Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Bioactivity of *Trichoderma harzianum* A peptaibols against *Zymoseptoria tritici* causal agent of septoria leaf blotch of wheat

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Vol. 63, No. 1: 59–67, 2023

DOI: 10.24425/jppr.2023.144504

Received: September 02, 2022
Accepted: October 27, 2022
Online publication: March 09, 2023

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Responsible Editor:
Andrea Toledo

Abstract

In this study *Trichoderma harzianum* strain A was isolated from the rhizosphere of an argan tree in southern Morocco. *Trichoderma harzianum* strain A had previously demonstrated a high antagonistic potential *in vitro* by direct confrontation and *in vivo* on wheat plants in pots under greenhouse conditions against *Zymoseptoria tritici*, the agent of septoria leaf blotch. In this study, the activity of filtrates prepared from the liquid culture of *T. harzianum* A alone and from the confrontation medium with two *Z. tritici* strains [G1-1 (durum wheat) and A5-1 (soft wheat)] on the inhibition of *Z. tritici* pycnidiospore germination was studied by nephelometry. The results of the antibiosis assay revealed that filtrate 0 (A in confrontation with G1-1) and F3 (A against A5-1) showed 95% of G1-1 and A5-1 pycnidiospore inhibition at 9/10 dilution of the undiluted filtrates after 4 days of incubation. To understand and explain the antifungal activity of these filtrates, the extraction and identification of secondary molecules of peptaibiotic nature secreted by *T. harzianum* A in the three studied filtrates were performed. According to the results of high-performance liquid chromatography-mass spectrometry (HPLC-MS) analyses, 38 peptaibiotic molecules reported in the literature for their antifungal activity were identified in the different extracts at high concentrations (high peak intensities). These molecules are divided into nine groups, namely: Trichocryptin, Trichobrevin, Triochocryptin, Hypocompactin, Hyporodicin, Trichocompactin, Alamethicine, Trichoferin, and Trichokonin. It was also shown that the presence of the pathogen induces the production of peptaibols by the antagonistic strain of *Trichoderma*.

Keywords: antibiosis, nephelometry, peptaibols, *Trichoderma harzianum*, *Zymoseptoria tritici*

Introduction

The antagonistic properties of *Trichoderma* spp. (Teleomorph: Hypocrea) against some phytopathogenic agents were first reported by Vuillemin in 1887 (cited by Mouria *et al.* 2008). Some strains of *Trichoderma* have been used as effective biological protective agents to control fungal and bacterial diseases in plants (Harman *et al.* 2004; Dubey *et al.* 2007; Sriram *et al.* 2013). Many *Trichoderma* strains exhibit the ability to parasitize fungi, including a very wide range of plant pathogens (Chet 1993). In addition, *Trichoderma* strains establish vigorous and long-lasting colonizations of parasitized plants by penetrating the epidermis and adjacent cell layers.

Currently, more than 200 species belonging to the *Trichoderma* genus have been identified and classified by molecular phylogenetic analyses (Atanasova *et al*. 2013). This high taxonomic diversity of *Trichoderma* is not only in the increasing number of species (Kim *et al*. 2012, 2013; Yamaguchi *et al*. 2012; Li *et al*. 2013; Yabuki *et al*. 2014), but also in the structure of their secondary metabolites. Mainly, these metabolites can be classified into three categories: volatile compounds, diffusible compounds, and peptaı-bols (Jelen *et al*. 2013; Blaszczyk *et al*. 2014; Vinale *et al*. 2014).

Metabolic analysis exhibited that the *Trichoderma* genus is the most prolific source of non-ribosomal peptide antibiotics, known as peptaibols or peptaibiotics (Degenkolb and Brückner 2008; Brückner *et al*. 2009). As a typical case, 950 out of nearly 1300 identified peptaibiotic molecules (Ren *et al*. 2009; Ayers *et al*. 2012; Röhrich *et al*. 2012, 2013a, b; Carroux *et al*. 2013; Figueroa *et al*. 2013; Kimonyo and Brückner 2013; Panizel *et al*. 2013; Stoppacher *et al*. 2013) were obtained from *Trichoderma* species. Nevertheless, in most cases, the specific activity of these molecules remains unknown (Reino *et al*. 2008; Mukherjee *et al*. 2012; Crutcher *et al*. 2013).

