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MANAGEMENT OF WHEAT AND BARLEY ROOT ROT THROUGH SEED TREATMENT WITH BIOPESTCIDES AND FUNGICIDES

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ABSTRACT

Biological and chemical treatments of seeds with a Trichoderma suspension or solutions of Thirame with 10% gelatin, 4% superphosphate, and 0.1% sucrose were able to reduce the severity, incidence and index of root rot in wheat and barley plants growing on a substrate inoculated with three pathogens responsible for root rot, *Curvularia spicifera, Bipolaris sorokiniana,* and *Fusarium roseum.* In general, the S4 severity class was not observed in wheat plants from Trichoderma treated seeds or the fungicide. Sometimes even S3 and S2 classes were not found, in the case of durum wheat plants from treated seeds with Trichoderma growing on a substrate inoculated with *B. sorokiniana.* Barley plants from treated grain are much more protected than wheat plants, Classes S3 and S4 have not been observed and sometimes protection against *B. sorokiniana* is total in the case of plants from seeds treated with *Trichoderma.* In soft and hard wheat plants from *Trichoderma* or Thiram treatment, incidences and indices of root rot ranged respectively from 58.3% to 25%, compared to 100% and 83%, and between 51.6% and 23.3%, compared with 78.3% and 50%. In barley plants, incidences and indices of root rot ranged respectively from 50 to 16.7%, compared with 83.3 and 75% for controls and 30 to 18.3 and 51.6 to 27.7 for controls. Similarly, the development of *C. spicifera* and *B. sorokiniana* in the upper levels of wheat and barley plants decreased and leaf area indices were very low in plants from Trichoderma-treated seedlings. Treatment with this biocontrol agent has sometimes prevented wheat and barley from being infected by the tested pathogens, re-isolation from roots and foliar lesions was negative, and some symptoms developed in response to the age of seedlings do not harbor pathogens. The re isolation of *Trichoderma* was also positive from the roots of all the plants derived from the grains treated with this fungus.

The potential of *Trichoderma* to reduce the effect of pathogens responsible for root rot in cereals is probably a function of the seed treatment technique adopted in this study. This technique can be improved by providing a sufficient quantity of seed-borne antagonist inoculum capable of developing in the early stages of germination and preventing the penetration and installation of pathogens in seedlings root tissues.

Keywords: Barley; wheat; seeds; treatment; *Trichoderma*; fungicide; root rot; biocontrol.

INTRODUCTION

The study of root mycoflora of wheat and barley revealed the presence of several pathogenic fungi (*Bipolaris sorokiniana*, *Curvularia spicifera, Fusarium culmorum, Fusarium solani, Fusarium nivale, Fusarium poae, Fusarium oxysporum, Fusarium graminearum, Fusarium* sp. and *Fusarium roseum*), able to infect the roots of these cereals thus posing a threat to their culture in Morocco [1,2]. In general, grain farmers use systemic fungicides regularly and systematically on seeds before cultivation to prevent diseases caused by soil and foliar pathogens [3]. However, continued and repeated use of identical families of fungicides or chemical pollutants may have unwanted and undesired effects on plants and their environment [4] and on non-target organisms [5,6]. The risk of the appearance of strains resistant to these fungicides and the potential risk posed by the use of pesticides on health and the environment encourage the search for alternative mean of disease control including biological control. Indeed, other telluric microorganisms may have a beneficial effect in controlling root pathogenic fungi [7,8].

The discovery of biocontrol microorganisms and the demonstration of their ability to reduce the incidence and severity of disease have paved the way for many promising research [9,10,11,12,13].

Among the biological control agents, filamentous fungi of the *Trichoderma* genus, known for their antagonistic properties, have been used against a broad spectrum of plant pathogens [14]. They have been used in Morocco against various telluric fungi such as *Verticillium dahliae* [15,16,17] and *Fusarium oxysporum* f sp. *albedinis* [18,19] against leaf pathogens such as *Botrytis cinerea* [20,21], *Pyricularia oryzae* [22,23,24,25], *H. oryzae* [26], *H. sativum*, *H. spiciferum* and *H. australiensis* [25,27] and against those transmitted by rice seeds [28,29]. *Trichoderma* is used in biological control to compete with soil phytopathogenic fungi [30]. Their effectiveness depends on their ability to compete saprophytically and the amount of inoculum incorporated into the soil [31].

The present study was undertaken with the objective of reducing root and foliar infection [32] through seed treatment with a biocontrol agent.

MATERIALS AND METHODS

Plant Material

Five grams seeds of each of the Oussama varieties of barley, Amjad durum wheat, and Wafia common wheat were first disinfected by soaking for 5 minutes in a 70% ethanol solution, rinsed three times with sterile distilled water, and dried on filter paper for 24 hours.

