

The Impact of a Hexaconazole Fungicide on Agronomic, Biochemical Parameters and Yield Components of Green Beans *Phaseolus Vulgaris* cv. Djedida

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Abstract

The use of synthetic phytosanitary products has proved to be very effective in controlling crop diseases and pests in a significant number of cases, however, many studies and studies concern the presence of these molecules in the soil, contamination of the waters we have to say confirmed the degradation of living ecosystems.

*The aim of this work is to verify the effect of the fungicide Hexaconazole on agronomic, biochemical markers and enzymatic activity of a legume green bean *Phaseolus vulgaris*, cv. Djedida. Four treatment doses were chosen with control namely Dose1 (0.4ml/l), Dose2 (0.8ml/l), dose3 (1.2ml/l), Dose4 (1.6ml/l). The obtained results show that all variables studied agronomic, physiologic and enzymatic are relatively stable for plants treated with low doses 0.4ml/l and 0.8ml/l. against, the plants treated by highest doses D3 and D4 recorded the lowest values. The dose effect is decisive and proportional to the applied level. The analysis of the mechanisms of response to oxidative stress or oxidative stress-xenobiotic interactions may therefore provide a better understanding of the processes of response to xenobiotic.*

Keywords

I. INTRODUCTION

Algeria is among the countries that use large amounts of pesticides. Thus, about 400 pesticides are registered including forty varieties are widely used by farmers [1].

Given this situation, all stakeholders (production units, producers, technical staff and researchers) must anticipate future changes and increase their responsiveness by continually seeking information as a basic tool for effective phytosanitary protection and respect for the environment. [2] [3] [4], but the environmental consequences have continued to increase. Many works and studies related to the presence of these molecules in the soil [5], water contamination, On the other hand, the degradation of living ecosystems [6] [7], their use as a source of carbon by microorganisms present in the soil and

finally the identification of residues that apparently seem to be related to pesticides [8]. Advances in crop protection are remarkable, but created environmental imbalances have a close dependence on pesticides [9].

The latter inhibits root development [10], induced chlorosis of leaves [11], reduced photosynthesis [12] and inhibited or activated severely enzymes [13]. The pesticides enter the leaves by the stomata are transported inside the cells and can thus harm the normal respiration is also to the mechanisms of photosynthesis by altering chlorophyll, Electron transport and oxidative phosphorylation. They can also disrupt biochemical activity, including the synthesis of carbohydrates and some amino acids under unfavourable environmental conditions, the plant is weakened and therefore unable to make the most of its environment [14].

The objective of this work is to know the answers agronomic parameters, biochemical and green bean yield components subjected to chemical stress based systemic fungicide: the Hexaconazole.

II. MATERIALS AND METHODS

The systemic fungicide is Anvil original (Zeneca), based Hexaconazole, which is the active ingredient belongs to the chemical family of triazoles. The chemical formula is $C_{14}H_{17}C_{12}N_3O$. Available in Algeria used for vegetable crops and fruit trees. The experiment was conducted under greenhouse with a completely random device. Seeded with five seeds per pot, on a balanced soil texture, at a depth of 2cm Four (4) selected doses of the fungicide are dissolved in distilled water with five repetitions. The doses used for this work are the dose 1 (0.4 ml/l), the dose 2 (0.8 ml/l), the dose 3 (1.2 ml/l), 4 dose (1.6 ml/l) and the reference dose, dose field or DC (0.8 ml/l). Two fungal treatments were applied by spraying, from the 6th week after emergence for the first, and in the 9th week after emergence for the second treatment. After each treatment analyzes of agronomic and physiological parameters are performed. The experiment of planting until harvest lasted four months.

III. PARAMETERS STUDIED

A. The agronomic parameters

The agronomic parameters studied are: the length of the aerial part of the plant (LAP) and leaf area (LA) by the method described by Paul and al [15].

B. Determination of Chlorophyll and Carotenoid Pigments

The dosage of the chlorophyll and carotenoid pigments is performed by the method of Holden [16]. The idea isto be weighed (0.2g) of green leaves, grind the min a mortar with a pinch of calcium carbonate (CaCO₃) to neutralize vacuolar acidity and 25ml 80% acetone. Chlorophyll isdetermined by the spectrophotometric method, which allows the reading of optical densities(OD) of the extract sat 645 and 663nm, then solving the equation system of Arnon [17] give us the contents of chlorophylls (a) and (b) and carotenoids in mg /g of FM (Fresh matter).

C. Total Protein Content Preparation of the Sample:

(0.1 g) of the sample of each concentration is ground in a mortar with 5 ml of distilled water and then filtered and poured into tubes with distilled water.

