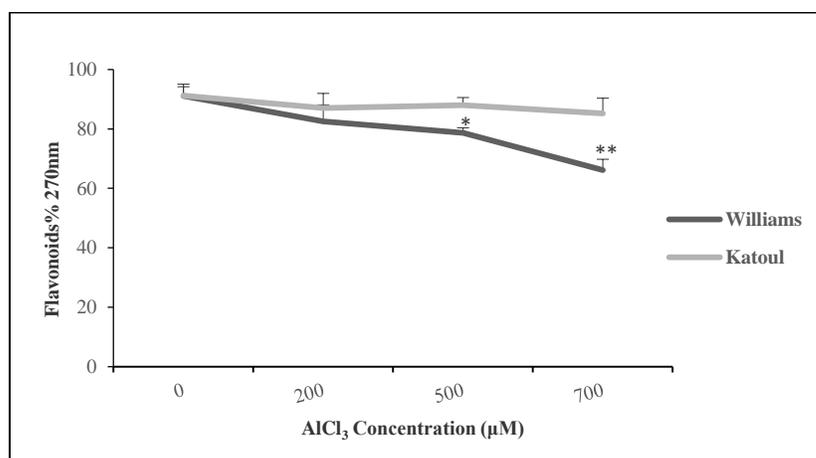


Figure 4. Effect of different AlCl₃ concentrations on flavonoids percent at a wavelength of 270 nm in two soybean cultivars (Williams and Katoul), Values are mean of four replicates ± SD. Different letters represent a significant difference $P \leq 0.05$ between treatments.

Treatment with Al causes significant reduce in sugar content of the williams cultivar. In addition, treatment by Al causes decrease in sugar content of the katoul cultivar, which is not significant statistically (Figure 5). The environmental conditions can affect the sugars metabolism and distribution of photosynthesis in growing plants. Studies on the *Glycine max* showed that the soluble sugars content reduced under aluminum stress [33], which corresponded with the result of this research. Probably, Aluminum reduces the activity of the enzyme invertase saccharose, causes reducing photosynthesis.

Hence, the starch accumulation increase in the leaves; resulting soluble sugar content decrease [34]. Aluminum with binding to phosphate compounds reduces the access Rubisco enzyme into the phosphate and therefore reduces the activity of the enzyme Rubisco. This enzyme is a key enzyme of soluble carbohydrates synthesis [35].

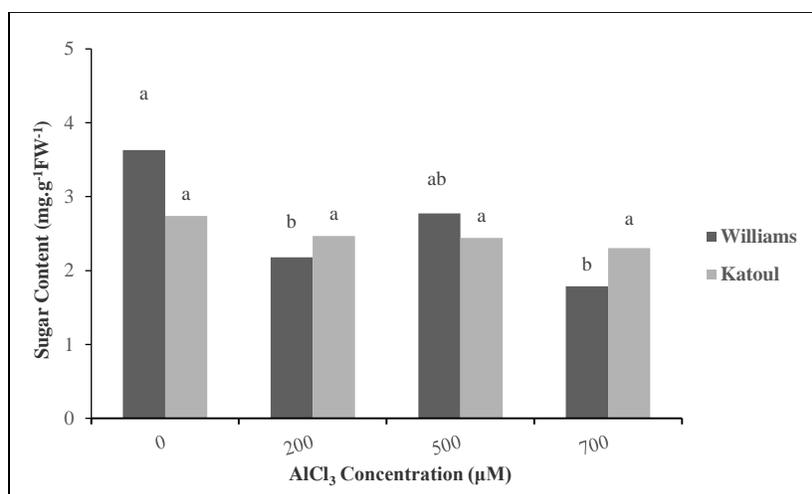


Figure 5. Effect of different AlCl₃ concentrations on Sugar content of two soybean cultivars (Williams and Katoul). Values are mean of four replicates ± SD. Different letters represent a significant difference $P \leq 0.05$ between treatments.