Seed Treatment Technique

The isolate of *Trichoderma*, TR-B 98 (3), obtained from the roots of plants growing on sludge from phosphate washing plants was tested in this study [33]. The identification of this isolate was carried out based on macro and micromorphological characteristics: Colony growth, pigmentation, conidia, conidiophores and philiades [34]. This isolate was cultured on Potato Sucrose Agar medium (PSA: 200 g potato, 20 g sucrose, 15 g agar-agar, 1000 mL distilled water) and incubated at 25°C for 8 days in the dark. The culture surface is then washed with sterile distilled water and the concentration of the conidial suspension adjusted to 10^7 conidia / milliliter.

Thiramic 80WP (80% Thiram) was used at the registered rate of 250 g / quintals for seed treatments [35]. The used fungicide solution for seed treatment was obtained by diluting the stock solution prepared by solubilizing the commercial

product in sterile distilled water at a concentration of 2000 ppm Thiram.

The used seed treatment solution contains 10% of gelatin, 4% superphosphate, 0.1% sucrose and 10 mL of treatment solution (*Trichoderma* /fungicide /EDS). The wheat and barley seeds at 5 g are soaked in Petri dishes containing the treatment solution, and then the seeds are placed in Petri dishes containing the clay and stirred gently. They are placed in sterile dishes and dried for 72 hours at room temperature.

Control wheat and barley seeds underwent the same treatment but without the addition of antagonist or fungicide. The treated seeds are stored at 4°C and at room temperature and their viability has been checked according to the preservation time.

Inoculation of Soils

The soil inoculum was prepared from the pure cultures of *Fusarirum roseum, Bipolaris sorokiniana, Curvularia spicifera.* The three isolates are grown on PSA medium (Potato-sucrose agar). After 15 days of incubation for all species, the spore-laden surface is sterilely scraped with a metal spatula and sterile distilled water. The resulting suspension is filtered through muslin to separate the spores from the mycelium. The resulting spore solution is adjusted with distilled water containing 0.05% Tween so as to have a final concentration of 10^6 spores / milliliter.

Each pot is inoculated, by watering, with a spore suspension of 10^6 spores / mL of one the tested fungal species isolate. Twenty four hours after the inoculation of the cultivation substrate, the grains of the different varieties of durum wheat, wheat and barley that have undergone biological or chemical treatments are cultured on these substrates. The pots of the control grains were sprinkled with distilled water.

Rating of the Results

After 75 days, the root part of the plants was examined visually and the symptoms developed in the whole root system, and the sub-collar of the plants was noted. The severity of the disease at the flowering stage made it possible to evaluate six severity classes according to the types of symptoms observed according to the Greaney et al. [36].

S0 No infection
S1 Small necrotic lesions dispersed at the sub-crown and root
S2 Distinct necrotic lesions on the basal part of the plant, particularly at the sub-collar and roots
S3 Large necrotic lesions on the neck, sub-collar and roots with decreased vigor of the plant
S4 Rots of the basal part, chlorosis of the plant, often dwarfing and wilting.
S5 Dead plant.

The incidence of the disease was calculated according to the following formula:

$$I = 100 [Nm / Nt]$$

I is the percentage of diseased plants
Nm: Number of diseased plants
Nt: Total number of examined plants.

The root rot index was calculated according to the formula:

$$IM = 100 \Sigma (Ni\ Si) / (5\ Nt)$$

Ni: Number of plants of severity class i.
Nt: Total number of plants.
Si: Severity class i.

The degree of leaf alteration was estimated after 20, 40 and 60 days of culture according to the following scale:

0 Healthy leaf area
1 1 to 20% of the diseased leaf area
2 21 to 40% of the diseased leaf area
3 41 to 60% of the diseased leaf area
4 61 to 80% of the diseased leaf area
5 81-100% of the diseased leaf area

The notes relative to the number of observed leaves constitute the Foliar Alteration Index (FIA), calculated according to the formula, the average index is then calculated for each batch of plants.

$$IAF = [\Sigma (i \times xi)] / [6 \times NtF]$$

IAF: Leaf alteration index.
i: Appearance notes of leaves 0 - 5.
xi: Number of leaves presenting the note i.
NtF: Total number of leaves.

The re-isolation of *Fusarium roseum, Bipolaris sorokiniana,* and *Curvularia spicifera* was carried out at the ripening stage from the control and inoculated plants. The plants were dug up and removed from their growing medium by washing them thoroughly with running water. Cross-sections (2 cm) of infected root and collar were separately deposited in the alcohol at 90° for 1 to 2 minutes, rinsed thoroughly with sterile water, dried on a sterile filter paper and deposited on PSA medium.

Re-isolation was also performed from foliar lesions. Foliar lesion fragments of the wheat and barley plants were disinfected as before and incubated in Petri dishes containing two slices of filter paper soaked in sterile distilled water under a white light at 28°C. After 7 days of incubation, the cut fragments were examined under an optical microscope maintaining aseptic conditions to detect the presence or absence of the conidia of the fungus. The transfer of certain conidia under a microscope was done using a stretched glass capillary previously flame-sterilized and cooled in the culture medium. The transferred conidia were deposited on the surface of the PSA culture medium supplemented with 0.1% chloramphenicol.

