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Research Article

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The *In vivo* Antagonistic Activity of Fungi Antagonists towards *Septoria spp* Under Controlled Conditions and in the Field

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ABSTRACT

Spraying sporals suspensions prepared from the antagonist's cultivation of a Trichoderma harzianum strain on the seedlings of 24 tender wheat lines as a preventive treatment under controlled conditions, causes a decrease of severity for most lines towards the E1 strain of Septoria tritici compared to the control seedlings.

Keywords: *Trichoderma harzianum;* Tender wheat; *Septoria tritici, biocontrol; Antagonist;* Severity

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INTRODUCTION

Septoria is among the most dreaded diseases of wheat in different regions of the world. It results to large productivity losses: from 30 to 50% [1,2]. In order to address the threats posed by this disease, several control methods were tested. However, only chemical control is considered as the most effective weapon [3].However, the use of these chemicals has harmful consequences on the environment such as the accumulation of waste, soil pollution, ecological imbalance, and induced resistance of pathogens [4].On this basis, the objective of this study is to evaluate the in vivo antagonistic power under controlled conditions of *Trichoderma harzianum* strain against a virulent strain E1 of *Septoria tritici* originally from Gharb on 16 differential lines and 8 Moroccan varieties of tender wheat.

EXPERIMETAL SECTION

Test on tender wheat seedlings under controlled conditions

The pathogenic agent:

Preparation of inoculums: The cultures of antagonistic fungus and E1 strain of *Septoria tritici*are incubated for 10 days at a temperature of 22°C with a photoperiod of 12 hours that is a sufficient period to get Petri dishes completely covered by antagonistic fungus and pathogenic strain. The surface of each box loaded cultured of conidia was submerged in 50 ml of sterile distilled water and scraped sterilely with a metal spatula to obtain a suspension that will be vortexed for one minute. The resulting suspensions were filtered through muslin to separate conidia from the mycelial fragments, before determining their concentrations using a Malassez cell then adjusting them with sterile distilled water to give a final concentration of 10^6 spores/ml that is addedto0.05% of Tween 20 (polyxy éthylene sorbitan) and 0.5% gelatin. The latter is necessary for a successful inoculation [5]. In fact, it enables uniform deposition of water droplets on the leaf surface and without gelatin, the majority of drops slide from the leaves [6]

Inoculation: The seedlings were inoculated at stage 3 leaves. This inoculation is done by spraying 60 ml of the previously prepared suspension on the leaf surface of the seedlings. These seedlings are then placed under a plastic bag which contains two humidifiers on both sides for 72 hours in order to maintain a relative humidity of 100%thatpromotes germination and direct penetration of conidia (without injury).The pots are then transferred to the growth chamber in a temperature of 20°C and a12hours photoperiod.

Figure1: Seedlings of tender wheat under a plastic bag containing 2 humidifiers after inoculation

The antagonistic agent:

A type of *Trichoderma harzianum* (T.H) was isolated and purified from a Moroccan soil. This antagonist is also multiplied on a PDA culture site.

The varieties of tender wheat:

The vegetal material used is made of 16 differential lines and 7 Moroccan varieties of tender wheat to which we added the indicator represented by the susceptible variety Achtar. (Table 1)

Table 1: Moroccan varieties and differential lines of tender wheat

Designation	Variety
A	Arrehane
B	Achtar
C	Rajae
D	Amal
E	Kanz
F	Salama
G	Tigre
H	wafia
1	BT04# 01
6	BT04# 06
8	BT04# 08
9	BT04# 09
10	BT04# 10
11	BT04# 11
12	BT04# 12
13	BT04# 13
15	BT04# 15
16	BT04# 16
18	BT04# 18
21	BT04# 21
22	BT04# 22
24	BT04# 24
25	BT04# 25
28	BT04# 28

Field trial:

The used vegetal material is previously mentioned and has been sown in Guich's domain. INRA experimental station in Rabat includes two plots, each covering 80 $m^2$of surface at a rate of 3 lines of 1m per variety with an indicator line separating two adjacent varieties (Figure 2).

