*Available online* **www.ijpras.com**

**International Journal of Pharmaceutical Research & Allied Sciences, 2016, 5(4):159-168**


**Research Article**

**ISSN : 2277-3657**
**CODEN(USA) : IJPRPM**

# *Phytotoxic effects of a sulfonylurea herbicide on two varieties of durum wheat (Triticum durumDesf)*

***FERFAR MERIEM[1], MEKSEM AMARA LEILA[1], GRARA NOUDJOUD [2], MEKSEM NABILA[1] , BENSAID MAROUA[1] and DJEBAR MOHAMMED REDA[1].***

*[1] Cellular Toxicology Laboratory, Department of Biology, Faculty of Sciences, Badji Mokhtar-Annaba University, P.O. Box 12, 23000 Annaba, Algeria.*

*[2] Department of Biology, SNV-STU faculty, 8 Mai 1945 University, Guelma, BP 401,2400 Algeria*

*E-mail:grara120@yahoo.fr*

---

**ABSTRACT**

*Sulfonylureas (herbicides) act by inhibiting acetolactate synthase, an enzyme responsible for the biosynthesis of amino acids. Studies have shown that perspiration and metabolism in weeds became almost zero few hours after application of the herbicide. In this work, we have studied several parameters: percentage germination after 48 and 96 h of treatment, leaf surface, the water content of the leaf, Content of total protein and measuring the catalase activity in* ***Triticum durum Desf:*** *Varieties Semito and Cirta. The goal is to detect oxidative stress phenomena under the effect of different concentrations of a sulfonylurea: Sekator$^{OD}$, using the following concentrations: in vitro (0, 3, 6 and 12 nMol) and in vivo (0, 56, 112 and 225 nMol), after 14 days of treatment. Our results show that treatment (Sekator) disrupts the process of seed germination and plant growth and confirm the presence of oxidative stress, which results in a significant loss of water in the cells, causing a voltage between the plasma membrane and the cell wall. The plant responds to this dehydration by reducing the leaf area and the prolonged closure of the stomata, which causes disruption of the photosynthetic process while contributing to the conservation of water resources. We also recorded a reduction of the total protein content and a stimulation of catalase activity in a dose-dependent effect. The strong stimulation of proteolysis is generated to produce the AA necessary for the survival of the plant. Stimulation of catalase activity can be explained by the activation of detoxification mechanisms.*

**Keywords**: *herbicide, oxidative stress, sekator$^{OD}$, sulfonylurea, Triticum durum.*

---

## INTRODUCTION

Wheat is one of the major cereal crops in the temperate zones and represents an important component of the human diet because of the universal use of its grains for the production of flour and semolina, the basic ingredients of bread and other bakery product and pasta **(1)**. Besides starch, proteins, dietary fiber and minerals, whole wheat grains are known for their unique health value due to their high and peculiar content in non-nutrient biologically active compounds, known as phytochemicals. They include a wide variety of both waters- and fat-soluble compound: phenolic acids (belonging to the benzoic and cinnamic acid families), flavonoids, anthocyanidins, lignans, carotenoids, tocotrienols, tocopherols, phytosterols **(2).**

Cereals hold an important place in agricultural research programs. In Algeria, this place is more important because the country wants to achieve stable production of cereals, especially concerning wheat and barley **(3).** 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 **(4).**

In Algeria, the usage of quantitative and qualitative, but disorganized in general, of pesticides, is common as agricultural practice. This may be due to its climate and the biological activity in soils that these elements undergo a better dynamic **(5).** Among many homologous pesticides in Algeria; forty are widely used by farmers **(6).**

In recent years, several herbicides as individual or pre-mix formulations belonging to a different family group specially sulfonylurea were registered in Algeria for weed control of durum wheat. Sulfonylurea group shows extremely high herbicidal potency at a very low dose that reduces the amount of chemical applied to the field very lower than conventional herbicides. **(7).**

Herbicides that inhibit amino acid biosynthesis are useful tools in weed management and have been particularly successful because of their low toxicity in mammals, as these herbicides inhibit pathways that are lacking in mammals. There are several types of herbicides whose targets or primary sites of action are associated with the specific inhibition of enzymatic activity in the biosynthetic pathways for amino acids. One such group of herbicides comprises compounds that inhibit the biosynthesis of branched-chain amino acids (Valine, leucine, and Isoleucine) through the inhibition of acetolactate synthase (ALS, EC 4.1.3.18), also referred to as acetohydroxyacid synthase **(8)**

ALS inhibitors include the active ingredients of several classes of chemicals and have become one of the most widely used types of herbicides because of their wide-spectrum weed control activity, high crop selectivity, low required application rates and low toxicity in mammals **(9).**

