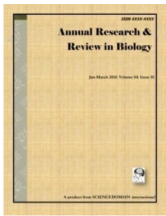

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Physiology and Biochemistry Effects of Herbicides Sekator and Zoom on Two Varieties of Wheat (Waha and HD) in Semi-Arid Region

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Authors' contributions

This work was carried out in collaboration between all authors. Author SH designed the study, performed the experiments, analyzed the data, wrote the protocol and wrote the manuscript. Author MAL performed the experiments, wrote the protocol and analyzed the data. Authors GN and DMR analyzed the data. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Herbicides are the most effective tool against the major weeds of cultivated plants, and are necessary in maintaining or increasing crop yields. However, most of these molecules are highly toxic for crop. Their massive and repeatly use can cause adverse effects on all components of the environment. The purpose of this study was to test the toxicity of two herbicides (Sekator and Zoom) on the physiology and biochemistry of hard wheat: *Triticum durum* Desf and common wheat: *Triticum aestivum* L in Tebessa. Both of the areas are considered as semi-arid zone of eastern Algeria.

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Methodology: The herbicides were applied at tillering stage in the dose full recommended doses (150 ml /ha of Sekator and 120 g/ ha of Zoom). After eight days of treatment leaves were collected to determine different physiological and biochemical parameters (chlorophyll, soluble sugar and proline).
Results: The results validated by statistical analysis revealed that the levels of chlorophyll *b* and soluble sugars in plants subjected to the effect of two herbicides (Sekator and Zoom) were significantly lower compared to untreated plants and the chlorophyll *a* were higher in the variety Waha treated with herbicide Zoom. There was a significant increase in proline content in plant samples treated with the herbicide Zoom in Hidhab variety.
Conclusion: Finally, the determination of herbicide in the leaves of wheat shows a greater diminution in chlorophyll *b* and soluble sugar for HD variety.

Keywords: Effect; herbicides; oxidative stress; physio-biochemical metabolites; hard wheat; common wheat.

ABBREVIATIONS

ROS: Reactive Oxygen Species, SO2: sulfur dioxide, cha: chlorophyll a, chb: chlorophyll b, FW: fresh weight, QA: quinone A, QB: quinone B, PSII: Photosystem II, PSI: Photosystem I, df : degrees of freedom, Mean Square : mean squared deviations from the mean = variance, F : is a ratio of the variance between groups to the variance within groups, the P value is determined from the F ratio and the two values for degrees of freedom shown in the ANOVA table.

1. INTRODUCTION

The culture of cereal is the dominant crop of the Algerian agriculture. It covers nearly three million hectares in a biennial system dominating fallow cereal [1]. Cereals constitute an important food resource of man and animal [2]. Among the cereal, the hard wheat (*Triticum durum* Desf) is the oldest species and a major part of the humanity food [3]. It sets all over the world, the fifth place after the common wheat, the rice, maize and the barley with a production of more than 30 million tons [4]. The common wheat (*T aestivum*) and the hard wheat *(T durum*) are economically the most important cereal species which adapted to varied natural conditions [5]. So, the low level of farmers technicality associated at the risk of obtaining low yields explain the poor soil preparation, the insufficient protection of the cultures against the predators, diseases and weeds and the low level of use of inputs [6]. To achieve the required standards and the economically viable levels of production, the farmers have to use products phytosanitary to protect crops against weed, pests or fungal diseases [7]. Since 1950, the development of agriculture and research to increase the yields, led to an increasing use of pesticides. These practices caused, further to a massive use, a contamination of the environment and in particular a diffuse pollution of a large number of aquifers on the whole territory [8]. Algeria uses approximately 6 000 in 10 000 tons of pesticides annually [9], approximately 400 phytosanitary products are approved in Algeria [10]. In addition, many organic xenobiotic, such as pesticides can pull an overproduction of Reactive Oxygen Species [11]. This accumulation can be due to an inhibition of the transport of electrons connected to the process of photosynthesis, and consequently to an inhibition of the activity of the photosystems [12,13,14]. Exposure to sulfur dioxide (SO_2), another atmospheric pollutant, also causes an increase of ROS in plant tissues [15]. The analysis of the mechanisms of response to the oxidative stress or xenobiotic interactions oxidative stress can thus allow a better understanding of the processes of response the xenobiotic [11]. [16] They showed that herbicidal treatments of Metribuzin and Isoproturon + Diflufenican produced smaller plants which can be due to their phytotoxic effect on wheat crop. [17] They showed that under field conditions, Isoproturon applied at 1,000 g/ha 32 d after sowing resulted in wheat grain yield decrease by 65% as compared to the weed-free control. The earlier work indicates that the application of herbicide 2,4-D at emergence (0-72 h treatment) to earlier seedling stage (3-5 leaf stage), boot stage and jointing stage to soft dough stage caused toxic effects on germination, cytological abnormalities (inhabitance of cell division and meristematic cells, chromosome aberrations), physiological abnormalities (enzymes, proteins and nucleic acids), and morphological parameters abnormalities (unfolded leaves, less tillers, reduced plant height, delay maturation, scattering of heads, sterility of reproductive structure and reduced grain yield) [18].