Since the first discovery of antibiotic peptide activity through the isolation of alamethicin from cultures of *T. arundinaceum* strain NRRL 3199 (originally identified as *T. viride*) (Reusser 1967), many biological activities have been assigned to paptaibols, including antifungal, antiviral, antibacterial, and insecticidal properties (Berg *et al*. 1996; Kim *et al*. 2000; Yun *et al*. 2000; Bandani *et al*. 2001; Leclerc *et al*. 2001; Landreau *et al*. 2002).

We have discovered a new strain of *Trichoderma harzianum* which potentially can be interesting for biocontrol approaches. In previous studies (Barakat *et al*. 2018, 2019a, b), we confirmed the antagonistic potential of this strain by direct confrontation tests and the induction of induced systemic resistance (ISR) assays, so the aim of this work was to study the antibiosis mechanism to confirm all the biocontrol mechanisms in our strain.

In the present study, the antibiosis effect of *T. harzianum* strain A was evaluated. The antifungal activity of three filtrates, prepared from the culture medium of *T. harzianum* A or from the confrontation medium of *Trichoderma* with *Z. tritici* A5-1 and G1-1 strains, on the pycnidiospores germination of A5-1 and G1-1 was studied by nephelometry. Also, the extraction of peptaibols produced by *T. harzianum* A was performed with two extraction methods. These extracts were analyzed by high-performance liquid chromatography-mass spectrometry (HPLC-MS) to identify the peptaibiotic molecules contained in the extracts and which can be related to the antifungal activity of *T. harzianum* against *Z. tritici*.

Materials and Methods

Antifungal activity of *Trichoderma harzianum* A filtrates

Trichoderma harzianum strain a was isolated from the rhizosphere of an argan tree in southern Morocco, and showed a high antagonistic potential against *Z. tritici* strains A5-1 and G1-1 (Barakat *et al*. 2018, 2019a, b).

Filtrate preparation

The filtrates of *T. harzianum* A were obtained from cultures grown in Potato Dextrose Broth (PDB) medium. From 200 ml of the medium, 16 pellets, 1 cm in diameter, were taken from a culture of *T. harzianum* on Potato Dextrose Agar (PDA) medium for 7 days. Also, *T. harzianum* cultures were prepared in confrontation with *Z. tritici* strains G1-1 and A5-1. The cultures were incubated in the dark under agitation at 25°C for 7 days. Then, the cultures were filtrated through 0.45 µm syringe filters of cellulose acetate (sterile).

Preparation of pathogen suspensions

Suspensions of the two *Z. tritici* strains (G1-1 and A5-1) were prepared from 7-day-old cultures grown on PDA medium and incubated in the dark at 18°C. The concentration of the suspensions was adjusted to 10^6 pycnidiospores · ml^{-1}.

Microtiter plate preparation

The three filtrates (F1 prepared with *T. harzianum* A alone, F2 prepared with A and G1-1, and F3 prepared with A and A5-1) were tested with three dilutions of the initial extract (d1 = 1/10, d2 = 1/2, and d3 = 9/10) on the two *Z. tritici* strains G1-1 and A5-1. In a tube, the filtrate (F1/F2/F3) was mixed with 100 µl of the aqueous suspension of *Z. tritici* and the volume of 1 ml was completed with the PDB medium. The mixture was vortexed to homogenize the suspension, and then the 96-well plate was filled with 300 µl/well of the final suspension with three repetitions. For the control, the filtrate volumes were replaced with sterile milliQ water. This test was repeated three times to confirm the results obtained.

Analysis parameters

This study was performed with a chronos type nephelometer which measures the turbidity in NTU, that is an indicator of fungal germination and growth at different dilutions of the filtrates. The incubation temperature of the plate during the analysis was 25°C. The number of analysis cycles was 16 cycles, with an interval of 6 hours between two successive cycles and a laser intensity of 80%. Before each reading, the plate underwent a double orbital shake for 300 seconds with a shaking speed of 150 rpm.

Statistical analysis

Turbidity data were analysed by analysis of variance (ANOVA 1) with the SPSS version 21 software. The factors studied were: filtrates (F1, F2, F3 and F4), dilutions (d1, d2 and d3) and *Z. tritici* strains (A5-1 and G1-1). The comparison of means was performed by Duncan's test.

