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Study of the Agronomic, Biochemical and Yield Components of Mirabelle Pea Varieties (*Pisum sativum subsp. sativum*), exposed to Oxidative Stress by Difenconazole and Responses of the Antioxidant Defense System

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ABSTRACT

The use of phytosanitary products is called into question, with the risks they can cause for the environment and human health. These chemical solutions can cause considerable damage to crops and can cause an overproduction of ROS (Reactive Oxygen Species) which results in chlorosis and necrosis visible to the naked eye, up to the premature death of plants. This scientific contribution aims to verify in the laboratory the effect of a Difenconazole on the agronomic parameters, the biochemical indicators and the components of the yield of a pea legume (*Pisum sativum subsp. Sativum var. Sativum*). Four treatment doses were chosen with control, namely Dose 1 (0.5 ml/l), Dose 2 (1 ml/l), Dose 3 (1.5 ml/l) and Dose 4 (2 ml/l). All the variables studied of the batches treated with the low doses D1 and D2 note a low antioxidant biochemical activity and higher yields, on the other hand the batches treated with the high doses D3 and D4 recorded a high biochemical activity, namely Proline, GSH, GST with apparent antioxidant stress and low yield components. The dose effect is decisive and proportional to the doses applied.

1. Introduction

Algeria is one of the countries that use large quantities of pesticides. Thus, about 400 phytosanitary products are registered in Algeria, of which about forty varieties are widely used by [14]. Protection against pests, diseases and weeds is provided by chemical control [12], The implementation of several chemical applications poses significant problems related to the resistance of pathogens and pests, the destruction of non-target species and the accumulation of these substances in natural environments, Isenring, (2010). The use of pesticides causes many physiological and metabolic disorders such as disruption of cell mitosis, inhibition of root development Liu et al. (2009), chlorosis on leaves [8], reduction of photosynthesis by inhibition of the [18]. Chlorophyll alteration, electron transport and oxidative phosphorylation. Finally, they generate oxidative stress through the production of reactive oxygen species (ROS) and free radicals [10]. On the other hand, at low doses, they can stimulate cellular metabolism and the activity of certain enzymes such as peroxidase, acid phosphatase and α-amylase [16]. Faced with this situation, research is oriented on the basis of effective phytosanitary protection that respects the environment.

The objective of this work is to know the responses of the antioxidant defense system of this legume by studying the Agronomic, Biochemical parameters and the components of the yield of the Pea of the Mirabelle variety, subjected to chemical stress by different doses of a fungicide. Systemic: Difenconazole.

2. Materials and methods

Difenconazole is an active ingredient, which has a systemic fungicidal effect with a wide spectrum of leaf diseases, and which belongs to the triazole family. Formula: C19H17Cl2N3O3 Molar mass: 406.26 g / mol, Crude formula: C19H17Cl2N3O3, PubChem CID: 86173

2.1 Experimental device

The experiment was carried out in a greenhouse at an average temperature of 24 ° C and a humidity of 80%, according to a device, totally random. Planting was done with five seeds per pot on a balanced textured soil at a depth of 2 cm. Four (04) doses of the fungicide are selected and solubilized in distilled water with 5 replicates plus control.

Dose 1 (0.5 ml / l), dose 2 (1 ml / l), dose 3 (1.5 ml / l) and dose 4 (2 ml / l). Two fungal treatments were carried out by spraying, starting from the 6th week after emergence for the first, and at the 9th week after the emergence for the second treatment. After each treatment, the analyzes of the Agronomical and Biochemical Parameters are carried out.

2.2 Parameters Studied

2.2.1 Parameters Agronomical

Measures of Agronomical parameters such as Dry matter of the leaves (DML) [1], and Number of knotted flowers.