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 **(10).**

All plants have evolved a series of non-enzymatic and enzymatic antioxidant systems to cope with herbicidal stress and avoid Photodynamic damage by either stress tolerance or stress avoidance. Nevertheless, little, if any, research has explored the antioxidant activities on neonicotinoid **(11).**

A wide variety of plant response assessment techniques have been used to measure effects on non-target plants. These included visual symptom, plant height, plant biomass, root, leaf growth and flowering response, and complex measurements of such as biochemical and nutritional response **(12).**

This study aims to evaluate the effect in the laboratory of the sulfonylurea herbicide Sekator on the seed germination percentage, the leaf area, the water content of the leaves and on the biochemical composition (total protein) and activity of a specific enzyme oxidative stress: catalase in two varieties of ***Triticum durum* Desf**: Semito and Cirta.

## Materials and Methods

### 2.1. Biological material

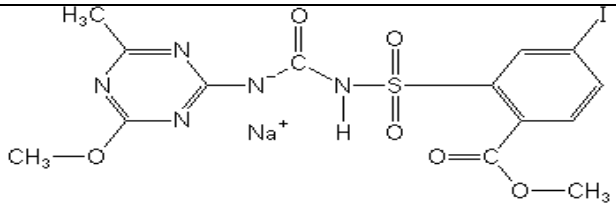
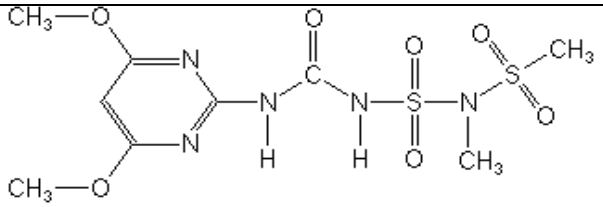
The biological material used in this work is durum family Poaceae specifically ***Triticum durum* Desf**. The samples come from the Interprofessional Algerian Office of Cereals (IAOC) El Hajar, Annaba, Algeria. We used two varieties: Semito and Cirta.

### 2.2. Herbicide

Sekator® OD offers unparalleled performance for the control of broadleaf weeds present in the wheat fields. It contains two active substances (Table 1): Iodosulfuron–methyl-sodium (25g/l) and Amidosulfuron (100g/l). This herbicide belongs to the sulfonylureas, it has an oily formulation miscible with water.

Iodosulfuron- methyl- sodium and Amidosulfuron are absorbed by the leaves, they are carried by ascending and descending systems engineering in the adventitia. They block the synthesis of essential branched-chain amino acids: Valine, leucine, and Isoleucine, responsible for cell division in the meristems of plants by inhibiting acetolactate synthase (ALS) **(13).**

**Table 1:** Chemical structure of active substance of herbicide Sekator (14).

| Active substance | Chemical structure | Chemical formula |
|---|---|---|
| Iodosulfuron-methyl-sodium |  | $C_{14}H_{13}IN_5NaO_6S$ |
| Amidosulfuron |  | $C_9H_{15}N_5O_7S_2$ |

**2.3. Analyzes and Measurements**

**2.3.1. Seed germination percentage**

Seeds of *Triticum durum*Desf: Semeto and Cirta, were sterilized with 5% sodium hypochlorite for 3 min. After being washed with distilled water several times, the seeds were incubated in Petri dishes containing 3, 6and 12 nMol of the systemic herbicide Sekator. The experiment was performed in 9 cm diameter sterilized Petri plates containing filter paper soaked in Herbicide solutions. 10 seeds were placed in each Petri plate separately. Untreated Petri plates served as control. The seeds were germinated under controlled conditions. Small amounts of respective herbicide's solutions were added when it was obvious that Petri dishes were beginning to dry out. The number of germinated seeds was scored after 48 and 96 hours. All tests were repeated three times **(15).**

**2.3.2. Performing the Physio-biochemical tests**

The culture was carried out in plastic pots filled with a mixture of soil, potting soil and gravel (5V: 4V: 1V). Using a pencil, we made holes about 2 cm, and we deposited 10 seeds per pot. A spray of 150 ml of the herbicide's solutions (0, 56, 112 and 225 nMol) per pot was performed three times per week for 14 days. (Faucet water for controls and the prepared solutions for treated)

**2.3.3. Measuring of the Leaf Area**

It is estimated by the method of **(16)**. Which consists in the determination of WF: the weight of the layer representing the leaf, Wq: corresponding to a known area Aq of a square of the same paper layer. It is calculated using the following formula:

LA ($cm^2$) = (WFxAq($cm^2$)) / Wq($cm^2$).