In this context, the objective of our research was to determine the effects of two herbicides on some physiological and biochemical activities of *Triticum aestivum* and *Triticum durum*. This aim was achieved by evaluation of important physiological parameter such as chlorophyll and biochemical parameters such as carbohydrates, and proline.

2. MATERIALS AND METHODS

2.1 Biological Material

The study concerned one variety of hard wheat (Waha) and one variety of common wheat (Hidhab: HD1220).

Waha is characterized by a high grain yield potential, strong ability to translocate carbohydrates stored in the collar of the ear [19] and a precocity of blooming which makes it sensitive to late frost [20].

HD is native of CIMMYT, half-dwarfish, premature, highly productive and is sensitive to the shelling [21]. It is also drought tolerant, highly appreciated by farmers because of its ability to yield high [22].

2.2 Herbicides

Sekator: It is a new selective herbicide for post-emergence control of dicotyledonous weeds in hard and common wheat and it is composed of two active substances (Table 1): amidosulfuron-sodium (100g/l) and 25g/l of iodosulfuron-methyl-sodium [23]. Sekator belongs to the sulfonylureas, which are broad-spectrum herbicides used in various cultures and with significant biological activity [24]. They exercise their action through blocking cell division at the meristem, by inhibiting the acetolactate synthase enzyme (ALS), which is crucial in the biosynthesis of essential branched-chain amino acids: valine, leucine and isoleucine[25,26].

Zoom: Is selective herbicide against annual and some perennial weeds dicotyledonous in wheat. It is absorbed by the roots and leaves. It acts primarily by inhibiting the growth of weeds which become necrotic and die within a few weeks [27]. Zoom is a combination of two active substance as shown in (Table 1), the Triasulfuron (4, 1%): $C_{14}H_{16}CN_5O_5S$ and Dicamba (65, 9%): ($C_8H_6C_{l2}O_3$) [28].

2.3 Conduct of the Experiment

The field experiments were conducted at two locations in Tebessa during 2012/2013. The sowing was conducted with an on-line leading seed drill solla, at the rate of 300 seeds /m ². The crop was fertilized with100 kgha^{-1}of urea 46% and sprayed by Sekator (150ml /ha) and Zoom (120 g/ ha) in March. Leaves of varieties Waha treated with herbicide Zoom were collected from the plot in El Houidjbat, while all other samples (untreated and treated) of both varieties Waha and HD were removed from the cultivated site at the plot in Ras El Aaiun (Fig. 1).

2.4 Analyses and Measurements

2.4.1 Chlorophyll *a* and *b* content

Chlorophyll was determined by the method of Holden (1975) [31]:100mg(for each test) collected from the median third of the youngest leaves at tillering stage were ground in the presence of calcium carbonate (to neutralize the acidity of the juice vacuolar) in acetone 80% and approximately 100mg of calcium bicarbonate (CaCO3). Milling was repeated several times to extract all chlorophyll pigments. The extract was filtered. The optical density of all filtrates was measured at 663 and 645nm. The formula relative to the solvent, to calculate the values of chlorophylls [32] is shown in Equation (1) and (2).