The percentage of re isolation (Pr %) was calculated according to the following formulation:

$$\mathbf{Pr = Ns\ Px\ /\ NT \times 100}$$

Ns Px: Number of segments containing the fungal species x.
NT: Total number of segments.

Statistical Analysis

The data processing was based on the analysis of the variance by the first-order ANOVA test. A comparison of the means is carried out by the PPDS test (smallest significant difference) if a significant difference is registered at the probability level of 5%.

RESULTS

Durum and soft wheat berries transplanted on a substrate inoculated with *Curvularia spicifera* yielded seedlings with the highest severity class (S4), respectively with 25 and 16.6% seedlings (Table 1). Barley grains gave plants with only class S3 as the highest severity class (8.33% plants). *Trichoderma* or fungicide treatment of wheat (soft and hard) and barley grains reduced the severity of disease in plants growing on a substrate inoculated with *C. spicifera*. S4 severity class was not observed in wheat plants and the highest severity class, noted in barley plants, is S2.

Soft and hard wheat plants growing on a substrate inoculated with *Bipolaris sorokiniana* had the highest S4 class, with percentages of the order of 8.33 and 16.6% respectively (Table 2). Barley plants, under these conditions, presented only the S3 severity class, with 8.33% of plants.

Table 1. Percentages of wheat and barley seedlings treated with *Trichoderma* or Thiram seed and growing on a substrate inoculated with *Curvularia spicifera* based on severity classes

Treatment	Varieties of wheat and barley	Severity class				
		S0	S1	S2	S3	S4
Inoculation of the soil	Soft wheat Wafia	8.33^{g}	16.6^{c}	25^{c}	33.3^{a}	16.6^{b}
	Durum wheat Amjad	0^{h}	25^{b}	25^{c}	25^{b}	25^{a}
	Barley Oussama	25^{f}	33.3^{a}	33.3^{b}	8.33^{d}	0^{e}
Trichoderma + soil inoculation treatment	Soft wheat Wafia	50^{c}	16.6^{c}	25^{c}	8.33^{d}	0^{e}
	Durum wheat Amjad	41.6^{d}	8.33^{d}	33.3^{b}	16.6^{c}	0^{e}
	Barley Oussama	75^{d}	16.6^{c}	8.33^{e}	0^{e}	0^{e}
Thiram treatment + soil inoculation	Soft wheat Wafia	41.6^{d}	25^{c}	16.6^{c}	16.6^{c}	0^{e}
	Durum wheat Amjad	33.3^{e}	0^{e}	41.6^{a}	25^{b}	0^{e}
	Barley Oussama	166.6^{b}	16.6^{c}	16.6^{d}	0^{e}	0^{e}

* Two values read on the same column followed by the same superscript letter are not significantly different at the 5% threshold

In soft and hard wheat from Trichoderma-treated grains growing on a substrate containing *B. sorokiniana* inoculum, severity classes S3 and S4 were not observed, class S2 was noted only in wheat plants, with a percentage of 8.33%. The wheat plants from the fungicide-treated grains did not also have the S3 and S4 severity classes; class S2, noted in these wheat plants, varies between 16 in wheat and 8.3% in durum wheat. On the other hand, barley plants derived from *Trichoderma* or fungicide treated grains are totally protected against the effect of *B. sorokiniana*, no severity was noted (S0 is 100%).

The durum and soft wheat and barley plants are grown on a substrate inoculated with *F. roseum* showed significant severity, the S4 severity class was noted with 33.3% plant percentages (soft and hard wheat) and 8.33% (barley) Table 3.

Soft wheat and durum wheat treated with *Trichoderma* reduced the disease severity induced by *F. roseum*. The highest severity classes S3 and S4 were not noted in these plants. The same result was observed in the wheat plants from grains treated with the fungicide, the S4 severity class was not detected and the percentages of the plants that showed the S3 severity class ranged from 8.33 (soft wheat) and 16.6% (durum wheat). Barley plants from grains treated with *Trichoderma* and fungicide did not show severity classes S3 and S4.

These results indicate that *Trichoderma* and Thiram treatment of wheat or barley grains helps to reduce the development of symptoms of root rot in these cereals induced by *C. spicifera, B. sorokiniana* and *F. roseum*, judging by the estimation of the different classes of severity. The effect induced by *Trichoderma* sometimes exceeds that of the fungicide (Fig. 1).

