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Induction of oxidative stress in a variety of durum wheat (*Triticum durum* Desf) exposed to recommended doses of pesticides

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Abstract. The objective of this study was to assess the toxicity of two pesticides (Prosaro® XRT and Decis® EC 25) widely used in the agricultural region of El-Tarf located in northeastern Algeria, as well as their combinations on a variety of durum wheat "*Triticum durum* Desf". The toxicity of these products was evaluated using physiological (chlorophyll) and biochemical parameters (proteins, glutathione, catalase activity and glutathione S-transferase, acetylcholine esterase, lipoxygenase). The recommended dose and its double were tested individually and in combination for this. It should be noted that the protocol used and the initial concentrations selected are the same as those used in the field. After D7 and D14 of exposure, all dosages were administered. The results obtained revealed a decrease in chlorophyll contents and Glutathione levels as well as an induction of total proteins and the differents enzymatic activity (catalase, glutathione S-transferase, lipooxygenase) and this for the two root and leaf compartments. Thus, it turns out that the concentrations used in open fields are not harmful to the plant but generate free radicals which are taken care of by the latter's defense system, thus allowing it to tolerate these stress conditions.

Keywords: toxicity, pesticides, *Triticum durum*, oxydative stress, stress biomarkers.

Introduction

The use of pesticides worldwide has increased dramatically coinciding with changes in agricultural practices and intensive farming (Konstantinou *et al.*, 2006). However, these chemical products are not without drawbacks, in particular by their toxic effects for non-target organisms such as beneficial insects, the contamination of soils and waterways, the pollution of groundwater as well as by their harmful effect on human health (Mebdoua *et al.,* 2017). Indeed, fungicides and insecticides are the most effective means of combating major diseases and pests of cultivated plants, which are necessary in maintaining or even increasing agricultural yields. However, most of these molecules are highly toxic and difficult to biodegrade. Their massive and repeated use can have harmful consequences for all components of the environment (Hafez *et al.,* 2020).

Application of chemical fungicides has been considered the primary method of protecting crops from many diseases due to their convenience and low cost (Xiao *et al.,* 2006). Although the effects of the latter are confirmed in controlling diseases and increasing crop yields, their toxic effects on crop plants have not been well studied, on the other hand, some studies have shown that they can affect plant respiration (Untiedt *et al.,* 2001), the synthesis of secondary metabolites (Mohamed *et al.,* 2017), the synthesis of plant hormones (Zhang *et al.,* 2020), chlorophyll synthesis and degradation and photosynthesis (Petit *et al.,* 2008). Similarly, insecticides have been shown to cause oxidative stress in plant cells, affecting various metabolic activities and plant growth components (Toscano *et al.,* 1982; Jones *et al.,* 1986). Several authors have demonstrated that pesticides in general induce oxidative stress in different species due to the production of reactive oxygen species (ROS) (Amamra *et al.,* 2014; Saillenfait *et al.,* 2015; Ferfar *et al.,* 2016; Sbartai and Sbartai, 2021; Belaid and Sbartai, 2021). To repair the damage induced by these ROS, plants have developed a complicated method of antioxidant enzyme system (superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX)), which can effectively maintain redox homeostasis in plant cells by removing excess ROS (Apel *et al.,* 2004).

Algeria is ranked among the countries that use a large amount of pesticides and their use continues to increase in many areas. Thus about 400 phytosanitary products are approved, of which about forty varieties are widely used by farmers (Bordjiba *et al.,* 2009). Among them are pyrethroids, which are a class of insecticide that has recently appeared to replace organophosphates and organochlorines (Horton *et al.,* 2011; Saillenfait *et al.,* 2015). The latter have a higher toxicity thanks to their lipophilic nature allowing them to accumulate in fatty tissues. They are also able to produce ROS during their metabolism, thus altering the

