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Study of the effect of a fungicide "the tachigazole" on some indicators of soil biological activity

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ABSTRACT This study tests the impact of a pesticide molecule (hymexazole) on, on the one hand, the physio-biochemistry of hard wheat *Triticum durum* Desf. (Poales Poaceae) and on the other hand, the indicators of soil biological activity. To do this, the analysis has focused on total proteins, proline, the total carbohydrates and total chlorophyll of wheat leaves. Total carbon and soil organic matter have been also determined. Results reveal that the levels of total chlorophyll are practically identical in the presence of different doses of the fungicide in comparison with those of the control dose. The contents of other parameters (total proteins, carbohydrates and proline) are slightly different from those obtained for witnesses doses. Finally, the analyzed soil samples show that the values of the total carbon are higher and exceed the standards in the samples treated with fungicide.

KEY WORDS fungicide; hymexazole; organic matter; physio-biochemical; soil.

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INTRODUCTION

The intensive use of chemical molecules to kill pests, weeds and fungi contributes significantly to the improvement of yields. However, this use leads parallel, negative consequences for the functioning of ecosystems. Pesticides cause severe damage to the environment due to the combined effects of their anarchical and abusive uses, their persistence and toxicity. In sum, pesticides have a large part in the degradation of natural resources. According to the method of application, they propagate into the atmosphere over large areas, and due to their persistence, they can persist in the environment for long periods. Thus, the components of the physical environment namely, water, soil and plants, are gravely polluted.

This alarming situation, generated due to the repeated use of a multitude of plant protection compounds, has show the interest of a reflection on approaches to improve the biological fertility and sustainable agricultural development.

It is in this context that this work has been done and that has the objective to verify the effect of multiple doses of a fungicide "the Tachigazole" on, on the one hand, the indicators of biological activity of treated soil, and on the other hand, some physiological and biochemical parameters of a hard wheat species *Triticum durum* Desf. (Poales Poaceae) developed in situ.

For this, an experiment has been done to try to verify the consequences resulting from the use of three different doses of the fungicide hymexazole and a control dose, on the soil and on the cultivated plant in situ in pots.

MATERIAL AND METHODS

The soil samples are selected at random to a depth of 0–20 cm in the forest of Edough (Algeria), considered unpolluted area. Geographic coordinates are 36°55' North and 07°40' East. The fungicide used is "Tachigazole", which hymexazole is the active substance of a systemic fungicide seed from Golden Agrochemical Union. It belongs to the chemical family of Triazines, whose chemical structure is shown in figure1.

The considered plant material is hard wheat Cirta variety of the grass family of the genus *Triticum* and the species *Triticum durum.*

Methods

The solutions of fungicide are prepared in a mixture of sterile distilled water and methanol at a rate of 20 parts/80 parts. Three different doses are chosen:

- a dose to the field divided by 5 (D_1) is 9µl/cm^2
- a dose to the field (D_2) is 45µl/cm^2
- a dose field multiplied by 10 (D_3) or 450 µl/cm^2
- a control dose (D_0) containing only sterile water without fungicide or methanol.

The solutions of different doses are pulverized directly on the soil before sowing.

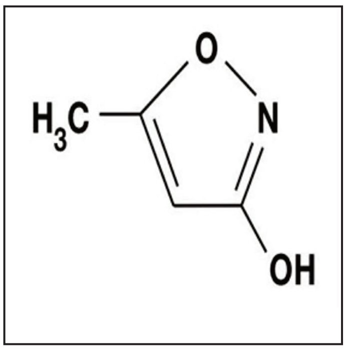

Figure 1. Hymexazole chemical structure

After 30 days, a hard wheat sowing is carried out at a depth of 2 cm, in previously prepared pots, with 10 grains of wheat per pot.

The experiment was performed on a total of 24 pots with repetition 6 pots per dose.

Data collection

The physio-biochemical parameters are measured and quantified from the leaves collected in the tillering of the plant.

The total chlorophyll is expressed in mg/g of fresh matter (F.M). It is extracted by the method of Mackiney (1941) improved by Holden (1975), by Hiscox & Israelsiam (1978) and this by means of a spectrophotometer.

The determination of total carbohydrates expressed in mg/g of fresh matter (F.M) is released by the method of Shields & Burnett (1960).

The extraction of total protein µg/g of fresh material (M. F.) is done according to the Bradford (1976) technique.