Table 2. Percentage of wheat and barley seedlings treated with *Trichoderma* or Thiram grown on a substrate inoculated with *Bipolaris sorokiniana* based on severity classes

Treatment	Varieties of wheat and barley	Severity class				
		S0	S1	S2	S3	S4
Inoculation of the soil	Soft wheat Wafia	8.33^{g}	25^{a}	33.3^{a}	25^{b}	8.33^{b}
	Durum wheat Amjad	16.6^{f}	8.33^{c}	25^{b}	33.3^{a}	16.6^{a}
	Barley Oussama	41.6^{e}	25^{a}	25^{b}	8.33^{c}	0^{c}
Trichoderma + soil inoculation treatment	Soft wheat Wafia	$83.3b^{c}$	8.33^{c}	8.33^{d}	0^{d}	0^{c}
	Durum wheat Amjad	91.6^{b}	8.33^{c}	0^{e}	0^{d}	0^{c}
	Barley Oussama	100^{a}	0^{d}	0^{e}	0^{d}	0^{c}
Thiram treatment + soil inoculation	Soft wheat Wafia	66.6^{d}	16.6^{b}	16.6^{c}	0^{d}	0^{c}
	Durum wheat Amjad	75^{c}	16.6^{b}	8.33^{d}	0^{d}	0^{c}
	Barley Oussama	100^{a}	0^{d}	0^{e}	0^{d}	0^{c}

* Two values read on the same column followed by the same superscript letter are not significantly different at the 5% threshold

Table 3. Percentages of wheat and barley seedlings from *Trichoderma* or Thiram treated seed growing on *Fusarium roseum* inoculated substrate according to severity classes

Treatment	Varieties of wheat and barley	Severity class				
		S0	S1	S2	S3	S4
Inoculation of the soil	Soft wheat Wafia	0^{e}	8.33^{d}	33.3^{a}	33.3^{a}	33.3^{a}
	Durum wheat Amjad	0^{e}	16.6^{c}	16.6^{b}	33.3^{a}	33.3^{a}
	Barley Oussama	16.6^{d}	33.3^{a}	33.3^{a}	8.33^{b}	8.33^{b}
Trichoderma + soil inoculation treatment	Soft wheat Wafia	50^{cd}	16.6^{c}	33.3^{a}	0^{d}	0^{c}
	Durum wheat Amjad	58.3^{c}	25^{b}	16.6^{b}	0^{d}	0^{c}
	Barley Oussama	83.3^{a}	8.33^{d}	8.33^{c}	0^{d}	0^{c}
Thirame treatment + soil inoculation	Soft wheat Wafia	41.6^{d}	25^{b}	16.6^{b}	16.6^{b}	0^{c}
	Durum wheat Amjad	50^{cd}	25^{b}	16.6^{b}	8.33^{c}	0^{c}
	Barley Oussama	75^{b}	16.6^{c}	8.33^{c}	0^{d}	0^{c}

* Two values read on the same column followed by the same superscript letter are not significantly different at the 5% threshold

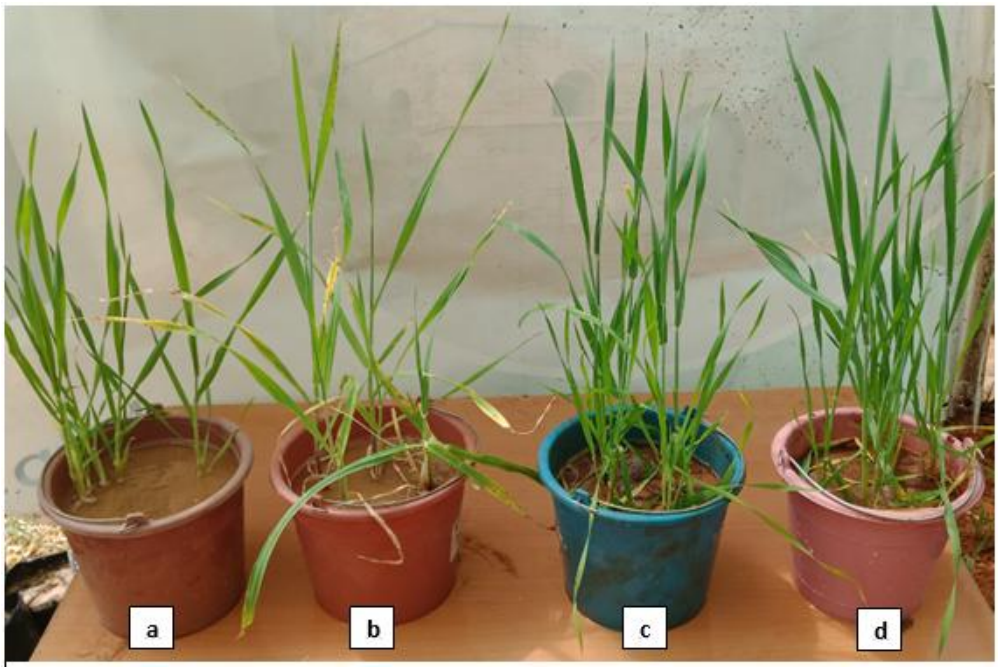

Fig. 1. Wheat seedlings derived from *Trichoderma* or Thiram treated grains and growing on a substrate inoculated or not with *B. sorokiniana*. a: Witness; b: Inoculated with *B. sorokiniana*; c: Treated with fungicide and inoculated with *B. sorokiniana*; d: *Trichoderma* treated and inoculated with *B. sorokiniana*