**2.3.4. Measuring the Water Content of the leaves**

The amount of water contained in the leaves is calculated from their fresh weight (FW) and dry weight (DW) determined after drying in an oven at 105 ° C.

The average grade is calculated using the formula of **(17)**:

WC (%) = (FW-DW) / FWx100

**2.3.5. Determination of the total protein content**

The total protein of *T.durum* is made by the method of **(18)**, using Coomassie blue (G250, Merck) as a reagent and bovine serum albumin (BSA, Sigma) as a standard protein. The absorbance reading is carried out at the wavelength of 595 nm.

### 2.4. Enzymatic assay

#### 2.4.1. Determination of catalase activity (CAT)

The spectrophotometric assay of catalase activity (CAT) is produced by the method of

**(19)**. The decrease in the DO is recorded for 1 min, for a wavelength of 240nm, and a linear molar extinction coefficient ξ = 39.400cm.M.L. To a final volume of 3ml. The reaction mixture contained 100µl of the crude enzyme extract, 50µl of hydrogen peroxide $H_2O_2$ was 0.3% and 2.8 ml Na K buffer (50 mM Na K, pH = 7.2). The reaction is initiated by the addition of hydrogen peroxide and catalase activity is expressed in nmol /min / mg protein.

### 2.5. Statistical analysis

The results were analyzed statistically using the Minitab16. The data are represented by the mean plus or minus the standard deviation (m ± SD). Means were compared in pairs with the Student t test. The significance level was $p \leq 0.05$.

## Results

### 3.1. Seed germination percentage

Figure 01 and 02, represents the effect of Sekator on the seed germination percentage. For Semito variety, a highly significant difference ($p \leq 0.01$) was recorded for the treated with the C3 (12 nMol) after 96h and a significant difference ($p \leq 0.05$) was observed in all the rest of the treaties compared to the control after 48 and 96 hours of treatment. For Cirta variety, after 48h of treatment, a non-significant difference ($p > 0.05$) were observed in the treated with the C1 (3 nMol), a significant diminution ($p \leq 0.05$) of the seed germination percentage of the treated with the C2 (6 nMol) and a highly significant diminution for the treated with the C3. After 96h, Cirta seeds present a non-significant diminution of the germination percentage of the treated with the C1 and C2 and a highly significant difference in the treated with the C3.

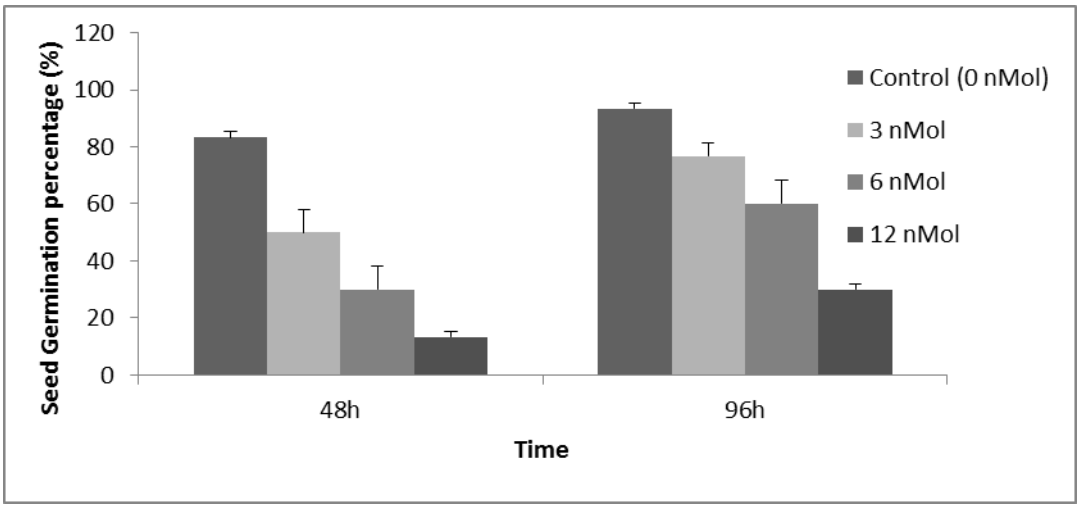



**Figure 01.** Effect of three concentrations of Sekator on the seed germination percentage in *T.durum*: Variety Semeto, after 48 and 96 hours (m±SD, n=3).