Production and identification of secondary metabolites

Preparation of the cultures

On Petri dishes containing 24-hour-old *Z. tritici* culture, 10 µl of *T. harzianum* A suspension, pre-cultured on PDA medium for 7 days was added to the center of the dishes. The confrontation dishes were incubated at 25°C in the dark for 4 days.

Liquid cultures were prepared according to the same protocol described in the section "Antifungal activity of *T. harzianum* A filtrates – Filtrate preparation".

Extraction of secondary metabolites

To extract secondary metabolites from the agar medium, three agar fragments of 8 mm diameter, were taken from the inhibition zones and mixed with 2 ml of acetonitrile in test tubes. The mixture was homogenized by vortex and incubated at 4°C for 4 hours. Then, it was centrifuged for 10 min at 4,000 rpm and the supernatant was recovered and filtered through a 0.45 µm syringe filter. Extraction of secondary metabolites produced by *T. harzianum* A without confrontation with the pathogen *Z. tritici* was performed from the biomass developed on the PDA dishes incubated for 10 days at 25°C in the dark. After collecting and adding the biomass of the three dishes to a tube, the tube was placed in a freezer for 4 days. Then in the removed and defrosted tube, 3 ml of ethyl acetate was added before shaking under rotary agitation at 200 rpm for 12 hours. The ethyl acetate was transferred to another tube to evaporate the solvent by rotavapor, and the extract was redissolved with 1 ml of acetonitrile. The solution was then filtered with a 0.45 µm syringe filter.

Concerning the secondary metabolites' extraction from the liquid medium, 15 ml of ethyl acetate were added to each 30 ml culture and shaken at 200 rpm for 12 hours. Then the ethyl acetate was collected and filtered through a Whatman PS1 filter paper, the solvent was evaporated, and the extract was redissolved with 1 ml of acetonitrile; and the solution was filtered through a 0.45 µm syringe filter.

Peptaibol identification by high-performance liquid chromatography-mass spectrometry (HPLC-MS)

The extracts obtained were analyzed by HPLC-MS (Thermo Scientific) using a C18 length capillary column. After injection of 10 µl of each extract diluted 1/100, elution was performed in a binary solvent system (solvent A: water + 0.1 % formic acid and solvent B: acetonitrile + 0.1 % formic acid) as follows: 30% B for 5 min, then a gradient from 30% B to 45% B for 5 min followed by a gradient from 45% B to 100% B for 25 min, with a flow rate of 0.5 ml · min^{-1} at 40°C. Peptaibols were identified according to their molar masses.

Results

Antibiosis effect of *Trichoderma harzianum* A on the germination of *Zymoseptoria tritici* pycnidiospores

The effect of filtrates F1, F2 and F3 was tested on *Z. tritici* G1-1 and A5-1 strains at different dilutions (1/10, 1/2 and 9/10). The results obtained showed that all filtrates had an inhibitory effect on the germination of *Z. tritici* pycnidiospores compared to the control F4 from the first hours of incubation. The percentages of inhibition were calculated with the turbidity data of the different studied conditions (filtrate and dilution) and compared to the control F4.

The three dilutions of the filtrate F1 prepared from the culture medium of the *T. harzianum* A showed significant inhibition rates, around 75% (89 KNTU) after 6 hours of incubation in the case of the strain G1-1. After 30 hours of incubation, the inhibition rates exceed 90% for the three dilutions; these rates were 95% in 96 hours for the 9/10 dilution and 93% for the 1/10 dilution (Fig. 1).

The same filtrate inhibited 90% of the *Z. tritici* A5-1 pycnidiospores germination within 24 hours and after 72 hours the inhibition rates were 94%, 95% and 96% for the 1/10, 1/2 and 9/10 dilutions, respectively (Fig. 2).

Concerning filtrate F2 prepared with *T. harzianum* A and *Z. tritici* G1-1, the inhibition of *Z. tritici* G1-1 pycnidiospores germination ranged from 75% after 6 hours of incubation to 95% for the three dilutions (1/10, 1/2, and 9/10) on the 4th day of incubation. It was the most effective filtrate that was classified in the first group according to Duncan's test of means comparison (Fig. 3).