The incidence and index of root rot calculated in soft and hard wheat plants grown on a substrate inoculated with *C. spicifera* ranged from 91.9 to 100% and 50 to 70% (Table 4). In barley plants, the incidence and index of root rot are respectively 75 and 21.7%

Wheat plants (soft and hard) derived from grains treated with *Trichoderma* or fungicide grew on a substrate inoculated with *C. spicifera*, showed incidences and indices of root rot that did not exceed respectively 58.3 and 45%. In barley plants, the incidence and index of root rot are 25 and 26.6%. Seedlings from seeds treated with the fungicide had slightly higher incidence and indices of root rot, the highest found in durum wheat, 66.6 and 51.6%, respectively.

Table 4. Incidence (%) and Root Rot Index (%) found in wheat and barley seedlings from *Trichoderma* or Thiram seed and growing on a substrate inoculated with *C. spicifera*

Treatment	Varieties of wheat and barley	*Curvularia spicifera*	
		Incidence	Index
Inoculation of the soil	Soft wheat Wafia	91.6^{b}	50^{b}
	Durum wheat Amjad	100^{a}	70^{a}
	Barley Oussama	75^{c}	21.7^{e}
Trichoderma + soil inoculation treatment	Soft wheat Wafia	50^{ef}	38.3^{cd}
	Durum wheat Amjad	58.3^{e}	45^{c}
	Barley Oussama	25^{g}	26.6^{de}
Thiram treatment + soil inoculation	Soft wheat Wafia	58.3^{e}	43.3^{c}
	Durum wheat Amjad	66.6^{d}	51.6^{b}
	Barley Oussama	33.3^{f}	30^{d}

* Two values read on the same column followed by the same superscript letter are not significantly different at the 5% threshold

Wheat and durum wheat plants are grown on a substrate inoculated with *Bipolaris sorokiniana* showed incidences of 91.6 and 89.3% and disease indices of 60 and 65% (Table 5). Barley plants had an incidence of 58.3% and a root rot index of 40%.

Seed treatment with *Trichoderma* or the fungicide may have reduced the incidence and index of root rot in wheat and barley plants growing on a substrate containing *B. sorokiniana* inoculum. The incidence and indices of root rot in wheat plants did not exceed 33.3% and 23.3% respectively. Barley plants from treatments with *Trichoderma* or the fungicide and growing under the same conditions did not show evidence of root rot, with the exception of barley plants derived from grains treated with a fungicide, which showed an index of root rot of around 20%.

Soft wheat and durum wheat plants are grown on a substrate inoculated with *Fusarium roseum* showed incidences of the order of 100% and root rot indices of 78.3 and 76.6% (Table 6). Barley plants had an incidence of 83.3% and a root rot index of 51.6%.

Wheat seedlings derived from grain treatment with *Trichoderma* or fungicide showed incidences and indices of root rot that did not exceed 58.3 and 41.7% respectively. The barley plants, also, showed incidences and indices that did not exceed 50 and 25% respectively.

From these results, it appears a second time that the *Trichoderma* or fungicide treatment of wheat and barley grains contributes to the protection of wheat and barley plants against the effects of pathogens responsible for root rot. The incidence and indices of this disease decreased in all plants resulting from treatment.

Table 5. Incidence (%) and Root Rot Index (%) found in wheat and barley seedlings from *Trichoderma* or Thiram seed and growing on a substrate inoculated with *B. sorokiniana*

Treatment	Varieties of wheat and barley	*Bipolaris sorokiniana*	
		Incidence	Index
Inoculation of the soil	Soft wheat Wafia	91.6^{a}	60^{ab}
	Durum wheat Amjad	83.3^{b}	65^{a}
	Barley Oussama	58.3^{c}	40^{b}
Trichoderma + soil inoculation treatment	Soft wheat Wafia	16.7^{e}	25^{d}
	Durum wheat Amjad	8.33^{f}	23.3^{d}
	Barley Oussama	0^{g}	0^{e}
Thiram treatment + soil inoculation	Soft wheat Wafia	33.3^{d}	30^{c}
	Durum wheat Amjad	25de	26^{d}
	Barley Oussama	0^{g}	20de

* Two values read on the same column followed by the same superscript letter are not significantly different at the 5% threshold

Table 6. Incidence (%) and Root Rot Index (%) found in wheat and barley seedlings treated with *Trichoderma* or Thiram grown on a substrate inoculated with *Fusarium roseum*

Treatment	Varieties of wheat and barley	*Fusarium roseum*	
		Incidence	Index
Inoculation of the soil	Soft wheat Wafia	100^{a}	78.3^{a}
	Durum wheat Amjad	100^{a}	76.6^{a}
	Barley Oussama	83.3^{b}	51.6^{b}
Trichoderma + soil inoculation treatment	Soft wheat Wafia	50^{cd}	36.6^{d}
	Durum wheat Amjad	41.6^{d}	31.6^{d}
	Barley Oussama	16.7^{f}	25^{e}
Thiram treatment + soil inoculation	Soft wheat Wafia	58.3^{c}	41.7^{c}
	Durum wheat Amjad	25^{e}	26.6^{e}
	Barley Oussama	50c^{d}	18.3^{g}