integrity and the function of the cell and its organelles, particularly the mitochondria which produces more ROS likely to generate an imbalance in the redox status causing a respiratory disturbance see even apoptosis or necrosis (Ambolet-camoit *et al.,* 2012; Hossain *et al.,* 2014). Deltamethrin is frequently used in our region to protect cereal crops against pests, it is considered among the most toxic pyrethroids (TianhuiJiaoa *et al.,* 2021). Indeed, the frequent use of this molecule increases the risk of contamination in humans through the inhalation of suspended particles as well as through food (Saillenfait *et al.,* 2015). Following this accumulation, it results in a disruption in the sodium channels of the axons thus causing irritation of the upper tract, dizziness, vomiting and paresthesia (Wolansky and Tornero-Vélez, 2013). At the same time, and among the most used fungicides, we find the triazoles, which have been a well-known family for thirty years now, having an action that is both preventive and to a certain extent curative. At the systemic level, the toxic effects of triazoles lead to hormonal imbalance (Yang *et al.,* 2014), nitrogen imbalance, lower germination rates, impaired root growth and development (Serra *et al.,* 2013, 2015) and the appearance of chromosomal abnormalities (Wandscheer *et al.,* 2017).

Countless studies have reported the toxic effects of these xenobiotics and more particularly tebuconazole and prothioconazole on the defense mechanism of different species such as tomato, wheat, sweet potato and soybean (Nagajothi and Jeyakumar, 2016; Maruthaiya Arivalagan and Ramamurthy Somasundaram, 2017; Shishatskaya *et al.,* 2018; Mohsin *et al.,* 2021; Wang *et al.,* 2023). Thus, the objective of this study consisted in the evaluation of the toxicity of two pesticides Prosaro® (tebuconazole + prothioconazole) and Decis® (deltamethrine), frequently used in agriculture, as well as their combinations, at the recommended doses in open fields, in a variety of durum wheat (*Triticum durum*). Toxicity was monitored through the monitoring of certain stress biomarkers in order to confirm whether these doses are really not harmful and do not affect this plant and thus highlight the capacity of the latter to tolerate the stress conditions represented by our pesticides.

Materials and methods

Biological material

The biological material used in our work was a variety of durum wheat: *Triticum durum* Desf. The variety chosen is Siméto (Italian variety) from the Algerian Inter-professional Office of Cereals (O.A.I.C.) of El Hadjar-Annaba. It is an early variety with average productivity, it is recommended in semi-arid and intermediate arid zones, resistant to rain and drought and tolerates cold.

Chemical material

Two pesticides (Prosaro® XRT and Decis® EC 25) commonly used in the agricultural region of El-TARF in northeastern Algeria were used for this study. Prosaro® XRT is a triazole drug that combines the effects of two active ingredients: prothioconazole and tebuconazole at equal concentration ($125gL^{-1}$). It is a fungicide known for its high efficiency, versatility, and persistence against various cereal diseases. Decis® EC 25 (deltamethrin), a pyrethroid insecticide, is used as an insecticide and snake repellent due to its neurotoxic properties.

Experimental protocol

The wheat seeds used were first disinfected (1mL of 10V hydrogen peroxide with 9mL of distilled water) for 5 min then rinsed thoroughly with distilled water. To facilitate and accelerate germination, the seeds are put in distilled water in the refrigerator for 24 hours (vernalisation). The seeds were then sown in cells filled with a sand / compost mixture (2 volumes of sand / 1 volume of compost) at the rate of 3 seeds for each cell. It should be noted that the bottom of the cells is lined with a layer of gravel to ensure drainage. Watering was carried out twice a week at the rate of 20mL of distilled water per cell until the development of the seedlings. At the same time, the medium was enriched with nutrients thanks to a nutrient solution (Hoshang, 1988) added every 15 days for the various trials. The treatment with the different concentrations of Prosaro® and Decis® alone as well as their combinations (Prosaro®/Decis®) was carried out after 4 weeks from sowing at the 2-3 leaf stage. Regarding the combined treatment, the protocol followed in vitro is identical to that used in the field where it was a question of applying the Prosaro® first and then the deltamethrin at an interval of one month. Concerning the concentrations, we have chose those used by the farmers as well as the double of these. For Prosaro® XRT, the dose used is 0.8 L h^{-1} (P1: 0.66mg kg^{-1} of dry soil) and its double (P2: 1.33mg kg^{-1} of dry soil), for Decis® EC25 1L/h (D1: 0.83mg kg^{-1} of dry soil) and its double (D2: 1.66mg kg^{-1} of dry soil). As for the combined treatment, we used: P1/D1 and P2/D2. All assays were performed after 7 and 14 days of treatment.