The dosage of proline expressed as µg/g of fresh matter (F.M) is effected by the method of Monneveux & Nemmar (1986).

The four parameters were obtained by using calibration curves.

The physico-chemical soil parameters are organic matter (O.M), which is obtained by the method Anne (Dabin, 1966). It is expressed in g/kg of dry matter (D.M). The total carbon that is deducted from the O.M expressed as g/kg of D.M.

Statistical analysis methods of data

The description of different studied characteristics of the plant and soil is made by calculating the average (m), standard deviation (s) and the minimal values (Xmin) and maximal (Xmax) for each dose of fungicide.

The analysis of variance (ANOVA) of the general linear model (GLM) of Minitab software for data statistical analysis (Minitab Inc., 2014) is used to compare averages, between them, of the four doses of the fungicide and this for each characteristic of interest (Dagnelie, 2009). We consider that there are significant differences between the means of four doses when the probability value (p) is less than or equal to the risk $\alpha = 0.05$ ($p \leq \alpha = 0.05$); highly significant differences when $p \leq \alpha = 0.01$ and very highly significant differences when p ≤ α = 0.001 (Dagnelie, 2009).

The test TUKEY (Dagnelie, 2009) was used to determine the doses of homogeneous groups characteristic of the plant or the soil (Minitab Inc., 2014).

Dunnett's test (Dagnelie, 2009) was used to compare the average of the control dose with each average of other doses for each parameter of the plant and soil (Minitab Inc., 2014).

RESULTS

The following Table 1 presents statistical parameter values obtained by characteristic and per dose of the fungicide to the plant and the soil. Average values and standard deviations are plotted as histograms in the various figures 2–7; which follow.

The results of the analysis of variance (ANOVA) are given in Table 2. Examination of the results of analysis of variance (Table 2) shows that there are only significant differences between the averages of 4 doses fungicide total chlorophyll of the plant and for the O.M and the total carbon in the soil; then, we notice highly significant differences for total protein and very highly significant differences for contents of the proline and the total carbohydrates.

The TUKEY's test used, after the rejection of the hypothesis of equality of averages, by the ANOVA shows that there exist two homogeneous dose groups, respectively, total chlorophyll, proline, organic matter and carbon total, and 3 homogeneous groups to total protein and carbohydrates. Alphabetic letters a, b, c in graphics from figures 2 to 7 designate these groups. The alphabetical letter indicates that the doses in question give consistent results on average.

Nature of samples	Variable	Doses	n	m	s	x_{min} — x_{max}
SOIL	ORGANIC MATTER	D_0	6	1.283	0.498	0.528 __ 1.869
		D_1	6	3.693	1.093	1.477 __ 4.257
		D_2	6	3.047	1.878	0.583 __ 4.404
		D_3	6	2.032	1.223	0.495 __ 4.180
	CARBONE TOTAL	D_0	6	0.745	0.289	0.306 __ 1.082
		D_1	6	2.147	0.636	0.858 __ 2.475
		D_2	6	1.779	1.097	0.340 __ 2.560
		D_3	6	1.257	0.739	0.289 __ 2.431
	CHLOROPHYLL	D_0	6	52.500	21.900	30.600 __ 74.400
		D_1	6	37.600	5.710	31.890 __ 43.310
		D_2	6	44.000	32.900	11.100 __ 76.900
		D_3	6	109.430	14.010	95.420 __ 123.440
PLANT	PROTEINS	D_0	6	2.769	0.298	2.476 __ 3.071
		D_1	6	2.172	0.178	2.000 __ 2.357
		D_2	6	2.988	0.357	2.571 __ 3.285
		D_3	6	1.817	0.274	1.547 __ 2.095
	PROLINE	D_0	6	0.073	0.056	0.009 __ 0.106
		D_1	6	0.684	0.036	0.644 __ 0.713
		D_2	6	0.046	0.007	0.038 __ 0.530
		D_3	6	0.009	0.001	0.008 __ 0.010
	CARBOHYDRATES	D_0	6	4.165	0.242	3.920 __ 4.404
		D_1	6	2.479	0.039	2.447 __ 2.522
		D_2	6	3.610	0.251	3.329 __ 3.812
		D_3	6	3.307	0.191	3.092 __ 3.458

Table 1. The values of basic statistical parameters based on soil samples and samples of the wheat plant: the number of samples (n), the mean (m), standard deviation (s), minimum values (X_{min}) and maximum (X_{max}).