Proteins were quantified according to the method of Bradford [18],using Coomassie G250 brilliant blue (BBC) and bovine serum albumin (BSA) as the standard.

D. Total Sugar Content Preparation of the Sample:

The sugars are determined by the method Shields and Burnett [19],which uses anthrone in a sulfuric medium, extraction of the soluble sugars is carried out after maceration of (0.1 g) of vegetable matter in 3 ml of 80% ethanol for 45 hours. Then pass the tubes to the rotavapor for evaporation of the ethanol, and then put 20 ml of distilled water in the whole of the extract.

E. Measurement of yield Components

Regarding yield components such as the number of knotted flowers, Number of pods per plant, Weight of 100 Seeds, using a graduated scale and a precision scale.

IV. RESULTS

A. The Agronomic Parameters

a. Length Of Theaerial Partof The Plant (LAP)

Fig (1) shows the growth in length of the plants over time after treatments with the fungicide Hexaconazole. This characteristic varies from highly significant manner (P<0.001) (Table.1). The results show that the highest average were observed in plants treated with the lowest dose1 (0.4ml/l) and the control with 36.5 cmon average, by against, doses (D₃andD₄) have induced the lowestvalueswith35 and34cmin succession for the first treatmentand36.5and34.8cmfor the second treatment, respectively.

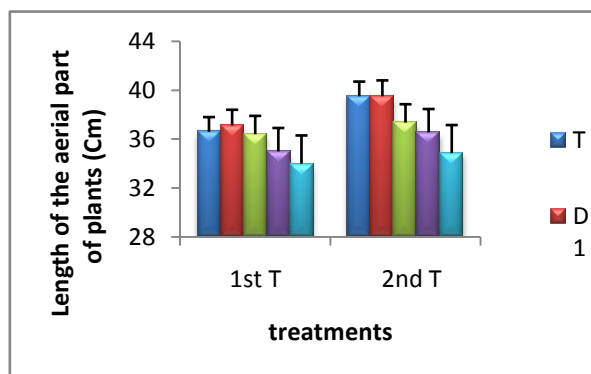


Fig. 1. Effect of Hexaconazoleon length aerial part of plants of green beans treated with different concentrations: D1(0.4ml / l), D₂(0.8ml / l), D₃(1.2ml / l), D₄(1.6ml / l), conducted in two processing operations at the 6th and 9thweek.

b. Leaf Area

The observation of the evolution of the leaf area over time after two fungal treatment operations by different doses shows that this character does not vary significantly in the plants for the first treatment although slight differences are noted between Mean values of plants treated with dose D1, The doses D2, D3, D4 with respect to the controls and representing 44.4, 46.8, 43.8 and 45.8 cm² respectively, then it becomes significant for the second treatment, the D1 records the highest value with a value of 47.8 cm² followed by the control With a value of 45.8 cm², the plants treated with the highest doses recorded the lowest value of 39.3 cm².

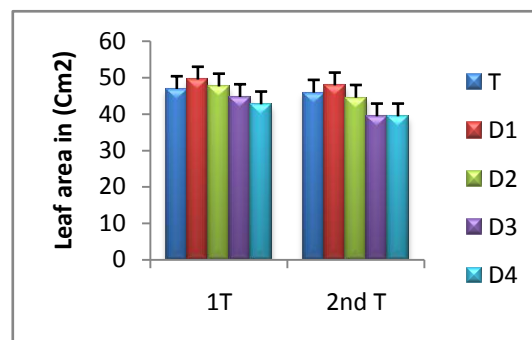


Fig. 2. Effect of Hexa conazoleon leaf area of green beans treated with different concentrations: D₁(0.4ml / l), D₂(0.8ml / l), D₃(1.2ml / l), D₄(1.6ml / l), conducted in two processing operations at the 6th and 9th week.

Table 1. Statistical values of Fobserved (Fobs) analysis of variance ANOVA.1 for agronomic parameters during the first and second treatment.

Source of variation	DF	LAP	LA
Treatments		T ₁ T ₂	T ₁ T ₂
Concentration	4	5,4 ^b 5,5 ^b	0,46 ^{ns} 2,7 ^c
Error	45		
Total	49		

Abreaction:

DF: degree of freedom; LAP: length of the aerial part; LA: leaf area; a) P <0.05: Significant differences; b) P

<0.01: Highly significant differences; c)P <0.001: Very highly significant differences; ns = Not significant. T₁: First treatment, T₂: second treatment.