For *Z. tritici* A5-1, the inhibition rates were 89%, 91%, and 92% for 1/10, 1/2, and 9/10 dilutions, respectively. At 66 hours, the inhibition was 94% for the 1/10 dilution, and 95% for the 1/2 and 9/10 dilutions (Fig. 4).

Regarding filtrate F3 prepared with *T. harzianum* A and *Z. tritici* A5-1, the 1/2 and 9/10 dilutions effectively inhibited the germination of *Z. tritici* pycnidiospores with an inhibition rate of 95% after 96 hours of incubation. However, with the 1/10 dilution, inhibition rates ranged from 65% to 84% during the first 48 hours for G1-1 and from 70% to 82% for A5-1. On the 3rd day of incubation, the rates started to decrease to 57% and 55% for G1-1 and A5-1 pycnidiospores, respectively (Figs. 5 and 6).

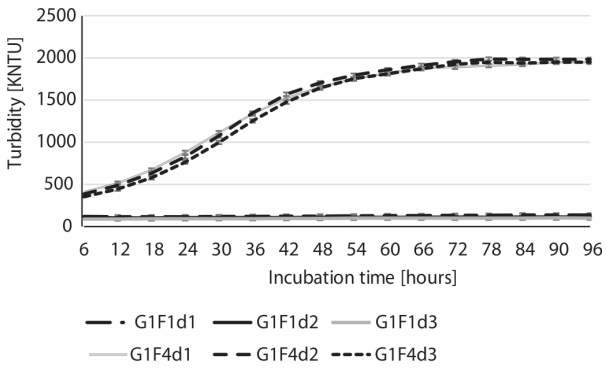

Fig. 1. Effect of filtrate F1 (prepared with *Trichoderma harzianum* A) on the germination of *Zymoseptoria tritici* pycnidiospores strain G1-1 in comparison to control F4. NTU – nephelometric turbidity unit

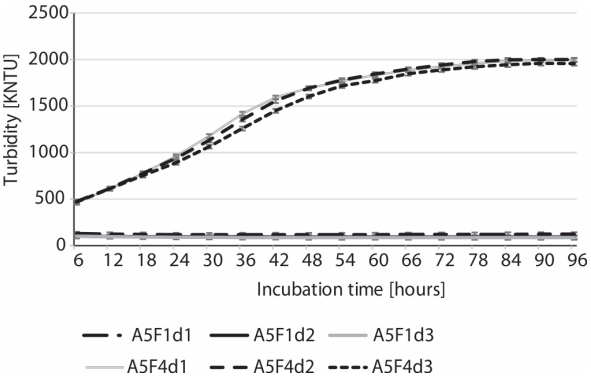

Fig. 2. Effect of filtrate F1 (prepared with *Trichoderma harzianum* A) on the germination of *Zymoseptoria tritici* pycnidiospores strain A5-1 in comparison to control F4. NTU – nephelometric turbidity unit

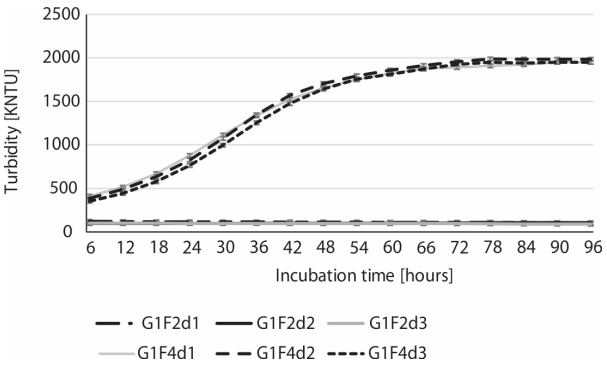

Fig. 3. Effect of filtrate F2 (prepared with *Trichoderma harzianum* A and *Zymoseptoria tritici* G1-1) on the germination of *Zymoseptoria tritici* pycnidiospores G1-1 in comparison to control F4. NTU – nephelometric turbidity unit