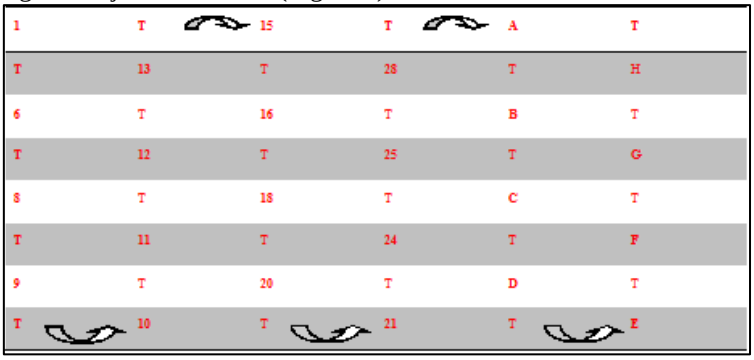

Figure 2: Plan of the planting area l'Guich

Inoculation plots:
A first inoculation was performed at the stage Z26 (tillering) depending to Zadoks (annex) where we have proceeded to spread the infected straw of wheat by septoria for both plots.
One of the two plots was inoculated to spray with a suspension of *Trichoderma harzianum*in Z32 stage (the second node emergence), then once arrived at stage Z39 (emergence of the last leaf), the two plots were inoculated to spray by a suspension of *Septoria tritici.*

Notation of the disease:
The estimation key of the severity of *Septoria tritici* on the leaves of tender wheat was taken into consideration to make the notation of the disease [7] (Table 2).

Table 2: Estimation key of *Septoria tritici*s everity on wheat leaves

Class	Characteristics
Resistant(R)	**Few** (lower leaves)
	Dissipated (2^{nd} level + 1^{st} light lower leaves)
	Light(1/3 plant +1^{st}moderatedlower leaves)
Moderately resistant(MR)	**Dissipated** (50% plant)
Moderately sensitive (MS)	**Severe**(low light leaves at 50% + dissipated in the upper dace**)**
	Severe(1/3 to the lower side of the plant + 50% dissipated to the upper side)
Sensitive (S)	**Severe**(50%was dissipated to the lower and upper side of the plant)
	Moderate to severe(1/3 on the upper side)
Very sensitive	**Severe**(the plant epi)

Scale is developed as part of the European scientific cooperation **(COST 817).**

RESULTS AND DISCUSSION

The Case tests of tender wheat on seedlings under controlled conditions
The independent samplet-test showed that a strong dependence (Phi Cramer = 0.613) between the lines and severity caused by *Septoria tritici* (isolate E1) for the different treatments ($\chi 2 = 21.64$, $P = 0.000$). The figure below shows the sensitivity level (severity) of each of the eight Moroccan varieties and the 16 differential lines towards E1 strain of *Septoria tritici* both before and after the treatment with 5 antagonists: TV, TH, AR, AT, TF. In the case of treatment with antagonists of *Trichoderma* genre, the results revealed a reduction in the level of sensitivity for the varieties and lines that were very sensitive (VS), sensitive (S) and moderately sensitive (MS) before treatment.This decreasing level of sensitivity proved to be more significant for treatments based on *Trichoderma harzianum* spores than treatments based on *Trichoderma viride* spores for varieties C, D and H and the lines 9, 15, 20 and 25 (Figure 3A). Figure 3B shows that the treatment by antagonists of the genus Acremonium didn't cause any effect on the entire vegetal material varieties tested except C, G and H and the lines 9 and 16.These lines have moved from a level of sensitivity (S) to a level (MS). In addition, treatment based on *Talaromyces flavus* led to a decline in the level of sensitivity of the majority of lines (Figure 3C). The treatments realized based on *Trichoderma* spores and *Talaromyces flavus* appear more efficient on the E1 strain under controlled conditions than the treatments based on *Acremonium* spores.
Figure 4 shows the distribution of tender wheat lines in relation to classes of sensitivity before and after treatment. It shows that only the percentage of lines belonging to the class of R sensitivity is more significant than the antagonists TH, TV and TF compared to the control. In parallel, no line has marked its presence in the VS class after treatment for these three antagonists.