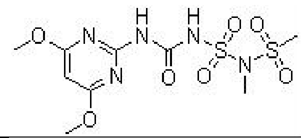
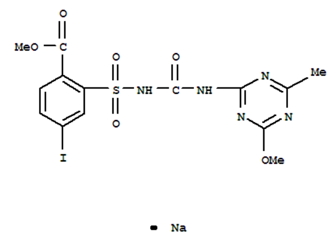
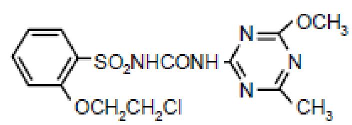
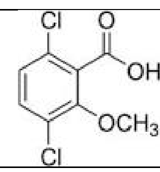
$$\text{Chl a} = 12,70.\text{DO}(663) - 2,69.\text{DO}(645) \quad (1)$$

$$\text{Chl b} = 22,90.\text{DO}(645) - 4,60.\text{DO}(663) \quad (2)$$

2.4.2 Prolinecontent

The method used is that of Troll and Lindsley [33], modified by Dreier and Goring [34]. 100 mg (for each test) taken from the middle third of the youngest leaves were immediately weighed and then placed in a test tube. A volume of 2 ml of 40% methanol was added to the sample and the whole was heated for 1 h in a water bath at 85ºC. After cooling, 1 ml of the extraction solution was added to 1 ml of acetic acid, 25 mg of ninhydrin and 1 ml some mixture distilled water, acetic acid, orthophosphoric acid at density of 1,7 (120, 300, 80: v / v / v).

Table 1. Chemical structure of active substance of herbicides Sekator and Zoom [29, 30].

Active substance	Chemical structure
Amidosulfuron-sodium	
Iodosulfuron-methyl-sodium	
Triasulfuron	
Dicamba	

The whole was boiled for 30 minutes in a water bath, then cooled and mixed with 5mlof toluene. After vortexing, two phases separate, the upper phase containing the proline was recovered and dehydrated by adding Na_2SO_4 and its optical density was determined at 528 nm. The obtained values were reported on a confirmed solutions ranging from 0 to 0.2 mg/ml of proline standard curve.

2.4.3 Soluble Sugars content

The total soluble sugars (sucrose, glucose, fructose, and their methylated derivatives polysaccharides) were measured by the method of Schields and Burnet [35], which uses the anthrone reagent as in a sulfuric medium. One hundred mg of plant material was macerated for 48 h in 3ml of 80% ethanol at room temperature to ensure the extraction of soluble sugars. At the time of dosing and after evaporation of the alcohol, 20 ml of distilled water were added to the extract, heated in a water bath at 70ºC for 30 min, and 2 ml of extract were removed for assay. The concentration of soluble sugars was determined after reading of the optical density measured with a spectrophotometer at 585 nm.

Three replicates were studied for each assay of chlorophyll, proline and soluble sugar content.

3. RESULTS AND DISCUSSION

3.1 Effect of herbicides Sekator and Zoom on Chlorophyll *a* and *b* Content.

The effects of Sekator on the chlorophyll content (*a* and*b*) are shown in (Fig. 2). The values of the probability of the analysis of variance (Table 2) show the existence of highly significant differences ($p \leq 0.01$) in HD wheat variety and chlorophyll *b*. While in the case of herbicide Zoom (Fig. 3 and Table 3), the comparison of the averages of each chlorophyll (*a, b)* and varieties of wheat using the variance analysis shows the highly significant ($p \leq 0.01$) differences in ch*a* for Waha variety and in ch *b* in HD variety only.