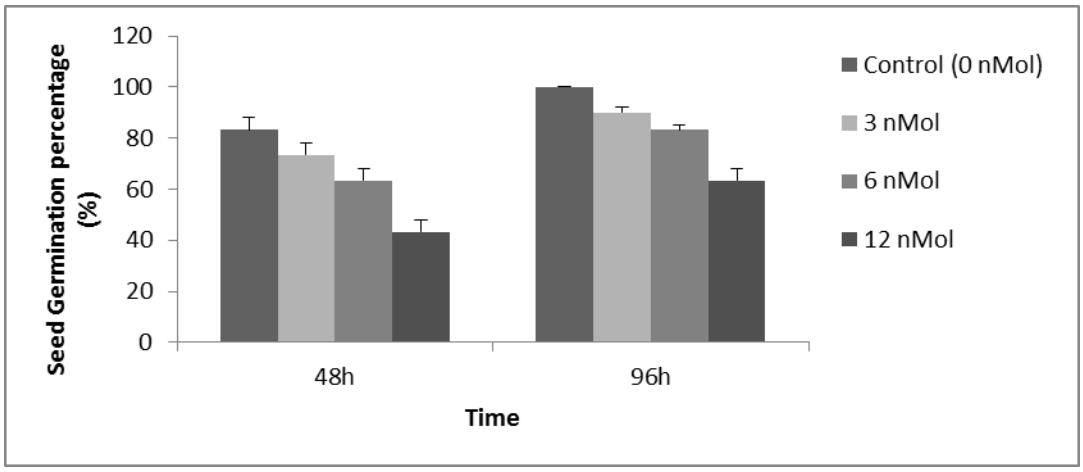



**Figure 02.** Effect of three concentrations of Sekator on the seed germination percentage in *T.durum*: Variety Cirta, after 48 and 96 hours (m±SD, n=3).

### 3.2. Measuring of the Leaf Area

The results of the measurement of the leaf area after 14 days of treatment by the Sekator on ***T. durum*** are represented in figure 03. For Semito variety, a significant ($p \leq 0.05$) decrease of the leaf area was recorded when we treated with the C1 (56nMol) and a highly significant ($p \leq 0.01$) decrease for the treated with C2 (112 nMol) and C3 (225nMol) compared to the control. For Cirta variety, we recorded a very highly significant ($p \leq 0.001$) decrease of the leaf area for all the treated.

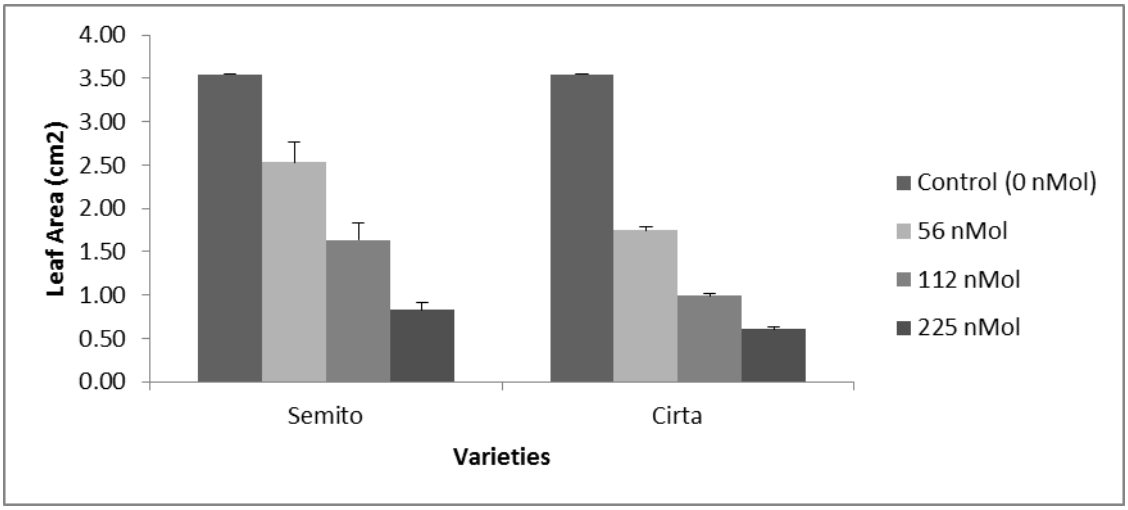



**Figure 03.** Effect of three concentrations of Sekator on the Leaf Area in *T.durum* Varieties: Semito and Cirta, after 14 days of treatment (m ± SD, n=3).