The effect of aluminum on the amount of anthocyanins was significant (Figure 6). In *G. max* var. williams, the highest concentrations of anthocyanin content obtained in concentration of 200μM Al. However, treatment by Al causes decrease in anthocyanin content of *G. max* var. katoul which is a significant decrease statistically. Anthocyanins, which possess potential health benefits that may be ascribed to their high antioxidant activities, are a group of widely distributed plant pigments. Anthocyanins have been raised as compounds that are involved in metal accumulation or tolerance, although

metal complex plays a role in determining anthocyanin color [36]. Anthocyanins are produced in response to metal stresses [37] and believed that by increasing the antioxidant response of plants in order to amplify the regular physiological status to biotic or abiotic stresses [38]. The reduction for anthocyanin in this study cannot be attributed to lower soybean resistance against stress aluminum. Studies on the *Ocimum basilicum* showed that the anthocyanin content reduced under aluminum stress that corresponds with our results [39].

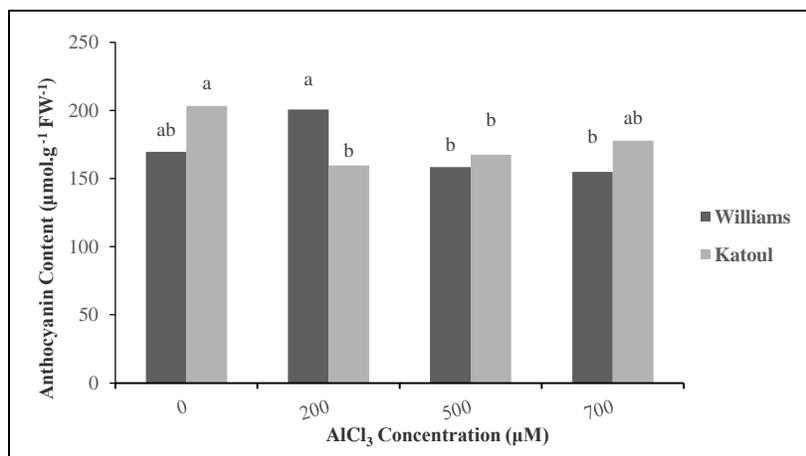


Figure 6. Effect of different AlCl₃ concentrations on Anthocyanin content of two soybean cultivars (Williams and Katoul), Values are mean of four replicates ± SD. Different letters represent a significant difference $P \leq 0.05$ between treatments.

Compared to control, treatments by Al also causes decrease in phenol compounds of the plant, which is a significant decrease statistically, in both *G. max* cultivars (Figure 7). Phenolic compounds especially hydroxylcinnamic acids exist so much in the epidermis. Aluminum affects the enzymes as phenylalanine ammonia

lyase, CHS and other enzymes in phenyl propanoids path. The enzyme of phenylalanine ammonia lyase (PAL) alters phenylalanine into trans-cinnamic acid and causes formation of phenolic compounds like flavonoids tannins and lignin [40]. Aluminum may causes decrease of phenolic compounds by affecting this enzyme [40].

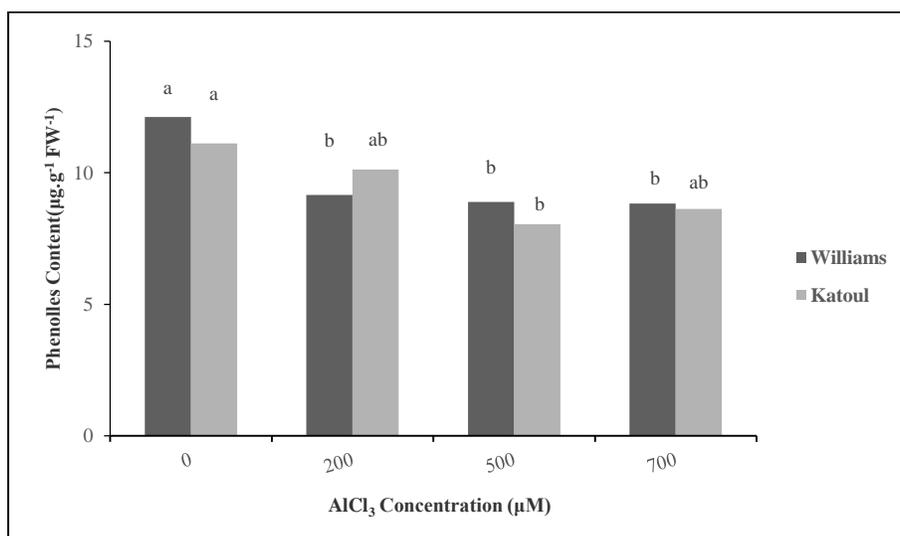


Figure 7. Effect of different AlCl₃ concentrations on Phenolics content of two soybean cultivars (Williams and Katoul), Values are mean of four replicates ± SD. Different letters represent a significant difference $P \leq 0.05$ between treatments.