Table 3 presents the results of the Dunnett's test calculated on the different characteristics of the plant and soil. From this Table 3 it can be seen that there is, each time, two fungicide doses, which give on average the results identical to those of the control dose, and this for, respectively, the total chlorophyll, proteins, proline, organic matter and the total carbon. Moreover, for contents of the carbohydrates, all doses of the fungicide on average give lower results compared to controls.

- Figure 2 relative to the total chlorophyll (a + b) shows that the D_3 dose of the fungicide provides the higher level with an average of 109.43 µg/g F.M. This result is, moreover, confirmed by the test TUKEY which classifies that this dose D_3 separately and the other doses D_0, D_1 and D_2; in a single homogeneous group.

- For protein contents represented by figure 3, the TUKEY test shows an overlapping 3 homogeneous groups of doses. The first group consists of doses D_0 and D_2, the second group consists of doses D_0 and D_1 and the third group D_1 and D_3. We notice that the dose D_0 is similar to D_1 and D_2 but different from dose D_3, and the dose D_1 is also identical to D_0 and D_3, doses but different from the dose D_2. The dose D_2 fungicidally induced a stimulation of the synthesis of proteins with the highest average 2.98 µg/g M.F.

- The total carbohydrates are given in figure 4. The TUKEY test shows 3 groups of distinct doses. D_0 dose is the first group with an average rate equal to the highest 4.16 µg/g F.M. D_1 dose alone represents a second group with the lowest rate (2.48 µg/g F.M), and D_2 and D_3 doses form a third homogenous group with no significant difference between their average. We note that the rates obtained from the leaves treated with all three doses are lower than those obtained from the reference dose.

- The levels of proline represented by figure 5 show two dose groups obtained by the TUKEY test. The first group consists of doses D_0, D_2 and D_3 with very low values and, particulary, almost zero for the D_3 dose (0.009 mg/g F.M). The second group consists only of the D_2 dose that gives the higher average (0.684 mg/g F.M).

- Figure 6 is related to organic matter content (O.M). In this figure, we noticed that two dose groups overlap. The first group consists of doses D_0, D_2 and D_3 and the second group consists of doses D_1, D_2 and D_3. This shows that the doses D_0 and D_1 are identical, each at doses D_2 and D_3, but are different from each other. D_0 control dose gives, on average, the lowest value (1.283 g/kg) O.M and the dose D_1 gives the higher value (3.693 g/kg).

- Finally, in Figure 7 related to the total carbon 2 dose groups. The doses D_0, D_2 and D_3 form the first group and, D_1 and D_2 doses form the second group. However, we note that the dose D_2 is common to both groups. The dose witness D_0 has given the lowest value (0.745 g/kg) of total carbon; while D_1 dose has given the higher value (2.147 g/kg).

DISCUSSION

In the present study, we tried to compare indicators of biological activity in soil submitted to the effect of a fungicide "the Tachigazole" and untreated control soil to establish a relationship between the repeated use of the fungicide, environmental parameters and soil fertility. The parameters analysed show variability in the results. The treated soils contain more total carbon but with slight variations between concentrations. The contents recorded in control samples are the lowest.

The organic material influences the distribution of the biomass. This latter is of great benefit for the microorganisms constituting the source of carbon and energy, which are transformed into new bodies and products of metabolism, and also play a role in the solubility of the pesticide (Bordjiba, 2003). The adsorption of the fungicide on the organic material increases its solubility and stability in the soil profile (Chiou et al., 1986).

The organic matter in the soil greatly influences the adsorption of pesticides in causing a decrease in the adsorption coefficient (Kd) and increased absorption coefficient (Koc) (Graber et al., 2010).