### 3.3. Measuring the Water content of the leaves

Figure 04; show the results of the water content of the leaves after 14 days of treatment with Sekator. For the Semito variety, we observed a significant ($p>0.05$) decrease in the water content when we treated with the C1 (56 nMol) and a very highly significant decrease for the treated with C2 (112nMol) and highly significant decrease for the treated with C3 (225nMol). For the Cirta variety, we noticed a highly significant decrease ($p >0.01$) of the water content for the treated with the C1, C2, and the C3.

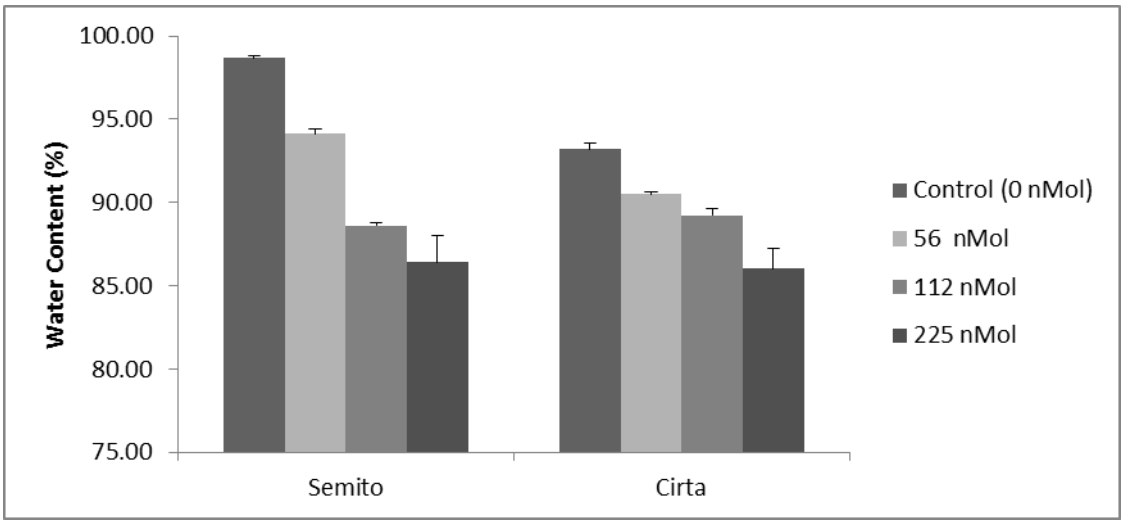



**Figure 04.** Effect of three concentrations of Sekator on the water content of the leaves in *T.durum* Varieties: Semito and Cirta, after 14 days of treatment (m ± SD, n=3).

### 3.4. Effects of Sekator on the content of total protein Content (µg/mg of MF)

The figures 04, illustrate the effects of the Sekator on the total protein content among the two varieties, we note a non-significant stimulation of total protein ($p > 0.05$) of the treated with the C1 (56nMol) by contribution to the control, for the two varieties. For Semito, a highly significant ($p=0.01$) and very highly significant ($P= 0.000$) decrease was observed in the leaves of the treated with C2 and C3 respectively. For Cirta, a significant ($p < 0.05$) decrease was noticed for the C2 treaties and a highly significant decrease ($p < 0.01$) for the C3 treaties.