2.3 The Biochemical Indicator Parameters

2.3.1 Glutathione (GSH) assay

The glutathione assay has been performed according to the [19]. The principle of this assay is based on measuring the optical absorbance of the 2-nitro-5- mercapturic acid. This results from the reduction of 5, 5-bis-2-nitrobenzoic acid (Elman's reagent, DTNB) by groups (-SH) of glutathione. For this deproteinization of the homogenate (supernatant) is essential to keep only specific glutathione thiol groups.

2.4 Enzymatic activity of glutathione S-transferase (GST)

The activity of glutathione S-transferase is produced by the method of [7]. The samples are homogenized in phosphate buffer (pH 6.5 to 100mM) and centrifuged at 9000G for 30 min. The method comprises reacting the GSTs on a mixture of CDNB (20mM) -GSH (100mM) the variation of the optical density due to the appearance of CDNB-GSH complex is measured every 15 seconds for 2 min at 340 nm. The GST activity is expressed in nmoles / min / mg of protein.

2.5 Measuring Yield Components

For yield components such as pod length, pod weight, use a ruler and precision balance.

2.6 Statistical Analysis:

All results have been validated by a statistical analysis, analysis of variance one classification criterion [4]. Data are average values (average ± standard deviation).

3. RESULTS AND DISCUSSION

3.1 The Agronomic Parameters

3.1.1 Dry Matter of leaves

The figure 1, watches the evolution of the dry matter of the leaves in time. After two treatment operations with Hexaconazole, the analysis of the variance with one classification criterion (ANOVA1) shows that there is a difference between significant and very highly significant for the two treatments. This parameter whenever the dose increases the weight of the lowest dry matter is recorded in the doses D3 and D4. The highest value of the recorded leaf dry matter characterizes the plants treated with the lowest doses.

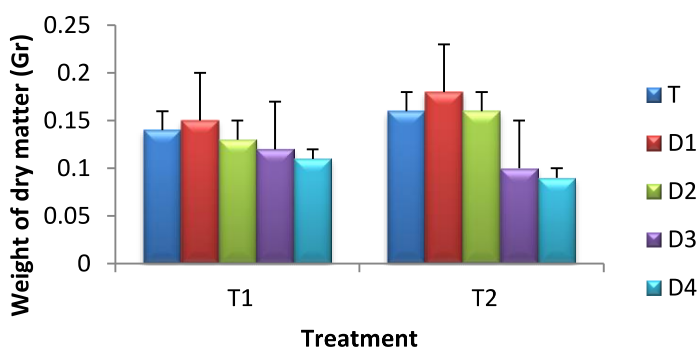

Figure. 1. Effect of Difenconazole on the weight of dry matter of pea legume leaves treated at different concentrations: D1 (0.4 ml / l), D2 (0.8 ml / l), D3 1.2 ml / l) D4 (1.6 ml / l) At the 6th and 9th week

3.2 Number of knotted flowers

Figure 2 shows a decrease of the knotted flowers each time the dose increases. The highest number is that of the control with 18 pods, the lowest values are those of D3 and D4 with 13 and 10 pods respectively, the number of knotted flowers of the treated plants having received two treatments is lower compared to the plants Which received only one treatment. The analysis of the variance proves the existence of very highly significant differences (Table 1).

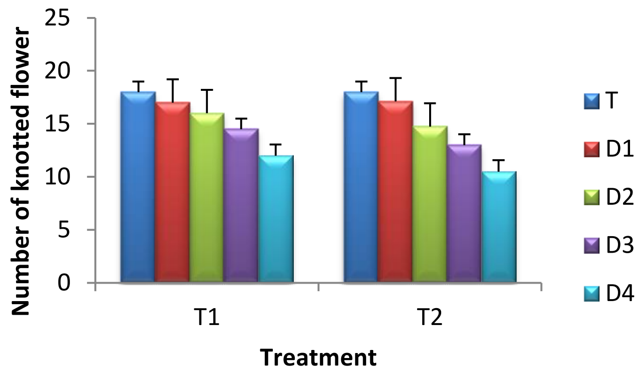

Figure.2. Effect of Difenconazole on the number of knotted flower of Pea treated at different concentrations: D1 (0.5 ml / l), D2 (1ml / l), D3 (1.5 ml / L) D4 (2 ml / l) At the 6th and 9th week (T1and T2)

Table 1. Statistical values of the observed F (Fobs) of the analysis of variance with 1 criterion of classification of the agronomic parameters during the first and second treatment.