We note that treated plants with high doses of the fungicide registered a significantly lower effect on chlorophyll a and b. The letter accords perfectly with the results obtained by Hammou (2001) and Boutamine & Lahmar (2016), showing that very few fungicides affect the vital functions of the plant and in particular on the development of chlorophyll. We find that there is a close correlation between the rate of carbohydrate and chlorophyll content. Carbohydrate levels are lower in treated plants versus the control. During photosynthesis, electron

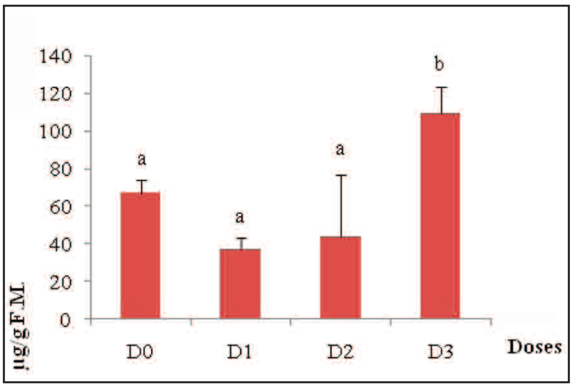

Figure 2. Total chlorophyll content in the presence of the four doses of the fungicide (hymexazole). The doses that have the same alphabetical letter constitute a homogeneous group according to the test TUKEY.

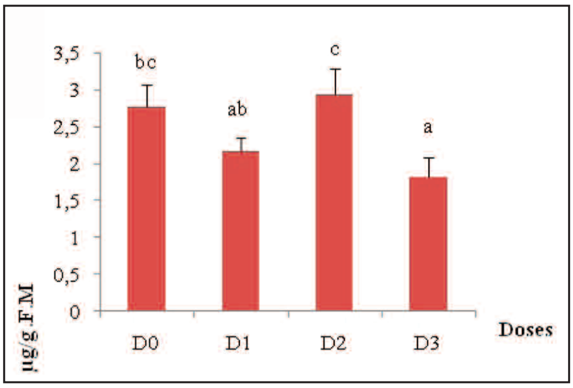

Figure 3. Content of proteins in the presence of four fungicide doses (hymexazole). The doses that have the same alphabetical letter constitute a homogeneous group according to the test TUKEY.

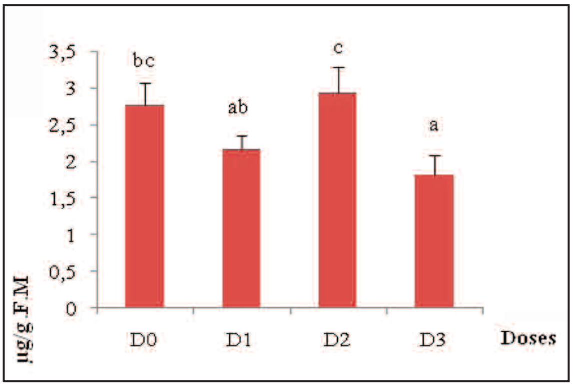

Figure 4. Content of proteins in the presence of four fungicide doses (hymexazole). The doses that have the same alphabetical letter constitute a homogeneous group according to the test TUKEY.

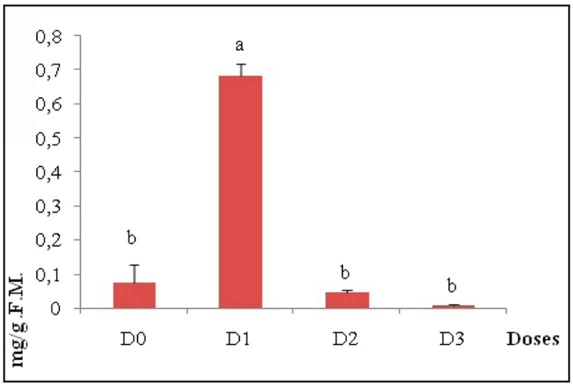

Figure 5. Content of proline in the presence of four fungicide doses (hymexazole). The doses that have the same alphabetical letter constitute a homogeneous group according to the test TUKEY.

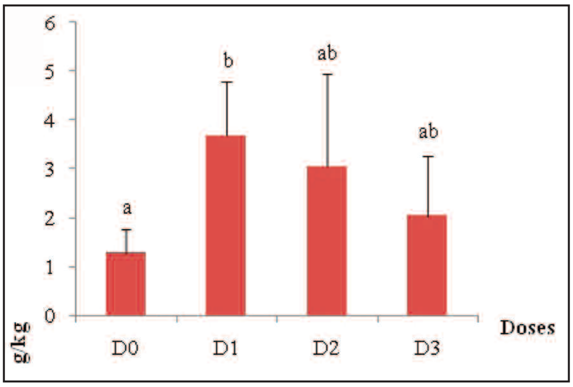

Figure 6. The organic matter in the presence of four fungicide doses (hymexazol). The doses that have the same alphabetical letter constitute a homogeneous group according to the test TUKEY.