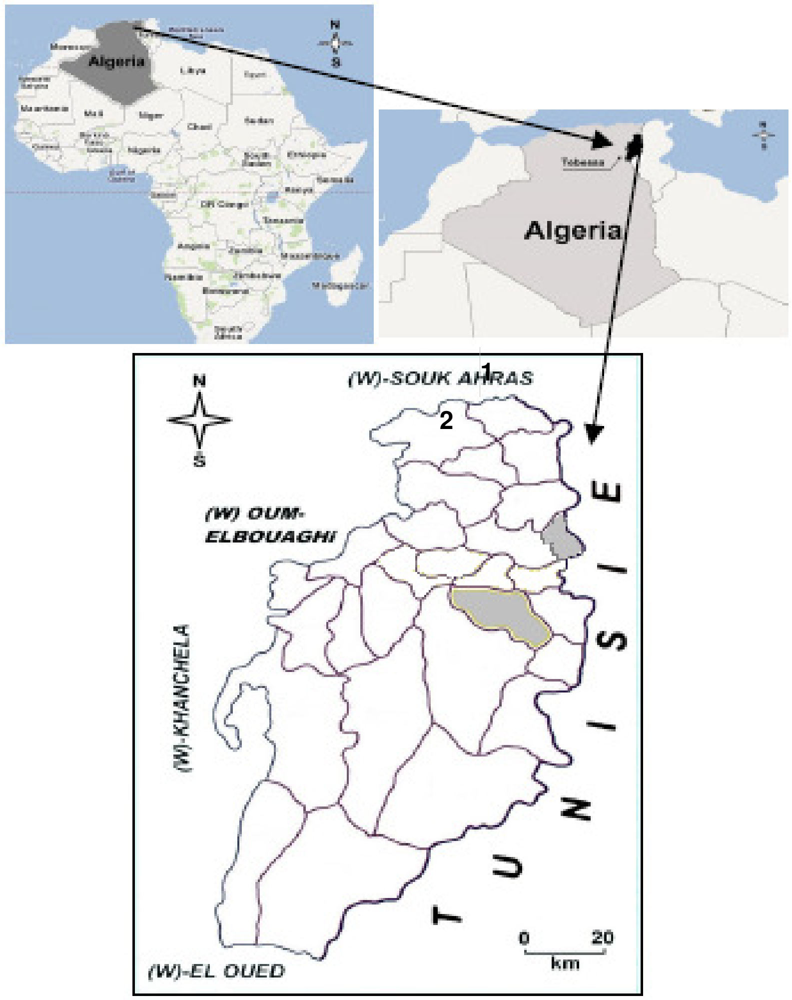


Fig. 1. The sampling sites on the Wilaya and the plain of Tebessa (Algeria)

Site (1): RasElAyoun (Situated in 30 km in the North of Tebessa, and being of the common of El Kouif).Site (2): El Houidjbat (Situated in 29,1 km in the South of Tebessa, and being of the common of El MaLabiod)

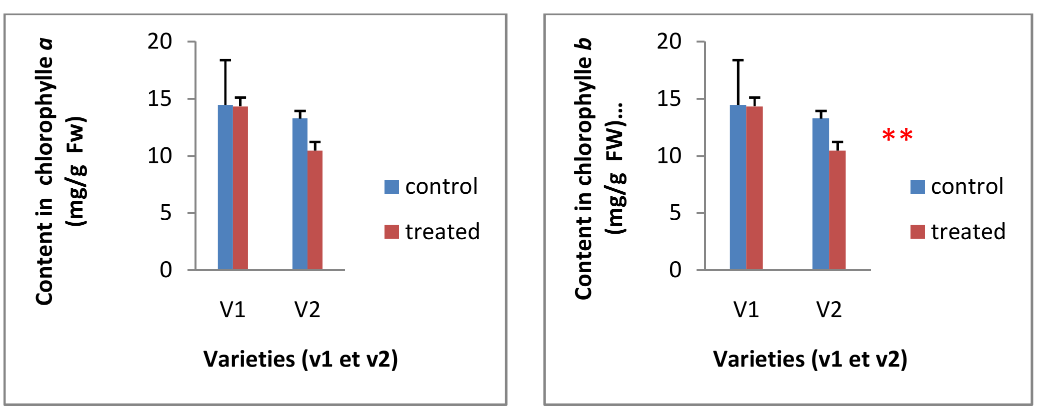


Fig. 2. Effect of Sekator on chlorophyll *a* and *b* content (mg/ gFW) in two wheat varieties Waha (V1) and HD (V2)