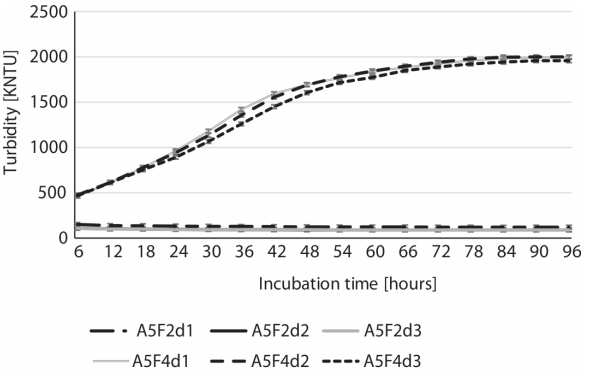

Fig. 4. Effect of filtrate F2 (prepared with *Trichoderma harzianum* A and *Zymoseptoria tritici* G1-1) on the germination of *Zymoseptoria tritici* pycnidiospores A5-1 in comparison to control F4. NTU – nephelometric turbidity unit

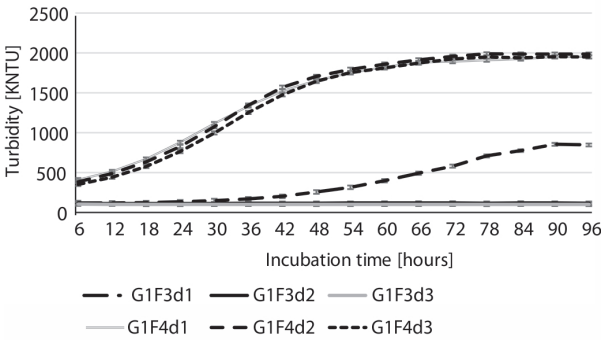

Fig. 5. Effect of filtrate F3 (prepared with *Trichoderma harzianum* A and *Zymoseptoria tritici* A5-1) on the germination of *Zymoseptoria tritici* pycnidiospores G1-1 in comparison to control F4. NTU – nephelometric turbidity unit

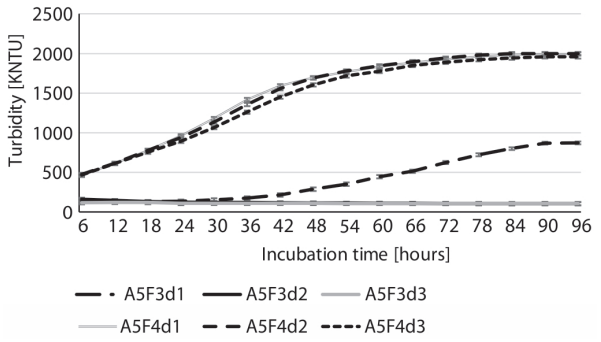

Fig. 6. Effect of filtrate F3 (prepared with *Trichoderma harzianum* A and *Zymoseptoria tritici* A5-1) on the germination of *Zymoseptoria tritici* pycnidiospores A5-1 in comparison to control F4. NTU – nephelometric turbidity unit

Secondary metabolites produced by *Trichoderma harzianum* A

The results of HPLC-MS analysis permitted the identification of 38 peptaibiotic molecules classified into nine groups: Trichocryptin, Trichobrevin, Triochocryptin, Hypocompactin, Hyporodicin, Trichocompactin, Alamethicin, Trichoferin, and Trichokonin (Table 1). *Trichoderma harzianum* A produced several peptaibiotic molecules under all the studied conditions, including: liquid and agar culture medium, cultures of strain

Table 1. Peptaibols produced by *Trichoderma harzianum* A. A5-1: *Zymoseptoria tritici* strain