Studied parameters

Chlorophyll assay

The extraction of chlorophylls was carried out according to the method of (Holden, 1975), which consisted of macerating the plant in acetone. The samples were treated as follows: 1g of the leaves of the plant cut into small pieces and ground with 20ml of 80% acetone and approximately 100mg of calcium

bicarbonate ($CaCo_3$). After total grinding, the solution is then filtered and put in black boxes to avoid the oxidation of chlorophylls by light. The reading is done at the two wavelengths 645nm and 663nm, after calibrating the device with the 80% acetone control solution.

Total protein content

Total protein content was determined using Bradford's (1976) method, which employs Coomassie Brilliant Blue (G250) as a reagent and Bovine Serum Albumin (BSA 1mg ml^{-1}) as a reference standard for a calibration of spectrophotometer at a wavelength 595nm.

Glutathione levels (GSH)

The GSH level was measured using the Weckberker and Cori (1988) method. The optical density of 2-nitro-5-mercapturic acid was measured due to the reduction of 5,5'-dithiol-2-nitrobenzoic acid (Ellman's reagent or DTNB) by glutathione's (-SH) groups. After 5 min of rest, absorbance readings at 412nm were taken for color stabilization against a blank where the 500µL of the supernatant are replaced by 500µL of distilled water.

Monitoring of catalase activity (CAT)

The method of Cakmak and Horst (1991) was used to calculate CAT. For a final volume of 3mL, the reaction mixture contains: 100µL of the crude enzymatic extract, 50µL of H_2O_2 at 0.1% and 2850µL of phosphate buffer (50 mM, pH 7.2). The decrease in absorbance is recorded for one minute for a wavelength of 240nm. The calibration of the device is done in the absence of the enzymatic extract. The reaction is triggered by the addition of H_2O_2.

Monitoring of glutathione S-transferase activity (GST)

GST determination was carried out according to the method of Habig *et al.,* (1974). The enzyme source represented the fraction obtained after homogenization and centrifugation of leaves and roots. A 200µL aliquot of the supernatant was mixed with 1.2mL phosphate buffer containing 1 mM CDNB (0.1 M, pH 6). At a wavelength of 340nm, absorbance readings were taken every minute for 5 min.

Monitoring of lipoxygenase activity (LOX)

The method of Axelrod *et al.*, (1981) was used to monitor lipoxygenase activity. The leaves and roots were ground in the presence of an extraction buffer composed of 50 mM phosphate buffer (KH_2PO_4/K_2HPO_4; pH 7), 5 mM cysteine

and 10 mM EDTA. The homogenate obtained was centrifuged at 14000 g for 20 min and the supernatant was recovered for the LOX activity assay. The reaction medium, with a final volume equal to 1mL, is composed of 0.16% tween-20 (v/v), 0.2 M glycine buffer (pH 10.0), 100 mM linoleic acid and the enzymatic extract. This activity was determined by measuring the absorbance of the hydroperoxides at 234nm.

Statistical analysis

The results obtained were statistically analyzed using Minitab software (Version 14.0). Datawere represented by the mean plus or minus the standard deviation (m±SD). We used two-way analysis of variance (ANOVA) to evaluate differences related to the effects of two independent variables (concentration and time) on a dependent variable (parameter). P≤0.05 was established as a significant difference.

Results

Effects of different treatments on chlorophyll level

According to the table below (Tab. 1), we have noted a slight variation in the level of chlorophyll **a** in the leaves treated for 7 days by the low concentrations of two pesticides P1, D1 (21.20; 19.17) compared to the control leaves (21.71), while this same rate decreases significantly (p≤.0.05) for the leaves treated by the rest of the concentrations of Prosaro® and Decis® (P2, D2, P1/D1 et P2/D2).

However, the significant decrease (p≤0.05) in the level of chlorophyll **b** is recorded according to the exposure time and the different treatments compared to the control leaves except for Prosaro® P1 (18.39) where this decrease is very low compared to the controls (19.80). As for the values of chlorophyll (**a+b**) recorded after 7 days of exposure, they follow the same direction as for chl**a** and chl **b** with a reduction of almost half for the combined treatment Prosaro®/Decis® (P2/D2) with a value of (23.51) compared to controls (41.51). After 14 days of exposure, there is a significant decrease in chl **a, b, a+b** in the leaves treated at the different concentrations where the lowest levels are recorded for the highest doses of the combined treatment P2/D2. Regarding the **a/b** ratio, there is a significant increase as a function of time and of the concentrations of the different treatments. it is 1.09 in the controls and reaches the value of 1.26 in the leaves treated after 7 days at the Posaro®/Decis® concentrations. After 14 days of exposure, this ratio is at most P2/D2 (1.33), D2 (1.32) and P2 (1.28).