***Are highly significant (p≤ α=0.01).NS: not significant (p> α=0.05)*

Table 2. Analysis of variance results for varieties of *Triticum* treated with herbicide Sekator and content of chlorophyll *a* and *b*

Chlorophyll	Varieties	statistical parameters			
		df	Mean square	F_{obs}	P
Chl*a*	Waha	1	26	0,01	0,926 NS
	HD	1	19437	2,34	0,201 NS
Chl*b*	Waha	1	48241	1,39	0,304 NS
	HD	1	302065	22,71	0,009**

Table 3. Analysis of variance results for varieties of *Triticum* treated with herbicide zoom and content of chlorophyll *a* and *b*

Chlorophyll	Varieties	statistical parameters			
		df	Mean square	F_{obs}	P
Chl*a*	Waha	1	3151	25,19	0,007**
	HD	1	15813	4,56	0,099 NS
Chl*b*	Waha	1	25555	1,80	0,251 NS
	HD	1	246341	21,47	0,010**

Most herbicides induce inhibition of Photosystem II activity but their effects on D1 polypeptides appear to be variable. Urea-type herbicides/triazine block the oxidation of QA' (reduced quinone A) in QB (quinone B) binding site, preventing its association with the plastoquinone [36,37], inducing an inhibition of electron transfer between the PSII and PSI. However, these herbicides induce little effect on the protein DI and photoinhibition of PSII compared to phenolic-type herbicides [13]. The photoinhibition of PSII herbicide urea/triazine is supposed to be associated with a delay in the formation of QA in the reduced state, and low production of singlet oxygen [38,14]. Also in plants and green algae, herbicides can induce an inhibition of the synthesis of chlorophyll by inhibition of protochlorophyllide reductase enzyme [39]. The increase in chlorophyll *a* of variety Waha treated with herbicide Zoom was also observed with the herbicide "Atrazine". Atrazine stimulates growth and chlorophyll content in green algae (*Chlamydomonas*) and a diatom (*Synedraaeus*). Stimulation of growth observed during exposure to low concent rations of toxins might be the result of an adaptation of photosynthetic pigments [40,41]. Rioboo et al. [42] found that the exposure of the algae to low concentrations of Triazine herbicides, causes an increase in cell density and growth rate after 96 hours. This stimulation of growth, showing the ability of algal cells, such as *Chlorella vulgaris*, to adapt and to resist inhibitory effects of herbicides. On the other hand, these effects disappear at higher concentrations. Gonzalez Barreiro and al. [43] reported as a stimulation of the content of carotenoids and chlorophyll with Atrazine to 48 hours, followed by a reduction of pigment content to 96 hours in the *cyanobacterium* (*Syneehocoeeus elongates)*. These stimulatory effects have been noted with other species and different herbicides [44,45].

3.2 Effect of Sekator and Zoom on Proline Content.

The effect of Sekator and Zoom on the content of proline (µg/g of Fw) illustrated in (Fig. 4) and the variance analysis for each herbicides and variety of wheat (Table 4), showed significant differences ($p \le 0.05$) for HD variety treated with Zoom and non-significant differences ($p > 0.05$) for variety Waha and HD weeded by Sekator.

Table 4. Analysis of variance results comparing proline obtained for each herbicides applied to the two varieties of *Triticum*

Herbicides	Varieties	Statistical parameters			
		df	Mean square	F_{obs}	P
Sekator	Waha	1	0,0204	0,54	0,504 NS
	HD	1	0,0048	0,33	0,596 NS
Zoom	Waha	1	0,0006	0,02	0,903 NS
	HD	1	0,1634	13,69	0,021 *