* Two values read on the same column followed by the same superscript letter are not significantly different at the 5% threshold

Wheat and barley plants growing on a substrate inoculated with *C. spicifera* and *B. sorokiniana* also showed leaf symptoms (Table 7). Foliar lesions were observed after 40 days of plant development (Figs. 2 and 3). Foliar indices after 60 days of cultivation ranged from 0.4 to 0.53 in wheat plants and 0.38 to 0.44 in barley plants.

Foliar indices decreased significantly in seedlings from Thiram and *Trichoderma*. They vary respectively between 0.32 and 0.18 and between 0.26 and 0.23 in seedlings derived from seeds treated with the fungicide and growing respectively on substrates inoculated with *C. spicifera* and *B. sorokiniana*. In barley plants growing under the same conditions, the leaf indices vary between 0.24 and 0.12.

Wheat and barley seedlings grown from *Trichoderma* grown on a substrate inoculated with an inoculum of *C. spicifera* and *B. sorokiniana* showed foliar indices ranging from 0.18 to 0.02. In barley plants, these indices vary between 0.12 and 0.09.

Based on these results, *Trichoderma* and Thiram seed treatment appeared to influence the occurrence of symptoms of *C. spicifera* and *B. sorokiniana* on the leaves of wheat and barley plants. These symptoms decreased and foliar indices increased from 0.5, the highest leaf number in untreated wheat plants, to 0.09 in barley plants from grains treated with *Trichoderma*.

F. roseum, C. spiciferum and *B. sorokiniana,* inoculated to culture substrates, were re-isolated from root symptoms observed in wheat and barley plants growing on these substrates (Table 8). *F. roseum* was not isolated from the aerial parts of wheat and barley plants. In contrast, *C. spiciferum* and *B. sorokiniana* infected the roots and upper levels of the seedlings. The percentages of re-isolation from the roots and foliar lesions of wheat plants growing on a substrate inoculated with *C. spiciferum* and *B. sorokiniana* vary between 75 and 100% and between 75.6 and 86.7% respectively. The percentages of re-isolation from the roots and foliar lesions of wheat plants growing on a substrate inoculated with *C. spiciferum* and *B. sorokiniana* vary between 75 and 100% and between 75.6 and 86.7% respectively.

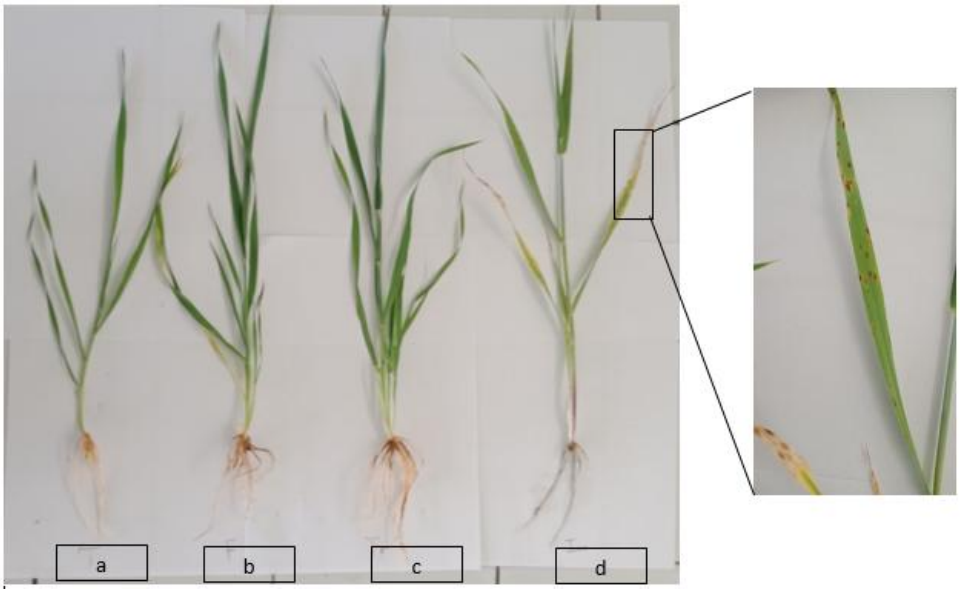

Fig. 2. Vegetative apparatus of wheat plants derived from *Trichoderma* or Thiram treated grains and growing on a substrate inoculated or not with *B. sorokiniana*. a: Witness; b: Treated with the fungicide and inoculated with *B. sorokiniana*; c: *Trichoderma* treated and inoculated with *B. sorokiniana*; d: Inoculated by *B. sorokiniana*