Source of variation	DF	DML		Number of knotted flower	
Treatments		T_1	T_2	T_1	T_2
Conc	4	5.4^b	5.5^b	13^a	14^a
Erreur	45				
Total	49				

DF: Degree of freedom: DML : Dry matter of leaves; c P< 0.05 : Significant differences; b P< 0.01 : Highly significant differences; a P< 0.001 : Very significant differences; ns : Not significant; T1 : traitement 1, T2 : traitement 2.

3.3 The Biochemical Indicator Parameters

3.3.1 Glutathione content (GSH)

The analysis of the levels of glutathione in leaves treated after the first application of treatment, shows that this fungicide has a highly significant effect between the different measured medium, the D_3 and D_4 has the highest values with 54.2 and 56.3 followed by 39.9 lot of D_2 by the control recorded against the lowest value 37.8 µM/ mg Protein. After a second processing operation, the contents of GSH levels of glutathione decreased for all lots with the exception of D_1, the control recorded the lowest values, analysis of variance reveals a significant difference between different average measured, the D_4 records the highest value of 45.06, with a regression values for wholes lots, the control always shows the lowest value 33.2 µkat / g of FM.

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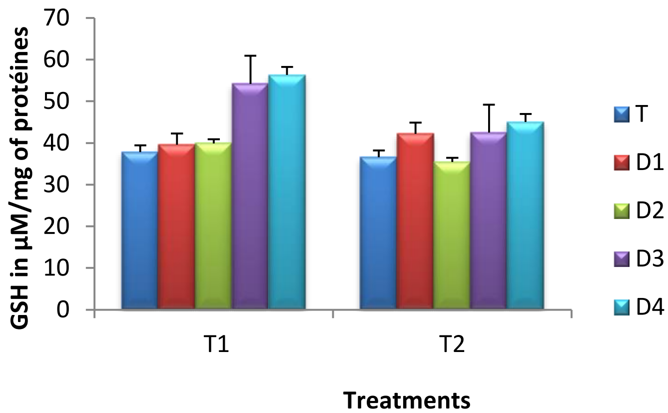

Figure.3. Effect of Difenconazole on glutathione content of Pea treated with different concentrations: D1 (0.5 ml / l), D2 (1ml / l), D3 (1.5 ml / L) D4 (2 ml / l) At the 6th and 9th week (T1and T2)

3.3.2 Glutathione sulfo-transférase activity (GST)

Analysis of GST enzyme concentrations in leaves treated after two applications of treatment shows that there is a very highly significant difference (Figure.4) between the different averages measured. D_3 and D_4 has the highest values with 14.2 and 14.5 followed by the lot D_1 with 10.3 and control recorded the lowest value 4.09 nmoles / min / mg of protein, after the first treatment. After a second application of treatment, the D_3 still retains the highest value µkat 14.1 / g of FM, compared to the control.