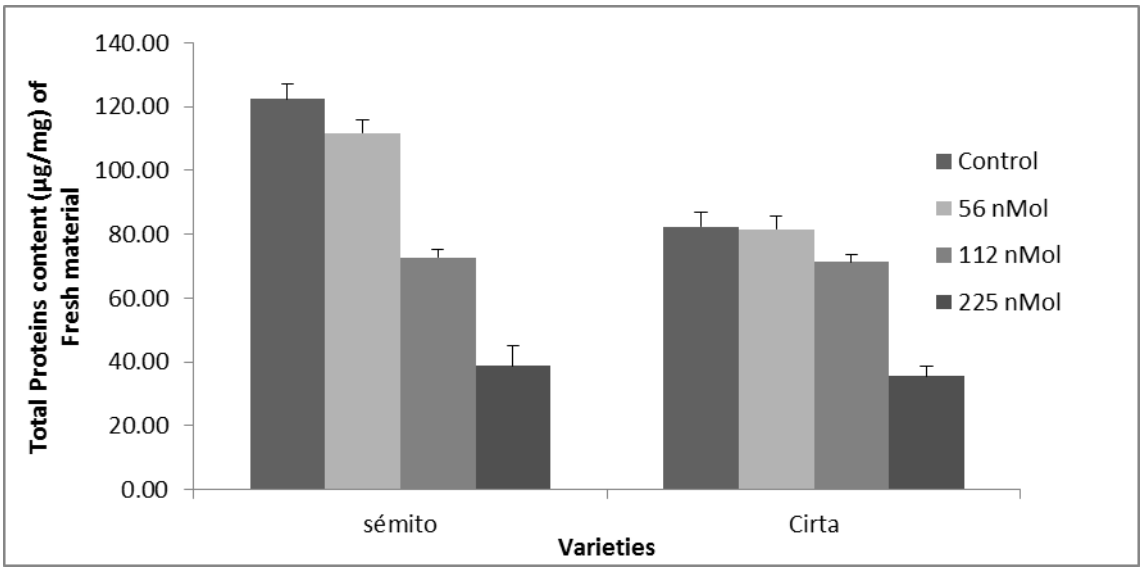



**Figure 05.** Effect of three concentrations of Sekator on the total protein content in *T.durum* Varieties: Semito and Cirta, after 14 days of treatment (m ± SD, n=3).

### 3.5. Measuring of the Catalase activity

The figure 06, represent the effect of Sekator on the catalase activity. The obtained results reveal that the catalase activity increases significantly ($p < 0.05$) for the treated with the C1 (56 nMol) for the Cirta variety but it is not significant for the treated with the C1 and C2 for Semito variety and also increases highly significantly for the

treated with the C2 ( 112 nMol) for Cirta variety, and increasses with highly significant differences for the treated with C3 (225nMol) by the contribution to the control of the both varieties.

(6).

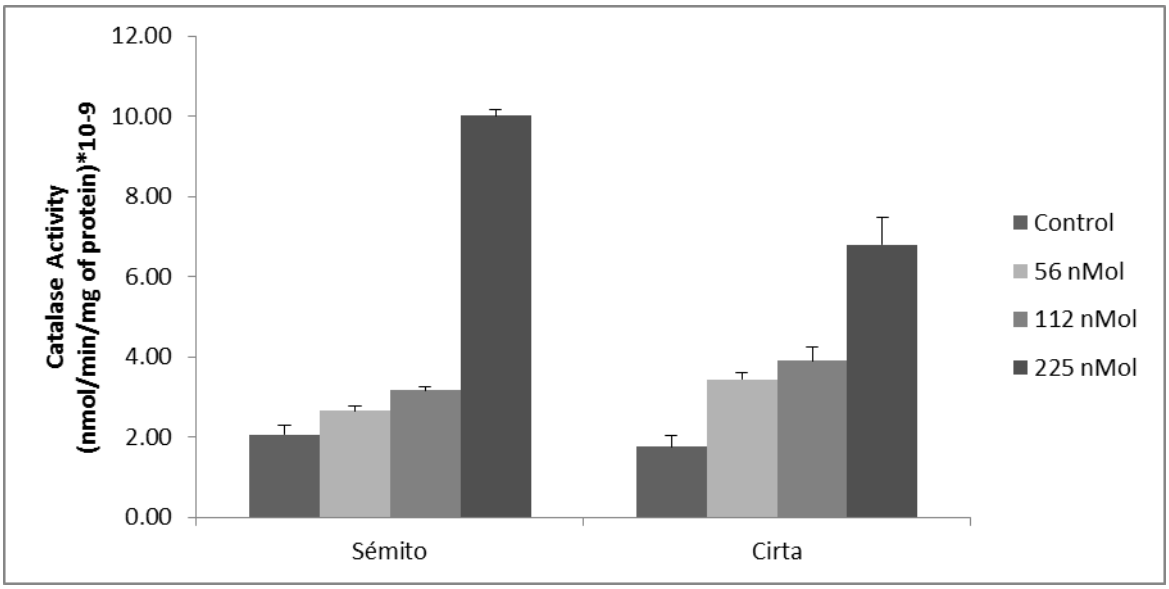



**Figure 06.** Effect of three concentrations of Sekator on the Catalase activity in *T.durum* Varieties: Semito and Cirta, after 14 days of treatment (m ± SD, n=3).

**Discussion**

In plant cells, an antioxidant defense mechanism has been developed for protection against reactive oxygen species and avoids the failure of the control system. To analyze the level of oxidative stress induced by treatment with Sekator in leaves of ***T. durum***, we measured several parameters related to stress, with a counting of seed germination percentage, the measuring of the leaf area, the water content on leaves, the rate of total protein, and the activity of a detoxification enzyme, namely Catalase (CAT).

The application of the three concentrations of Sekator has a harmful effect on the seed germination percentage, and a significant decrease was recorded for the two varieties. The results of **(15); (20); (21)** show that under abiotic stress the germination processes are disrupted and a decrease of the seed germination percentage was noticed. However, the results of **(22)** indicate that seed pre-soaking for 2 and 4 h with $La^{3+}$ and a with a mixture of different REEs at low concentrations has no effects on the germination of ***T. durum*** seeds.