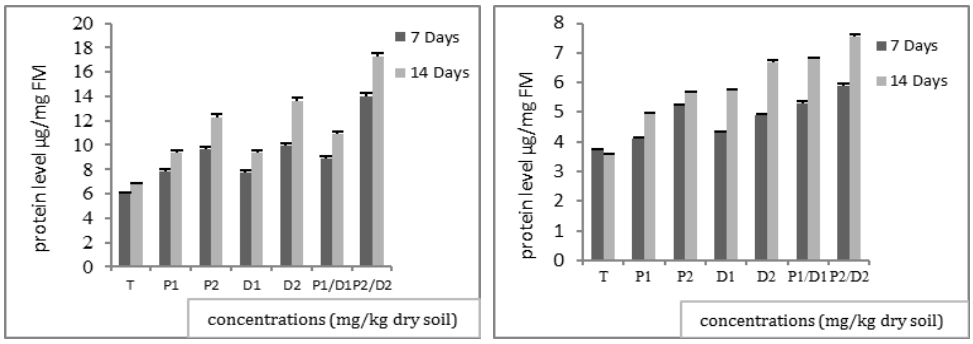
Table 1. Variation in chlorophyll level in weat leaves treated with different concentrations. Standard deviations are obtained from averages corresponding to three replicates ± SE and significant differences were established according to a two-way ANOVA (P≤ 0.05).

Concentrations	***Chl a***		***Chl b***		***Chla+b***		***Chl a/b***	
(mg kg^{-1} dry soil)	**7 days**	**14 days**	**7 days**	**14 days**	**7 days**	**14 days**	**7 days**	**14 days**
Control	21.71± 0.012	22.01± 0.315	19.80± 0.037	19.75± 1.028	41.51± 0.717	41.76± 0.201	1.09± 0.011	1.11± 0.208
P1	21.20± 0.109	20.13± 0.221	18.39± 0.146	16.71± 0.116	39.59± 0.632	36.84± 0.056	1.15± 0.105	1.20± 0.017
P2	18.50± 0.168	15.52± 0.511	15.43± 0.098	12.06± 1.03	33.93± 0.391	27.58± 1.031	1.19± 0.028	1.28± 0.005
D1	19.17± 0.092	16.94± 0.122	15.49± 0.136	13.45± 0.255	34.66± 0.088	30.39± 0.975	1.23± 0.033	1.25± 0.230
D2	15.57± 0.157	13.33± 0.116	12.92± 0.471	10.06± 0.521	28.49± 0.111	23.39± 1.012	1.20± 0.098	1.32± 0.102
P1/D1	16.84± 0.09	13.97± 0.344	13.58± 0.160	11.04± 0.219	30.42± 0.320	25.01± 0.058	1.23± 0.066	1.26± 0.154
P2/D2	13.13± 0.118	10.74± 0.608	10.38± 0.032	8.07± 0.014	23.51± 0.084	18.81± 0.167	1.26± 0.103	1.33± 0.083

Effect of different treatment on the level of total proteins

According to our results (Fig. 1a, 1b), we have observed a significant increase (p ≤0.05) in protein contents in wheat leaves (a) as a function of time and concentrations used, compared to the controls. The most marked values were reported after 7 days for the highest concentration of D2 (9.92 µg mg^{-1} of FM) and for the combination P2/D2 (13.99 µg mg^{-1} of FM) compared to control values (5.96 µg mg^{-1} of FM). Similarly, after 14 days where the highest protein levels were reported at D2 (13.63 µg mg^{-1} of FM) which is twice the control (6.72 µg mg^{-1} of FM) and the combination P2/D2 (17.23 µg mg^{-1} FM) which is almost three times the control (6.72 µg mg^{-1} FM).

The same observations were retained for the quantity of protein in wheat roots which increases significantly (p ≤0.05) as a function of time and the concentrations used of the two pesticides. This quantity reaches its maximum after 14 days with a value of 7.54 µg mg^{-1} FM at P2/D2 which is almost double the value recorded in control roots (3.55 µg mg^{-1} FM).