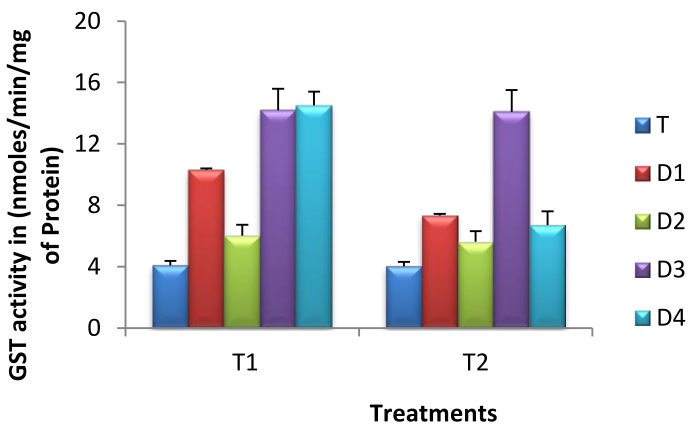

Figure.4. Effect of Difenconazole on Sulfo-transférase activity of Pea treated with different concentrations: D1 (0.5 ml / l), D2 (1ml / l), D3 (1.5 ml / L) D4 (2 ml / l) At the 6th and 9th week (T1and T2)

Table 2. Observed value (Fobs) analysis of variance ANOVA of the Biochimical parameters of the two treatments.

Source of variation	D.F	GSH		GST	
Treatments		T1	T2	T1	T2
Conc	4	6.1^{b}	4.3^{c}	16.6^{a}	83.5^{a}
Error	25				
Total	29				

D.F: degree of freedom: GSH: Glutathione; GST: glutathione sulfo-transférase. ^{c}) P <0.05: significant differences; ^{b}) P <0.01: Highly significant differences; ^{a}) P <0.001: very highly significant differences; ns:

not significant; T_1: 1st treatment, T_2: 2nd treatment.

3.4 Yield Components parameters

3.4.1 The pod length

This figure represents the length of the pods according to the different treatments with two fungal applications (Difenconazole), The analysis of the variance with one classification criterion shows that there are very highly significant differences (P <0.001). The D1 (half-dose) records the highest length with an average of 11.59 cm. The control (without treatment) occupies the second position with 10.03cm, the doses D3 and D4 always give low values and come last with 8.6 and 7.2 cm respectively.

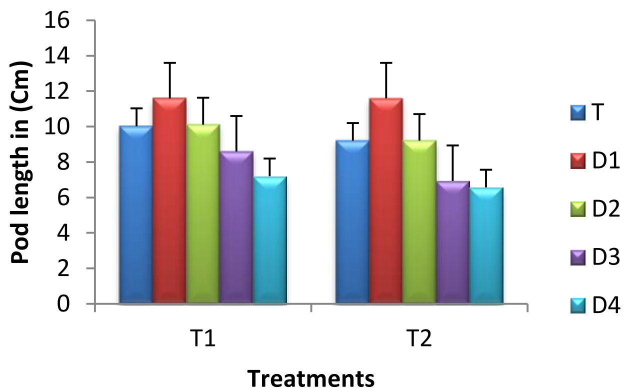

Figure. 5. Effect of Difenconazole on pod length of Pea treated at different concentrations: D1 (0.5 ml / l), D2 (1ml / l), D3 (1.5 ml / L) D4 (2 ml / l) at the 6th and 9th week (T1and T2)

3.4.2 Pod weight

The Figure. 6 show a low pod weight after two treatment operations. And each time the dose of treatment increases, the weight as well as pod number decreases. The analysis of the variance to one classification criterion shows that there are very highly significant differences between the different average values of this measured variable, Tab.3 (P <0.001); In fact, the pods of plants treated with dose 1 (6.21 g) are the highest; The control comes in second position with 4.5g, on the other hand, those who have undergone the D4 come in last position with 2.02 g.

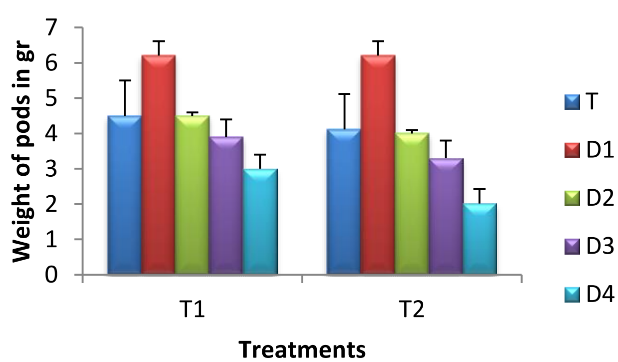

Figure.6. Difenconazole the effect on the weight of the pods pea treated at different concentrations: D1 (0.5 ml / l), D2 (1ml / l), D3 (1.5 ml / L) D4 (2 ml / l) At the 6th and 9th week (T1and T2)

Table 3. Statistical values of the observed F (Fobs) of the analysis of variance with 1 criterion of

classification of the yield components during the first and second treatment.