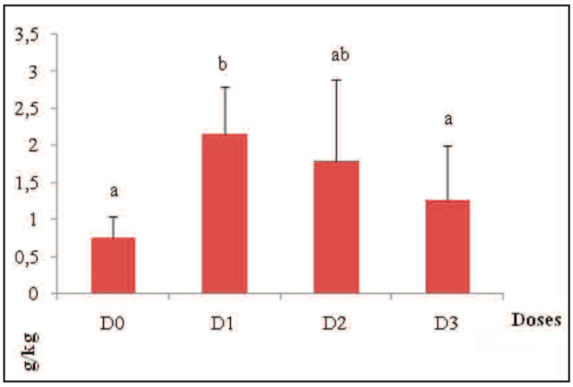

Figure 7. The total carbon levels in the presence of four fungicide doses (hymexazole). The doses that have the same alphabetical letter constitute a homogeneous group according to the test TUKEY.

Nature of samples	Variable	Source de variation	DF	Seq SS	MS	F	p
SOIL	ORGANIC MATTER	Doses	3	20.535	6.845	4.230	0.018*
	TOTAL CARBONE	Doses	3	6.746	2.249	4.020	0.022*
	CHLOROPHYLL	Doses	3	9760.50	3253.5	7.270	0.011 *
PLANT	PROTEINS	Doses	3	2.415	0.805	9.970	0.004 **
	PROLINE	Doses	3	0.932	0.310	279.58	0.000 ***
	CARBOHYDRATES	Doses	3	4.489	1.486	37.220	0.000 ***

Table 2. Results of the analysis of variance (ANOVA) to criteria for the soil and the plant. The number of degree of freedom (DF), the sum of squared differences (Seq SS), the middle square (MS), the observed value of the variable F Fisher (F) and the probability (p). *: Significant differences. **: Highly significant differences. ***: Very highly significant differences.

NATURE OF SAMPLE	VARIABLES	DOSES AND MEANS			
SOIL	ORGANIC MATTER	D_0 1.283	D_3 2.032	D_2 3.047	D_1 3.693
	CARBONE	D_0 0.745	D_3 1.257	D_2 1.779	D_1 2.147
	CHLOROPHYLL	D_0 37.600	D_3 44.000	D_2 52.500	D_1 109.430
PLANT	PROTEINS	D_3 1.817	D_1 2.172	D_0 2.769	D_2 2.988
	PROLINE	D_3 0.009	D_2 0.046	D_0 0.073	D_1 0.684
	CARBOHYDRATES	D_1 2.479	D_3 3.307	D_2 3.610	D_0 4.165

Table 3. Results of DUNNETT's test. Averages doses at once underlined bold are identical to the average of the control dose (D_0) for the soil and the plant.

transport along the photosynthetic chain is ensured by chlorophyll a and b. From there, the electrons are transferred to different carriers in the chain to the levels of photosystems I and II till the reduction of NADP to $NADPH_2$ necessary to the transformation of CO_2 into organic molecules, such as carbohydrates (Berkaloff et al., 1997).

Finally, the amounts of total protein are almost similar in samples treated with all doses of fungicide and are not very far from those of controls. The same was reported by Kloskowski (1992) and Dec & Bollog (1997), who claim that moderate concentrations of pesticides do not greatly affect the level of proteins.

CONCLUSIONS

The development of agriculture is accompanied by the use of plant protection products worldwide. This use has shown particular advantages in increasing production yields by eliminating or reducing crop predators. However, behind its misdeeds can hide insidious effects on the different components of the environment or human health. The study, we conducted, was to target soil fertility under conditions of considerable pollution by different doses of a pesticide molecule. This fertility was assessed using several indicators of biological activity, such as total carbon and organic matter.

We have tried to show the influence of a molecule of hymexazole with different concentrations on the physio-biochemistry durum wheat *Triticum durum* and some indicators of soil biological activity.

Due to the quasi-homogeneity and non-variability of the results recorded in the different samples treated and control samples, we believe that the assessment of the chemical fertility of soils polluted by pesticides seems difficult because of multiple interactions between the nature of the pollutant, the physio-chemical soil characteristics and environmental factors.

In addition, the lack of data on soils and norms of quality of biochemical and organic chemical and biological quality fertile soils makes assessment of soil quality and fertility difficult and not obvious.

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