Fig. 3. Foliar lesions observed in barley plants growing on a substrate inoculated with *B. sorokiniana*

Table 7. Foliar Alteration Indices (IAF) in wheat and barley plants from *Trichoderma* and Thiram-treated seed developed on substrates inoculated with *C. spicifera* and *B. sorokiniana*

Treatment	Varieties of wheat and barley	*C. spicifera*			*B. sorokiniana*		
		20 days	40 days	60 days	20 days	40 days	60 days
Inoculation of the soil	Soft wheat Wafia	0^{a}	0.09^{b}	0.53^{a}	0^{a}	0.07^{d}	0.4^{b}
	Durum wheat Amjad	0^{a}	0.1^{a}	0.51^{a}	0^{a}	0.1^{a}	0.43^{b}
	Barley Oussama	0^{a}	0.08^{c}	0.49^{b}	0^{a}	0.08^{c}	0.38^{c}
Trichoderma + soil inoculation treatment	Soft wheat Wafia	0^{a}	0.02^{f}	0.18^{e}	0^{a}	0.07^{d}	0.26^{d}
	Durum wheat Amjad	0^{a}	0.05^{d}	0.32^{c}	0^{a}	0.09^{b}	0.23^{d}
	Barley Oussama	0^{a}	0.05^{d}	0.24^{d}	0^{a}	0.04^{e}	0.12^{e}
Thiram treatment + soil inoculation	Soft wheat Wafia	0^{a}	0.05^{d}	0.1^{e}	0^{a}	0.03^{f}	0.18^{e}
	Durum wheat Amjad	0^{a}	0.03^{e}	0.16^{e}	0^{a}	0.02^{g}	0.15^{e}
	Barley Oussama	0^{a}	0.01^{g}	0.12^{e}	0^{a}	0.02^{g}	0.09^{a}

* Two values read on the same column followed by the same superscript letter are not significantly different at the 5% threshold

The re-isolation percentages were reduced in plants coming from grains treated with fungicide or *Trichoderma.* In wheat (durum and soft wheat) treated with the fungicide, the re-isolation percentages of *C. spicifera* and *B. sorokiniana* from roots and foliar lesions ranged respectively

from 45% to 28.5% and 26.7% and 16.8%. In barley plants, the percentages of re-isolation of these two pathogens from the roots are 33.5 (*C. spicifera*) and 16.6% (*B. sorokiniana*) and from the leaf lesions are of the order 15.5 (*C. spicifera*) and 9.5% (*B. sorokiniana*). The re-isolation percentages of *F. roseum* are zero from the roots of barley plants and vary between 15.7 and 9.5% from the roots of wheat plants treated with Thiram.

In plants grown from Trichoderma-treated wheat or barley, the percentages of re-isolation of the three pathogens tested from roots and foliar lesions were even lower than those observed in the same Thiram-treated plants. Re-isolations of the three tested pathogens from the roots and lesions of barley plants were negative. The percentages of *F. roseum* re-isolation from the roots of wheat plants ranged from 6.5 to 9.5%, those of *C. spicifera* and *B. sorokiniana* are of the order of 8.5%. *B. sorokiniana* was not reisolated from the roots of durum wheat plants. The re-isolation percentages of *C. spicifera* vary between 8.5 (common wheat) and 10.6% (durum wheat).

The percentages of re-isolation of *C. spicifera* and *B. sorokiniana* from foliar lesions of wheat plants ranged from 10.6 to 12.5%. In contrast, the re-isolation of these two pathogens was negative from leaf lesions in durum wheat plants.

The reisolation of *Trichoderma* was positive from the roots and rhizosphere of all the seedlings treated by this fungus.

These results indicate that seed treatments with *Trichoderma* and Thiram reduced plant infection by the different pathogens tested. Sometimes Trichoderma seed treatments prevented this infection, to judge by the negative reisolation observed from the roots and foliar lesions. Some symptoms developed according to the age of the plants do not harbor the pathogens. Similarly, *Trichoderma* was able to colonize plant roots and the rhizosphere of seedlings treated with this biological control agent.

DISCUSSION

Treatment of wheat and barley grains with *Trichoderma* spores or fungicide resulted in a significant reduction in the development of rot symptoms, estimated by the severity, incidence, and index of root rot. Sometimes the effect of *Trichoderma* in reducing disease in wheat and barley exceeded that of the tested fungicide. Hervás et al. [37] observed that soil treatment with a *T. harzianum* bio fungicide significantly reduced the incidence of fusarium wilt in chickpea. Dubey et al. [38] showed the efficacy of a few isolates belonging to the species: *T. viride, T. harzianum* and *T. virens*. Dubey et al. [39] and Uoti [40] reported that *Trichoderma harzianum* has inhibitory action against *Fusarium culmorum* and *Bipolaris sorokiniana*, a root rot agent in cereals.