The antagonistic effect of *Trichoderma harzianum* on the field
The figure below shows the sensitivity level (severity) of each of the eight Moroccan varieties and 16 differential lines before and after treatment with the spores of *Trichoderma harzianum.* In consequence, this made the variety F and the line 6 resistant to the E1 strain before treatment in contrary to the variety B and lines 21 and 24 which were very sensitive after inoculation with E1. However, the results obtained after treatment reveal a reduction in the sensitivity level of all the lines and varieties towards septoria except the varieties A, F and the lines 6, 8, 13 and 18 that remain in the same level of sensitivity. In contrast, the line 9 has marked a significant decrease going from the sensitive level (S) towards the resistant level (R).
Figure 6 represents the frequency of tender wheat lines in relation to the classes of sensitivity before and after the treatment. According to the rating scale of sensitivity level of CSE [7], the figure shows that the percentage of lines representing a sensitivity level R, MS and TS were, respectively, from the order of 8%, 33% and 12% before treatment. In parallel, the percentages of lines from the same sensitivity classes were in the order of 21%, 46% and 0% after treatment.

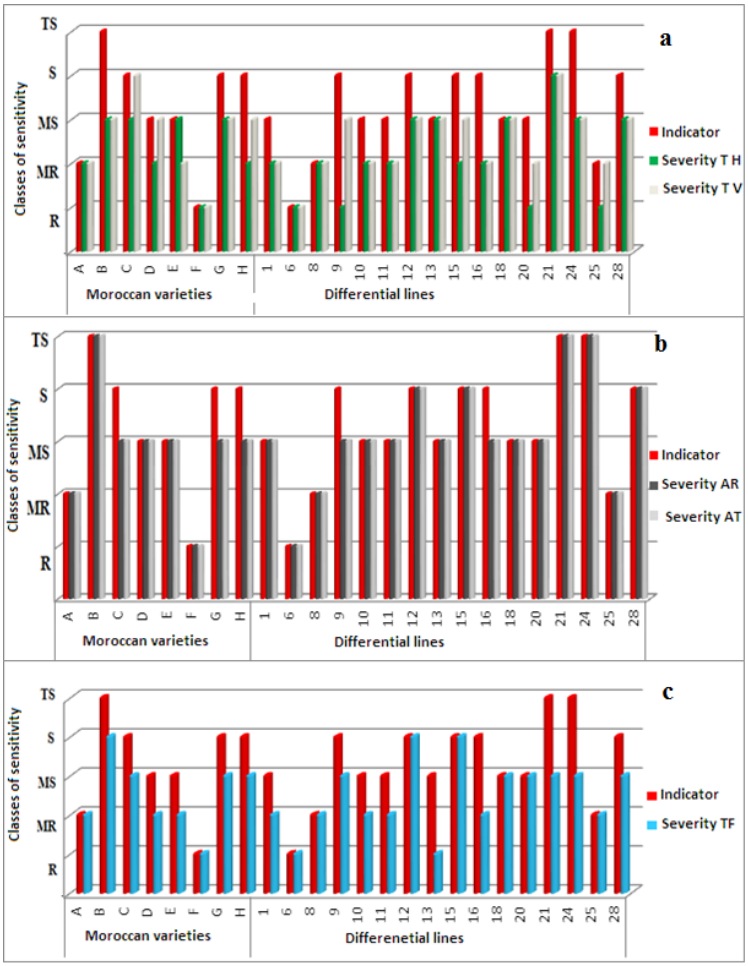

Figure 3: The sensitivity level of Moroccan varieties and differential lines of tender wheat towards the E1 strain of *Septoria tritici*both before and after treatment by different antagonists: A: *Trichoderma*; B: *Acremonium* and C: *Talaromyces flavus*

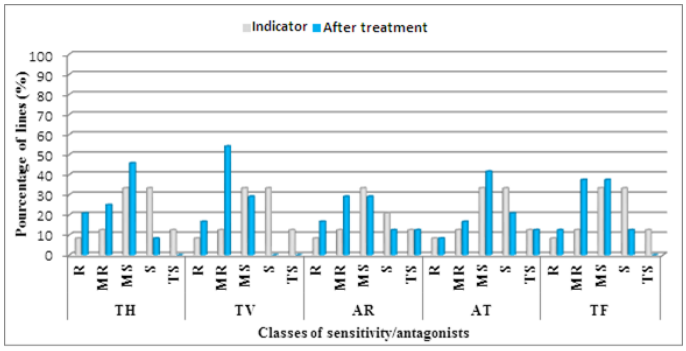

Figure 4: Frequency (%) of tender wheat lines depending to the classes of sensitivities towards septoria (E1) before and after treatment for each antagonist