The results show that the treatment with the herbicide significantly reduced the leaf area in the two varieties of ***T.durum.*** This can be the response of vegetables to the dehydration; it contributes to the preservation of water resources, what allows the survival of the plant **(23).** Our results are similar to those in works of **(24)** where the leaf area of durum wheat was significantly reduced under a water stress. The results of **(25)** are also similar to these results.

The measurement of the water content in leaves shows a significant decrease in Semito and cirta. This decrease resulted from the reduction of water loss through stomatal closure; this action is a means of plant adaptation to unfavorable environmental factors **(26).** These results are in agreement with those of **(24)** which states that water stress showed a sizable drop in the water content of leaves.

The observed results for the determination of total protein in leaves indicate a significant decrease of the protein during treatment with high concentrations. An increase in the amino acid content and a decrease in the soluble protein content decrease is very well-known effects of herbicides inhibiting amino acid biosynthesis. The increased amino acid pool is thought to be derived from a rise in protein turnover, suggesting that proteases might be involved

in protein degradation to provide plants with amino acids that cannot otherwise be synthesized due to herbicide inhibition **(8).** However, the work of **(21)** shows a significant stimulation of protein synthesis due to salt stress in the variety Benbachir, unlike Semito range where no effects were recorded**. (27)** have also recorded a very significant increase in protein levels by treating Elodea canadensis and Lemna minor by the Callifop 36EC which suggests a protein availability in the leaf tissue can essentially be used in the development of the reproductive process.

The stimulating effect observed in both varieties following treatment with the Sekator, on catalase activity, key antioxidant enzymes in the cell, is a response to the overproduction of $H_2O_2$, thus absorbing active ingredients in the pesticide would be responsible for the synthesis of free radicals in large quantities thus generating a real oxidative stress **(15).** It is well known that many abiotic stresses induce an increase in the antioxidant systems that may not be able to counteract the negative effects on plant growth and/or metabolism **(28); (29); (27); (20); (21).**

The results obtained in our study show that the herbicides that inhibit amino acid biosynthesis cause a slow plant death, characterized with a several physiological effects and disequilibria of the plant metabolism. The application of different concentrations of Sekator on the durum wheat varieties: Semito and Cirta seriously affect the seed germination. The treatment with the stronger concentration causes an important decrease of the leaf area and the water content of the leaves to preserve water resources and avoid water loss through stomatal closure. To provide plants with amino acids that cannot be synthesized due to herbicide inhibition a reduce in the content of total protein is explained by their degradation. The potential for the accumulation of sekator in *T.durum* and the capacity of this plant survived the xenobiotics in developing antioxidant enzymes that protect cells metabolizing the pesticide in question. A stimulation of the activity catalase has been recorded to help plant to survive the oxidative stress.

**References**

1. Adom, KK., Sorrells, ME., Liu, RH. (2003). Phytochemical profiles and antioxidant activity of wheat varieties. Journal of Agricultural and Food Chemistry 51: 7825e7834.

2. Liu, R.H. (2007). Whole grain phytochemicals and health. Journal of Cereal Science 46: 207-219.

3. Issaad, G. (2013). Statut nutritionnel et plasticite de réponses aux stress chez un modèle végétal: Triticum durum Desf. Thèse de doctorat LMD. UniversitéBadji Mokhtar Annaba.

4. Samuel, O., Saint-Laurent, L. (2001). Prevention guide for users of pesticides in vegetable farming.

5. Zouaoui, A., Cheloufi, R., Messaadia, H. (2013). The impact of herbicides on the dynamic of the organic carbon in soil. Albanian j. Agric. Sci. University of Tirana 12 (2): 223-228.

6. Bouziani, M. (2007). L'usage immodéré des pesticides. De graves conséquences sanitaires. Le guide de la médecine et de la santé. Santé Maghreb.

7. Bahrampor, T., SharifiZiveh, P. (2013). Effects of Residue Sulfonylurea Herbicides on Wheat. International Journal of Agronomy and Plant Production4 (10): 2707-2713.

8. Zulet, A., Gil-Monreal, M., Villamor, J.G., Zabalza, A., van der Hoorn, RAL. *et al* (2013). Proteolytic Pathways Induced by Herbicides That Inhibit Amino Acid Biosynthesis. PLoS ONE 8 (9): e73847 do: 10.1371/ journal. Phone. 0073847.

9. Zhou, Q., Liu, W., Zhang, Y., Liu, K. (2007). Action mechanisms of acetolactate synthase inhibiting herbicides. PesticBiochemPhysiol89: 89–96.