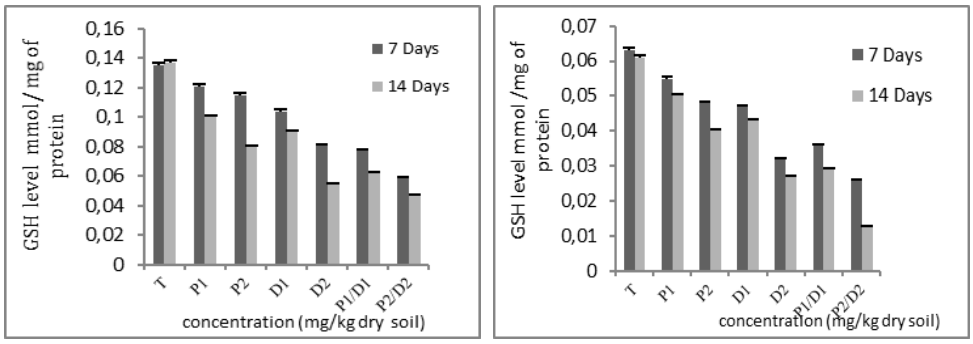


(a) (b)

Figure 1. Effect of different treatments on the variation of total protein level in leaves (a) and roots (b) of wheat . Significant differences were established according to a two-way ANOVA (P≤0.05).

Effects of different treatments on glutathione level (GSH)

According to (Fig. 2a, 2b), a significant decrease (p≤0.05) in the GSH level was recorded as a function of the time of exposure of wheat leaves and roots to different concentrations of the two pesticides as well as the combined treatment compared to the control. After 7 days of exposure, the greatest reduction (56%) in the level of GSH was recorded in the leaves treated with combination P2/D2 (0.059 µmol mg^{-1} of Prot) compared to the control value (0.135 µmol/mg of Prot). Similarly for the GSH levels recorded after 14 days where 66% reduction was noted for the combination P2/D2 (0.047 µmol mg^{-1} of Prot) compared to the control leaves (0.137 µmol mg^{-1} of Prot). It should be noted that after 14 days of exposure a strong reduction was also noted in wheat leaves treated with highest concentration of D2 (0.049 µmol mg^{-1} of Prot) which is almost equivalent to the values found for combination P2/D2. In the roots, the lowest content (0.016 µmol mg^{-1} of Prot) was reported for P2/D2 after 14 days of exposure compared to the control roots (0.063 µmol mg^{-1} of proteins), i.e. a reduction of 75%.

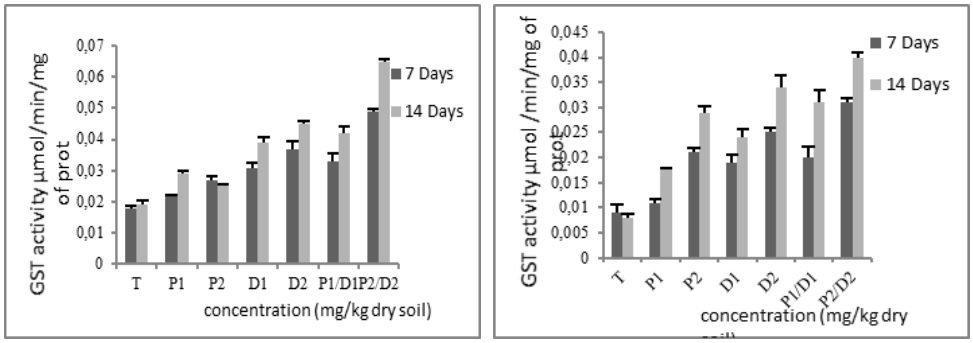


(a) (b)

Figure 2. Effect of different treatments on the variation ofGSH level in leaves (a) and roots (b) of wheat. Significant differences were established according to a two-way ANOVA (P≤0.05).

Effects of different treatments on glutathione S-transferase activity (GST)

According to (Fig. 3a, 3b), we have noted a significant induction (p≤0.05) of GST activity as a function of time and the concentrations of the different treatments compared to the control leaves and roots. Indeed, GST activity was at its maximum in the leaves (0.045 and 0.065 µmol min^{-1} mg^{-1} of Prot) after 14 days of treatment for D2 and combination (P2/D2) compared to controls (0.019 µmol min^{-1} mg^{-1} of Prot). The same observations were recorded in wheat roots but it should be noted that the values recorded in the leaves were higher than those recorded in the roots.