**: Significant differences ($p \le \alpha = 0.05$).NS: non-significant differences ($p > 0.05$)*

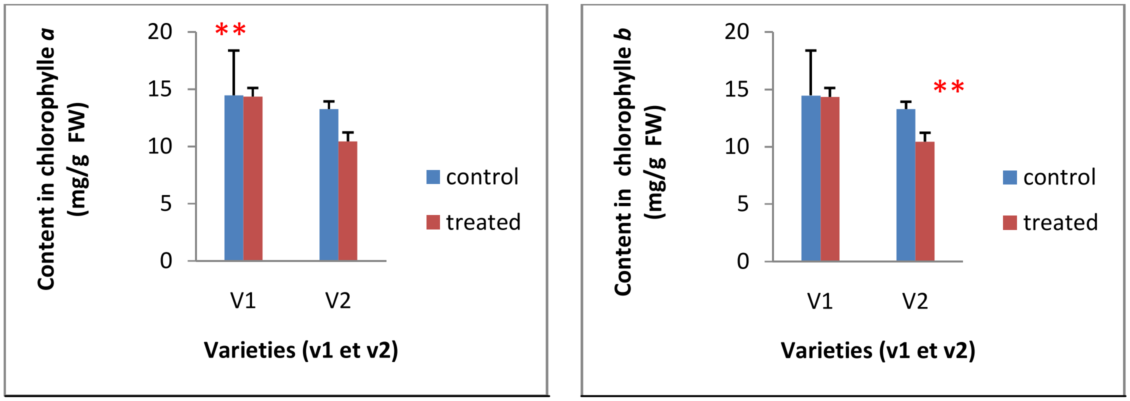


Fig. 3. Effect of zoom on chlorophyll *a* and *b* content (mg/ g FW) in two wheat varieties Waha (V1) and HD (V2)

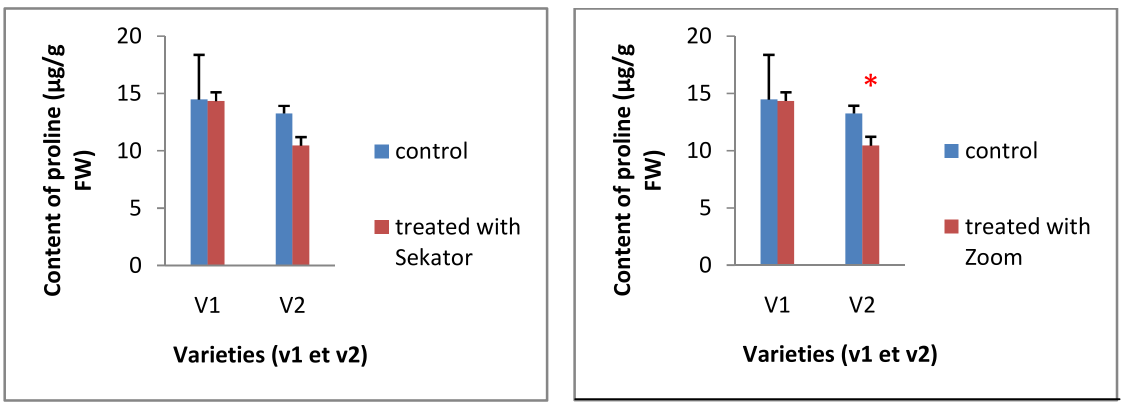


Fig. 4. Effect of Sekator and zoom on proline content (µg / g FW) in two wheat varieties Waha (V1) and HD (V2)

The accumulation of proline is one of adaptive strategies activated by the plant side, the constraints of the environment [46]. While the explanation of the accumulation of proline is different according to the authors [47,48,49,50,51] they showed that the increase in leaf proline content under the stress, followed by the reduction in total chlorophyll pigments (chlorophylls *a* and *b*) content. Results of [48] show some proportionality, but inverse, between the contents of accumulated proline and chlorophyll pigments contents lost and the variety that accumulates more proline is also the one who is the fastest decrease in levels of chlorophyll pigments and vice versa. These results suggest the existence of a connection between the likely pathways of biosynthesis of chlorophyll pigments and proline [52]. Competition between these two compounds on their common precursor, glutamate, maybe the cause of this development [53,54].