Source of variation	D F	P. leng		P. weig	
Treatments		T1	T2	T_1	T_2
Conc	4	13.3[a]	14[a]	66.4[a]	47[a]
Error	10				
Total	14				

D.F: degree of freedom: P. Leng: The pod length; P.weig: Pod weight; Num s / p; [c)] P <0.05: significant differences; [b]) P <0.01: Highly significant differences; [a]) P <0.001: very highly significant differences; ns: not significant; T_1: 1st treatment, T_2: 2nd treatment.

4. DISCUSSION

The results obtained from this work show that the Dry matter of the leaves, Number of knotted flowers during the vegetative cycle seems to have a difference between the various treatments, especially after the second application, the dry matter of the leaves is influenced by the fungicide. It is observed that the doses used D3 and D4 cause a regression of the agronomic parameters, with a cumulative effect after the second application (Figure 1. 2, Table 1). These results seem to corroborate those obtained by [17], the recorded Agronomic Parameters of regressions compared to controls after triazoles treatments, [11].

The activity of antioxidant enzymes is considered bio-markers of oxidative stress [6], antioxidant enzymes such as GST (Glutathione sulfo-transférase) in plants treated with D3 and D4 doses registers the highest values and this is explained by the stress of the plants, GST is an important enzyme that enters the process of detoxification of ROS [9]. The content of the GST enzyme and Glutathione increases under treatment with Difenconazole, these molecules are involved in the metabolism of oxidative stress [16]. The observation of the results shows the cumulative effect that the fungicide has on the enzymatic activity of plants, these results were found by [20], The combined action of GST / GSH is essential in mitigating effects of oxidative stress, such as acts against the superoxide anion, conversion to another reactive intermediate (H2O2) and the last act against H2O2, converting it to water and oxygen, [5].

Yield components such as pod length, pod weight, seem to have differences between different treatment doses, at a certain fungicide dose these yield parameters are negatively influenced, [13], D3 and D4 cause a regression of these parameters with a cumulative effect on the second application. These results seem to have been obtained by [2], these yield components treated with Dose 1 gave some advantage over Control and D2, plants use the fungicide as a source of energy and carbon at low doses [3], since difenconazole is an active member of the Triazoles family and has properties in plant growth regulation, [21].

5. CONCLUSION

It can be said that the action of the fungicide Difenconazole on the Pois Mirabelle variety under semi-controlled conditions has allowed us to have some interesting information on the effect and behavior of this species with respect to the concentrations applied. high concentrations have a negative impact on agronomic parameters such as (leaf dry matter, and number of set flowers/plants). In contrast, enzyme activity like GST and GSH molecule to support free radicals and detoxifies cell hyaloplasm by removing hydrogen peroxide (H2O2). Indeed, whatever the treatment (first and second), a significant detoxifying activity was determined. For the yield components, they are influenced by the high doses of fungicide, D3 (1.2ml/l) and D4 (1.6ml/l) with a cumulative effect after a second treatment application, the plants treated with the weak doses D1 and D2 do not seem to be influenced by the treatment, on the other hand, the batches of dose1 represent the highest values, therefore a higher yield. The use of the fungicide can be beneficial for improving yield, plant resistance to diseases and the quality of harvested crops, but on one condition: use of the Ideal dose otherwise it would generate oxidative stress and a depressive effect on irréversible plants.

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