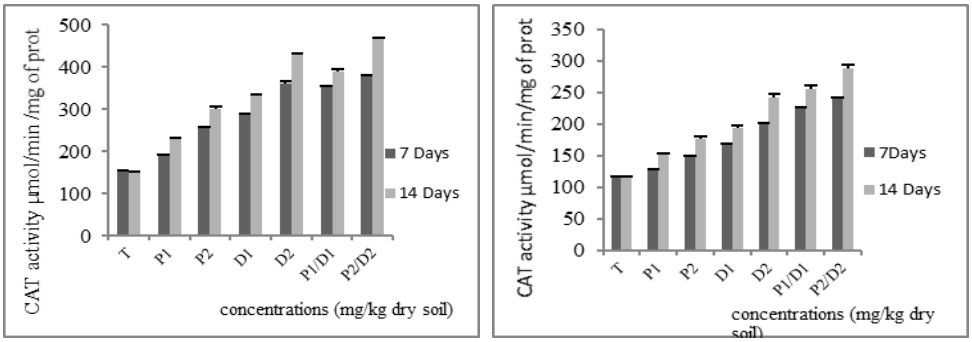


(a) (b)

Figure 3. Effect of different treatments on the variation of GST activity in leaves (a) and roots (b) of wheat. Significant differences were established according to a two-way ANOVA (P≤0.05).

Effects of different treatments on catalase activity (CAT)

According to the results mentioned in (Fig. 4a, 4b), we have observed a significant increase ($p \leq 0.05$) in CAT activity as a function of exposure time and increasing concentrations compared to control leaves and roots.



(a) (b)

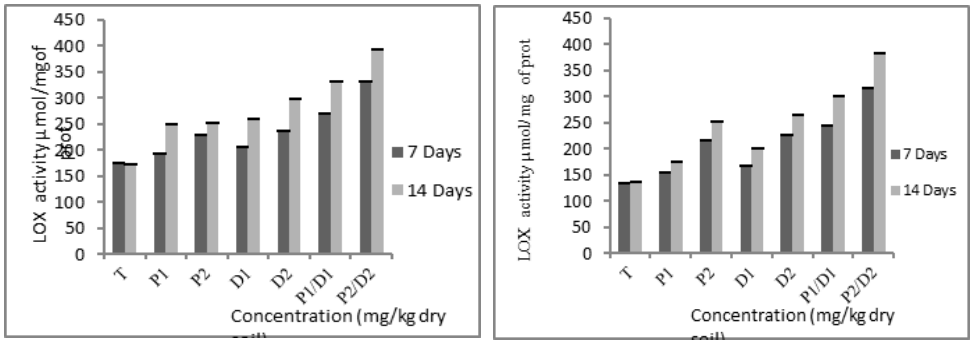
Figure 4. Effect of different treatments on the variation of CAT activity in leaves (a) and roots (b) of wheat. Significant differences were established according to a two-way ANOVA ($P \leq 0.05$).

After 7 and 14 days of exposure, the maximum values recorded in the leaves were reported for concentrations D2, P1/D1 and P2/D2 compared to the control values (150 µmol min^{-1} mg^{-1} Prot; 149.8 µmol min^{-1} mg^{-1} of Prot). In the roots, the maximum value was recorded after 14 days of exposure to the combination P2/D2 (288 μmol^{-1} min^{-1} mg^{-1} of Prot) compared to the control values (115 µmol min^{-1} mg^{-1} of Prot).

Effects of different treatments on lipoxygénase activity (LOX)

According to the figure below (Fig. 5a, 5b), wehave observed a significant increase ($P \leq 0.05$) in LOX activity as a function of the exposure time of the wheat leaves to the increasing concentrations of the different treatments compared to the controls. However, this activity was more stimulated after 14 days than after 7 days of exposure with maximum values reported for the high concentrations of the different treatments P2, D2, P2/D2 (250 µmol mg^{-1} of Prot; 295 µmol mg^{-1} of Prot and 390 µmol mg^{-1} of Prot) compared to control values which are equivalent to 171 µmol mg^{-1} of Prot.