3.3 Effect of Sekator and Zoom on the Sugar Content

The graphical representation of results obtained in the case of wheat treatment by the molecule of herbicide illustrated in (Fig. 5) and ANOVA test (Table 5) revealed no significant differences for all comparisons except for the variety HD treated with Zoom and Sekator where we can observe a significant difference.

Concerning the dosages of total sugars, the results are consistent with those of [55], which recorded a decrease in sugar content in *Chlorella vulgaris* under the effect of the herbicide Diuron. According to the research of [56] on the effect of three Pesticides (Hexaconazole, Bromuconazole and Fluazifop-p butyl) on some biochemical metabolites of the hard wheat: *Triticum durum*, a close correlation between the rate of

Table 5. Analysis of variance results comparing soluble sugar obtained for each herbicide applied to the two varieties of *Triticum*

Herbicides	Varieties	Statistical parameters			
		df	Mean square	F_{obs}	P
Sekator	Waha	1	17,20	03,75	0,125 NS
	HD	1	01,48	11,05	0,029*
Zoom	Waha	1	04,20	01,45	0,295 NS
	HD	1	03,49	12,88	0,023*

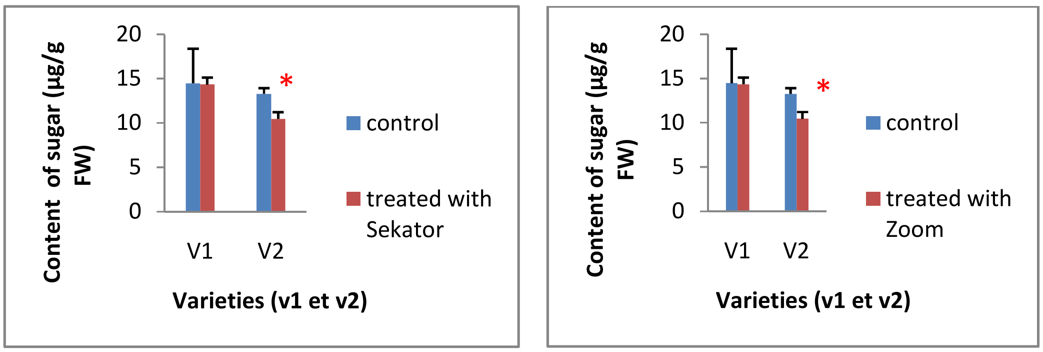


Fig. 5. Effect of Sekator and zoom on sugar content (µg / g FW) in two wheat varieties Waha (V1) and HD (V2)

carbohydrate and chlorophyll was found. Indeed, carbohydrate derived from photosynthesis process involves molecules of chlorophyll a, b and carotenoids for collecting light energy which is transmitted to various of the photosynthetic electron transport chain in order to reduce the NADP to NADPH+H+ and ATP to phosphorylate ADP+Pi. NADPH and ATP so produced are used to reduce CO_2 in the organic molecules such as carbohydrates. More the number of molecules of chlorophyll *a*, chlorophyll *b* and carotenoid are important, more the absorbed bright energy is important and the photosynthetic chain can work at the most of its capacities.

4. CONCLUSION

This work shows that oxidative stress exerts in both varieties of wheat. A depressive effect on the physiological and metabolic parameters studied, but it does not affect in the same manner. The degree of affection depends on the intensity of the stress and variety. Indeed, we showed that levels of chlorophyll *a* and *b* are very sensitive parameters, which can provide information on the tolerance of wheat varieties to the toxicity of herbicides. Waha variety showed reduction rate of chlorophyll *b* 17, 79% for Sekator and 29, 45% for herbicide Zoom against reduction rates ranging from 40, 43% for Sekator and 28, 98% for herbicide Zoom in HD variety. Moreover, it should be noted that the chlorophyll *b* content is more sensitive to the effect of herbicide stress than chlorophyll *a*. The evolution of the proline content in different varieties also confirmed the results previously indicated. Waha variety showed a slight non-significant increase of proline content. In contrast, in the sensible variety (HD), proline content showed a significant increase indicating some metabolic perturbation. Overall, hard wheat appear to be more tolerant than common wheat. However, the order of tolerance varies depending on the evaluation criteria used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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