De Mayer et al. [41] noted the treatment effect of *Trichoderma harzianum* on the reduction of symptoms of *Botrytis cinerea* gray mold on tomato, lettuce, pepper, beans and tobacco. Dubey and Patel [42] and Poddar et al. [43] reported that seed and soil treatment with *Trichoderma* species led to a decrease in disease incidence and increased yields of cultivated plant species. In this sense, Harman et al. [44] reported that corn seed treatment with *T. harzianum* was able to reduce the development of anthracnose symptoms in plants subsequently inoculated with *Colletotrichum graminicola*. Orakç et al. [45] and Khezri et al. [46] noted that the application of natural antagonists on crop residues, directly on plant organs or by seed coating greatly reduces the severity of root rot. Sghir et al. [18] also highlighted the effect of *T. harzianum* on the development of *Fusarium* date palm blight, caused by *F. oxysporum* f sp. *albedinis*. In fact, *T. harzianum* treatment of the plants prevented the installation of the pathogen at the root level and its migration to the higher levels of the plants.

Trichoderma has been reported to be highly effective against a broad spectrum of fungal diseases, eggplant seedling burns [47], gray mold on many crops [44] and damping induced by *Phytophthora* [48], *Rhizoctonia solani* [49]. In other studies, Montaser et al. [50] reported that *Trichoderma viride* and *T. harzianum* are the best biocontrol agents because of their ability to significantly reduce the incidence of disease and increase plant yield. Srivastava et al. [51] and Mouria et al. [14] noted that the colonization of plant roots by *Trichoderma* and their competitiveness in the rhizosphere against the

Table 8. Re-isolation of pathogens from different parts of wheat and barley plants from Trichoderma and Thiram treated seeds and grown on substrates inoculated with *C. spicifera, B. sorokiniana* and *F. roseum*

Treatment	Varieties of wheat and barley	Root part			Arial part	
		C. spicifera	*B. sorokiniana*	*F. roseum*	*C. spicifera*	*B. sorokiniana*
Inoculation of the soil	Soft wheat Wafia	100^{a}	100^{a}	75.6^{b}	86.7^{a}	76.9^{a}
	Durum wheat Amjad	95.5^{a}	75^{c}	100^{a}	75.6^{b}	78.9^{a}
	Barley Oussama	93.5^{a}	83.6^{b}	67.5^{c}	63.5^{c}	53.5^{b}
Trichoderma + soil inoculation treatment	Soft wheat Wafia	45^{b}	36.9^{d}	10.4^{e}	25^{d}	16.8^{c}
	Durum wheat Amjad	43.6^{b}	28.5^{e}	20^{d}	26.7^{d}	19.5^{c}
	Barley Oussama	33.5^{c}	16.7^{f}	0^{g}	15.7^{e}	9.5^{d}
Thiram treatment + soil inoculation	Soft wheat Wafia	8.5^{e}	8.5^{g}	6.5^{f}	12.5^{e}	10.6^{cd}
	Durum wheat Amjad	10.6^{d}	0^{h}	9.5^{f}	0^{f}	0^{e}
	Barley Oussama	0^{f}	0^{h}	0^{g}	0^{f}	0^{e}

* Two values read on the same column followed by the same superscript letter are not significantly different at the 5% threshold

soil microflora are responsible for protecting the roots against pathogens and improving the growth and development of roots of these plants. Sghir et al. [19] showed that the treatment of two varieties of date palm "Majhoul" and "Boufegouss" with *Trichoderma harzianum* decreased the effect of Fusarium wilt. This decrease was accompanied by a positive effect on the growth of treated plants of both varieties by *T. harzianum* and inoculated with *F. oxysporum* f. sp. *albedinis* of both varieties compared to control plants inoculated only by the pathogen. The same result was reported by Duffy et al. [52] which have shown that *Trichoderma koningii* better promotes the development of wheat plants even in the absence of the pathogen on two substrates, a natural soil and another sterile.

Trichoderma seed treatment greatly influenced the development of symptoms of *C. spicifera* and *B. sorokiniana* on the higher seedling levels, foliar indices were very low and the isolation of these two pathogens from foliar lesions is sometimes negative. This result seems to be important. Indeed, *C. spicifera* and *B. sorokiniana* can attack the roots and foliage of cereals. Rotem [53] reported that the most infectious species are those capable of affecting a larger part of the host tissue and allowing the multiplication of the inoculum. Qostal et al. [1,2] reported that these two pathogens sporulate well on root necrotic lesions and foliar lesions and formed spores can initiate other cycles of the disease. It is therefore advisable to reduce the amount of aerial inoculum from sporulated necrotic lesions or to prevent the seeds from being infected if the transmission is panicles to grains in a systemic way.

CONCLUSION

In the light of these greenhouse investigations, *Trichoderma*, biological control agents, is treating wheat and barley seeds, is effective and has reduced the severity of root and foliar pathogens for those able to grow on higher plant levels. Improvement of the seed treatment technique may lead to improved plant protection against the effects of pathogens responsible for root rot in cereals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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