B. The biochemical parameters

a. Pigment Content

The analysis of variance ANOVA shows that there are very highly significant differences between the averages measured various different doses of treatment. Chlorophyll (a), plants of lot D₁, D₂ and control, recorded the highest values with 2.45, 2.02 and 2.26 µg / g of FM respectively. For the first treatment, for the 2nd treatment it becomes weaker with 1.63 µg / g for the control and 1.43 µg / g for the D₁ and D₂. Lots D₃ and D₄ have the lowest values. For Chlorophyll (b) there were no large differences between the D₁, D₂ and control plants, while the lowest values were D₃ and D₄ with 0.45 and 0.3 µg / g FM The first and 0.52 and 0.46 for the second treatment. On the other hand, the highest carotenoid contents are present in lot D₂, whereas D₄ has the lowest contents. After a second application of the treatment, all the activity of the chlorophylls was lowered, the activity of the carotenoid pigments is greater compared to the chlorophyll.

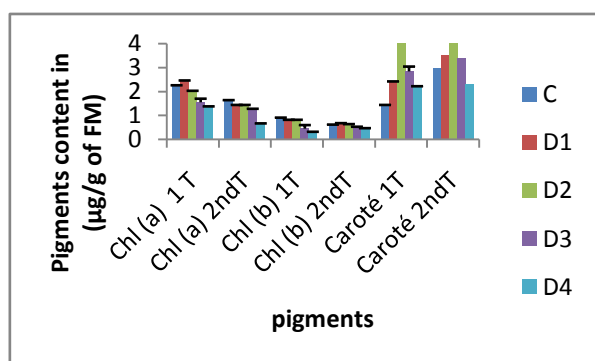


Fig. 3. Effect of Hexaconazole on pigment content chlorophylls and carotenoids of green bean treated with different concentrations: D₁(0.4ml / l), D₂(0.8ml / l), D₃(1.2ml / l), D₄ (1.6ml / l), conducted in two processing operations at the 6th and 9th week.

b. Total protein content

ANOVA.1 shows the existence of very highly significant differences in protein content (Tab.2). In fact, the contents of the leaves treated with dose D₃ are the highest value 5.36 µg / mg of MF followed by D₂ with 4.4 µg / mg of FM. Conversely, D₄ recorded the lowest value (3.5 µg / mg FM) for the first treatment and 3.1 µg / mg FM for the second treatment.

c. Total sugar content

The results concerning the effect of Hexaconazole on the sugar content are shown in Figure 5. Indeed, the results obtained show that this characteristic varies in a very highly significant way (Table 2).

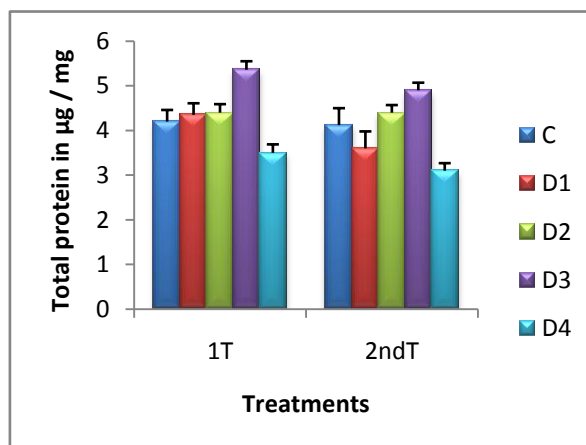


Fig. 4. Effect of Hexaconazole on total proteins content of green beans treated with different concentrations: D₁(0.4ml / l), D₂(0.8ml / l), D₃(1.2ml / l), D₄(1.6ml / l), conducted in two processing operations at the 6th and 9th week.

It is noted that the most important content of sugars was found in the plants treated with the lowest dose (D₁) which seems to favor the synthesis of the carbohydrates with a value of 10.64 µg / mg of FM. The doses D₃ and D₄ cause an inhibition of the synthesis of these compounds since the lowest values are 0.51 and 0.4 for the first treatment and 0.31, 0.22 µg / mg for the second treatment.

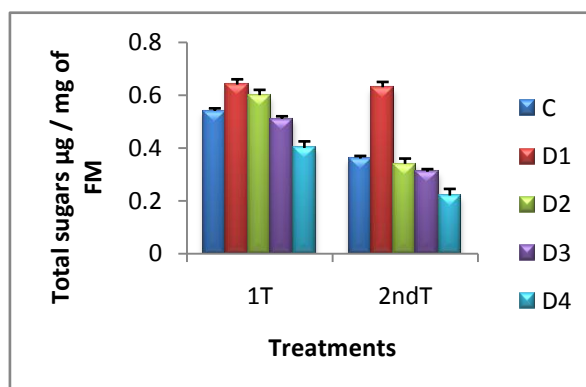


Fig. 5. Effect of Hexaconazole on total sugar content of green beans treated with different concentrations: D₁(0.4ml / l), D₂(0.8ml / l), D₃(1.2ml / l), D₄(1.6ml / l), conducted in two processing operations at the 6th and 9th week.