Treatment by Al causes significant increase in proline of *G. max* var. katoul, which is a significant increase in 500

and 700 µM concentration of Al (Figure 8). These results are corresponding with the studies that showed by

Al treatment proline content increase in *Brassica napus* [31]. Compared to control, proline content decreased with increasing Al for *G. max* var. Williams as studies on sorghum plant showed that proline content reduce under aluminum stress [41]. Accumulation of proline, an amino acid, can play beneficial and adaptive roles in plant tolerance exposed to various stress conditions [31]. In addition, the accumulation of proline in cells cytoplasm acting as an excellent osmolyte Proline [42]. The major role of proline during stress, are as a metal chela-

tor, a molecule of antioxidative defense and a signaling molecule. Proline act as a sweeper of free radicals that play a role protect for enzymes and cellular [43-45]. When cell exposed to Al stress, proline accumulation will increase in plant regarding the Proline biosynthesizing path [42]. Proline accumulation under abiotic stress is generally higher in plants more tolerant than in plants more sensitive [46]. Increase in content of proline may be related to either de novo synthesis or decreased degradation or both [47].

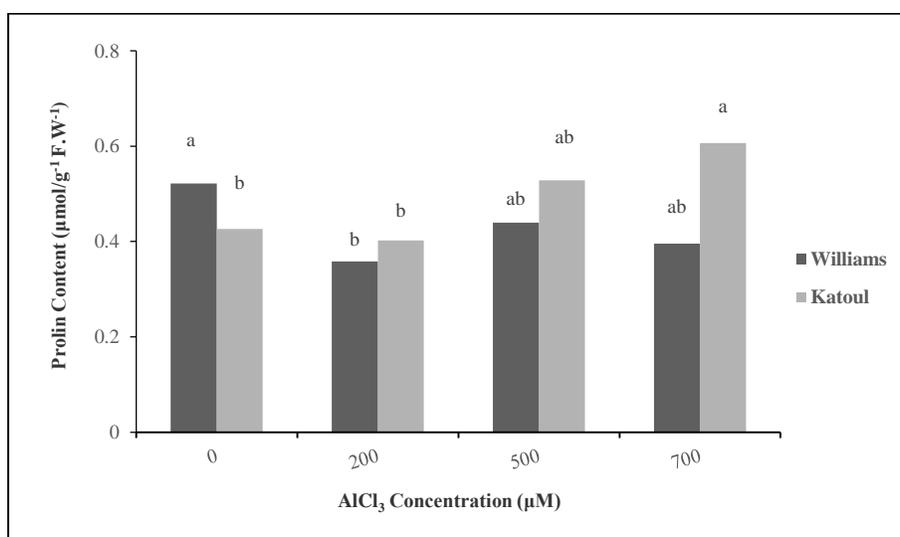


Figure 8. Effect of different AlCl₃ concentrations on Proline content of two soybean cultivars (Williams and Katoul). Values are mean of four replicates ± SD. Different letters represent a significant difference $P \leq 0.05$ between treatments

CONCLUSIONS

At different concentrations of Al, the soybean cultivars studied here suffered from Al stress. However, Al elicited different responses on the biochemical parameters of both cultivars. Given that aluminum effect was felt more on the varieties William, it seems that *G. max* var. katoul suffers less than var. Williams. As regards, proline accumulation under Al stress to be generally higher in *G. max* var. katoul; hence, these results suggest that var. katoul is more resistance than var. Williams. The adverse effects of Al stress can induce antioxidant de

fense activity in plants to remove the possible toxic effects of free radicals, making the plants more resistant to metal toxicity. More studies under controlled environmental conditions are required for evaluating the tolerance ability of these cultivars to Al toxicity.

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