Peptaibol	Molar mass (Dalton)	Peak area			
		agar medium		liquid medium	
		A	A + A5-1	A	A + A5–1
Alamethecin 1	1964	–	1.5E + 5	–	1.5E + 5
Alamethecin 31	1950	6.0 E + 3	–	4.2E + 3	–
Hypocompactin 108	752	8.5E + 5	–	1.5E + 6	–
Hypocompactin 109	766	4.6E + 5	–	3.0E + 5	–
Hypocompactin 111	714	1.0E + 5	3.1E + 5	2.5E + 5	3.0E + 5
Hypocompactin 112	728	–	5.2E + 4	–	5.0E + 4
Hyporodicin 117	1394	7.1E + 4	–	6.0E + 4	–
Hyporodicin 113	1437	4.6E + 6	3.0E + 5	3.2E + 6	3.6E + 5
Hyporodicin 114	1451	5.4E + 6	1.7E + 5	3.7E + 6	2.0E + 5
Trichocryptin-A_IIc 90	1116	–	1.0E + 6	–	1.0E + 6
Trichocryptin-A_IIIc 91	1130	–	6.0E + 4	–	8.2E + 4
Trichocryptin-A_If 89	1102	3.5E + 4	4.2E + 4	3.5E + 4	4.2E + 4
Trichocryptin-B_IIIe 85	1215	1.7E + 7	9.1E + 5	1.3E + 7	1.1E + 6
Trichocryptin-C Ia 92	1097	2.1E + 5	1.5E + 5	1.5E + 5	1.5E + 5
Trichocryptin-C IIa 93	1111	3.2E + 5	4.4E + 5	3.2E + 5	4.4E + 5
Trichocryptin-C IIIa 94	1125	3.6E + 5	4.6E + 5	3.6E + 5	4.6E + 5
Trichocryptin-D Ia 96	1196	5.2E + 4	1.8E + 6	4.8E + 4	1.2E + 5
Trichocryptin-D_IIa 96	1187	9.4E + 5	7.8 E + 5	6.6E + 5	1.1E + 6
Trichocryptin-D_IIIa 98	1201	1.5E + 7	1.1 E + 6	1.2E + 7	1.4E + 6
Trichobrevin 48	1113	–	8.8 E + 4	–	1.1 E + 5
Trichobrevin 50	1127	–	9.2 E + 5	–	1.2 E + 6
Trichobrevin 55	1129	–	2.2 E + 5	–	3.0E + 5
Trichobrevin 54	1141	–	1.4 E + 7	–	1.8E + 7
Trichobrevin 58	1143	–	3.4E + 6	–	4.4E + 6
Trichobrevin 60	1157	–	6.3E + 4	–	1.3E + 5
Trichobrevin 104	1171	–	2.0E + 5	–	2.4E + 5
Trichocompactin 5	726	1.2E + 5	1.7E + 5	1.2E + 5	1.7E + 5
Trichocompactin 9	754	–	8.8E + 4	–	1.7E + 5
Trichocompactin 39	770	–	4.3E + 4	–	1.1E + 5
Trichoferin 65	1193	–	1.1E + 6	–	1.1E + 6
Trichoferin 64	1207	5.2E + 4	1.2E + 5	2.6E + 4	1.6E + 5
Triochocryptin-B 15	1238	9.0E + 4	5.0E + 5	7.8E + 4	7.1E + 5
Triochocryptin-B 17	1252	5.2E + 4	4.6E + 5	3.5E + 4	4.6E + 5
Triochocryptin-B 33	1266	8.0E + 4	9.7E + 4	8.0E + 4	9.7E + 4
Triochocryptin-A 22	1139	–	8.1E + 4	–	7.0E + 4
Triochocryptin-A 26	1167	–	1.6E + 5	–	2.2E + 5
Triochocryptin-A 29	1181	–	1.6E + 5	–	2.2E + 5
Trichokonin 118	1866	1.8E + 4	–	5.2E + 3	–

(–) – unidentified metabolite

A alone, and in confrontation with *Z. tritici* A5-1. It is important to note that the same groups of molecules were identified in both culture media (liquid and agar), while in the control, no molecules were detected.

The major group of identified molecules is the Trichocryptin with ten molecules, which includes four subgroups: A, B, C and D. These differ in the number of residues making up the chain of each molecule.

Trichocryptin-A_IIc and A_IIIc with molar masses of 1116 Da and 1130 Da, respectively, were produced only in the culture medium of confrontation between A and A5-1 while Trichocryptin-A_If, B_IIIe, C_Ia, C_IIa, D_Ia, D_IIa and D_IIIa with molar masses ranging from 1097 Da to 1215 Da were produced in cultures of both *T. harzianum* alone and in confrontation with *Z. tritici*.

The Trichobrevin group was classified as the second abundant group of peptaibols. Seven molecules were identified in the culture extracts of strain A in confrontation with *Z. tritici* A5-1. The molar masses of these molecules ranged from 1113 Da to 1171 Da. Neither of these molecules was reported in cultures of *T. harzianum* alone.