In the roots, a significant increase ($p \leq 0.05$) in this activity was observed as a function of time and the concentrations of the different treatments where the maximum values were recorded after 14 days at the combination P2/D2 (381 µmol mg^{-1} of Prot) which represent three times the control value (134 µmol mg^{-1} of Prot).



(a) (b)

Figure 5. Effect of different treatments on the variation of LOX activity in leaves (a) and roots (b) of wheat. Significant differences were established according to a two-way ANOVA (P≤0.05).

Discussion

The impact of different treatments (Prosaro®, Decis®, Prosaro®/Decis®) on *Triticum durum* was evaluated using a biological approach at different scales of a cellular organization by examining the variations of several physiological and biochemical parameters. In our work we first tested the level of chlorophyll which is considered an excellent biomarker of plant toxicity knowing that there is a strong correlation between cell densities and photosynthetic fluorescence parameters in environmental pollution (Dewez *et al.,* 2007). Our results clearly show a decrease in leaf chlorophyll levels. This decrease can be attributed to the inhibition of its biosynthesis and photo-destruction of pesticides by reducing the formation of aminolevulinic acid (ALA) as a precursor of plant porphyrin essential for photosynthesis. Many studies have reported the negative effect of pesticides on chlorophyll levels in wheat leaves exposed to fungicides (Artea, Punch and Paclobutrazol) and herbicides (Cossack and Sékator) (Berova *et al.,* 2002; Ferfar *et al.,* 2016). Similarly, Liu *et al.,* (2021) showed that foliar exposure of common wheat (*Triticum aestivum* L) to difenoconazole induced a reduction in chlorophyll contents leading to a reduction in photosynthesis and the subsequent inhibition of plant growth.

At the same time, we focused on the response and regulation of the wheat defense system to these two xenobiotics. Indeed, the proportional increase in the total protein level observed as a function of increasing concentrations of the two pesticides as well as combinations in wheat leaves and roots tells us about the stress state of the plant. Gardés-Albert *et al.*, (2003) link this increase to the

fact that the plant seeks to protect its morpho-physiological integrity in response to damage induced by xenobiotics. In other words, protein accumulation is a molecular stress tolerance strategy that is directly linked to overproduction of ROS (Mishra et *al*., 2006). Thus, oxidative damage can be reduced by activation of the antioxidant defense system to eliminate these ROS (Pompeu *et al.,* 2017; Arfaoui *et al.,* 2018) hence the induction of total proteins. Indeed, to deal with the generation of ROS, plants reinforce their antioxidant action of the enzymatic and non-enzymatic defense system (Hasanuzzaman *et al.,* 2020). Thus, the decrease in GSH recorded in both leaf and root compartments of wheat supports the hypothesis of the induction of the plant's defense system to allow it to tolerate this state of stress. GSH, a non-enzymatic antioxidant, is a low molecular weight thiol involved in a wide range of metabolic processes and constitutes an important plant defense system against environmental stresses, including pesticides (Hossain *et al.,* 2012). GSH is the substrate of GPx which is involved in the elimination of H_2O_2 (Lu, 2013, Mailloux *et al.,* 2014). The recycling of GSSG in GSH is catalyzed by glutathione reductase (GR) using NADPH as an electron donor. NADPH is indispensable for GSH recycling by GR and high GSH levels can lead to reducing stress. NAD(P)H and GSH are reducing equivalents essential for the response to oxidative stress. Paradoxically, excessive accumulation of cellular NAD(P)H and/or GSH leads to reductive stress and cellular dysfunction. In addition, it is also involved in the modulation of cellular redox signaling, in the regulation of proliferation and cell death as well as in the detoxification of xenobiotics and their metabolites (Fratelli *et al.,* 2005; Lu, 2013; Aquilano *et al.,* 2014).

This decrease could be explained by the fact that GSH could establish a direct bond with pesticides or their metabolites (Galaris *et al.,* 2002). This glutathione-pesticide interaction takes place thanks to the intervention of GST which allows this conjugation during phase II of metabolism (Belaid and Sbartai, 2021)**,** this is confirmed by our results which indicate an induction of GST in the presence of pesticides tested. Indeed, GST is a multifunctional phase II enzyme which plays an essential role in the conjugation of electrophilic compounds (phase I metabolites) and catalyzes the conjugation of GSH with substances of an endogenous or exogenous nature. The increase in GST activity indicates both a high concentration of xenobiotics present in the environment and the induction of oxidative stress following the increasing production of ROS (Bhagat *et al.*, 2016). This production of ROS can be favored by the installed reductive stress which has been proposed according to certain researchers as an inducer of oxidative stress depending on the redox couples in which these ROS are engaged (Shen *et al.,* 2005; Yan *et al.,* 2014; Korge *et al.,* 2015; Xiao *et al.,* 2019). Much like oxidative stress, reductive stress also impairs cellular functions (Handy and Loscalzo, 2016).