10. Ramel, F. (2009). The implication of soluble sugars in response to xenobiotic and oxidative stress in *Arabidopsis thaliana*. Thesis status in Life Sciences and Environmental Ph.D. University of Rennes.

11. Yao, X., Min, H., Lü, Z., and Yuan, H. (2006). Influence of acetamiprid on soil enzymatic activities and respiration. European Journal of Soil Biologie, 2:120-126.

12. Obrigawitch, T.T., Cook, G., Wetherington, J. (1998). Assessment of Effects on Nontarget Plants from Sulfonylurea Herbicides Using Field Approaches. Pestic. Sci52:199-217.

13. Bayer Crop Sciences, fiche données de sécurités conformément au règlement (CE) N°1907/2006. Version 1 / EU, 2011.

14. Souahi, H., Meksem Amara, L., Grara, N., Djebar, M.R. (2015). Physiology and biochemistry Effects of Herbicides Sekator and Zoom on Two Varieties of Wheat (Waha and HD) in Semi-Arid Region. Annual Research & Review in Biologie, 5 : 449-459.

15. Meksem, L. (2007). Effects of two fungicides: Flamenco SC and Tilt 250 EC on the physiology, growth, and energy metabolism of the roots of *Triticum durum* Desf: GTA varieties and Vitron. Ph.D., Badji Mokhtar University, Annaba, Algeria.

16. Paul, M.H., Planchton, C., Ecochard, R. (1976). Etude des relations entre le développement foliaire, le cycle de développement et la productivité chez le soja. Amélio plants 29 : 479 -492.

17. Monneveux, Ph., Nemmar, M. (1986). Contribution à l'étude de la résistance à la sécheresse chez le blé tendre et le blé dur ; étude de l'accumulation de la proline au cours de cycle de développement. Agronomie6: 583-590.

18. Bradford, M. (1976). A Rapid and Sensitive Method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochem* 72: 248-254.

19. Cakmak, Horst, W.J., (1991). Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidases activities in root tips of soybean (Glycine max), physiology plant 83: 463-468.

20. Issaad, G., Djebar, M.R., Berrebbah, H. (2013). Ros and redox signaling in the response of stems of wheat durum to abiotic stress. International Journal of Biosciences 3 (10): 298-305.

21. Graibia, H., Souiki, L., Derbel, N., Berrebbah, H., Djebar, M.R. (2014). Tolerance to salt stress applied on two local varieties of Durum Wheat, Benbachir, and Sémito. Journal of Biodiversity and Environmental Sciences, Vol. 4, No. 1: 54-58.

22. D'Aquino, L., Concetta de Pinto, M., Nardi, L., Morgana, M., Tommasi, F., (2009). Effect of some rare earth elements on seed germination, seedling growth and antioxidant metabolism in Triticum durum. Chemosphere 75: 900-905.

23. Lebon, E., Pellegrino, A., Tardieu, F., Lecoeur, J. (2014). Shoot development in grapevine is affected by the modular branching pattern of the stem and intra and inter-shoot trophic competition. *Annals of Botany ,* 93: 263 -274.

*24.* Mouellef, A. (2010). Caractères physiologiques et biochimiques de tolérance du blé dur (TriticumdurumDesf.*)* au stress hydrique. Thèse de magistère. UniversitéMentouri Constantine.

25. Sbartai, H., Djebar, M.R., Sbartai, I., Berrabbah, H. (2012). Bioaccumulation du Cd et du Zn chez les plants de tomates (*Lycopersiconesculentum L.)*. Laboratoire de toxicologie cellulaire. Université Badji Mokhtar Annaba. *Comptes rendus –Biologies* 335; 9 ; 585-593.

26. *Djekoun,* A., Planchon, C. (1992). Stomatal conductance photosynthesis and acetylene reduction rate in Soybean genotypes.*Can. J. Plantsci*72: 383 - 390.

27. Tlidjen, S., Meksem Amara, L., Bouchelaghem, S., Sbartai, H., Djebar, M.R. (2012). Effects of the herbicide Calliofop 36EC on growth, antioxidant enzyme response and respiratory metabolism in two aquatic plant *Elodea canadensis* and *Lemna minor*. Advances in Environmental Biology 6 (9): 2524-2530.

28. Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci9: 405–410.

29. Ippolito, M.P., Paciolla, C., d'Aquino, L., Morgana, M., Tommasi, F. (2007). Effect of rare earth elements on growth and antioxidant metabolism in *Lemna minor* L. Caryologia60: 125–128.