Also, three molecules of Triochocryptin A with molar masses of 1139 Da, 1167 Da and 1181 Da were identified in the confrontation cultures between the antagonist and *Z. tritici*. Three molecules from the group of Triochocryptin-B were produced in both types of cultures. The molar masses of these molecules were: 1238 Da, 1252 Da and 1266 Da.

Four molecules of the Hypocompactin group were identified of which two molecules in the *T. harzianum* A culture with molar masses of 752 Da and 766 Da, one molecule with a mass of 728 Da in the confrontation culture and one molecule with a molar mass of 714 Da were produced in both cultures but in higher abundance in the confrontation cultures.

In the Hyporodicin group, the molecule with a molar mass of 1394 Da was produced only in cultures of *T. harzianum* A while the two molecules with molar masses of 1437 Da and 1451 Da were produced in both types of cultures, with higher intensities in cultures of strain A than in the confrontation cultures between A and A5-1.

Two molecules of the Trichocompactin and Trichoferin groups with molar masses of 726 Da and 1207 Da, respectively, were produced in the two types of studied cultures. However, the molecule with the molar mass 1193 Da of the Trichoferin group and the two molecules with molar masses 754 Da and 770 Da were identified only in the confrontation cultures of *T. harzianum* A and *Z. tritici* A5-1.

Among the most studied peptaibols, we find the Alamethicin group, which is characterizd by a diversity of subgroups. The abundance of the latter depends on the acidity of the culture medium. In our study, two molecules of Alamethicin were identified, one with a molar mass of 1964 Da in the confrontation cultures with a high intensity and one molecule with a molar mass of 1950 Da in the cultures of *T. harzianum* A but with a low intensity.

The last group of peptaibols identified in our study is Trichokonin. Just one molecule of this group with a molar mass of 1866 Da was identified only in the cultures of *T. harzianum* A.

Discussion

Our study focused on the bioactivity of three different filtrates types of *T. harzianum* strain A and the identification of the main peptaibols produced by this strain which has shown significant antagonistic potential against *Z. tritici* in vitro and in vivo (Barakat *et al.* 2018, 2019a, b). The biocontrol mechanism of *T. harzianum* is a complex process mediated by the secretion of extracellular enzymes, such as chitinases (de la Cruz *et al.* 1992), b-glucanases (Lorito *et al.* 1994) and proteinases (Geremia *et al.* 1993), and secondary metabolites (Sivasithamparam and Ghisalberti 1998). The role of *Trichoderma* antibiotics in biological control is still under discussion. While some antibiotics may be the primary factor in the biocontrol activity of a particular strain, this may not be the case for others (Harman 2000). *Trichoderma harzianum* is a well-known producer of antibiotics (Lorito *et al.* 1996) such as Dermadin, Alamethicin, Trichoticin, and Trichoziamine (Vial 1989 cited in Vinale *et al.* 2006). In addition, the bioactivity of *T. harzianum* on several pathogens is already known, but to our knowledge its activity against *Z. tritici* has not yet been described.

Antibiosis tests show that the three filtrates of *T. harzianum* A in the presence/absence of *Z. tritici* strains G1-1 and A5-1 can inhibit the germination of *Z. tritici* pycnidiospores (Figs. 1–6). Filtrate F1 inhibited 96% of A5-1 strain pycnidiospores with the 9/10 dilution after 96 hours of incubation (Fig. 2). The 9/10 dilution of the F2 and F3 filtrates resulted in an inhibition rate of G1-1 and A5-1 pycnidiospores around 95% after 4 days of incubation (Figs. 3–6). These results agree with the study of Dubey *et al.* (2007) who demonstrated that *T. harzianum* isolates T7 and T8 inhibited the growth for four races of *F. oxysporum* f. sp. *ciceris* significantly through the production of non-volatile antibiotic substances; the same *T. harzianum* isolates inhibited the growth of *F. solani* f. sp. *pisi* causing crown rot of peas by the production of non-volatile substances (Dubey *et al.* 2007). Similarly, Boureghda and Bouznad (2009) reported the complete inhibition of chickpea *Fusarium* by culture filtrate of *T. atroviride*. According to the study of Ommati and Zaker (2012), *T. harzianum*, T. longibrachiatum, *T. brevicompactum*, *T. asperellum* and *T. atroviride* showed promising ability to inhibit the growth of *Fusarium oxysporum*, the causal agent of potato wilt, through the production of non-volatile inhibitors. Vinale *et al.* (2006) demonstrated the efficacy of secondary metabolites of *T. harzianum* strains T22 and T39 against *Rhizoctonia solani*,