However, and in the case of deltamethrin, tebuconazole and prothioconazole, metabolism occurs by hydroxylation, oxidation or hydrolysis reactions resulting in different metabolites followed by conjugation to glucuronic acid or sulfate (Ruzo et *al*., 1979; IPCS, 1990) and not to GSH, which implies that GST is not involved in this biotransformation.Thus, the increase in GST could therefore be explained by the fact that it is also involved in the transport and elimination of reactive compounds that perform other antioxidant functions (Sies, 1993; Livingstone, 2003) such as CAT, GSH and SOD and also in the defense against oxidative damage to lipids and DNA induced by peroxide products (Van der Oost *et al*., 2003). However, it was noted that at the end of treatment, GSH expression seems to be very sensitive to xenobiotics where it is strongly declined at high concentrations (D2, P2/D2) compared to controls but which are almost equivalent thus suggesting that Decis® (deltamethrin) alone has the same effect as the combined treatment (Prosaro®/Decis®) at these concentrations probably due to an antagonistic effect between the two pesticides. On the other hand, we recorded the induction of CAT activity with the different treatments, which testifies to the state of oxidative stress par excellence. The latter is an important enzyme in the defense system (antioxidant). It catalyzes, extremely quickly, the disproportionation of oxygen peroxide (H_2O_2) into oxygen and water, thus protecting cells from oxidative effects. The change in CAT activity is explained by cellular damage caused by exposure to contaminants (Shi *et al.,* 2011). Our results are in agreement with those obtained by Ferfar *et al.,* (2016) who demonstrated an increase in CAT activity in two varieties of wheat (Simeto and Cirta) exposed to two sulfonylurea herbicides, in leaves and roots of wheat "*Triticum aestivum* L". Similarly, the results of Belahcene *et al.,* (2015) which highlight the influence of oxidative stress caused by a systemic herbicide Cossack on the CAT activity of three varieties of durum wheat (Sersou, Carioca and Wersenis) where a variability very important genotypic was noted resulting from the response of each variety towards the applied stress.

Finally, the increase in LOX activity observed during our study could be due to the peroxidation of linoleic and linolenic acids. The formation of oxidation derivatives in the lipid bilayer, such as 4-hydroxy-2-nonenal, malondialdehyde or phytoprostanes, leads to disturbances in the micro-architecture of membranes, alters their permeability and can act with amine functions, lipids, proteins and DNA, as well as with the thiol functions of proteins.Indeed, these lipid peroxidation products are reactive electrophilic species (RES) which can bind covalently to proteins and thus damage them (Farmer *et al*., 2007). Lipid peroxidation by forming aldehydes leads to the destruction of structures, inhibits cellular functions and potentially accelerates cell senescence (Reich and Amundson, 1985; Dann and Pell, 1989).

Conclusions

The results obtained in this study clearly revealed the toxicity of the pesticides (Prosaro®/Decis®) used on *Triticum durum* even at regulatory concentrations. Indeed, the application of pesticides (fungicide and insecticide) directly to durum wheat causes disturbances at the foliar level and indirectly a negative effect on the chlorophyll and GSH content as well as an accumulation of total proteins and an induction of enzymatic activities (CAT, GST and LOX). These physiological and biochemical results thus suggest the establishment of a defense mechanism in order to neutralize the free radicals generated by the stress applied to this variety of wheat. In conclusion, the regulatory doses disturb the plant but the latter manages to overcome this stress, on the other hand the high concentrations clearly affect the leaves and roots of wheat. Thus, Decis® (deltamethrin) alone appears more toxic than Prosaro® (prothioconazole/ tebuconazole) and has the same effect as the combined treatment (Prosaro®/ Decis®). All of this information could help us formulate countermeasures to reduce the risk of pesticide contamination in agricultural production.

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