Table 2. Observed value F observed (Fobs) Analysis of variance ANOVA 1 for the biochemical parameters of two treatments.

Source of variation	d.f	Chl(a)	Chl(b)	Carot	Total proteins	Total sugars
Treatments		T ₁ T ₂	T ₁ T ₂	T ₁ T ₂	T ₁ T ₂	T ₁ T ₂
Conc	4	130 ^a 713,3 ^a	180 ^a 53,3 ^a	111,3 ^a 87,1 ^a	6.3 ^a 8.1 ^a	25 ^a
Error	25					140.6 ^a
Total	29					

Abreacation:

d.f: degree of freedom; Ch.: Chlorophyll; Carot: Carotenoids; POD: peroxidase; CAT: catalase; GST: glutathione-sulfo-transferase; GSH: Glutathione a) P <0.05: significant differences; b) P<0.01: Highly significant

differences; c) $P < 0.001$: very highly significant differences; ns: not significant; T_1 : 1st treatment, T_2 : 2nd treatment.

C. Yield Components

a. Number Of Flower Knotted

Figure 6 shows knotted flower decay 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 treated plants having received two treatments is lower compared to plants which received only one treatment. The analysis of the variance proves the existence of very highly significant differences (Table 3).

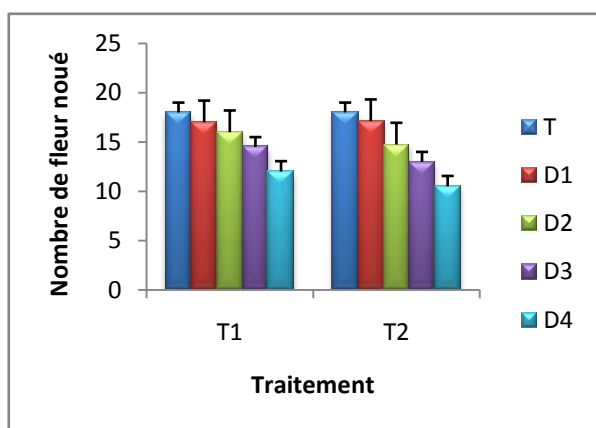


Fig. 6. Effect of Hexaconazole on the number of knotted flower of green beans treated at different concentrations: D_1 (0.4 ml / l), D_2 (0.8 ml / l), D_3 (1.2 ml / L), D_4 (1.6 ml / l) At the 6th and 9th week.

b. Number of pods / plants

Figure 7 shows once again the effect of the fungicide on the number of pods. The analysis of the variance to a classification criterion shows that this characteristic varies in a very highly significant way (Tab.3) since ($P < 0.001$). The control plants recorded the highest averages 23.5 and the plants treated with D3 and D4 were characterized by the lowest values 15.75 and 11.4 pods respectively.

c. Weight of 100 seeds

Figure 8 illustrates the weight averages of 100 seeds after application of treatments with different doses plus control, analysis of the one-criterion variance shows that there is a very highly significant difference between the mean ($P < 0.001$) (Tab.3), for the second treatment a high value of D1 was recorded with 110 g, D2 with 80.6, D3 and D4 last with 34.3 and 21.3 g.

VI. DISCUSSION

The results obtained are validated by a statistical study, the analysis of the variance with a single classification criterion according to Dagnelie [20]. The results obtained from this work show that the length of the plants during the vegetative cycle seems

to have a difference between the various treatments, especially after the second application, the leaf area are 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 (Fig 1. 2, Table 1). These results seem to corroborate those obtained by authors [21][22][23] the recorded agronomic parameters of regressions by treatments with triazoles [24][25].

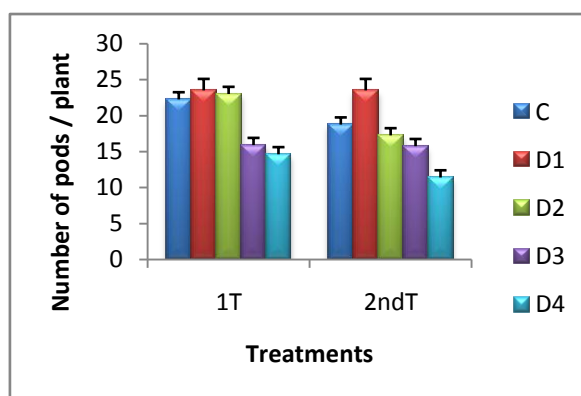


Fig. 7. Hexaconazole the effect on the number of pod / plant green beans treated at different concentrations: D_1 (0.4 ml / l), D_2 (0.8 ml / l), D_3 (1.2 mL / L), D_4 (1.6 ml / l) At the 6th and 9th week.