Pythium ultimum and *Gaeumannomyces graminis* var. *tritici*. Benouzza (2012) showed that among the five strains of *Trichoderma* spp. studied, the filtrate of *T. harzianum* strain Tma15 was the most active against *Verticillium dahliae* from the olive tree. Also, Naglot *et al*. (2015) reported that *T. viride* filtrate showed a significant level of inhibition activity against *Pestalotia theae* and *F. solani*, demonstrating the presence of an extracellular antifungal metabolite mixture. More recently, the study conducted by Almi (2016) showed that the two strains 127a and 127b of *T. harzianum* can produce diffusible substances inhibiting the mycelial growth of both pathogens *Fusarium solani* and *Cylindrosporium* sp.

To understand the antifungal activity of *T. harzianum* strain A filtrates, the extraction and identification of peptaibols produced in these filtrates was performed. Also, the intensity of peptaibols secreted by strain A in filtrates F1, F2 and F3 was compared with those produced in the agar medium and the effect of the confrontation of *T. harzianum* A with *Z. tritici* on the stimulation/inhibition of peptaibol production was studied.

According to the literature, *T. harzianum* is recognized by the importance of its filtrates on the development and growth inhibition of many fungal species (Ghisalberti *et al*. 1990). In addition, Reino *et al*. (2008) included peptaibols among the antimicrobial metabolites produced by *Trichoderma* spp. The evolution of peptaibol formation appears to be too complex to predict the type of peptaibols to be produced based on phylogenetic relationships (Neuhof *et al*. 2007; Degenkolb *et al*. 2008). Thus, a wide variety of peptaibols has been identified in the Trichoderma genus (Degenkolb *et al*. 2007, 2008; Stoppacher *et al*. 2008) since the identification of Alamethicin (Reusser 1967) and paracelsin (Bruckner and Graf 1983).

According to the results obtained in this study (Table 1), 38 peptaibiotic molecules from nine groups of peptaibols (Trichocryptin, Trichobrevin, Triochocryptin, Hypocompactin, Hyporodicin, Trichocompactin, Alamethicin, Trichoferin and Trichokonin) were identified in extracts from cultures of strain A in the presence and absence of *Z. tritici*. The same results showed that the agar or liquid culture medium had no effect on the production of peptaibols while the confrontation between strain A and *Z. tritici* promoted the production of some peptaibiotic molecules including Trichocryptin A IIc and IIc, Trichobrevins, Triochocryptin A, Hypocompactin 112, Trichocompactin 9 and 39, Alamethecin 1 and Trichoferin 65. Similarly the presence of *Z. tritici* with *T. harzianum* strain A inhibited the production of some molecules such as: Hypocompactin 108 and 109, Hyporodicin 117, Alamethecin 31 and Trichokonin 1.

The groups of: Trichocryptin, Trichobrevin, Triochocryptin, Hypocompactin, Hyporodicin, Trichocompactin, Alamethicin, and Trichoferin which were detected in extracts from confrontation cultures between *T. harzianum* A and *Z. tritici*, could contribute significantly to the inhibition of *Z. tritici* strains G1-1 and A5-1 pycnidiospores germination. Also, it is important to note that the distribution of peptaibols between taxonomic groups/species of Trichoderma is currently under investigation to explain their antagonistic properties (Degenkolb *et al*. 2006).

From these results, it is important to note that the three filtrates of *T. harzianum* A studied were very effective on the pycnidiospore germination of the two selected strains of *Z. tritici*, which allows us to affirm that the presence or absence of the pathogen strains in the *T. harzianum* A cultures did not affect the efficacy of the metabolites produced by the antagonist. Also, this efficacy was correlated to the importance and diversity of the identified secondary metabolites in the filtrates.

Acknowledgements

The authors would like to thank the Ministry of National Education, Vocational Training, Higher Education, and Scientific Research of the Kingdom of Morocco for funding the Arimnet research project entitled "Bacplant," and, we acknowledge the French ANR (Agence National de la Recherche) project Lab -Com, ESTIM for the financial support.

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