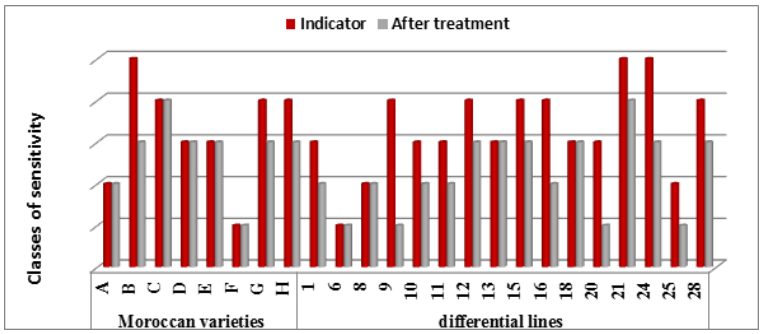

Figure 5: The sensitivity level of Moroccan varieties and differential lines of tender wheat towards the E1 strain of Septoria tritici before and after treatment with Trichoderma harzianum (TH)

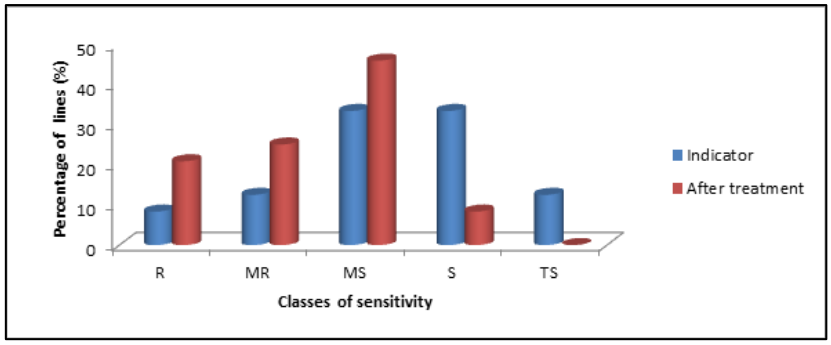

Figure 6: Frequency (%) of tender wheat lines according to the classes of sensitivities in relation to the septoria (E1) before and after treatment

DISCUSSION

The use of specific micro-organisms that interfere with both pathogens and parasites of plants is a respectful approach to nature and ecological for overcoming problems caused by conventional chemical methods of plant protection. Research has repeatedly shown that various fungal micro-organisms can act as natural antagonists of different plant pathogens [8]. The spraying spirals suspensions prepared under the antagonist's cultivation on seedlings of 24 tender wheat lines preventive treatment in controlled conditions causes a reduction in severity for most lines towards the E1 strain of *Septoria tritici* compared to control seedlings.

These results are consistent with several studies that have shown the decrease of some diseases after treatment with spores antagonists [9]. The spores' treatment of *Trichoderma harzianum* in the field shows a decrease in the level of severity of *Septoria tritici* for all the lines tested. Similar results were observed in Argentina for the control of *Septoria tritici* by preventive treatment of *Trichoderma harzianum* spores.

REFERENCES

[1] JE King ; RJ Cook ; SC Melville. *Ann App Biol*. **1983**, 103(2), 345-373.
[2] Z Eyal. The Septoria diseases of wheat: concepts and methods of disease management. Cimmyt, **1987**.
[3] M Bessa.,**1996**. Lutte contre les maladies cryptogamiques foliaires du blé et de l'orge par traitements fongicides des semences et en pleine végétation . Mémoire de 3ème cycle en Agronomie, IAV Hassan II, Rabat. 92p.
[4] Y Thakore. *Ind Biotechnol*, **2006**, 2(3), 194-208.
[5] S Ranomenjanahary. Etude de l'acide ténuazonique dans les relations riz-Pyricularia oryzae. Thèse de 3ème cycle. Université Paris XI, Orsay, France, **1987**,179.
[6] F Bouslim, B Ennaffah, A Ouazzani Touhami, A Douira, NE El Haloui. Pathogénie comparée de quelques isolats marocains de Helminthosporium oryzae vis-à-vis de certaines variétés du riz (Oryza sativa L.). Al Awamia, **1997**, 98, 74-56.
[7] Michel. Echelle élaborée dans le cadre de la coopération scientifique européenne, (action COST 817), **2001**.
[8] RJ Cook. *Annu Rev Phytopathol*, **2000**, 38(1), 95-116.
[9] AE Perelló; MV Moreno; C Mónaco; MR Simón; C Cordo. *Bio Control*, **2009**, 54(1), 113-122.