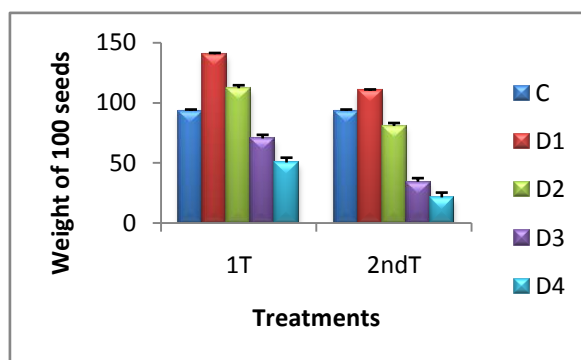


Fig. 8. Hexaconazole effect on Weight of 100 seeds of green beans treated at different concentrations: D_1 (0.4 ml / l), D_2 (0.8 ml / l), D_3 (1.2 mL / L), D_4 (1.6 ml / l) at the 6th and 9th week.

Photoreceptor pigments (chlorophylls and carotenoids) play an important role in the capture of light energy and its transformation into potential chemical energy for the reduction of atmospheric CO_2 into organic molecules such as carbohydrates and proteins. Under the influence of the fungicide, the synthesis of these pigments was decreased.

Drastically in plants treated with high doses and the effect of the fungicide is cumulative after application of the second treatment. These results are in perfect agreement with those found by authors [26][27]. Pigment reduction is important using the highest doses of the fungicide [28], this decrease in pigments results

in a reduction in net photosynthesis [29]. It affects the thylakoidal membrane of photosystem1 [30]. And modifies the phytosterol profile and thylakoidal functions [31][32]. Cucumber plants treated with atriazole fungicide showed the same finding. [33] While Dose 1, which is considered the recommended half-dose of the fungicide, appears to favour the synthesis of chlorophyll (a) and Carotenoids. Similar results have been found by [34]. This can be explained by the fact that Hexaconazole is an active member of the triazole family with properties involved in the regulation of plant growth [35] and inhibits peroxidation Lipids [36][37]. Concerning the total sugars it was noted that the lower doses D1 and D₂ induced more synthesis compared to the controls of the sugars. This leads us to say that the low-dose fungicide can cause a respiratory exaltation by slightly activating the cellular metabolism Which will become, as it were, "a source of energy and carbon" for plants [38][39]. On the other hand, the application of high doses leads to a fall in metabolic activities, which generally results in a decrease in photosynthesis, for proteins their contents increase with the dose applied, the more the dose increases the more the content increases [40][41].

The yield components such as knotted flower number during the vegetative cycle appear to have a difference between the various treatments. It is observed that the doses used D₃ and D₄ cause a regression of knotted flower number with a cumulative effect at the second application. These results appear to be similar to those obtained by Lakshmanan in 2007 [42], which explain that a certain amount of fungicide can be influenced by agronomic parameters.

The number of pods and the weight of 100 seeds of plants treated with Dose 1 resulted in some advantage over the Control and D₂. Plants use the fungicide as a low-dose source of energy and carbon [43], Knowing that Hexaconazole is an active member of the triazole family and has properties in the regulation of plant growth [44].

V. Conclusion

As a general conclusion, we can say that this study of the action of the fungicide Hexaconazole on the green bean variety Djedida, allowed us to know that the high concentrations in relation to the ideal concentration, recommended by the laboratory supplier product had a negative impact on agronomic parameters made (elongation of plants, leaf area)throughout the test with the higher doses.

Furthermore, with regard to photoreceptor pigments, chlorophylls, carotenoids and sugars, a slight stimulation of the synthesis of these pigments was found by the low doses applied as compared to the controls. For total proteins, on the other hand, it was noted that only high doses resulted in a high accumulation of these proteins.

For yield components, knotted flower number, number of pods / plant and weight of 100 seeds show

that plants are influenced by high doses of fungicide D3 (1.2ml / l) and D4 (1.6 ml / l) and revealed differences between highly and very highly significant with a cumulative effect after a second application of treatment, plants treated with low doses D1 and D2 did not appear to be influenced by treatment.

To conclude, it can be said that the use of the fungicide may be beneficial for yield improvement, plant resistance to diseases and the quality of crops harvested, but only on one condition: use of the dose Ideal otherwise it would generate oxidative stress and a depressive effect on irreversible plants.

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